Tumor immune microenvironment and the current immunotherapy of cholangiocarcinoma (Review)

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Abstract. Cholangiocarcinoma (CCA) is a highly heterogeneous malignancy originating from the epithelial system of the bile ducts, and its incidence in recent years is steadily increasing. The immune microenvironment of CCA is characterized by diversity and complexity, with a substantial presence of cancer-associated fibroblasts and immune cell infiltration, which plays a key role in regulating the distinctive biological behavior of cholangiocarcinoma, including tumor growth, angiogenesis, lymphangiogenesis, invasion and metastasis. Despite the notable success of immunotherapy in the treatment of solid tumors in recent years, patients with CCA have responded poorly to immune checkpoint inhibitor therapy. The interaction of tumor cells with cellular components of the immune microenvironment can regulate the activity and function of immune cells and form an immunosuppressive microenvironment, which may cause ineffective immunotherapy. Therefore, the components of the tumor immune microenvironment appear to be novel targets for immune therapies. Combination therapy focusing on immune checkpoint inhibitors is a promising and valuable first-line or translational treatment approach for intractable biliary tract malignancies. The present review discusses the compositional characteristics and regulatory factors of the CCA immune microenvironment and the possible immune escape mechanisms. In addition, a summary of the advances in immunotherapy for CCA is also provided. It is hoped that the present review may function as a valuable reference for the development of novel immunotherapeutic strategies for CCA.

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1. Introduction

Cholangiocarcinoma (CCA) originates from the bile duct epithelium and is the second most common malignant tumor of the hepatobiliary system, which accounts for ~3% of all digestive tract tumors. CCA is characterized by a specific anatomical position, insidious clinical symptoms and an early tendency for neural-vascular invasion and lymph node metastases. Therefore, the majority of patients are diagnosed at an advanced stage with either locally advanced tumors or distant metastases, precluding them from undergoing surgical intervention. While a minority of patients may qualify for surgical resection, the disease often exhibits a propensity for recurrence and metastasis even after radical surgery. Furthermore, the 5-year survival rate of patients remains dismally low, at <10%, accompanied by a staggering 1-year recurrence rate of ~60% (1-3). Additionally, CCA has exhibited resistance to systemic therapies, such as chemotherapy and targeted treatments. Consequently, there is an urgent need for the development of innovative treatment approaches.

The progress in cancer immunology holds significant promise for the development of novel treatment approaches for CCA. Recent studies have revealed a close association between the majority of CCA cases and the biliary system, which is characterized by persistent, long-term chronic inflammation. The tumor immune microenvironment (TIME) refers to the spatial organization and abundance of immune cells, which play a pivotal role in tumorigenesis and development. The TIME of CCA is characterized by significant interstitial fibrosis and infiltration of abundant cancer-associated fibroblasts (CAFs), as well as pro-cancer and pro-inflammatory immune cells, such as tumor-associated macrophages (TAMs), tumor-associated neutrophils (TANs) and tumor-infiltrating lymphocytes (TILs). Through interactions with tumor cells,

these immune cells play a crucial role in regulating specific biological behaviors of CCA, including tumor growth, angiogenesis, lymphangiogenesis, invasion and metastasis (4). Additionally, these immune cells have the potential to serve as prognostic factors associated with the clinical outcomes of patients with CCA (5,6).

Cancer immunoediting is founded on the concept that the immune system has the dual capacity to suppress tumor growth and alter tumor immunogenicity. This concept encapsulates the dynamic interactions between the immune system and tumors throughout various stages, which can be delineated into three successive phases: Elimination, equilibrium and escape (7,8). In the elimination phase, the innate and adaptive immune systems cooperate to eradicate tumor cells, rendering the tumor undetectable. However, in the event that certain subclonal tumor cells succeed in evading the cytotoxic effects of the immune system, they progress to the following phase. The equilibrium phase represents the efforts of the adaptive immune system to modify the immunogenicity of tumor cells, allowing them to evade immune surveillance and avoid destruction. It is important to note that at this stage, the growth of tumor cells is restricted or may even come to a halt. These immunogenically reduced (immuno-edited) tumor cells then progress to the escape phase, where they exhibit typical tumor characteristics, such as unlimited growth and can be detected through clinical means. Cancer immunoediting is characterized by the recognition of antigens expressed by tumor cells by T-cells, which can lead to either the death of tumor cells or a reduction in their immunogenicity. As a result, T-cells play a predominant role in cancer immunoediting (9). During all stages of cancer immunoediting, the components of the TIME interact with each other, which may impact or inhibit the activation and/or function of T-cells and eventually influence their antitumor effects. The TIME plays a crucial role in the cancer immunoediting process, with its components actively participating in all stages of cancer immunoediting.

With the application of immunotherapy in solid tumors, immune-mediated primary or adjuvant therapy is increasingly recognized as having immense potential in CCA, which functions by enhancing the immune response against tumors, involving both innate and adaptive immune cells. The inhibition of signaling pathways mediated by immune checkpoints has been proposed as a potential therapeutic strategy for CCA. Based on this premise, immune checkpoint inhibitors (ICIs) have been widely used in patients with CCA and have shown promising results for the treatment of CCA (10-12). However, the response rate to immunotherapy is relatively low, and only a small portion of patients can benefit from it. Mounting evidence has indicated a connection between the TIME and the response to immunotherapy, and the failure of immunotherapy may be partially attributed to the high heterogeneity and intricate TIME of CCA.

The present review aimed to provide insight into the compositional characteristics and regulatory factors governing the TIME of CCA, shedding light on possible immune escape mechanisms. Furthermore, the recent advances in immunotherapy for CCA are summarized. The aim of the present review was to provide a valuable reference point for the development of innovative immunotherapeutic strategies tailored to the unique challenges posed by CCA.

2. Tumor immune microenvironment of cholangiocarcinoma

CAFs. CAFs are activated myofibroblasts and are characterized by the expression of α-smooth muscle (α-SMA) actin and Tenascin C protein (13,14). They constitute the primary cell population responsible for the fibrotic stroma in CCA. Current evidence suggests that CAFs are a heterogeneous group of cells derived from various lineages, including pericytes, mesenchymal stem cells, adipocytes, liver resident hepatic stellate cells (HSCs), portal fibroblasts and bone marrow-derived precursor cells (15-17). In the study by Affo *et al* (18), it was demonstrated that HSCs are a major source of CAFs, and among the CAF subpopulations, HSC-derived CAFs engage in the most significant ligand-receptor interactions with CCA cells. CAFs influence tumor progression by tumor extracellular matrix (ECM) remodulation, and by interacting with tumor cells and immune cells. In previous study using a syngeneic orthotopic rat model of CCA, the induction of CAF apoptosis using the BH3 mimetic navitoclax resulted in reduced primary tumor growth, as well as in the inhibition of tumor lymphatic vascularization, regional lymph node metastases and peritoneum metastases (19). HSC-derived CAFs can trigger the secretion of hepatocyte growth factor (HGF) from inflammatory CAFs. This process occurs through a direct interaction involving the HSC-CAF-tumor pathway, which subsequently promotes the proliferation of intrahepatic CCA (iCCA) cells by means of mesenchymal-epithelial transition (MET) factor expressed by the tumor (18). Furthermore, it has been reported that high expression of α-SMA is associated with poor survival outcomes in patients with CCA (20,21).

CAFs secrete various soluble cytokines, including HGF, transforming growth factor β1 (TGF-β1), epidermal growth factor (EGF), connective tissue growth factor and stromal cell-derived factor-1 (SDF-1), which can enhance the malignant phenotype of CCA cells (22). Heparin-binding EGF, released by CAFs, interacts with EGF receptor (EGFR) on the plasma membrane of CCA cells to activate EGFR. This activation, in turn, stimulates ERK1/2 and STAT3, leading to the nuclear translocation of β-catenin and disruption of the adherens junction complexes with E-cadherin internalization. The nuclear translocation of β-catenin triggers a transcriptional program that promotes tumor progression (23). Additionally, it has been demonstrated that the disruption of E-cadherin-mediated adherens junctions leads to epithelial-to-mesenchymal transition (EMT), a cellular process strongly associated with cancer progression (24). A previous study proved that SDF-1 (also known as CXCL12) released by WI-38 fibroblasts promoted iCCA cell migration (25). CAF-derived SDF-1 binds to the C-X-C chemokine receptor (CXCR)4 on CCA cells in a paracrine manner, stimulating ERK1/2 and AKT signaling to increase the invasive ability of CCA (26). A recent study divided CAFs into inflammatory and growth factor-enriched and myofibroblastic (myCAF) subpopulations, which exhibited different ligand-receptor interactions (18). myCAFs synthesize and secrete hyaluronan synthase 2 (Has2) after interacting with CCA cells. Has2 exerts pro-tumorigenic effects via binding to non-tumor cells or receptors other than CD44. Therefore, myCAFs promote tumor growth through Has2, but not type I collagen (18). In addition, hyaluronan (HA) is associated with tumor promotion, treatment resistance and a poor prognosis

in pancreatic, head and neck, colorectal, gastric and liver cancer (27). The molecular size and degradation of HA are also factors in its bioactivity; high-molecular-weight HA is considered to be antitumorigenic, whereas low-molecular-weight HA is pro-inflammatory and tumor-promoting (28,29). Furthermore, vascular CAFs express high levels of interleukin (IL)-6, leading to notable changes in the epigenetics of iCCA cells. These alterations notably include the increased expression of enhancer of zeste homolog 2, consequently intensifying the malignancy of the tumor cells (30).

CCA cells recruit and activate fibroblasts or precursor cells of myofibroblasts via platelet-derived growth factor (PDGF)-D and TGF-β1. PDGF-D released by CCA cells contributes to fibroblast aggregation (31). In turn, the binding of CAF-secreted PDGF-BB to PDGFRβ in CCA cells decreases the susceptibility of CCA cells to tumor necrosis factor (TNF)-α-related apoptosis-inducing ligand, inducing tumor growth and metastasis (32). In addition, PDGF-D can stimulate fibroblast to secrete vascular endothelial growth factor (VEGF)-A and VEGF-C that increase the markedly generation of tumor lymphangiogenesis and lead to the invasion of tumor cells in lymphatic vessels (33). Therefore, interacting paracrine loops exist between CAFs and CCA cells, establishing a bidirectional reinforcing relationship (Fig. 1).

CAFs can secrete major ECM components, such as periostin (PN) and various matrix metalloproteinases (MMPs) (22). PN can interact with components, such as collagen type I, Tenascin C and integrins (34). Previous studies have highlighted that when PN combines with integrins, it activates various signaling pathways, influencing downstream molecules, and thereby contributing to tumor progression (35-38). In the context of CCA, PN has been shown to enhance invasion through the ITGα5β1/PI3K/AKT pathway (39). Furthermore, it induces EMT, promoting CCA migration, primarily through the integrin α5β1/TWIST-2 axis (40). MMPs play a crucial role in degrading and remodeling the ECM, thereby contributing to tumor progression. Among these MMPs, CAFs have been shown to produce MMP1, MMP2, MMP3 and MMP9, all of which collectively increase tumor aggressiveness (41).

Innate immune cells

Macrophages. Macrophages play a pivotal role in regulating tumor cell proliferation and progression by releasing various inflammatory factors and cytokines. Notably, they are the most commonly encountered immune infiltrating cells within the tumor microenvironment. Of particular concern in CCA is the presence of high levels of M2 macrophages, which have been shown to be strongly associated with carcinogenesis and poor outcomes in with CCA, as evidenced by prior studies (42-44). TAMs, a subtype of M2 macrophages, exert potent pro-tumor effects. The shift of macrophages toward this alternative M2 phenotype is primarily orchestrated by the actions of specific signaling pathways, notably involving IL6/STAT3 and PCAT6/miR-326/RohA pathway (43,45). Notably, Kitano *et al* demonstrated an association between TAM infiltration, increased levels of Tregs and TANs, and a poor recurrence-free survival (RFS) (46). TAMs participate in remodeling the ECM via secretion of MMPs and the release IL-4, IL-8, IL-10, chemokine ligand (CCL)2, CCL22 and CCL17 to recruit immunosuppressive cells, such as TANs, myeloid-derived suppressor cells (MDSCs) and regulatory T-cells (Tregs), to form the suppressive immune microenvironment (47).

TAMs play a multifaceted role in promoting tumorigenesis and can exert their tumor-promoting effects by interacting with CCA cells and TANs (Fig. 2). CCA cells can produce IL-6 and TGF-β, which are involved in the activation of TAMs. In a reciprocal interaction, TAMs release significant amounts of IL-10, which, can activate the STAT3 pathway in CCA cells. This activation, in turn, enhances the migration and invasion of tumor cells, largely through the process of EMT (48). Additionally, activated TAMs are capable of producing molecules, including VEGF-A, angiopoietin, IL-8, cyclooxygenase-2 and inducible nitric oxide synthase, to promote tumor angiogenesis (43,49). Furthermore, CCA cells express certain Wnt ligands, including Wnt3, Wnt5a, and Wnt7b, which have the capability to recruit and activate TAMs. Subsequently, TAMs release Wnt, which in turn stimulates the Wnt/β-catenin pathway, leading to increased tumor cell proliferation (50,51). Moreover, *in vitro* experiments involving the inhibition of Wnt signaling using a Wnt inhibitor have revealed a significant reduction in CCA proliferation and an increase in apoptosis. These effects have been observed in mouse and rat models of CCA, ultimately resulting in tumor regression (52). TAMs secret IL-6 to promote the activation of TANs and CCA cells can express epithelial-derived neutrophil-activating peptide-78 (ENA-78) to recruit TANs mediated by the PI3K-AKT and ERK1/2 signaling pathways (53). The interaction between TANs and TAMs leads to the production of oncostatin M (OSM) and IL-11 by TANs and TAMs, respectively. Both OSM and IL-11 have been found to stimulate the STAT3 pathway in CCA cells, resulting in increased tumor cell proliferation and invasion. Of note, when STAT3 is knocked down, it mitigates the pro-tumor effects of TANs and TAMs in iCCA (54).

TANs. The role of TANs in tumorigenesis and development is still under investigation. TANs are likely to assume the N2 subtype, which is distinct from N1 neutrophils that are activated in normal tissue (55,56). The activation of the N2 subtype neutrophils is mainly induced by TGF-β and granulocyte colony-stimulating factor (CSF) (57). Activated N2 neutrophils can release various growth factors, enzymes and cytokines to promote tumor growth, shape the TIME and stimulate angiogenesis (58). The high level of TAN infiltration tends to be associated with a poor prognosis, as it is related to decreased overall survival (OS) and RFS of patients with CCA (46,59). However, a recent study proposed a contrasting view, suggesting that patients with biliary tract cancer (BTC) with a higher neutrophil infiltration exhibit an improved prognosis (60). Owing to the limited amount of evidence and the conflicting findings, it remains challenging to arrive at a definitive conclusion regarding the prognostic significance of TAN infiltration. High levels of TAN infiltration have been shown to promote the growth and invasion of tumors *in vivo*, although they do not appear to alter the *in vitro* proliferative and invasive abilities of iCCA cells (53).

MDSCs. MDSCs are a group of heterogeneous immune cells which exert potent immunosuppressive effects that can inhibit various immune cell activities. Chronic inflammation functions as a stimulus for MDSCs, prompting them to synthesize molecules such as arginase, reactive oxygen

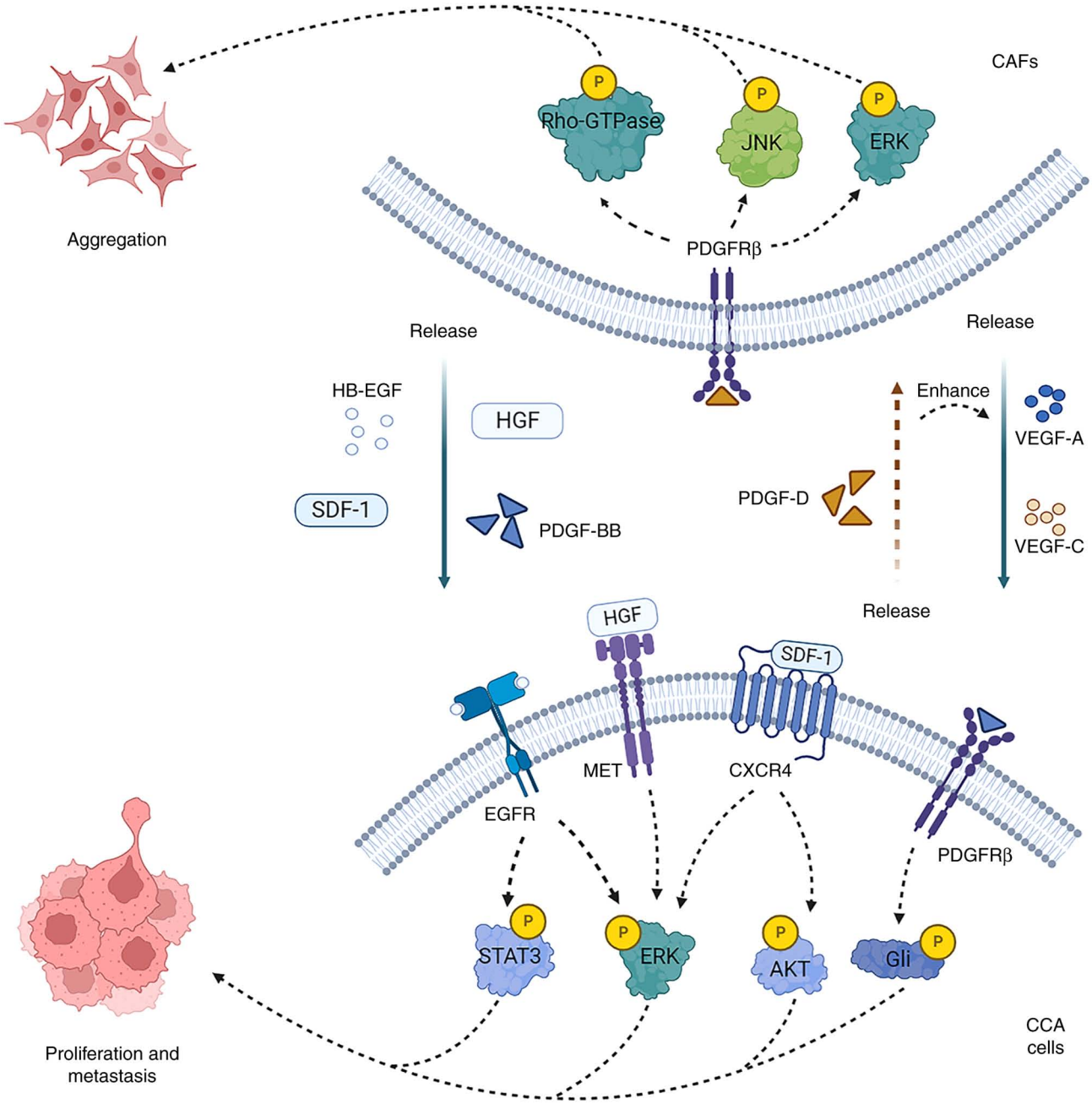

Figure 1. Interactions between CAFs and CCA cells. CAFs release HGF, TGF-β1, HB-EGF and SDF-1 to enhance the malignant phenotype of CCA cells. HB-EGF, released by CAFs, can interact with EGFR on the plasma membrane of CCA cells to activate EGFR and stimulate ERK and STAT3, leading to the nuclear translocation of β-catenin and the disruption of adherens junction complexes with E-cadherin internalization, triggering a transcriptional program involved in tumor progression. CAF-derived SDF-1 binds to CXCR4 on CCA cells in a paracrine manner, which stimulates ERK1/2 and AKT signaling to increase the invasive ability of CCA. The HGF interaction with MET ligand expressed by CCA cells can activate ERK signaling pathway to accelerate tumor growth. PDGF-D released by CCA cells interacting with the cognate receptor PDGFRβ presenting in fibroblasts contributes to fibroblast aggregation. PDGF-D can stimulate fibroblast to secrete VEGF-A and VEGF-C, that increase the generation of tumor lymphangiogenesis pronouncedly and lead to invasion of tumor cells in lymphatic vessels. In turn, binding of CAF-secreted PDGF-BB to PDGFRβ in CCA cells can activate the Hedgehog signaling to promote tumor proliferation and metastasis. CCA, cholangiocarcinoma; CAF, cancer-associated fibroblasts; HGF, hepatocyte growth factor; HB-EGF, heparin-binding epidermal growth factor; SDF-1, stromal cell-derived factor-1; CXCR4, C-X-C chemokine receptor 4; PDGF, platelet-derived growth factor; VEGF, vascular endothelial growth factor.

species, inducible nitric oxide synthase and indoleamine 2,3-dioxygenase. Furthermore, MDSCs release immunosuppressive factors, such as TGF-β and IL, which act to curtail the function of cytotoxic T-lymphocytes, natural killer (NK) cells and their respective subpopulations, thereby achieving a state of immunosuppression (61). The accumulation of MDSCs in the TIME has been linked to heightened immune evasion and increased resistance to immunotherapy in various types of cancers (62,63). Studies on primary hepatocellular carcinoma (HCC) have shown that MDSCs play a role in promoting the development of Tregs, the inactivation of $CD8^+$ T-cells, and the suppression of the cytotoxic activity of NK cells. Furthermore, elevated levels of mononuclear MDSCs in the peripheral blood have been shown to

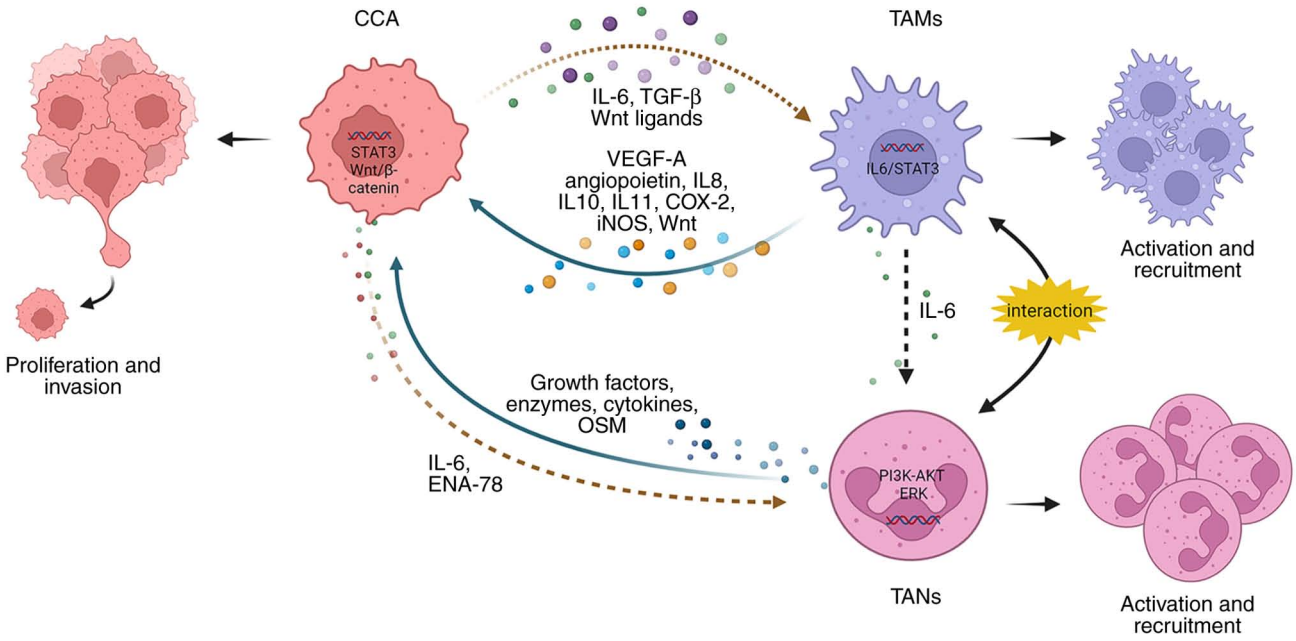

Figure 2. Associations between CCA, TAMs and TANs. CCA can produce IL-6 and TGF-β to activate TAMs. In response, TAMs release IL-10 to activate the STAT3 pathway and enhance the migration and invasion of the tumor cells. Moreover, activated TAMs contribute to tumor angiogenesis by producing VEGF-A, angiopoietin, IL-8, COX-2 and iNOS. CCA cells express certain Wnt ligands, which assemble and activate TAMs. These TAMs, in turn, secrete Wnt, thereby stimulating the Wnt/β-catenin pathway, ultimately resulting in increased tumor cell proliferation. TANs release a variety of growth factors, enzymes and cytokines that collectively enhance tumor growth. CCA express ENA-78 to recruit TANs. Furthermore, the interaction between TANs and TAMs induces the production of OSM by TANs and IL-11 by TAMs, which leads to increased tumor cell proliferation and invasion via STAT3 pathway. CCA, cholangiocarcinoma; TAMs, tumor-associated macrophages; TANs, tumor-associated neutrophils; VEGF, vascular endothelial growth factor; COX-2, cyclooxygenase 2; iNOS, inducible nitric oxide synthase; ENA-78, epithelial-derived neutrophil-activating peptide-78; OSM, oncostatin M.

be associated with the poor OS of patients with HCC (64). Nevertheless, the precise functions of MDSCs in the context of CCA remain incompletely understood. Recent research has indicated that the gut microbiome can induce the accumulation of $CXCR2^+$ polymorphonuclear MDSCs through TLR4-dependent CXCL1 production, thus facilitating the establishment of an immunosuppressive environment that promotes CCA progression (65). The level of $CD33^+$ MDSCs in the blood and tumor tissues of patients with iCCA has been found to be increased and to be associated with a poor clinical outcome (66). The relevance between CAFs and MDSCs has been brought to light. Specifically, CAFs have been found to secrete IL-6 and IL-33, which in turn stimulate MDSCs to upregulate the expression of 5-lipoxygenase (5-LO). Notably, one of the metabolites of 5-LO, known as LTB4, has been shown to activate the PI3K/Akt-mTORC1 signaling pathway in iCCA cells through the interaction with BLT2. This activation ultimately contributes to the promotion of cancer stemness in iCCA (66). Additionally, Loeuillard *et al* (67) highlighted the significance of the interaction between TAMs and MDSCs. They identified an abundance of programmed death ligand 1 (PD-L1)-positive TAMs in both human CCA samples and CCA mouse models. Elevated levels of $PD\text{-}L1^+$ TAMs were associated with an enhanced cancer progression. However, attempts to block TAMs alone have not effectively reduced CCA tumor burden (67). This lack of a response may be attributed to the compensatory accumulation of granulocyte MDSCs (G-MDSCs), which impair the T-cell response and induce immune evasion. Notably, the simultaneous inhibition of G-MDSCs and TAMs has shown promise in enhancing the efficacy of anti-PD-1 therapy for CCA (67). Collectively, that study underscored the role of MDSCs as mediators that collaborate with other components within the CCA TIME to promote tumor progression (67).

NK cells. NK cells are known for their potent antitumor activity, as they possess the ability to induce tumor cell apoptosis directly by releasing molecules, such as perforin, cytotoxic factors and TNF (68). Previous findings have suggested that enhancing NK cell function or increasing the numbers of NK cells delays CCA progression, which may be a potential therapeutic target for CCA. Anti-Globo H antibody VK9 can enhance the activation and increase the presence of NK cells in the TIME, resulting in the inhibition of iCCA rat tumor growth (69). *In vitro* studies have demonstrated that the cytotoxic effect of activated NK cells on CCA cell lines may be augmented by cetuximab and cordycepin, effectively restraining CCA cell growth (70,71). Additionally, in a xenograft mouse model of CCA, the transplantation of *ex vivo*-expanded human NK cells has been found to inhibit tumor growth (72). Fukuda *et al* (73) observed that low numbers of tumor-infiltrating NK, cells regulated by endogenous CXCL9, were associated with a large tumor size and the poor survival of iCCA (73). The activating receptor natural-killer group 2D (NKG2D), predominantly expressed on NK cells, holds promise as a therapeutic target. A high expression of the NKG2D receptor in patients with CCA has been linked to an improved prognosis (74). Moreover, it is worth noting that variations in NKG2D have been found to be associated with bile duct tumorigenesis in patients with primary sclerosing cholangitis (75).

Adaptive immune cells

TILs. The adaptive immune system is the predominant defense system against tumors. The major components of the adaptive immune system in the CCA TIME are TILs, including B-lymphocytes, $CD4^+$ helper T-lymphocytes, $CD8^+$ cytotoxic T-cells and Tregs. While various studies have examined the spatial distribution of TILs in CCA tissue (49,76,77), it remains a topic of debate and discussion. According to the current literature, it appears that $CD3^+$, $CD4^+$ and $CD8^+$ T-cells predominantly reside in the peritumoral region, regardless of the CCA subtype. However, as for $Foxp3^+$ T-cells and B-cells, the exact distribution location has not been definitively determined (6). Recent studies have demonstrated that $Foxp3^+$ T-cells have been observed to accumulate in the tumor border area (76,77). Another previous study indicated that $Foxp3^+$ T-cells were distributed in the intratumoral area (78), while another study failed to found the distribution difference of $Foxp3^+$ T-cells (79). In the case of B-cells, two separate studies have reported their presence in the peritumoral area (78,80). These varying observations regarding the distribution of $Foxp3^+$ T-cells and B-cells may be attributed to distinct contexts or factors, warranting further investigation for a comprehensive understanding. The potential association between TILs and various signaling pathways in tumor promotion has been revealed by numerous studies. Recent research has demonstrated that a low abundance of tissue-resident memory T-cells is associated with a significantly increased expression of genes related to the Wnt/β-catenin and TGF-β signaling pathways (81). Carnevale *et al* (82) made an intriguing discovery where the expression of cellular FADD-like IL-1β-converting enzyme-inhibitory protein in iCCA cells significantly increased following co-culture with human peripheral blood mononuclear cells. This led to the activation of the Fas/FasL pathway, inducing the apoptosis of T-cells and NK cells, ultimately resulting in tumor immune escape (82). Another study highlighted the significance of the B7-H1/PD-1 signaling pathways in the induction of $CD8^+$ TIL apoptosis and the inhibition of antitumor immune responses (83). Isocitrate dehydrogenase 1 mutations drive the activation of interferon γ (IFN-γ)-responsive genes in iCCA cells through a TET2-dependent mechanism. This occurs by impeding the recruitment of activated $CD8^+$ T-cells and the expression of IFN-γ (84).

It has been proven that the number or density of TILs in the TIME of CCA affects prognosis. High levels of $CD8^+$ T-cells have consistently been associated with an improved survival and reduced invasion into surrounding tissues in several studies (76,78,85,86). A substantial infiltration of $CD4^+$ T-cells has similarly been linked to a favorable OS and RFS (77,78). $Foxp3^+$ T cell infiltration, however, has generated mixed results in terms of prognosis. While the majority of studies suggest that it is associated with a poor prognosis of patients with CCA (49,76,87), Goeppert *et al* (78) indicated that patients with $Foxp3^+$ T cell infiltration experience improved outcomes. Thus, the prognostic value of $Foxp3^+$ T-cells remains uncertain, necessitating further studies to clarify their specific impact on long-term results. Current studies have revealed that patients with CCA benefited from high levels of B-cell infiltration (78,88). Nevertheless, due to limited available evidence, the association between B-cells and the prognosis of patients with CCA also warrants further investigation through additional research.

Of note, Tregs play an essential role in establishing the suppressive TIME (Fig. 3). Tregs are the major immune cells in the TIME of advanced-stage cancer, overexpressing FoxP3 and cytotoxic T-lymphocyte-associated antigen-4 (CTLA-4) (89-91). The transcription factor Forkhead box M1 has been shown to increase FoxP3 expression, leading to the recruitment of Tregs. This recruitment attenuates the killing ability of $CD8^+$ T-cells on iCCA cells, contributing to immune escape and the progression of CCA (92). Recent studies have shown that Tregs can adapt to the glucose-depleted tumor microenvironment by shifting their glucose metabolism to fatty acid metabolism, allowing them to survive in the TIME (93). The activation of the EGFR/PI3K/Akt signaling pathway, triggered by mucin protein 1 (MUC1), induces the enrichment of Tregs to bolster the malignant phenotypes, including tumor growth and metastasis (94). Moreover, natural Treg-like $CD4^+CD25^-$ cells activated by CCA cells can produce numerous TGF-β, and elevated levels of TGF-β downregulate the expression of miR-29a, which, in turn, suppresses antitumor immune responses and eventually causes adverse clinical outcomes (95).

Dendritic cells (DCs). DCs are essential components of the immune system, functioning as professional antigen-presenting cells (APCs) that bridge innate and adaptive immune responses. Their presence in tumor tissues can trigger robust antitumor immune responses, potentially improving cancer patient outcomes. The abundance of DCs in the peripheral blood of patients is significantly decreased compared to healthy individuals (96). It has been shown that patients with a higher number of DCs infiltrating the tumor margin experience a lower rate of lymph node metastasis and better prognosis (97). DCs may affect the number of TILs within the TIME. Junking *et al* (98) found that DCs pulsed by CCA cells induced the differentiation of PBMCs into DCs. This process increased and activated $CD3^+$ $CD8^+$ T-cells, empowering them to induce tumor cell apoptosis (98). DCs generated by the stimulation of CCA cells can utilize activated lymphocytes as anti-CCA effector cells. IL-12 and TGF-β in TIME can impair the functions of DCs. The application of specific neutralizing antibodies that block the IL-10 and TGF-β receptors on DCs, or the knockdown of TGF-βRII and IL-10RA mRNA, has been shown to enhance the performance of effector T-cells (99,100). Sung *et al* (101) discovered that ABL501, a bispecific antibody targeting lymphocyte-activation gene 3 (LAG-3) and PD-L1, induced $CD8^+$ T-cell activation by promoting DC maturation. This activation ultimately amplifies the cytotoxic effects of $CD8^+$ T-cells against tumor cells (101). CTLA-4 is a transmembrane protein encoded by the CTLA-4 gene as well as a homologous protein of CD28, which is expressed in activated $CD4^+$ and $CD8^+$ T-cells (102), which presents with higher affinity competes with CD28 for binding to B7 (CD80/86) to impair T-cells. Mature DCs have been demonstrated to significantly release CTLA-4 into the extracellular compartment by vesicular transport, which can inhibit the antibody binding of B7, eventually resulting in the dysfunction of T-cells (103). In addition, the activation of the CD40/CD40L pathway can enhance the function of DCs and induce cytotoxic effects to CCA cell (104). CD40/CD40L is significantly

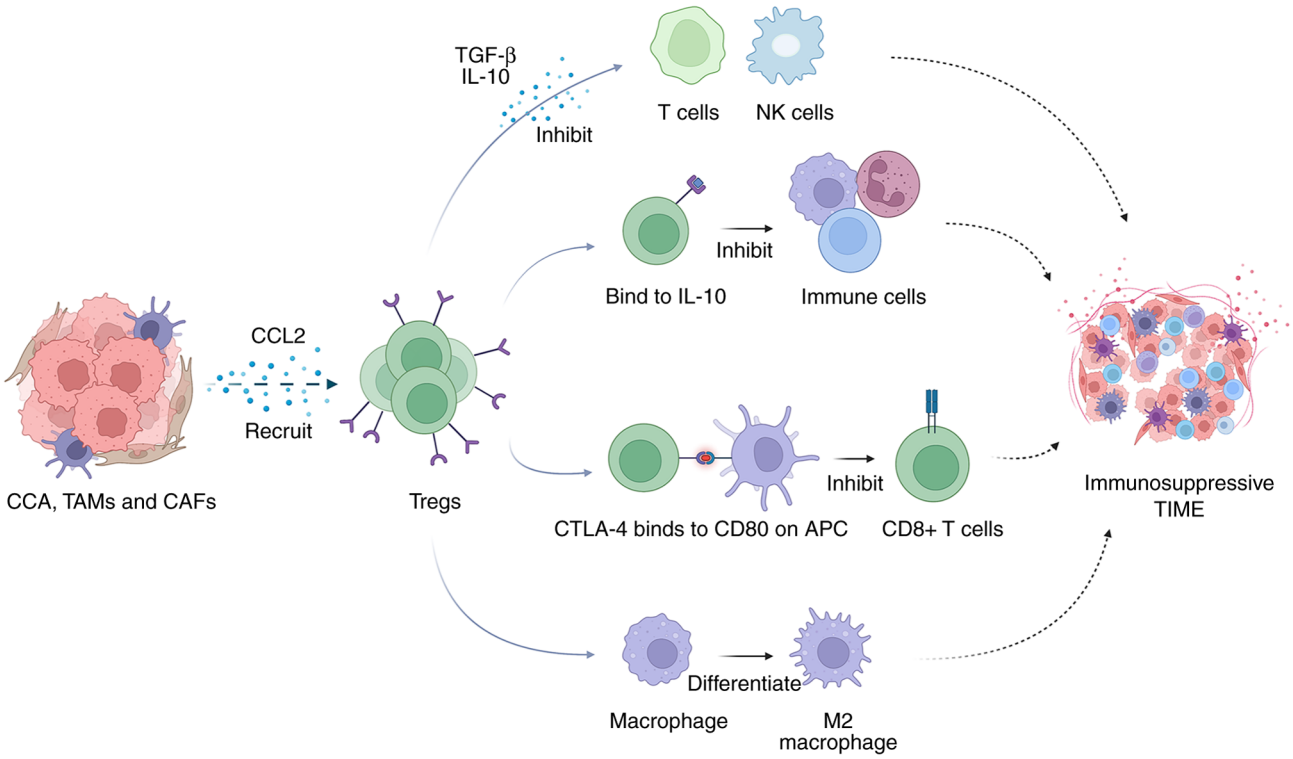

Figure 3. Roles of Tregs in the establishment of the immunosuppressive tumor immune microenvironment. Tregs originate from tumor-infiltrating T-cells under the stimulation of CCL2 produced by tumor cells, TAMs and CAFs. They play a suppressive role mainly through the following mechanisms: i) Tregs suppress T-cell and NK cell immune activity by releasing abundant TGF-β and IL-10; ii) they bind to IL-2 to inhibit the activation of other immune cells; iii) CTLA-4 expressed by Tregs binds to CD80 on antigen-presenting cells to attenuate the cytotoxic effects of CD8+ T-cells; iv) they induce macrophage differentiation toward the tumor-promoting M2 subtype. Tregs, regulatory T-cells; CCL2, chemokine ligand 2; TAMs, tumor-associated macrophages; CAF, cancer-associated fibroblasts; NK, natural killer; CTLA-4, cytotoxic T-lymphocyte-associated antigen-4; APC, antigen-presenting cell; TIME, tumor immune microenvironment.

involved in the maturation, proliferation and survival of DCs, which were triggered by activating p38 MAPK, PI3K-Akt and NF-κB pathways (105). In addition, CD40 signaling triggered the expression of Bcl2l1 to reduce the caspase activation and apoptosis, eventually contributing to maintaining classical type 1 DCs survival during the initiation of anti-tumor immune responses (106). The signaling pathways associated with DCs exerting antitumor effects are summarized in Table SI. In summary, these findings underscore the potential of DC-based immunotherapies as promising approaches for improving outcomes in the context of CCA treatment.

3. Immune escape of cholangiocarcinoma

Evading immune surveillance is one of the features of tumor cells. Although CCA cells express immunogenic tumor-associated antigens (TAAs), the body generates immunosuppressive signals that effectively neutralize the tumor-killing effect. CCA cells employ a strategy of recruiting immunosuppressive cells by releasing factors, such as TGF-β and IL-10. These recruited cells not only enhance tumor activity, but also contribute to the formation of an immunosuppressive TIME, effectively hindering the antitumor immune response. To counter this immunosuppression, inhibiting IL-10 and TGF-β or down-regulating their expression could significantly amplify the cytolytic activity of effector T-cells, potentially reinvigorating the immune response against the tumor (99,100). The intricate interaction between these immunosuppressive cells grants CCA the ability to acquire and deploy immunosuppressive mechanisms (107). Consequently, blocking the recruitment of these immunosuppressive cells emerges as a promising avenue for CCA immunotherapy. Notably, the simultaneous inhibition of TAMs and G-MDSCs has demonstrated the potential to enhance the effectiveness of anti-PD-1 treatment, while suppressing tumor growth (67). Moreover, blocking granulocyte macrophage-CSF signaling has been shown to reduce the accumulation of bone marrow-derived monocytes, impair TAM viability and promote the repolarization of both TAMs and MDSCs. This concerted action leads to an increased infiltration and activation of cytotoxic T-lymphocytes, further strengthening the antitumor immune response (108).

In the usual course, the acquisition of adaptive immunity hinges on activating DCs and macrophages as APCs. Consequently, a TIME deficient in APCs renders T-cells ineffective (109). In CCA, M2 macrophages play a significant role in immune evasion by hampering DC maturation and impairing the function of T-cell effectors. Furthermore, Tregs also inhibit APCs, which disrupt metabolic pathways directly through their cytotoxic effects, leading to the suppression of immune responses (110). Tumor cells employ various strategies to evade the immunosurveillance and innate immune system elimination. Tumor cells upregulate the expression of CD47, which interacts with signal regulatory protein α (SIRPα) on macrophages, facilitating the escape of tumors

from phagocytosis (111). Targeting CD47 and disrupting the CD47-SIRPα interaction can enhance macrophage phagocytosis in all macrophage subtypes, effectively suppressing CCA growth and metastasis (112). Tumor cells block the antitumor effects of NK cells by preserving major histocompatibility complex class I molecules, downregulating NKG2D ligands on tumor cells and releasing immunoregulatory factors (such as TGF-β, prostaglandin E2 and indoleamine 2,3-dioxygenase) that compromise NK cell activity (113). An example of an approach to counteract this is the use of the monoclonal antibody 7C6, which can inhibit the cleavage of major histocompatibility complex-class I chain related proteins A and B and subsequently lead to NKG2D-dependent activation of NK cells (114).

CCA cells employ immune checkpoint manipulation to achieve immune evasion, notably targeting immune checkpoints, such as PD-1 and CTLA-4. PD-1 is expressed on activated T-cells. When PD-1 binds to its ligand PD-L1, it assembles protein tyrosine phosphatase, which inhibits the downstream PI3K-Akt-mTOR and Ras-MEK-ERK signaling pathways. This leads to an altered metabolism, the exhaustion of peripheral T-cells, the suppression of the tumoricidal immune response, and ultimately, to tumor progression (115,116). Additionally, PD-L1 has been found to prevent tumor cells from cytotoxic T-lymphocyte-induced apoptosis and interfere with interferon-mediated cytotoxicity (117,118). CTLA-4 mediates inhibitory signaling in several ways to block the proliferation and activation of T cells (119). These mechanisms include the following: i) Inducing the production of indoleamine 2,3-dioxygenase; ii) hindering the establishment of a zeta-associated protein of 70 kDa; iii) increasing the expression of Casitas-B-lineage lymphoma-b protein; and iv) repressing the NF-κB and PI3K/Akt pathways, CDK4/CDK6 and cyclin D3.

CCA exhibits regional variation, leading to differences in the positive rate of PD-L1 expression among patients. In the Western world, the positive rate is ~11.6% (120), whereas in the East, it ranges from 28 to 45% (79,121-123). In addition, PD-L1 expression is significantly higher in tumor tissues than in paraneoplastic tissues. An elevated expression of PD-L1 has been linked to tumor progression and a poorer prognosis. CCA tumors with a high PD-L1 expression tend to display more aggressive features and shorter survival times (124,125). CTLA-4 has also been observed to be upregulated in CCA, and of note, a significant positive correlation exists between the expression levels of PD-1 and CTLA-4 (126). An increased expression of CTLA-4 in TILs has been shown to be associated with malignant characteristics and poor survival outcomes in iCCA. However, a high expression of CTLA-4 in CCA cells does not appear to predict a poor patient prognosis (127).

4. Immunotherapy for cholangiocarcinoma

ICIs. Tumor immunotherapy mainly uses monoclonal antibodies to enhance endogenous antitumor activity. These monoclonal antibodies predominantly focus on immune checkpoint regulators, collectively known as ICIs (128). CTLA-4 and PD-1 represent the most classical T-cell immune checkpoints and are the most extensively studied targets for ICIs. Moreover, ongoing research is exploring ICIs that target additional immune checkpoints, such as LAG-3, TIM-3, TIGIT and B7-H3 (129). Favorable therapeutic responses to ICIs have been documented in several types of solid tumors (130-132). Presently, pembrolizumab and nivolumab have received approval from the Food and Drug Administration (FDA) for the treatment of advanced malignancies (133,134).

The response of tumor cells to ICIs appears to be closely related to the extent of $CD8^+$ T-cell infiltration and the expression of immune checkpoint molecules within the tumor (135). Tumors characterized by a high presence of $CD8^+$ T-cell infiltration and elevated immune checkpoint molecule expression are often termed immunologically 'hot' tumors and exhibit high response rates to ICIs. However, almost half of all patients with CCA have immunologically 'cold' tumors and have low response rates to treatment with ICIs (136). Genetic abnormalities in tumor cells, such as defective DNA mismatch repair (dMMR) and microsatellite instability-high (MSI-H), can also influence the responsiveness of tumors to ICIs (137-139). Studies have suggested that patients with solid tumors that feature abundant $CD8^+$ T cells, significant PD-L1 expression, MSI-H, high levels of dMMR and a high tumor mutation burden (TMB) may exhibit sensitivity to immunotherapy (140,141). Consequently, $CD8^+$ T-cell infiltration, PD-L1 expression, MSI and TMB are employed as biomarkers to predict immunotherapy response rates. Nevertheless, the accuracy of these biomarkers still requires refinement. There is a pressing need for more precise biomarkers and improved protocols for personalized treatment.

ICI monotherapy. The clinical trial NCT01876511 exhibited that 86 (including CCA) patients with dMMR or MSI-H were treated with pembrolizumab and achieved satisfactory treatment outcomes (139). In the phase 1b trial KEYNOTE-028, 20 patients with advanced-stage CCA and 4 patients with advanced-stage gallbladder cancer (GBC), all of whom tested positive for PD-L1, received pembrolizumab monotherapy (142). A total of 3 patients with CCA and 1 patient with GBC achieved stable disease (SD). Grade 3 toxicities were observed in 17% of cases, with no grade 4 events reported. The objective response rate (ORR) was 17%, and the median progression-free survival (mPFS) and median OS (mOS) were 1.8 and 6.2 months, respectively. Notably, that study confirmed that pembrolizumab was well-tolerated, displayed excellent antitumor activity, and exhibited manageable safety and effectiveness (142).

In a phase 2 study involving 54 patients with BTC pre-treated with at least one line, but no more than three lines of systemic therapy, the anticancer activity of nivolumab in advanced refractory BTC was evaluated (143). More than half of the patients had well-controlled conditions, resulting in a mOS of 14.22 months and a mPFS of 3.68 months. However, Ueno *et al* (144) suggested a poor response rate to nivolumab monotherapy. In summary, the effectiveness of nivolumab monotherapy for CCA remains uncertain due to the cohort size, and further research is required to provide a clearer picture. Durvalumab, atezolizumab and avelumab are also approved by the FDA for the treatment for various solid tumors. However, studies have indicated that these monoclonal antibodies have limited efficacy when used as monotherapy for CCA. For instance, Doki *et al (*145) reported that among 42 patients with BTC treated with durvalumab alone, the

median OS was 1.5 months, the mPFS was 8.1 months and the ORR was 4.8%.

Apart from ICIs targeting a single immune checkpoint, there is growing interest in ICIs that can simultaneously act on two immune checkpoints. A promising example is ABL501, which can inhibit both LAG-3 and PD-L1 concurrently, demonstrating higher antitumor activity compared to a combination of anti-LAG-3 and anti-PD-L1 treatments (101). ABL501 has emerged as a promising candidate in cancer immunotherapy and is currently undergoing its initial human trial (NCT05101109). Another innovative approach is represented by M7824, a novel bifunctional fusion protein. M7824 comprises a monoclonal antibody against PD-L1 fused to the extracellular domain of human TGF-β receptor II. This design allows M7824 to serve a dual function by blocking PD-L1 and sequestering TGF-β molecules (146). Research has explored the response of M7824 in Asian patients with CCA, revealing an ORR of 23%. However, it is essential to note that treatment-related adverse events (TRAEs) have been observed in 63% of patients (147). Further investigations are warranted to assess the overall safety and efficacy of this approach in CCA immunotherapy.

Dual ICI combination therapy. Given the limited effectiveness of ICI monotherapy for CCA, there is a growing focus on exploring combination immunotherapies to enhance treatment responses and overcome immune tolerance. Combination therapy involving two ICIs has demonstrated promising outcomes in various solid tumors. In a phase 2 study evaluating the combination of nivolumab and ipilimumab in 39 patients with advanced-stage BTC (148), the trial reported an ORR of 23% and a disease control rate (DCR) of 44%, highlighting the potential superiority of dual ICI combination therapy compared to monotherapy. A phase 1 study investigated the combination of durvalumab and tremelimumab in advanced-stage BTC (145), revealing an ORR of 10.8% in a cohort of 65 BTC patients. The study reported a mPFS of 1.6 months and a mOS of 10.1 months. However, grade 3 or higher TRAEs were observed in 23.1% of patients. Nevertheless, it is worth mentioning that durvalumab plus tremelimumab combination therapy, when tested in Japanese patients with HCC and BTC, yielded less encouraging response rates and survival outcomes (149). Considering the mixed results, the efficacy of durvalumab and tremelimumab combination therapy in patients with CCA remains unclear, and the safety profile of this approach warrants further refinement. It is important to exercise caution when interpreting these conclusions due to the limited sample sizes in these studies. Therefore, while combination immunotherapies hold promise, particularly in the context of dual immune checkpoint inhibition, continued research with larger and more diverse patient cohorts is essential to establish the true effectiveness and safety of these approaches in the treatment of CCA.

ICIs plus chemotherapy. The trial NCT03046862 classified 124 patients with advanced-stage BTC into three treatment cohorts and revealed that ICIs combined with gemcitabine and cisplatin (GS) hold promise as an effective treatment for advanced BTC (150). Another study supported the strategy of GS plus immunotherapy for BTC. The TOPAZ-1 trial represents a significant advancement in the field, being the first phase 3 study designed to investigate PD-L1 inhibitors in combination with chemotherapy for progressive CCA (151). In that trial, 685 patients were randomly assigned to either the durvalumab + GS group or the placebo + GS group. The interim analysis revealed encouraging results, with a mOS of 12.8 months and a mPFS of 7.2 months in the durvalumab + GS group, compared to 11.5 and 5.7 months in the placebo + GS group, respectively. Notably, while TRAEs were observed in 62.7% of patients in the treatment group and 64.9% of patients in the control group, the combination of chemotherapy regimens for advanced-stage BTC with ICIs appeared to enhance survival and other efficacy outcomes without significantly increasing the risk of treatment toxicity. This suggests a promising avenue for improving treatment outcomes in advanced-stage BTC.

In addition, several trials have evaluated the therapeutic efficacy and safety of GS in combination with nivolumab (11,144,152), pembrolizumab plus capecitabine and oxaliplatin (CAPOX) (12) and paclitaxel with durvalumab and tremelimumab (153) in BTC (Table I). Apart from the trial NCT03704480, the results from these studies have been consistently positive. This collective body of evidence suggests a promising treatment approach that combines chemotherapy with ICIs in patients with BTC, offering not only enhanced efficacy, but also an acceptable safety profile.

ICIs plus targeted therapy. The combination of molecular targeted therapy and immunotherapy has demonstrated synergistic effects. The capacity of immunotherapy to eliminate immunosuppression can extend the remission effect induced by molecular targeted therapy, thereby enhancing the overall effectiveness of targeted therapy.

The trial NCT02443324 assessed the benefits of pembrolizumab in combination with ramucirumab (a VEGFR-2 monoclonal antibody) in 26 patients with locally advanced, unresectable or metastatic BTC (154). However, the results were quite disappointing, with only one patient achieving a partial response, and a mOS of 6.4 months. The trial NCT03201458 compared the efficacy of atezolizumab monotherapy with atezolizumab in combination with cobimetinib (MEK inhibitor) in patients with BTC who had failed first/second line therapy (155). While the combined treatment group exhibited a slightly better mPFS, both groups had extremely low ORRs. Similarly, a single-arm study investigated the efficacy and tolerability of pembrolizumab + lenvatinib in the treatment of patients with BTC who had failed prior systemic treatments (156). That study reported that 25% of patients responded to treatment and the DCR was 78.1%, with a clinical benefit rate of 40.5% (156). Additional studies exploring the combination of ICIs with targeted therapies are listed in Table I.

ICIs plus other therapies. Local treatment may be an effective option for patients with BTC who are not eligible for surgery or have advanced lesions. Techniques such as local ablative therapy and radiation therapy can effectively kill tumor cells, boost the production of tumor neoantigens and enhance the immune recognition response. Consequently, there is a theoretical synergy between local therapy and immunotherapy. The trial NCT01853618 revealed that in the evaluable patients who received combination therapy with tremeliumab + microwave ablation, 12.5% achieved a PR, 31.2% achieved SD, with a mPFS of 3.4 months, and a mOS

Table I. Clinical trials of immunotherapy regimens for CCA.

	Trial number	Phase	Line of treatment	Type of BTC	Estimated enrollment	Allocation	Study arms		Pathways targeted	Primary outcomes	Status
							Arm A	Arm B			
Monotherapy	NCT04157985	3	First-line	CCA	578	Randomized	Pembrolizumab or nivolumab or atezolizumab or ipilimumab or cemiplimab	N/A	PD-1/ PD-L1	Time to next treatment, PFS	Recruiting
	NCT03110328	2	Second-line	CCA	33	N/A	Pembrolizumab	N/A	PD-1	Best overall response, PFS, OS	Completed
	NCT02054806	1b	Second- or later-line	CCA, GBC	477	N/A	Pembrolizumab	N/A	PD-1	Best overall response	Completed
	NCT02829918	2	Second- or later-line	CCA, GBC	54	N/A	Nivolumab	N/A	PD-1	ORR After 4 Cycles of Treatment	Active, not recruiting
	NCT03999658	2	Second- or later-line	CAA, GBC	220	Non-randomized	STI-3031	N/A	PD-L1	ORR	Not yet recruiting
	NCT02628067	2	Second- or later-line	CCA, GBC	1,609	Non-randomized	Pembrolizumab	N/A	PD-1	ORR	Recruiting
	NCT03201458	2	Second- or later-line	CCA, GBC	76	Randomized	Atezolizumab	Atezolizumab plus cobimetinib	PD-L1, MEK1	PFS	Active, not recruiting
	NCT04238637	2	Second- or later-line	ICC	50	Randomized	Durvalumab	Durvalumab plus tremelimumab	PD-L1, CTLA-4	ORR	Recruiting
	NCT04440943	1	Second- or later-line	CCA	40	N/A	CDX-527	N/A	PD-L1xCD27	Safety, tolerability	Recruiting
	NCT05101109	1	Second- or later line	N/A	36	N/A	ABL501	N/A	LAG-3xPD-L1	Safety, tolerability	Recruiting
	NCT03849469	1	Second- or later-line	ICC	242	Non-Randomized	XmAb® 22841	XmAb® 22841 plus pembrolizumab	CTLA-4xLAG-3, PD-1	Safety, tolerability	Active, not recruiting
	NCT03517488	1	Second- or later-line	CCA	154	N/A	XmAb® 22842	N/A	CTLA-4xLAG-3	Safety, tolerability	Active, not recruiting

Table I. Continued.

	Trial number	Phase	Line of treatment	Type of BTC	Estimated enrollment	Allocation	Study arms		Pathways targeted	Primary outcomes	Status
							Arm A	Arm B			
	NCT03219268	1	Second- or later-line	CCA	353	N/A	Tebotelimab plus margetuximab	PD-1xLAG-3, HER2	Safety, tolerability	Active, not recruiting	
	NCT04802876	2	N/A	CCA	141	N/A	Spartalizumab	N/A	PD-1	ORR	Recruiting
ICIs + chemotherapy	NCT04066491	2/3	First line	CCA, GBC	309	Randomized	M7824 plus GC	GC	Placebo plus L1xTGF-β	PD-	Safety, OS recruiting	Active, not
	NCT03046862	2	First-line	CCA, GBC	31	N/A	Durvalumab or tremelimumab plus GC	N/A	PD-L1, CTAL-4	ORR	Active, not recruiting	
	NCT03875235	3	First-line	CCA, GBC	810	Randomized	Durvalumab plus GC	Placebo plus GC	PD-L1	OS	Active, not recruiting
	NCT04003636	3	First-line	CCA, GBC	1,048	Randomized	Pembrolizumab plus GC	Placebo plus GC	PD-1	OS	Active, not recruiting
	NCT03260712	2	First-line	CCA, GBC	50	N/A	Pembrolizumab plus GC	N/A	PD-1	PFS at 6 months	Active, not recruiting
	NCT03796429	2	First-line	CCA, GBC	40	N/A	Toripalimab plus GS	N/A	PD-1	PFS, OS	Recruiting
	NCT04172402	2	First-line	CCA, GBC	48	N/A	Nivolumab plus GS	N/A	PD-1	ORR	Active, not recruiting
	NCT04027764	2	First-line	CCA, GBC	30	N/A	Toripalimab plus S1 plus albumin paclitaxel	N/A	PD-1	ORR	Recruiting
	NCT03478488	3	First-line	CCA, GBC	480	Randomized	KN035 plus GEMOX	GEMOX	PD-L1	OS	Recruiting
	NCT03785873	1b/2	Second-line	CCA, GBC	34	N/A	Nivolumab plus 5-FU plus Nal-Irinotecan	N/A	PD-1	Safety, tolerability, PFS	Active, not recruiting
	NCT03704480	2	Second-line	CCA, GBC	102	Randomized	Durvalumab plus tremelimumab	Durvalumab plus tremelimumab plus Paclitaxel	PD-L1, CTLA-4	PFS	Recruiting

Table I. Continued.

	Trial number	Phase	Line of treatment	Type of BTC	Estimated enrollment	Allocation	Study arms		Pathways targeted	Primary outcomes	Status
							Arm A	Arm B			
	NCT03111732	2	Second- or later-line	CCA, GBC	11	N/A	Pembrolizumab plus oxaliplatin plus capecitabine	N/A	PD-1	PFS	Completed
	NCT03101566	2	N/A	CCA, GBC	75	Randomized	Nivolumab plus GC	Nivolumab plus ipilimumab	PD-1, CTLA-4	PFS at 6 months	Active, not recruiting
	NCT04295317	2	N/A	ICC	65	N/A	SHR-1210 plus capecitabine	N/A	PD-1	RFS	Recruiting
	NCT03311789	1/2	N/A	CCA, GBC	30	N/A	Nivolumab or SHR-1210 plus GC	N/A	PD-1	PFS at 6 months	Recruiting
ICIs + targeted therapy	NCT04361331	2	First-line	ICC	60	Randomized	Toripalimab plus lenvatinib	Lenvatinib plus GEMOX	PD-1, TKI	ORR	Active, not recruiting
	NCT03895970	2b	Second-line	CCA, GBC	50	N/A	Pembrolizumab plus lenvatinib	N/A	PD-1, TKI	ORR, DCR, PFS	Recruiting
	NCT02443324	1	Second- or later-line	CCA, GBC	298	N/A	Pembrolizumab plus ramucirumab	N/A N/A	PD-1, VEGFR-2 PD-1,	Safety, Tolerability	Completed
	NCT05174650	2	Second- or later-line	ICC	37	N/A	Atezolizumab plus derazantinib		FGFR1/ 2/3	ORR	Recruiting
	NCT04298021	2	Second- or later-line	CCA, GBC	74	Non-Randomized	Durvalumab plus AZD6738	AZD6738 + Olaparib	PD-L1, ATR/ATM, PARP	DCR	Recruiting
	NCT03475953	1/2	Second- or later-line	CCA, GBC	482	Non-Randomized	Avelumab plus regorafenib	N/A	PD-L1, TKI	RP2D, ORR	Recruiting
	NCT04550624	2	Second- or later-line	CCA, GBC	40	N/A	Pembrolizumab plus lenvatinib	N/A	PD-2, TKI	ORR	Recruiting
	NCT04298008	2	Third-line	CCA, GBC	26	N/A	Durvalumab + AZD6738	N/A	PD-L1, ATR/ATM	DCR	Recruiting
	NCT05010681	2	N/A	ICC	25	N/A	Sintilimab plus lenvatinib	N/A	PD-1, TKI	ORR	Recruiting

Table I. Continued.

	Trial number	Phase	Line of treatment	Type of BTC	Estimated enrollment	Allocation	Study arms		Pathways targeted	Primary outcomes	Status
							Arm A	Arm B			
	NCT03639935	2	Maintenance after first line platinum-based systemic chemo-therapy	CCA, GBC	35	N/A	Nivolumab plus rucaparib	N/A	PD-1, PARP	Proportion of patients alive and without radiological or clinical progression at 4 months	Recruiting
ICIs + Chemo-therapy+ Target therapy	NCT04300959	2	First-line	CCA, GBC	80	Randomized	Sintilimab plus anlotinib plus GC	N/A	PD-1, PDGFR, FGFR, VEGFR and c-KIT kinase	12 months OS rate	Recruiting
	NCT05342194	3	First-line	ICC	480	Randomized	Toripalimab plus lenvatinib plus GEMOX/ GC	Placebo plus GEMOX/GC	PD-1, TKI	OS	Not yet recruiting
	NCT03951597	2	First-line	ICC	30	N/A	JS001 plus Lenvatinib plus GEMOX	N/A	PD-1, TKI	ORR	Active, not recruiting
	NCT05211323	2	First-line	ICC	88	Randomized	Atezolizumab plus bevacizumab plus GC	GC	PD-1, VEGFR	PFS	Recruiting
	NCT04506281	2	NAT	ICC	128	Randomized	Toripalimab plus lenvatinib plus GEMOX	Capecitabine	PD-1, TKI	Event-free survival	Recruiting
ICIs + other therapy	NCT03937895	1/2a	First-line	CCA, GBC	40	N/A	Pembrolizumab plus allogeneic natural killer cell	N/A	PD-1	Safety, Tolerability, ORR	Completed
	NCT04866836	2	Second	CCA, GBC	20	N/A	Tislelizumab plus radiotherapy	N/A	PD-1	ORR	Recruiting

Table I. Continued.

	Trial number	Phase	Line of treatment	Type of BTC	Estimated enrollment	Allocation	Study arms		Pathways targeted	Primary outcomes	Status
							Arm A	Arm B			
	NCT04068194	1/2	Second- or later-line	CCA, GBC	39	Randomized	Avelumab plus peposertib plus hypofractionated RT	Avelumab plus hypofractionated RT	PD-L1, DNA-PK	Safety, Tolerability, ORR	Recruiting
	NCT05010668	2	Second- or later-line	ICC	25	N/A	Sintilimab plus lenvatinib plus cryoablation	N/A	PD-1,TKI	ORR	Recruiting
	NCT04299581	2	Second- or later-line	ICC	25	N/A	Camrelizumab plus cryoablation	N/A	PD-1	ORR	Recruiting
CAR-T	NCT03633773	1/2	N/A	ICC	9	N/A	MUC-1 CART plus fludarabine plus cyclophos-phamide	N/A	MUC-1	DCR	Recruiting
	NCT04951141	1	Second- or later-line	CCA	10	N/A	anti-GPC3 CAR-T	N/A	GPC3	Safety	Recruiting
	NCT05194735	1/2	Second- or later-line	CCA	180	Non-randomized	TCR-T-cell drug product	TCR-T-cell drug product plus IL-2	Neoantigens	Safety-ORR	Recruiting
Cancer vaccines	NCT04853017	1	AT	CCA, GBC	18	N/A	ELI-002 2P Amph-CpG-7909 admixed with Amph modified KRAS peptides	N/A	KRAS	MTD, Safety	Recruiting
	NCT03942328	1	N/A	CCA	26	N/A	EBRT, autologous DCs, pneumococcal 13-valent conjugate vaccine	N/A	Dendritic cells	Safety	Recruiting

CCA, cholangiocarcinoma; BTC, biliary tract cancer; GBC, gallbladder cancer; PD-1, programmed cell death protein 1; PD-L1, programmed cell death ligand 1; CTLA-4, cytotoxic T-lymphocyte-associated antigen-4; LAG-3, lymphocyte-activation gene 3; DCR, disease control rate; 5-FU, 5-fluorouracil; GC, gemcitabine and cisplatin; GEMOX, gemcitabine and oxaliplatin; GS, gemcitabine and S-1; MTD, highest dose level for which <33% of subjects had a dose-limiting toxicity; N/A, not available; AT, adjuvant therapy; ORR, objective response rate; OS, overall survival; PFS, progression-free survival; RFS, recurrence-free survival; RP2D, recommended phase 2 dose; RT, radiotherapy.

of 6 months (157). Furthermore, studies have suggested that the immune response triggered by antigens released from dead tumor cells following radiation therapy can extend to distant metastatic lesions (140). This phenomenon underscores the potential of combining local therapies with immunotherapy for more comprehensive cancer treatment.

The dense fibrotic matrix found in CCA tissue can impede the effectiveness of antitumor drugs and immune cell infiltration. Therefore, reducing stromal fibrosis in CCA may enhance the response to therapy. The trial NCT03267940 is currently investigating the effectiveness of hyaluronidase when combined with ICIs and chemotherapy for progressive BTC. As aforementioned, increasing the number of NK cells can inhibit tumor growth. A phase 1/2a trial explored the safety and efficacy of pembrolizumab combining allogeneic NK cells in chemotherapy-refractory BTC patients (158). That study reported an overall ORR and DCR of 17.4 and 30.4%, all without severe TRAEs. That study demonstrated that pembrolizumab plus allogeneic NK cells represents a promising therapeutic approach, exhibiting an improved efficacy and a favorable safety profile (158).

Adoptive immune cell therapy. Chimeric antigen receptor (CAR) T-cells, derived from peripheral blood and modified *in vitro*, express CARs formed by merging antigen recognition sites from tumor-specific antibodies with costimulatory molecules, such as CD28. These CAR T-cells can selectively target tumor antigens and activate antitumor responses. While CAR T-cell therapy has achieved success in hematological malignancies, particularly gaining FDA approval for B-lymphoblastic leukemia in 2017 (159), there is growing interest in its application against solid tumors. Capitalizing on the overexpression of EGFR and CD133 in CCA, studies have devised treatment strategies involving EGFR or CD133 CAR T-cells. *In vitro* experiments with anti-CD133 CAR T-cells demonstrated significant and potent cytolytic activity against CCA cells (160). However, a phase 1 clinical study evaluating patients with EGFR-positive metastatic or recurrent BTC found insignificant benefits (161). Another study investigated the effectiveness of EGFR-targeted and CD133-targeted CAR T-cell sequential therapy in a patient with advanced-stage CCA, yielding a partial response of 8.5 and 4.5 months after CAR T-cell EGFR and CAR T-cell CD133 treatments, respectively (162). In addition to CAR T-cell therapy, the efficacy of adoptive cellular transfer of TILs in CCA has been substantiated by various studies. Case reports have revealed that immune cell adoptive transfer exhibits encouraging efficacy in patients with CCA, which a reduced tumor load and prolonged survival (163,164). Moreover, the effectiveness and safety of immune cell adoptive transfer therapy in combination with other treatments have been investigated. Zhang *et al* (165) combined local treatment with the adoptive transfer of allogeneic γδ T-cells for CCA and found no significant survival benefit for patients receiving the combination therapy, despite a favorable safety profile. The combination of the DC vaccine and activated T-cell transfer was proven to be an adjuvant immunotherapy that significantly prolonged the survival of patients with iCCA and HCC undergoing surgery (166,167). Although numerous studies have shown that immune cell adoptive transfer therapy is a potential treatment modality for BTC, the use of this therapy in BTC is still in its infancy and needs to be validated in more high-quality clinical trials.

CD40 agonist. CD40, a member of the TNF receptor superfamily, plays a pivotal role in the immune response. Upon interaction with its ligands, CD40 can stimulate DCs to initiate T-cell-dependent antitumor responses and induce the macrophage-mediated destruction of the tumor stroma. CD40 agonists hold the potential to transform 'cold' tumors into 'hot' tumors, rendering them more responsive to immunotherapy. *In vitro* research has illustrated that CD40 agonists can activate DCs, leading to tumor cell killing (168). Furthermore, combining CD40 agonists with immunotherapy has been shown to increase the number of DCs and restore their function, enhancing the antitumor response of T-cells *in vivo* (169). The combination of CD40 agonists with ICIs has shown significant promise in treating various solid tumors (170-173). However, there has been limited exploration of CD40 agonists in CCA. Humphreys *et al* (174) demonstrated the effectiveness of CD40 agonists in inducing the apoptosis of CCA cells (174). A recent study provided compelling evidence that combining CD40 agonists with anti-PD-1 therapy yielded robust antitumor activity in iCCA mouse models and significantly improved OS with good tolerability (175). The findings of that study suggest that the triple combination of CD40 agonists, ICIs and chemotherapy holds promise as an effective therapy for CCA. This triple combination therapy was tested in pancreatic cancer. Notably, a similar triple combination therapy was evaluated in pancreatic cancer, demonstrating exciting therapeutic effects in a phase 1b clinical trial. That trial investigated gemcitabine/nab-paclitaxel combined with a CD40 agonist (APX005M), with or without nivolumab, for the treatment of untreated metastatic pancreatic cancer (176).

Tumor vaccines. Vaccine therapy represents a promising avenue for enhancing the immune microenvironment in cancer treatment. It functions by eliciting a pre-existing immune response against target antigens within the body, ultimately triggering potent, specific cellular immunity. CCA poses a challenge for immunotherapy due to its low tumor mutation burden and limited expression of neoantigens (177). Therefore, vaccine therapy holds particular appeal as a treatment option for CCA. These cancer vaccines can be broadly categorized into three groups: Cancer antigen peptide or protein vaccines, cellular vaccines and tumor antigen gene vaccines (178).

Peptide or protein-based vaccines commonly incorporate antigens that are overexpressed to enhance immunogenicity. For CCA, Wilm's tumor protein 1 (WT1) and MUC1 are widely expressed (179). Many single peptide-based cancer vaccines for CCA have targeted WT1 or MUC1. Despite being well-tolerated, these tumor vaccines often exhibit limited effectiveness when used as monotherapy (180-182).

The efficacy of single peptide-based vaccines is constrained by the heterogeneity of CCA, stemming from the uneven distribution of TAAs. The response of the immune system interacting with TAAs varies widely from one patient to another. The immune system's response to TAAs can vary significantly from one patient to another. Immune cells induced by individual peptide vaccines specifically target tumor cells expressing that single peptide (protein). However,

when these tumor cells downregulate or silence the targeted peptide (protein), the single peptide vaccines tend to lose their efficacy. By contrast, vaccines designed to target multiple antigenic peptides have the potential to address these limitations. Aruga *et al* (183) conducted investigations into the safety, immune responses and antitumor effects of a four-peptide vaccination in patients with advanced-stage refractory BTC. The results of their study revealed detectable peptide-specific T-cell immune responses in 7 out of 9 vaccinated patients, indicating that the four-peptide vaccine was both safe and associated with a survival benefit (183). Subsequently, the same cohort was treated with a three-peptide vaccine, which yielded similar results in terms of safety and effective immune responses (184). Additionally, a phase 2 trial identified four peptides for the development of personalized multiple-peptide vaccines based on patients' immunological profiles (185). These personalized vaccines were found to induce robust immune responses with favorable tolerability. While these studies demonstrate the feasibility and safety of multi-peptide approaches for refractory BTC, their impact on survival warrants further investigation in larger prospective studies. However, it is worth noting that in the limited immune space, immune cells compete with each other. In cases where an inappropriate peptide vaccine induces an immune response, it may inadvertently suppress the function of pre-existing memory immune cells. This phenomenon may contribute to accelerated disease progression and even premature mortality among patients.

Cellular vaccines are designed to expose the immune system to antigens, thereby stimulating the generation of memory lymphocytes and facilitating a robust immune response against tumors. DCs, modified autologous cancer cells and allogeneic tumor cell lines are commonly used cell types in cell-based tumor vaccines (178). DC-based tumor vaccines loaded with TAAs have demonstrated favorable tolerability and potential efficacy in CCA. In a phase 1/2 study, 12 patients with CCA or pancreatic cancer received a DC vaccine loaded with MUC1 following primary tumor resection, resulting in a mOS of 26 months with good tolerability (186). Another study involving 65 patients with unresectable or recurrent BTC utilized DC vaccines pulsed with WT1 and/or MUC1, which proved to be well-tolerated, with 15% of patients experiencing an attenuated disease progression (187). A recent study employed Listeria monocytogenes expressing antigen of interest (LmAIO) for prophylactic vaccination in a mouse model of CCA (188). This approach successfully induced potent tumor-specific Th1 immunity, leading to reduced tumor burden, delayed disease progression, and prolonged survival (188). That study employed an attenuated strain of Listeria monocytogenes as a TAA presentation vehicle, shedding new light on the development of cell-based tumor vaccines.

Nucleic acid-based cancer vaccines, which leverage genetic material, have emerged as a focal point in tumor immunotherapy. These vaccines offer several advantages compared to other types. They can concurrently deliver multiple TAAs, mitigating the risk of resistance, and encode full-length TAAs to stimulate broader T-cell responses. Moreover, these vaccines have demonstrated good tolerability and safety profiles across various digestive system tumors (189). Huang *et al* (190) applied bioinformatics techniques to identify three potential TAAs for CCA mRNA vaccines. They further stratified patients with CCA based on immunophenotyping and suggested that those with an 'immune-cold' phenotype may derive more substantial benefits from mRNA vaccine therapy (190). However, the application of genetic vaccines in CCA remains at a preliminary stage, necessitating further research to establish their safety and true efficacy.

5. Current limitations and future perspectives

CCA is a highly malignant tumor and characterized by a poor prognosis. Risk factors for CCA include hepatitis viral infection, parasitic infection, cirrhosis, primary sclerosing cholangitis and cholelithiasis (191). Furthermore, studies using animals have suggested that exposure to dioxin-like compounds can increase the incidence of CCA in mice (192,193); however, this association has not been definitively confirmed in humans. At present, the main treatment option for CCA is radical surgery, supplemented by chemotherapy and radiotherapy. However, even with these interventions, the survival rates of patients with CCA remain disoncertingly low. The emergence of immunotherapy as a treatment modality for solid tumors has garnered increasing attention, holding significant promise for CCA. Nevertheless, the progress of immunotherapy for CCA remains in its infancy, a circumstance that may be attributed to several factors. Firstly, the majority of CCA cases are immunologically 'cold' tumors with a suppressive TIME and present with a low response rate to immunotherapies. Secondly, CCA is a heterogenous disease and the molecular characteristics of CCA derived from different/same regions of bile duct differ from each other. Thirdly, for unselected patients with CCA, single ICI therapy is less effective and patients are more likely to exhibit resistance to therapy. These multifaceted challenges underscore the need for a more in-depth understanding of the immune landscape in CCA and the development of tailored, combinatorial immunotherapeutic approaches that can overcome the complexities posed by this aggressive malignancy.

The TIME of CCA is highly intricate and dynamic, associated with CCA progression, metastasis and treatment failure. Within the TIME of CCA lies a wealth of potential drug targets for the development of innovative immune-based therapies. To pave the way for more precise and efficacious treatments for CCA, future research endeavors should harness cutting-edge techniques, such as single-cell sequencing, transcriptomics, proteomics and metabolomics. These approaches will enable a comprehensive exploration of the intricate mechanisms governing the interplay between CCA and its TIME. By elucidating the TIME landscape of CCA in its entirety, invaluable insight can be obtained into novel therapeutic avenues targeting specific components of the TIME, heralding a new era in CCA treatment.

CCA can be classified into distinct subtypes based on its anatomical origin, primarily as iCCA and extrahepatic CCA (eCCA), which further includes perihilar and distal CCA. Notably, the molecular profile of cancerous tissues varies significantly across different biliary system sites. For instance, mutations in genes such as IDH1, BAP1 and PBRM1 are prevalent among patients with iCCA, whereas KRAS, CDKN2A and BRCA1 mutations are more commonly observed in eCCA cases (194,195). Even within the same anatomical region,

CCAs with distinct histological features may exhibit differing gene mutations (196). This inherent heterogeneity poses a challenge in predicting responses to immunotherapies. Studies have indicated that ICI treatments tend to be more effective for iCCA compared to eCCA (195). Therefore, conducting comprehensive multi-omics investigations of CCA using genomic, proteomic, metabolomic and colonyomic technologies holds the promise of providing detailed insight into the characteristics relevant to CCA immunotherapies. This approach may shift the classification of CCA from anatomical and morphological criteria to molecular typing, offering a more accurate reflection of the tumor's biological essence. Ultimately, this may enable a more precise diagnosis and may lead to the development of treatment strategies tailored to the specific molecular profile of CCA.

Combination therapy focused on ICIs is a promising and valuable first-line or translational treatment approach for intractable biliary tract malignancies. Dual ICI treatment targeting different immune checkpoints has also shown prospective synergistic therapeutic effects. However, there remain several caveats for ICI combination therapy in clinical practice. Notably, the majority of patients are insensitive to ICI combination therapy, and the overall ORR is relatively low. The ICI combination therapy has a lower overall ORR and risks of therapeutic resistance. The therapeutic resistance phenomenon is also observed. Therefore, future prospective precision immunotherapy should focus on developing more well-established and definite personalized treatment for patients with CCA with different subtypes. With the greater understanding of the molecular features of CCA, the issue of identifying more accurate and reliable biomarkers of immunotherapy effects needs to be solved imminently. Although the favorable safety profile of ICI combination therapy has been proven in several clinical trials, it is necessary and crucial to evaluate the treatment-related adverse effects. On the one hand, the liver function of the majority of patients with CCA is impaired, affecting metabolic detoxification. On the other hand, the sample volume of existing studies is minimal, which is likely to lead to bias in conclusions. Further large-volume, high-quality, prospective and randomized controlled trials are required to identify the safety, as well as the therapeutic effects of different immune combination regimens.

6. Conclusion

CCA stands out as an exceptionally malignant tumor, marked by its often-grim prognosis. In the quest for precision immunotherapy, future efforts should harness advanced techniques to delve deeper into the intricate mechanisms governing the interplay between CCA and the TIME. Such endeavors should ultimately yield a comprehensive TIME landscape specific to CCA. This knowledge may serve as the foundation for developing more robust and tailored personalized treatments, accounting for the diversity of CCA subtypes. One particularly promising avenue is the exploration of combination therapy, with a specific focus on ICIs. This approach holds substantial potential as a first-line or translational treatment strategy, particularly for the challenging realm of intractable biliary tract malignancies.

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Authors' contributions

SY contributed to data acquisition and drafted the manuscript. YH, RZ and YD contributed to data acquisition. FL and HH contributed to the study design and the revision of the manuscript. All authors have read and approved the final manuscript. Data authentication is not applicable.

Ethics approval and consent to participate

Not applicable.

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Not applicable.

Competing interests

The authors declare that they have no competing interests.

References

1. Banales JM, Cardinale V, Carpino G, Marzioni M, Andersen JB, Invernizzi P, Lind GE, Folseraas T, Forbes SJ, Fouassier L, *et al*: Expert consensus document: Cholangiocarcinoma: Current knowledge and future perspectives consensus statement from the European network for the study of cholangiocarcinoma (ENS-CCA). Nat Rev Gastroenterol Hepatol 13: 261-280, 2016.
2. Rizzo A, Carloni R, Frega G, Palloni A, Di Federico A, Ricci AD, De Luca R, Tavolari S and Brandi G: Intensive follow-up program and oncological outcomes of biliary tract cancer patients after curative-intent surgery: A twenty-year experience in a single tertiary medical center. Curr Oncol 29: 5084-5090, 2022.
3. Cai Y, Cheng N, Ye H, Li F, Song P and Tang W: The current management of cholangiocarcinoma: A comparison of current guidelines. Biosci Trends 10: 92-102, 2016.

4. Fabris L, Perugorria MJ, Mertens J, Björkström NK, Cramer T, Lleo A, Solinas A, Sänger H, Lukacs-Kornek V, Moncsek A, *et al*: The tumour microenvironment and immune milieu of cholangiocarcinoma. Liver Int 39 (Suppl 1): S63-S78, 2019.
5. Xia T, Li K, Niu N, Shao Y, Ding D, Thomas DL, Jing H, Fujiwara K, Hu H, Osipov A, *et al*: Immune cell atlas of cholangiocarcinomas reveals distinct tumor microenvironments and associated prognoses. J Hematol Oncol 15: 37, 2022.
6. Liu D, Heij LR, Czigany Z, Dahl E, Lang SA, Ulmer TF, Luedde T, Neumann UP and Bednarsch J: The role of tumor-infiltrating lymphocytes in cholangiocarcinoma. J Exp Clin Cancer Res 41: 127, 2022.
7. Mittal D, Gubin MM, Schreiber RD and Smyth MJ: New insights into cancer immunoediting and its three component phases-elimination, equilibrium and escape. Curr Opin Immunol 27: 16-25, 2014.
8. O'Donnell JS, Teng MWL and Smyth MJ: Cancer immunoediting and resistance to T cell-based immunotherapy. Nat Rev Clin Oncol 16: 151-167, 2019.
9. Gubin MM and Vesely MD: Cancer immunoediting in the era of immuno-oncology. Clin Cancer Res 28: 3917-3928, 2022.
10. Kelley RK, Ueno M, Yoo C, Finn RS, Furuse J, Ren Z, Yau T, Klümpen HJ, Chan SL, Ozaka M, *et al*: Pembrolizumab in combination with gemcitabine and cisplatin compared with gemcitabine and cisplatin alone for patients with advanced biliary tract cancer (KEYNOTE-966): A randomised, double-blind, placebo-controlled, phase 3 trial. Lancet 401: 1853-1865, 2023.
11. Sahai V, Griffith KA, Beg MS, Shaib WL, Mahalingam D, Zhen DB, Deming DA and Zalupski MM: A randomized phase 2 trial of nivolumab, gemcitabine, and cisplatin or nivolumab and ipilimumab in previously untreated advanced biliary cancer: BilT-01. Cancer 128: 3523-3530, 2022.
12. Monge C, Pehrsson EC, Xie C, Duffy AG, Mabry D, Wood BJ, Kleiner DE, Steinberg SM, Figg WD, Redd B, *et al*: A phase II study of pembrolizumab in combination with capecitabine and oxaliplatin with molecular profiling in patients with advanced biliary tract carcinoma. Oncologist 27: e273-e285, 2022.
13. Sirica AE and Gores GJ: Desmoplastic stroma and cholangiocarcinoma: Clinical implications and therapeutic targeting. Hepatology 59: 2397-2402, 2014.
14. Montori M, Scorzoni C, Argenziano ME, Balducci D, De Blasio F, Martini F, Buono T, Benedetti A, Marzioni M and Maroni L: Cancer-associated fibroblasts in cholangiocarcinoma: Current knowledge and possible implications for therapy. J Clin Med 11: 6498, 2022.
15. Okabe H, Beppu T, Hayashi H, Horino K, Masuda T, Komori H, Ishikawa S, Watanabe M, Takamori H, Iyama K and Baba H: Hepatic stellate cells may relate to progression of intrahepatic cholangiocarcinoma. Ann Surg Oncol 16: 2555-2564, 2009.
16. Dranoff JA and Wells RG: Portal fibroblasts: Underappreciated mediators of biliary fibrosis. Hepatology 51: 1438-1444, 2010.
17. Quante M, Tu SP, Tomita H, Gonda T, Wang SS, Takashi S, Baik GH, Shibata W, Diprete B, Betz KS, *et al*: Bone marrow-derived myofibroblasts contribute to the mesenchymal stem cell niche and promote tumor growth. Cancer Cell 19: 257-272, 2011.
18. Affo S, Nair A, Brundu F, Ravichandra A, Bhattacharjee S, Matsuda M, Chin L, Filliol A, Wen W, Song X, *et al*: Promotion of cholangiocarcinoma growth by diverse cancer-associated fibroblast subpopulations. Cancer Cell 39: 866-882, 2021.
19. Mertens JC, Fingas CD, Christensen JD, Smoot RL, Bronk SF, Werneburg NW, Gustafson MP, Dietz AB, Roberts LR, Sirica AE and Gores GJ: Therapeutic effects of deleting cancer-associated fibroblasts in cholangiocarcinoma. Cancer Res 73: 897-907, 2013.
20. Zhang XF, Dong M, Pan YH, Chen JN, Huang XQ, Jin Y and Shao CK: Expression pattern of cancer-associated fibroblast and its clinical relevance in intrahepatic cholangiocarcinoma. Hum Pathol 65: 92-100, 2017.
21. Itou RA, Uyama N, Hirota S, Kawada N, Wu S, Miyashita S, Nakamura I, Suzumura K, Sueoka H, Okada T, *et al*: Immunohistochemical characterization of cancer-associated fibroblasts at the primary sites and in the metastatic lymph nodes of human intrahepatic cholangiocarcinoma. Hum Pathol 83: 77-89, 2019.
22. Sirica AE: The role of cancer-associated myofibroblasts in intrahepatic cholangiocarcinoma. Nat Rev Gastroenterol Hepatol 9: 44-54, 2011.
23. Clapéron A, Mergey M, Aoudjehane L, Ho-Bouldoires TH, Wendum D, Prignon A, Merabtene F, Firrincieli D, Desbois-Mouthon C, Scatton O, *et al*: Hepatic myofibroblasts promote the progression of human cholangiocarcinoma through activation of epidermal growth factor receptor. Hepatology 58: 2001-2011, 2013.
24. Clapéron A, Mergey M, Nguyen Ho-Bouldoires TH, Vignjevic D, Wendum D, Chrétien Y, Merabtene F, Frazao A, Paradis V, Housset C, *et al*: EGF/EGFR axis contributes to the progression of cholangiocarcinoma through the induction of an epithelial-mesenchymal transition. J Hepatol 61: 325-332, 2014.
25. Ohira S, Sasaki M, Harada K, Sato Y, Zen Y, Isse K, Kozaka K, Ishikawa A, Oda K, Nimura Y and Nakanuma Y: Possible regulation of migration of intrahepatic cholangiocarcinoma cells by interaction of CXCR4 expressed in carcinoma cells with tumor necrosis factor-alpha and stromal-derived factor-1 released in stroma. Am J Pathol 168: 1155-1168, 2006.
26. Gentilini A, Rombouts K, Galastri S, Caligiuri A, Mingarelli E, Mello T, Marra F, Mantero S, Roncalli M, Invernizzi P and Pinzani M: Role of the stromal-derived factor-1 (SDF-1)-CXCR4 axis in the interaction between hepatic stellate cells and cholangiocarcinoma. J Hepatol 57: 813-820, 2012.
27. McCarthy JB, El-Ashry D and Turley EA: Hyaluronan, cancer-associated fibroblasts and the tumor microenvironment in malignant progression. Front Cell Dev Biol 6: 48, 2018.
28. Cyphert JM, Trempus CS and Garantziotis S: Size matters: Molecular weight specificity of hyaluronan effects in cell biology. Int J Cell Biol 2015: 563818, 2015.
29. Tian X, Azpurua J, Hine C, Vaidya A, Myakishev-Rempel M, Ablaeva J, Mao Z, Nevo E, Gorbunova V and Seluanov A: High-molecular-mass hyaluronan mediates the cancer resistance of the naked mole rat. Nature 499: 346-349, 2013.
30. Zhang M, Yang H, Wan L, Wang Z, Wang H, Ge C, Liu Y, Hao Y, Zhang D, Shi G, *et al*: Single-cell transcriptomic architecture and intercellular crosstalk of human intrahepatic cholangiocarcinoma. J Hepatol 73: 1118-1130, 2020.
31. Cadamuro M, Nardo G, Indraccolo S, Dall'olmo L, Sambado L, Moserle L, Franceschet I, Colledan M, Massani M, Stecca T, *et al*: Platelet-derived growth factor-D and Rho GTPases regulate recruitment of cancer-associated fibroblasts in cholangiocarcinoma. Hepatology 58: 1042-1053, 2013.
32. Fingas CD, Bronk SF, Werneburg NW, Mott JL, Guicciardi ME, Cazanave SC, Mertens JC, Sirica AE and Gores GJ: Myofibroblast-derived PDGF-BB promotes Hedgehog survival signaling in cholangiocarcinoma cells. Hepatology 54: 2076-2088, 2011.
33. Cadamuro M, Brivio S, Mertens J, Vismara M, Moncsek A, Milani C, Fingas C, Cristina Malerba M, Nardo G, Dall'Olmo L, *et al*: Platelet-derived growth factor-D enables liver myofibroblasts to promote tumor lymphangiogenesis in cholangiocarcinoma. J Hepatol 70: 700-709, 2019.
34. Wang Z, An J, Zhu D, Chen H, Lin A, Kang J, Liu W and Kang X: Periostin: An emerging activator of multiple signaling pathways. J Cell Commun Signal 16: 515-530, 2022.
35. Yue H, Li W, Chen R, Wang J, Lu X and Li J: Stromal POSTN induced by TGF-β1 facilitates the migration and invasion of ovarian cancer. Gynecol Oncol 160: 530-538, 2021.
36. Chen G, Wang Y, Zhao X, Xie XZ, Zhao JG, Deng T, Chen ZY, Chen HB, Tong YF, Yang Z, *et al*: A positive feedback loop between periostin and TGFβ1 induces and maintains the stemness of hepatocellular carcinoma cells via AP-2α activation. J Exp Clin Cancer Res 40: 218, 2021.
37. Yu B, Wu K, Wang X, Zhang J, Wang L, Jiang Y, Zhu X, Chen W and Yan M: Periostin secreted by cancer-associated fibroblasts promotes cancer stemness in head and neck cancer by activating protein tyrosine kinase 7. Cell Death Dis 9: 1082, 2018.
38. Ma H, Wang J, Zhao X, Wu T, Huang Z, Chen D, Liu Y and Ouyang G: Periostin promotes colorectal tumorigenesis through integrin-FAK-Src pathway-mediated YAP/TAZ activation. Cell Rep 30: 793-806.e6, 2020.
39. Utispan K, Sonongbua J, Thuwajit P, Chau-In S, Pairojkul C, Wongkham S and Thuwajit C: Periostin activates integrin α5β1 through a PI3K/AKT-dependent pathway in invasion of cholangiocarcinoma. Int J Oncol 41: 1110-1118, 2012.
40. Sonongbua J, Siritungyong S, Thongchot S, Kamolhan T, Utispan K, Thuwajit P, Pongpaibul A, Wongkham S and Thuwajit C: Periostin induces epithelial-to-mesenchymal transition via the integrin α5β1/TWIST-2 axis in cholangiocarcinoma. Oncol Rep 43: 1147-1158, 2020.
41. Peng H, Zhu E and Zhang Y: Advances of cancer-associated fibroblasts in liver cancer. Biomark Res 10: 59, 2022.

42. Kunk PR, Dougherty SC, Lynch K, Whitehair R, Meneveau M, Obeid JM, Winters K, Ju JY, Stelow EB, Bauer TW, *et al*: Myeloid cell infiltration correlates with prognosis in cholangiocarcinoma and varies based on tumor location. J Immunother 44: 254-263, 2021.
43. Hasita H, Komohara Y, Okabe H, Masuda T, Ohnishi K, Lei XF, Beppu T, Baba H and Takeya M: Significance of alternatively activated macrophages in patients with intrahepatic cholangiocarcinoma. Cancer Sci 101: 1913-1919, 2010.
44. Charbel A, Tavernar L, Albrecht T, Brinkmann F, Verheij J, Roos E, Vogel MN, Köhler B, Springfeld C, Brobeil A, *et al*: Spatiotemporal analysis of tumour-infiltrating immune cells in biliary carcinogenesis. Br J Cancer 127: 1603-1614, 2022.
45. Tu J, Wu F, Chen L, Zheng L, Yang Y, Ying X, Song J, Chen C, Hu X, Zhao Z and Ji J: Long non-coding RNA PCAT6 induces M2 polarization of macrophages in cholangiocarcinoma via modulating miR-326 and RhoA-ROCK signaling pathway. Front Oncol 10: 605877, 2021.
46. Kitano Y, Okabe H, Yamashita YI, Nakagawa S, Saito Y, Umezaki N, Tsukamoto M, Yamao T, Yamamura K, Arima K, *et al*: Tumour-infiltrating inflammatory and immune cells in patients with extrahepatic cholangiocarcinoma. Br J Cancer 118: 171-180, 2018.
47. Paillet J, Kroemer G and Pol JG: Immune contexture of cholangiocarcinoma. Curr Opin Gastroenterol 36: 70-76, 2020.
48. Yuan H, Lin Z, Liu Y, Jiang Y, Liu K, Tu M, Yao N, Qu C and Hong J: Intrahepatic cholangiocarcinoma induced M2-polarized tumor-associated macrophages facilitate tumor growth and invasiveness. Cancer Cell Int 20: 586, 2020.
49. Bai R, Li Y, Jian L, Yang Y, Zhao L and Wei M: The hypoxia-driven crosstalk between tumor and tumor-associated macrophages: Mechanisms and clinical treatment strategies. Mol Cancer 21: 177, 2022.
50. Loilome W, Bungkanjana P, Techasen A, Namwat N, Yongvanit P, Puapairoj A, Khuntikeo N and Riggins GJ: Activated macrophages promote Wnt/β-catenin signaling in cholangiocarcinoma cells. Tumour Biol 35: 5357-5367, 2014.
51. Cheng H and Li Q: Sevoflurane inhibits cholangiocarcinoma via Wnt/β-catenin signaling pathway. BMC Gastroenterol 23: 279, 2023.
52. Boulter L, Guest RV, Kendall TJ, Wilson DH, Wojtacha D, Robson AJ, Ridgway RA, Samuel K, Van Rooijen N, Barry ST, *et al*: WNT signaling drives cholangiocarcinoma growth and can be pharmacologically inhibited. J Clin Invest 125: 1269-1285, 2015.
53. Zhou SL, Dai Z, Zhou ZJ, Chen Q, Wang Z, Xiao YS, Hu ZQ, Huang XY, Yang GH, Shi YH, *et al*: CXCL5 contributes to tumor metastasis and recurrence of intrahepatic cholangiocarcinoma by recruiting infiltrative intratumoral neutrophils. Carcinogenesis 35: 597-605, 2014.
54. Zhou Z, Wang P, Sun R, Li J, Hu Z, Xin H, Luo C, Zhou J, Fan J and Zhou S: Tumor-associated neutrophils and macrophages interaction contributes to intrahepatic cholangiocarcinoma progression by activating STAT3. J Immunother Cancer 9: e001946, 2021.
55. Pandey G: Tumor-associated macrophages in solid tumor: Friend or foe. Ann Transl Med 8: 1027, 2020.
56. Brandau S, Dumitru CA and Lang S: Protumor and antitumor functions of neutrophil granulocytes. Semin Immunopathol 35: 163-176, 2013.
57. Ohms M, Möller S and Laskay T: An attempt to polarize human neutrophils toward N1 and N2 phenotypes in vitro. Front Immunol 11: 532, 2020.
58. Jaillon S, Ponzetta A, Di Mitri D, Santoni A, Bonecchi R and Mantovani A: Neutrophil diversity and plasticity in tumour progression and therapy. Nat Rev Cancer 20: 485-503, 2020.
59. Mao ZY, Zhu GQ, Xiong M, Ren L and Bai L: Prognostic value of neutrophil distribution in cholangiocarcinoma. World J Gastroenterol 21: 4961-4968, 2015.
60. Branchi V, Jürgensen B, Esser L, Gonzalez-Carmona M, Weismüller TJ, Strassburg CP, Henn J, Semaan A, Lingohr P, Manekeller S, *et al*: Tumor infiltrating neutrophils are frequently found in adenocarcinomas of the biliary tract and their precursor lesions with possible impact on prognosis. J Pers Med 11: 233, 2021.
61. Parker KH, Beury DW and Ostrand-Rosenberg S: Myeloid-derived suppressor cells: Critical cells driving immune suppression in the tumor microenvironment. Adv Cancer Res 128: 95-139, 2015.
62. Desai R, Coxon AT and Dunn GP: Therapeutic applications of the cancer immunoediting hypothesis. Semin Cancer Biol 78: 63-77, 2022.
63. Qin G, Liu S, Liu J, Hu H, Yang L, Zhao Q, Li C, Zhang B and Zhang Y: Overcoming resistance to immunotherapy by targeting GPR84 in myeloid-derived suppressor cells. Signal Transduct Target Ther 8: 164, 2023.
64. Kalathil S, Lugade AA, Miller A, Iyer R and Thanavala Y: Higher frequencies of GARP(+)CTLA-4(+)Foxp3(+) T regulatory cells and myeloid-derived suppressor cells in hepatocellular carcinoma patients are associated with impaired T-cell functionality. Cancer Res 73: 2435-2444, 2013.
65. Zhang Q, Ma C, Duan Y, Heinrich B, Rosato U, Diggs LP, Ma L, Roy S, Fu Q, Brown ZJ, *et al*: Gut microbiome directs hepatocytes to recruit MDSCs and promote cholangiocarcinoma. Cancer Discov 11: 1248-1267, 2021.
66. Lin Y, Cai Q, Chen Y, Shi T, Liu W, Mao L, Deng B, Ying Z, Gao Y, Luo H, *et al*: CAFs shape myeloid-derived suppressor cells to promote stemness of intrahepatic cholangiocarcinoma through 5-lipoxygenase. Hepatology 75: 28-42, 2022.
67. Loeuillard E, Yang J, Buckarma E, Wang J, Liu Y, Conboy C, Pavelko KD, Li Y, O'Brien D, Wang C, *et al*: Targeting tumor-associated macrophages and granulocytic myeloid-derived suppressor cells augments PD-1 blockade in cholangiocarcinoma. J Clin Invest 130: 5380-5396, 2020.
68. Chiossone L, Dumas PY, Vienne M and Vivier E: Natural killer cells and other innate lymphoid cells in cancer. Nat Rev Immunol 18: 671-688, 2018.
69. Hung TH, Hung JT, Wu CE, Huang Y, Lee CW, Yeh CT, Chung YH, Lo FY, Lai LC, Tung JK, *et al*: Globo H is a promising theranostic marker for intrahepatic cholangiocarcinoma. Hepatol Commun 6: 194-208, 2022.
70. Morisaki T, Umebayashi M, Kiyota A, Koya N, Tanaka H, Onishi H and Katano M: Combining cetuximab with killer lymphocytes synergistically inhibits human cholangiocarcinoma cells in vitro. Anticancer Res 32: 2249-2256, 2012.
71. Panwong S, Wathikthinnakon M, Kaewkod T, Sawasdee N, Tragoolpua Y, Yenchitsomanus PT and Panya A: Cordycepin sensitizes cholangiocarcinoma cells to be killed by natural killer-92 (NK-92) cells. Molecules 26: 5973, 2021.
72. Jung IH, Kim DH, Yoo DK, Baek SY, Jeong SH, Jung DE, Park SW and Chung YY: In Vivo study of natural killer (NK) cell cytotoxicity against cholangiocarcinoma in a nude mouse model. In Vivo 32: 771-781, 2018.
73. Fukuda Y, Asaoka T, Eguchi H, Yokota Y, Kubo M, Kinoshita M, Urakawa S, Iwagami Y, Tomimaru Y, Akita H, *et al*: Endogenous CXCL9 affects prognosis by regulating tumor-infiltrating natural killer cells in intrahepatic cholangiocarcinoma. Cancer Sci 111: 323-333, 2020.
74. Tsukagoshi M, Wada S, Yokobori T, Altan B, Ishii N, Watanabe A, Kubo N, Saito F, Araki K, Suzuki H, *et al*: Overexpression of natural killer group 2 member D ligands predicts favorable prognosis in cholangiocarcinoma. Cancer Sci 107: 116-122, 2016.
75. Melum E, Karlsen TH, Schrumpf E, Bergquist A, Thorsby E, Boberg KM and Lie BA: Cholangiocarcinoma in primary sclerosing cholangitis is associated with NKG2D polymorphisms. Hepatology 47: 90-96, 2008.
76. Asahi Y, Hatanaka KC, Hatanaka Y, Kamiyama T, Orimo T, Shimada S, Nagatsu A, Sakamoto Y, Kamachi H, Kobayashi N, *et al*: Prognostic impact of CD8+ T cell distribution and its association with the HLA class I expression in intrahepatic cholangiocarcinoma. Surg Today 50: 931-940, 2020.
77. Kim HD, Kim JH, Ryu YM, Kim D, Lee S, Shin J, Hong SM, Kim KH, Jung DH, Song GW, *et al*: Spatial distribution and prognostic implications of tumor-infiltrating FoxP3-CD4+ T cells in biliary tract cancer. Cancer Res Treat 53: 162-171, 2021.
78. Goeppert B, Frauenschuh L, Zucknick M, Stenzinger A, Andrulis M, Klauschen F, Joehrens K, Warth A, Renner M, Mehrabi A, *et al*: Prognostic impact of tumour-infiltrating immune cells on biliary tract cancer. Br J Cancer 109: 2665-2674, 2013.
79. Ueno T, Tsuchikawa T, Hatanaka KC, Hatanaka Y, Mitsuhashi T, Nakanishi Y, Noji T, Nakamura T, Okamura K, Matsuno Y and Hirano S: Prognostic impact of programmed cell death ligand 1 (PD-L1) expression and its association with epithelial-mesenchymal transition in extrahepatic cholangiocarcinoma. Oncotarget 9: 20034-20047, 2018.
80. Kasper HU, Drebber U, Stippel DL, Dienes HP and Gillessen A: Liver tumor infiltrating lymphocytes: Comparison of hepatocellular and cholangiolar carcinoma. World J Gastroenterol 15: 5053-5057, 2009.

81. Kim HD, Jeong S, Park S, Lee YJ, Ju YS, Kim D, Song GW, Lee JH, Kim SY, Shin J, *et al*: Implication of $CD69^+$ $CD103^+$ tissue-resident-like $CD8^+$ T cells as a potential immunotherapeutic target for cholangiocarcinoma. Liver Int 41: 764-776, 2021.
82. Carnevale G, Carpino G, Cardinale V, Pisciotta A, Riccio M, Bertoni L, Gibellini L, De Biasi S, Nevi L, Costantini D, *et al*: Activation of Fas/FasL pathway and the role of c-FLIP in primary culture of human cholangiocarcinoma cells. Sci Rep 7: 14419, 2017.
83. Ye Y, Zhou L, Xie X, Jiang G, Xie H and Zheng S: Interaction of B7-H1 on intrahepatic cholangiocarcinoma cells with PD-1 on tumor-infiltrating T cells as a mechanism of immune evasion. J Surg Oncol 100: 500-504, 2009.
84. Wu MJ, Shi L, Dubrot J, Merritt J, Vijay V, Wei TY, Kessler E, Olander KE, Adil R, Pankaj A, *et al*: Mutant IDH inhibits IFNγ-TET2 signaling to promote immunoevasion and tumor maintenance in cholangiocarcinoma. Cancer Discov 12: 812-835, 2022.
85. Lu JC, Zeng HY, Sun QM, Meng QN, Huang XY, Zhang PF, Yang X, Peng R, Gao C, Wei CY, *et al*: Distinct PD-L1/PD1 profiles and clinical implications in intrahepatic cholangiocarcinoma patients with different risk factors. Theranostics 9: 4678-4687, 2019.
86. Tian L, Ma J, Ma L, Zheng B, Liu L, Song D, Wang Y, Zhang Z, Gao Q, Song K and Wang X: PD-1/PD-L1 expression profiles within intrahepatic cholangiocarcinoma predict clinical outcome. World J Surg Oncol 18: 303, 2020.
87. Vigano L, Soldani C, Franceschini B, Cimino M, Lleo A, Donadon M, Roncalli M, Aghemo A, Di Tommaso L and Torzilli G: Tumor-infiltrating lymphocytes and macrophages in intrahepatic cholangiocellular carcinoma. Impact on prognosis after complete surgery. J Gastrointest Surg 23: 2216-2224, 2019.
88. Goeppert B, Roessler S, Renner M, Singer S, Mehrabi A, Vogel MN, Pathil A, Czink E, Köhler B, Springfeld C, *et al*: Mismatch repair deficiency is a rare but putative therapeutically relevant finding in non-liver fluke associated cholangiocarcinoma. Br J Cancer 120: 109-114, 2019.
89. Whiteside TL: What are regulatory T cells (Treg) regulating in cancer and why? Semin Cancer Biol 22: 327-334, 2012.
90. Tan YS, Sansanaphongpricha K, Xie Y, Donnelly CR, Luo X, Heath BR, Zhao X, Bellile E, Hu H, Chen H, *et al*: Mitigating SOX2-potentiated immune escape of head and neck squamous cell carcinoma with a STING-inducing nanosatellite vaccine. Clin Cancer Res 24: 4242-4255, 2018.
91. Ma C, Peng C, Lu X, Ding X, Zhang S, Zou X and Zhang X: Downregulation of FOXP3 inhibits invasion and immune escape in cholangiocarcinoma. Biochem Biophys Res Commun 458: 234-239, 2015.
92. Ma K, Sun Z, Li X, Guo J, Wang Q and Teng M: Forkhead box M1 recruits $FoxP3^+$ Treg cells to induce immune escape in hilar cholangiocarcinoma. Immun Inflamm Dis 10: e727, 2022.
93. Sarkar T, Dhar S and Sa G: Tumor-infiltrating T-regulatory cells adapt to altered metabolism to promote tumor-immune escape. Curr Res Immunol 2: 132-141, 2021.
94. Zhang G, Zheng G, Zhang H and Qiu L: MUC1 induces the accumulation of $Foxp3^+$ Treg cells in the tumor microenvironment to promote the growth and metastasis of cholangiocarcinoma through the EGFR/PI3K/Akt signaling pathway. Int Immunopharmacol 118: 110091, 2023.
95. Wang H, Li C, Jian Z, Ou Y and Ou J: TGF-β1 reduces miR-29a expression to promote tumorigenicity and metastasis of cholangiocarcinoma by targeting HDAC4. PLoS One 10: e0136703, 2015.
96. Martín-Sierra C, Martins R, Laranjeira P, Abrantes AM, Oliveira RC, Tralhão JG, Botelho MF, Furtado E, Domingues R and Paiva A: Functional impairment of circulating $FcεRI^+$ monocytes and myeloid dendritic cells in hepatocellular carcinoma and cholangiocarcinoma patients. Cytometry B Clin Cytom 96: 490-495, 2019.
97. Böttcher JP and Reis e Sousa C: The role of type 1 conventional dendritic cells in cancer immunity. Trends Cancer 4: 784-792, 2018.
98. Junking M, Grainok J, Thepmalee C, Wongkham S and Yenchitsomanus PT: Enhanced cytotoxic activity of effector T-cells against cholangiocarcinoma by dendritic cells pulsed with pooled mRNA. Tumour Biol 39: 1010428317733367, 2017.
99. Thepmalee C, Panya A, Sujjitjoon J, Sawasdee N, Poungvarin N, Junking M and Yenchitsomanus PT: Suppression of TGF-β and IL-10 receptors on self-differentiated dendritic cells by short-hairpin RNAs enhanced activation of effector T-cells against cholangiocarcinoma cells. Hum Vaccin Immunother 16: 2318-2327, 2020.
100. Thepmalee C, Panya A, Junking M, Chieochansin T and Yenchitsomanus PT: Inhibition of IL-10 and TGF-β receptors on dendritic cells enhances activation of effector T-cells to kill cholangiocarcinoma cells. Hum Vaccin Immunother 14: 1423-1431, 2018.
101. Sung E, Ko M, Won JY, Jo Y, Park E, Kim H, Choi E, Jung UJ, Jeon J, Kim Y, *et al*: LAG-3xPD-L1 bispecific antibody potentiates antitumor responses of T cells through dendritic cell activation. Mol Ther 30: 2800-2816, 2022.
102. Zeng FL and Chen JF: Application of immune checkpoint inhibitors in the treatment of cholangiocarcinoma. Technol Cancer Res Treat 20: 15330338211039952, 2021.
103. Halpert MM, Konduri V, Liang D, Chen Y, Wing JB, Paust S, Levitt JM and Decker WK: Dendritic cell-secreted cytotoxic T-lymphocyte-associated protein-4 regulates the T-cell response by downmodulating bystander surface B7. Stem Cells Dev 25: 774-787, 2016.
104. Sadeghlar F, Vogt A, Mohr RU, Mahn R, van Beekum K, Kornek M, Weismüller TJ, Branchi V, Matthaei H, Toma M, *et al*: Induction of cytotoxic effector cells towards cholangiocellular, pancreatic, and colorectal tumor cells by activation of the immune checkpoint CD40/CD40L on dendritic cells. Cancer Immunol Immunother 70: 1451-1464, 2021.
105. Djureinovic D, Wang M and Kluger HM: Agonistic CD40 antibodies in cancer treatment. Cancers (Basel) 13: 1302, 2021.
106. Wu R, Ohara RA, Jo S, Liu TT, Ferris ST, Ou F, Kim S, Theisen DJ, Anderson DA III, Wong BW, *et al*: Mechanisms of CD40-dependent cDC1 licensing beyond costimulation. Nat Immunol 23: 1536-1550, 2022.
107. Najafi M, Goradel NH, Farhood B, Salehi E, Solhjoo S, Toolee H, Kharazinejad E and Mortezaee K: Tumor microenvironment: Interactions and therapy. J Cell Physiol 234: 5700-5721, 2019.
108. Ruffolo LI, Jackson KM, Kuhlers PC, Dale BS, Figueroa Guilliani NM, Ullman NA, Burchard PR, Qin SS, Juviler PG, Keilson JM, *et al*: GM-CSF drives myelopoiesis, recruitment and polarisation of tumour-associated macrophages in cholangiocarcinoma and systemic blockade facilitates antitumour immunity. Gut 71: 1386-1398, 2022.
109. Feng M, Jiang W, Kim BYS, Zhang CC, Fu YX and Weissman IL: Phagocytosis checkpoints as new targets for cancer immunotherapy. Nat Rev Cancer 19: 568-586, 2019.
110. Grinberg-Bleyer Y, Oh H, Desrichard A, Bhatt DM, Caron R, Chan TA, Schmid RM, Klein U, Hayden MS and Ghosh S: NF-κB c-Rel is crucial for the regulatory T cell immune checkpoint in cancer. Cell 170: 1096-1108.e13, 2017.
111. Li Z, Li Y, Gao J, Fu Y, Hua P, Jing Y, Cai M, Wang H and Tong T: The role of CD47-SIRPα immune checkpoint in tumor immune evasion and innate immunotherapy. Life Sci 273: 119150, 2021.
112. Vaeteewoottacharn K, Kariya R, Pothipan P, Fujikawa S, Pairojkul C, Waraasawapati S, Kuwahara K, Wongkham C, Wongkham S and Okada S: Attenuation of CD47-SIRPα signal in cholangiocarcinoma potentiates tumor-associated macrophage-mediated phagocytosis and suppresses intrahepatic metastasis. Transl Oncol 12: 217-225, 2019.
113. Morvan MG and Lanier LL: NK cells and cancer: You can teach innate cells new tricks. Nature reviews Cancer 16: 7-19, 2016.
114. Oliviero B, Varchetta S, Mele D, Pessino G, Maiello R, Falleni M, Tosi D, Donadon M, Soldani C, Franceschini B, *et al*: MICA/B-targeted antibody promotes NK cell-driven tumor immunity in patients with intrahepatic cholangiocarcinoma. Oncoimmunology 11: 2035919, 2022.
115. Boussiotis VA: Molecular and biochemical aspects of the PD-1 checkpoint pathway. N Engl J Med 375: 1767-1778, 2016.
116. Sharpe AH and Pauken KE: The diverse functions of the PD1 inhibitory pathway. Nat Rev Immunol 18: 153-167, 2018.
117. Azuma T, Yao S, Zhu G, Flies AS, Flies SJ and Chen L: B7-H1 is a ubiquitous antiapoptotic receptor on cancer cells. Blood 111: 3635-3643, 2008.
118. Gato-Cañas M, Zuazo M, Arasanz H, Ibañez-Vea M, Lorenzo L, Fernandez-Hinojal G, Vera R, Smerdou C, Martisova E, Arozarena I, *et al*: PDL1 signals through conserved sequence motifs to overcome interferon-mediated cytotoxicity. Cell Rep 20: 1818-1829, 2017.
119. Hosseini A, Gharibi T, Marofi F, Babaloo Z and Baradaran B: CTLA-4: From mechanism to autoimmune therapy. Int Immunopharmacol 80: 106221, 2020.
120. Walter D, Herrmann E, Schnitzbauer AA, Zeuzem S, Hansmann ML, Peveling-Oberhag J and Hartmann S: PD-L1 expression in extrahepatic cholangiocarcinoma. Histopathology 71: 383-392, 2017.

121. Yu F, Gong L, Mo Z, Wang W, Wu M, Yang J, Zhang Q, Li L, Yao J and Dong J: Programmed death ligand-1, tumor infiltrating lymphocytes and HLA expression in Chinese extrahepatic cholangiocarcinoma patients: Possible immunotherapy implications. Biosci Trends 13: 58-69, 2019.
122. Ma K, Wei X, Dong D, Wu Y, Geng Q and Li E: PD-L1 and PD-1 expression correlate with prognosis in extrahepatic cholangiocarcinoma. Oncol Lett 14: 250-256, 2017.
123. Kim H, Kim J, Byeon S, Jang KT, Hong JY, Lee J, Park SH, Park JO, Park YS, Lim HY, *et al*: Programmed death ligand 1 expression as a prognostic marker in patients with advanced biliary tract cancer. Oncology 99: 365-372, 2021.
124. Kitano Y, Yamashita YI, Nakao Y, Itoyama R, Yusa T, Umezaki N, Tsukamoto M, Yamao T, Miyata T, Nakagawa S, *et al*: Clinical significance of PD-L1 expression in both cancer and stroma cells of cholangiocarcinoma patients. Ann Surg Oncol 27: 599-607, 2020.
125. Xian F, Ren D, Bie J and Xu G: Prognostic value of programmed cell death ligand 1 expression in patients with intrahepatic cholangiocarcinoma: a meta-analysis. Front Immunol 14: 1119168, 2023.
126. Cai Z, Ang X, Xu Z, Li S, Zhang J, Pei C and Zhou F: A pan-cancer study of PD-1 and CTLA-4 as therapeutic targets. Transl Cancer Res 10: 3993-4001, 2021.
127. Guo XJ, Lu JC, Zeng HY, Zhou R, Sun QM, Yang GH, Pei YZ, Meng XL, Shen YH, Zhang PF, *et al*: CTLA-4 synergizes with PD1/PD-L1 in the inhibitory tumor microenvironment of intrahepatic cholangiocarcinoma. Front Immunol 12: 705378, 2021.
128. Perkhofer L, Beutel AK and Ettrich TJ: Immunotherapy: Pancreatic cancer and extrahepatic biliary tract cancer. Visc Med 35: 28-37, 2019.
129. Andrews LP, Yano H and Vignali DAA: Inhibitory receptors and ligands beyond PD-1, PD-L1 and CTLA-4: breakthroughs or backups. Nat Immunol 20: 1425-1434, 2019.
130. Robert C, Long GV, Brady B, Dutriaux C, Maio M, Mortier L, Hassel JC, Rutkowski P, McNeil C, Kalinka-Warzocha E, *et al*: Nivolumab in previously untreated melanoma without BRAF mutation. N Engl J Med 372: 320-330, 2015.
131. Sharma P, Retz M, Siefker-Radtke A, Baron A, Necchi A, Bedke J, Plimack ER, Vaena D, Grimm MO, Bracarda S, *et al*: Nivolumab in metastatic urothelial carcinoma after platinum therapy (CheckMate 275): A multicentre, single-arm, phase 2 trial. Lancet Oncol 18: 312-322, 2017.
132. El-Khoueiry AB, Sangro B, Yau T, Crocenzi TS, Kudo M, Hsu C, Kim TY, Choo SP, Trojan J, Welling TH Rd, *et al*: Nivolumab in patients with advanced hepatocellular carcinoma (CheckMate 040): An open-label, non-comparative, phase 1/2 dose escalation and expansion trial. Lancet 389: 2492-2502, 2017.
133. Casak SJ, Marcus L, Fashoyin-Aje L, Mushti SL, Cheng J, Shen YL, Pierce WF, Her L, Goldberg KB, Theoret MR, *et al*: FDA approval summary: Pembrolizumab for the first-line treatment of patients with MSI-H/dMMR advanced unresectable or metastatic colorectal carcinoma. Clin Cancer Res 27: 4680-4684, 2021.
134. Nakajima EC, Vellanki PJ, Larkins E, Chatterjee S, Mishra-Kalyani PS, Bi Y, Qosa H, Liu J, Zhao H, Biable M, *et al*: FDA approval summary: Nivolumab in combination with ipilimumab for the treatment of unresectable malignant pleural mesothelioma. Clin Cancer Res 28: 446-451, 2022.
135. Jenkins L, Jungwirth U, Avgustinova A, Iravani M, Mills A, Haider S, Harper J and Isacke CM: Cancer-associated fibroblasts suppress CD8+ T-cell infiltration and confer resistance to immune-checkpoint blockade. Cancer Res 82: 2904-2917, 2022.
136. Job S, Rapoud D, Dos Santos A, Gonzalez P, Desterke C, Pascal G, Elarouci N, Ayadi M, Adam R, Azoulay D, *et al*: Identification of four immune subtypes characterized by distinct composition and functions of tumor microenvironment in intrahepatic cholangiocarcinoma. Hepatology 72: 965-981, 2020.
137. Ikeda Y, Ono M, Ohmori G, Ameda S, Yamada M, Abe T, Fujii S, Fujita M and Maeda M: Successful pembrolizumab treatment of microsatellite instability-high intrahepatic cholangiocarcinoma: A case report. Clin Case Rep 9: 2259-2263, 2021.
138. Mody K, Jain P, El-Refai SM, Azad NS, Zabransky DJ, Baretti M, Shroff RT, Kelley RK, El-Khouiery AB, Hockenberry AJ, *et al*: Clinical, genomic, and transcriptomic data profiling of biliary tract cancer reveals subtype-specific immune signatures. JCO Precis Oncol 6: e2100510, 2022.
139. Le DT, Durham JN, Smith KN, Wang H, Bartlett BR, Aulakh LK, Lu S, Kemberling H, Wilt C, Luber BS, *et al*: Mismatch repair deficiency predicts response of solid tumors to PD-1 blockade. Science 357: 409-413, 2017.
140. Liu X, Yao J, Song L, Zhang S, Huang T and Li Y: Local and abscopal responses in advanced intrahepatic cholangiocarcinoma with low TMB, MSS, pMMR and negative PD-L1 expression following combined therapy of SBRT with PD-1 blockade. J Immunother Cancer 7: 204, 2019.
141. Mou H, Yu L, Liao Q, Hou X, Wu Y, Cui Q, Yan N, Ma R, Wang L, Yao M and Wang K: Successful response to the combination of immunotherapy and chemotherapy in cholangiocarcinoma with high tumour mutational burden and PD-L1 expression: A case report. BMC Cancer 18: 1105, 2018.
142. Piha-Paul SA, Oh DY, Ueno M, Malka D, Chung HC, Nagrial A, Kelley RK, Ros W, Italiano A, Nakagawa K, *et al*: Efficacy and safety of pembrolizumab for the treatment of advanced biliary cancer: Results from the KEYNOTE-158 and KEYNOTE-028 studies. Int J Cancer 147: 2190-2198, 2020.
143. Kim RD, Chung V, Alese OB, El-Rayes BF, Li D, Al-Toubah TE, Schell MJ, Zhou JM, Mahipal A, Kim BH and Kim DW: A phase 2 multi-institutional study of nivolumab for patients with advanced refractory biliary tract cancer. JAMA Oncol 6: 888-894, 2020.
144. Ueno M, Ikeda M, Morizane C, Kobayashi S, Ohno I, Kondo S, Okano N, Kimura K, Asada S, Namba Y, *et al*: Nivolumab alone or in combination with cisplatin plus gemcitabine in Japanese patients with unresectable or recurrent biliary tract cancer: A non-randomised, multicentre, open-label, phase 1 study. Lancet Gastroenterol Hepatol 4: 611-621, 2019.
145. Doki Y, Ueno M, Hsu CH, Oh DY, Park K, Yamamoto N, Ioka T, Hara H, Hayama M, Nii M, *et al*: Tolerability and efficacy of durvalumab, either as monotherapy or in combination with tremelimumab, in patients from Asia with advanced biliary tract, esophageal, or head-and-neck cancer. Cancer Med 11: 2550-2560, 2022.
146. Lan Y, Zhang D, Xu C, Hance KW, Marelli B, Qi J, Yu H, Qin G, Sircar A, Hernández VM, *et al*: Enhanced preclinical antitumor activity of M7824, a bifunctional fusion protein simultaneously targeting PD-L1 and TGF-β. Sci Transl Med 10: eaan5488, 2018.
147. Yoo C, Oh DY, Choi HJ, Kudo M, Ueno M, Kondo S, Chen LT, Osada M, Helwig C, Dussault I and Ikeda M: Phase I study of bintrafusp alfa, a bifunctional fusion protein targeting TGF-β and PD-L1, in patients with pretreated biliary tract cancer. J Immunother Cancer 8: e000564, 2020.
148. Klein O, Kee D, Nagrial A, Markman B, Underhill C, Michael M, Jackett L, Lum C, Behren A, Palmer J, *et al*: Evaluation of combination nivolumab and ipilimumab immunotherapy in patients with advanced biliary tract cancers: Subgroup analysis of a phase 2 nonrandomized clinical trial. JAMA Oncol 6: 1405-1409, 2020.
149. Floudas CS, Xie C, Brar G, Morelli MP, Fioravanti S, Walker M, Mabry-Hrones D, Wood BJ, Levy EB, Krishnasamy VP and Greten TF: Combined immune checkpoint inhibition (ICI) with tremelimumab and durvalumab in patients with advanced hepatocellular carcinoma (HCC) or biliary tract carcinomas (BTC). J Clin Oncol 37 (4 Suppl): S336, 2019.
150. Oh DY, Lee KH, Lee DW, Yoon J, Kim TY, Bang JH, Nam AR, Oh KS, Kim JM, Lee Y, *et al*: Gemcitabine and cisplatin plus durvalumab with or without tremelimumab in chemotherapy-naive patients with advanced biliary tract cancer: An open-label, single-centre, phase 2 study. Lancet Gastroenterol Hepatol 7: 522-532, 2022.
151. Oh DY, Lee KH, Lee DW, Kim TY, Bang JH, Nam AR, Lee Y, Zhang Q, Rebelatto M, Li W and Kim JW: Phase II study assessing tolerability, efficacy, and biomarkers for durvalumab (D) ± tremelimumab (T) and gemcitabine/cisplatin (GemCis) in chemo-naïve advanced biliary tract cancer (aBTC). J Clin Oncol 38 (15 Suppl): S4520, 2020.
152. Feng K, Liu Y, Zhao Y, Yang Q, Dong L, Liu J, Li X, Zhao Z, Mei Q and Han W: Efficacy and biomarker analysis of nivolumab plus gemcitabine and cisplatin in patients with unresectable or metastatic biliary tract cancers: Results from a phase II study. J Immunother Cancer 8: e000367, 2020.
153. Boilève A, Hilmi M, Gougis P, Cohen R, Rousseau B, Blanc JF, Ben Abdelghani M, Castanié H, Dahan L, Tougeron D, *et al*: Triplet combination of durvalumab, tremelimumab, and paclitaxel in biliary tract carcinomas: Safety run-in results of the randomized IMMUNOBIL PRODIGE 57 phase II trial. Eur J Cancer 143: 55-63, 2021.
154. Arkenau HT, Martin-Liberal J, Calvo E, Penel N, Krebs MG, Herbst RS, Walgren RA, Widau RC, Mi G, Jin J, *et al*: Ramucirumab plus pembrolizumab in patients with previously treated advanced or metastatic biliary tract cancer: Nonrandomized, open-label, phase I trial (JVDF). Oncologist 23: 1407-e136, 2018.

155. Yarchoan M, Cope L, Ruggieri AN, Anders RA, Noonan AM, Goff LW, Goyal L, Lacy J, Li D, Patel AK, *et al*: Multicenter randomized phase II trial of atezolizumab with or without cobimetinib in biliary tract cancers. J Clin Invest 131: e152670, 2021.
156. Lin J, Yang X, Long J, Zhao S, Mao J, Wang D, Bai Y, Bian J, Zhang L, Yang X, *et al*: Pembrolizumab combined with lenvatinib as non-first-line therapy in patients with refractory biliary tract carcinoma. Hepatobiliary Surg Nutr 9: 414-424, 2020.
157. Xie C, Duffy AG, Mabry-Hrones D, Wood B, Levy E, Krishnasamy V, Khan J, Wei JS, Agdashian D, Tyagi M, *et al*: Tremelimumab in combination with microwave ablation in patients with refractory biliary tract cancer. Hepatology 69: 2048-2060, 2019.
158. Leem G, Jang SI, Cho JH, Jo JH, Lee HS, Chung MJ, Park JY, Bang S, Yoo DK, Cheon HC, *et al*: Safety and efficacy of allogeneic natural killer cells in combination with pembrolizumab in patients with chemotherapy-refractory biliary tract cancer: A multicenter open-label phase 1/2a trial. Cancers (Basel) 14: 4229, 2022.
159. Sterner RC and Sterner RM: CAR-T cell therapy: Current limitations and potential strategies. Blood Cancer J 11: 69, 2021.
160. Sangsuwannukul T, Supimon K, Sujjitjoon J, Phanthaphol N, Chieochansin T, Poungvarin N, Wongkham S, Junking M and Yenchitsomanus PT: Anti-tumour effect of the fourth-generation chimeric antigen receptor T cells targeting CD133 against cholangiocarcinoma cells. Inte Int Immunopharmacol 89: 107069, 2020.
161. Guo Y, Feng K, Liu Y, Wu Z, Dai H, Yang Q, Wang Y, Jia H and Han W: Phase I study of chimeric antigen receptor-modified T cells in patients with EGFR-positive advanced biliary tract cancers. Clin Cancer Res 24: 1277-1286, 2018.
162. Feng KC, Guo YL, Liu Y, Dai HR, Wang Y, Lv HY, Huang JH, Yang QM and Han WD: Cocktail treatment with EGFR-specific and CD133-specific chimeric antigen receptor-modified T cells in a patient with advanced cholangiocarcinoma. J Hematol Oncol 10: 4, 2017.
163. Alnaggar M, Xu Y, Li J, He J, Chen J, Li M, Wu Q, Lin L, Liang Y, Wang X, *et al*: Allogenic Vγ9Vδ2 T cell as new potential immunotherapy drug for solid tumor: A case study for cholangiocarcinoma. J Immunother Cancer 7: 36, 2019.
164. Tran E, Turcotte S, Gros A, Robbins PF, Lu YC, Dudley ME, Wunderlich JR, Somerville RP, Hogan K, Hinrichs CS, *et al*: Cancer immunotherapy based on mutation-specific CD4+ T cells in a patient with epithelial cancer. Science 344: 641-645, 2014.
165. Zhang T, Chen J, Niu L, Liu Y, Ye G, Jiang M and Qi Z: Clinical safety and efficacy of locoregional therapy combined with adoptive transfer of allogeneic γδ T cells for advanced hepatocellular carcinoma and intrahepatic cholangiocarcinoma. J Vasc Interv Radiol 33: 19-27.e3, 2022.
166. Shimizu K, Kotera Y, Aruga A, Takeshita N, Takasaki K and Yamamoto M: Clinical utilization of postoperative dendritic cell vaccine plus activated T-cell transfer in patients with intrahepatic cholangiocarcinoma. J Hepatobiliary Pancreat Sci 19: 171-178, 2012.
167. Shimizu K, Kotera Y, Aruga A, Takeshita N, Katagiri S, Ariizumi S, Takahashi Y, Yoshitoshi K, Takasaki K and Yamamoto M: Postoperative dendritic cell vaccine plus activated T-cell transfer improves the survival of patients with invasive hepatocellular carcinoma. Hum Vaccin Immunother 10: 970-976, 2014.
168. Vonderheide RH: CD40 agonist antibodies in cancer immunotherapy. Annu Rev Med 71: 47-58, 2020.
169. Hegde S, Krisnawan VE, Herzog BH, Zuo C, Breden MA, Knolhoff BL, Hogg GD, Tang JP, Baer JM, Mpoy C, *et al*: Dendritic cell paucity leads to dysfunctional immune surveillance in pancreatic cancer. Cancer Cell 37: 289-307.e9, 2020.
170. Zhang J, Li Y, Yang S, Zhang L and Wang W: Anti-CD40 mAb enhanced efficacy of anti-PD1 against osteosarcoma. J Bone Oncol 17: 100245, 2019.
171. Leblond MM, Tillé L, Nassiri S, Gilfillan CB, Imbratta C, Schmittnaegel M, Ries CH, Speiser DE and Verdeil G: CD40 agonist restores the antitumor efficacy of anti-PD1 therapy in muscle-invasive bladder cancer in an IFN I/II-mediated manner. Cancer Immunol Res 8: 1180-1192, 2020.
172. Ma HS, Poudel B, Torres ER, Sidhom JW, Robinson TM, Christmas B, Scott B, Cruz K, Woolman S, Wall VZ, *et al*: A CD40 agonist and PD-1 antagonist antibody reprogram the microenvironment of nonimmunogenic tumors to allow T-cell-mediated anticancer activity. Cancer Immunol Res 7: 428-442, 2019.
173. Moreno V, Perets R, Peretz-Yablonski T, Fourneau N, Girgis S, Guo Y, Hellemans P, Verona R, Pendás N, Xia Q, *et al*: A phase 1 study of intravenous mitazalimab, a CD40 agonistic monoclonal antibody, in patients with advanced solid tumors. Invest New Drugs 41: 93-104, 2023.
174. Humphreys EH, Williams KT, Adams DH and Afford SC: Primary and malignant cholangiocytes undergo CD40 mediated Fas dependent apoptosis, but are insensitive to direct activation with exogenous Fas ligand. PLoS One 5: e14037, 2010.
175. Diggs LP, Ruf B, Ma C, Heinrich B, Cui L, Zhang Q, McVey JC, Wabitsch S, Heinrich S, Rosato U, *et al*: CD40-mediated immune cell activation enhances response to anti-PD-1 in murine intrahepatic cholangiocarcinoma. J Hepatol 74: 1145-1154, 2021.
176. O'Hara MH, O'Reilly EM, Rosemarie M, Varadhachary G, Wainberg ZA, Ko A, Fisher GA, Rahma O, Lyman JP, Cabanski CR, *et al*: Abstract CT004: A phase Ib study of CD40 agonistic monoclonal antibody APX005M together with gemcitabine (Gem) and nab-paclitaxel (NP) with or without nivolumab (Nivo) in untreated metastatic ductal pancreatic adenocarcinoma (PDAC) patients. Cancer Res 79 (13 Suppl): CT004, 2019.
177. Lin Y, Peng L, Dong L, Liu D, Ma J, Lin J, Chen X, Lin P, Song G, Zhang M, *et al*: Geospatial immune heterogeneity reflects the diverse tumor-immune interactions in intrahepatic cholangiocarcinoma. Cancer Discov 12: 2350-2371, 2022.
178. Morse MA, Gwin WR III and Mitchell DA: Vaccine therapies for cancer: Then and now. Target Oncol 16: 121-152, 2021.
179. Goldstein D, Lemech C and Valle J: New molecular and immunotherapeutic approaches in biliary cancer. ESMO Open 2 (Suppl 1): e000152, 2017.
180. Koido S, Kan S, Yoshida K, Yoshizaki S, Takakura K, Namiki Y, Tsukinaga S, Odahara S, Kajihara M, Okamoto M, *et al*: Immunogenic modulation of cholangiocarcinoma cells by chemoimmunotherapy. Anticancer Res 34: 6353-6361, 2014.
181. Kaida M, Morita-Hoshi Y, Soeda A, Wakeda T, Yamaki Y, Kojima Y, Ueno H, Kondo S, Morizane C, Ikeda M, *et al*: Phase 1 trial of Wilms tumor 1 (WT1) peptide vaccine and gemcitabine combination therapy in patients with advanced pancreatic or biliary tract cancer. J Immunother 34: 92-99, 2011.
182. Yamamoto K, Ueno T, Kawaoka T, Hazama S, Fukui M, Suehiro Y, Hamanaka Y, Ikematsu Y, Imai K, Oka M and Hinoda Y: MUC1 peptide vaccination in patients with advanced pancreas or biliary tract cancer. Anticancer Res 25: 3575-3579, 2005.
183. Aruga A, Takeshita N, Kotera Y, Okuyama R, Matsushita N, Ohta T, Takeda K and Yamamoto M: Long-term vaccination with multiple peptides derived from cancer-testis antigens can maintain a specific T-cell response and achieve disease stability in advanced biliary tract cancer. Clin Cancer Res 19: 2224-2231, 2013.
184. Aruga A, Takeshita N, Kotera Y, Okuyama R, Matsushita N, Ohta T, Takeda K and Yamamoto M: Phase I clinical trial of multiple-peptide vaccination for patients with advanced biliary tract cancer. J Transl Med 12: 61, 2014.
185. Yoshitomi M, Yutani S, Matsueda S, Ioji T, Komatsu N, Shichijo S, Yamada A, Itoh K, Sasada T and Kinoshita H: Personalized peptide vaccination for advanced biliary tract cancer: IL-6, nutritional status and pre-existing antigen-specific immunity as possible biomarkers for patient prognosis. Exp Ther Med 3: 463-469, 2012.
186. Lepisto AJ, Moser AJ, Zeh H, Lee K, Bartlett D, McKolanis JR, Geller BA, Schmotzer A, Potter DP, Whiteside T, *et al*: A phase I/II study of a MUC1 peptide pulsed autologous dendritic cell vaccine as adjuvant therapy in patients with resected pancreatic and biliary tumors. Cancer Ther 6: 955-964, 2008.
187. Kobayashi M, Sakabe T, Abe H, Tanii M, Takahashi H, Chiba A, Yanagida E, Shibamoto Y, Ogasawara M, Tsujitani S, *et al*: Dendritic cell-based immunotherapy targeting synthesized peptides for advanced biliary tract cancer. J Gastrointest Surg 17: 1609-1617, 2013.
188. Hochnadel I, Hoenicke L, Petriv N, Neubert L, Reinhard E, Hirsch T, Alfonso JCL, Suo H, Longerich T, Geffers R, *et al*: Safety and efficacy of prophylactic and therapeutic vaccine based on live-attenuated Listeria monocytogenes in hepatobiliary cancers. Oncogene 41: 2039-2053, 2022.
189. Miao L, Zhang Y and Huang L: mRNA vaccine for cancer immunotherapy. Mol Cancer 20: 41, 2021.
190. Huang X, Tang T, Zhang G and Liang T: Identification of tumor antigens and immune subtypes of cholangiocarcinoma for mRNA vaccine development. Mol Cancer 20: 50, 2021.

191. Izquierdo-Sanchez L, Lamarca A, La Casta A, Buettner S, Utpatel K, Klümpen HJ, Adeva J, Vogel A, Lleo A, Fabris L, *et al*: Cholangiocarcinoma landscape in Europe: Diagnostic, prognostic and therapeutic insights from the ENSCCA Registry. J Hepatol 76: 1109-1121, 2022.
192. Walker NJ, Crockett PW, Nyska A, Brix AE, Jokinen MP, Sells DM, Hailey JR, Easterling M, Haseman JK, Yin M, *et al*: Dose-additive carcinogenicity of a defined mixture of 'dioxin-like compounds'. Environ Health Perspect 113: 43-48, 2005.
193. National Toxicology Program: Toxicology and carcinogenesis studies of 2,3',4,4',5-pentachlorobiphenyl (PCB 118) (CAS No. 31508-00-6) in female harlan Sprague-Dawley rats (gavage studies). Natl Toxicol Program Tech Rep Ser: 1-174, 2010.
194. Lowery MA, Ptashkin R, Jordan E, Berger MF, Zehir A, Capanu M, Kemeny NE, O'Reilly EM, El-Dika I, Jarnagin WR, *et al*: Comprehensive molecular profiling of intrahepatic and extrahepatic cholangiocarcinomas: Potential targets for intervention. Clin Cancer Res 24: 4154-4161, 2018.
195. Weinberg BA, Xiu J, Lindberg MR, Shields AF, Hwang JJ, Poorman K, Salem ME, Pishvaian MJ, Holcombe RF, Marshall JL and Morse MA: Molecular profiling of biliary cancers reveals distinct molecular alterations and potential therapeutic targets. J Gastrointest Oncol 10: 652-662, 2019.
196. Kendall T, Verheij J, Gaudio E, Evert M, Guido M, Goeppert B and Carpino G: Anatomical, histomorphological and molecular classification of cholangiocarcinoma. Liver Int 39 (Suppl 1): S7-S18, 2019.