Immunotherapy in Advanced Biliary Tract Cancers

Subjects: Oncology

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Biliary tract cancers (BTC) are a heterogeneous group of rare but highly lethal carcinomas that originate from the gallbladder, intrahepatic bile ducts, and extrahepatic (perihilar and distal) bile ducts. Cholangiocarcinoma (CCA), or cancer arising from the bile ducts, accounts for 3% of all gastrointestinal malignancies and is the second most common primary liver cancer after hepatocellular carcinoma (HCC). Incidence of CCA varies greatly by geographic region, with the highest rates observed in Southeast Asia, including Thailand, where cases are as high as 113 per 100,000 in men and 50 per 100,000 women.

Keywords: biliary tract cancer ; cholangiocarcinoma ; gallbladder cancer ; immunotherapy

1. Current Treatment Paradigm of Advanced Biliary Tract Cancers (BTC)

1.1. First-Line Systemic Therapy

GEMCIS is the current standard of care first-line treatment for advanced BTC. In the phase III ABC-02 trial, 410 patients with locally advanced or metastatic BTC were randomized to GEMCIS or gemcitabine alone. Doublet chemotherapy had superior survival outcomes compared to monotherapy, with a median OS of 11.7 months versus 8.1 months (hazard ratio [HR] 0.64, $p < 0.001$), median progression-free survival (PFS) of 8 months versus 5 months ($p < 0.001$), and a disease control rate (DCR) of 81.4% versus 71.8% ($p = 0.049$). Toxicity profiles were similar between both groups except for neutropenia, which was more frequent in the combination treatment arm, although not associated with higher rates of neutropenic fever or infection [1]. The ABC-02 trial findings were supported by the Japanese randomized phase II BT22 study that also demonstrated an improved median OS of 11.2 months with GEMCIS compared to 7.7 months with gemcitabine monotherapy [2].

Given the modest survival benefit with GEMCIS, many trials have attempted to develop new first-line treatment strategies. The phase III FUGA-BT trial showed noninferior survival outcomes with gemcitabine plus oral fluoropyrimidine S-1 (tegafur, gimeracil, oteracil) compared to GEMCIS in 354 Japanese patients with chemotherapy-naïve recurrent or unresectable BTC (median OS 15.1 months with S-1 vs. 13.4 months with cisplatin, HR 0.95). Both treatments were generally well tolerated, but adverse events, including cytopenias and peripheral neuropathy, were more common with cisplatin, whereas oral mucositis and diarrhea were more frequent with S-1 [3]. Although S-1 is available in many Asian countries, its application in Western countries, including the United States, is not as widespread due to the increased gastrointestinal toxicity observed in Caucasian compared to East Asian patients [4]. Gemcitabine plus oxaliplatin (GEMOX) could be another reasonable alternative for first-line therapy in cisplatin-ineligible patients based on multiple phase II studies [5][6][7]. In one phase III trial, median OS with GEMOX versus GEMCIS was numerically higher with the former regimen (9 months vs. 8.3 months, $p = 0.057$), but did not meet the criteria for equivalence [8]. Capecitabine plus oxaliplatin (CAPOX) was noninferior to GEMOX for 6-month PFS rates in a randomized phase III study, but has not been directly compared to first-line GEMCIS [9]. Other doublet regimens that have shown efficacy and safety in phase II studies include gemcitabine plus capecitabine [10], gemcitabine plus nab-paclitaxel [11], and nanoliposomal-irinotecan (nal-IRI) plus 5-fluorouracil (5-FU)/leucovorin [12]. The phase Ib ABC-08 study showed encouraging efficacy using the combination of cisplatin and novel agent NUC-1031, a phosphoramidate transformation of gemcitabine designed to overcome drug resistance [13], prompting the ongoing phase III NuTide:121 trial comparing this regimen to GEMCIS (NCT04163900).

More intensive triplet therapy regimens have also been investigated in the first-line setting for advanced BTC. Modified FOLFIRINOX (5-FU, irinotecan, oxaliplatin) failed to improve 6-month progression-free survival (PFS) compared to GEMCIS (44.6% vs. 47.3%) in the phase II/III PRODIGE 38 AMEBICA trial [14]. In Japan, GEMCIS plus S-1 improved survival outcomes compared to GEMCIS in 246 patients with advanced BTC, with a median OS of 13.5 months versus 12.6 months (HR 0.79, $p = 0.046$) [15]. Promising results from a phase II study [16] of the combination of gemcitabine, nab-paclitaxel, and cisplatin (GAP) that demonstrated a remarkable median OS of 19.2 months, although with frequent rates (58%) of grade 3 or higher toxicity, provide a basis for the ongoing phase III SWOG-1815 trial comparing this triplet

regimen with GEMCIS (NCT03768414). In general, alternative cytotoxic chemotherapy regimens have not significantly improved on GEMCIS, which remains the preferred first-line systemic therapy. This is likely to change with the potential approval of durvalumab plus GEMCIS regimen, as discussed [17].

2. Emerging Treatment Options with ICIs and a Potential New Firstline Therapy

2.1. ICI Monotherapy

The success of ICIs for malignancies such as HCC has led to an increasing number of studies evaluating their use in BTC. KEYNOTE-028 was a phase I basket trial with 20 different solid tumor cohorts, including 24 patients with advanced and relapsed BTC, who received the anti-PD-1 monoclonal antibody (mAb) pembrolizumab (10 mg/kg every 2 weeks). PD-L1 positivity, defined by ≥1% expression of tumor and tumor-associated cells, was required for trial enrollment. The ORR was 13% (3/23, all PR) with a median PFS of 1.8 months and median OS of 5.7 months. Of the three responders, two (one MSI-H, the other unknown MSI status) achieved response durations lasting more than 4 years by the time of data cutoff. The majority of patients (91.7%) discontinued treatment, primarily due to disease progression and one case related to toxicity. Common treatment-related side effects included fevers (16.7%), nausea (12.5%), and pruritis (12.7%) with no grade 4 or higher toxicities. KEYNOTE-158 was a larger phase II study that also evaluated pembrolizumab (200 mg every 3 weeks) in 104 recurrent and advanced BTC patients. Unlike KEYNOTE-028, this trial did not require PD-L1 positivity for enrollment, but retrospectively analyzed tumor biomarkers and found 61 patients (58.7%) with PD-L1-positive tumors although none were MSI-H. Positive PD-L1 expression was associated with higher ORR (6.6% vs. 2.9%), which was 5.8% (6/104, all PR) for the entire cohort. However, PD-L1 positivity did not correlate with superior survival outcomes, with no significant differences in median PFS (1.9 vs. 2.1 months) and OS (7.2 vs. 9.3 months) between PD-L1-expressing and non-expressing subgroups [18].

In contrast to the KEYNOTE-028 results, a phase I study evaluating nivolumab (anti-PD-1) with and without GEMCIS in a Japanese cohort with relapsed and unresectable BTC found that PD-L1 expression in at least 1% of tumor cells correlated with longer median OS (11.6 vs. 5.2 months) and PFS (2.8 vs. 1.4 months) in the nivolumab monotherapy group [18][19]. A multi-center phase II study of nivolumab (240 mg every 2 weeks for 16 weeks, then 480 mg every 4 weeks) in 46 patients with advanced refractory BTC demonstrated an ORR 22% (10/46) and DCR of 59% (27/46) by investigator assessment. Median PFS was 3.7 months and median OS was 14.2 months. None of the responders were dMMR, but positive PD-L1 status (≥1% expression) was associated with significantly longer PFS (HR 0.23, $p < 0.001$), albeit not OS. Common side effects of nivolumab included increased alkaline phosphatase, lymphopenia, and fatigue [20].

Durvalumab (anti-PD-L1) monotherapy has also been studied in advanced BTC. A phase I study evaluated the safety and efficacy of durvalumab with and without tremelimumab (anti-CTLA-4) in previously treated advanced BTC. In the monotherapy cohort of 42 patients, treatment with durvalumab (10 mg/kg every 2 weeks) was tolerable with no observed unexpected toxicities or treatment-related deaths. Only two patients achieved PR (4.8%) and disease control rate was 16.7% at 12 weeks. Median duration of response was 9.7 months and median OS was 8.1 months [21].

Bintrafusp alfa (M7824) is a novel bifunctional fusion protein that binds both TGF-β and PD-L1, thereby neutralizing their activities involved in tumor cell proliferation. Thirty patients with refractory BTC received bintrafusp alfa for a median of 8.9 weeks in a phase I open-label study. Frequent toxicities observed were rash, fever, and increased lipase. The ORR was 20% (6/30), median PFS 2.5 months, and median OS 12.7 months. Only 1 of the 6 responders was MSI-H and there was no correlation between treatment response and PD-L1 expression or TMB [22]. Based on these findings, the phase II INTR@PID BTC 047 trial evaluated bintrafusp alfa for 159 patients with platinum-refractory advanced BTC. Although an ORR of 10.1% was observed, the study did not meet the predefined threshold that would have allowed for regulatory filing for its use in the second-line setting [23].

2.2. ICI-Based Combinations

2.2.1. Dual ICIs

In order to improve efficacy and overcome potential resistance with ICI monotherapy, the addition of other agents, including different ICIs, chemotherapy, and targeted agents for advanced BTC, is under investigation. Multiple preclinical studies indicate that the combination of CTLA-4 and PD-1 inhibitors is more effective than monotherapy, potentially due to synergistic effects resulting in increased numbers of TILs, decreased Tregs, and overall improved inhibition of tumor growth [24][25]. The multi-center phase II CA209-538 trial studied the combination of nivolumab plus ipilimumab (anti-CTLA-4) in advanced rare cancers including 39 patients with BTC (16 iCCA, 10 eCCA, 13 GBC). Among the BTC

subgroup, the primary endpoint of disease control rate was 44%, with an ORR of 23% (9/39) and median duration of response that was not reached. None of the treatment responders had eCCA or MSI-H tumors, and they all had received prior treatment. The median PFS was 2.9 months and OS was 5.7 months [26]. A similar dual ICI regimen evaluated for relapsed and advanced BTC was durvalumab (20 mg/kg every 4 weeks) plus tremelimumab (1 mg/kg every 4 weeks) in the aforementioned durvalumab phase I study. This regimen resulted in an ORR of 10.8% (7/65, all PR), disease control rate of 32.2% at 12 weeks, and OS was 10.1 months. Grade ≥ 3 treatment-related adverse events occurred in 23% of patients [21].

2.2.2. ICI + Chemotherapy: A New Frontline Standard of Care?

Emerging evidence indicates that cytotoxic chemotherapy works synergistically with immunotherapy by enhancing anti-tumor immunity [27]. Certain chemotherapy agents can modulate the tumor microenvironment through two major mechanisms: the induction of immunogenic tumor cell death and inhibition of mechanisms utilized by tumors for immune evasion [28]. Most chemoimmunotherapy trials in advanced BTC applied GEMCIS as the chemotherapy backbone, although oxaliplatin-based regimens were also used. EORTC-1607 (NCT03260712) and KEYNOTE-966 (NCT04003636) are ongoing phase II/III trials evaluating the addition of pembrolizumab to GEMCIS for first-line therapy in unresectable and advanced BTC. The combination of nivolumab plus GEMCIS was tested in a phase II study that enrolled 32 chemotherapy-resistant and naïve BTC patients. Two patients achieved responses (1 CR, 1 PR, ORR 33%) among the six patients in the chemotherapy-resistant cohort, whereas the ORR in the chemotherapy-naïve cohort was 61.9% (13/21, 4 CR, 9 PR). However, there were no significant differences in median PFS (3.5 vs. 6.2 months) and OS (6.7 vs. 8.6 months) between those who were previously treated versus untreated. PD-L1 expression also did not correlate with overall responses or survival outcomes, but higher TMB tumors showed a trend towards better clinical responses [29]. Final results from another similar phase II study (BilT-01) of nivolumab plus GEMCIS are pending [30]. Clinical trials evaluating nivolumab plus other chemotherapy regimens, including S-1 plus gemcitabine (NCT04172402) and nal-irinotecan plus 5-FU/leucovorin (NCT03785873) [31], are also underway.

Toripalimab, a novel anti-PD-1 mAb, has shown safety and efficacy when combined with chemotherapy in advanced BTC. A phase II study of toripalimab plus S-1 and gemcitabine in 48 treatment-naïve BTC patients had promising survival benefits, with a median PFS of 7 months and median OS of 16 months. The ORR was 27.1% and disease control rate was 87.5% (13 PR, 29 stable disease [SD]). Biomarker analysis revealed common mutations in *TP53*, *KRAS*, *CDKN2A*, and *SMAD4*. TMB was not associated with treatment response or survival outcomes, but patients with PI3K signaling pathway activation had significantly shorter PFS. Patients generally tolerated treatment well, and frequent side effects included leukopenia, anemia, and rash [32]. Multiple phase II studies have evaluated another anti-PD-1 mAb camrelizumab plus the oxaliplatin-based regimens GEMOX and FOLFOX in untreated, advanced BTC. Response rates ranged from 16.3 to 54%, median PFS from 5.3 to 6.1 months, and median OS from 11.8 to 12.4 months [33][34]. One of the studies reported an association between PD-L1 expression and treatment response in patients who received camrelizumab plus GEMOX; ORR was 80% in patients with PD-L1 tumor proportion score (TPS) ≥ 1% versus 53.8% in PD-L1 TPS < 1%. TMB was not predictive of response and survival, but positive post-treatment circulating tumor DNA (ctDNA) correlated with shorter PFS (HR 2.83, $p = 0.007$) [33].

The most promising results of ICI plus chemotherapy combination regimens have come with the use of durvalumab. GEMCIS plus durvalumab, with and without tremelimumab, demonstrated significant efficacy for chemotherapy-naïve BTC in a phase II study. Forty-five of the 121 enrolled patients who received durvalumab plus GEMCIS achieved an ORR of 73.4%, DCR of 100%, and median response duration of 9.8 months. Median PFS was 11 months and OS 18.1 months in this subgroup, comparable to survival outcomes seen with the four-drug combination (median PFS 11.9 months, median OS 20.7 months). Although baseline tissue TMB did not correlate with survival benefit, PD-L1 expression after one cycle of treatment trended with improved PFS. Frequently detected mutations on analysis were in genes involved with DNA damage repair (DDR), cell cycle regulation, and genomic instability, such as *ATM*, *BRCA2*, *CDKN2A*, and *MSH2*. The most commonly observed adverse events were nausea (59.5%), pruritis (55.4%), and neutropenia (54.5%) [35]. Other chemoimmunotherapy trials with durvalumab were less successful, including the addition of paclitaxel to durvalumab plus tremelimumab, which resulted in unexpected serious anaphylactic reactions in the phase II IMMUNOBIL PRODIGE 57 trial [36]. Notably, recent data from the randomized phase III TOPAZ-1 trial demonstrated that durvalumab plus GEMCIS significantly improved survival outcomes compared to chemotherapy alone as a first-line treatment in advanced BTC. The addition of ICI to standard first-line GEMCIS resulted in superior OS (12.8 vs. 11.5 months; HR 0.80, $p = 0.021$), PFS (7.2 vs. 5.7 months; HR 0.75, $p = 0.001$), and ORR (26.7% vs. 18.7%) compared to chemotherapy alone. Patients in the experimental arm received 1500 mg of durvalumab every 3 weeks with GEMCIS for up to eight cycles, followed by durvalumab 1500 mg every 4 weeks, until disease progression or unacceptable toxicity. Treatment with the chemoimmunotherapy combination was generally well tolerated and rates of grade 3 or 4 adverse events were similar

between both groups (62.7% with durvalumab vs. 64.9% with placebo) [17]. Final reports of the trial data that may identify which subgroups most benefited from immunotherapy, such as BTC subtypes, patient characteristics, and predictive biomarkers, are eagerly awaited. Based on the positive results for TOPAZ-1, GEMCIS plus durvalumab will most likely become a new standard first-line systemic therapy option for advanced BTC, signaling a pivotal change in the frontline treatment landscape more than a decade after the ABC-02 trial.

2.2.3. ICI + Anti-Angiogenic Agents

The overexpression of neo-angiogenic pathways such as vascular endothelial growth factor (VEGF) is common in BTC, prompting the evaluation of angiogenesis inhibitors for these cancers [37]. Previous studies of anti-angiogenic therapies with mAb and tyrosine kinase inhibitors (TKIs) including bevacizumab, ramucirumab, sorafenib, and regorafenib, have shown mixed efficacy, both alone and with chemotherapy in untreated and relapsed advanced BTC [38][39][40][41][42]. However, the increased research focus on identifying mechanisms of resistance to ICIs has led to growing awareness of the important role that angiogenesis plays in immune suppression. Angiogenesis factors directly inhibit APCs and effector cells as well as activating inhibitory cells, including Tregs and tumor-associated macrophages (TAMs), which, in turn, secrete factors that support angiogenesis and contribute to a highly immunosuppressive tumor microenvironment. Thus, the simultaneous blockade of immune checkpoints and angiogenesis pathways could potentially enhance anti-tumor immunity [43].

Pembrolizumab plus the anti-VEGFR-2 mAb ramucirumab was well tolerated but showed limited clinical activity for relapsed, advanced BTC in a phase I study [44]. The phase II LEAP-005 trial evaluated the combination of pembrolizumab plus lenvatinib, an anti-angiogenic multikinase inhibitor, in advanced solid tumors, including 31 patients with previously treated, advanced BTC. DCR was 68% (3 PR, 18 SD) and ORR was 10%, while median PFS was 6.1 months and median OS was 8.6 months. The most frequent adverse events were hypertension, dysphonia, and diarrhea. Based on these results, enrollment in the BTC cohort was expanded to 100 patients, with final analysis pending [45]. Another phase II study showed an improved median PFS of atezolizumab (anti-PD-L1) plus cobimetinib (MEK inhibitor) compared to atezolizumab alone (3.65 vs. 1.87 months); however, response rates were low in both groups (1 PR each) [46]. Other combinations of anti-angiogenic drugs and ICIs, including toripalimab plus lenvatinib (NCT04211168), are being evaluated in ongoing studies.

The addition of chemotherapy to the combined angiogenesis/checkpoint blockade has the potential to further augment the anti-tumor immune response. A phase II study examined the combination of toripalimab and levatinib with GEMOX as first-line treatment for advanced iCCA. Among the 30 enrolled patients, the ORR was 80% (24/30), with one patient achieving CR and three patients with locally advanced tumors that were successfully downstaged and then underwent resection. The median duration of response was 9.8 months, median PFS was 10 months, and median OS, remarkably, had not been reached at a median follow-up of 16.6 months. Responses correlated with positive PD-L1 expression and DDR-related mutations. Non-hematologic side effects were jaundice (10%), rash (6.7%), and proteinuria (6.7%), with no observed grade 5 toxicities [47]. The phase II IMbrave 151 trial (NCT04677504) plans to randomize 150 patients with treatment naïve, advanced BTC to GEMCIS plus atezolizumab with or without bevacizumab (anti-VEGF). PFS per RECIST 1.1 is the primary endpoint and biomarker analysis will be performed on collected tissue, blood, and stool samples [48].

2.2.4. Other ICI-Based Combinations

As previously mentioned, approximately 20% of BTCs express either IDH1 or FGFR2 mutations, which can be targeted by novel molecular agents. Recent early-phase studies are examining the tolerability and effectiveness of FGFR (NCT02393248) and IDH1 (NCT03684811) inhibitors combined with ICIs in advanced solid malignancies including BTC. BRCA 1/2 mutations occur in only 1–7% of BTCs, but DDR mutations have been reported in up to 63.5% of BTC tumors [49]. Tumors that harbor either of these genetic alterations seem highly vulnerable to poly ADP-ribose polymerase inibitors (PARPi), which result in genomic instability and cell death. Prior studies suggest that PARPi may promote responsiveness to ICIs by increasing neoantigens and TMB, recruiting T cells through activation of the cGAS-STING signaling pathway, and upregulating PD-L1 expression [50]. Some phase II studies are currently investigating different combinations of PARPi and ICI, such as nivolumab plus rucaparib (NCT03639935) and dostarlimab (ant-PD-1) plus niraparib (NCT04895046). Based on preclinical data supporting the potent immunoregulatory effects of epigenetic modulators, including histone deacetylase inhibitors (HDACi) and DNA methyltransferase inhibitors (DNMTi), the combination of these agents with ICIs for relapsed advanced CCA is being explored in clinical trials (NCT03257761, NCT03250273) [51].

2.3. Other Immunotherapy Options

2.3.1. Cancer Vaccines

Cancer vaccines utilize tumor-specific antigens based on peptides and DCs to prime T cells and enhance the anti-tumor immune response. The most commonly used targets for vaccine therapy are Wilms tumor-1 (WT-1) and Mucin-1 (MUC-1), which are both overexpressed in BTC and associated with worse prognosis [17]. A phase I study of WT-1 vaccine and gemcitabine for advanced pancreatic cancer and BTC was found to have tolerable toxicity but only modest clinical efficacy, with a median OS of 9.5 months [52]. Sixty-five patients with relapsed or unresectable BTC received DC-based vaccines targeting WT-1 and MUC-1 in one retrospective study. Most patients (77%) received chemotherapy simultaneously, and the combination of chemotherapy and DC-based immunotherapy led to better response rates and survival outcomes compared to vaccines alone (HR 0.51, p = 0.025). It was concluded that DC vaccines were safe, although insufficient without the addition of chemotherapy to achieve meaningful clinical responses [53]. Despite some encouraging results in early phase studies, vaccine therapies in BTC remain investigational.

2.3.2. Adoptive Cell Therapy

Adoptive cell therapy is an immunotherapeutic strategy in which T cells are genetically modified to express chimeric antigen receptors (CAR) or tumor antigen-specific T cell receptors (TCR) in order to enhance their ability to recognize and kill cancer cells. The tyrosine kinase receptor EGFR is a promising therapeutic target, since it is commonly expressed in BTCs, although clinical trials of EGFR inhibitors in advanced BTC have generally been unsuccessful, as previously mentioned [54][55][56]. One phase I study enrolled 19 patients with EGFR-positive (>50%) advanced BTC, who were infused with T cells expressing EGFR-specific CAR after conditioning with nab-paclitaxel and cyclophosphamide. CAR T cell infusion was well tolerated, but cutaneous and mucosal side effects expected with anti-EGFR therapy were observed. Among the 17 evaluable patients, the disease control rate was 65%, with 1 CR achieved for 22 months and 10 SD for from 2.5 to 15 months after the first cycle. The median PFS was 4 months [57]. Another phase I study used HER2 CAR T cells in HER2-positive (>50%) advanced pancreaticobiliary malignancies, including 9 BTC. No cases of severe cytokine release storm (CRS) and treatment-related deaths were reported. DCR was 55% (1 PR, 5 SD) and median PFS was 4.8 months. [58] Adoptive cell transfer has generally not been as successful in solid tumors compared to hematologic malignancies, besides the use of sipuleucel-T in metastatic castration-resistant prostate cancer [59]. However, more studies have suggested that the addition of PD-1/PD-L1 blockade could improve the anti-tumor efficacy of CAR T cells in solid tumors, representing another potential strategy that warrants further evaluation [60].

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