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Immune Checkpoint Inhibitors: The Emerging Cornerstone in Cholangiocarcinoma Therapy?

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Abstract

Background: Cholangiocarcinoma (CCA) encompasses a heterogeneous group of malignant tumors with dismal prognosis and increasing incidence worldwide. Both late diagnosis due to the lack of early symptoms and the refractory nature of these tumors seriously compromise patients' welfare and outcomes. ***Summary:*** During the last decade, immunotherapy and, more specifically, modulation of immune checkpoints-mediated signaling pathways have been under the spotlight in the field of oncology, emerging as a potential therapeutic approach for the treatment of several cancers, including CCA. Generally, high expression levels of immune checkpoints in patients with CCA have been associated with worse clinical outcomes, particularly with shorter overall survival and relapse-free survival. Thus, immune checkpoint inhibitors (ICIs), which mainly constitute different monoclonal antibodies, have been developed in order to hamper the immune checkpoint-mediated pathways. Interestingly, chemotherapy may increase the expression of immune checkpoints, while other therapeutic approaches such as ablative and targeted therapies may enhance their antitumor activity. In this sense, several clinical trials evaluated the safety and efficacy of ICIs for CCA, both as a monotherapy and in combination with other ICIs or loco-regional and systemic therapies. Additionally, many other clinical trials are currently ongoing and results are eagerly awaited. Here, we summarize the key aspects of immune checkpoint molecules as prognostic factors and therapeutic targets in CCA, highlighting the most recent advances in the field and future research directions. ***Key Messages:*** (1) Effective therapeutic approaches for CCA are urgently needed. (2) Expression levels of immune checkpoints in patients with CCA have been proposed to be related with clinical outcomes.

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(3) Combination of different ICIs may outperform the efficacy of ICI monotherapy for CCA treatment. (4) Recent studies point toward the combination of ICIs and other common therapies, especially chemotherapy, as a promising strategy for treatment of CCA patients.

Introduction

Cholangiocarcinoma (CCA) encompasses a group of biliary malignant tumors with dismal prognosis. Different cells, including hepatic stem/progenitors cells, cholangiocytes, hepatocytes, and/or multipotent stem cells within peribiliary glands are susceptible to undergo neoplastic transformation, partially explaining the high heterogeneity of these tumors [1]. According to its anatomical site of development, CCAs can emerge at any point of the biliary tree, being classified as intrahepatic cholangiocarcinoma (iCCA), perihilar cholangiocarcinoma (pCCA), and distal cholangiocarcinoma (dCCA) [1]. Incidence (0.3–85/100,000 person-years) [2] and mortality (0.02–2.8/100,000 person-years) [3] rates have been progressively increasing worldwide over the past few decades, currently placing CCA as the second most common primary liver neoplasm after hepatocellular carcinoma (HCC), accounting for ~15% and ~3% of all primary hepatic malignancies and gastrointestinal cancers, respectively [1].

Since CCA etiology is usually uncertain, it is conceivable that this increasing incidence trends could be attributed to emerging and still undefined etiological factors [2]. In this regard, several conditions such as primary sclerosing cholangitis, cirrhosis, viral and parasitic infections, type 2 diabetes mellitus, and genetic landscape have been described to increase the odds for cholangiocarcinogenesis, while the role of others, including non-alcoholic fatty liver disease and alcohol/tobacco consumption, remains controversial [2]. Furthermore, the lack of symptomatology at initial stages of the disease and the absence of precise screening strategies hamper the early diagnosis of CCA. Therefore, most patients are diagnosed at advanced stages when the disease is already disseminated, thus compromising the effectiveness of curative therapeutic options, ultimately resulting in dismal prognosis and low life expectancy [4].

To date, the only potentially curative therapy for patients with CCA is surgical resection of the tumor [5, 6]. However, only ~20% of patients are eligible for surgery due to the late diagnosis and, even when curative resection is achieved, relapse has been reported to occur in ~60–70% of patients, regardless of CCA subtype [7]. Therefore, patients with unresectable, metastatic, or recurrent tumors are only amenable to receive palliative chemotherapy. Nowadays, the combination of gemcitabine and cisplatin (GemCis) is widely accepted as the first-line standard of care [4]. However, many efforts to identify more effective first-line therapeutic regimens have been made by combining different compounds with the reference standard of care, albeit with no conclusive results so far [1]. The existence of diverse and complex mechanisms of chemoresistance in tumor cells and microenvironment seriously compromises GemCis efficacy, which has led to the development of second-line treatment strategies (i.e., folinic acid + fluorouracil + oxaliplatin) intended for a non-negligible minority of CCA patients who progress after first-line regimens [1]. Furthermore, the applicability of other therapeutic approaches, such as loco-regional procedures or liver transplantation, is conditioned by the CCA subtype and their benefits need to be confirmed [1]. A better understanding of the molecular biology driving biliary tract malignancies has contributed to the development of novel and tailored therapies based on the mutational status of CCA driver genes (i.e., mainly *isocitrate dehydrogenase1/2* mutations and *fibroblast growth factor receptor 2* gene fusions) and the immunological tumor microenvironment [4]. Regarding immunotherapies, modulation of immune checkpoints has been gaining relevance in clinical oncology, currently being considered as a potential strategy for the treatment of several cancers. Herein, we provide a state-of-the-art summary focused on both the clinical relevance of immune checkpoints in CCA and the current and emerging therapeutic strategies aiming to modulate the immunological tumor microenvironment in this malignancy.

Immune Checkpoint Expression and Clinical Outcomes in CCA

During tumorigenesis, immune cells can recognize tumor-specific and tumor-associated antigens expressed by malignant cells, triggering specific responses to control tumor growth. However, both tumoral and immune cells may also express high levels of inhibitory immune checkpoints which, through different pathways, prevent T cells from exerting their effector functions (i.e., cytokine release and direct cytotoxicity mostly) [8]. Cancer immunotherapy modulates immune components and tumor

microenvironment to restore an effective immune surveillance that controls tumor growth and reverts the evasion capacity of neoplastic cells. Interestingly, different strategies may contribute to boost antitumor immune response, including the use of monoclonal antibodies (mAbs) directed against immune checkpoints [8]. Depending on their physiological inhibitory or stimulating role in the immune response, immune checkpoints require a fine-tuning antagonistic or agonistic modulation, respectively, to exert therapeutic effects [8]. In the last decade, it has been demonstrated that at least a subgroup of CCAs present suitable genomic and transcriptomic features for this type of treatment, such as high tumor mutational burden, which promotes the expression of neoantigens recognizable by T cells, and the overexpression of genes encoding inhibitory immune checkpoints [9]. In order to understand the basis for immune checkpoint targeting as a novel treatment for CCA, the expression of multiple immune checkpoints and the relationship between such expression with prognosis and other clinical outcomes have been reported.

Programmed Death 1 and Programmed Death-Ligand 1

Programmed death 1 (PD-1) is a cluster of differentiation (CD) 28 family member expressed on activated T and B lymphocytes, monocytes, dendritic cells (DCs), regulatory T cells (Tregs), and natural killer T cells, whereas its ligand programmed death-ligand 1 (PD-L1) belongs to the B7 superfamily and is expressed on resting B cells, T cells, macrophages (including Kupffer cells), DCs, and various tumor cells (shown in Fig. 1) [10]. Upon PD-L1 binding to PD-1, T-cell effector functions are inhibited and apoptosis is induced [10]. Drugs targeting the PD-1/PD-L1 pathway have been approved by the Food and Drug Administration for the treatment of several malignancies, being expression of PD-L1 an explored potential predictive biomarker of efficacy for this therapeutic approach in some of them [10].

Currently, the expression rate of these immune checkpoints in bile duct carcinomas remains controversial. Some of the first studies were performed in medium-size cohorts of patients with iCCA (i.e., 27–54), reporting PD-L1 expression in all patients [11, 12]. Noteworthy, Sabbatino et al. [12] and Fontugne et al. [13] reported lower tumor cell-specific PD-L1 expression (29.6% and 8.62%, respectively), whereas Gani and colleagues [11] showed 72.22% of PD-L1-expressing cells within the tumor front. Similarly, expression of PD-L1 in extrahepatic CCA (eCCA) tumor cells was heterogeneous, ranging from 7.1% to 45% [14–16]. Some authors have reported a frequency of ~70% of PD-L1$^+$ eCCAs, regardless of the cell type that expresses this immune checkpoint [16]. Moreover, 2 recent studies with larger cohorts detected expression and high expression of PD-L1 in 42% and 31% of iCCA patients, respectively, albeit the cellular type being analyzed was not specified [17, 18]. In parallel, some of these studies have also reported the presence of PD-1 in tumor-infiltrating T lymphocytes (TILs) [12, 16]. The striking observed differences in PD-L1 expression rates among published studies might be explained by the following: (1) differences in immunohistochemical staining procedures and/or in the sensitivity of the anti-PD-L1 antibody clones employed [15]; (2) lack of consensus in the interpretation of the results; (3) the size of the tumor tissue sample used for evaluating PD-L1 expression [15]; (4) differences in ethnicity and in environmental risk factors [17]; and (5) the potential bias associated with small patient cohorts (online supplementary Table 1, see www.karger.com/doi/10.1159/000518104).

PD-1/PD-L1 signaling pathway is involved in T cell exhaustion [10], suggesting that its hyperactivation promotes evasion from immune surveillance and increases aggressiveness of cancer cells. However, the clinical significance of this axis in CCA is still unclear. In fact, enrichment of PD-1$^+$ TILs has been associated with worse overall survival (OS) and relapse-free survival (RFS) [17]. Likewise, most of the reviewed studies have correlated a high expression of PD-L1 in CCA tissue with worse clinical outcomes [11, 15, 17, 18]. Similar results have been obtained in a study where class I human leukocyte antigen (HLA-I) expression was also measured. In this case, only when HLA-I was conserved within the tumor, PD-L1 expression was associated with worse prognosis [12], revealing a functional relationship between HLA-I and PD-1/PD-L1 pathway in tumor immune surveillance and evasion. HLA expression on tumor cell membranes is required for antigen recognition by T cell receptors, and thus, when HLA is downregulated in the tumor, T cells do not recognize tumoral antigens successfully, even if T cell activity is restored. This is a point to keep in mind, since HLA-I has shown to be downregulated in around half of the samples [12, 16]. On the other hand, Yu et al. [16] reported that, in comparison with negative PD-L1 tumors, positive expression of PD-L1 in CCA cells was significantly associated with better OS and progression-free survival (PFS), as well as with the absence of vascular invasion in patients who had undergone curative-intended surgery. Of note, PD-L1 may be overexpressed in tumor cells in response to an inflammatory environment

and high T cell infiltration within the tumor, potentially reflecting an active immunological response against cancer cells [19].

Human Endogenous Retrovirus-H Long Terminal Repeat-Associating Protein 2

Human endogenous retrovirus-H long terminal repeat-associating protein 2 (HHLA2) is a recently described human immune checkpoint which belongs to the B7 family. It is constitutively expressed in monocytes, but its expression can also be induced in B cells upon stimulation and observed in some cancer cells (shown in Fig. 1) [20, 21]. Interestingly, while HHLA2 seems to exert co-stimulating effects when binding to CD28 family members, its interaction with other putative receptors expressed on $CD4^+$ and $CD8^+$ T lymphocytes, as well as on antigen-presenting cells (APCs), leads to a reduction of T-cell effector functions [20]. Recently, the killer cell immunoglobulin-like receptor, 3 immunoglobulin domains, and long cytoplasmic tail 3 has been identified as an inhibitory receptor for HHLA2 in both T and natural killer cells. Importantly, antibodies blocking this interaction have been proved to abolish the co-inhibitory role of HHLA2, while preserving its stimulating function [22].

A recent and interesting study found that 49% of patients with iCCA undergoing curative resection presented high tumor levels of HHLA2, while PD-L1 expression in tumoral cells was only detected in 28.1% of tumor samples [23]. In addition, 50% of samples with negligible PD-L1 expression were positive for HHLA2, suggesting that this immune checkpoint might be a promising target for patients who are not eligible for PD-L1 inhibition. Moreover, high HHLA2 expression was associated with a higher ratio of Treg/$CD8^+$ TILs and with shortened OS, indicating that the expression of this immune checkpoint may also serve as prognosis predictor in iCCA [23]. Nevertheless, more preclinical studies are needed in order to investigate the effects of HHLA2 blockade and its potential therapeutic efficacy.

Cytotoxic T Lymphocyte Antigen 4

Similar to PD-1, cytotoxic T lymphocyte antigen 4 (CTLA4) is a co-inhibitory receptor present on T cells' surface. It competes with CD28 for binding the ligands CD80 and CD86 expressed on APCs' membrane, but contrary to the effects triggered by CD28, their interaction with CTLA4 inhibits T-cell activation, making the blockade of this checkpoint attractive for oncological therapy [24]. Transcriptomic analysis of 770 immune-related genes performed in 22 bile duct cancer samples (i.e., CCA and gallbladder cancer [GBC]) reported an inverse correlation between *CTLA4* expression and RFS [25]. In addition, a recent publication described that ex vivo exposure of CCAs-isolated TILs with high expression of CTLA4 to anti-CTLA4 antibody promoted T cell maturation and activation [26]. On the other hand, Lim et al. [27] studied 77 patients diagnosed with eCCA, detecting prolonged OS and disease-free survival 5-year rates in those with high expression of CTLA4 in comparison with patients with low levels (36.8% vs. 0.0% and 30.9% vs. 0.0%, respectively), only when tumors with hilar location (n = 29) were considered. Furthermore, high expression of CTLA4 on tumor cells was associated with greater $CD8^+$ TILs density within the tumor [27].

Glucocorticoid-Induced Tumor Necrosis Factor Receptor-Related Protein

Glucocorticoid-induced tumor necrosis factor receptor-related protein (GITR) is a co-stimulatory immune checkpoint belonging to the tumor necrosis factor receptor family, whose expression is rapidly induced upon activation in $CD8^+$ and $CD4^+$ T cells and particularly in Tregs (shown in Fig. 1). Binding of ligand to GITR increases cytotoxic and helper T cell effector functions [28]. Paradoxically, this agonistic interaction ligand-GITR in Tregs abolishes their immunosuppressive function (shown in Fig. 1) [28]. A recent study found greater expression of GITR, PD-1, and CTLA4 on TILs from surgically resected CCA pieces in comparison with T cells from blood or tumor-free liver tissue [26]. Additionally, ex vivo targeting of any of the aforementioned immune checkpoints in CCA TILs increased the production of effector molecules and the proliferation of effector T cells, which is intimately related to the control of tumor growth and spread [26].

CD40 and CD40 Ligand

Some of the most recent evidence point toward other less-explored immune checkpoints. Particularly, CD40/CD40 ligand (CD40L) axis has been the focus of in vitro and in vivo studies published during 2020 [29, 30]. CD40L is a co-stimulatory molecule mainly expressed on the membrane of T helper lymphocytes (shown in Fig. 1) that, through interaction with CD40 on APCs triggers their maturation, cytokine production, and indirect activation of cytotoxic T lymphocytes [30]. In this regard, Sadeghlar et al. [30] transduced in vitro DCs from healthy donors with CD40L adenovirus and pulsed them with tumor cell lysates, increasing the expression not only of CD40L but also of maturation markers and co-stimulato-

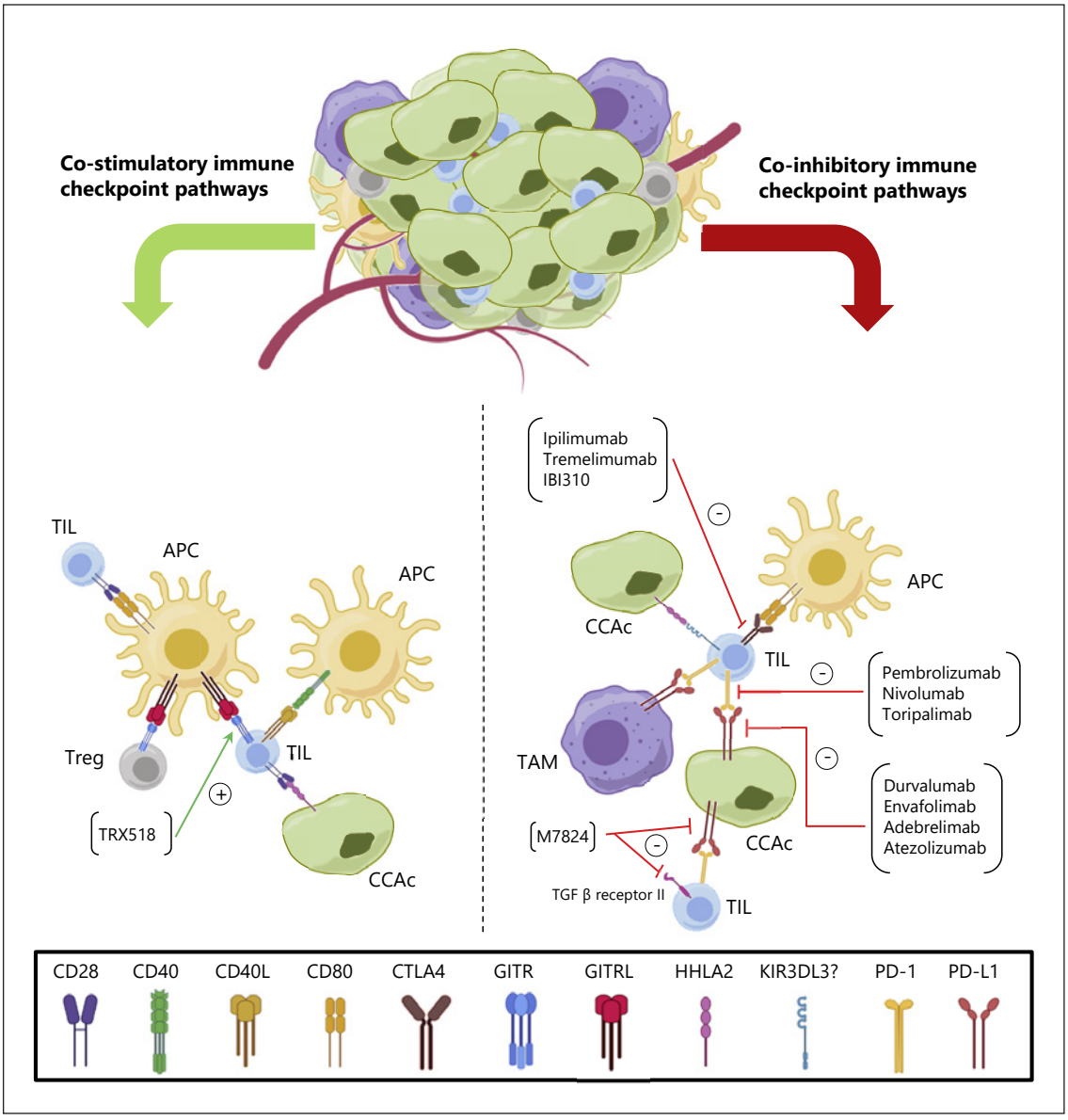

Fig. 1. Interactions and therapeutic targeting of immune checkpoints in CCA. Schematic representation showing the co-inhibitory (red) and co-stimulatory (green) immune checkpoint pathways studied in CCA and main cell interactions implicated in their signaling. In addition, drugs targeting these immune checkpoints, which have been studied in CCA, are indicated in the figure. APC, antigen-presenting cell; CCAc, cholangiocarcinoma cell; CD28, cluster of differentiation 28; CD40, cluster of differentiation 40; CD40L, CD40 ligand; CD80, cluster of differentiation 80; CTLA4, cytotoxic T lymphocyte antigen 4; HHLA2, human endogenous retrovirus-H long terminal repeat-associating protein 2; GITR, glucocorticoid-induced tumor necrosis factor receptor-related protein; GITRL, GITR ligand; KIR3DL3, killer cell immunoglobulin-like receptor, 3 immunoglobulin domains and long cytoplasmic tail 3; PD-1, programmed death 1; PD-L1, programmed death-ligand 1; TAM, tumor-associated macrophage; TGF β, transforming growth factor β; TIL, tumor-infiltrating T lymphocyte; Treg, regulatory T cell. Created with BioRender.com.

ry molecules on DCs, Th1 cytokine/chemokine production, and the proliferation and stimulation of cytotoxic cells against the eCCA cell line EGI-1.

These results were consistent with a study where different murine models of iCCA were treated with a CD40 agonistic antibody. Interestingly, the efficacy of a CD40 agonist was compared with anti-PD-1 antibody adminis-

tration, combination of both antibodies, and combination of CD40 agonist, anti-PD-1 antibody, and chemotherapy [29]. In this setting, anti-PD-1 and CD40 agonist monotherapies resulted in modest beneficial effects, while the combination of both molecules markedly reduced tumor burden [29]. Similarly, combination of anti-PD-1 and CD40 agonist with chemotherapy improved mice survival when compared with GemCis alone [29]. Importantly, such benefits were demonstrated to be dependent on macrophages, DCs, $CD4^+$, and $CD8^+$ lymphocytes [29].

Immune Checkpoint-Targeted Therapies for CCA

Administration of immune checkpoint inhibitors (ICIs) might stimulate TILs and help to eliminate cancer cells, thus controlling tumor growth and recurrence of many neoplasms, such as CCA [31]. Considering the clinical applicability of ICIs as a potential treatment for this tumor, several clinical trials have been completed and, importantly, much more are currently ongoing, albeit with a limited number of patients and only preliminary results available. Noteworthy, most studied cohorts only included patients with advanced CCA previously treated with different therapies and further studies are warranted.

ICI Monotherapies

PD-1 Inhibitors

Several human mAbs have been designed to block PD-1 and hamper its binding to specific ligands (shown in Fig. 1). In this regard, the efficacy of the anti-PD-1 pembrolizumab has been evaluated in small, non-randomized studies for different types of tumors, including biliary tract cancers (BTCs) [32]. The phase II KEYNOTE-158 (NCT02628067) and phase Ib KEYNOTE-028 (NCT02054806) clinical trials were developed to evaluate the antitumor activity and safety of pembrolizumab in patients with advanced BTCs (i.e., CCA and GBC), including 104 and 24 patients, respectively [32]. The main difference between both clinical trials was the proportion of patients with PD-L1 expression. Therefore, all the patients enrolled in the KEYNOTE-028 study were positive for this immune checkpoint, while tumor expression of PD-L1 was only detected in 58.6% of the subjects included in the KEYNOTE-158 trial [32]. This had minimal impact on some clinical outcomes, observing an objective response rate (ORR) of 13% and 5.8% with a corresponding estimated duration of response of ≥18 months and ≥6 months, a median OS (mOS) of 5.7 months and 7.4 months, and a median PFS (mPFS) of 1.8 months and 2.0 months in the patients enrolled in the KEYNOTE-028 and KEYNOTE-158 trials, respectively (shown in Table 1) [32]. Therefore, these data suggest that pembrolizumab exerts durable antitumor activity regardless of PD-L1 expression [32].

Contrarily, the therapeutic response to the anti-PD-1 nivolumab was conditioned by PD-L1 expression in an American phase II (NCT02829918) and a Japanese phase I (JapicCTI-153098) clinical trials. Both study cohorts were constituted by patients with CCA and GBC that were refractory or intolerant to previous systemic therapy (shown in Table 1) [33, 34]. Despite differences in ethnicity and environmental risk factors, treatment with nivolumab exerted an antitumor effect with durable response for at least 1 year, with minor adverse events (AEs) [33, 34]. Currently, the safety and efficacy of toripalimab are being tested in an ongoing phase Ib/II clinical trial (NCT03867370) for resectable iCCA with no preliminary results available yet (shown in Table 1).

PD-L1 Inhibitors

In the case of PD-L1 blocking agents, the efforts have been mainly focused on the development of immunoglobulin (Ig) G1 mAbs. This IgG subclass, unlike IgG4, binds fragment crystallizable (Fc) receptors with high affinity, triggering antibody-dependent cellular cytotoxicity (ADCC). This feature is a double-edged sword though, as it may enable lysis of PD-L1-expressing tumor cells, but also the death of T cells expressing this immune checkpoint. For this reason, different approaches have been assumed in the development of anti-PD-L1 IgG1 antibodies.

In this way, durvalumab is a selective, high-affinity PD-L1 inhibitor (shown in Fig. 1) with an inactivated Fc [35]. This feature restrains the activation of both ADCC and complement, while overcoming PD-L1-mediated exhaustion of TILs [35]. Durvalumab monotherapy resulted in a mOS of 8.1 months and 16.7% of disease control rate (DCR) in an Asian BTC cohort (NCT01938612), although 19% of patients developed significant AEs (shown in Table 1) [36]. According to preclinical data, an ongoing phase I clinical trial (NCT03101488) on Chinese patients with advanced solid tumors aims to analyze the impact of envafolimab, a novel camelid-derived chimeric anti-PD-L1 nanobody with human IgG1 Fc (shown in Table 1) [37]. Interestingly, partial response (PR) has been observed in the 2 patients with CCA included in this cohort, at a dosage of both 5 and 10 mg/kg [37].

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Table 1. Clinical trials with ICIs in monotherapy and in combination (finished and ongoing)

Identifier	Design	ICIs	mAb type	Patients enrolled	Drug administration		Main outcomes	AEs	Status	Refs
					dose	period				
Anti-PD-1										
NCT02628067 (KN158)	Phase II non-randomized	Pembrolizumab	IgG4	Advanced BTCs (CCA among them) after any number of prior standard treatment regimens (n = 104)	200 mg/kg	Every 3 weeks for 2 years	ORR: 5.8% (2.1–12.1)[b] mOS: 7.4 mo (5.5–9.6)[b] mPFS: 2.0 mo (1.9–2.1)[b]	Fatigue, rash, pruritus, immune-mediated AEs, infusion reaction	Finished	[32]
NCT02054806 (KN028)	Phase Ib single group assignment	Pembrolizumab	IgG4	Advanced BTCs (CCA among them) after any number of prior standard treatment regimens and positive PD-L1 expression (n = 24)	10 mg/kg	2 weeks for up to 24 mo	ORR: 13% (2.8–33.6)[b] mOS: 5.7 mo (3.1–9.8)[b] mPFS: 1.8 mo (1.4–3.7)[b]	Immune-mediated AEs, infusion reaction	Finished	[32]
JapicC-TI-153098	Phase I multicenter	Nivolumab	IgG4	Unresectable or recurrent BTCs (i.e., iCCA, eCCA, GBC, AMPAC) after gemcitabine treatment failure (n = 30)	240 mg/kg	Every 2 weeks (10 days between doses during 5.1 mo)	ORR: 3.3% (0.7–13.6)[a] mOS: 5.2 mo (4.5–8.7)[a] mPFS: 1.4 mo (1.4–1.4)[a]	Decreased appetite, malaise, pruritus, rash, maculopapular rash, amylase increase, depressed level of consciousness, pleurisy	Finished	[33]
NCT02829918	Phase II multicenter	Nivolumab	IgG4	Histologically confirmed BTCs (CCA among them) with disease progression previously treated with systemic treatment (n = 54)	240 mg/kg 480 mg/kg	Every 2 weeks (during 16 weeks) Every 4 weeks (during 17 weeks)	PR: 22% DCR: 59% mOS: 14.2 mo (5.98-not reached)[b] mPFS: 3.7 mo (2.30–5.69)[b]	Hyponatremia, increased alkaline phosphatase	Finished	[34]
NCT03867370	Phase Ib/II single center	Toripalimab	IgG4	Resectable HCC or iCCA (n = 20)	480 mg; then 240 mg after surgery	Once every 3 weeks	NA	NA	Ongoing	NA
Anti-PD-L1										
NCT01938612	Phase I multicenter non-randomized	Durvalumab	IgG1 inactivated Fc domain	Asian cohort with advanced solid tumors (n = 269; BTCs = 42)	10 mg/kg	2 weeks	DCR: 16.7% mOS: 8.1 mo (5.6–10.1)[b]	Severe or medically significant, but not immediately life threatening (i.e., grade 3 AEs)	Finished	[36]
NCT03101488	Phase I	Envafolimab	IgG1 single domain (nanobody)	Chinese cohort with advanced solid tumors (n = 60; CCA = 2)	0.1–10 mg/kg	Weekly	NA	NA	Ongoing	[37]
NCT03201458 (arm A)	Phase II randomized	Atezolizumab	IgG1	Unresectable BTCs (n = 37; iCCA = 21, eCCA = 7, and GBC = 11)	840 mg	Every 2 weeks	mPFS: 1.9 mo PR: 2.9% SD: 29.4%	Grade 3–4 AEs	Ongoing	[38]

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Table 1 (continued)

Identifier	Design	ICIs	mAb type	Patients enrolled	Drug administration		Main outcomes	AEs	Status	Refs
					dose	period				
Anti-PD-1 + anti-CTLA4										
NCT02923934 (CA209-538)	Phase II multicenter non-randomized	Nivolumab + ipilimumab	IgG4 IgG1	Advanced BTCs (n = 39; CCA = 26)	3 mg/kg 1 mg/kg	Every 2 weeks for 4 doses	ORR: 23% DCR: 44% mOS: 5.7 mo (2.7–11.9)[b] mPFS: 2.9 mo (2.2–4.6)[b]	Immune-related toxic events, infusion reactions	Finished	[40]
NCT02834013	Phase II nonrandomized	Nivolumab + ipilimumab	IgG4 IgG1	Rare tumors previously treated (n = 818); iCCA and eCCA among them	30 min 60 min	Every 42 days for up to 2 years	NA	NA	Ongoing	NA
		Nivolumab	IgG4		30 min					
Anti-PD-L1 + anti-CTLA4										
NCT01938612	Phase I multicenter non-randomized	Durvalumab + tremelimumab	IgG1 inactivated Fc domain IgG2	Asian cohort with advanced solid tumors (n = 269; BTCs = 65)	20 mg/kg 1 mg/kg	4 weeks	DCR: 32.2% mOS: 10.1 mo (6.2–11.4)[b]	Severe or medically significant but not immediately life threatening (i.e., grade 3 AEs)	Finished	[36]
NCT04634058	Phase II single group assignment	Adebrelimab + IBI310	IgG4 IgG1	Advanced iCCA that progressed after chemotherapy (n = 40)	4 cycles	NA	NA	NA	Ongoing	NA

AEs, adverse events; AMPAC, ampullary cancer; BTCs, biliary tract cancers; CCA, cholangiocarcinoma; CTLA4; cytotoxic T lymphocyte antigen 4; DCR, disease control rate; eCCA, extrahepatic CCA; Fc, fragment crystallizable; GBC, gallbladder cancer; HCC, hepatocellular carcinoma; iCCA, intrahepatic CCA; ICIs, immune checkpoint inhibitors; Ig, immunoglobulin; KN028, keynote-028; KN158, keynote-158; mAb, monoclonal antibody; min, minutes; mo, months; mOS, median overall survival; mPFS, median progression-free survival; n, number of patients; NA, not available; ORR, objective response rate; PD-1, programmed death 1; PD-L1, programmed death-ligand 1; PR, partial response; Refs, references; SD, stable disease. [a] 90% confidence interval. [b] 95% confidence interval.

Table 2. Clinical trials with ICIs in combination with other therapies (finished and ongoing)

Identifier	Design	Treatment	Patients enrolled	Drug administration		Main outcomes	AEs	Status	Refs
				dose	period				
Ablative therapies + ICIs									
NCT01853618 (arm E)	Phase I/II non-randomized	Tremelimumab + MWA	Advanced BTCs that progressed after chemotherapy (n = 20; CCA = 18)	3.5 mg/kg or 10 mg/kg	Monthly (6 doses); then every 3 months for 2 years On day 36	ORR: 12.5% mOS: 6.0 mo (3.8–8.8)[b] mPFS: 3.4 mo (2.5–5.2)[b] SD: 37.5%	Diarrhea, edema limbs, nausea, vomiting, flank pain, rash maculopapular	Finished	[43]
NCT02821754	Phase II non-randomized	Tremelimumab + durvalumab + RFA or CA	Histologically or cytologically confirmed diagnosis of HCC (n = 40) or CCA (n = 30)	75 mg 1,500 mg	Every week up to 4 doses On day 36	NA	NA	Ongoing	NA
Chemotherapy + ICIs									
NCT03311789	Phase II single center	Gemcitabine/cisplatin + nivolumab	Histologically confirmed unresectable or metastatic BTCs (n = 32; iCCA = 11, pCCA = 6, dCCA = 9, and GBC = 6)	1,000 mg/m^2 75 mg/m^2 3 mg/kg	On days 1 and 5 (every 3 weeks up to 6 cycles) On day 1 (every 3 weeks up to 6 cycles) On day 3 (every 3 weeks up to 6 cycles)	ORR: 55.6% DCR: 92.6% mOS: 8.5 mo (5.0–12.5)[b] mPFS: 6.1 mo (3.4–8.2)[b] SD: 37%	Grade 3 or higher AEs were thrombocytopenia and neutropenia	Finished	[45]
JapicCTI-153098	Phase I multicenter	Gemcitabine/cisplatin + nivolumab	Chemotherapy-naive Japanese cohort with unresectable or recurrent BTCs (i.e., iCCA, eCCA, GBC, AMPAC) (n = 30)	1,000 mg/m^2 25 mg/m^2 240 mg	On days 1 and 8 of a 3-week cycle Every 2 weeks	ORR: 36.7% mOS: 15.4 mo (11.8-not estimable)[a] mPFS: 4.2 mo (2.8–5.6)[a]	Platelet count decrease, febrile neutropenia anemia, anaphylactic reaction, decreased appetite, pyrexia, and myocarditis	Finished	[33]
NCT03796429	Phase II single center	Toripalimab + gemcitabine/S1	Histologically confirmed advanced BTCs (n = 39; iCCA = 16, eCCA = 5, and GBC = 18)	240 mg/kg 1,000 mg/m^2 40–60 mg	Once every 3 weeks On days 1 and 8 of each 21-day cycle	ORR: 20.6% DCR: 85.3% mPFS: 6.7 mo	Leukopenia, anemia, rash, infection, thrombocytopenia	Ongoing	NA
NCT03101566	Phase II multicenter randomized	Gemcitabine/cisplatin + nivolumab	Advanced unresectable BTCs; iCCA and eCCA among them (n = 75)	1,000 mg/m^2 25 mg/m^2 360 mg	On days 1 and 8 every 3 weeks On day 1 every 3 weeks	NA	NA	Ongoing	NA
NCT03875235	Phase III multi-regional randomized	Gemcitabine/cisplatin + durvalumab or placebo	First-line advanced BTCs; iCCA and eCCA among them (n = 757)	NA	Every 3 weeks with chemotherapy up to 8 cycles followed by ICI/placebo every 4 weeks until DP	NA	NA	Ongoing	NA
NCT03478488	Phase II multicenter randomized	Envafolimab + gemcitabine/oxaliplatin	Previously untreated locally advanced or metastatic BTCs (including CCA) (n = 390)	2.5 mg/kg 1,000 mg/m^2 85 mg/m^2	Weekly of each 21-day cycle On days 1 and 8 of each 21-day cycle	NA	NA	Ongoing	NA

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Table 2 (continued)

Identifier	Design	Treatment	Patients enrolled	Drug administration		Main outcomes	AEs	Status	Refs
				dose	period				
NCT03260712	Phase II multicenter	Pembrolizumab + gemcitabine/cisplatin	Histologically or cytologically confirmed diagnosis of unresectable or recurrent BTCs; iCCA and eCCA among them (n = 50)	200 mg/kg 1,000 mg/m^2 25 mg/m^2	On day 1 of a 21-day cycle On days 1 and 8 of a 21-day cycle	NA	NA	Ongoing	NA
NCT03111732	Phase II open label	Pembrolizumab + capecitabine/ oxaliplatin	Histologically confirmed advanced BTCs; iCCA and eCCA among them (n = 11)	200 mg 750 mg/m^2 130 mg/m^2	On day 1 of each 21-day cycle Twice a day on days 1–14 of cycles 1–6 On day 1 of cycles 1–6	NA	NA	Ongoing	NA
NCT03257761	Phase Ib open label	Guadecitabine + durvalumab	Multiples advance digestive cancers, including histologically or cytologically documented iCCA, eCCA, and GBC (n = 90)	NA	On days 1–5 (every 28 days until DP or toxicity) On day 8 (every 28 days until DP or toxicity)	NA	NA	Ongoing	NA
NCT03704480	Phase II open label randomized	Durvalumab + tremelimumab± paclitaxel	Histologically or cytologically proven advanced BTCs; iCCA and eCCA among them	1,500 mg 300 mg or 75 mg 80 mg/m^2	On day 1 of each 4-week cycle until DP or toxicity On day 1 at cycle 1 only or for 4 cycles Weekly during 3 weeks	NA	NA	Ongoing	NA
Other therapies + ICIs									
NCT03201458 (arm B)	Phase II multicenter	Atezolizumab + cobimetinib	Metastatic or unresectable BTCs (n = 38; iCCA = 22, eCCA = 8, and GBC = 8)	840 mg 60 mg	On days 1 and 15 of each 28-day cycle until DP or toxicity Daily (21 days on/7 days off)	mPFS: 3.65 mo PR: 3.2% SD: 41.9%	Grade 3 AEs were similar in both arms	Finished	[38]
NCT04550624	Phase II multicenter	Lenvatinib + pembrolizumab	Histologically confirmed advanced CCA (n = 40)	20 mg 200 mg	Daily during each 21-day cycle On day 1 of every 21-day cycle	NA	NA	Ongoing	NA
NCT04677504	Phase II randomized multicenter	Atezolizumab + bevacizumab + gemcitabine/cisplatin	Previously untreated recurrent/metastatic or locally advanced unresectable BTCs; iCCA and eCCA among them	1,200 mg 15 mg/kg 1,000 mg/m^2 25 mg/m^2	On day 1 of each 21-day cycle On day 1 of each 21-day cycle On days 1 and 8 of each 21-day cycle for cycles 1–8	NA	NA	Ongoing	NA
NCT02699515	Phase I open label multiple-ascending dose multicenter	M7824	Expansion cohort of pre-treated Asian BTC patients (n = 30)	1,200 mg	Once every 2 weeks for up to 12 mo	ORR: 20% mPFS: 2.5 mo mOS: 12.7 mo	11 patients had grade ≥3 treatment-related AEs	Ongoing	[49]

Table 2 (continued)

Identifier	Design	Treatment	Patients enrolled	Drug administration		Main outcomes	AEs	Status	Refs
				dose	period				
NCT04066491	Phase II/III randomized multicenter	M7824 + gemcitabine/ cisplatin	Chemotherapy and immunotherapy-naïve participants with locally advanced/metastatic BTCs (i.e., CCA and GBC)	2,400 mg 1,000 mg/m^2 25 mg/m^2	Once every 3 weeks On days 1 and 8 of 21-day cycle, for 8 cycles	NA	NA	Ongoing	NA
NCT03833661	Phase II open label multicenter	M7824	Locally advanced/metastatic BTCs (i.e., CCA and GBC) previously treated or intolerant to first-line platinum-based chemotherapy (n = 159)	1,200 mg	Once every 2 weeks until DP, toxicity, or death	NA	NA	Ongoing	NA
NCT03250273 (arm A)	Phase II non-randomized	Entinostat + nivolumab	Previously treated unresectable or metastatic CCA and pancreatic adenocarcinoma (n = 44)	5 mg 240 mg	Once a week Every 2 weeks	NA	NA	Ongoing	NA
NCT03785873	Phase Ib/II multicenter	Nivolumab + nal-irinotecan + 5-fluorouracil + leucovorin	Advanced BTCs (i.e., iCCA, eCCA, GBC) previously treated with one systemic therapy (n = 40)	NA	NA	NA	NA	Ongoing	NA
NA	Observational single center	Lenvatinib + pembrolizumab or nivolumab	Advanced iCCA previously treated with ≥2 anticancer therapy (n = 14)	NA	NA	ORR: 21.4% DCR: 92.9% CBR: 64.3% mPFS: 5.9 mo (4.2–6.2)[b]	Hypertension, aminotransferase elevation, and fatigue. Grade 3 AEs occurred at 14%	NA	[47]

AEs, adverse events; AMPAC, ampullary cancer; BTCs, biliary tract cancers; CA, cryoablation; CBR, clinical benefit rate; CCA, cholangiocarcinoma; CR, complete response; DCR, disease control rate; dCCA, distal CCA; DP; disease progression; eCCA, extrahepatic CCA; GBC, gallbladder cancer; HCC, hepatocellular carcinoma; iCCA, intrahepatic CCA; ICIs, immune checkpoint inhibitors; mDOR, median duration of response; mo, months; mOS, median overall survival; mPFS, median progression-free survival; MWA, microwave ablation; n, number of patients; NA, not available; ORR, objective response rate; pCCA, perihilar CCA; PR, partial response; Refs, references; RFA, radiofrequency ablation; S1, tegafur/gimeracil/oteracil; SD, stable disease. [a] 90% confidence interval. [b] 95% confidence interval.

In addition to the ICIs mentioned above, there are other PD-L1 blocking IgG1 mAbs including atezolizumab, avelumab, CK-301, CBT-502, and BGB-A33, as well as IgG4 as BMS-936559, CS-1001, and adebrelimab. However, CBT-502 (NCT03825705), atezolizumab (NCT03201458) [38], and adebrelimab (NCT04634058) are the only ones in which safety and efficacy are being tested in patients with CCA so far (shown in Tables 1, 2).

GITR Agonists

The majority of clinical trials evaluating ICIs are focused on agents directed against TILs' inhibitory immune checkpoints, but there are also few studies trying to activate these lymphocytes through stimulatory molecules such as GITR [26]. Based on preclinical evidence, a recent phase I clinical trial (NCT01239134) was the first to determine the safety, pharmacokinetics, pharmacodynamics, and the maximum tolerated dose of TRX518, an agonistic mAb against GITR (shown in Fig. 1) which has a dysfunctional Fc abolishing ADCC and complement-mediated lysis [39]. Currently, TRX518 monotherapy is being tested as second-line treatment in 43 patients with different advanced solid tumors including pancreatic cancer, which shares many features with eCCA subtypes [39]. Of note, the results obtained so far corroborate previous findings on the effect of GITR agonistic stimulation over effector lymphocytes and Tregs, but no clinical response has been achieved [39]. For this reason, and based on preclinical evidence, the combination of GITR and ICIs will be evaluated in upcoming clinical trials [39].

ICI Combined Therapies

PD-1+CTLA4 Inhibitors

A phase II clinical trial (NCT02923934), in which nivolumab and the CTLA4 targeting inhibitor ipilimumab were administered in combination, could not outperform the efficacy of anti-PD-1 monotherapy in a cohort of patients with CCA and GBC. Thus, the ORR and DCR of the entire cohort were 23% and 44%, respectively, being further reduced to 19.2% and 30.8% when only patients with CCA were considered [40]. Moreover, a mOS of 5.7 months and a mPFS of 2.9 months were reported (shown in Table 1), indicating that the impact of the dual therapy was similar to that obtained in clinical trials evaluating the efficacy of anti-PD-1 monotherapy [40]. Nevertheless, an ongoing phase II study (NCT02834013) compares this combination (i.e., nivolumab + ipilimumab) with nivolumab as a monotherapy for CCA (shown in Table 1), among other rare tumors, which will allow to determine whether these similarities between anti-PD-1 monotherapy and anti-PD-1/anti-CTLA4 dual therapy are real.

PD-L1 + CTLA4 Inhibitors

The NCT01938612 clinical trial also evaluated the impact of durvalumab combined with tremelimumab (i.e., anti-CTLA4 mAb) in the same cohort of Asian patients. Interestingly, mOS and the DCR slightly increased (i.e., 10.1 months in dual therapy vs. 8.1 months in monotherapy and 32.2% in dual therapy vs. 16.7% in monotherapy, respectively) (shown in Table 1), although the number of patients with any grade AEs increased, with 23% of patients developing severe side effects. In addition, the average duration of the response diminished 1.2 months [36].

Finally, other clinical trials combining novel anti-PD-L1 and anti-CTLA4 mAbs are now under investigation. In this regard, the NCT04634058 study aims to evaluate the efficacy and safety of adebrelimab combined with IBI310, a PD-L1, and a CTLA4 inhibitor, respectively, in patients with advanced iCCA who have progressed after systemic treatment (shown in Table 1).

Local and Systemic Therapies Combined with ICIs for CCA Treatment

Combination of Radiotherapy and ICIs

To date, the use of radiotherapy in CCA remains controversial [5]. Lately, its combination with pembrolizumab or nivolumab (i.e., anti-PD-1 inhibitors) has been analyzed in 4 patients with advanced iCCA [41, 42]. Even though none of them were ideal candidates for receiving this immunotherapy according to the national comprehensive cancer network guidelines, the combination of both therapeutic strategies (i.e., radiotherapy + ICI) either achieved PR with reduction of the sum of lesion diameters or maintained complete response (CR) for 11 and 26 months [41, 42]. This unexpected beneficial antitumor effect of immunotherapy combined with radiotherapy in iCCA could be the result of the radiotherapy's ability to sensitize the tumor to ICIs by improving the presentation of tumor-associated antigens and increasing PD-L1 expression in tumor cells [41]. However, more studies should be conducted to confirm this statement, since the only available data are derived from case reports.

Combination of Ablative Therapy and ICIs

Tumor ablation techniques may also enhance the effects of ICIs and, consequently, the antitumor immune response [43]. Thereby, one of the arms (i.e., arm E) of a

phase I/II clinical trial (NCT01853618) combined microwave ablation plus tremelimumab (i.e., anti-CTLA4 mAb) for the treatment of 20 patients with advanced BTCs (i.e., iCCA [*n* = 12], eCCA [*n* = 6], and GBC [*n* = 2]) (shown in Table 2) [43]. A total of 16 patients had lesions that were evaluable for response, but only 2 responding patients (i.e., 12.5%; all eCCA) were identified [43]. Moreover, 37.5% of the patients (6/16) achieved stable disease (SD) lasting up to 6.2 months, while the remaining 50% (8/16) underwent disease progression [43]. Overall, treatment was well tolerated by the majority of patients as <10% of them experienced severe AEs, albeit some low-grade treatment-related AE appeared in all study patients [43]. Interestingly, the authors also reported a significant increase in $CD8^+$ T cell activation mediated by tremelimumab [43]. However, this was the arm of the clinical trial with the lowest mOS and mPFS (i.e., 6 months and 3.4 months, respectively) in comparison with the other arms, where tremelimumab was combined with radiofrequency ablation (RFA) and other ablative therapies. This could be a reason why a pilot study (NCT02821754) is currently ongoing to evaluate the efficacy of RFA or cryoablation combined with tremelimumab and durvalumab (i.e., anti-PD-L1 mAb) in a similar, but larger, cohort of patients with BTC (shown in Table 2).

Combination of Chemotherapy and ICIs

To date, the clinical response achieved by exclusively chemotherapy-based treatment for CCA is unsatisfactory. Nevertheless, treatment with gemcitabine and other chemotherapeutic agents revealed to increase PD-L1 expression, consequently maximizing PD-1/PD-L1 axis signals [44]. Therefore, this supports the idea of combining chemotherapy with ICIs to increase treatment effectiveness. In this regard, in a phase II clinical trial (NCT03311789) the combination of GemCis plus nivolumab (i.e., anti-PD-1 mAb) was evaluated in 32 patients with unresectable or metastatic BTCs (i.e., iCCA [*n* = 11], pCCA [*n* = 6], dCCA [*n* = 9], and GBC [*n* = 6]) (shown in Table 2) [45]. Unfortunately, 5 patients were excluded from the study due to a rapid deterioration as a consequence of tumor-related complications (*n* = 4) and AEs unrelated to study drugs (*n* = 1) [45]. Thus, response-evaluable patients achieved an ORR of 55.6% (5 patients with CR and 10 patients with PR) with a mOS and a mPFS of 8.5 months and 6.1 months, respectively [45]. Moreover, 6 patients previously defined as resistant to GemCis-based therapy were included in the trial, in order to evaluate whether the combination of ICIs and chemotherapy could re-sensitize BTCs [45]. Surprisingly, one of these patients achieved CR, while another presented PR [45]. Similarly, a Japanese study (JapicCTI-153098) employed the same therapeutic approach (i.e., GemCis + nivolumab) in patients with non-resectable CCA [33]. Compared to what was observed when nivolumab was administered as monotherapy (shown in Table 1), patients treated with the dual therapy experienced a marked increase of mOS, mPFS, and response rate (i.e., 15.4 months, 4.2 months, and 36.7%, respectively) (shown in Table 2) [33]. However, the proportion of patients with treatment-related serious AEs also increased [33]. Noteworthy, the NCT03101566 is an ongoing clinical trial bearing important similarities with the aforementioned study, but in a Western population (shown in Table 2).

On the other hand, a phase II (NCT03046862) study including CCA and GBC patients detected no significant differences between GemCis + durvalumab and GemCis + durvalumab + tremelimumab schemes in terms of mOS (i.e., 18.1 months vs. 20.7 months, respectively), mPFS (i.e., 11.0 vs. 11.9 months, respectively), and ORR (i.e., 73.4% vs. 73.3%, respectively) [46]. Nevertheless, these therapeutic regimens improved the mOS and the ORR obtained when both ICIs were combined with the standard of care chemotherapy and administered after 1 cycle of GemCis (i.e., 15.0 months and 50.0%, respectively) [46]. According to these promising results, the combination of GemCis + durvalumab versus GemCis + placebo is being investigated in a phase III clinical trial (NCT03875235) (shown in Table 2). Currently, there are other clinical trials evaluating potential therapeutic combinations of ICIs with different chemotherapeutic agents, as both first- and second-line treatments, but without any reported findings so far. Some details about these studies are described in Table 2.

Combination of Other Therapies and ICIs

The therapeutic value of dual treatment based on ICIs and targeted therapies or epigenetic modulators is also being analyzed for CCA therapy. In this line, an observational study combining lenvatinib (i.e., a vascular endothelial growth factor 1–3 and fibroblast growth factor receptor 1–4 inhibitor) with pembrolizumab or nivolumab (i.e., anti-PD-1 mAbs) has shown promising results in patients with iCCA, in whom 2 or more anticancer therapies had previously failed [47]. Thus, 3 out of the 14 patients enrolled in the study achieved PR with a mPFS of 5.9 months after treatment [47]. Importantly, the DCR and the clinical benefit rate (i.e., ORR + SD ≥5 months) were 92.9% and 64.3%, respectively [47]. According to these

data, an Asian phase II clinical trial (NCT04550624) has been recently launched using the aforementioned angiogenic inhibitor plus pembrolizumab in a cohort with similar characteristics (shown in Table 2). Interestingly, another vascular endothelial growth factor inhibitor, called bevacizumab, has been combined with atezolizumab (i.e., anti-PD-L1 mAb), obtaining promising results for the treatment of patients with unresectable HCC [48] and promoting the development of an ongoing phase II clinical trial (NCT04677504) to evaluate the safety and efficacy of bevacizumab in combination with atezolizumab and GemCis in previously untreated BTC patients (shown in Table 2). Recently, a fusion protein containing the extracellular domain of the human transforming growth factor β receptor II and an IgG1 anti-PD-L1 antibody, called M7824, has been developed and tested in a phase I trial for metastatic or locally advanced solid tumors (NCT02699515). An expansion cohort of this trial including 30 Asian BTC patients showed an ORR, mPFS, and mOS of 20%, 2.5 months, and 12.7 months, respectively [49]. Importantly, treatment response was unrelated to PD-L1 expression, as well as durable with 83% ongoing responses at data cutoff (12.5 + to 14.5 + months) [49]. Based on these data, a a phase II/III (NCT04066491) and a a phase II (NCT03833661) clinical trials are under development for locally advanced or metastatic BTC patients, in order to evaluate M7824 as first-line treatment in combination with GemCis and as second-line after chemotherapy failure, respectively (shown in Table 2).

Similarly, ICIs have been tested in combination with epigenetic modulating drugs such as entinostat, a histone deacetylase inhibitor, which has been described to promote both the expression of major histocompatibility complex class II and the function of Tregs [31]. Therefore, an ongoing phase II clinical trial (NCT03250273) is evaluating the combination of nivolumab with entinostat for the treatment of patients with unresectable or metastatic CCA and pancreatic adenocarcinoma (shown in Table 2).

Conclusion and Future Directions

Certainly, effective therapeutic approaches for CCA still remain a challenge and, therefore, development of novel and promising alternatives is urgently needed. In this regard, immunotherapy and, more specifically, immune checkpoint modulation, has emerged in recent years as a potential strategy in oncology. Particularly in CCA, overexpression of immune checkpoint molecules has generally been associated with worse clinical outcomes, emerging as potential prognosis predictors. Hence, regulation of immune checkpoints-mediated signaling pathways opens avenues to control the progression of CCA and maybe even to eliminate it. Thus, therapies combining different ICIs with the standard of care chemotherapy have shown a relevant therapeutic value as first-line therapy. Importantly, this treatment regimen achieved better response than ICI monotherapy as second line, which unfortunately has not provided the expected results. Moreover, ICIs in combination seem to exert similar or slightly higher therapeutic effects than ICI monotherapy, although this should be confirmed in the future studies. It should be kept in mind that the majority of clinical trials developed so far have been focused on evaluating the safety, tolerability, and efficacy of these compounds. For this reason, further research is still needed to determine their advantages over standard treatments, as well as to expand the knowledge about other less studied ICIs and to investigate new combinations that could provide greater benefits to these patients. In this regard, and as previously mentioned, ICIs have been combined with loco-regional therapies or targeted therapies, also exhibiting synergic effects. Hence, studies comparing the efficacy of this approach with GemCis + ICI treatment could be interesting.

Since the response of patients to immune checkpoint modulation is variable and the prognostic and predictive value of PD-L1 expression remains controversial, upcoming studies should be focused on the identification of more accurate biomarkers. Unfortunately, little evidence is available so far about the role of other immune checkpoints in determining the clinical outcome of CCA. This unmet need requires future research to elucidate the specific relationship between the expression of immune checkpoints in CCA (either alone or in combination) and both prognosis and response to ICIs, as recently suggested for other solid tumors in a systematic study [50]. Undoubtedly, this will help to identify patients who could benefit from immunotherapy-based regimens, leading toward a more personalized medicine. Finally, guidelines indicating the recommended ICIs-based therapy for CCAs with different immune checkpoint expression signatures should be developed.

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