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Advancing immunotherapy for intrahepatic cholangiocarcinoma: exploring the tumor immune microenvironment and innovative treatments

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Abstract

Intrahepatic cholangiocarcinoma (ICC), a highly malignant tumor characterized by poor prognosis, has shown limited response to conventional treatments. Recently, advances in immunotherapy have offered new hope for treating such tumors. This article reviews the tumor immune microenvironment (TIME) of ICC, its pivotal role in immunotherapy, and methods to enhance ICC treatment by converting 'cold tumors' to 'hot tumors' through immune activation. Additionally, the article examines the characteristics of immune checkpoint inhibitors and their essential role in immunotherapy. Recent research and clinical trial outcomes for various immunotherapeutic approaches - namely immune checkpoint inhibitors (ICIs), cancer vaccines, and adoptive cell therapy (ACT) - are detailed, highlighting challenges in patient variability, side effect management, cost, and treatment accessibility. Furthermore, the article explores future research directions such as identifying new immunotherapy targets, applying precision medicine, and developing integrated therapeutic strategies to enhance immunotherapy efficacy and improve survival rates for ICC patients.

Keywords: Intrahepatic cholangiocarcinoma, biliary tract cancer, immunotherapy, tumor immune microenvironment, immune checkpoint inhibitors

INTRODUCTION

Intrahepatic cholangiocarcinoma (ICC), a malignant tumor originating from the intrahepatic secondary bile ducts and their branches, has seen a rising incidence over recent decades, particularly in Asia[1]. The complex pathophysiology and absence of early symptoms typically lead to diagnoses at advanced stages for most patients[2]. Traditional treatments such as surgical resection, chemotherapy, and radiotherapy are often limited in effectiveness due to the tumor's complex biology and high heterogeneity, resulting in a 5-year survival rate of approximately 30% after radical resection - a prognosis significantly poorer than that of hepatocellular carcinoma (HCC)[3]. Particularly for advanced cases, the likelihood of successful surgical resection is low, and chemotherapy and radiotherapy are often ineffective and associated with significant side effects[4]. Consequently, developing more effective treatments has become a critical research focus in this field[5].

In recent years, immunotherapy has emerged as a groundbreaking cancer treatment by activating or enhancing the patient's immune system, demonstrating significant potential across various tumors[6,7]. Despite the significant advances in immune checkpoint inhibitors (ICIs) for various malignancies, their efficacy in biliary tract cancer (BTC) remains limited. This limitation may be due to the unique tumor microenvironment and immune escape mechanisms specific to BTC[4], with most supporting evidence derived from small trials and subgroup analyses. Future research should prioritize combining ICIs with other therapies and exploring biomarkers to enhance the prediction of patient responses to immunotherapy[8].

This review aims to systematically examine the progress of ICC immunotherapy research, analyze current challenges, and anticipate future research directions, with the goal of providing a scientific basis and new ideas for clinical treatment. Through in-depth exploration, we aim to offer valuable references for future research and treatment strategies, thereby contributing to the advancement of ICC immunotherapy.

FUNDAMENTALS OF ICC IMMUNOTHERAPY

The immune system and cancer

The human immune system, a sophisticated and complex defense mechanism, is specifically designed to identify and eliminate foreign or abnormal cells, including cancer cells[9]. It comprises two primary components: innate immunity and adaptive immunity. Innate immunity, serving as the first line of defense, includes macrophages, neutrophils, and natural killers (NKs) that swiftly identify and neutralize non-specific targets. Conversely, adaptive immunity depends on the specific responses of T and B cells to particular antigens, with memory functions enabling rapid and robust responses to similar subsequent attacks[10]. The immune system monitors cellular activity, effectively identifying and eliminating cancerous cells through a sophisticated network of cell signaling. However, cancer cells can evade immune surveillance by mechanisms such as altering antigen expression or secreting immunosuppressive molecules[11].

The relationship between cancer and the immune system, termed "immunoediting", encompasses three phases: elimination, equilibrium, and escape[12]. During the elimination phase, the immune system identifies and eradicates most abnormal cells. In the subsequent equilibrium phase, some cancer cells may temporarily evade immune detection and achieve a dynamic balance with the immune system[13]. Eventually, during the escape phase, cancer cells advance their immune evasion strategies through genetic and epigenetic modifications, such as impairing antigen presentation or amplifying the immunosuppressive environment - for example, by inducing T cell depletion or activating regulatory T cell (Treg) - thereby facilitating their survival and proliferation[14]. Understanding these mechanisms is crucial for developing targeted immunotherapeutic strategies for specific cancers.

The tumor immune microenvironment in ICC

The tumor immune microenvironment (TIME) of ICC comprises a highly complex and dynamic array of cellular and non-cellular elements[15]. Cellular elements include cancer cells, immune cells, fibroblasts, and endothelial cells[16]. These cells contribute to tumor growth and proliferation and interact via various cytokines, chemotactic factors, and growth factors that influence tumor growth and invasion. Non-cellular elements, including the extracellular matrix (ECM) and soluble signaling mediators like platelet-derived growth factor (PDGF), also play crucial roles[17]. A distinctive feature of ICC is the abundant fibrous stroma within its TIME, which is highly reactive and exhibits immunosuppressive and protumorigenic functions, impacting the efficacy of cancer therapy[18]. Therefore, a thorough understanding of both the cellular and non-cellular components of the TIME and their interactions is crucial for developing effective immunotherapeutic strategies, as illustrated in Figure 1.

Cellular elements of TIME

Cancer-associated fibroblasts

Cancer-associated fibroblasts (CAFs) contribute to both tumor fibrosis formation and tumor progression through various mechanisms[19]. CAFs promote angiogenesis, lymphangiogenesis, and a pro-inflammatory tumor environment by secreting growth factors, cytokines, and chemotactic factors, including vascular endothelial growth factor (VEGF), fibroblast growth factor (FGF), and C-X-C motif chemokine ligand 12 (CXCL12)[20]. Additionally, CAFs exacerbate tumor invasiveness and growth by altering the ECM and releasing matrix metalloproteinases (MMPs) along with other stromal proteins[21]. This modified, more rigid ECM serves as a barrier, restricting immune cell penetration and correlating with decreased cytotoxic T cell infiltration, increased tumor-associated macrophages (TAMs) infiltration, and a poorer clinical prognosis.

In ICC, CAFs are activated from various cells, including hepatic stellate cells, portal fibroblasts, bone marrow mesenchymal stem cells, and fibroblasts derived from epithelial-mesenchymal transition (EMT) and endothelial-mesenchymal transition (EndMT)[22]. Recent studies indicate that inducing apoptosis in CAFs with specific drugs like the BH3 mimetic navitoclax significantly slows tumor growth and inhibits lymphovascular invasion and metastasis[23]. Furthermore, CAFs interact complexly with tumor cells and immune cells, particularly through the CCL2-STAT3 signaling pathway, displaying immunosuppressive properties like T cell proliferation and function inhibition, thereby promoting tumor immune escape[24]. Consequently, research and therapeutic approaches targeting CAFs and their specific signaling pathways not only deepen our understanding of tumor biology but also pave the way for new immunotherapeutic developments.

Tumor-associated macrophages

Tumor-associated macrophages (TAMs), particularly the M2-type, play a crucial role in the TIME[25]. These macrophages are primarily involved in tissue repair and angiogenesis, thereby contributing to tumor development[26]. M2-type TAMs exacerbate tumor invasiveness and metastasis by secreting pro-inflammatory and pro-angiogenic factors, including TNF-α, IL-6, TGF-β, and VEGF-A[27]. Specifically, these macrophages actively remodel the TIME by interacting with cancer cells, thereby enhancing tumor growth and local invasiveness[28]. Furthermore, M2-type TAMs inhibit the immune system's phagocytic functions by expressing inhibitory receptors like PD-1 and Siglec-10, which bind to ligands on tumor cells, aiding in tumor immune escape[29].

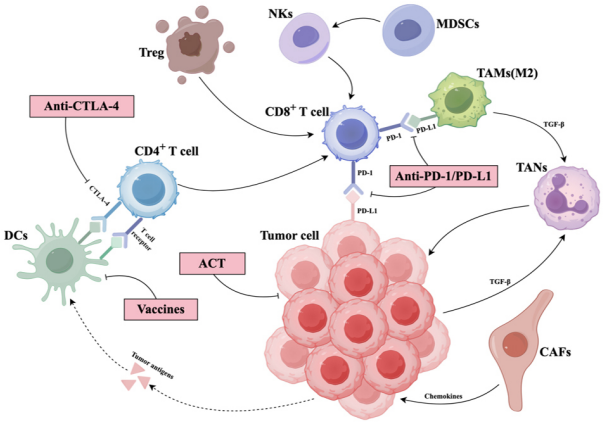

Figure 1. Biological principles of tumor immune microenvironment and immunotherapy in intrahepatic cholangiocarcinoma. NKs: Natural killers; MDSCs: myeloid-derived suppressor cells; TAMs: tumor-associated macrophages; TANs: tumor-associated neutrophils; Treg: regulatory T cell; PD-1: programmed cell death protein 1; PD-L1: programmed death-ligand 1; CTLA-4: cytotoxic T-lymphocyte-associated protein 4; DCs: dendritic cells; ACT: adoptive cell therapy; CAFs: cancer-associated fibroblasts; TGF-β: transforming growth factor β.

Studies indicate that a high density of M2-type TAMs correlates strongly with ICC aggressiveness, increased Treg infiltration, and poor prognosis[30,31]. To counteract this immunosuppressive state, novel therapeutic strategies are under development. These include using IFN-γ or activating the Notch pathway to repolarize M2-type TAMs to M1-type, enhancing their antitumor capabilities[32]. Recent studies have explored genetically engineered macrophages, known as chimeric antigen receptor macrophages (CAR-M), that express specific tumor antigen receptors. These CAR-M cells target and phagocytose tumor cells and activate T cell responses, thereby enhancing the efficacy of immunotherapy[33]. This interdisciplinary and innovative approach provides new avenues for ICC therapy and shows significant potential in addressing the limitations of conventional immunotherapy.

Tumor-associated neutrophils

The role of tumor-associated neutrophils (TANs) in the TIME and their impact on patient prognosis is complex and controversial. High levels of TAN infiltration are typically linked to a poor prognosis, notably marked by reduced overall survival (OS) and recurrence-free survival (RFS)[34,35]. However, recent studies suggest that, in some cases, high neutrophil infiltration may correlate with a better prognosis in patients with BTC[36]. This discrepancy could stem from the varied phenotypes of TANs and their behaviors within TIME[37]. Studies indicate that the polarized phenotypes of TANs, N1 and N2, function differently across tumor progression stages: N1 exhibits antitumor properties in early stages, while N2 becomes immunosuppressive and promotes tumor growth and metastasis in later stages[38].

The function and polarization of TANs are primarily regulated by tumor cells and factors secreted by TAMs, including TGF-β[39]. TGF-β facilitates the conversion of N1-type TANs to N2-type and boosts tumor growth and metastatic potential by upregulating factors like arginase 1 (ARG1) and CCL2[40]. Moreover, single-cell RNA sequencing has uncovered the role of TANs in enhancing the metastatic capabilities of circulating tumor cells. These N2-type TANs stimulate angiogenesis via the release of growth factors and cytokines and inhibit effector T cell function, thereby further promoting tumor immune escape[41]. Therapeutically, using TGF-β receptor inhibitors or radiotherapy to expose tumor-specific antigens, in conjunction with granulocyte colony-stimulating factor, can effectively modify TAN polarization and boost the antitumor activity of CD8+ T cells[42]. As research into TANs continues, neutrophil-based therapeutic

strategies are poised to become a rapidly evolving field in cancer therapy.

Myeloid-derived suppressor cells

Myeloid-derived suppressor cells (MDSCs) are a heterogeneous group of immature myeloid cells within the TIME that primarily promote tumor growth and metastasis by suppressing both innate and adaptive immune responses, particularly targeting CD8+ T cells[43]. Their accumulation in TIME not only impedes T cell activation and function but also facilitates tumor angiogenesis through the secretion of VEGF and other pro-angiogenic factors[44]. Furthermore, MDSCs contribute to T cell depletion and promote immune escape by highly expressing programmed death ligand 1 (PD-L1), which interacts with PD-1 on T cells[45].

The functional diversity of MDSCs makes them significant targets in immunotherapy[46]. Studies indicate that in tumor patients, MDSCs block T cell activation by catabolizing or depleting arginine and cysteine, which inhibits the metabolic activity of T cells and reduces the expression of homing receptors on their surfaces[47,48]. In cholangiocarcinoma patients, circulating levels of MDSCs (CD11b+CD14+HLA-DR-) are significantly elevated, strongly correlating with disease severity and prognosis[49]. Strategies targeting MDSCs, like reducing their numbers using anti-granulocyte differentiation antigen-1 specific antibodies, have demonstrated the potential to inhibit tumor growth in animal studies[50]. Furthermore, reducing the aggregation and activation of MDSCs at the tumor site could enhance the effectiveness of immune checkpoint inhibitors, potentially offering more effective treatment options for ICC patients[51].

Natural killers

Natural killers (NKs), as a key component of the innate immune system, demonstrate significant antitumor activity within the TIME. NKs can directly kill cancer cells through non-specific recognition, without the need for antigenic pretreatment[52]. In the liver, NKs constitute about 30%-40% of all lymphocytes. Besides releasing cytotoxic factors like TRAIL and FasL to perform antitumor functions, NKs regulate cytotoxic responses by activating and inhibiting surface receptors to directly kill tumor cells. Additionally, interferon-γ (INF-γ) and tumor necrosis factor-α (TNF-α) secreted by NKs are crucial in tumor immune responses[53]. However, the tumor microenvironment can alter the phenotype and function of NKs, reducing their cytotoxicity and even promoting tumor growth by producing pro-angiogenic factors. Furthermore, NKs are vital in tumor control by inducing apoptosis and inhibiting the proliferation and metastasis of tumor cells[54]. Recent studies suggest that enhancing the number or function of NKs can effectively delay cholangiocarcinoma progression, highlighting NKs as a potential therapeutic target[55].

Studies have indicated that the expression of the NKs activating receptor NKG2D is closely linked to the prognosis of ICC patients[56]. Patients exhibiting high levels of NKG2D expression generally have a better prognosis, likely due to NKG2D's role in enhancing NKs' ability to recognize and eliminate cancer cells[57]. Additionally, NKs demonstrate potential in precision immunotherapy by inducing tumor cell apoptosis and modulating adaptive immune responses, reducing off-target effects through cytokine release[58]. Experimental studies have shown significant inhibition of tumor growth following the infusion of human NKs into a xenograft mouse model using the human cholangiocarcinoma cell line HuCCT-1, underscoring the promise of NKs-based immunotherapeutic strategies[59].

Tumor-infiltrating lymphocytes

Tumor-infiltrating lymphocytes (TILs) perform a complex immunomodulatory role within the TIME, comprising various subpopulations like CD8-positive cytotoxic T cells and CD20-positive B cells, which directly engage in the immune response against tumor cells[60]. Studies have demonstrated that TILs can detect tumor antigens and elicit antitumor immune responses[61]. Specific signaling pathways, including Wnt/β-catenin, TGF-β, and PD-1/PD-L1, contribute to tumor immune escape by promoting the apoptosis of TILs[62]. High levels of CD8+ TILs are generally associated with improved patient survival, and extensive infiltration of CD4+ T cells correlates with longer overall and recurrence-free survival[63,64].

The types and functions of TILs exhibit considerable heterogeneity within the TIME. For instance, CD8+ TILs typically concentrate in the tumor's inner regions, while CD4+ T cells are more commonly found in the peripheral areas[65]. CD8+ T cells tend to accumulate at sparse fibrous junctions and the collagen-rich peripheral matrix; however, they struggle to penetrate dense tumor tissue due to their inability to produce proteases that degrade the extracellular matrix. Additionally, the distribution of TILs within TIME can be influenced by the density and orientation of the matrix structures.

Tumor-associated antigens (TAAs) identified in ICC serve as potential targets for cancer vaccine candidates, aiming to enhance the immune responses of TILs[60]. Recent transcriptomic studies have highlighted variations among T cell populations in ICC, particularly noting that CD8+ TILs from highly heterogeneous tumors exhibit diminished cytotoxic capabilities[66]. It was also observed that CD8+ TILs primarily localize at the invasive margins of tumors, while IL-17-positive immune cells (likely Th17 cells) and Tregs are more prevalent inside the tumor, a distribution that correlates with poorer prognosis[67].

Additionally, FOXP3, a transcription factor predominantly overexpressed by Tregs, is associated with increased CTLA-4 expression and may indicate the potential for tumor recurrence and chemoresistance[68]. Within the Tumor Immune Microenvironment (TIME), Tregs modify the cellular phenotype by inducing CCL2, resulting in the secretion of immunosuppressive factors like TGF-β and IL-10[69]. This suppresses effector T cell function and promotes tumor immune escape. A deep understanding of the functional and regulatory mechanisms targeting TILs, particularly in overcoming their immunosuppressive state in ICC, is crucial for developing more effective immunotherapeutic strategies[70].

Dendritic cells

Dendritic cells (DCs), as specialized antigen-presenting cells, play a central role in the TIME of ICC. These cells are adept at uptaking, processing, and presenting antigens. Immature DCs exhibit strong migratory capabilities, whereas mature DCs are effective in activating initial T and B cells, which are crucial for initiating, modulating, and sustaining the immune response. In ICC, mature DCs (CD83+) are predominantly found at the invasive tumor margins, whereas the tumor interior hosts a significant number of immature DCs (CD1a+). The presence of mature DCs correlates positively with the number of CD4+/CD8+ cells in TIME, enhancing T cell activation and the antitumor immune response, closely linked to a favorable prognosis[71].

However, in TIME, the density of DCs is notably lower, possibly due to reduced concentrations of chemokines (such as CCL4 and CCL5) that recruit DCs[72]. Typically, these chemokines are produced by NKs and other lymphocytes. Elevated levels of hypoxia, adenosine, IL-10, and TGF-β in TIME can induce an immunosuppressive phenotype in DCs[73,74]. This phenotype not only negates the tumor-fighting effects of effector T cells through immune checkpoint ligands, leading to T cell depletion, but also, by secreting IL-6 and inducing TGF-β production, fosters the recruitment of MDSCs, M2-type TAMs, N2-type TANs, and

the polarization of Tregs.

The biological properties of DCs offer significant opportunities for tumor vaccine technology. Tumor vaccine technologies that exploit DC properties to load tumor antigens and activate immune effector cells have been clinically used to treat various tumors, including Provenge, a DC-based vaccine for advanced prostate cancer[75]. In animal models of ICC, enzyme-loaded DCs have increased CD3+ lymphocyte infiltration, effectively inhibiting tumor growth and metastasis[76]. These findings indicate that enhancing the antigen presentation and immune activation capabilities of DCs, particularly in combination with other immunomodulatory strategies, may further improve immunotherapeutic efficacy against ICC.

Non-cellular elements of TIME

Extracellular matrix

Extensive reorganization of the Extracellular Matrix (ECM) is crucial for tumorigenesis and progression, facilitating structural remodeling of tumor tissues through the release of key components like MMPs, osteoprotegerin, junctional protein-C, and osteonectin. Overexpression of these components is closely linked to tumor growth, increased lymph node metastasis, and OS[77]. Specifically, increased expression of osteonectin in the TIME of ICC significantly correlates with tumor size, local and distant invasion, and advanced cancer stages, influencing tumor behavior and progression through activation of the RAS-RAF-MEK-ERK and Wnt/β-catenin signaling pathways[78]. Additionally, osteonectin enhances the development of NKs and the survival of T cells, further illustrating the multifaceted roles of the ECM in regulating the TIME.

In studies on ICC, increased stiffness of the ECM is identified as a distinctive feature of tumor progression. The increased stiffness of the ECM not only drives tumor progression by promoting cell proliferation, survival, migration, and differentiation but also influences tumor behavior through extracellular vesicles, which contain nucleic acids, lipids, and proteins secreted by tumor cells[79]. These vesicles contribute to the reconstitution of the TIME, immune regulation, and tumor angiogenesis, representing a complex and crucial mechanism in tumor progression. Current research is investigating the therapeutic potential of targeting the ECM, particularly aiming to enhance the outcomes and prognosis of ICC patients by modulating key ECM components to influence TIME.

Chemokines

Chemokines, a class of small molecule cytokines, direct the chemotactic migration of leukocytes such as monocytes, macrophages, and T-lymphocytes[80]. They play a crucial role in tumor development in ICC, influencing cancer cell migration, invasion, and immune escape[81]. Chemokines such as CXCL1-CXCL3, CXCL5-CXCL6, CXCL8, CXCL12, CCL2, CCL11, and CCL16 promote angiogenesis directly or indirectly, facilitating the necessary nutrient and oxygen supply to tumors[82]. Specifically, in cholangiocarcinoma, CXCL12 is positively regulated by angiotensin II and negatively by TGF-β. Meanwhile, TNF-α, secreted by TAMs, promotes the expression of CXCR4.

These chemokines promote tumor cell survival and proliferation by activating several signaling pathways, including the phosphatidylinositol 3-kinase/protein kinase B (PI3K/AKT), extracellular signal-regulated kinase (ERK1/2), and Wnt/β-catenin pathways, through interactions with their specific receptors[83,84]. For instance, CXCL5 serves as a chemokine for neutrophils, and CCL2, induced by fibroblast activating protein (FAP), regulates the migration of macrophages and MDSCs. Current clinical trials targeting chemokines

extend beyond hematological malignancies and breast cancer to include solid tumors such as cholangiocarcinoma, actively exploring the clinical utility of chemokines in oncology treatment. The progress in these studies could offer vital biological insights and therapeutic targets for developing new immunotherapeutic strategies against cholangiocarcinoma.

Extracellular vesicles

Extracellular vesicles (EVs) are small membrane structures secreted by cells that function as intercellular messengers and play a crucial role in the TIME[85]. These vesicles are categorized by size into exosomes (30-100 nm in diameter), microvesicles (0.1-1 μm in diameter), and apoptotic bodies (greater than 1 μm in diameter), with the latter typically being cleared by phagocytosis soon after their release. The components of EVs include proteins, lipids, messenger RNAs (mRNAs), microRNAs (miRNAs), long noncoding RNAs (lncRNAs), and circular RNAs (circRNAs), all of which are shielded from enzymatic degradation by the vesicles' lipid bilayer[86].

In ICC, EVs promote the migration of mesenchymal stem cells and the secretion of various cytokines and chemokines that contribute to tumor progression[87]. For example, EVs derived from cholangiocarcinoma can induce the expression of markers like α-smooth muscle actin[88]. Additionally, EVs facilitate interactions between cholangiocarcinoma cells and are considered potential new therapeutic targets[89]. Studies have shown that the downregulation of miRNA-15a in CAFs leads to increased secretion of plasminogen activator inhibitor 2 (PAI-2), subsequently promoting tumor cell migration. In animal models, the infusion of miRNA-195-enriched EVs has been shown to reduce tumor size and improve survival, highlighting the therapeutic potential of miRNAs and the EVs that transport them. These findings underscore the potential of extracellular vesicles in ICC immunotherapy strategies and could lead to the development of novel therapies targeting these molecular messengers.

Platelet-derived growth factor

Platelet-derived growth factor (PDGF) plays a key role in promoting tumor growth, angiogenesis, and fibrosis. As a potent mitogen, it stimulates the proliferation of peripheral support cells, including fibroblasts and smooth muscle cells, enhancing the structural support and vascular density of tumors. Recent studies have demonstrated that PDGF promotes tumor cell survival and proliferation through direct actions on tumor cells and by activating signaling pathways like PI3K/Akt and MAPK[90]. Additionally, high levels of PDGF in the TIME are closely linked to the prognosis of ICC patients, with increased levels typically indicating disease progression and poorer survival[90]. Consequently, targeted therapeutic strategies against PDGF and its receptors are actively being pursued to inhibit this growth factor's pathway, block tumor growth signals, and explore new treatment avenues for ICC. These strategies include the use of specific inhibitors like Imatinib, a small molecule drug effective in inhibiting the PDGF receptor, which has shown potential efficacy in clinical trials for various cancers[91].

Immune evasion in ICC

ICC utilizes a complex array of immune escape mechanisms that allow tumor cells to evade immune surveillance and clearance. Specifically, ICC cells counteract immune attacks by altering surface antigens and upregulating immunosuppressive molecules[92]. For instance, ICC cells often exhibit increased expression of PD-L1, which binds to PD-1 on T cells, inhibiting their activation and suppressing immune responses[93]. Additionally, there is elevated expression of cytotoxic T-lymphocyte-associated protein 4 (CTLA-4) in tumor-infiltrating lymphocytes (TILs), associated with aggressive tumor behavior and poor

prognosis[94]. The expression of these checkpoint molecules not only disrupts cell signaling and metabolic processes but also contributes to peripheral T cell depletion and fosters a state of immune tolerance.

In the TIME, the interaction between tumor cells and immunosuppressive cells like TAMs and MDSCs strengthens the immune escape mechanisms. These cells hinder T cell metabolism and signaling by releasing inhibitory molecules, including arginase and inducible nitric oxide synthase (iNOS). Additionally, studies indicate that high expression of PD-L1 in ICC correlates with more severe clinical outcomes, including shortened survival times[92]. For instance, research by Nakamura *et al.* found that immune checkpoint molecules were upregulated in about 45% of ICC samples, signaling a poorer prognosis[95].

Additional research has demonstrated that the regulation of PD-1 and PD-L1 is a crucial mechanism for immune escape in ICC. For instance, a study by Gani *et al.* revealed that 72% of ICC samples with PD-L1 expression experienced a 60% reduction in overall survival compared to those without PD-L1 expression[96]. Additionally, FOXP3 overexpression in ICC cells, often accompanied by increased CTLA-4 levels, was linked to lymph node metastasis and poorer survival, underscoring CTLA-4's negative prognostic impact in cholangiocarcinoma. These findings highlight the pivotal role of immune checkpoint molecules in ICC's immune escape and identify them as potential targets for developing immunotherapies.

Immunophenotyping in ICC

The TIME of ICC is complex and variable. Studies on its immunophenotype have increasingly demonstrated how different immune subtypes are linked to the tumor's biological behavior and prognosis. Recent advances in gene expression profiling have classified ICC into four immune subtypes: immune-indifferent (I1), with very low levels of immune cell infiltration; immune-activated (I2), marked by substantial infiltration of CD4+, CD8+, and CD45RO+ lymphocytes; myeloid-derived (I3), primarily composed of monocytes and macrophages; and mesenchymal (I4), noted for minimal immune infiltration but significant activation of CAFs[97]. Among these, the immune-activated subtype (I2) is associated with a relatively better prognosis and displays significant immunotherapeutic potential. This classification enables the possibility of personalized immunotherapy tailored to the specific immune profiles of ICC patients.

Moreover, comprehensive multi-omics-based analyses have further refined the molecular and immunophenotypes of ICC. For instance, a large-sample genome sequencing study categorized ICC into two types: inflammatory, which is linked to bold ductal ICC and characterized by enriched immune signals like interleukins and chemokines, suggesting a better prognosis; and proliferative, marked by heightened activity in tumor proliferation signaling pathways, indicating a poorer prognosis[98]. These findings lay a theoretical foundation for more targeted treatments tailored to the distinct molecular subtypes of ICC. Subsequent proteomic analyses have identified molecular types based on protein expression, including inflammatory (S1), mesenchymal (S2), metabolic (S3), and differentiated (S4). Identifying these subtypes aids in the precise diagnosis and treatment of tumors[99]. These studies indicate that integrating molecular biology and immunology data can enhance our understanding of ICC's biology and facilitate the development of personalized treatment plans for patients.

The relationship between molecular subtypes, immune microenvironment subtypes, and immunotherapy of ICC

In recent years, high-throughput sequencing technology has significantly advanced the molecular typing of ICC. Molecular typing of ICC typically includes various classification methods based on gene mutations (e.g., *IDH1/2*, *FGFR2*, *TP53*), gene expression profiles, and epigenetic modifications[100]. For example, studies have shown that ICC with *IDH1/2* mutations has unique metabolic features and a distinct tumor microenvironment[95]. ICC with *FGFR2* fusion genes exhibit distinct growth patterns and drug

responsiveness[101]. The immune microenvironment can be broadly classified as "inflammatory" and "non-inflammatory" based on the degree of immune cell infiltration, expression of immunosuppressive molecules, and cytokine secretion in the tumor tissue. An inflammatory immune microenvironment is usually characterized by high levels of T cell infiltration and immunoreactivity, whereas a non-inflammatory immune microenvironment is marked by enhanced immune escape mechanisms, such as high PD-L1 expression and T cell depletion[101].

Different molecular subtypes of ICC are closely associated with specific immune microenvironmental phenotypes. For example, *IDH1/2* mutant ICC is typically accompanied by lower T cell infiltration and higher expression of immunosuppressive molecules, suggesting that this subtype may respond poorly to conventional immune checkpoint inhibitors. In contrast, ICC with the *FGFR2* fusion gene shows higher levels of immune cell infiltration and may be more sensitive to immunotherapy[102]. Clarifying the relationship between ICC molecular subtypes and immune microenvironment phenotypes can guide the development of individualized immunotherapy strategies. For patients with the *IDH1/2* mutant phenotype, a combination of metabolic inhibitors and immunotherapy can be considered[103]. For patients with the FGFR2 fusion genotype, the use of immune checkpoint inhibitors can be prioritized[104]. The development of such precise treatment strategies is expected to significantly improve clinical outcomes for ICC patients.

ADVANCES IN ICC IMMUNOTHERAPY

Since 2010, immunotherapy has marked the advent of a new era in cancer treatment, emerging as a revolutionary anti-cancer strategy. In 2013, the journal *Science* named immunotherapy the scientific breakthrough of the year[105]. Since then, it has become the fifth pillar of tumor treatment, following surgery, radiotherapy, chemotherapy, and targeted therapy. In the realm of ICC therapy, where options are limited, treatments including ICIs like PD-1, PD-L1, and CTLA-4, as well as cancer vaccines (such as monopeptide, individualized peptide, and dendritic cell vaccines) and adoptive cell therapy (ACT) - either alone or combined with targeted therapies or chemotherapy - are advancing, as illustrated in Table 1. However, despite its potential to rapidly and durably eliminate large numbers of tumor cells, immunotherapy's effectiveness varies among individuals due to differences in the TIME, with only about 10% to 35% of patients experiencing lasting benefits[106]. Numerous clinical trials exploring immunotherapy for ICC are currently underway. These studies promise to further optimize treatment regimens and enhance efficacy. For detailed data, refer to Tables 2 and 3.

Immune checkpoint inhibitors

Key immune checkpoints targeted

Immune checkpoint inhibitors (ICIs) have become a pivotal therapeutic strategy in the treatment of ICC. Currently, PD-1 and CTLA-4 are the most extensively researched T cell immune checkpoints[107]. PD-1, a member of the CD28 superfamily, regulates T cell activity through interactions with its ligands PD-L1 and PD-L2. Approximately 35% of cholangiocarcinoma patients in China express PD-L1, an expression closely linked to tumor stage and prognosis[108]. Patients positive for PD-L1 generally experience better OS and RFS compared to those who are negative. Similar to PD-1, CTLA-4 is a co-suppressive receptor on T cells that inhibits their activation by competitively binding to CD80 and CD86, making CTLA-4 a compelling target for therapeutic strategies. Studies have demonstrated that targeting cholangiocarcinomas with high CTLA-4 expression using anti-CTLA-4 antibodies significantly enhances T cell maturation and activation, correlating with improved prognosis[109].

The research and application of ICIs are rapidly evolving. Beyond PD-1 and CTLA-4, other immune checkpoints like LAG-3, TIM-3, TIGIT, and B7-H3 are under investigation[110,111]. ICIs have shown

Table 1. Clinical outcomes of immunotherapy in BTC, including ICC

Regimen	Mechanism	Clinical trial identifier	No. of patients	Phase	Treatment line	Outcomes			Report year
						ORR (%)	mPFS (months)	mOS (months)	
Pembrolizumab	Anti-PD-1	NCT02628067	104	II	2nd	5.8	2.0	7.4	2020
Pembrolizumab	Anti-PD-1	NCT02054806	24	Ib	2nd	13	1.8	5.6	2020
Pembrolizumab + GemCis	Anti-PD-1	NCT04003636	533	III	1st	N/A	6.5	12.7	2023
Pembrolizumab + ramucirumab	Anti-PD-1 + Anti-VEGFR2	NCT02443324	26	I	2nd	4	1.6	6.4	2018
Pembrolizumab/nivolumab + lenvatinib	Anti-PD-1 + Anti-MTK	NCT03892577	56	I	2nd	30	5	6.4	2023
Nivolumab	Anti-PD-1	JapicCTI-153098	30	I	2nd	3	1.4	5.2	2019
Nivolumab	Anti-PD-1	NCT02829918	54	II	2nd	22	3.7	14.2	2020
Nivolumab + GemCis	Anti-PD-1	JapicCTI-153098	30	II	1st	37	4.2	15.4	2019
Nivolumab + GemCis	Anti-PD-1	NCT03311789	30	II	1st	55.6	6.1	8.5	2020
Nivolumab + ipilimumab	Anti-PD-1 + Anti-CTLA-4	NCT02923934	39	II	1st/2nd	23	2.9	5.7	2020
Camrelizumab + GemOx	Anti-PD-1	NCT03486678	37	II	1st	54	6.1	11.8	2020
Camrelizumab + GemOx/FOLFOX	Anti-PD-1	NCT03092895	92	II	1st	16.3	5.3	12.4	2021
Toripalimab + Gem/S-1	Anti-PD-1	NCT03796429	39	II	1st	27	7	16	2022
Toripalimab + lenvatinib + GemOx	Anti-PD-1 + Anti-MTK	NCT03951597	30	II	1st	80	10	N/A	2023
Lenvatinib + PD-1 inhibitors	Anti-PD-1 + Anti-MTK	ChiCTR2100044476	38	II	1st	42.1	8	17.7	2021
Durvalumab	Anti-PD-L1	NCT01938612	42	II	2nd	N/A	1.5	8.1	2022
Durvalumab + GemCis	Anti-PD-L1	NCT03875235	341	III	1st	26.7	7.2	12.8	2022
Durvalumab + tremelimumab + RT	Anti-PD-L1 + Anti-CTLA-4	NCT03482102	15	I	2nd	25	N/A	N/A	2022
Durvalumab + tremelimumab + GemCis	Anti-PD-L1 + Anti-CTLA-4	NCT03046862	121	II	1st	50-73	11-13	15-21	2022
Tremelimumab + RFA	Anti-CTLA-4	NCT01853618	32	I	2nd	N/A	7.4	12.3	2017
Bintrafusp alfa	Anti-PD-L1 + Anti-TGFβ-RII	NCT02699514	30	I	2nd	20	2.5	12.5	2020
Bintrafusp alfa	Anti-PD-L1 + Anti-TGFβ-RII	NCT02699515	30	I	2nd	20	N/A	12.7	2020
Bintrafusp alfa	Anti-PD-L1 + Anti-TGFβ-RII	NCT03833661	159	II	2nd	10.7	1.8	7.6	2023

BTC: Biliary tract cancer; ICC: intrahepatic cholangiocarcinoma; ORR: objective response rate; mPFS: median progression-free survival; mOS: median overall survival; PD-1: programmed cell death protein 1; NCT: National Clinical Trial; GemCis: gemcitabine + cisplatin; N/A: not available; VEGFR2: vascular endothelial growth factor receptor 2; MTK: multi-tyrosine kinase; JapicCTI: Japan Pharmaceutical Information Center Clinical Trials Information; CTLA: cytotoxic T-lymphocyte-associated protein; GemOx: gemcitabine + oxaliplatin; FOLFOX: folinic acid + 5-fluorouracil + oxaliplatin; Gem: gemcitabine; S-1: oral 5-fluorouracil; ChiCTR: Chinese Clinical Trial Registry; PD-L1: programmed death-ligand 1; RT: radiotherapy; RFA: radiofrequency ablation; TGFβ-RII: transforming growth factor beta receptor 2.

significant efficacy in treating various solid tumors. Notably, Pembrolizumab and Nivolumab have received U.S. Food and Drug Administration (FDA) approval for treating advanced malignant tumors. The mechanism of action for ICIs involves restoring the immune system's ability to destroy cancer cells by blocking the inhibitory signals from immune checkpoints. Ipilimumab, an anti-CTLA-4 monoclonal antibody, was the first ICI approved by the FDA in 2011 for malignant melanoma treatment. Since then, various ICIs have been approved, revolutionizing cancer therapy.

Table 2. Ongoing clinical trials of ICIs in BTC

Clinical trial identifier	Phase	No. of patients	Status	Primary outcomes	Target	Regimen
NCT02628067	II	1,609	Recruiting	ORR	PD-1	Pembrolizumab
NCT02465060	II	6,452	Active, not recruiting	ORR	PD-1	Nivolumab
NCT03101566	II	75	Active, not recruiting	PFS	PD-1, CTLA-1	Nivolumab + ipilimumab
NCT02834013	II	818	Active, not recruiting	ORR	PD-1, CTLA-1	Nivolumab + ipilimumab
NCT02821754	II	53	Active, not recruiting	PFS	PD-1, CTLA-4	Durvalumab + tremelimumab + TACE/RFA/cryoablation
NCT03898895	II	36	Recruiting	PFS	PD-1	Pembrolizumab + chemotherapy
NCT04003636	III	1,069	Active, not recruiting	OS	PD-1	Pembrolizumab + chemotherapy
NCT03482102	II	70	Recruiting	ORR	PD-1, CTLA-4	Durvalumab + tremelimumab + RT
NCT03046862	II	31	Active, not recruiting	ORR	PD-1, CTLA-4	Durvalumab + tremelimumab + chemotherapy
NCT03704480	II	106	Active, not recruiting	OS	PD-1, CTLA-4	Durvalumab + tremelimumab + chemotherapy
NCT03875235	III	810	Active, not recruiting	OS	PD-1	Durvalumab + chemotherapy
NCT03257761	Ib	55	Active, not recruiting	TEAEs, ORR	PD-1	Durvalumab + chemotherapy
NCT03785873	I/II	34	Active, not recruiting	DLTs, PFS	PD-1	Nivolumab + chemotherapy
NCT03478488	III	480	Recruiting	OS	PD-L1	KN035 + chemotherapy
NCT03201458	II	86	Active, not recruiting	PFS	PD-L1, MEK	Atezolizumab + cobimetinib
NCT03639935	II	32	Active, not recruiting	PFS	PD-1, PARP	Nivolumab + rucaparib
NCT03991832	II	58	Recruiting	ODCR, ORR	PD-1, PARP	Durvalumab + olaparib
NCT03797326	II	590	Active, not recruiting	ORR	PD-1, VEGFR-2	Lenvatinib + pembrolizumab

ICIs: Immune checkpoint inhibitors; BTC: biliary tract cancer; NCT: national clinical trial; ORR: objective response rate; PD-1: programmed cell death protein 1; PFS: progression-free survival; CTLA: cytotoxic T-lymphocyte-associated protein; TACE: transarterial chemoembolization; RFA: radiofrequency ablation; OS: overall survival; RT: radiation therapy; TEAEs: treatment-emergent adverse events; DLTs: dose-limiting toxicities; PD-L1: programmed death-ligand 1; PARP: poly (ADP-ribose) polymerase; ODCR: overall disease control rate; VEGFR: vascular endothelial growth factor.

The successful application of ICIs, particularly in treating immunosuppressive tumors like ICC, offers new therapeutic directions and hope. Ongoing optimization and development of new therapeutic strategies, integrating genomic and immunophenotypic data, may yield more effective and personalized treatments for ICC patients. Additionally, an increasing number of studies are focused on overcoming resistance to existing ICIs and enhancing efficacy through the combination of various treatment modalities, such as radiotherapy, chemotherapy, and other targeted therapies. These are crucial areas in ongoing and future ICC treatment research.

Monotherapy with ICIs

In immunotherapy studies for ICC, PD-1 inhibitor monotherapies like Pembrolizumab and Nivolumab showed some efficacy, yet overall results were modest. For instance, in two key studies, Pembrolizumab yielded a median progression-free survival of just 1.8 months and a median overall survival of 5.7 months, despite a 13% objective remission rate in PD-L1-positive cholangiocarcinoma patients[112]. Additionally,

Table 3. Ongoing clinical trials of cancer vaccines and ACT in BTC

Clinical trial identifier	Phase	No. of patients	Status	Primary outcomes	Regimen
NCT05986981	II	20	Active, not recruiting	TEAEs, DLTs, RP2D	MUC-1 therapeutic tumor vaccine
NCT05916248	I	60	Recruiting	ORR, DCR, DLTs	Personalized tumor vaccines mRNA-0217/S001 + pembrolizumab
NCT06195293	I	30	Active, not recruiting	DLTs	Neoantigen polypeptide vaccine
NCT06195384	I	30	Active, not recruiting	DLTs	Neoantigen mRNA vaccine
NCT03820310	II	20	Recruiting	OS, PFS	Autologous central memory T cell therapy + radiotherapy/chemotherapy
NCT03633773	I/II	9	Recruiting	DCR	Autologous MUC-1 CAR-T cell therapy + fludarabine/cyclophosphamide
NCT03801083	II	59	Recruiting	ORR	Autologous TIL
NCT02482454	II/III	50	Active, not recruiting	PFS	Autologous cytokine-induced NK cells + RFA
NCT03412877	II	270	Recruiting	ORR	TIL-based adoptive T cell therapy + pembrolizumab + chemotherapy
NCT01174121	II	332	Recruiting	ORR	Autologous TIL + pembrolizumab + chemotherapy

ACT: Adoptive cell therapy; BTC: biliary tract cancer; NCT: national clinical trial; TEAEs: treatment-emergent adverse events; DLTs: dose-limiting toxicities; RP2D: recommended phase 2 dose; MUC-1: mucin 1; ORR: objective response rate; DCR: disease control rate; OS: overall survival; PFS: progression-free survival; CAR-T cell: chimeric antigen receptor T cell; TIL: tumor-infiltrating lymphocytes; NK: natural killers; RFA: radiofrequency ablation.

Nivolumab demonstrated sustained antitumor responses exceeding one year in clinical trials conducted in the U.S. and Japan, though variations in PD-L1 expression were noted across different racial and environmental conditions[113,114]. Durvalumab monotherapy achieved a disease control rate of 16.7% in advanced cholangiocarcinoma patients, with a median time to effectiveness of 9.7 months and a median overall survival of 8.1 months[115]. Moreover, several investigational PD-L1 inhibitors, including the atezolizumab monoclonal antibody and Avelumab, are currently in clinical trials assessing their safety and efficacy in treating cholangiocarcinoma[116].

Although ICIs have demonstrated better efficacy in other solid tumors, their effects as monotherapies in cholangiocarcinoma have been relatively limited[117]. Future research should investigate combination therapies or tailored treatments for specific subgroups, such as Pembrolizumab for patients with mismatch repair (MMR) deficiency or high microsatellite instability, indicating that those with particular genetic profiles may derive greater benefits from ICI therapy. Additionally, the development of novel immunotherapeutic agents and combination treatment strategies could provide new avenues for enhancing survival rates in cholangiocarcinoma patients.

Combination therapy with ICIs

In the treatment of ICC, monotherapy with ICIs has shown some success, though its efficacy remains limited. Consequently, researchers are exploring the combination of ICIs with other immunotherapies or conventional treatments. The combination of CTLA-4 and PD-1/PD-L1 inhibitors leverages their complementary roles in the immunomodulatory process. CTLA-4 primarily modulates the early immune response, while PD-L1 regulates the immune response in late-stage peripheral tissues. Clinical studies have demonstrated that combining CTLA-4 and PD-1 inhibitors is more effective than using them alone, likely due to a synergistic effect that increases TILs and reduces regulatory T cells, thereby enhancing tumor growth inhibition[118]. For instance, in certain studies, cholangiocarcinoma patients treated with this

combination therapy achieved an overall efficacy rate of 10.8%, a disease control rate of 32.2%, and an overall survival of 10.1 months[119].

Ongoing research into ICIs has shown that combining these with chemotherapy or targeted therapies offers potential therapeutic benefits. For instance, the TOPAZ-1 trial, a first-of-its-kind Phase III study, investigated the efficacy of combining a PD-L1 inhibitor with chemotherapy (cisplatin and gemcitabine) for treating advanced cholangiocarcinoma[120]. Following this study, multiple regulatory agencies approved the combination therapy as a first-line treatment for patients with untreated unresectable or metastatic bile duct cancer. Additionally, the synergistic use of localized treatments, such as radiation therapy or local ablation, with immunotherapy provides new options for treating patients with advanced or surgically unresectable cholangiocarcinoma.

Considering current data and ongoing clinical trials, combination therapy with ICIs introduces new therapeutic opportunities in cholangiocarcinoma treatment. For instance, ongoing clinical trials are assessing M7824, a bifunctional fusion protein that targets PD-L1 and TGF-β, both as a monotherapy and in combination with chemotherapy[121]. Additionally, combining PARP inhibitors with ICIs for tumors with specific mutations, like DDR gene mutations, has demonstrated potential benefits[122]. This approach leverages the synthetic lethal properties of the tumors and their immunomodulatory effects to enhance therapeutic efficacy. As more research findings emerge, new combination therapeutic strategies are poised to significantly transform the treatment paradigm for cholangiocarcinoma.

Cancer vaccines

Single-peptide vaccines

Cancer vaccines show significant potential as emerging therapeutic tools that activate the immune response using tumor-specific antigens. TAAs like Wilms tumor gene 1 (WT1) and mucin 1 (MUC1) have been identified as potential targets in ICC therapy[123]. In a phase I trial, the MUC1 peptide vaccine demonstrated a favorable safety profile, though it did not significantly enhance survival[124]. However, the trial provided valuable data for future vaccine strategies. Despite challenges like tumor cell heterogeneity and the reduced expression of major histocompatibility complex class I molecules (MHC-I), which can facilitate T cell immunosurveillance escape, the single peptide vaccine remains promising for ICC treatment.

Personalized multi-peptide vaccines

Individualized peptide vaccines have shown significant potential in the field of personalized therapy for ICC. In 2020, Prof. Shu-Qing Chen's team published results from the first clinical trial of a novel antigenic individualized peptide vaccine, iNeo-Vac-P01, for advanced solid tumors. The trial demonstrated high rates of disease control and significantly prolonged patient survival, underscoring the safety and efficacy of individualized vaccines[125]. Specifically, a female patient with advanced ICC, who underwent multiple surgeries and received an antigen-specific vaccine targeting her HLA-I expression, achieved a durable immune response[126]. Additionally, a phase II trial involving six ICC patients assessed the feasibility of HLA-matched vaccine peptides, revealing that low levels of IL-6 were linked to significantly improved overall survival. This finding offers further biomarker guidance for personalized vaccine therapy[127]. Building on these results, ongoing early-stage clinical trials are exploring if blocking the IL-6-mediated inflammatory response with tocilizumab can enhance the immune response to an individualized peptide vaccine. These studies hold new therapeutic promise for patients with advanced cholangiocarcinoma.

Dendritic cell vaccines

Dendritic cell vaccines typically use tumor lysates to activate and harness the patient's immune system to fight cancer. While in vitro studies have shown early efficacy, clinical applications of this strategy are still under investigation[128]. In an early clinical trial with 36 ICC patients, investigators used DCs derived from the patients' own tumor lysates, pulsed them, and combined them with CD3-activated T cells. This combination therapy significantly improved RFS (18.3 months *vs.* 7.7 months, $P = 0.005$) and OS (31.9 months *vs.* 17.4 months, $P = 0.022$) compared to a control group that underwent only surgery[129]. These results suggest that combining dendritic cell vaccines with T cell therapy could offer a more effective treatment option for ICC patients.

Combination therapy

The combined use of cancer vaccines with other therapies is gradually demonstrating potential efficacy in the treatment of ICC. For instance, a phase I/II trial by Lepisto *et al.* employed a MUC1-loaded dendritic cell vaccine as adjuvant therapy for patients with early pancreatic cancer and stage II ICC[130]. Notably, one ICC patient experienced no recurrence, highlighting the vaccine's initial effectiveness in tumor immunotherapy. Additionally, a retrospective study involving 65 patients with recurrent or unresectable cholangiocarcinoma revealed that 77% of the patients who received WT-1 and MUC-1 dendritic cell vaccines combined with chemotherapy exhibited improved response rates and survival outcomes compared to those receiving only the vaccines[131]. Moreover, a Japanese study tested the combination of a WT1 peptide vaccine with gemcitabine in patients with unresectable or recurrent BTC, achieving a median overall survival of 9.5 months and good tolerability[132]. These findings suggest that cancer vaccines, particularly when combined with chemotherapy or targeted therapies, could offer an effective treatment for ICC patients. Ongoing clinical trials are expected to further clarify the long-term effects and underlying mechanisms of these therapeutic combinations.

Adoptive cell therapy

Adoptive cell therapy (ACT), which uses genetically engineered and modified T cells, has shown notable potential in the treatment of ICC. Utilizing chimeric antigen receptor (CAR) technology, scientists can engineer T cells to express specific receptors that recognize and destroy cancer cells. While this technology has been highly effective in treating certain hematological malignancies, its application in solid tumors like ICC is challenging due to the lack of effective targets and significant tumor heterogeneity[133]. Recent studies, however, indicate that combining CAR-T cell therapy with PD-1 or PD-L1 inhibitors can significantly enhance the antitumor effects in solid tumors, including ICC[134]. For instance, Feng *et al.* observed clinical remission in some patients with advanced ICC treated with CAR-T cells targeting EGFR and CD133[135].

Additionally, autologous TILs, a form of ACT, have been successful in treating other cancers, such as melanoma[136]. In the treatment of ICC, both TILs and CAR-T cell therapies targeting specific tumor antigens like MUC1 have shown promise across multiple clinical trials. Patients in these trials received T cells targeting specific antigens, demonstrating good tolerability and notable efficacy. For instance, in a phase I trial, CAR-T cell therapy targeting HER-2 exhibited promising clinical activity in patients with advanced cholangiocarcinoma and pancreatic cancer[137]. Although still in the early stages and facing multiple challenges, ACT in ICC has shown promising initial results in enhancing immune response and potentially improving survival. As technology advances and more clinical trials are conducted, ACT could become a key therapeutic strategy for challenging cancers like ICC.

CHALLENGES AND LIMITATIONS

Immunotherapy for ICC faces multiple challenges. First, the unique immune microenvironment of ICC, characterized by abundant immunosuppressive signals and a lack of effective biomarkers, significantly limits the efficacy of immunotherapeutic strategies[138]. Although some patients respond to immune checkpoint inhibitors like anti-PD-1 antibodies, the overall success rate of these therapies is low, with significant variation in response between individuals. These variations may be due to the "cold" and "hot" nature of tumors, which refers to the degree and type of immune cell infiltration within the patient's tumor microenvironment[139]. Furthermore, the clinical use of ICIs has raised safety concerns, particularly treatment-related adverse effects like autoimmune symptoms, which require control through comprehensive management programs[138].

Economic burden and treatment accessibility pose significant barriers to the widespread adoption of immunotherapy for ICC. High treatment costs and unequal availability across different regions restrict broader use. Additionally, although initial clinical trials show promising results, the long-term efficacy and safety of these therapies require validation through larger randomized controlled trials (RCTs). Current therapeutic strategies, including cancer vaccines and ACT, require further development and standardization in technological maturity, immune activity maintenance, and production processes to enhance therapeutic efficacy and patient survival.

FUTURE DIRECTIONS IN RESEARCH

Immunotherapeutic research for ICC is rapidly advancing and shows great promise, but it continues to face significant challenges. Ongoing exploration of novel immunotherapeutic targets, particularly the discovery and application of new immune checkpoints, will lead to more diverse ICC treatment strategies. Precision medicine technologies, including genomics and immunomics analysis, are expected to advance individualized treatment plans, allowing immunotherapy to more accurately activate the patient's immune response and convert "cold tumors" into "hot tumors" to enhance immune activity. Immunotherapy advancements have enabled more precise activation of the immune response and the conversion of "cold tumors" into "hot tumors," thereby enhancing immune activity. However, the efficacy of ICC immunotherapy remains constrained by tumor heterogeneity and immune escape mechanisms. Optimizing treatment strategies for different patient groups remains a critical focus for future research.

Future ICC immunotherapy research will emphasize developing integrated treatment strategies, combining immunotherapy with chemotherapy, targeted therapy, and other approaches to overcome tumor resistance. For instance, combining immune checkpoint inhibitors with other antitumor agents may enhance efficacy and better manage treatment-related adverse effects. However, challenges related to the safety, economic burden, and global accessibility of immunotherapy cannot be overlooked. To benefit more patients, future research should focus on scaling up these advanced therapies across different regions. Additionally, the lack of effective predictive biomarkers is a major bottleneck. Future research should focus on developing and validating these markers to better guide therapeutic decisions and improve efficacy. With comprehensive efforts, immunotherapy is expected to become a key strategy for improving the prognosis of ICC patients and may also offer insights for other BTC subtypes. Nevertheless, achieving this goal requires further exploration, particularly in optimizing treatment regimens, overcoming drug resistance, and improving patients' quality of life.

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Authors' contributions

Performed literature search, article organization, drafting, and data collection: Wei P
Provided guidance on the article's conceptual framework and innovation, and revised the manuscript: Li Z

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All authors declared that there are no conflicts of interest.

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