

Immunotherapeutic approaches in biliary tract carcinoma: Current status and emerging strategies

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Abstract

For biliary tract carcinoma (BTC), complete surgical

resection of tumor is only feasible in a minority of patients, and the treatment options for patients with unresectable or metastatic disease are limited. Advances in cancer immunology have led to identification of tumor-infiltrating immune cells as indicators of prognosis and response to treatment in BTC. This has also facilitated development of immunotherapy that focuses on enhancing the immune system against biliary tumors. This includes peptide- and dendritic cell-based vaccines that stimulate *in-vivo* immune responses against tumor-specific antigens. Adoptive immunotherapy, which entails the *ex-vivo* expansion of tumor-infiltrating immune cells for subsequent reintroduction, and cytokine-based therapies have been developed in BTC. Clinical studies indicate that this type of therapy is generally well tolerated. Combination therapy with dendritic cell-based vaccines and adoptive immunotherapy has shown particularly good potential. Emerging strategies through discovery of novel antigen targets and by reversal of tumor-associated immunosuppression are expected to improve the efficacy of immunotherapy in BTC. Collaborative efforts by integration of targeted immunotherapeutics with molecular profiling of biliary tumor will hopefully make a positive impact on advancing towards the goal of developing precision treatment of patients with this highly lethal disease.

Key words: Adoptive immunotherapy; Cancer vaccines; Biliary tract carcinoma; Cholangiocarcinoma; Gallbladder carcinoma; Immunotherapy; Precision treatment

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Core tip: Advances in cancer immunology have led to development of novel therapeutics that focuses on enhancing the immune system against biliary tract cancer. These include peptide- or dendritic cell-based vaccines, adoptive immunotherapy, and immunostimulatory cytokines. Immunotherapy is generally well tolerated with good potential for developing into treatment. The efficacy of immunotherapy may be improved by

reversal of tumor-associated immunosuppression and through discovery of novel antigen targets. Integration of targeted immunotherapeutics with molecular profiling of biliary tumor is expected to make a positive impact on advancing towards the goal of developing precision treatment of patients with this highly lethal disease.

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INTRODUCTION

Cholangiocarcinoma and gallbladder adenocarcinoma are the most common primary malignancies of the biliary tract. Collectively referred to as biliary tract carcinoma (BTC), these diseases are a cause of substantial morbidity and mortality. Each year in the United States alone, approximately 11000 patients are diagnosed with BTC and 3700 lives are claimed by the disease^[1].

Until recently, the treatment options available to patients with BTC primarily involved surgery, radiation, and systemic chemotherapy. Complete surgical resection is potentially curative, but it can only be achieved in the 10% of patients who present with localized disease without vascular invasion^[2]. Patients with BTC that is locally advanced, metastatic, or recurrent are typically offered single agent or combination chemotherapy, depending upon performance status. Typical regimens consist of gemcitabine, 5-fluorouracil, and platinum-based agents^[3]. Despite these interventions, clinical outcomes in BTC are generally poor. Fewer than 5% of patients with cholangiocarcinoma^[2] and 13% with gallbladder cancer^[4] survive longer than two years following diagnosis.

Advances in cancer immunology and immunotherapy have facilitated the development of additional treatment options that bring new hope to patients with BTC. This new generation of therapeutics seeks to strengthen the patient's immune system in combating malignancy, typically by priming it against tumor-specific antigens. Such treatments are more selective against malignant cells and therefore tend to be less toxic than traditional chemotherapy. Furthermore, by exerting an antitumor effect indirectly through the immune system rather than *via* direct activity against malignant cells, these therapeutic approaches can produce durable responses that persist long after the drug itself has been metabolized.

In this article, we concisely review cancer immunology as it relates to malignancies of the biliary tract. The immunotherapeutic approaches that are being investigated for use in BTC will be described, along with the data from clinical trials that have been completed thus far. We will also discuss ongoing clinical trials and

emerging strategies for immunotherapy in BTC.

CANCER IMMUNOLOGY IN BILIARY TRACT CANCER

Focusing and enhancing the antineoplastic effects of the immune system as treatment for BTC has only recently become a subject of concerted investigation. Evidence suggests that at the earliest stages of tumor development, the host immune system is capable of both detecting and controlling the disease. Over time, however, this generates evolutionary pressure that favors the proliferation of cancer cells that are less immunogenic or otherwise capable of suppressing the host immune response^[5-9]. Despite this, there often persists a small cohort of immune cells that remain able to identify and invade the tumor. The characteristics of this immune infiltrate are of prognostic value in a variety of malignancies, including BTC^[10,11]. The frequency and clinical significance of tumor infiltration by the cellular mediators of the host immune response is summarized in Table 1.

Tumor infiltration by the innate immune system

The innate immune system, consisting of the complement cascade, natural killer (NK) cells, granulocytes, and phagocytes, mounts an initial non-specific defense against infections and malignancy. The frequency of tumor infiltration by the cellular components of the innate immune system is highly variable. While fewer than half of biliary tumors are penetrated by NK cells^[12,13] or mast cells^[13], macrophages are observed in the majority of BTC^[13].

Despite correlating with outcomes in a host of other malignancies^[16-20], infiltration of BTC by the innate immune system appears to be of little clinical significance. Neither the presence of intratumoral NK cells nor mast cells is correlated with clinical outcomes^[12]. The density of tumor-infiltrating macrophages, however, appears to increase as lesions progress from pre-malignant precursors to invasive malignancy and later to metastatic disease^[13]. This is believed to be the result of activated macrophages releasing pro-inflammatory and pro-angiogenic cytokines that facilitate tumor growth. These include tumor necrosis factor- α , vascular endothelial growth factor A, and granulocyte macrophage colony-stimulating factor^[21,22].

Tumor infiltration by the adaptive immune system

The adaptive immune response is initiated by the consumption of foreign material by antigen presenting cells, most often dendritic cells. After processing the antigen for presentation, dendritic cells migrate to lymph nodes where they stimulate the proliferation of antigen-specific lymphocytes and recruit CD4⁺ T-helper cells. Activated CD4⁺ cells release cytokines that induce the differentiation of B-lymphocytes into antibody-releasing plasma cells, and activate cytotoxic CD8⁺

Table 1 Cellular mediators of innate and adaptive immune system in biliary tract carcinoma

Cell type	Frequency of infiltration	Clinical significance	Ref.
Natural killer cells	19.1%-33% overall 20% of ICC, 21% of ECC, 16% of GBC	No correlation with disease stage, grade, or survival	[12,13]
Mast cells	2% of ICC, 2.5% of ECC, 8.5% of GBC	No correlation with survival	[13]
Macrophages	87% of ICC, 70% of ECC, and 71% of GBC	Associated with more advanced disease	[13]
Dendritic cells	Not determined	Associated with improved survival	[12,14]
CD4 ⁺ helper T-lymphocytes	43% of ICC, 30% of ECC, and 34%-51% of GBC	Associated with reduced probability of metastases and improved survival in ECC	[12,13]
CD8 ⁺ cytotoxic T-lymphocytes	46% of ICC, 49%-55% of ECC, and 38%-51% of GBC	Associated with reduced probability of metastases and improved survival in ECC	[12,13,15]
B-lymphocytes / plasma cells	4.5% of ICC, 6.7% of ECC, and 10.1% of GBC	Associated with improved survival	[13]

ECC: Extrahepatic cholangiocarcinoma; GBC: Gallbladder carcinoma; ICC: Intrahepatic cholangiocarcinoma.

T-lymphocytes (CTL). After clearing the antigen, both CD4⁺ and CD8⁺ T cells may differentiate into memory T-cells that organize an expedited secondary immune response if the offending antigen is encountered again. It is these memory cells that form the physiologic basis for vaccination.

Like the innate immune system, there is considerable variability in the frequency of tumor infiltration by cells of the adaptive immune system. Although the exact percentage of BTC that contains dendritic cells is not clear, their presence appears to be nearly universal in both GBC^[12] and cholangiocarcinoma^[14]. Approximately 30%-50% of BTC is infiltrated with CD4⁺ or CD8⁺ T-lymphocytes^[12,13]. Tumor infiltration by B-lymphocytes or plasma cells is seldom observed^[13], which may be attributed to the tendency for these cells to rarely migrate outside of lymph nodes.

Tumor infiltration by the cellular mediators of the adaptive immune response is generally correlated with improved outcomes in BTC. The presence of dendritic cells^[12,14], CD4⁺ T-cells^[12], CD8⁺ T-cells^[12,15], or plasma cells^[13] within a biliary tumor is predictive of improved OS. This trend towards more favorable prognosis is consistent with findings in other malignancies, such as colorectal^[23] and esophageal carcinoma^[24]. Though it has not been reported in BTC, the subset of CD3⁺ T-cells in colorectal cancer suggests that these cells are possibly involved in vitamin D-mediated immunoprevention^[25].

IMMUNOTHERAPEUTIC APPROACHES IN BTC

While the endogenous immune response is initially successful in slowing the growth of BTC, the malignancy eventually becomes capable of evading the immune system. This occurs through intense evolutionary pressure that confers a survival advantage to cancer cells that lack foreign antigens, secrete immunosuppressive substances, or otherwise limit the effectiveness of the host immune system^[5-9]. Several approaches for potentiating or redirecting the immune response to BTC are being investigated. Vaccines based upon either peptides or dendritic cells seek to sensitize the immune

system against tumor-specific antigens. The extraction, amplification, and reintroduction of a patient's own tumor-infiltrating immune cells *via* adoptive immunotherapy is being evaluated. Treatment using immunostimulatory cytokines has been attempted.

Targets of vaccination

Through the controlled presentation of a particular antigen, vaccination primes the immune system to respond swiftly and accurately to repeat exposures in the future. This occurs, in part, through the production of memory T-cells that orchestrate this secondary response. As a result, the effectiveness of vaccination is a function of both the immune system's strength and the selection of a proper target antigen. Ideally, the target should be highly specific to malignant cells and strictly conserved within the tumor. This ensures that collateral damage to normal tissues will be minimized, while also reducing the likelihood that an antigen-negative cancer cell will arise to repopulate the tumor.

One antigen that largely fulfills these criteria is Wilm's Tumor protein 1 (WT1)^[10], a transcription factor that is normally involved in urogenital development. This protein also functions as a tumor suppressor through interactions with platelet derived growth factor receptor, epithelial growth factor receptor, c-MYC, and B-cell lymphoma 2^[26]. Approximately 68%-80% of biliary tumors harbor mutations of WT1^[26]. While the clinical significance of mutated WT1 in BTC remains unclear, similar mutations are known to correlate with poor prognosis in testicular cancer^[27], breast cancer^[28], and squamous cell carcinoma of the head and neck^[29].

Another potential target for immunization is the glycoprotein, mucin protein 1 (MUC1)^[10]. Consisting of a large and heavily glycosylated extracellular domain, MUC1 forms the hydrophilic barrier that is characteristic of BTC and other types of adenocarcinoma. This mucinous shell repels hydrophobic chemotherapeutics and obstructs immune cells, while also allowing the tumor to immerse itself in growth factors^[30]. MUC1 is over-expressed in 90% of gallbladder carcinoma^[31] and 59%-77% of cholangiocarcinoma^[31-34]. Excessive production of MUC1 in BTC is typically indicative of more

advanced disease^[32] and impaired OS^[31-33].

Peptide-based vaccines and personalized peptide vaccination

Peptide-based vaccines are among the most investigated class of cancer immunotherapy. The vaccine typically contains one or more antigens that are heavily expressed by malignant cells and often emulsified in Freund's adjuvant to increase immunogenicity. The goal of immunization is to stimulate mass-production of memory lymphocytes that can generate a strong secondary immune response against cancer cells that bear the particular antigen.

The efficacy of any single peptide-based vaccine is intrinsically limited, however, by the heterogeneity of BTC. Although the overall expression of certain antigens, such as WT1 and MUC1, is often increased within biliary tumors, the distribution of these antigens is non-uniform. While some cells over-express the antigen, there are often others from which it is entirely absent. Furthermore, the tenacity with which the immune system responds to these antigens varies widely between patients, even among those with similar HLA types^[35]. This is due, in part, to differences in the number of lymphocyte precursors that are maximally sensitive to the particular antigen^[36].

Personalized peptide vaccination seeks to overcome these limitations by immunizing patients against multiple antigens simultaneously. While it is likely that a tumor will harbor cells that lack any single antigen, the odds are exponentially less that any single cell will lack each of 3 to 4 antigens that are individually quite common. This has the additional benefit of theoretically counteracting the pressure of selection for tumor cells that lack the target antigens^[35]. To bypass individual differences in sensitivity to particular antigens, it is possible to measure the frequency of antigen-sensitive CTL precursors within each patient. They may then be vaccinated against only the antigens to which they will most likely respond^[36].

Dendritic cell-based vaccines

Similar to their peptide-based counterparts, dendritic cell-based vaccines expose the immune system to an antigen with the goal of generating memory lymphocytes that will produce a robust secondary immune response. Rather than simply introducing a peptide that requires subsequent processing and presentation to the adaptive immune system, these vaccines contain dendritic cells that are already loaded with antigen. These vaccines may be prepared against a particular antigen or more generally against a tumor lysate. While the latter approach stimulates the immune system against a larger number of antigens and theoretically produces a greater antitumor response, it may also carry a risk of autoimmunity. While the use of dendritic cells-based vaccines against BTC remains in its infancy, the success of sipuleucel-T in treating prostate cancer^[37]

demonstrates the promise that these therapeutics may someday fulfill.

Adoptive immunotherapy

Unlike the treatments described previously, adoptive immunotherapy is not intended to produce an *in-vivo* immune response. Instead, a patient's own tumor-infiltrating lymphocytes are extracted, modified, and induced to clonally proliferate *ex-vivo*. This expanded population of tumor-specific immune cells is then reintroduced, and they migrate back to the tumor and continue to combat its growth. The effectiveness of this treatment may be further increased by depleting the patient's existing lymphocyte population with cytotoxic chemotherapy in advance of returning the grafted lymphocytes. This is believed to prolong the lifespan of the transplanted cells.

Immunostimulating cytokines

The cytokine, interleukin-2 (IL2) is a potent anti-neoplastic agent due to its ability to stimulate the proliferation and cytotoxic effects of CD8⁺ T-lymphocytes^[38-40]. Administering IL2 as a monotherapy or in combination with adoptive immunotherapy is an effective treatment for certain malignancies, such as melanoma^[41,42] and renal cell carcinoma^[42,43]. Treatment with IL2 is associated with a substantial side effect profile that includes nephrotoxicity, extravasation of fluid secondary to increased vascular permeability, and rarely transient myocarditis^[40,41].

CLINICAL STUDIES OF IMMUNOTHERAPY IN BTC

Each type of immune-based approach described above has been evaluated for therapeutic efficacy in patients with BTC. Many of these agents have been studied as monotherapy as well as in combination with traditional chemotherapy or targeted therapeutics. The completed clinical trials of immunotherapy in BTC are described below and the compiled data are summarized in Table 2.

Peptide-based vaccines

To date, most clinical studies of immunotherapy in BTC have focused on peptide-based vaccines, often targeted against WT1 or MUC1. This type of treatment is generally well tolerated; however it appears to exert only a modest anti-neoplastic effect when administered as monotherapy.

Vaccines against WT1 are often administered in combination with gemcitabine based chemotherapy. Preclinical studies suggest that gemcitabine upregulates the expression of WT1, thereby theoretically enhancing the effect of immunization^[53]. In a phase I trial, anti-WT1 vaccination and gemcitabine were administered to patients with unresectable gallbladder cancer, cholangiocarcinoma, or pancreatic adenocarcinoma^[44]. This regimen increased the number of WT1-specific

Table 2 Trials of immunotherapy in biliary tract carcinoma

Immunotherapy	Treatment regimens	Phase	n	Types of BTC	OS (mo)	PFS (mo)	Ref.
Peptide-based vaccine (WT1)	Peptide vaccine + gemcitabine	I	25	Pancreatic, GBC, ICC, ECC	9.3	--	[44]
Peptide-based vaccine (WT1)	Peptide vaccine monotherapy	I	9	Pancreatic, CC	--	--	[45]
Peptide-based vaccine (NUF2, CDH3, KIF20A)	Peptide vaccine triple therapy	I	9	GBC, ICC, ECC	9.7	3.4	[46]
Peptide-based vaccine (LY6K, TTK, IGF2BP3, DEPDC1)	Peptide vaccine quadruple therapy	I	9	GBC, ICC, ECC	12.3	5	[47]
Peptide-based vaccine (Many)	Personalized peptide vaccination +/- chemotherapy	II	25	GBC, ICC, ECC	6.7	--	[48]
Dendritic cell-based vaccine (MUC1)	Dendritic cell vaccination +/- chemotherapy +/- radiotherapy	I / II	12	Pancreatic, CC	26	--	[49]
Dendritic cell-based vaccine (WT1, MUC1)	Peptide vaccine +/- chemotherapy	--	65	GBC, ICC, ECC	--	--	[50]
Dendritic cell-based vaccine, adoptive immunotherapy	Surgery + dendritic cell vaccine + T-cell transfer <i>vs</i> surgery alone	--	36	ICC	31.9	18.3	[51]
Interleukin-2	Induction cisplatin + gemcitabine, consolidation capecitabine + radiation, and maintenance IL-2 + 13-cis-retinoic acid	II	54	Pancreatic, GBC, CC	> 27.5	16.2	[52]

CC: Cholangiocarcinoma; OS: Overall survival; PFS: Progression-free survival; WT1: Wilm’s tumor 1; NUF2: Cell division cycle associated protein 1; CDH3: Cadherin 3; DEPDC1: DEP domain containing 1; ECC: Extrahepatic cholangiocarcinoma; GBC: Gallbladder cancer; ICC: Intrahepatic cholangiocarcinoma; IGF2BP3: Insulin-like growth factor-II mRNA binding protein 3; KIF20A: Kinesin family member 20A; LY6K: Lymphocyte antigen 6 complex locus K; MUC1: Mucin 1.

lymphocytes in circulation, but it did not improve clinical outcomes or increase toxicity over that which is expected from gemcitabine monotherapy. At the present time, a phase II study of WT1 vaccination as an adjunct to combination chemotherapy with gemcitabine plus cisplatin is underway^[53]. This study aims to establish the 1-year OS rate for patients receiving treatment.

Similar to WT1, peptide-based immunization against MUC1 is well tolerated but it lacks definite proof of clinical efficacy. In a phase I trial of nine patients with advanced stage cholangiocarcinoma or pancreatic adenocarcinoma, monotherapy with peptide-based vaccines against MUC1 produced only a single instance of stable disease^[45]. Despite failing to influence outcomes, vaccination did generate a robust anti-MUC1 IgG response in 78% of patients with negligible toxicity. In the future, vaccination against MUC1 could fill a niche in addition to gemcitabine or fluorouracil-based chemotherapy. This is because preclinical studies have found that these agents increase the expression of MUC1 in cholangiocarcinoma cells^[53]. Further research is indicated to determine the safety and efficacy of such regimens.

The prospect of combination therapy with multiple peptide-based vaccines has been explored. Triple therapy with vaccines against cell division cycle associated protein 1 (NUF2), cadherin 3 (CDH3), kinesin family member 20A in patients with GBC, ICC, and ECC was investigated in a phase I clinical trial^[46]. This treatment stimulated peptide-specific T-cell responses in all patients and 55% achieved stable disease. A four vaccine regimen against lymphocyte antigen 6 complex locus K (LY6K), TTK protein kinase, insulin-like growth factor-II mRNA binding protein 3, and DEP domain containing 1 has also been tested in a phase I trial of

nine patients with BTC^[47]. Peptide specific T-cell responses were generated in 78% of patients receiving this regimen and clinical responses were observed in 67%. In both trials of combination therapy with peptide-based vaccines, the presence of an injection site reaction correlated with OS^[46,47]. This underscores the reliance of this treatment upon provoking a strong immune response to generate an anti-tumor effect. Aside from these local dermatologic reactions, treatment-associated toxicity was minimal.

The efficacy of combination vaccination may be refined by individualizing the process by which targets are selected. This approach of personalized peptide-based vaccination was assessed in a phase II trial of 25 patients with either gallbladder adenocarcinoma or cholangiocarcinoma^[48]. Patients received as many as 4 of 31 possible vaccines in addition to systemic chemotherapy, if their performance status could support such treatment. This regimen produced stable disease in 80% of patients and negligible toxicity beyond that which is typically associated with chemotherapy.

Dendritic cell-based vaccines

Immunotherapy with antigen-pulsed dendritic cells is exceptionally well tolerated, and it appears to be efficacious against BTC. In a combined phase I / II trial, 12 patients with BTC or pancreatic adenocarcinoma received an anti-MUC1 dendritic cell-based vaccine following tumor resection and, in some instances, chemoradiation^[49]. A median OS of 26 mo was observed, while 33% of patients survived longer than 50 mo without evidence of disease recurrence. While this study was not designed to differentiate between durable responses that occur due to vaccination and those that arise from complete surgical resection, it is conceivable

Table 3 Ongoing clinical trials of immunotherapy in biliary tract carcinoma

Agent	Treatment regimen	Phase	Estimated date of completion	Sponsoring Institution	Identification number
Cytokine induced killer cells	Cytokine induced killer cell monotherapy	I / II	May, 2016	Siriraj Hospital	NCT01868490
Tumor infiltrating lymphocytes	Tumor infiltrating lymphocytes + IL-2 + cyclophosphamide + fludarabine	II	December, 2019	National Cancer Institute	NCT01174121
Poly-ICLC	Cyclophosphamide + radiation therapy + TACE + poly-ICLC	I / II	July, 2014	Rutgers, the State University of New Jersey	NCT00553683

IL-2: Interleukin-2; Poly-ICLC: Polyinosinic-polycytidylic acid polylysine carboxymethylcellulose; TACE: Transcatheter arterial chemoembolization.

that the combination of adjuvant chemotherapy, radiation therapy, and immunotherapy eliminated microscopic residual disease after surgery.

In another trial, dendritic cell-based vaccines against WT1 and/or MUC1 in combination with chemotherapy was evaluated in 65 patients with unresectable, metastatic, or recurrent BTC^[50]. This regimen was well tolerated and 15% of patients had stable disease following 6 mo of treatment. Although the response rate did not differ between patients who were vaccinated against one or both targets, the correlation between post-immunization fever and improved survival does suggest the responses generated by this regimen may be at least partially attributed to immune activation.

Adoptive immunotherapy

Direct transfer of cellular immunity *via* adoptive immunotherapy has also been investigated for use in BTC. In a study of 36 patients with intrahepatic cholangiocarcinoma, surgery alone was compared to surgery followed by combination adoptive immunotherapy with tumor-lysate pulsed dendritic cells and transfer of activated T-cells^[51]. Patients who received adjuvant immunotherapy experienced nearly double the OS of those treated with surgery alone with minimal toxicity. Among the 16 patients who produced the largest injection site reaction, median OS was 95.5 mo.

Similar durable and dramatic responses to combined immunotherapy with dendritic cell-based vaccines and activated T cell transfer have been described in case reports of patients with cholangiocarcinoma^[54] and gallbladder cancer^[55]. Anecdotal evidence also suggests that combining T-cell based adoptive immunotherapy with cetuximab may have activity against malignant ascites and peritoneal carcinomatosis due to metastatic cholangiocarcinoma^[56].

IL2 maintenance therapy

The use of IL2 as a maintenance therapy was explored in a multicenter phase II trial of 54 patients with pancreatic adenocarcinoma or BTC^[52]. These patients initially received 3 cycles of combination chemotherapy with cisplatin and gemcitabine as induction therapy. Patients who remained progression-free were subsequently treated with concurrent capecitabine and radiotherapy as consolidation, followed by maintenance

IL2 and 13-cis-retinoic acid. The progression-free survival (PFS) and overall survival (OS) for all patients enrolled in this study was 6.8 and 12.1 mo, respectively. Outcomes were notably better when considering only the subset of patients who were able to complete the entire course of treatment, however, with median PFS of 16.2 mo and OS that had not yet been reached after a median follow-up of 27.5 mo. Further investigation will be needed to determine whether this differential survival is truly due to a response to treatment, or if those patients simply had more indolent disease independent of therapy.

ONGOING CLINICAL TRIALS OF IMMUNOTHERAPY IN BTC

Currently, several clinical trials of immunotherapy in malignancies of the biliary tract are ongoing and as listed in Table 3. These studies utilize different immunotherapeutic approaches. In one study, cytokine induced killer cells are employed as monotherapy. In another study, adoptive transfer of tumor-infiltrating lymphocytes is combined with IL2 and chemotherapy. In attempt to reverse systemic immunosuppression, the immunomodulatory agent, polyinosinic-polycytidylic acid polylysine carboxymethylcellulose, is used in combination with chemotherapy and radiation therapy. In those two studies involving chemotherapy, low-dose metronomic cyclophosphamide is used to eliminate the immunosuppressive regulatory T lymphocytes (T_{reg}) and prevent tumor-associated angiogenesis.

CONCLUSION

Immunotherapy in BTC has been under active investigation and tremendous opportunities exist for developing it into a safe and effective treatment of patients with this disease. Clinical studies indicate that this type of therapy is generally well tolerated. The efficacy of immune-based treatment of BTC is improving as the complex interactions between the immune system and biliary tumors are better understood. Combination therapy with dendritic cell-based vaccines and adoptive immunotherapy has shown particularly good potential. Several directions for future investigation of immunotherapy that may improve the clinical

outcomes of patients with this disease are described as follows.

Preliminary studies suggest that the distribution and types of immune cells that infiltrate biliary tumors may be used to predict the likelihood that an individual tumor will respond to a particular chemotherapy regimen^[57]. Further characterizing these associations could be clinically beneficial, as it would provide a physiologic basis for selecting therapy as an adjunct to the current paradigm that relies upon tumor histology and stage. On the other hand, application of mass spectrometry and genomic sequencing to discover new antigens^[58] may help facilitate development of novel strategies for targeted immunotherapy in BTC. Furthermore, evidence suggests that increased inflammatory signaling *via* IL6 is associated with reduced response to vaccination^[36,48]. The hypothesis that addition of the IL6 receptor antagonist tocilizumab enhances the effects of vaccination remains to be tested.

Besides, tumor evasion of the immune system is often mediated by cytotoxic T-lymphocytes associated antigen 4 (CTLA4) or the interaction between programmed cell death 1 (PDCD1, also known as PD1 or CD279) and its ligand (PDCD1LG1, also known as PDL1 or CD274)^[59]. It will be important to investigate the potential of blocking these immunosuppressive pathways with monoclonal antibodies in conjunction with the currently used immunotherapeutic approaches in BTC. The anti-CTLA4 antibody ipilimumab has shown great promise in other malignancies such as melanoma^[59], but it has not yet been studied in BTC. Similarly, pembrolizumab and nivolumab, monoclonal antibodies that target PD1/CD279 signaling have been found to improve anti-tumor T-cell response and induce tumor regression in subsets of patients with melanoma, renal cell carcinoma, and non-small-cell lung cancer^[8,60,61]. Preclinical studies suggest that immunohistochemical analysis for PDL1/CD274 in biliary tumors may help identify the patients who are likely to benefit from such therapeutics^[62].

The synergistic relationships between cytotoxic chemotherapy and immunotherapy deserve further investigation for treatment of BTC. In one study, gemcitabine, which is a mainstay of treatment in BTC, was found to enhance cell-mediated immunity *via* increased expression of HLA on malignant cells^[63]. Platinum-based agents have a similar effect on HLA expression, while also reducing PDL2/CD273-mediated suppression of antigen-specific T-lymphocytes^[64]. It is plausible that the addition of gemcitabine and cisplatin to immunotherapy could further improve the treatment responses.

Ultimately, the goal is to combine the advances in cancer immunotherapy with those of targeted therapy and molecular profiling to develop precision treatment for improving the clinical outcomes of patients with this highly lethal disease.

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