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# Research progress of T cells in cholangiocarcinoma

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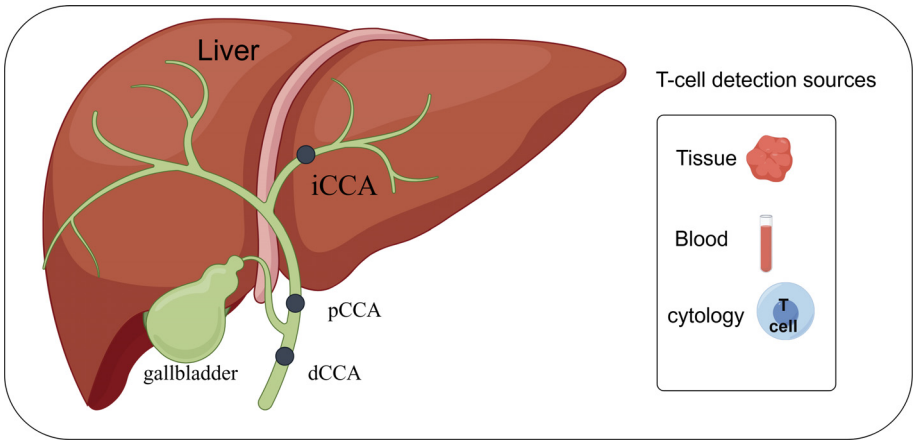
Cholangiocarcinoma (CCA), a malignant tumor, is typically challenging to detect early and often results in a poor prognosis. In recent years, research interest has grown in the potential application of immunotherapy for CCA treatment. T cells, as a crucial component of the immune system, play a significant role in immune surveillance and therapy for cholangiocarcinoma. This article provides a review of the research advancements concerning T cells in cholangiocarcinoma patients, including their distribution, functional status, and correlation with patient prognosis within the tumor microenvironment. It further discusses the potential applications and challenges of immunotherapy strategies targeting T cells in CCA treatment and anticipates future research directions. A more profound understanding of T cells' role in cholangiocarcinoma can guide the development of clinical treatment strategies, thereby enhancing patient survival rates and quality of life. Finally, we explored the potential risks and side effects of immunotherapy for T-cell cholangiocarcinoma.

KEYWORDS

**cholangiocarcinoma, T lymphocytes, tumor microenvironment, immunotherapy, immunization checkpoints**




GRAPHICAL ABSTRACT
Depending on the anatomical location, cholangiocarcinoma can be categorized into intrahepatic cholangiocarcinoma (iCCA), periportal cholangiocarcinoma (pCCA), and distal cholangiocarcinoma (dCCA). Research related to T-lymphocytes in cholangiocarcinoma can be carried out in depth by detecting tissues, blood and cells. By FigDraw.

# 1 Introduction

CCA is a highly malignant neoplasm that arises from the biliary epithelium and is characterized by its late presentation and aggressive course. CCA has numerous subtypes with different origins. Intrahepatic CCA (iCCA) originates within the liver parenchyma; perihilar CCA (pCCA) occurs at the confluence of the left and right hepatic ducts; and distal CCA (dCCA) develops in the lower portion of the bile duct near the duodenum (1). Asia has the highest incidence of CCA in the world, with the proportion of CCA-related deaths ranging from 2.88% to 4.65% (2–4), posing a serious threat to public health. The etiology of CCA is multifactorial, with a range of risk factors contributing to its development. Chronic inflammation of the bile ducts, often associated with conditions such as primary sclerosing cholangitis (PSC) and chronic biliary infections, is a well-established risk factor (5). Additionally, exposure to toxins, such as certain chemicals and liver flukes, genetic predisposition, and underlying liver diseases, including cirrhosis, can heighten the risk of CCA (6). One of the greatest challenges in managing CCA lies in its insidious nature, with symptoms often remaining undetectable until the disease has advanced to later stages. Common clinical presentations include jaundice, abdominal pain, unexplained weight loss, and changes in stool or urine color (7). Recognizing these signs and symptoms early on is pivotal for timely diagnosis and intervention. Despite advancements in medical research, treatment options for CCA have been still limited to surgical intervention, chemotherapy, and radiation therapy. For patients who undergo resection, reported 5-year survival rates are low, ranging from 21 to 35% (8). Currently, the first-line chemotherapy regimen for advanced or recurrent CCA is gemcitabine plus cisplatin. However, the efficacy of chemotherapy for CCA is low compared to other cancers (9, 10). While traditional treatments like surgery, chemotherapy, and radiation have limited benefits in certain patient populations, the development of novel immunotherapeutic approaches, have begun to show potential in improving survival rates and quality of life for patients that leverages the immune system.

The effectiveness of CCA immunotherapy depends largely on the fitness and distribution of immune cells within the tumor microenvironment(TME). These factors are critical in determining which patients may benefit from such treatments. The TME of CCA consists of a diverse range of cells, including stromal cells like cancer-associated fibroblasts (CAFs), endothelial cells, and immune cells from both the innate and adaptive immune systems such as tumor-associated macrophages (TAMs), neutrophils, natural killer cells, and T and B lymphocytes (11). In CCA, T cells constitute the major subset of the TME (12). The success of some T cell-related immunotherapies developed for this purpose in cholangiocarcinoma depends on whether T cells can effectively recognize and respond to tumor antigens to attack cancer cells. Given these complexities, a comprehensive understanding of T cells' mechanism in cholangiocarcinoma patients and related treatment strategies holds significant potential for improving patient prognosis and extending survival time. This review will encapsulate the research advancements of T cells in cholangiocarcinoma, investigate their application potential in tumor immunotherapy, and anticipate future research directions.

# 2 The basic knowledge of T cells in cancer

T lymphocytes originate from bone marrow (BM) progenitors and subsequently migrate to the thymus. After differentiating and maturing in the thymus, T lymphocytes are distributed to immune organs and tissues throughout the body, where they play a crucial role in immune responses through the circulation of lymphatic vessels, blood, and tissue fluid (13). Over the past few decades, we have been studying T cells more and more, and our knowledge of T

cells has become clearer and clearer. In this section, we describe several of the major T cell subsets to aid in the understanding of this review.

$CD4^+$ T helper (Th) cells represent a heterogeneous group of T cells that play central roles in almost all aspects of immune responses. These cells can be activated by the peptide-MHC class II complex on antigen-presenting cells (APCs), along with costimulatory signals and cytokine signaling, differentiating into several subsets characterized by distinct surface molecules and cytokine profiles, including Th1, Th2, Treg, Th17, etc (14, 15).

Th1 cells predominantly exert anti-tumor activity. The frequency of the Th1 subset and the production of IFN-γ in the TME correlate positively with better clinical outcomes across multiple tumor types including melanoma, breast, ovarian, lung, colorectal, and laryngeal cancers (16–22). Th1 cells promote tumor rejection by shaping an anti-tumor immune environment and indirectly supporting the effector functions of other immune cells (23). They are an important subset of CD4 T cells that provide help for CD8 T cell responses and functions. The migration of effector CD8 T cells in the TME depends on the chemokine receptor CXCR3 and its ligands, CXCL9 and CXCL10, which are predominantly expressed by Th1-related, IFN-γ-activated macrophages, cancer-associated fibroblasts (CAFs), and tumor cells (24). Additionally, IFN-γ and IL-2 produced by Th1 cells enhance the survival, proliferation, and cytolytic function of CD8 cytotoxic T lymphocytes (CTLs) (25). IFN-γ can significantly enhance MHC class I and II expression, as well as tumor-derived antigen presentation on tumor cells (26).

The role of Th2 cells in tumor progression remains controversial, exhibiting both favorable and deleterious effects. Previous studies have shown that Th2 cells can suppress tumor growth by activating eosinophils as cytotoxic effector cells in murine plasmacytoma and melanoma (27). The adoptive transfer of tumor-specific Th2 cells induces a massive accumulation of M2-type macrophages at the tumor site, triggering an inflammatory immune response to eliminate myeloma cells (28). However, Th2-associated IL-4 signaling in monocytes and macrophages promotes breast cancer metastasis (29). Th2 cells can also attenuate Th1-associated anti-tumor responses through IL-4 signaling (30). The discrepancies in Th2-mediated tumor immunity may be attributed to different tumor types and distinct Th2 cell states. For example, studies suggest that tumor-promoting Th2 cells exhibit high levels of IL-10 and TGF-β, whereas Th2 cells with elevated expression of IL-3, IL-5, and IL-13 demonstrate anti-tumor immunity (31, 32). Regulatory T (Treg) cells are a specialized subset of CD4 T cells that maintain immune tolerance by suppressing immune responses. Treg cells are characterized by high expression of the IL-2 receptor alpha chain (IL-2Rα, CD25), inhibitory cytokines IL-10, TGF-β, and IL-35, as well as the master transcription factor Foxp3 (33). Two major subsets of Treg cells are identified based on their developmental origin: thymic Treg (tTreg) cells, also known as natural Treg (nTreg) cells that derive from the thymus, and induced Treg (iTreg) cells that differentiate from conventional CD4 T cells in the periphery following antigen stimulation in the presence of TGF-β and IL-2 (34). Treg cells are significantly infiltrated in many solid tumors (35, 36), and a high frequency of Treg cells is mainly associated with worse clinical outcomes in the majority of tumor types.

$CD8^+$ T cells play critical roles in combating intracellular pathogens and eliminating malignant cells in cancer (37). Upon antigen stimulation, naïve $CD8^+$ T cells undergo robust expansion, giving rise to effector and memory T cells. Effector $CD8^+$ T cells, known as $CD8^+$ cytotoxic T lymphocytes (CTLs), can directly induce target cell death through the interaction between Fas and its ligand, as well as the secretion of the cytolytic mediator perforin, which creates pores in target cells and allows the delivery of granule serine proteases (granzymes) to induce apoptosis. Memory $CD8^+$ T cells provide rapid and strong protection upon antigen re-encounter, which is critical for effective and long-term immunity. During $CD8^+$ T cell differentiation, heterogeneous effector and memory populations have been identified, including short-lived effector $CD8^+$ T cells (TE), exhausted $CD8^+$ T cells (Tex), long-lived memory $CD8^+$ T cells (TM), memory precursor $CD8^+$ T cells (TMP), central memory $CD8^+$ T cells (TCM), effector memory $CD8^+$ T cells (TEM), and tissue-resident memory (TRM) cells, named for their phenotype, differentiation potential, and functionality (38, 39). With tumor progression, $CD8^+$ T cells gradually lose their production of IL-2 and TNF-α, as well as their cytotoxic function (40). A key hallmark of Tex cells is the upregulated and sustained expression of multiple immune checkpoints(ICs), such as PD-1, CTLA-4, TIGIT, Tim-3, LAG-3, and GITR. Tumors undergo immune escape via these immune checkpoints by destroying $CD8^+$ T cells or inhibiting their immune function, thus achieving tumor immune escape for tumor metastasis or progression (41–45). Figure 1 shows the currently known $CD8^+$ T cell-related immune checkpoints and their receptors in CCA. The extent and co-expression of ICs directly correlate with the severity of exhaustion (46). On the other hand, Tex cells also express costimulatory molecules, which can promote T cell exhaustion in the tumor microenvironment. For example, costimulation of CD27 and CD28 enhances T cell exhaustion (47). CD28 signaling is compromised due to loss of competition with CTLA-4 for B7 family ligands (48). PD-1 signaling further suppresses T cell function by specifically inducing CD28 dephosphorylation (49).

Tissue-resident $CD8^+$ T cells, identified as $CD103^+$ $CD8^+$ T cells, are essential for the anti-tumor immune response in regional tissue immunity (50). E-cadherin is an important ligand for CD103 (integrin alpha E, ITGAE) (51). In the tumor microenvironment (TME), epithelial cancer cells can express E-cadherin, interact with $CD103^+$ $CD8^+$ T cells, and maintain the interaction with cancer cells, leading to the residence of tumor antigen-reactive $CD8^+$ T cells and a persistent anti-tumor effect in tumor tissues (52). It was found that patients with high infiltration of tissue-resident $CD8^+$ T cells in ICC tumor tissues had better overall survival (OS) and prognosis (53).

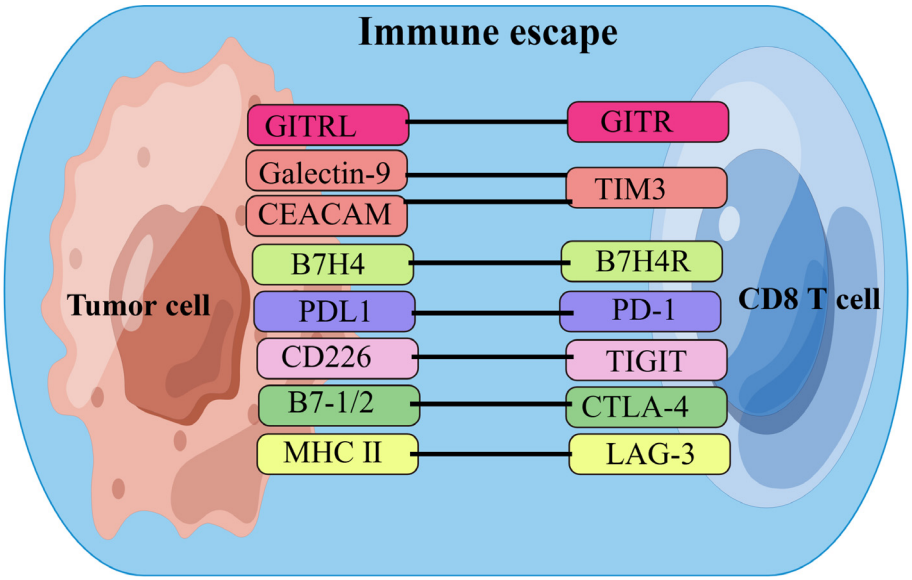



FIGURE 1
List of ICs and their receptors in CCA. Cholangiocarcinoma cells achieve immune escape by interacting with immune checkpoints on $CD8^+$T cells. ICs, immune checkpoints;B7H4, B7 homolog 4; B7H4R, B7H4 receptor; PD1, programmed cell death protein-1;PDL1, programmed cell death ligand-1; CEACAM, the carcinoembryonic antigen-related adhesion molecules; MHC II, major histocompatibility complex class II; GITR, Glucocorticoid-Induced TNF-related protein; GITRL, GITR ligand; TIM3, T cell immunoglobulin and mucin domain-containing protein 3;CTLA-4, cytotoxic T-lymphocyte associated protein 4; TIGIT, T-cell immunoglobulin and ITIM domain; LAG-3, lymphocyte activation gene 3. By FigDraw.

## 3 The proportion and distribution of T cells in cholangiocarcinoma

Studies have demonstrated that the proportion and distribution of T cell subsets significantly alter in patients with cholangiocarcinoma. Specifically, the study discovered an increase in exhausted and regulatory T cells, a reduction in cytotoxic T cells, and the appearance of tumor-specific T cells in cholangiocarcinoma tissue (54, 55). The proportion of total lymphocytes decreased, while the percentages of activated T cells as well as $CD4^+CD25^+$ regulatory T cells (Tregs) increased in peripheral blood of patients with CCA (56). Additionally, tumor-infiltrating immune cells were found to be concentrated in the tumor stroma and infiltration margins, yet scarce in the tumor epithelium and tumor core. Overall, CD8 T cells showed high expression of suppressive markers (PD-1, TIM-3, LAG-3, TIGIT, and NKG2A), indicating depletion of cytotoxic effector cells, along with high infiltration of immune-suppressing tumor-infiltrating Tregs (CD4FOXP3) (57), and also the impaired function of tumor-specific $CD8^+$ T cells and enhanced immunosuppression by $CD4^+$ regulatory T cells (58).Regarding the spatial distribution of T cell subsets, the study discovered that the density of $CD8^+$ T cells, $FoxP3^-CD4^+$ helper T cells, and $FoxP3^+$ $CD4^+$ regulatory T cells in the tumor edge area was considerably higher than that in the tumor stroma and tumor core (59). Additionally, the density of tissue-resident $CD8^+$ tumor-infiltrating lymphocytes (TILs) expressing $CD69^+CD103^+$ was noticeably higher in the tumor edge zone and tumor core zone than in the stromal zone (53, 60). Spatial heterogeneity is one of the key features of the tumor microenvironment (61), and the composition and localization of the immune infiltrate varies significantly according to its dynamic interactions with tumor and/or stromal cells (62, 63). According to the above studies, the peritumor region rather than the tumor core itself is the main site of active infiltration of T cell subsets such as $CD8^+$ T cells and $FoxP3^-$ $CD4^+$ T cells, while Tregs infiltrate into the tumor. Thus, CCA must be considered an immune-rejecting tumor in which the majority of effector T cells are isolated at the tumor margin (64).These results highlight the complex changes of T cells in the cholangiocarcinoma microenvironment and provide potential immunotherapy directions, providing an important reference for optimizing immunotherapy strategies for cholangiocarcinoma. An overview of the different cell subsets and their spatial distribution is presented in Table 1.

## 4 Molecular pathogenesis of T cell-associated cholangiocarcinoma

The molecular pathogenesis of T cell-associated cholangiocarcinoma encompasses several facets. Chronic inflammation is a major contributor to cancer promotion and progression. A plethora of clinical and epidemiological observations have validated the link between a prolonged inflammatory state and cancer incidence. Primary sclerosing cholangitis (PSC) and primary biliary cholangitis (PBC) are major chronic inflammatory diseases that damage bile duct epithelial cells. Although PSC predisposes individuals to bile duct cancer, its incidence is low in the autoimmune setting of PBC. Type 1 T helper (Th1) and T cytotoxic (Tc1) effector cells are critical mediators of both autoimmunity and cancer immunosurveillance (65). In PBC mice, Th1/Tc1 and Th2/Tc2 cell subsets were notably enriched in the liver, detected in

TABLE 1 Characteristic distribution of T lymphocytes in CCA.

| Ref | Year | Country | Location of TILs | Experimental materials | Experimental methods and assessment of TILS | Outcomes |
|---|---|---|---|---|---|---|
| (54) | 2023 | China | IT vs PT | ICC tissue | ScRNA and scTCR sequencing | 1.exhausted and regulatory T cells:IT>PT<br>2.cytotoxic T cells:IT<PT<br>3.IT: new tumor-specific T cell |
| (57) | 2023 | China | IT vs TM | ICC tissue | multiplexed immunofluorescence | tumor-infiltrating immune cells:TM>IT |
| (58) | 2022 | Italy | IT vs TM | ICC tissue | high-dimensional single-cell technologies | 1.tumor-specific $CD39^+$ $CD8^+$ T cells:TM>IT<br>2.hyperactivated $CD4^+$ Tregs: TM>IT |
| (59) | 2021 | Korea | IT vs TM | CCA tissue | mIHC | 1.$FoxP3^-$ $CD4^+$ helper T cells, $FoxP3^+$ $CD4^+$ regulatory T cells and $CD8^+$ T cells: TM>IT<br>2.$FoxP3^-CD4^+$ helper T cell and $LAG3^+TIM3^+CD8^+$ T cell: TM>IT |
| (53) | 2023 | China | IT vs PT | ICC tissue | mIHC | Tissue-resident CD8T cells (CD103CD8T cells): IT>PT |
| (60) | 2021 | Korea | Blood vs ICC tissue | Blood<br>ICC tissue | multicolor flow cytometry<br>mIHC<br>RNA sequencing | $CD69^+CD103^-$and $CD69^+CD103^+$tissue-resident memory (TRM) -like $CD8^+$T cells: Blood < ICC tissue |

tumor-draining lymph nodes, and concentrated in CCA tissues compared with PSC mice or mice without cholangitis (66). This suggests that protection against PBC depends on both type 1 and type 2 T cell responses.

Cholangiocarcinoma cells evade immune surveillance by obstructing Fas receptor (FasR) signaling or augmenting Fas ligand (FasL) expression to trigger apoptosis in T cells. Further research revealed that decreasing the expression of FLICE inhibitory protein (I-FLICE) in cholangiocarcinoma cells reinstates Fas-mediated cell apoptosis. I-FLICE is homologous to cystatinase 8 and expresses a death effector structural domain but has no catalytic activity. Therefore, it competitively prevents the binding of cystatinase 8 to the FasR complex by binding to FADD (Fas-associated with death domain protein) through its death effector domain, thus preventing Fas-mediated apoptosis. Hence, suppressing the expression of I-FLICE could prove beneficial for cholangiocarcinoma treatment (67–69).

In addition, studies have discovered that cholangiocarcinoma cells overproduce mucin 1 (MUC1), which interacts with EGFR, thereby activating the EGFR/PI3K/Akt signaling pathway. Simultaneously, this interaction provokes the accumulation of $Foxp3^+$ regulatory T cells in the tumor microenvironment, enhancing the malignant phenotype of cholangiocarcinoma cells and promoting tumor initiation. Consequently, this process intensifies the growth and metastasis of cholangiocarcinoma (70). However, it is unclear how MUC1 regulates the enrichment of $Foxp3^+$ Treg cells in the TME. Many questions, including which cytokines are involved, how $Foxp3^+$ Treg cells respond to these induced signals, and the source of $Foxp3^+$ Treg cells, need to be further explored.

Furthermore, the expression of MMP14 in cholangiocarcinoma tissue is significantly elevated compared to adjacent tissues. MMPs (matrix metalloproteinases) are a group of proteinases intimately linked with angiogenesis and tumor progression (71).MMP14, the first transmembrane protein identified in this group, is strongly correlated with the infiltration of various immune cells. The number of central memory CD8 T cells, neutrophils, monocytes, and central memory CD4 T cells was significantly decreased in patients with ICC with high MMP14 expression. MMP14 may accelerate the progression of ICC by interfering with the abundance of monocytes and CD4 T cells (72).

Mutations in isocitrate dehydrogenase 1 (mIDH1) are prevalent in cholangiocarcinoma (73–75). The mIDH1 enzyme produces (R)-2-hydroxyglutarate, which in turn supports the maintenance of cholangiocarcinoma tumors with an immune evasion program centered on a dual mechanism mediated by (R)-2-hydroxyglutarate (suppression of $CD8^+$ T cell activity and tumor cell-autonomous inactivation of TET2 DNA demethylase) (76, 77).

Lastly, a substantial upregulation of secreted phosphoprotein 1 (SPP1) has been observed in the tumor epithelial cells of ICC. CD44 was identified as a ligand for osteopontin (OPN), a protein encoded by SPP1, which is primarily expressed in T cells. SPP1 is thought to inhibit T cell activation, however, how the SPP1-CD44 combination affects T cell anti-tumor immunity and clinical outcomes in patients remains unclear (78). SPP1 interacts with T cells via SPP1-CD44 interaction, inhibiting the sustained proliferation of T cells. However, immunosuppressive T cells in the TME may evade this inhibition by reducing CD44 expression (79). Collectively, these research findings uncover the molecular mechanisms intimately linked with T cells and cholangiocarcinoma pathogenesis, offering vital insights for the formulation of new immunotherapy strategies and prognostic markers. Table 2 provides a summary of the molecular pathogenesis of CCA associated with T lymphocytes collected for this review.

## 5 T cells play a pivotal role in the prognosis of cholangiocarcinoma

In CCA patients, the extent of CD8 T cell infiltration in tumor tissues exhibits a negative correlation with serum alpha fetoprotein (AFP)levels, tumor size, and lymph node metastasis (80). Low-level CD8 T-cell infiltration corresponds to shortened OS and shortened disease-free survival (DFS) (80–83).Among patients with ICC, those

TABLE 2 Molecular pathogenesis of CCA associated with T lymphocytes.

| Ref | Year | Country | Study of genes or molecular pathways | Experimental materials | Experimental methods and assessment of TILS | Tumor type | Main findings |
|---|---|---|---|---|---|---|---|
| (67) | 2017 | Italy | Fas/ FasL pathway | cell | cell culture, Western blot, IHC | iCC | iCCA cells have immunomodulatory properties and mediate T cell apoptosis through the Fas/FasL pathway. |
| (70) | 2023 | China | EGFR/PI3K/ Akt signaling pathway | Cell tissue | cell culture, Western blot, IHC | CCA | MUC1 interacts with EGFR and activates the EGFR/ PI3K/Akt signaling pathway, thereby inducing the aggregation of Foxp3$^+$Treg cells, enhancing the malignant phenotype of cholangiocarcinoma cells, and ultimately promoting the growth and metastasis of cholangiocarcinoma. |
| (72) | 2023 | China | MMP14 | tissue | Sangshin database analysis | iCC | MMP14 affects the infiltration of activated-memory CD4$^+$T cells, resting-memory CD4$^+$T cells, and other immune cells, and is strongly associated with the expression of CD200, CTLA-4, CD14, CD44, and other immune checkpoints. |
| (79) | 2023 | China | SPP1 | tissue | Sangshin database analysis | iCC | SPP1 expression is upregulated in ICCA tumor epithelial cells, and SPP1-CD44 interactions impede T cell proliferation, but immunosuppressive T cells in the TME may escape this suppression by reducing CD44 expression. |
| (76) | 2022 | America | IDH1 | tissue | RNA-seq IHC,HE,ELISA | iCC | Mutant-IDH1 inhibits cytotoxic T cell function through the IFNγ-TET2 axis |

with a higher ratio of CD8$^+$ PD-1$^{High}$ in CD8$^+$ PD-1$^+$ cells experience poorer postoperative survival (84).This might be due to the expression of PD-1$^{High}$ suggesting highly activated CD8$^+$ T cells, which, however, demonstrate severe functional dysregulation and impaired IFN-γ secretion, leading to negative clinical outcomes (85). A high proportion of CD8$^+$ PD-1$^{High}$ in activated CD8$^+$ PD-1$^+$ cells leads to CD8$^+$ T cell exhaustion (84). Research also indicates that late recurrence patients with ICC have higher levels of regulatory T cell infiltration in the TME and lower CD8$^+$ T cell infiltration compared to early recurrence patients (86).Moreover, the expression levels of T cell chemokines, such as CXCL9, CXCL10, and CXCL11, are lower in the TME of late recurrence patients (86).

In ICC patients, the FoxP3 to CD8 tumor-infiltrating lymphocytes ratio (FCR) is linked with poor prognosis and lymph node metastasis (87).ICC patients with a higher FCR show poorer recurrence-free survival and OS, and those with lymph node metastasis have a higher FCR in tumor-free lymph nodes (TFLN) compared to patients without lymph node metastasis (87).FoxP3$^+$ Treg cells can be categorized into three subtypes: Treg I (CD45RA$^+$FoxP3$^{low}$), Treg II (CD45RA$^-$FOXP3$^{high}$), and Treg III (CD45RA$^-$FoxP3$^{low}$) (88, 89).The Treg III subtype within regulatory T cells (Tregs) may significantly influence the prognosis of ICC patients. Studies have found that the Treg III subtype is predominant in the peripheral blood and tumor tissues of ICC patients and is associated with higher rates of recurrence-free survival. However, Treg I and Treg II are not associated with ICC recurrence (86). In previous studies, FoxP3$^+$ Treg cells have always been reported to be associated with poor outcomes in cancer patients. However, there are some opposite findings in hepatocellular carcinoma and vulvar melanoma (90, 91). The roles of FoxP3 expression levels and FoxP3$^+$ Treg cells in predicting prognosis of biliary malignancies have rarely been investigated, and thus need to be abundantly confirmed by more studies. Further, analysis of peripheral blood mononuclear cells from CCA patients and healthy volunteers revealed that lower levels of helper T cells (HT), higher levels of effector regulatory T cells (eTregs), and lower levels of CD80$^+$ eTregs are associated with shorter overall survival. Recurrence in CCA patients is associated with higher frequencies of CD4$^+$ T cells, CCR6$^+$ nTregs, and CXCR3$^+$ nTregs, and lower frequencies of PD-1$^+$ HT, OX40$^+$ HT, CD8$^+$ T cells, and CTLA-4$^+$ CD8$^+$ T cells (92).

Mucosal associated invariant T (MAIT) are cytotoxic innate T cells that are highly enriched in the human liver near the biliary epithelium, and are reduced in tumors of patients with intrahepatic and perihepatic CCA. The researchers found that patients who retained large numbers of MAIT cells in their tumors and surrounding liver tissue had a higher likelihood of long-term survival (93).In conclusion, T cells are intimately linked with the prognosis of cholangiocarcinoma patients. Predictions about patient outcomes can be made based on their functional status, infiltration level, and subtype distribution. Table 3 shows the relationship between T-lymphocytes and CCA prognosis collected for this review.

# 6 Immunotherapeutic approaches for T-cell-associated CCA

## 6.1 ICIs

Immune checkpoint inhibitors(ICIs) are monoclonal antibodies that focus primarily on immune checkpoint regulatory molecules.

TABLE 3 Relationship between T-lymphocyte CCA prognosis.

| Ref | Year | Country | Tumor type | Assessment Of TIL | Follow-up (months) | Endpoint | Prognostic significance |
|---|---|---|---|---|---|---|---|
| (80) | 2021 | China | ICC(140) | IHC | 25(median) | OS/RFS | Patients with high CD73 expression in ICC tissue or too few tumor-infiltrating $CD8^+$ T cells exhibit shorter OS and higher DFS |
| (81) | 2018 | Japan | ECC(114) | IHC | 62.6 (median) | OS | Low $CD8^+$ T cells and high $Foxp3^+$ Treg cell infiltration in ECCA tumor tissue are associated with poorer OS |
| (82) | 2022 | China | CCA(104) | mIHC | >36 | OS | 1. High Treg cells in CCA tissues are significantly associated with poor prognosis<br>2. High granzyme-BCD8 effector T cells in ICC and dCCA tissues were significantly associated with better OS |
| (84) | 2020 | China | ICC(322) | mIHC | 27 (median) | OS/TTR | Higher proportion of $CD8^+$ $PD\text{-}1^{High}$ in $CD8^+$ $PD\text{-}1^+$ cells had poorer OS |
| (86) | 2023 | China | ICC(99) | mIHC | Not reported | RFS | 1. Patients with high $FoxP3^+$ Treg cell infiltration in ICC tumor tissues have longer RFS, which is an independent favorable prognostic factor.<br>2. TregIII in peripheral blood correlates with RFS in patients with ICC. |
| (87) | 2022 | Japan | ICC(61) | IHC | 27.5 (median) | OS | Intratumoral $FoxP3^+$ Treg is associated with $CD8^+$ T-cell infiltration, and a high FoxP3/CD8 ratio (FCR) is an important marker of poor survival |
| (92) | 2021 | Japan | CCA(41) | flow cytometry (peripheral blood) | 20(median) | OS/RFS | Low infiltration of helper T cells (HT), high infiltration of effector regulatory T cells (eTregs), and low infiltration of $CD80^+$ eTregs were associated with shorter OS |
| (93) | 2022 | Sweden | ICC | IHC | 20(median) | OS | High tumor infiltration of MAIT cells is associated with good immune adaptation and predicts long-term survival in CCA patients |

CTLA-4 and PD-1 represent the most classical T-cell immune checkpoints and are the most widely studied targets for ICIs (94). PD-1 is a typical representative with intrinsic and extrinsic mechanisms of induction, in which the extrinsic mechanism, also known as adaptive resistance, refers to the adaptation of PD-L1-expressing tumors to antitumor immunity (95). PD-L1 primarily limits the ability of T cells to mount an immunological defense by attaching to PD-1. The binding of PD-L1 to PD-1 on T cells is how this occurs. Depletion of T cells results from this process; however, PD-L1/PD-1 inhibition can also be used to restart the antitumor response (96). CTLA-4 is particularly aberrantly strongly expressed in Tregs and is frequently expressed on activated $CD4^+CD8^+$ T cells. In order to prevent T cell activation, active T cells produce CTLA-4, a CD28 homolog, which competes with CD80/86 for binding to CD28. Blocking the CTLA-4 signaling pathway significantly improves the immune response and lessens T cells' tendency to become suppressive.

Gemcitabine treatment with CCA cells upregulates the expression of an immune checkpoint protein (PD-L1), thereby inhibiting the cytotoxicity of T lymphocytes. To overcome this challenge and take advantage of PD-L1 upregulation after gemcitabine treatment, investigators produced a recombinant PD-L1xCD3 bispecific T-cell attractor that specifically binds to CD3 on T-lymphocytes as well as PD-L1 overexpressed on CCA cells after gemcitabine treatment, thereby simultaneously blocking PD-1/PD-L1 signaling and recruiting T-lymphocytes to eliminate CCA cells. The results showed that the cytotoxicity of T lymphocytes against CCA cells was significantly enhanced, especially after gemcitabine treatment, and the cytotoxicity was positively correlated with the level of PD-L1 expression. The combination of gemcitabine and PD-L1xCD3 conjugate has been shown to be a potential alternative therapy for the treatment of CCA (97) (Figure 2B).

## 6.2 Advanced cell therapy

Advanced cell therapy (ACT), a type of cancer immunotherapy that uses a patient's own immune cells to locate and destroy tumor cells, was developed as a result of advancements in solid cancer research and technological discoveries. Its main objective is to destroy cancer cells by altering or triggering the immune system of patients. Tumor-infiltrating T lymphocytes are considered involved in ACT, among other processes. Tumor-infiltrating lymphocytes(TILs) are specifically taken from surgically removed tumor samples, activated and grown in a laboratory, and then returned to the patient. TIL ACT, as a therapeutic agent, has been shown to have objective anticancer effects in numerous solid cancers (98–100), including CCA (98). Chimeric antigen receptor-T (CAR-T) cell therapy is becoming increasingly well known as a cutting-edge method for treating cancer (101). Immunotherapy using chimeric antigen receptor-modified T cells (CARTs) is a unique approach for treating a variety of malignant tumors. Choosing the right antigen on cancer cells is crucial for designing a CAR-T-cell strategy that works and avoids side effects.

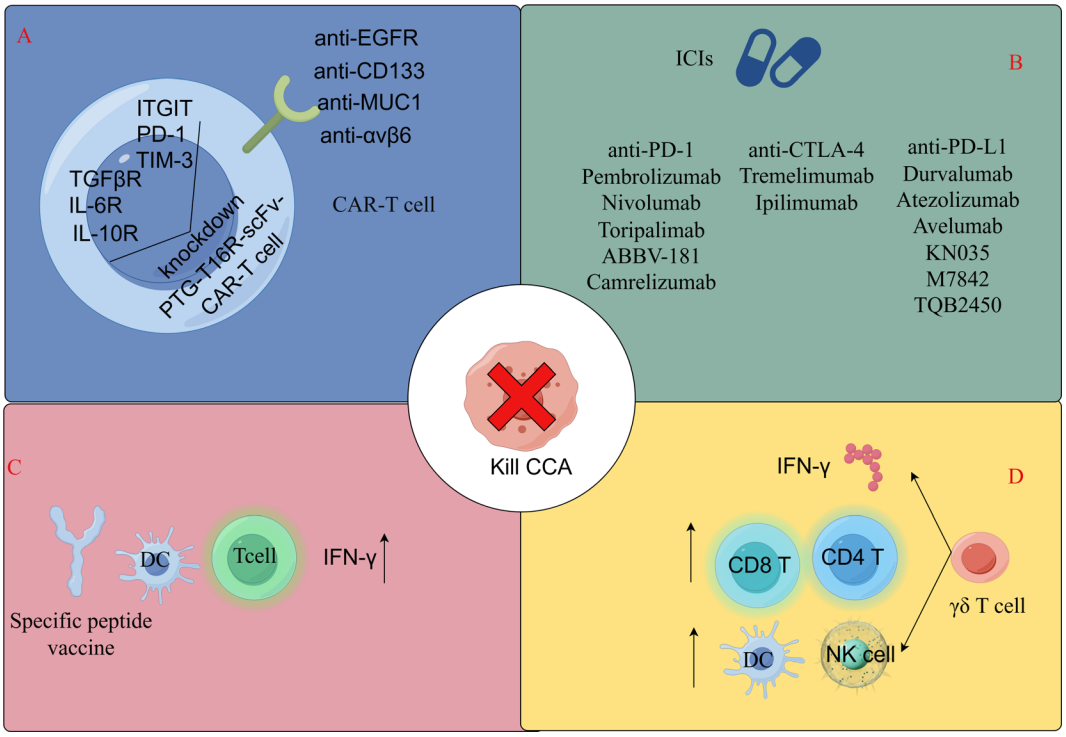



FIGURE 2
Immunotherapeutic approaches for T-lymphocyte-associated CCA. **(A)** CAR-T cell therapy. **(B)** Immune checkpoint inhibitors for cholangiocarcinoma in clinical studies or early stage trials. **(C)** Specific peptide vaccination therapy. **(D)** AllogeneicγδT cell immunotherapy. By FigDraw.

CAR-T cells (Figure 3), specifically targeting the epidermal growth factor receptor, can be employed in the treatment of advanced cholangiocarcinoma cases (102).CD133, a recognized cancer stem cell marker, is highly expressed and linked with cancer progression. Anti-CD133-CAR4 T cells demonstrate high potency against CD133-expressing CCA cells, leading to tumor cell lysis in a dose- and CD133 antigen-dependent manner (102, 103). MUC1, an overexpressed protein in CCA cells, is a potential target antigen for CART cell therapy. Integrin αvβ6 is upregulated in CCA but expressed at a low level in normal epithelial cells (104, 105), suggesting that integrin αvβ6 is an attractive target antigen for CAR T cell immunotherapy in CCA. Research has found that CAR-T cells targeting integrin αvβ6 and mucin 1, expressed on bile duct cancer cells, can be utilized in adoptive T cell therapies for bile duct cancer (106–109).However, MUC1 overexpression is also linked with the upregulation of PD-L1, an immune checkpoint protein that inhibits the antitumor function of T cells, which may lead to reduced efficacy of MUC1-targeting CART cell therapy for cholangiocarcinoma. To address this, researchers developed an anti-MUC1-CART cell line, αM.CAR/SRT, which contains a PD-1-CD28 switch receptor (SR) that targets MUC1 and engages the inhibitory PD-1/PD-L1 interaction to trigger CD28 signaling. Compared to αM.CAR cells, the αM.CAR/SRT cells display augmented cytotoxic function against CCA cells (110). Three immune checkpoints with the highest expression of PD-1, Tigit and Tim-3, as well as three key soluble immunosuppressive cytokines, TGFβR, IL-10R and IL-6R, were screened from cholangiocarcinoma tissues. PTG-T16R-scVF-CAR-T cells were designed based on these six tumor immunosuppressive targets, and both *in vivo* and *in vitro* experiments showed that this T-cell therapy has a strong inhibitory effect on CCA tumor growth (111) (Figure 2A).

## 6.3 Specific peptide vaccination therapy

Dendritic cells (DCs) are antigen-presenting cells that take up antigens and present them to adaptive immune cells. CCA tumor tissues have a higher population of activated DCs compared to normal tissues, suggesting that DCs are involved in CCA (112). DCs play an important role in enhancing antitumor responses, and the absence of DCs or the presence of dysfunctional DCs can lead to adverse outcomes. Therefore, increasing DC density and/or restoring DC function can be considered as a potential therapeutic approach for treating malignancies including CCA. Vaccines targeting DCs are another strategy to promote antitumor immunity (113). DC vaccines are usually pulsed with tumor-associated antigens (TAAs) *in vitro* and then injected *in vivo*. Several TAAs have been studied in CCA (114–116). The researchers chose three cholangiocarcinoma driver mutations (TP53, KRAS, and RNF43) to design antigenic peptides and used the antigenic peptides to stimulate DCs during DC differentiation. Peptide treatments had no effect on the differentiation of DCs to monocytes but increased the gene expression levels of the CD80 and CD86 costimulatory molecules,

which play a role in regulating the interactions between DCs and T cells and in activating T cell function. Increases in CD80 and CD86 following peptide stimulation enhance T cell-DC interactions and function. DC-activated T cells stimulated by antigenic peptides had higher populations of IFN-γ-positive $CD4^+$ and $CD8^+$ cells, which enhanced the killing ability of cholangiocarcinoma cells (117) (Figure 2C).

## 6.4 γδ T cells

A major immune evasion strategy found in advanced cancers is the downregulation of MHC molecules that are required for αβ T cell activation upon presentation of somatically mutated "neoantigens" to the αβ T cell receptor (118). However, this limitation does not apply to T cells expressing γδ TCR (γδ T cells), which, although rare in human peripheral blood, are enriched in epithelial tissues where many cancers develop and have been shown to actively participate in antitumor immunity (119). γδ T cells make up a small fraction, ranging from 1% to 10%, of the total human $CD3^+$ T-cell population. These cells express a lineage-specific TCR, containing one of seven Vγ chain isotypes (Vγ2, 3, 4, 5, 8, 9, and 11) paired with one of four Vδ chain types (Vδ1, 2, 3, and 5), which can be highly diverse due to the stochastic nature of the TCR somatic recombination process (120). Researchers sorted and cultured Vγ9Vδ2T cells from healthy human peripheral blood PBMCs and co-cultured them with cholangiocarcinoma cell lines, and showed that Vγ9Vδ2T cells could mediate cholangiocarcinoma apoptosis via lysosome-associated membrane protein (LAMP-1), suggesting that Vγ9Vδ2T cells may be useful in facilitating the development of new strategies for adoptive immunotherapy of cholangiocarcinoma (121, 122). This is related to the fact that γδ T cells recognize antigen in a non-MHC-restricted manner and that γδ T cells provide an early source of IFN-γ in the tumor microenvironment, γδ T cells can enhance the function of $CD4^+$, $CD8^+$ T cells, mature dendritic cells and activate neutrophils (123–125). Among human γδ T cell subsets, $Vδ2^+$ T cells (especially those expressing Vγ9Vδ2 TCR) have been more extensively studied because they are the most abundant subset in peripheral blood. However, in skin cancer tissue infiltration, the number of Vδ1 T cells exceeds that of Vδ2 T cells. Compared with Vδ2 TIL cultures, Vδ1 tumor-infiltrating lymphocyte (TIL)-derived cell cultures can exhibit superior *in vitro* cancer killing ability (126, 127), and Vδ1 T cells can exist as tumor-reactive lymphocytes for a long time (128), so the study of Vδ1 T cells in the treatment of cholangiocarcinoma needs to be carried out (Figure 2D). Table 4 and Figure 2 demonstrate the immunotherapeutic approach to T-lymphocyte-associated CCA.

# 7 Potential risks and side effects of immunotherapy for T-cell-associated cholangiocarcinoma

Potential risks and side effects are inherent in T-cell cholangiocarcinoma immunotherapy (129). These include Cytokine Release Syndrome (CRS), characterized by elevated levels of inflammatory cytokines, particularly interleukin (IL)6, due to immune activation (130). Symptoms range from high fever and flu-like symptoms to life-threatening complications such as organ failure (131). Neurotoxicity is another severe side effect, with patients potentially experiencing impaired consciousness, speech difficulties, balance loss, and in extreme cases, seizures, hallucinations, and coma (131–135).3. Tumor lysis syndrome (TLS) is a metabolic disorder caused by rapid tumor necrosis, resulting in conditions like hyperuricemia and hyperkalemia (136, 137). There's also the risk of damage from attacks on normal tissues due to minimal expression of tumor-associated antigens (also known as off-target effect) (138). Therefore, enhancing the safety and efficacy of T-cell immunotherapy for cholangiocarcinoma is a significant challenge in cancer treatment.

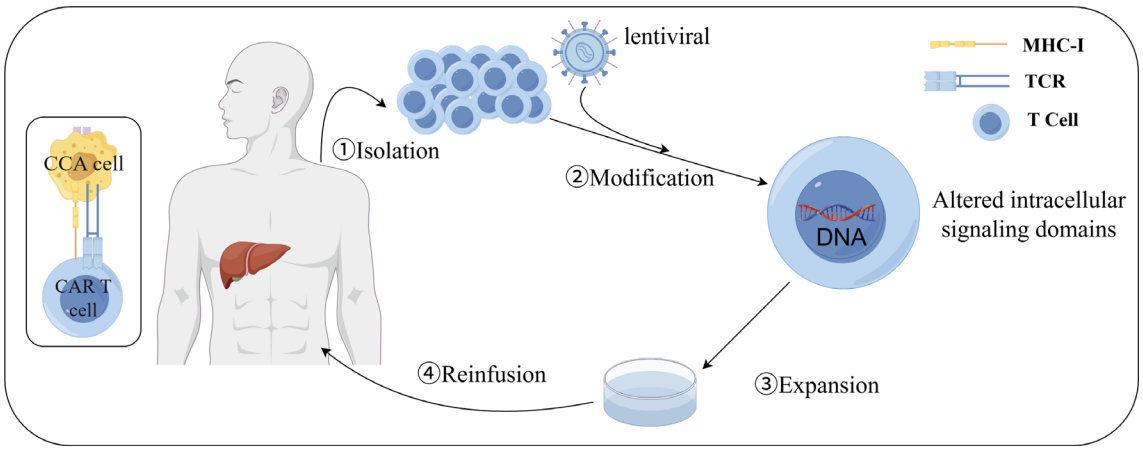



FIGURE 3
Preparation of CAR-T cells. **(1)** isolation: PBMCs were collected from peripheral blood of patients or donors; **(2)** modification: T cells were activated and CAR was transduced into activated T cells by lentivirus; **(3)** expansion: modified T cells were expanded *in vitro* to obtain clinically relevant cell counts; **(4)** reinfusion: modified T cells at the desired dosage were reinfused into patients who were previously lymphocyte-depleted. By FigDraw.

TABLE 4 Potential T-lymphocyte-associated immunotherapies for CCA.

| Ref | Year | Country | Experimental methods | Tumor type | Treatment | Outcomes |
|---|---|---|---|---|---|---|
| (102) | 2017 | China | clinical trial | CCA | CART cells targeting EGFR and CD133 | This patient achieved longer progression-free survival with CART-EGFR and CART133 therapy. |
| (103) | 2020 | Thailand | Cell culture experiment | CCA | CART cells targeting CD133 | Anti-CD133-CAR4T Cell Immunotherapy available to treat patients with CD133-Positive CCA tumor cells |
| (106) | 2021 | Thailand | Cell culture experiment | CCA | CART cells targeting MUC1 | Anti-MUC1-CAR4 T cells achieve anticancer effects on MUC1-expressing CCA cells by increasing the production of anti-tumor cytokines (TNF-α and IFN-γ), pro-apoptotic proteins (granzyme B), and by inducing lysis of CCA cells. |
| (107) | 2021 | Thailand | Cell culture experiment | CCA | CART cells targeting integrin αvβ6 | Anti-αvβ6-CAR T cells effectively kill αvβ6-positive CCA cells |
| (108) | 2023 | China | Cell culture experiment | ICC | CART cells targeting Tn-MUC1(5E5) | Anti-5E5-CAR T cells effectively eliminate Tn-MUC1-positive ICC cells *in vitro* and *in vivo* |
| (110) | 2023 | Thailand | Cell culture experiment | CCA | aM.CAR/SR T cells: anti-MUC1-CAR (aM.CAR) T cells containing SR molecules (PD-1-CD28) | aM.CAR/SR T cells were significantly more cytotoxic to CCA cells expressing MUC1 and PD-L1 |
| (111) | 2023 | China | Cell culture experiment | CCA | PTG-T16R-scFv-CAR-T cells | PTG-T16R-scFV-CAR-T cells knocking down the hexameric inhibitory molecule are highly immune to cholangiocarcinoma cells *in vivo* and *in vitro* |
| (97) | 2022 | Thailand | Cell culture experiment | CCA | Combination gemcitabine and PD-L1xCD3 bispecific T cell engager (BiTE) | BiTE significantly enhanced the cytotoxicity of T lymphocytes against CCA cells, especially after gemcitabine treatment, and the magnitude of cytotoxicity was positively correlated with the expression level of PD-L1 |
| (117) | 2023 | Thailand | Cell culture experimen | CCA | peptide vaccine | Peptide-pulsed DC-activated autologous HLA-A* 11:01-restricted T cells efficiently lysed KKU-213A (HLA-A*11:01) CCA cells compared to conventional tumor lysate-pulsed DC. |
| (121) | 2019 | China | clinical trial | CCA | allogenic γδ T cell immunotherapy | Allogeneic γδ T cell therapy positively modulated the peripheral blood immune function of the patients, reduced CCA tumor cell activity, and prolonged the life span of the patients. |
| (122) | 2024 | Thailand | Cell culture experimen | CCA | Vγ9Vδ2 T cells | Vγ9Vδ2 T cells can mediate cytotoxic effects on cholangiocarcinoma |

## 8 Metabolic reprogramming of T cells in cholangiocarcinoma

A review of the literature reveals a limited number of studies focusing on the metabolic reprogramming of T cells in cholangiocarcinoma. However, research on other tumors has demonstrated that resting $CD8^+$ T cells undergo dynamic shifts in metabolism, transitioning from oxidative metabolism to aerobic glycolysis upon activation. This transition is essential for supporting growth and differentiation into cytotoxic T cells, which can divide every 6–8 hours and produce inflammatory cytokines as well as cytolytic granules, including perforin and granzyme B (139). Tumor glucose consumption metabolically restricts T cells, leading to diminished mammalian target of rapamycin (mTOR) activity, reduced glycolytic capacity, and decreased IFN-γ production (140). For regulatory T (Treg) cells, the transcription factor Foxp3 reprograms T cell metabolism by suppressing Myc, a nuclear phosphoprotein involved in cell cycle progression, apoptosis, cellular transformation, and glycolysis. This reprogramming enhances mitochondrial oxidative phosphorylation (OXPHOS) and increases nicotinamide adenine dinucleotide oxidation (141). These adaptations confer a metabolic advantage to Tregs in low-glucose, lactate-rich environments. This metabolic phenotype may explain how Tregs promote peripheral immune tolerance during tissue injury and how cancer cells evade immune destruction in the tumor microenvironment. Thus, it is crucial to conduct studies targeting the metabolic reprogramming of T cells in cholangiocarcinoma.

## 9 Conclusion

From reading the literature published so far studying cholangiocarcinoma and T lymphocytes, we can assume that regardless of CCA subtype, $CD8^+$ and $CD4^+$ T cells are mainly located in the peri-tumor area, and $Foxp3^+$ T cells mainly infiltrate in the tumor center, but for some The contrary reports may be related to different sample sizes and research methods, so more research is

needed to confirm. We found that under the same conditions, PBC with chronic inflammation of the bile ducts with an autoimmune background are less susceptible to cholangiocarcinoma, which is related to their greater type 1 and type 2 T cell responses. The Fas/FasL signaling pathway and EGFR/PI3K/Akt signaling pathway related to T lymphocytes are involved in the progression of cholangiocarcinoma. I-FLICE, MUC1, MMP14, mIDH1, and SPP1 were found to be highly expressed in cholangiocarcinoma tissues, and they promoted cholangiocarcinoma progression by affecting T cell interactions or T cell immune infiltration. The more detailed mechanism remains to be elucidated, which has considerable potential for precise tumor treatment. Different immune cells and their subtypes have different prognostic effects on the long-term outcome of CCA. High levels of $CD8^+$ T-cell infiltration in CCA are associated with a better prognosis, and high levels of Tex cells are associated with a poor prognosis. High density of $CD4^+$ T cells at the tumor edge also seems to be associated with good DFS and OS. In contrast, a high number of Tregs is likely to be associated with worse OS. Future studies are definitely needed to elucidate the prognostic relevance of TILs in the long-term outcome of CCA. Currently, the main treatments for CCA include surgery and chemotherapy. Problems such as poor surgical results and chemotherapy resistance pose challenges to the treatment of cholangiocarcinoma. The availability of immunotherapies, including ICIs, cancer vaccines, and adoptive T-cell therapies, holds great potential to enable precision oncology treatment. But the side effects of immunotherapy also need to be taken seriously, and it is important to improve the safety of treatment for patients. Research on metabolic reprogramming of cholangiocarcinoma T cells also needs to be carried out.

## Author contributions

ZW: Writing – original draft. YD: Writing – original draft. YPZ: Writing – review & editing. YW: Writing – review & editing. PC: Writing – review & editing. YL: Writing – review & editing. YFZ: Writing – review & editing. XW: Writing – review & editing. YH: Writing – review & editing. HL: Writing – review & editing. GL: Writing – review & editing. YJ: Writing – review & editing.

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## Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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# Glossary

| | |
|---|---|
| CCA | Cholangiocarcinoma |
| eCCA | extrahepatic cholangiocarcinoma |
| iCCA | intrahepatic cholangiocarcinoma |
| DFS | disease-free survival |
| OS | overall survival |
| RFS | relapse-free survival |
| TTR | Time to recurrence |
| IT | intratumoral |
| PT | peritumoral |
| TM | tumor margin |
| IHC | Immunohistochemistry |
| mIHC | multiplexed immunohistochemistry |
| HE | hematoxylin-eosin staining |
| ELISA | Enzyme-Linked Immunosorbnent Assay |
| ScRNA | single-cell RNA sequencing |
| scTCR | single-cell T cell receptor |
| DC | dendritic cell |
| TILs | tumor-infiltrating lymphocytes |
| CAR T cells | chimeric antigen receptor-T cells |
| CAR4 T cells | Fourth-generation chimeric antigen receptor (CAR4) T cells |
| KKU-213A | CCA cell lines |
| PD-1 | programmed death ligand 1 |
| TIM-3 | T-cell Ig and mucin domain-3 protein |
| TIGIT | T-cell Ig and immunoreceptor tyrosine-based inhibitory motif domain |
| NKG2A | NK group 2 member A |
| CTLA-4 | cytotoxic T lymphocyte antigen-4 |
| CCR6 | C-C chemokine receptor 6 |
| CXCR3 | C-X-C motif chemokine receptor 3 |
| LAG-3 | lymphocyte-activation gene 3 |
| FOXP3 | forkhead box P3 |
| MMP | matrix metalloproteinases |
| FLICE | caspase 8 |
| EGFR | Epidermal growth factor receptor |
| PI3K | Phosphatidylinositol-3 kinase |
| Akt | Protein kinase B |
| TET2 | Tet Methylcytosine Dioxygenase 2 |
| IFN-γ | interferon-γ |
| CXCL | chemokine [C-X-C motif] ligand 9 |
| Tigit | T cell immunoglobulin and ITIM domain |
| Tim-3 | T-cell immunoglobulin and mucin domain-3 |
| TGFβ | Transforming Growth Factor Beta 1 |
| scVF | single-chain fragment variable |
| TP53 | Tumor Protein P53 |
| KRAS | KRAS Proto-Oncogene, GTPase |
| RNF43 | Ring Finger Protein 43 |
| HLA | Human leukocyte antigen. |