Review

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Immunotherapy in Cholangiocarcinoma: Can We Turn the Tide Against This Silent Killer?

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Cholangiocarcinoma (CCA) is a highly aggressive malignancy of the biliary tract with limited therapeutic options and a dismal prognosis. Although immune checkpoint inhibitors (ICIs) have transformed treatment paradigms for several solid tumors, their impact on CCA remains minimal. The unique immunobiology of CCA, including its immune excluded phenotype, low tumor mutational burden, and paucity of actionable biomarkers, has constrained meaningful immunotherapy responses. This review outlines the key immunologic barriers that define the tumor microenvironment in CCA, including the abundance of myeloid-derived suppressor cells, tumor-associated macrophages, regulatory T cells, metabolic reprogramming, and immune checkpoint overexpression. Given the modest clinical outcomes of ICI monotherapy in CCA, we highlight the rationale for combination strategies incorporating chemotherapy, anti-angiogenic agents, epigenetic modulators, and metabolic inhibitors. Additionally, we assessed the emerging roles of adoptive cell therapy, tumor vaccines, and gut microbiome modulation as novel immunologic interventions. We also discussed how adaptive trial designs and real-time circulating tumor DNA monitoring support dynamic therapy optimization. Although the immunologic silence of CCA has historically limited the efficacy of immunotherapy, growing insights into the tumor microenvironment (TME) and advances in biomarker-guided, personalized strategies offer a compelling roadmap forward. Overcoming immune resistance in CCA will require multidimensional innovation combining biology, technology, and trial design to shift this malignancy from immune evasion to immune engagement.

Keywords: cholangiocarcinoma; immunotherapy; immune checkpoint inhibitors; tumor microenvironment; biomarker-guided therapy; radiomics; artificial intelligence; precision oncology

Introduction

Cholangiocarcinoma (CCA) comprises primary malignancies arising from the biliary epithelium and represent approximately 3% of all gastrointestinal cancers [1]. Based on anatomical location, CCA is classified into intrahepatic (iCCA), which originates above the second-order bile ducts and constitutes the second most common primary liver cancer after hepatocellular carcinoma, and extrahepatic (eCCA). The latter includes perihilar (pCCA) and distal (dCCA) subtypes, defined by their relationship to the cystic duct [2,3]. Despite a shared epithelial origin, these subtypes exhibit marked molecular and genetic heterogeneity [4], resulting in distinct epidemiological patterns, clinical presentations, and therapeutic considerations. Many studies have reported that the incidence and mortality of CCA are still increasing year by year, and it has a high recurrence rate [2,5,6], while the global incidence of CCA shows marked geographic variation. Rates are highest in Asia [7], particularly in Northeast Thailand (85 per 100,000), South Korea (8.8 per 100,000) [2]. Western countries report substantially lower incidence (0.5–3.4 per 100,000), with Italy at the upper range. Mortality patterns for iCCA and eCCA parallel incidence trends, and iCCA mortality has risen across Europe over the past decade [8], with Ireland, the UK, Portugal, and Spain showing particularly high male and female mortality. The most pronounced recent increases in iCCA incidence occur in the Baltic states. Overall CCA mortality is higher in older patients than in younger patients, in men than in women, and in Asian countries than in Western countries [9].

Several recognized risk factors have increased globally over recent decades and could be contributing to increasing CCA rates. For instance, high alcohol consumption, tobacco smoking and viral infections (hepatitis B virus and hepatitis C virus) have been reported to increase the risk of CCA development [10]. Surgical resection is an option for CCA and is feasible mainly in the localized and potentially resectable stages shown in Fig. 1. Staging laparoscopy can detect occult metastatic or unresectable diseases in up to 27.2% of patients [11]. Advances in major hepatectomy, bile-duct resection, and vascular reconstruction have expanded resectability [12], with 5-year survival reaching 32.5% overall [13]. As reflected in Fig. 1, surgery is limited to Stage 1–2 disease, whereas locally advanced vascular invasion and metastatic spread (Stage 3–4) are not

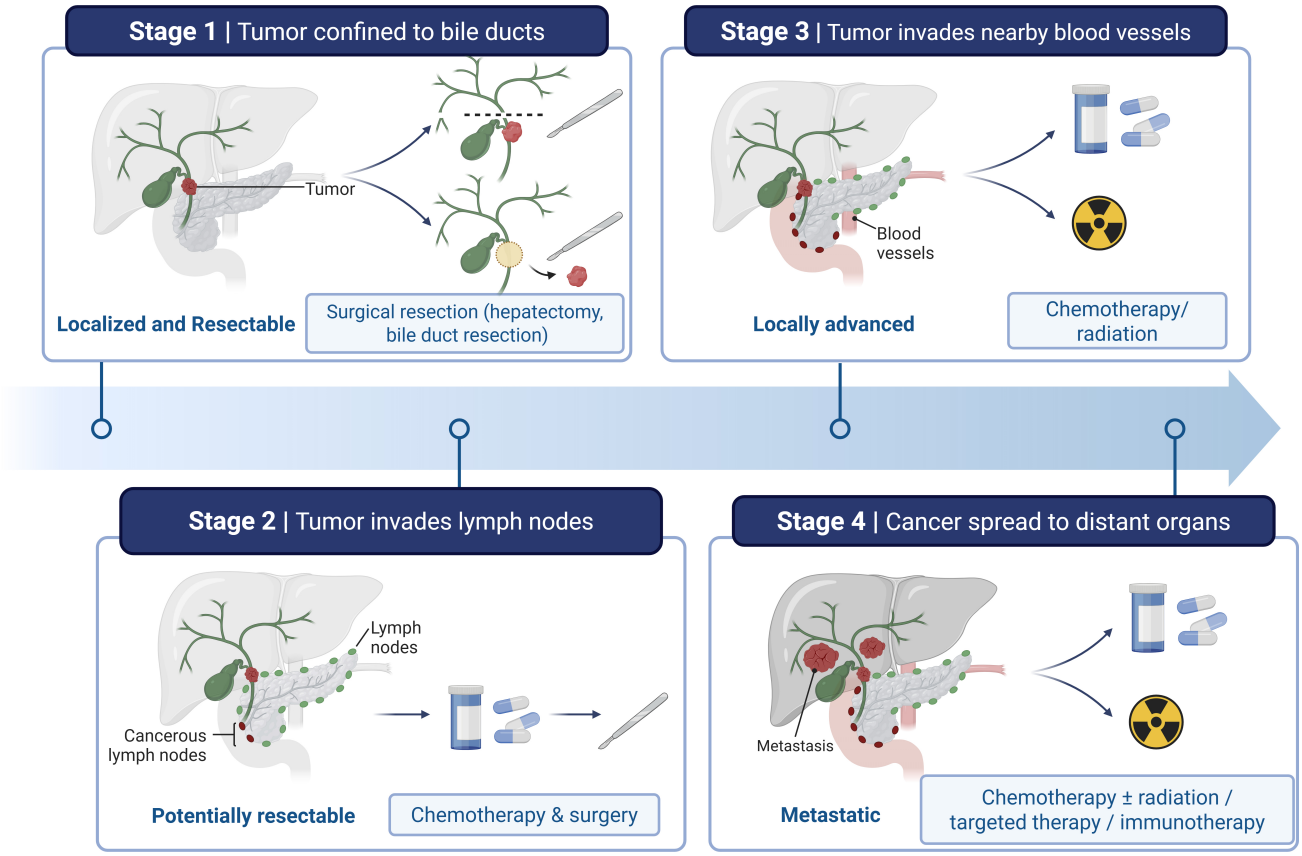

Fig. 1. Stage-based overview of CCA management. This schematic illustrates a simplified, stage-based summary of treatment pathways for CCA adapted from contemporary international guidelines. Stage 1 depicts localised, resectable disease confined to the bile ducts, for which curative-intent surgical resection is the recommended standard. Stage 2 represents regional lymph-node involvement/potentially resectable disease, where multimodality strategies combining systemic therapy and surgery may be considered. Stage 3 corresponds to unresectable, locally advanced disease with major vascular invasion, typically managed with systemic therapy ± chemoradiation according to guideline recommendations. Stage 4 illustrates metastatic disease, for which palliative systemic therapy (chemotherapy, targeted therapy and/or immunotherapy in selected patients) and clinical-trial enrolment are advised. This figure is intended as a conceptual framework rather than a formal staging system. Created with https://www.biorender.com/. CCA, cholangiocarcinoma.

amenable to curative intent resection. Most patients, however, present with disease that is not amenable to surgery, and even among those who undergo curative-intent resection, recurrence is frequent and overall survival remains poor [14].

For patients with advanced-stage CCA who are not candidates for surgery or locoregional therapy, first-line systemic treatment consists of cisplatin plus gemcitabine. The ABC-02 trial established this regimen as standard of care, demonstrating a median overall survival of 11.7 months compared with 8.1 months for gemcitabine alone, underscoring the urgent need for more effective strategies [15]. Targeted therapy has emerged as an option for molecularly selected patients. iCCA frequently carries IDH1/IDH2 mutations (around 15%) and alterations in fibroblast growth factor receptor (FGFR) pathways, as well as BRCA1-associated protein 1 (BAP1) and other genetic changes [16]. In a phase III trial of chemotherapy-refractory IDH1-mutant iCCA, the IDH1 inhibitor Ivosidenib (AG-120) significantly improved progression-free survival compared with placebo (2.7 vs. 1.4 months; hazard ratio (HR) 0.37, 95% confidence interval (CI) 0.25–0.54; one-sided $p < 0.0001$) [17].

Currently, Immunotherapy is emerging as a potentially pivotal strategy in CCA. In KEYNOTE-028 & 158, pembrolizumab achieved an objective response rate of just 5.8% in advanced biliary tract cancer [18], underscoring the limited efficacy of programmed cell death protein 1 (PD-1) blockade in an unselected population, but with higher activity observed in mismatch repair–deficient tumors [19]. Subsequent trials have reported more encouraging signals: in a phase II study of previously treated biliary tract cancer, nivolumab produced an objective response rate of 22% and a median overall survival of 14.2 months [20], while dual PD-1/CTLA-4 blockade yielded a median overall survival of 5.7 months (95% CI, 2.7–11.9 months) and progression-free survival of 2.9 months (95% CI, 2.2–4.6 months), with responses predominantly seen in iCCA or gallbladder can-

cer [21]. These data suggest that, once the immune landscape of CCA is better defined and biomarker-driven or combination strategies are optimized, immunotherapy may become a key component. The phase III KEYNOTE-966 trial has now demonstrated that adding pembrolizumab to gemcitabine–cisplatin significantly improves overall survival compared with chemotherapy alone (median 12.7 vs. 10.9 months; HR 0.83, 95% CI 0.72–0.95; $p = 0.0034$), with a safety profile consistent with known immune-related toxicities [22]. Moreover, KEYNOTE-966 demonstrated that health-related quality of life remained stable with the addition of pembrolizumab to gemcitabine–cisplatin, reinforcing the clinical value of this combination as a first-line therapy for advanced biliary tract cancer [23]. Despite these advances, the overall efficacy of immunotherapy in CCA still falls short of expectations, largely due to the unique biological barriers inherent to this disease. Mounting evidence has indicated a connection between the mor immune microenvironment (TIME) and the response to immunotherapy, and the failure of immunotherapy may be partially attributed to the high heterogeneity and intricate TIME of CCA [24–27]. As immunotherapy becomes increasingly integrated into CCA management, biomarker-guided patient selection is emerging as a critical strategy for improving therapeutic outcomes. Recent studies have identified a broad spectrum of potential biomarkers, including genomic alterations, circulating tumor DNA, exosomal cargo, microRNAs, cytokines, and immune-cell signatures that may help stratify immunologically distinct subgroups of CCA and predict responsiveness to immune checkpoint inhibitors [26,28]. However, traditional biomarkers commonly used in other malignancies, such as PD-L1 expression, microsatellite instability-high (MSI-H), and high tumor mutational burden (TMB) are rare in CCA, markedly limiting their predictive utility [27]. These limitations underline why only a small subset of patients currently experiences meaningful benefit from immunotherapy and further highlight the importance of developing multi-omics-based, TIME-informed biomarkers to enable more precise patient stratification in this highly heterogeneous disease [24,25].

To overcome these TIME-related barriers, multiple investigational strategies are being developed, including combinations of ICIs with chemotherapy, anti-angiogenic agents, epigenetic therapies, and metabolic modulators, all aiming to remodel the TIME and restore antitumor immunity [29,30]. Concurrently, advances in single-cell multi-omics profiling and spatial transcriptomics also enable the identification of predictive biomarkers and mechanistic insights that inform precision immuno-oncology [31,32].

Building on these advances, Fig. 2 synthesizes the major immune-evasion mechanisms in CCA, immunosuppressive cells, stromal barriers, metabolic reprogramming, low TMB/MSS status and checkpoint overexpression, and outlines emerging strategies to reprogram the TIME, including rational ICI-based combinations, metabolic and epigenetic modulators, cancer vaccines and AI-assisted patient stratification. To assess whether we can truly turn the tide, we integrate CCA immune biology with clinical evidence to define practical, biomarker-guided strategies that may unlock durable benefit from immunotherapy.

The Tumor Immune Microenvironment in CCA

The TIME in CCA is profoundly immunosuppressive characterized by sparse cytotoxic T-cell infiltration with enrichment of M2-polarized macrophages, Tregs, and MDSCs, a CAF-dense stromal barrier, metabolic reprogramming, impaired antigen presentation, and checkpoint overexpression, altogether fostering progression and resistance to ICIs [24,25,30]. As a concise schema, Fig. 3 highlights these pro-tumor vs. anti-tumor immune compartments and the principal therapeutic levers to reprogram the TIME that are increasingly supported by multi-omics and biomarker-guided strategies [20,26].

Immune Cell Components and Their Immunosuppressive Roles

Several immune cell populations collectively enforce the immunosuppressive nature of the CCA tumor microenvironment (TME). Myeloid-derived suppressor cells are prominent mediators that dampen cytotoxic T-cell activation and promote Treg expansion in CCA [30,33], in part through L-arginine metabolism, arginase and iNOS dependent depletion of L-arginine impairs T-cell receptor signaling and antigen-specific priming [34,35]. Tumor-associated macrophages in CCA frequently display an M2-like program with anti-inflammatory, pro-tumor functions [36], while single-cell profiling demonstrates prominent $CD8^+$ T-cell exhaustion and Treg enrichment in intrahepatic CCA [37]. Together with a dense stromal milieu, these myeloid-dominant suppressive circuits create effective barriers to antitumor immunity and underpin therapeutic resistance [24,30]. These macrophages secrete cytokines such as IL-10 and TGF-β, dampening pro-inflammatory immunity, and they further drive CCA progression by fostering angiogenesis, extracellular matrix remodeling and epithelial mesenchymal transition. Regulatory T cells constitute another dominant suppressive axis; they curb effector T-cell proliferation and function and impair dendritic-cell maturation/antigen presentation via IL-10/TGF-β–mediated mechanisms [24,38,39]. Single-cell profiling corroborates this landscape, showing T-cell exhaustion with Treg enrichment as a hallmark of intrahepatic CCA [37].

In parallel, cancer-associated fibroblasts deposit collagen and fibronectin and orchestrate a dense, chemokine-rich stroma that excludes cytotoxic T cells and sustains an immunosuppressive niche [40–42]. Collectively, these circuits establish a hostile immunologic microenvironment that limits immune surveillance and promotes tumor growth and dissemination.

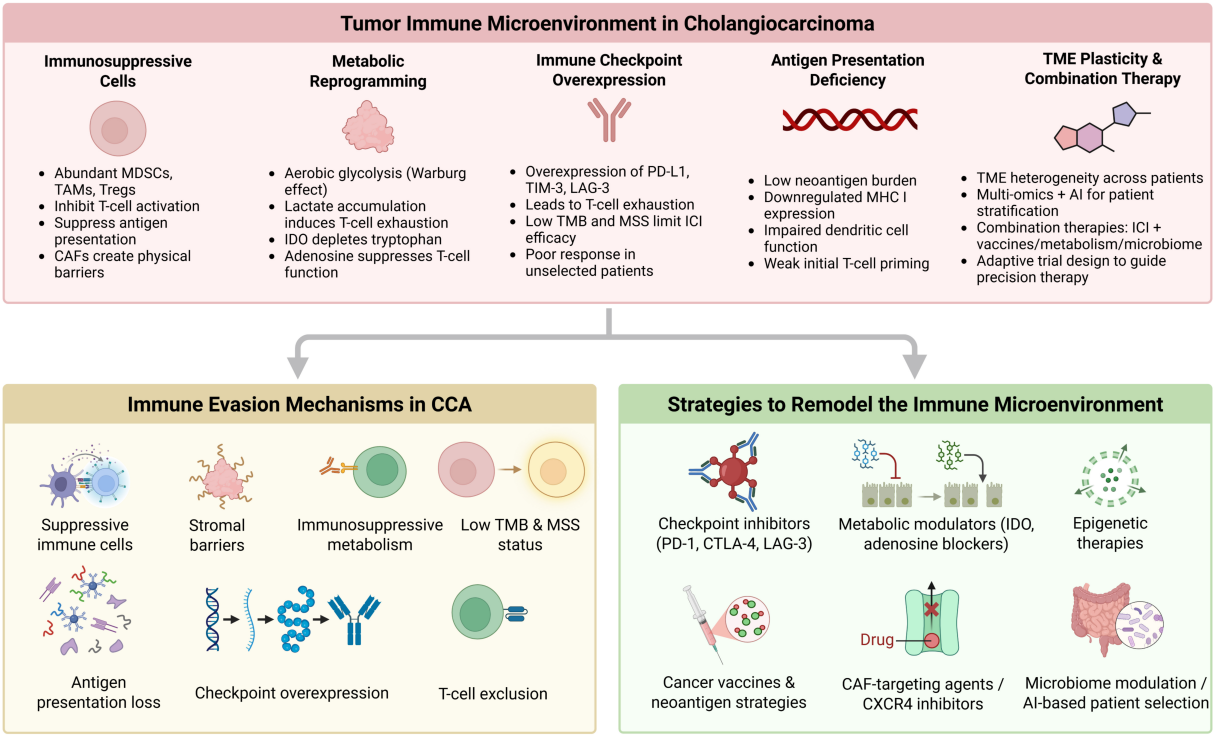

Fig. 2. Tumor immune microenvironment and emerging strategies to remodel immune resistance in CCA. The immune microenvironment of CCA is characterized by multiple layers of immunosuppression, including the accumulation of suppressive immune cells (MDSCs, TAMs, Tregs), metabolic reprogramming (aerobic glycolysis, IDO activation, adenosine signaling), overexpression of immune checkpoints (PD-L1, TIM-3, LAG-3), and defective antigen presentation. These alterations contribute to T-cell exhaustion, immune exclusion, and low responsiveness to immune checkpoint inhibitors, particularly in tumors with low TMB and microsatellite stability. Therapeutic strategies to remodel the immune microenvironment include checkpoint blockade, metabolic and epigenetic modulation, stromal or CAF-targeted therapy, cancer vaccination, and microbiome-based or AI-assisted patient stratification approaches. Created with https://www.biorender.com/. MDSCs, myeloid-derived suppressor cells; TAMs, tumor-associated macrophages; Tregs, regulatory T cells; IDO, indoleamine 2,3-dioxygenase; PD-L1, programmed death-ligand 1; TIM-3, T-cell immunoglobulin and mucin domain-containing protein 3; LAG-3, Lymphocyte activation gene 3; TMB, tumor mutational burden; CAF, cancer-associated fibroblasts.

Metabolic Reprogramming in the CCA Tumor Microenvironment

Metabolic reprogramming within the CCA TME plays a key role in immune evasion and therapy resistance. Tumor cells preferentially engage aerobic glycolysis, a phenomenon known as the Warburg effect, which leads to excessive lactate production [43]. Tumor-derived lactic acid directly impairs dendritic-cell functions, blunting activation/type-I IFN signaling and diminishing antigen cross-presentation [44,45]. Elevated lactate concentrations interfere with T-cell metabolism and effector programs, suppressing proliferation, cytokine production, cytotoxicity, and motility, thereby fostering dysfunctional/exhausted states [46–48]. Glycolytic lactate production and the resultant acidic milieu attenuate antigen presentation and adaptive immunity in CCA, with clear implications for resistance to immune checkpoint therapy [43].

In parallel, indoleamine 2,3-dioxygenase (IDO) activity within the CCA TME depletes tryptophan, an essential amino acid necessary for T-cell proliferation. IDO-mediated tryptophan depletion contributes to immune suppression by inducing T-cell anergy and promoting the kynurenine-pathway metabolites further skew differentiation toward regulatory T cells via stress/AhR pathways [49]. Hypoxia further elevates extracellular adenosine via the CD39/CD73 axis (HIF-1–dependent), which signals through A2A/A2B receptors to blunt T-cell activation/cytotoxicity, expand Tregs/MDSCs and impair antigen-presenting functions [50].

Notably in CCA, CD73 is upregulated and portends poor outcome, and CD73 blockade augments anti-PD-1 activity in preclinical iCCA models; a potent CD73 inhibitor restores T-cell function and potentiates PD-1 blockade *in vivo*, supporting clinical translation [51].

Metabolic reprogramming in the CCA TME-driven by glycolytic lactate acidification, IDO/kynurenine/AhR signaling, and CD39/CD73-mediated adenosine-suppresses DC/T-cell function, expands Tregs/MDSCs, and thereby

fuels immune evasion and ICI resistance, highlighting metabolism-targeted combinations as actionable strategies.

Immune Checkpoint Molecule Overexpression and Immune Escape

Immune checkpoint molecule overexpression plays a crucial role in immune escape mechanisms within the CCA TME. PD-L1 is frequently expressed on both tumor cells and infiltrating immune cells, and engagement of PD-1 on $CD8^+$ T cells drive an exhausted, hypofunctional state that suppresses antitumor immunity [52–54]. Single-cell profiling further confirms an exhausted $CD8^+$ T-cell phenotype with high inhibitory-receptor expression within the iCCA microenvironment [37].

Elevated PD-L1 expression in CCA is consistently associated with worse survival, as shown by a 2023 meta-analysis and supporting cohort studies [55]. Although PD-1/PD-L1 inhibitors have demonstrated activity in other malignancies, pooled analyses of KEYNOTE-158/-028 indicate only modest response rates in unselected biliary tract cancers, with clear benefit largely confined to rare MSI-H/dMMR subsets [18,56,57].

Beyond PD-L1, multiplexed immunofluorescence in 50 CCA cases mapped a broader checkpoint landscape, PD-L2, TIM-3, LAG-3, TIGIT, ICOS and CTLA-4 with enrichment in the sclerotic tumor region and recurrent co-expression signatures that are associated with nodal metastasis, underscoring the need for multi-target strategies [58]. Consistent with these data, TIGIT is detectable in biliary tract cancer and correlates with immunosuppressive Tumor Infiltrating Lymphocytes (TIL) features, supporting exploration of multi-checkpoint blockade in this disease [59]. Mechanistically, LAG-3 engagement of stable peptide MHC-II acts as a functional inhibitory axis that dampens T-cell receptor signaling [60]; this is now supported by structure-level evidence defining the LAG-3/MHC-II interface [61] and by cryoEM showing the therapeutic antibody favezelimab binds the MHC-II–binding site on LAG-3 [62].

The concurrent upregulation of these checkpoints underscores the rationale for next-generation, biomarker-guided combinations that target multiple inhibitory pathways simultaneously [63].

Immunotherapeutic Approaches in CCA

Immune Checkpoint Inhibitors

In BTC cohorts with substantial CCA representation, pembrolizumab monotherapy delivered low response rates: KEYNOTE-158 (NCT02628067) ORR 5.8%, PFS 1.8 months, OS 7.4 months; KEYNOTE-028 (NCT02054806) ORR 13.0%, PFS 1.8 months, OS 5.7 months. PD-L1 enrichment offered only a modest ORR difference in KEYNOTE-158 (6.6% vs. 2.9%), and safety was manageable (grade $\geq$3 TRAEs 13–17%) together underscoring limited monotherapy efficacy in largely MSS CCA [18]. By contrast, MSI-H/dMMR CCA can be highly responsive to PD-1 blockade; for example, a patient with MSI-H/dMMR CCA achieved a deep and durable response to pembrolizumab despite low PD-L1 and limited T-cell infiltration, illustrating the biomarkers predictive value in this setting [64]. Nivolumab monotherapy in refractory BTC (NCT02829918) yielded an investigator-assessed ORR 22% (10/46) vs. 11% (5/46) by central review, median PFS 3.68 months (95% CI 2.30–5.69) and median OS 14.24 months (95% CI 5.98–NR). Treatment was generally tolerable; the most common grade 3–4 events were hyponatremia (6%) and elevated alkaline phosphatase (4%) [20].

Chemo-immunotherapy as first-line standard for advanced CCA. A phase-3 trial (NCT03875235) has established gemcitabine and cisplatin plus PD-L1 blockade as a new benchmark applicable to CCA. TOPAZ-1 showed durvalumab plus gemcitabine and cisplatin improved overall survival versus placebo plus gemcitabine and cisplatin (median 12.9 vs. 11.3 months; HR 0.76), with consistent benefit across prespecified subgroups and no new safety signals on longer follow-up [65,66]. KEYNOTE-966 (NCT04003636) similarly demonstrated an OS advantage for pembrolizumab plus gemcitabine and cisplatin compared with gemcitabine and cisplatin alone (12.7 vs. 10.9 months; HR 0.83), and prespecified patient-reported outcomes show maintenance of health-related quality of life with the addition of pembrolizumab [22].

Dual-checkpoint and non-cytotoxic combinations. Intensifying immunotherapy beyond single-agent PD-L1 has yielded signal-generating results in BTC cohorts containing many CCA cases. In the CA209-538 (NCT02923934) rare-cancers phase-2 subgroup, nivolumab plus ipilimumab achieved ORR 23% with durable responses in pretreated, predominantly MSS disease, the median progression-free survival was 2.9 months (95% CI, 2.2–4.6 months), and overall survival was 5.7 months (95% CI, 2.7–11.9 months), supporting further CCA-specific randomized testing rather than immediate routine use [21].

Outside gemcitabine and cisplatin, multiple early-phase or single-arm studies (NCT03951597, NCT03486678) in unresectable CCA suggest biological synergy when PD-1 inhibitors are paired with anti-angiogenic agents and/or oxaliplatin-based chemotherapy (GEMOX), with reported ORRs around 30–50% in selected cohorts; however, heterogeneity, small sample sizes, and non-randomized designs mandate confirmatory trials before broad adoption [67]. This regimen is notable not only for prolonging survival but also for a favorable safety profile, making it suitable for broader implementation. This chemoimmunotherapy combination has become the new first-line standard, and ongoing trials continue to explore related regimens (Table 1 (Ref. [18,20–22,65,66,68–79]) and Table 2).

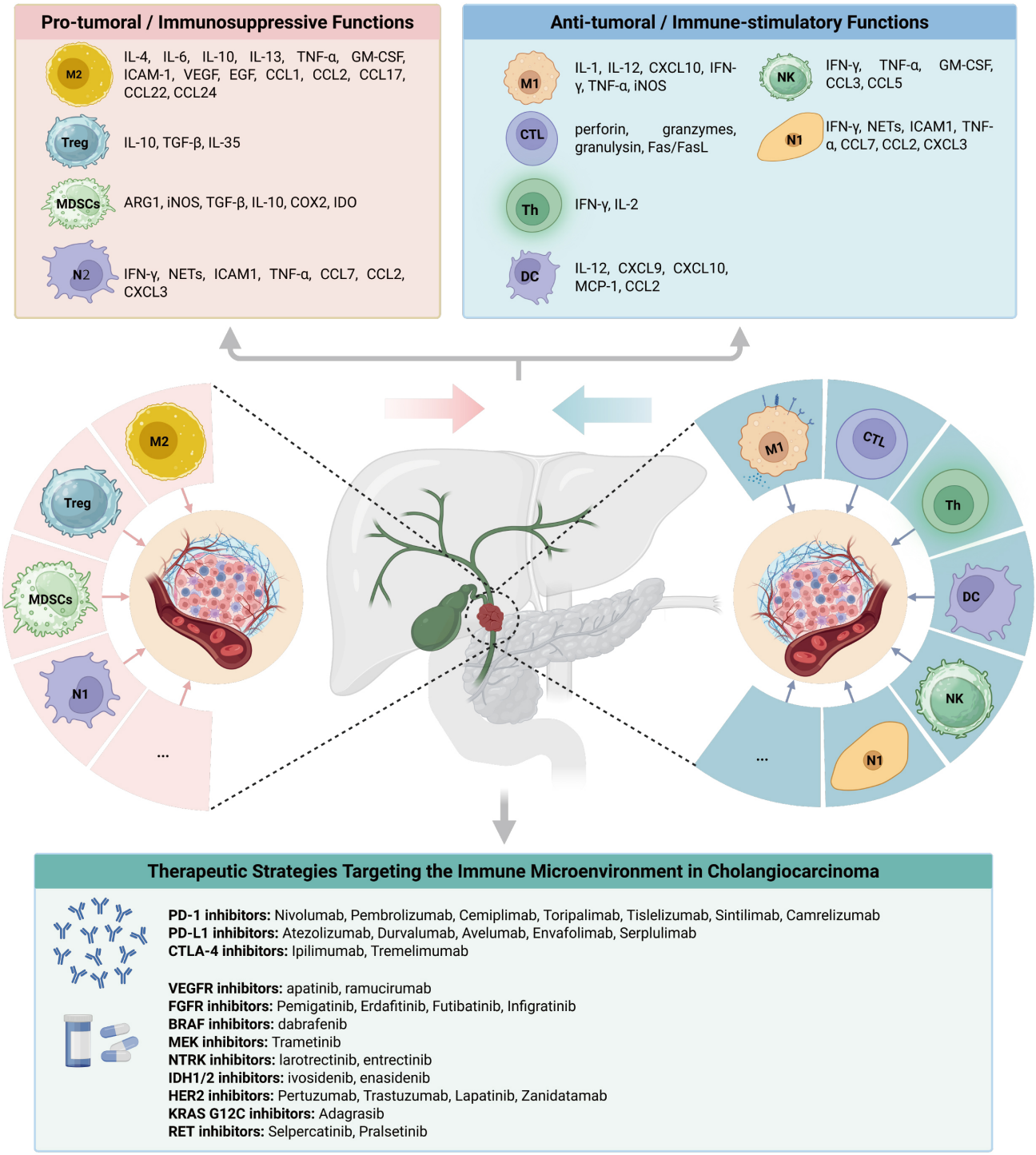

Fig. 3. Immune cell polarization and therapeutic targeting of the tumor microenvironment in CCA. The immune landscape of CCA includes a dynamic balance between pro-tumoral and anti-tumoral immune cell subsets. Immunosuppressive populations—such as M2 macrophages, regulatory T cells (Tregs), myeloid-derived suppressor cells (MDSCs), and N2-type neutrophils—secrete inhibitory cytokines and metabolic enzymes (e.g., IL-10, TGF-β, IDO), fostering immune evasion and tumor progression. In contrast, anti-tumoral components—including M1 macrophages, cytotoxic T lymphocytes (CTLs), natural killer (NK) cells, dendritic cells (DCs), and T helper cells—produce effector molecules such as IFN-γ, CXCL9/10, and perforin, supporting immune activation. Immunotherapeutic strategies seek to shift this balance by targeting immune checkpoints (PD-1, PD-L1, CTLA-4) and oncogenic pathways, aiming to reprogram the tumor microenvironment and enhance anti-tumor immunity in CCA. Created with https://www.biorender.com/. IL-10, interleukin 10; TGF-β, transforming growth factor beta; IFN-γ, interferon gamma; CXCL9/10, C-X-C motif chemokine ligand 9/10; PD-1, programmed cell death protein 1; CTLA-4, cytotoxic T-lymphocyte–associated protein 4.

Table 1. Representative clinical trials investigating ICI-based combinations with immunotherapy, chemotherapy, anti-angiogenic agents, or local therapies in CCA.

Line of Treatment	Trial Number	Phase	Treatment	Patients (n)	ORR (%)	PFS (months)	OS (months)
1st	NCT03875235 [65,66]	III	Durvalumab + Gemcitabine + Cisplatin	341	26.7	7.2	12.9 vs. 11.3
	NCT04003636 [22]	III	Pembrolizumab + Gemcitabine + Cisplatin	533	29	6.5	12.7 vs. 10.9
	NCT03486678 [68]	II	Camrelizumab + Gemcitabine + Oxaliplatin (GEMOX)	37	54	6.1	11.8
	NCT03092895 [69]	II	Camrelizumab + FOLFOX4/GEMOX	92	16.3	5.3	12.4
	ChiCTR2000036652 [70]	II	Sintilimab + Gemcitabine + Cisplatin	30	36.7	5.1	15.9
	NCT03796429 [71]	II	Toripalimab + Gemcitabine + S-1	50	30.6	7	15
	NCT03951597 [72]	II	Toripalimab + Lenvatinib + GEMOX	30	80	10.2	22.5
	NCT03101566 [73]	II	Nivolumab + Gemcitabine + Cisplatin	35	NR	6.6	10.6
≥2nd	NCT02628067 [18]	II	Pembrolizumab	104	5.8	2	7.4
	NCT02054806 [18]	Ib	Pembrolizumab	24	13	1.8	5.7
	NCT03695952 [74]	-	Pembrolizumab	40	10	1.5	4.3
	Multicenter retrospective cohort study [75]	-	Pembrolizumab	51	9.8	2.1	6.9
	NCT02829918 [20]	II	Nivolumab	54	22	3.7	14.2
	NCT01938612 [76]	I	Durvalumab	42	4.8	NR	NR
	NCT01938612 [76]	I	Durvalumab + Tremelimumab	65	10.8	NR	NR
	NCT03704480 [77]	II	Durvalumab + Tremelimumab	106	9.7	2.5	8
	NCT02923934 [21]	II	Nivolumab + Ipilimumab	39	23	2.9	5.7
	NCT04642664 [78]	II	Camrelizumab + Apatinib	22	19	4.4	13.1
	NCT03895970 [79]	II	Pembrolizumab + Lenvatinib	32	25	4.9	11

ICI, immune checkpoint inhibitor; CCA, cholangiocarcinoma; ORR, objective response rate; PFS, progression-free survival; OS, overall survival; FOLFOX4, leucovorin calcium, fluorouracil, and oxaliplatin regimen.

Table 2. Ongoing studies of ICI-based combination strategies in CCA.

Line of Treatment	Trial Number	Treatment	Target	Patients	Phase
Neoadjuvant	NCT04308174	Gemcitabine + Cisplatin ± Durvalumab	Anti-PD-L1, chemotherapy	45	II
	NCT03695952	Nivolumab or pembrolizumab	Anti-PD-1	100	prospective
	NCT02091141	Atezolizumab	Anti-PD-L1	673	II
	NCT03898895	Camrelizumab + radiation	Anti-PD-1, radiation	36	II
	NCT03796429	Toripalimab + Gemcitabine + S1	Anti-PD-1, chemotherapy	50	II
	NCT04172402	Nivolumab + Gemcitabine + TS1	Anti-PD-1, chemotherapy	48	II
	NCT03260712	Pembrolizumab + Gemcitabine + Cisplatin	Anti-PD-1, chemotherapy	50	II
	NCT04003636	Pembrolizumab + Gemcitabine + Cisplatin vs. Gemcitabine + Cisplatin	Anti-PD-1, chemotherapy	1069	III
	NCT04191343	Toripalimab + GEMOX	Anti-PD-1, chemotherapy	20	II
	NCT04413734	Triprilumab + Gemcitabine + Cisplatin	Anti-PD-1, chemotherapy	120	II
	NCT05771480	Durvalumab + Gemcitabine-based Chemotherapy	Anti-PD-L1, chemotherapy	142	III
	NCT03478488	Gemcitabine + Oxaliplatin ± KNO35	Anti-PD-L1, chemotherapy	480	III
	NCT04454905	Camrelizumab + Apatinib	Anti-PD-1, TKI	50	II
	NCT05742750	Camrelizumab + Apatinib + Gemcitabine + Cisplatin	Anti-PD-1, TKI, chemotherapy	48	Ib/II
	NCT05451290	Camrelizumab + Apatinib + GEMOX	Anti-PD-1, TKI, chemotherapy	30	II
	NCT04300959	Sintilimab + Anlotinib + Gemcitabine + Cisplatin	Anti-PD-1, TKI, chemotherapy	80	II
1st	NCT04720131	Camrelizumab + Apatinib + Capecitabine	Anti-PD-1, TKI, chemotherapy	28	II
	NCT05156788	Tislelizumab + Lenvatinib + GEMOX	Anti-PD-1, TKI, chemotherapy	40	II
	NCT05668884	GEMOX + Donafenib + Tislelizumab	Anti-PD-1, TKI, chemotherapy	93	II
	NCT05410197	Envafolimab + Lenvatinib + Gemcitabine + Cisplatin	Anti-PD-L1, TKI, chemotherapy	43	II
	NCT05749900	Nivolumab + Trastuzumab + Gemcitabine + Cisplatin	Anti-PD-1, Anti-HER2, chemotherapy	44	Ib/II
	NCT03829436	Nivolumab + TPST-1120	Anti-PD-1, Anti-PARP	38	I
	NCT03473574	Durvalumab + Tremelimumab + Gemcitabine ± Cisplatin vs. Gemcitabine + Cisplatin	Anti-PD-L1, anti-CTLA-4, chemotherapy	128	II
	NCT03046862	Durvalumab + Tremelimumab + Gemcitabine + Cisplatin	Anti-PD-L1, anti-CTLA-4, chemotherapy	31	II
	NCT03991832	Durvalumab + Olaparib	Anti-PD-L1, Anti-PARP	58	II
	NCT04677504	Atezolizumab +/− Bevacizumab + Gemcitabine + Cisplatin	Anti-PD-L1, Anti-VEGF, chemotherapy	162	II
	NCT04984980	Gemcitabine + Oxaliplatin + Sintilimab + Bevacizumab	Anti-PD-L1, Anti-VEGF, chemotherapy	37	II
	NCT04217954	HAIC (Oxaliplatin + 5-FU) + Bevacizumab + Toripalimab	Anti-VEGF, Anti-PD-1	32	II
	NCT04066491	Gemcitabine-Cisplatin ± M7824	Chemotherapy, Anti-TGF-β/PD-L1	309	II/III
	NCT03633773	MUC-1 CAR-T cell	ACT	9	I/II
	NCT03801083	Tumor Infiltrating Lymphocytes (TIL)	ACT	59	II
	NCT02482454	Cytokine-induced killer cells (CIK) + Radiofrequency ablation (RFA)	ACT	50	III

Table 2. Continued.

Line of Treatment	Trial Number	Treatment	Target	Patients	Phase
2nd	NCT02829918	Nivolumab	Anti-PD-1	54	II
	NCT02628067	Pembrolizumab	Anti-PD-1	1609	II
	NCT04299581	Cryoablation + Camrelizumab	Anti-PD-1	25	II
	NCT05056116	Toripalimab + Surufatinib	Anti-PD-1, TKI	30	II
	NCT04550624	Pembrolizumab + Lenvatinib	Anti-PD-1, TKI	40	II
	NCT04211168	Toripalimab + Lenvatinib	Anti-PD-1, TKI	44	II
	NCT03797326	Pembrolizumab + Lenvatinib	Anti-PD-1, TKI	603	II
	NCT05781074	Cryoablation + Sintilimab + Lenvatinib	Anti-PD-1, TKI	25	II
	NCT04781192	Durvalumab + Regorafenib	Anti-PD-L1, TKI	40	I/II
	NCT03475953	Avelumab + Regorafenib	Anti-PD-L1, TKI	747	I/II
	NCT03785873	Nivolumab + nanoliposomal-irinotecan + 5-fluorouracil + leucovorin	Anti-PD-1, chemotherapy	34	Ib/II
	NCT03257761	Durvalumab + Guadecitabine	Anti-PD-L1, chemotherapy	55	Ib
	NCT05653180	IBI310 + Sintilimab	Anti-PD-1, Anti-CTLA-4	20	I/II
	NCT02834013	Nivolumab + Ipilimumab	Anti-PD-1, Anti-CTLA-4	818	II
	NCT03668119	Nivolumab ± Ipilimumab	Anti-PD-1, Anti-CTLA-4	212	II
	NCT02866383	Nivolumab + radiation +/− Ipilimumab	Anti-PD-1, Local +/− Anti-CTLA-4	160	II
	NCT03704480	Durvalumab + Tremelimumab ± Paclitaxel	Anti-PD-L1, anti-CTLA-4, chemotherapy	106	II
	NCT03482102	Durvalumab + Tremelimumab + radiation	Anti-PD-L1, Anti-CTLA-4, Local	70	II
	NCT04238637	Durvalumab + Tremelimumab + Y90 SIRT	Anti-PD-L1, Anti-CTLA-4, Local	50	II
	NCT03937830	Durvalumab + Bevacizumab + Tremelimumab + TACE	Anti-VEGF, Anti-PD-L1, Anti-CTLA-4	27	II
	NCT04010071	Axitinib + Toripalimab	Anti-VEGFR, Anti-PD-1	60	II
	NCT04057365	Nivolumab + DKN-01	Anti-PD-1, Anti-DKK1	15	II
	NCT03095781	Pembrolizumab + XL888	Anti-PD-1, Anti-HSP90	49	Ib
	NCT04306367	Pembrolizumab + Olaparib	Anti-PD-1, Anti-PARP	14	II
	NCT05222971	Olaparib ± Durvalumab	Anti-PARP, Anti-PD-L1	62	II
	NCT04298021	Durvalumab + Ceralasertib vs. Olaparib + Ceralasertib	Anti-PD-L1, Anti-ATR, Anti-PARP	74	II
	NCT04301778	Durvalumab + SNDX-6532	Anti-PD-L1, Anti-CSF-1R	5	II
	NCT03257761	Durvalumab + Guadecitabine	Anti-PD-L1, Anti-DNMT	55	Ib
	NCT03201458	Atezolizumab + Cobimetinib	Anti-PD-L1, Anti-MEK	86	II
	NCT05052099	Atezolizumab + Bevacizumab + mFOLFOX6	Anti-PD-L1, Anti-VEGF, chemotherapy	20	Ib/II
	NCT04708067	Hypofractionated Radiation Therapy + M7824	Anti-TGF-β/PD-L1	2	I
	NCT04298008	AZD6738 + Durvalumab	Anti-ATR kinase, Anti-PD-L1	26	II
	NCT04068194	Peposertib + Avelumab + Radiation	Anti-DNA-PK, Anti-PD-L1	103	I/II
	NCT05540483	Disitamab Vedotin + Zimberelizumab	Anti-HER2, anti-PD1	31	II

*All information was obtained from https://clinicaltrials.gov/ (accessed in June 2025).

S1, tegafur/gimeracil/oteracil; TS1, tegafur/gimeracil/oteracil; KNO35, anti–PD-L1 monoclonal antibody; TPST-1120, PARP inhibitor TPST-1120; HAIC, hepatic arterial infusion chemotherapy; 5-FU, 5-fluorouracil; MUC-1, mucin 1; CAR-T, chimeric antigen receptor T; IBI310, anti–CTLA-4 monoclonal antibody IBI310; SIRT, selective internal radiation therapy; TACE, transarterial chemoembolization; DKN-01, DKK1 monoclonal antibody DKN-01; XL888, heat shock protein 90 inhibitor XL888; SNDX-6532, CSF-1R inhibitor SNDX-6532; mFOLFOX6, modified fluorouracil/leucovorin/oxaliplatin regimen; AZD6738, ATR inhibitor AZD6738; PD-L1, programmed death-ligand 1; PD-1, programmed cell death protein 1; TKI, tyrosine kinase inhibitor; HER2, human epidermal growth factor receptor 2; PARP, poly(ADP-ribose) polymerase; VEGF, vascular endothelial growth factor; TGF-β, transforming growth factor beta; ACT, Adoptive cell therapy; CTLA-4, cytotoxic T-lymphocyte–associated protein 4; HSP90, heat shock protein 90; ATR, ataxia telangiectasia and Rad3-related protein kinase; CSF-1R, colony-stimulating factor 1 receptor; DNMT, DNA methyltransferase; MEK, mitogen-activated protein kinase kinase.

CCA transcriptomic and spatial atlases delineate an "inflamed" iCCA subgroup enriched for IFN-γ–responsive programs and co-expressed checkpoints, nominating IFN-γ–anchored signatures as hypothesis-generating biomarkers that warrant prospective validation in CCA immunotherapy trials [80]. At the tissue level, TIL metrics (especially $CD8^+$ density/spatial organization) carry prognostic and putative predictive relevance in CCA [81], while single-cell and multiplex mapping in iCCA delineate exhausted $CD8^+$ programs and co-expressed checkpoints (PD-1, PD-L1, PD-L2, LAG-3, ICOS, TIGIT, TIM-3, CTLA-4), nominating composite, mechanism-anchored biomarkers and rational multi-checkpoint combinations [58,82].

Non-invasive markers are also advancing, radiomics can non-invasively phenotype ICC and aid ICC–HCC discrimination [83], and ctDNA kinetics are emerging for response/ resistance monitoring in BTC with growing translational evidence [84]. Candidate biomarkers differ widely in maturity, priorities include assay harmonization, predefined cut-offs, and prospective, biomarker-stratified CCA trials to establish reproducible, clinically actionable predictive utility.

Taken together, ICIs have a defined yet qualified role in CCA, with the strongest evidence for chemo-immunotherapy backbones in the first-line setting, while single agent activity in unselected MSS disease remains limited. Durable benefit will depend on rational combinations that disrupt multiple inhibitory nodes and remodel the TIME, alongside biomarker guided selection that spans MSI-H/dMMR where present and emerging transcriptomic, spatial and liquid-biopsy signatures. Table 2 summarizes ongoing and planned trials that test these strategies and embed translational end points, including unified PD-L1 methodology and cut-offs, biomarker stratified randomization, central radiology and pathology review, consistent efficacy and quality-of-life end points, and prospective tissue and ctDNA collection. Standardized designs and harmonized assays will enable robust cross-study comparisons, support regulatory decision making, and help define a durable place for immunotherapy in CCA care. Beyond these methodological constraints, a substantial proportion of immunotherapy trials in BTC have yielded negative or neutral results, further highlighting the gap between mechanistic rationale and clinical benefit.

Negative or Neutral Trials

Several immunotherapy-based strategies in CCA have yielded negative or neutral results, underscoring biological complexity and trial-design limitations. In the randomized phase II BilT-01 study, neither gemcitabine-cisplatin plus nivolumab nor ipilimumab plus nivolumab exceeded the predefined 6-month PFS benchmark vs. ABC-02; ORR was 22.9% vs. 3.0%, with median PFS 6.6 vs. 3.9 months, respectively [26,85]. The phase II IMMUCHEC trial of durvalumab/tremelimumab-based regimens in the first line setting likewise failed its ORR primary endpoint, with response rates of only 4.6–28.6% and no clear advantage over gemcitabine-cisplatin alone [86]. In IMbrave151, adding bevacizumab to atezolizumab plus cisplatin-gemcitabine produced a modest PFS improvement (8.3 vs. 7.9 months; HR 0.67, 95% CI 0.46–0.95) but no OS gain (14.9 vs. 14.6 months; HR 0.97), while grade 3–4 adverse events occurred in 74% of patients in both arms [87]. PARP-inhibitor combinations have also been disappointing: the BilT-02 maintenance trial of rucaparib plus nivolumab did not meet its 4-month PFS primary endpoint (PFS4 54.8% vs. 63% null; ORR 6.4%; median PFS 4.6 months), olaparib plus pembrolizumab achieved an ORR of 15.4% with median PFS 5.45 months, and an olaparib plus durvalumab study in IDH-mutant CCA reported no objective responses and median PFS 1.97 months [88]. Taken together, these negative or neutral trials indicate that, outside of TOPAZ-1 and KEYNOTE-966, most immunotherapy approaches in CCA have conferred only incremental benefit, reinforcing the need for biomarker-enriched, mechanistically rational trial designs.

Adoptive Cell Therapy

Adoptive cell therapy (ACT) represents a rapidly advancing frontier in cancer immunotherapy, with applications in CCA currently under early-phase clinical investigation [89]. ACT modalities, including chimeric antigen receptor T (CAR-T) cells, TILs, and cytokine-induced killer cells are being tested for their ability to bypass tumor immune evasion and deliver personalized, tumor-reactive cytotoxicity.

CAR-T cell therapy, despite being highly effective in hematologic malignancies, faces significant barriers in solid tumors such as CCA. Preclinical studies targeting antigens like glypican-3 and mucin-1 have demonstrated cytotoxic potential [90], but clinical translation remains limited owing to challenges including poor trafficking to tumor sites, limited persistence, and the risk of on-target/off-tumor toxicity [91]. Notably, most ACT studies have yet to demonstrate meaningful tumor regression beyond transient disease stabilization, and several early phase trials were terminated prematurely due to limited efficacy or manufacturing constraints. Strategies such as chemokine receptor co-expression (e.g., CXCR3) and ICI co-administration are being investigated to improve intratumoral localization and functional durability [92].

TIL therapy harnesses polyclonal T cells derived from a patient's tumor, offering broad recognition of tumor antigens and the potential for durable responses. Approaches aimed at enhancing TIL efficacy, such as lymphodepletion, IL-2 support, and *ex vivo* expansion protocols are currently under evaluation [93].

In parallel, T-cell receptor (TCR)-engineered therapies are attracting increasing interest because they can rec-

ognize intracellular tumor antigens in an HLA-restricted manner, thereby overcoming the limitation of surface antigen targeting by CAR-T cells [94]. Early-phase clinical trials and preclinical studies are evaluating TCR-T cells directed against cancer-testis antigens such as NY-ESO-1 and WT1, as well as patient-specific neoantigens in solid tumors, including hepatobiliary malignancies and biliary tract cancer [95].

To overcome ACT resistance mechanisms, combinatorial strategies are being developed. These include concurrent administration of immune checkpoint inhibitors, cytokine-based agents (e.g., IL-15 super agonists) [96], and metabolic modulators (e.g., IDO inhibitors) [97], all aimed at reversing T-cell dysfunction and enhancing effector activity within the TME. Ultimately, the success of ACT in CCA will depend on addressing key hurdles such as immune exclusion, T-cell exhaustion, and the lack of predictive biomarkers [27].

In summary, ACT holds significant therapeutic potential in CCA. However, its clinical impact remains constrained by the immunosuppressive nature of the disease. Ongoing efforts to optimize cell engineering, refine patient selection, and rationally integrate combination regimens may help translate ACT into a more effective and durable treatment strategy. However, most ACT trials in CCA remain early-phase, single-arm, and exploratory, often with fewer than 20 participants and short follow-up. These design constraints hinder reliable efficacy estimation and cross-study reproducibility.

Tumor Vaccines

Tumor vaccines targeting tumor-associated antigens, such as Wilms' tumor 1 and New York esophageal squamous cell carcinoma 1 are currently under clinical investigation in CCA [98,99]. These therapeutic vaccines aim to enhance antitumor immunity by presenting tumor-specific antigens to the host immune system, thereby promoting the activation and expansion of cytotoxic T lymphocytes.

Personalized neoantigen vaccines represent a promising advancement in this field. These vaccines are tailored to the patient's individual tumor mutational profile, typically identified through next-generation sequencing [100]. Early-phase trials in gastrointestinal malignancies, including CCA, have demonstrated the feasibility of generating neoantigen-based vaccines with acceptable safety profiles and immunogenicity [101]. mRNA-based vaccine platforms, which gained significant momentum following the success of COVID-19 vaccines, are now being adapted for use in solid tumors, including CCA [102,103]. These platforms offer rapid development timelines, high scalability, and strong potential to elicit robust T-cell responses. Preliminary data suggest that mRNA-based personalized vaccines, especially when combined with immune checkpoint inhibitors, may synergistically enhance antitumor immunity in advanced biliary tract cancers [104].

Dendritic cell based vaccines also remain a subject of active exploration. In this approach, autologous dendritic cells are pulsed *ex vivo* with tumor antigens to prime antigen-specific T-cell responses following reinfusion [105]. Clinical evaluation of dendritic cell vaccines loaded with WT1 peptides in patients with biliary tract cancer patients has shown encouraging immune activation signals and favorable safety outcomes [106]. However, despite immunogenicity, objective clinical responses remain rare, and multiple peptide-based vaccine studies in biliary tract cancers have failed to translate immune activation into measurable survival benefit. To further enhance vaccine efficacy, combinatorial approaches are under investigation. These include pairing neoantigen vaccines with immune checkpoint blockade, particularly PD-1 inhibition, as well as with immunostimulatory adjuvants such as toll-like receptor agonists (e.g., poly-ICLC) [107]. Such combinations aim to improve vaccine-induced T-cell priming and sustain long-term antitumor responses. Nevertheless, several challenges remain in realizing the full potential of tumor vaccines in CCA. The immunosuppressive TME may attenuate vaccine-induced immune activation, necessitating rational combination strategies and improved delivery platforms [108]. Moreover, identifying the most immunogenic tumor antigens and optimizing vaccine design are critical areas of ongoing research. Looking ahead, integrating tumor vaccines into multimodal immunotherapy regimens, supported by biomarker-guided patient selection and advances in vaccine formulation, may significantly improve outcomes. By personalizing vaccine content and combining it with complementary immune interventions, therapeutic vaccination may become a viable strategy to overcome resistance and improve survival in patients with CCA [109]. In comparison, the current evidence for vaccine-based immunotherapy in CCA is derived mainly from pilot studies or mixed-BTC cohorts with small sample sizes and non-randomized designs, which limits definitive conclusions on efficacy and safety.

Novel Immunomodulatory Strategies

Several emerging immunomodulatory approaches are under investigation to augment the immune response in CCA, aiming to overcome tumor-induced immune suppression and improve the efficacy of existing therapies. These include agonists of the STING and CD40 pathways, inhibitors of IDO, and interventions targeting the gut microbiome.

STING (Stimulator of Interferon Genes) agonists activate innate immune signaling by promoting type I interferon production and enhancing antigen presentation, thereby facilitating robust antitumor immunity [110]. Preclinical studies have demonstrated that STING activation can remodel the TME by reducing immunosuppressive cell populations and increasing cytotoxic T-cell infiltration [111]. Based on these findings, STING agonists are being

tested clinically often in combination with immune checkpoint inhibitors to boost response rates in solid tumors, including biliary tract cancers [112]. Nonetheless, most available data for these emerging strategies stem from preclinical or phase I settings. The translational gap between mechanistic promise and clinical validation remains substantial, and safety profiles require further prospective evaluation.

CD40 agonists act by stimulating a key costimulatory receptor on antigen-presenting cells, enhancing dendritic cell activation and facilitating T-cell priming [113]. In CCA, CD40 targeting agents, such as monoclonal antibodies, are being explored to improve antigen presentation and synergize with checkpoint inhibitors. Preliminary trial data suggest that CD40 activation may potentiate T-cell mediated tumor clearance and broaden the benefit of immune-based therapies [114].

IDO inhibitors, which target the enzyme indoleamine 2,3-dioxygenase involved in tryptophan catabolism, aim to reverse T-cell anergy and suppressive metabolic reprogramming within the TME [115]. Agents such as epacadostat have been tested in combination with PD-1 inhibitors in biliary tract cancers. However, outcomes from larger trials have shown limited clinical benefit, highlighting the need for combinatorial regimens that incorporate additional immune-stimulatory mechanisms.

Gut Microbiome Modulation is also gaining traction as a novel strategy to influence systemic immune responses. The gut microbiota has been observed to affect immunotherapy responsiveness, and its modulation through interventions such as fecal microbiota transplantation or probiotic supplementation is currently being evaluated in hepatobiliary cancers [116]. Current studies assess whether altering microbial composition can enhance treatment efficacy by improving immune activation and reducing systemic immunosuppression.

Limitations of Current Clinical Evidence and Risk of Bias Considerations

Despite growing enthusiasm for immunotherapy in CCA and other biliary tract cancers, the current clinical evidence base is constrained by important methodological and statistical limitations. Most available data derive from small, single-arm phase II trials and retrospective series, with heterogeneous inclusion criteria and limited stratification by anatomical subtype, aetiology or biomarker profile, which introduces substantial selection bias and restricts external validity [117–119]. Recent pooled analyses and systematic reviews of 15–30 ICI-based cohorts highlight moderate pooled ORR and survival but also emphasize between-study heterogeneity in treatment line, backbone regimens and follow-up duration, as well as the predominance of Asian, single-center experiences over Western, real-world datasets [117,120]. Biomarker analyses are further hampered by variability in PD-L1 assays, scoring systems and cut-off thresholds, which significantly modifies the apparent predictive value of PD-L1 expression across studies and complicates cross-trial interpretation [121].

In addition, most reports often single-arm, two-stage phase II oncology designs, lack robust pre-specified statistical adjustment for multiplicity and are underpowered for key endpoints such as overall survival or durable response, raising concerns about type I error inflation and publication bias towards positive early-phase signals [122–124]. Collectively, these issues underscore the need for rigorously designed, biomarker-integrated randomized trials with standardized eligibility criteria, centralized assay platforms and harmonized endpoints to generate high-quality, low-bias evidence for immunotherapy in CCA.

Immune-Related Adverse Events (irAEs) in CCA Immunotherapy

Across first-line chemo-immunotherapy trials, toxicity is largely driven by the gemcitabine–cisplatin backbone, with only a modest incremental immune-related signal from PD-L1 blockade. In TOPAZ-1, maximum grade 3–4 AEs occurred in 74% (250/338) of patients receiving durvalumab plus gemcitabine–cisplatin vs. 75% (257/342) with gemcitabine–cisplatin alone; the most common grade 3–4 treatment-related events were decreased neutrophil count (21% vs. 25%), anemia (19% vs. 19%), and neutropenia (19% vs. 20%), without new safety signals [66]. In KEYNOTE-966, pembrolizumab plus gemcitabine–cisplatin led to grade 3–4 treatment-related adverse events in 369/529 patients (70%) vs. 367/534 (69%) with placebo plus gemcitabine–cisplatin, and immune-mediated events and infusion reactions of any grade occurred in 22% vs. 13% of patients, respectively [22]. Taken together, these large phase III studies indicate that adding PD-1/PD-L1 inhibitors does not substantially worsen high grade toxicity compared with chemotherapy alone.

Single-agent and intensified combination immunotherapy regimens show a more heterogeneous, but generally manageable, AE profile. Nivolumab monotherapy in refractory BTC yielded grade 3–4 treatment related toxicities in 17% of patients, most commonly hyponatremia (6%) and increased alkaline phosphatase (4%) [20]. Pembrolizumab monotherapy in KEYNOTE-158 and -028 reported grade 3–5 treatment-related AEs in 13.5% and 16.7% of patients, respectively, confirming a relatively favorable safety profile [18]. By contrast, dual checkpoint blockade with nivolumab + ipilimumab caused immune-related AEs in 49% of patients, with grade 3–4 immune-related events in 15% [21], and the triplet regimen toripalimab + lenvatinib + GEMOX was associated with grade $\geq$3 AEs in 56.7% of patients, mainly neutropenia (40.0%) and leukocytopenia (23.3%) [72]. Overall, CCA immunotherapy trials consistently report substantial haematologic and hepatic toxicities that are largely chemotherapy-mediated, while immune-related events remain mostly low frequency but clinically mean-

ingful, underscoring the need for vigilant monitoring and early management in routine practice.

Discussion

Immunotherapy has reshaped standards of care in several solid tumors, but its impact in CCA remains modest and highly context dependent. In the first line setting, the addition of PD-1/PD-L1 blockade to gemcitabine–cisplatin has established a new reference regimen: TOPAZ-1 and KEYNOTE-966 both demonstrated statistically significant but numerically small gains in median overall survival (around 1–2 months), with a tail of long-term survivors that supports true biological synergy in a subset of patients. In TOPAZ-1, durvalumab plus gemcitabine–cisplatin improved median OS to 12.9 vs. 11.3 months (HR 0.76) with similar rates of grade 3–4 toxicity, whereas KEYNOTE-966 reported median OS 12.7 vs 10.9 months with pembrolizumab plus gemcitabine–cisplatin (HR 0.83) [22,66].

Real-world cohorts and pooled analyses corroborate that chemo-immunotherapy modestly shifts the survival curve to the right without transforming CCA into a "big winner" of immunotherapy [125]. Beyond first-line, PD-1 monotherapy produces objective responses in only a small minority of patients, while early phase combinations with anti-angiogenic agents, GEMOX plus camrelizumab, or "triplet" regimens report higher response rates but remain largely single-arm, biomarker-heterogeneous, and prone to selection bias [68,79,126].

Taken together, current clinical data suggest that ICIs can incrementally improve outcomes in advanced CCA, but durable benefit is confined to a relatively small subgroup, and there is no consensus yet on the optimal immunotherapy backbone in the second-line or beyond. These clinical limitations mirror the underlying biology of CCA, which is among the most immunologically "cold" or immune excluded epithelial cancers. CCA arises within a dense desmoplastic stroma populated by cancer associated fibroblasts, myeloid derived suppressor cells, tumor associated macrophages, and regulatory T cells; this architecture physically and functionally excludes effector T cells, blunts antigen presentation, and promotes chronic immunosuppression [127].

Conventional biomarkers that are highly informative in other tumors-PD-L1 expression, MSI-H/dMMR, and TMB-high are uncommon in CCA and even when present show inconsistent association with benefit from ICIs. Large biomarker focused analyses and reviews converge on the conclusion that PD-L1 positivity, MSI-H, or TMB-high identify only a small fraction of BTC cases and are insufficient as stand-alone selection tools [128,129]. Recent genomic and immune profiling studies further highlight the biological heterogeneity of CCA: distinct genomic subtypes (for example, KRAS/TP53 co-altered vs. KRAS-alone) show divergent immune signatures and differential response to camrelizumab plus GEMOX, and integrated genomic–TME signatures more accurately stratify immunotherapy outcomes than either dimension alone [31,130]. Given these biological constraints, the failure of "one-size-fits-all" checkpoint blockade is not surprising; the central challenge is how to convert a stroma-rich, myeloid-dominated, heterogeneous tumor into one that can be productively engaged by the immune system.

Emerging multi-omics and spatial technologies provide a blueprint for such a shift. Single-cell RNA sequencing and spatial transcriptomics in intrahepatic CCA have begun to map the spatial organization of immune and stromal populations, revealing immune excluded vs. inflamed regions, tertiary lymphoid-like structures, and discrete myeloid and fibroblast niches that correlate with prognosis and potential ICI sensitivity [128,131]. Multi-omics studies integrating targeted sequencing, transcriptomics, and multiplex immunofluorescence now link specific genomic features (e.g., KRAS pathway activation, DNA-damage signatures) and T-cell infiltration patterns with response or resistance to PD-1 based regimens in advanced CCA [130]. In our view, these data argue that future biomarker strategies in CCA should move beyond static PD-L1 or TMB cut-offs toward composite, dynamic scores that incorporate genomic alterations, TIL/TLS architecture, and real-time ctDNA dynamics to capture the evolving immune fitness of each tumor. Such an approach is particularly important in a disease where anatomical subtype (intrahepatic vs. extrahepatic), etiology, and prior locoregional therapies all shape the TIME.

Artificial intelligence (AI) and quantitative imaging/pathology are rapidly becoming indispensable tools to operationalize this complexity at scale. Deep learning models applied to histopathology and radiologic imaging can extract immune related features that are not discernible by the human eye and have already shown promise in predicting response to systemic therapy in CCA and other hepatobiliary tumors [67,132,133]. In CCA specifically, an "immuno-genomic-radiomics" signature derived from patients treated with camrelizumab plus GEMOX achieved superior discrimination of responders vs. non-responders compared with clinical factors alone, suggesting that multimodal AI can help identify those few patients who stand to derive deep and durable benefit from PD-1 based combinations [67]. We anticipate that such models, once prospectively validated, will not only refine patient selection but also support adaptive treatment strategies.

Therapeutically, these insights support a shift from empiric "more drugs" toward rational, biology driven combinations designed to remodel the TIME. Chemotherapy-ICI regimens likely owe much of their benefit to chemotherapy induced immunogenic cell death and transient enhancement of antigen release and T-cell infiltration, as suggested by both clinical data and mechanistic reviews [120].

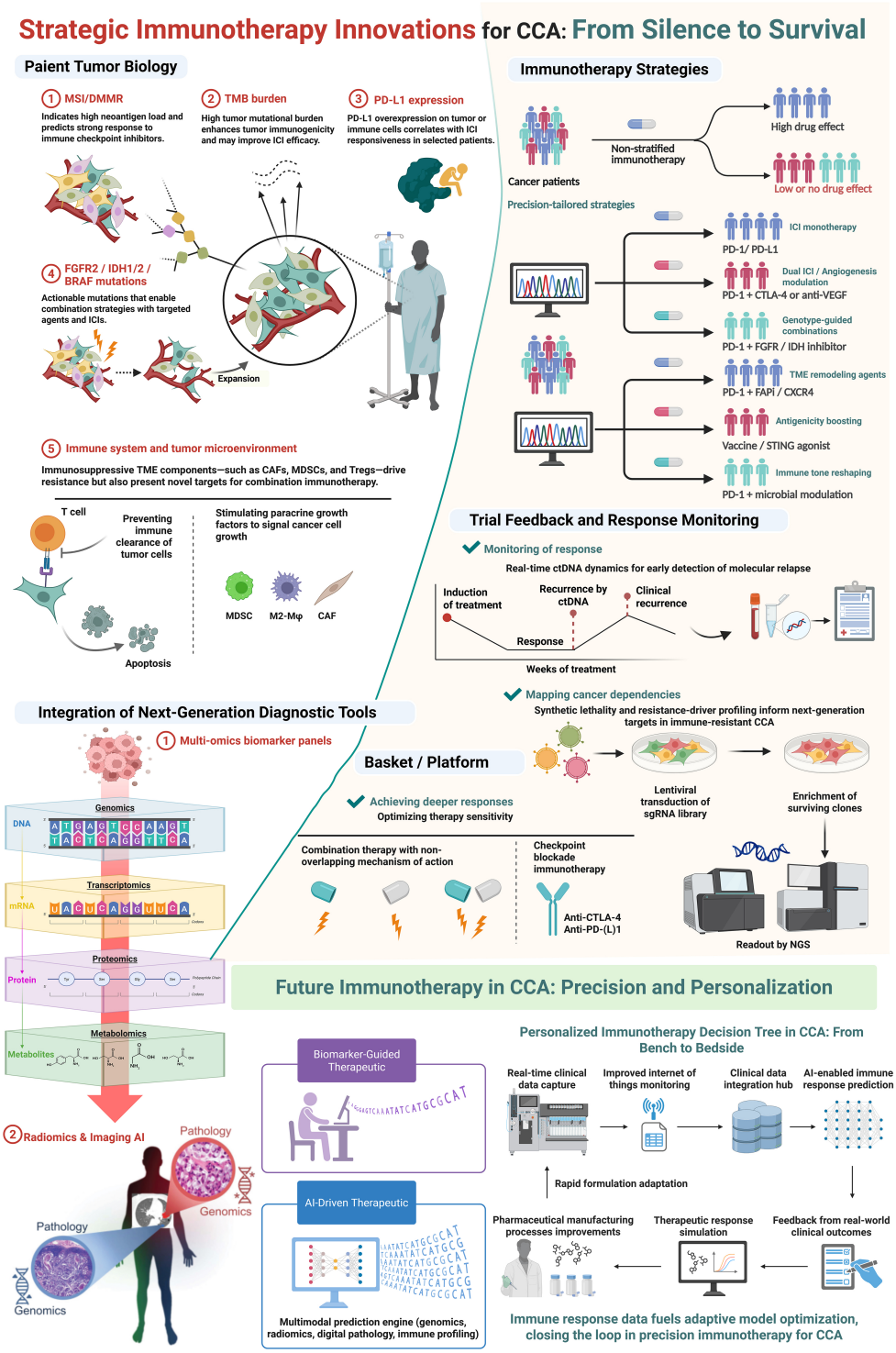

Fig. 4. Strategic immunotherapy innovations in CCA: from silence to survival. This conceptual model illustrates the evolving landscape of immunotherapy in CCA, integrating tumor-intrinsic biology, precision combination strategies, and next-generation diagnostic tools. Key determinants of immunotherapy response, including microsatellite instability, tumor mutational burden, PD-L1 expression, and an immunosuppressive microenvironment highlight the need for individualized treatment. Precision approaches under investigation include checkpoint inhibition combined with angiogenesis blockade, genotype-directed therapy, tumor microenvironment remodeling, vaccines, and microbiome modulation. Real-time ctDNA monitoring and functional genomics enable dynamic response tracking and early relapse detection. Multi-omics profiling, radiomics, and artificial intelligence driven analytics enhance patient stratification and therapeutic decision making. Together, these components converge toward a closed-loop, adaptive immunotherapy framework that transforms CCA management from static protocol to dynamic, personalized intervention. Created with https://www.biorender.com/.

Building on this, combinations of ICIs with anti-angiogenic agents (e.g., Lenvatinib plus PD-1 blockade), FGFR2/IDH1/HER2-targeted therapies, metabolic or epigenetic modulators, and radiotherapy are being explored to simultaneously relieve multiple layers of immune resistance; early-phase BTC cohorts have shown encouraging response rates, but they also highlight added toxicity and the risk of over treatment in unselected patients [79,134–136].

Parallel efforts in nanomedicine and the gut microbiome offer more experimental, but conceptually attractive, means of "priming" CCA for immunotherapy: laser-activated or PD-L1-targeting nanoparticles can rewire the TIME and enhance PD-1 blockade in preclinical CCA models, while microbiome-oriented interventions (diet, probiotics, fecal microbiota transplantation) have been shown in other solid tumors to modulate ICI efficacy and may be particularly relevant in hepatobiliary cancers along the gut–liver axis [137–141]. However, these innovative strategies will require carefully staged translation, with rigorous pharmacokinetic, safety, and biomarker endpoints, before they can be meaningfully integrated into standard CCA care.

Finally, we believe that progress in CCA immunotherapy will depend as much on how we test therapies as on which drugs we use. Traditional, histology-only, fixed-arm phase II/III trials are poorly suited to a rare, molecularly heterogeneous disease with a complex TIME. Adaptive, biomarker enriched basket or platform designs some already being piloted in precision oncology are better positioned to evaluate multiple immunotherapy combinations in parallel, drop futile arms early, and dynamically refine eligibility based on emerging multi-omics and AI-derived biomarkers [32,142–144]. Within this evolving therapeutic landscape, first-line chemo-immunotherapy regimens such as gemcitabine–cisplatin plus durvalumab or pembrolizumab (TOPAZ-1 [66] and KEYNOTE-966 [22]) should be viewed not as the endpoint of progress, but as the clinical backbone on which more personalized strategies are built. Our conceptual model (Fig. 4) therefore emphasizes a closed loop precision immuno-oncology paradigm that links deep tumor profiling (genomics, TIME, microbiome) with advanced analytics (radiomics, digital pathology, AI), rational combination design, and adaptive clinical trial feedback. Whether we can truly "turn the tide" against this silent killer will depend on our ability to implement this framework in CCA, transforming modest survival gains into durable, biologically grounded disease control for a far larger proportion of patients.

Conclusion

CCA has long stood as an archetype of therapeutic resistance silent in presentation, aggressive in progression, and refractory to most systemic interventions. Although immunotherapy has revolutionized treatment paradigms in several solid tumors, its efficacy in CCA remains constrained by a profoundly immunosuppressive TME, low immunogenicity, and a paucity of actionable biomarkers. Yet, this challenge can be overcome. As outlined in this review, a confluence of scientific breakthroughs is reshaping the immunotherapeutic landscape of CCA. From combination regimens designed to dismantle stromal and metabolic barriers to emerging cell therapies, tumor vaccines, and next generation immunomodulators, new strategies are actively being explored. Technological innovation, particularly multi-omics profiling, AI-enhanced imaging, and real-time treatment adaptation has enabled a more granular understanding of tumor immune dynamics and opened the door to precision-guided immunotherapy. Collectively, these advances support an integrated framework for biomarker-guided patient stratification, real-time monitoring, and adaptive treatment strategies.

The path forward lies in converging disciplines oncology, immunology, bioinformatics, systems biology and translating mechanistic insight into clinically meaningful outcomes. Turning the tide against this silent killer will not be achieved by any single breakthrough, but by coordinated, adaptive, and patient-specific strategies. With sustained momentum, CCA may finally move from a paradigm of inevitability to one of individualized, immunologically active control.

Availability of Data and Materials

All data generated or analyzed during this study are included in this published article.

Author Contributions

XFT conducted the comprehensive literature search, extracted and synthesized the evidence, and drafted the manuscript. HCH contributed to the conceptual framework, critical appraisal of the literature, and refinement of key arguments. JY contributed to the acquisition and interpretation of key evidence for the review and critically reviewed the manuscript for important intellectual content. JW conceived and designed the review, supervised the work throughout the writing process, and provided critical oversight. All authors contributed substantially to the work, participated in revising the manuscript critically, approved the final version for publication, and agreed to be accountable for all aspects of the work, ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

Ethics Approval and Consent to Participate

Not applicable.

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Conflict of Interest

The authors declare no conflict of interest. Figures were created with https://www.biorender.com/. Jian Wu is serving as an Editorial Board member of this journal. We declare that the handling Editor had no involvement in the peer review of this article and has no access to information regarding its peer review.

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