Biologics, Immunotherapy, and Future Directions in the Treatment of Advanced Cholangiocarcinoma

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Abstract

Gemcitabine plus cisplatin remains the standard first-line systemic therapy for advanced cholangiocarcinoma and offers a median survival of approximately 1 year. No standard regimens beyond the first line and no targeted or immunotherapy agents are approved yet in this disease. Development of molecular targeted therapy in this heterogenous and relatively rare malignancy continues to be a challenging area. The rapidly growing precision medicine efforts have uncovered the underlying mutational landscape of this lethal disease and paved the way for molecularly oriented clinical trials. The early results from such trials like those exploring IDH and FGFR2 derangements have highlighted its promising potential as alternative therapeutic options. Additionally, advances in cancer immunology have identified certain correlates as biomarkers of response to immune modulatory approaches. For instance, the presence of tumor DNA mismatch repair (MMR) deficiency and/or microsatellite instability (MSI), in 5% to 10% of cholangiocarcinoma, is associated with high rates and durability of responses to immune checkpoint blockade. Beside checkpoint inhibitors, other types of immune therapeutics like peptide and dendritic cell-based vaccines and adoptive cell therapies have been developed and are undergoing active evaluation in cholangiocarcinoma. With further research effort, the integration of tumor molecular profiling in trials exploring targeted immunotherapy will lead to better understanding of the predictive role of various molecular and immune biomarkers and ultimately shine the horizon for this patient population.

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Introduction

Cholangiocarcinoma (CCA) is a primary liver cancer with features of cholangiocyte differentiation, the epithelial cell lining the intra and extrahepatic portions of the biliary tree. Intrahepatic cholangiocarcinomas (iCCA) are located within the hepatic parenchyma. The second-order bile ducts serve as the point of separation between iCCA and extrahepatic CCA (eCCA), which is divided into perihilar CCAs (pCCAs) and distal CCAs (dCCAs). The cystic duct is the anatomical boundary between these latter 2 subtypes. pCCA is the most common type of CCA. In a large series of patients with bile duct cancer, 8% had iCCA, 50% had pCCA, and 42% had dCCA. Each subtype has distinct clinical feature, risk factors, molecular pathogenesis, therapeutic options, and prognosis.

Classically, CCA has been always regarded as a 'rare' tumor, at least in Western countries, but over the last 15 years, its incidence has steadily increased worldwide, and nowadays, it represents the second most common type of primary malignancy in the liver (15%-20% of cases) after hepatocellular carcinoma.[1,2]

CCA is an aggressive malignancy with a poor overall prognosis and median survival of less than 2 years in patients with advanced disease. Potentially curative surgical treatment options are limited to the small subset of patients with early stage disease. Presently, the available systemic medical therapies for advanced or metastatic CCA have limited therapeutic efficacy.[3,4]

In addition to the known clinical heterogeneity between iCCA and eCCA, recent investigations demonstrated significant genetic differences between the two. Churi et al performed a mutational profiling study and reported the nature and frequency of the genetic aberration (GA) differences between them. IDH1 and DNA repair

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gene alterations occurred more frequently in iCCA, whereas ERBB2 GAs occurred in the eCCA group. Commonly occurring GAs in iCCA were TP53 (35%), KRAS (24%), ARID1A (20%), IDH1 (18%), MCL1 (16%), and PBRM1 (11%). Most frequent GAs in eCCA (n = 20) were TP53 (45%), KRAS (40%), ERBB2 (25%), SMAD4 (25%), FBXW7 (15%), and CDKN2A (15%). In iCCA (n = 55), KRAS, TP53, or MAPK/mTOR GAs were significantly associated with a worse prognosis, whereas FGFR GAs correlated with a relatively indolent disease course. IDH1 GAs did not have any prognostic significance. GAs in the chromatin modulating genes, BAP1 and PBRM1, were associated with bone metastases and worse survival in eCCA.[5]

Another study by the International Cancer Genome Consortium has also confirmed the genetic heterogeneity of CCA. In this study, whole-genome and epigenomic analysis was performed on 489 tumors. Four distinct genetic clusters were identified, defined by mutation and copy number profiles, gene expression, and epigenetics. Cluster 1 was enriched in *TP53*, *ARID1A*, *BRCA1/2* mutations, and ERBB2 amplification. Cluster 2 was enriched with *TP53* mutations. Both clusters occurred equally as extrahepatic and intrahepatic tumors and were liver fluke-positive or fluke-negative. Cluster 4 was enriched in *BAP1* and *IDH1/2* mutations as well as FGFR alterations and was predominantly intrahepatic and fluke-negative, as was cluster 3. The prognosis was distinct among clusters, and it was best for cluster 4. Around 60% of patients in cluster 4 were alive at 7 years, compared with 0% to 40% of patients in the other clusters ($P < .0001$)[6] (Figure 1).

A more recent study by Lowery et al analyzed CCA tumor samples from 195 patients using targeted next-generation sequencing techniques. Per study results, the most frequently deranged genes in iCCA were IDH1 (30%), ARID1A (23%), BAP1 (20%), TP53 (20%), and FGFR2 fusions (14%). Alterations in CDKN2A/B and ERBB2 were associated with dismal prognosis. Actionable targets were found in around 50% of the study population. Sixteen percent of those patients were either treated with biologic targeted therapy or enrolled in a clinical trial.[7]

Those existing clinical and genetic variations between the different types of CCA present a challenging aspect when designing and interpreting clinical trials for this relatively rare malignancy. Most of the past and ongoing therapeutic trials were designed to consider a molecularly and anatomically unselected CCA population, and this has largely hindered the development of effective novel agents for this disease. The rapidly evolving comprehensive genomic profiling technologies have enhanced our understanding of the distinct biologic features of the different types of CCA, highlighting the unmet need for more personalized and molecularly oriented therapeutic trials.

In this article, we provide a concise review of biologic targeted therapy and immunotherapeutic approaches as it relates to malignancies of the biliary tract. We will highlight the data from clinical trials that have been completed and discuss ongoing trials and emerging therapeutic strategies.

Biologic Targeted Therapy

Recent large scale molecular profiling studies by Nakamura et al have highlighted genomic differences characterizing tumors of intrahepatic and extrahepatic bile duct and gallbladder.[5-8] They revealed potentially actionable alterations in IDH1, IDH2, FGFR2, KRAS, PTEN, and CDKN2A, among others. The most common alterations involved ARID1A, IDH1, IDH2, and TP53 (each identified in 36% of the tumors), as well as MCL1 (amplified in 21% of tumors).[9]

EGFR Inhibitors

The epidermal growth factor receptor (EGFR) family (RAS, RAF, MEK, ERK/MAPK) of polypeptide growth factors play a vital role in the pathogenesis and progression of different cancer types.[10] Therapeutic targeting of these receptors has been attempted in patients with various cancers and has shown efficacy in some cancers and potential in others. The agents that target EGFR can be classified into 2 groups: tyrosine kinase inhibitors (TKIs) such as gefitinib and erlotinib; and monoclonal antibodies like cetuximab or panitumumab. Blocking EGFR in advanced CCA has demonstrated favorable results in a small phase II study. In this study, 42 patients received erlotinib 150 mg daily. Three patients had partial response (PR), and 17 (43%) patients had stable disease (SD). The median time to progression (TTP) was 2.6 months, and the median overall survival (OS) was 7.5 months.[11] Combining EGFR with another novel agent or chemotherapy has been studied with conflicting results. Gemcitabine and oxaliplatin plus cetuximab initially showed an objective response rate of 63%, with 3 patients achieving a complete response.[12] Unfortunately, the larger randomized phase II data did not reproduce the survival advantage.[13]

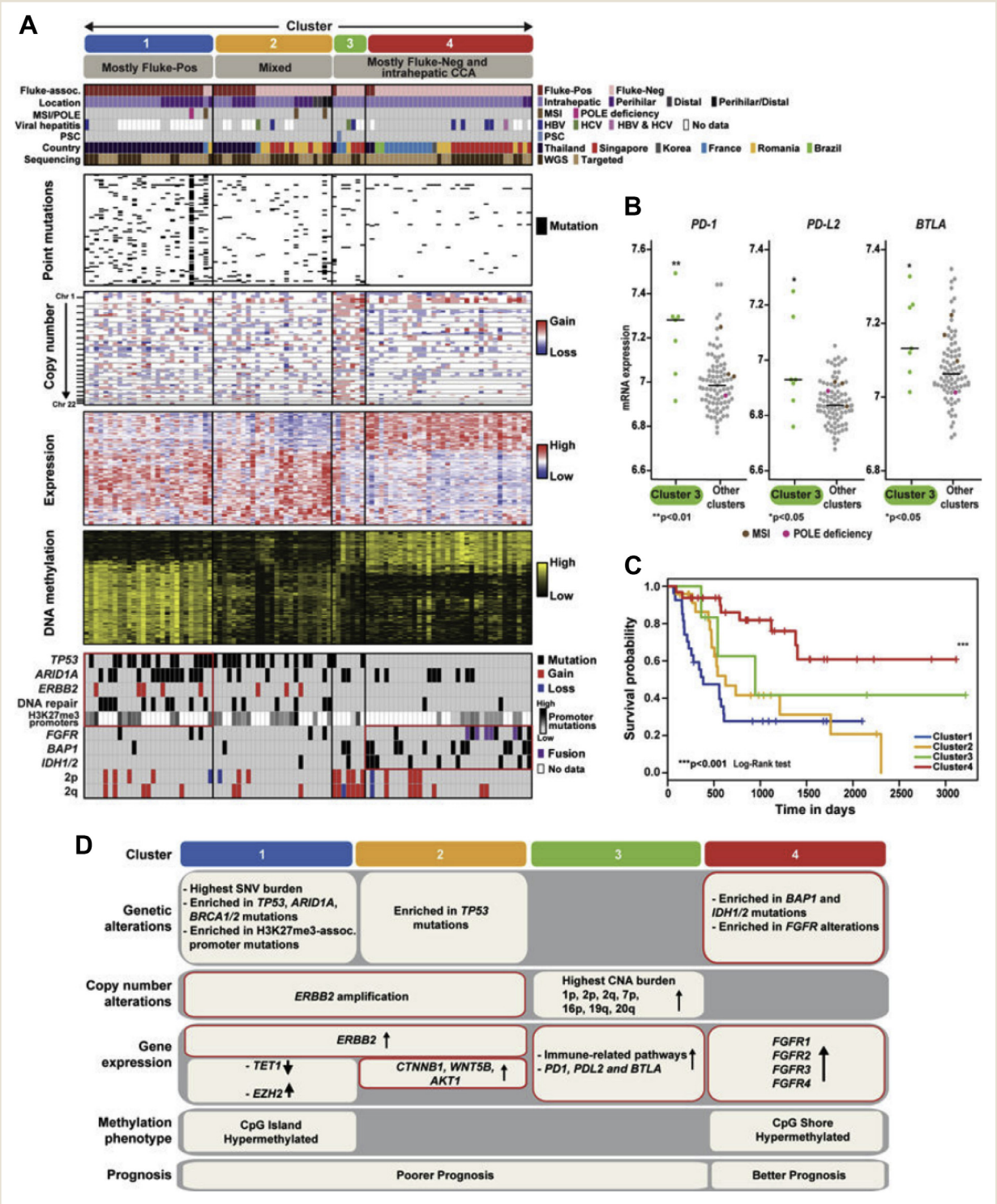
An estimated 14% of patients with gallbladder cancer have ERBB2 amplification or protein overexpression.[14] ERBB2 mutations or amplifications have also been reported in CCA.[15,16] A small single institute retrospective analysis suggested that biliary tract cancer with ERBB2 GAs or protein overexpression may respond to ERBB2 directed therapy.[17]

Receptor TKIs: FGFR2, MEK, and BRAF Inhibitors

Nakamura and colleagues revealed FGFR 2 fusion genes were exclusively identified in iCCA and were observed in up to 20% of patients. This resulted in ligand-independent activation of this receptor tyrosine kinase.[8] As a result, wild-type FGFR2 fusion, not mutant FGFR2, caused the autophosphorylation and activation of downstream MAPK.

Several selective and nonselective small-molecule inhibitors of FGFR are currently being investigated in early phase clinical trials in solid tumors including CCA. Oral FGFR inhibitors like NVP-BGJ398, erdafitinib, pemigatinib, and derazantinib have been tested in small phase II trials with very encouraging results and manageable side effects.[18-20] NVP-BGJ398 demonstrated potential in preclinical models of CCA.[21] This TKI is currently being investigated in a phase II study in patients with advanced-stage CCA harboring FGFR alterations (NCT02150967). An interim analysis of the study indicated that NVP-BGJ398 has impressive antitumor activity, with a disease-control rate (DCR) of 82%, and a manageable safety profile.[18] An interim analysis of the phase II trial with pemigatinib (INCB54828) in advanced unresectable CCAs that harbor a FGFR 2 translocation showed an overall response rate of 40% and a DCR of 85% with a median progression-free survival (PFS) of 9.2 months and a median OS of 15.8 months.[22] Ongoing trials are being conducted to validate the preliminary results (Table 1).

Figure 1 Integrative Clustering Defines 4 Molecular Subtypes of CCA. A, Heatmap Showing 4 Clusters Identified by iClusterPlus Based on Clustering of Mutation, Copy-number, Gene Expression, and Methylation Data. Top Rows Indicate Clinical Characteristics, Risk Factors, Geographical Region, and Sequencing Platform. MSI Status Was Defined by Indel Counts (≥ 6 Indels) in Simple Repeat Sequences. Bottom Rows Indicate Selected Genetic alterations. B, High Expression of PD-1, PD-L2 and BTLA in Cluster 3 Relative to Other Clusters. Brown Dots Indicate MSI Cases. Pink Dots Indicate Cases with DNA Polymerase Epsilon (POLE) Proofreading Deficiency. C, Survival Analysis Showing Improved Survival in Cluster 3 and 4 CCAs Compared to Other Clusters. Multivariate Analysis Confirmed this Difference Even After Accounting for Fluke Association, Anatomical Location, and Clinical Staging. D, Representative Genetic, Epigentic and Gene Expression Features of CCA Clusters.



Abbreviations: BTLA = B- and T- lymphocyte attenuator; CCA = cholangiocarcinoma; MSI = microsatellite instability; PD-1 = programmed cell death protein 1; PD-L2 = programmed death-ligand 2.

Mutations of the KRAS proto-oncogene are a frequent occurrence (11%-25%) in CCAs.[8] This leads to upregulation of the KRAS downstream signaling pathways, including the RAF–MEK–ERK (MAPK) pathway. This suggest that KRAS-mutant CCAs could be targeted by MEK inhibition. Results of a phase II study of selumetinib, a MEK TKI, in patients with metastatic biliary cancer, demonstrated a median PFS of 3.7 months and a median OS of 9.8 months.[23]

BRAF mutations can be found in CCA and occur in lower frequency (3%-5%).[5] In a very small study, in 8 patients with BRAF V600E-mutated CCAs, treatment with oral BRAF inhibitor vemurafenib led to a PR in 1 patient.[24]

Targeting Tumor Epigenetic Alterations: IDH1 Inhibitors

IDH1 mutation occurs in about 20% of iCCAs. This mutation results in the production of d-2-hydroxyglutarate (2-HG), an oncometabolite. Elevated 2-HG levels are implicated in epigenetic alterations and impaired cellular differentiation.[8]

Ivosidenib (AG-120) is a first-in-class oral, selective, reversible inhibitor of mutant IDH1. It was investigated in a phase II study of 73 heavily pretreated patients with CCA. Ivosidenib was well-tolerated with 6% PR and 56% SD. The median PFS was 3.8 months, and the 12-month PFS was 20.7%.[25] This medication is currently being investigated in an ongoing study of AG-120 in Previously Treated Advanced Cholangiocarcinoma With

Table 1 Ongoing Clinical Trials Evaluating Molecular Targeted Agents With or Without Chemotherapeutics in Advanced Stage Cholangiocarcinoma

Trial	Phase	Indication	Therapy	Status
VEGF inhibitors				
NCT02053376	II	Biliary tract carcinoma/ cholangiocarcinoma	Regorafenib	Active, not recruiting
NCT03251443	III	Cholangiocarcinoma	Apatinib	Recruiting
NCT01855724	II	Biliary tract carcinoma/ cholangiocarcinoma	Gemcitabine-pazopanib combination therapy	Recruiting
IDH or FGFR inhibitors				
NCT02924376	II	Cholangiocarcinoma	INCB054828	Recruiting
NCT03230318	II	Cholangiocarcinoma	ARQ 087	Recruiting
NCT02150967	II	Cholangiocarcinoma	BGJ 398	Recruiting
NCT03656536	III	Cholangiocarcinoma	INCB054828	Not yet Recruiting
NCT01752920	I/II	Solid tumors including cholangiocarcinoma	ARQ 087	Active, not recruiting
NCT02699606	II	Various cancers including cholangiocarcinoma	Erdafitinib (JNJ-42756493)	Recruiting
NCT02052778	I/II	Various cancers including cholangicarcinoma	TAS 120	Recruiting
NCT02272998	II	Various cancers including cholangiocarcinoma	Ponatinib hydrochloride	Recruiting
NCT02834780	I	Various cancers including cholangicarcinoma	H3B-6527	Recruiting
NCT03144661	I	Various cancers including cholangicarcinoma	INCB054828	Recruiting
NCT02393248	I/II	Various cancers including cholangicarcinoma	INCB054828	Recruiting
NCT03583125	I	Various cancers including cholangicarcinoma	EOC 317	Recruiting
NCT02989857	III	Cholangiocarcinoma	AG-120	Recruiting
NCT02073994	I	Various cancers including cholangicarcinoma	AG-120	Active, not recruiting
NCT03684811	I/II	Various cancers including cholangicarcinoma	FT-2102 as a single agent or in combination with other anticancer drugs	Not yet recruiting
NCT03207347	II	Various cancers including cholangicarcinoma	Niraparib	Recruiting
NCT02381886	I	Various cancers including cholangicarcinoma	IDH305	Active, not recruiting
Other noteworthy trials				
NCT03768414	III	Cholangiocarcinoma	Gemcitabine plus cisplatin with or without nab-paclitaxel	Recruiting

Abbreviations: FGFR = fibroblast growth factor receptor; IDH = isocitrate dehydrogenase; VEGF = vascular endothelial growth factor.

IDH1 Mutations (ClarIDHy), a phase III multicenter, randomized, double-blind study of AG-120 versus placebo in patients with non-resectable or metastatic CCA with an IDH1 mutation.

Targeting Tumor Angiogenesis

Angiogenesis and aberrant MET signaling are implicated in the pathogenesis and progression of invasive CCA. Because of the relevance of angiogenesis in the development of CCAs and based on the data from preclinical studies, the activity of many anti-angiogenetic treatments with both monoclonal antibodies (bevacizumab and ramucirumab) and TKIs (sorafenib, vandetanib, cabozantinib and regorafenib) were explored alone or in association with chemotherapy and/or anti-EGFR drugs in several phase I and II trials with contrasting results, possibly owing to unknown drug resistance mechanisms and lack of molecular enrichment.

Bevacizumab

Combining bevacizumab with erlotinib as a therapeutic alternative in patients with advanced chemotherapy-refractory CCA was explored. This combination demonstrated clinical activity with infrequent grade 3/4 adverse effects in this population. In a phase II trial of 49 evaluable patients treated with bevacizumab/erlotinib, 12% of patients had a confirmed PR, and 25 (51%) patients had an SD, with a median OS of 9.9 months, and a median TTP of 4.4 months.[26] Furthermore, the bevacizumab combination with gemcitabine and oxaliplatin (GEMOX-B) as the first-line therapy was studied in 35 patients with advanced CCA. The study showed antitumor activity with tolerable safety. The median PFS was 7.0 months (95% confidence interval [CI], 5.3-10.3 months), and the PFS at 6 months was 63%.[27] The efficacy of the combination of bevacizumab with FOLFIRI (fluorouracil, folinic acid, and irinotecan) was indicated by a single institutional retrospective collection from 13 patients after failing the first line of gemcitabine and oxaliplatin.[28]

Ramucirumab

Ramucirumab is a monoclonal anti-body directed against vascular endothelial growth factor receptor 2 (VEGFR2). It is being evaluated in phase II studies enrolling patients with pretreated CCA. Preclinical evidence suggests that a simultaneous blockade of VEGFR2 and programmed death 1 (PD-1) or programmed death-ligand 1 (PD-L1) enhances antitumor effects. However, combining ramucirumab and pembrolizumab, an immune checkpoint inhibitor, in advanced previously treated CCA in a phase II trial did not improve OS when compared with historical controls in biomarker-unselected patients. On other hand, the combination demonstrated an improvement in OS in the PD-L1-positive patients as compared with the PD-L1-negative population. The median OS in patients with PD-L1-positive and PD-L1-negative tumors was 11.3 months and 6.1 months, respectively. The study did not reveal any unexpected safety results.[29] Adding ramucirumab or merestinib, a MET kinase inhibitor, to chemotherapy is under active investigation.[30]

Sorafenib

Sorafenib is an inhibitor of the VEGF receptors 2 and 3 (VEGFR2/3) and the platelet-derived growth factor receptor-β. It has been shown to inhibit the proliferation of various human bile duct adenocarcinoma cell lines.[31] In the SWOG-0514 study, sorafenib was used as the first-line treatment for patients with CCA. Thirty-six patients were enrolled, and 10 (32%) patients had SD. The OS was 9 months, which is comparable to the combination of gemcitabine and capecitabine in the SWOG-0202 study.[32] However, sorafenib did not demonstrat significant anti-tumor activity as a second-line treatment in another study.[33] Furthermore, the combination of sorafenib with gemcitabine and cisplatin in patients with advanced CCA failed to show additional benefits over historic data, and was associated with higher toxicity.[34]

Regorafenib

Regorafenib is a TKI that inhibits VEGFRs and TIE2. Recent studies showed encouraging efficacy of regorafenib in patients with chemotherapy-refractory advanced CCA. A phase II study of regorafenib as a single agent in 37 previously treated patients with advanced CCA demonstrated a PFS of 15.6 weeks (90% CI, 12.9-24.7 weeks), and a median OS of 31.8 weeks (90% CI, 13.3-74.3 weeks), with survival rates of 40% at 12 months and 32% at 18 months. Five patients had PR, and 19 patients had SD.[35] The other phase II study performed in the same setting showed equivalent results: for 32 evaluable patients, the 6-month OS was 50.9% (95% CI, 32.1%-67.0%), the 12-month OS was 35% (95% CI, 16.2%-53.7%), and the 18-month OS was 35% (95% CI, 16.2%-53.7%). The median PFS was 3.7 months (95% CI, 2.3-5.5 months), and the median OS was 9.9 months (95% CI, 3.7-20.1 months). PR was achieved in 2 (6.2%) patients and SD in 18 (56.2%) patients with a DCR of 62.4%.[36] Combining regorafenib with conventional chemotherapy is another venue under active investigation in this patient population. A safety report from the ongoing phase Ib to II trial assessing the safety and activity of regorafenib in combination with modified gemcitabine-oxaliplatin (mGEMOX) demonstrated acceptable toxicity.[37]

Vandetanib

Vandetanib is a multitargeted TKI that affects VEGF, EGFR, and rearranged during transfection. In vitro studies of vandetanib in CCA, using cancer cell lines, showed promising results.[38,39] However, findings from the VanGogh study did not demonstrate any survival advantage of vandetanib alone or in association with gemcitabine over gemcitabine alone in patients with advanced CCA. The safety profile of vandetanib given alone or in combination with gemcitabine does not show any additional adverse effects (AEs) or worsening of already known AEs.[40]

Cabozantinib

A multikinase inhibitor with potent activity against VEGFR2 and MET, cabozantinib was studied in a phase II trial in patients with unresectable or metastatic CCA. It demonstrated limited activity and significant toxicity.[41] As this study been performed in an unselected molecular population, the heterogeneity of the study population was a likely contributor to the lack of efficacy.

As we learn more about the prognostic value and natural history of the different molecularly defined cohorts of CCA, additional biomarker-driven clinical trials remain crucial. The identification of patients with hyperactivation of the pro-angiogenic pathways or with specific genetic aberrations could identify defined groups of patients with CCA that could benefit from the treatment with certain combinations of biologic targeted agents.

Immune Therapeutics

Checkpoint Inhibitors

Rationale for the Use of Checkpoint Inhibitors in CCA. Certain tumor genetic aberrations have been associated with a likelihood of response to immune-checkpoint inhibitors, which might relate to the expression of neoantigens capable of eliciting an antitumor T-cell response. One example is the presence of tumor MMR deficiency and/or MSI, which is associated with high rates and durability of responses to immune-checkpoint blockade across multiple tumor types.[42,43] The anti-PD-1 antibody pembrolizumab has been approved by the United States Food and Drug Administration for previously treated patients with MMR-deficient and/or MSI-high advanced solid tumors, independent of histology − which would include those with CCA.[44] Notably, MMR deficiency has been reported to occur in 5% to 10% of CCAs.[45]

Beyond MMR deficiency, the cumulative tumor mutational burden has been correlated with responsiveness to immune-checkpoint inhibitors in some cancers, including melanoma, non−small-cell lung cancer, and urothelial carcinoma.[46-48] Nakamura et al performed a whole-exome-sequencing study of 231 cholangiocarcinoma tumor samples; a median of 39 and 35 somatic nonsynonymous mutations were identified in intrahepatic and extrahepatic CCAs, respectively. Overall, ∼6% of the CCAs had evidence of hypermutation (mutation rates of $>$ 11.13 per megabase; median number of 641 non-silent mutations), with concurrent MMR deficiency and/or MSI detected in about 36% of these hypermutated tumors.[7] Specifically, this subgroup was also found to have abundant tumor-specific neoantigens and enrichment for expression of immune-related genes, including genes encoding inhibitory immune-checkpoint proteins. Interestingly, this patient subgroup had the poorest prognosis.[8,48] These data suggest that immune-checkpoint blockade and other immune-modulating therapies could be promising options for this subgroup of patients with CCAs harboring high mutational loads.

Remarkably, checkpoint blockade and other immunotherapies have shown promising efficacy in malignancies commonly associated with viral infections, such as head and neck cancer, Hodgkin lymphoma, Merkel-cell carcinoma, and hepatocellular carcinoma,[49] and this relationship is thought to be partly mediated by the presentation of neoantigens associated with viral infections.[50,51] Several chronic infections, such as liver-fluke disease, viral hepatitis B and C, and bacterial pyogenic cholangitis, are established risk factors for CCA.[52] The group of patients with CCA with underlying chronic viral infections or chronic inflammation, like sclerosing cholangitis, might partly represent the subgroup of patients with CCA with high mutational and neoantigen burden who would possibly derive benefit from checkpoint inhibitory approach.

Moreover, there is preclinical evidence supporting the correlation between activated immune cell infiltration and better outcome in CCA, another reason why checkpoint modulators should be explored in this disease.[53]

Safety and Early Efficacy Results From Recent Checkpoint Inhibitor Trials. Patients with MMR-deficient CCA have demonstrated responsiveness to treatment with immune-checkpoint inhibitors.[42,43,54] Among 86 patients in KEYNOTE-016 with MMR-deficient tumors, 4 patients had CCA; the overall response rate was 53%, with 21% being CRs; the 2-year OS was 64%.[54] Among 94 patients with MMR-deficient tumors enrolled in KEYNOTE-158, there were 9 patients with CCA; the overall response rate was 37%, and as of the latest report, the median duration of response had not been reached. The unprecedented clinical results seen in those trials sharply underscore the importance of biomarker development to identify patients who are most likely to respond. Furthermore, other immunotherapeutic strategies are being explored in this group of patients to capitalize on the current findings. Such strategies include combinatorial approaches integrating checkpoint inhibitors with other checkpoints, chemotherapy, vaccines, or targeted agents.

A mutational profiling study on 321 CCA tumors revealed DNA repair mutations (*MSH6*, *BRCA1*, *BRCA2*, *ATM*, *MLH1*, or *MSH2* genes) occurring in 13% of iCCA, 26% of eCCA, and 6% of gall bladder cancer cases.[55] This subset of patients might benefit from a future approach where novel DNA repair inhibitors could be coupled with immune modulators.

The potential benefit of checkpoint blockade in the patients with CCA with microsatellite stable (MSS) tumors is under active evaluation through multiple trials. Interim safety and efficacy data from the KEYNOTE-028 basket trial of the anti-PD-1 antibody pembrolizumab have been reported for a small cohort of patients with PD-L1-positive biliary tract cancer.[56] Thirty-seven (41.6%) of 89 patients screened had PD-L1 expression on $\geq$ 1% of tumor cells by immunohistochemistry, 24 of whom enrolled in the study (20 with CCA, 4 with gallbladder carcinoma). Of these 24 patients, 4 (17%); 3 with CCA and 1 with gallbladder carcinoma had a PR, and 4 (17%) had SD. The duration of PR was protracted, with the median PFS not reached at the time of reporting. The rate of grade 3 toxicities was 16.7%, with no patients experiencing grade $\geq$ 4 toxicities, nor any marked hepatotoxicity. The promising safety and efficacy of pembrolizumab in the KEYNOTE-028 biliary cancer cohort prompted a successor biliary cancer cohort of 100 patients in the ongoing KEYNOTE-158 basket trial (NCT02628067).

The immune checkpoint inhibitor nivolumab also showed activity against CCAs that have progressed on prior systemic therapies. A phase II multi-institutional study by Kim et al explored the efficacy of single-agent nivolumab in patients with advanced CCA who have failed 1 prior systemic therapy. Among the 29 patients evaluable for response, 5 (17%) patients achieved PR, and 11 (38%) patients achieved SD. The DCR was 55%. Four patients who responded were all MSS and still remain on treatment (2 patients have durations of response $>$ 12 months). The median PFS was 3.5 months (95% CI, 2.1-7.6 months), and the median OS has not been reached. The 6-month OS was 76.3%.[57]

To build on the results seen with single-agent checkpoint inhibitors in patients with CCA, a multitude of clinical trials have been initiated looking at combination therapies with 2 checkpoint inhibitors or a checkpoint inhibitor in combination with other types of immunotherapy, or with chemotherapy or targeted therapy (Table 2).

One example of such trials is a Chinese single-center trial combining lenvatinib (a VEGF multi-TKI) with either nivolumab or pembrolizumab. Preliminary results from this trial showed a positive efficacy signal and a favorable toxicity profile for the drug combination. The ORR was 21.4%, consisting of 3 PRs. Eleven (79%) patients had SD, for a DCR of 93%. The clinical benefit rate (ORR + durable SD $\geq$ 5 months) was 64.3%. Eleven of 14 patients had tumor

shrinkage; 8 of the 10 who received pembrolizumab and 3 of the 4 who received nivolumab. One responder had a 399 bp deletion on MLH1, and was identified as MSI-H. Two patients with a low tumor burden had tumor progression, including 1 with an FGFR2 rearrangement. One other patient with low tumor burden and FGFR2 rearrangement had tumor shrinkage of 27%.[58]

Cancer Vaccines

Tumors may express antigens that can become targets of antigen-specific T cell responses that lead to tumor rejection. These tumor rejection antigens, which include tumor-specific antigens, cancer-testis antigens, and differentiation antigens, have been exploited for therapeutic purposes including for cancer vaccines, where patients are inoculated with one or more tumor rejection antigens to induce tumor responses. The types of cancer vaccines that have been studied to date in CCA are single-antigen and multi-antigen cancer vaccines and cell-based cancer vaccines. Wilm's tumor protein 1 (WT1) and mucin protein 1 (MUC1) are tumor rejection antigens that have been explored for use as cancer vaccines in CCA. About 68% to 80% of CCA express WT1, whereas about 44% to 95% of CCA express MUC1, and the expression of either of these proteins are associated with poor survival.[59,60] An open-labeled, dose-escalation phase I trial evaluated WT1 vaccine and gemcitabine combination therapy for patients with advanced biliary tract cancer and pancreatic cancer. The primary endpoints were toxicity, safety, and optimal dose. In 25 enrolled patients, 8 had CCA. Adverse events for the combination therapy were comparable with gemcitabine alone. Cell culture assays showed WT-1 specific T cells in 59% of patients and positivity to delayed type hypersensitivity after vaccination in 2 patients. The median OS for patients with CCA was 288 days, and the DCR at 2 months was 89%.[61] A phase I trial evaluated the safety of vaccination with MUC1 in 3 patients with biliary tract cancer and 6 patients with pancreatic cancer. No notable AEs were seen except for injection site reactions. Among 8 patients who were eligible for evaluation, 7 had progressive disease and 1 had SD. Although there were no significant changes in the titers of anti-MUC1 IgG antibodies before and after vaccination of all patients, the single patient with SD showed a non-significant increase in the titer.[62]

Because of the heterogeneity in tumor-rejection antigen expression, multi-antigen cancer vaccines may be more effective than single-antigen vaccines in eliciting anti-tumor immune responses and enhancing therapeutic benefit. Lymphocyte antigen 6 complex locus K, TTK protein kinase, insulin-like growth factor-II mRNA-binding protein 3, and DEP domain containing 1 are overexpressed in nearly all biliary tract cancers.[63] A phase I trial evaluated the safety, tumor response, PFS, and OS of inoculation with a cancer vaccine containing these 4 peptides. The treatment was well-tolerated without serious AEs. The median PFS and OS were 156 and 380 days, respectively. Tumor response was seen in 6 of 9 patients.[63] The same authors evaluated another cancer vaccine containing the cancer-testis antigens cell division cycle associated 1 (CDCA1), cadherin 3 (CDH3), and kinesin family member 20A (KIF20A), which are also overexpressed in nearly all biliary tract cancers.[64] The outcomes of interest were tumor response, PFS, and OS. There were 5 of 9 patients who achieved SD. The median PFS

Table 2 Ongoing Clinical Trials Evaluating Immunotherapy in Advanced Stage Cholangiocarcinoma

Trial	Phase	Therapy	Indication	Status
Checkpoint inhibitors				
NCT03473574	II	Durvalumab + tremelimumab with gemcitabine or gemcitabine + cisplatin vs. gemcitabine + cisplatin	Cholangiocarcinoma	Recruiting
NCT02982720	II	Pembrolizumab + sylatron	Cholangiocarcinoma	Active, not recruiting
NCT03250273	II	Nivolumab + entinostat	Cholangiocarcinoma and pancreatic cancer	Recruiting
NCT02834013	II	Nivolumab + ipilimumab	various cancers including cholangiocarcinoma	Recruiting
NCT02340975	I/II	FT-2102 + nivolumab	Various cancers with positive IDH-1 mutation including ICC	Not yet recruiting
NCT02628067	II	Pembrolizumab	Solid tumors including cholangiocarcinoma	Recruiting
NCT03095781	Ib	Pembrolizumab + XL888	Gastrointestinal malignancies including cholangiocarcinoma	Recruiting
NCT02393248	I/II	Pembrolizumab + INCB054828	various cancers including cholangiocarcinoma	Recruiting
NCT02821754	II	Durvalumab + tremelimumab alone or with RFA or cryoablation	Cholangiocarcinoma and hepatocellular carcinoma	Recruiting
NCT03257761	Ib	Durvalumab + guadecitabine	various cancers including cholangiocarcinoma	Recruiting
Cancer vaccines				
NCT03042182	II	Oral vaccination with V3-X	Cholangiocarcinoma	Recruiting
Adoptive cell transfer				
NCT01868490	I	Autologous cytokine-induced killer cells	Cholangiocarcinoma	Unknown
NCT02482454	III	Radiofrequency ablation combined with cytokine-induced killer cells	Cholangiocarcinoma	Active, not recuriting
NCT03633773	I/II	MUC-1 CAR T cells	Cholangiocarcinoma	Recruiting
NCT02757391	I	CD8+ T cells + Pembrolizumab	Advanced gastrointestinal tumors including cholangiocarcinoma	Not yet recruiting

and OS were 3.4 and 9.7 months, respectively. No grade 3 to 4 AEs were observed following vaccination.[64] In another study, a multi-antigen vaccine designed as personal vaccines with respect to the pre-existing host immunity of enrolled patients was evaluated in 25 patients with biliary tract cancer in a phase II trial. Vaccinations were given subcutaneously every week for the first 6 weeks and every 2 weeks thereafter. Tumor response was evaluable in only 10 of the 25 patients. SD was seen in 8 patients and progressive disease (PD) in 2 patients. No serious AEs were seen with vaccination. Antigen-specific IgG responses were seen in 7 of 20 patients and in 7 of 8 patients evaluated after the sixth and twelfth inoculations, respectively. Antigen-specific T cell responses were seen in 5 (23%) of 22 patients prior to vaccination. At the end of the sixth vaccination and the twelfth vaccination, antigen-specific T cell responses were seen in 8 (47%) of 17 and 4 (57%) of 7 patients, respectively.[65]

Cell-based vaccines, such as dendritic cell (DC) vaccines, are generally more effective than non−cell-based vaccines in eliciting cell-mediated immune responses against tumor cells. Indeed, mouse studies have shown that, out of several different vaccine formulations, dendritic cells loaded with MUC1 elicited the most robust anti-MUC1 CD8 T cell responses.[66] A phase I/II trial evaluated an MUC1 peptide-loaded DC vaccine in 12 patients with biliary tract cancer and pancreatic cancer. No serious AEs were seen with the treatments. The immunogenicity of the vaccine was evaluated using anti-MUC1 antibody titers and T cell activity. However, no conclusions could be drawn regarding the vaccine's immunogenicity as 3 patients had pre-existing anti-MUC1 antibody responses, and CD4 and CD8 T cells showed non-specifically increased activity following vaccination. Four of the 12 patients remain alive and without recurrence at 4-year follow-up.[67]

Adoptive Cell Transfer (ACT)

ACT therapy involves expanding tumor-specific T cells to large numbers then re-infusing them into patients to elicit anti-tumor immune responses. The novel treatment modality has been United States Food and Drug Administration-approved for use in hematopoietic malignancies such as in acute lymphoblastic leukemia. Several case series studies and case reports have demonstrated tumor response and long-term survival of patients with advanced CCA treated with a combination of ACT and cancer vaccines.[68-71] Such encouraging results have prompted clinical trials evaluating ACT in CCA. ACT in combination with DC vaccination was evaluated in 36 patients in the adjuvant treatment setting. Patients were either vaccinated with dendritic cells pulsed with autologous tumor lysate and anti-CD3 activated T cell transfer or given curative intent surgical therapy alone. The mPFS and OS were 18.3 and 31.9 months versus 7.7 and 17.4 months, respectively, in favor of the patients who received the adjuvant therapy. Among those treated, patients with robust injection-site reactions showed a significantly better mPFS and OS.[72] Further clinical trials are under way to evaluate the safety and efficacy of ACT for the treatment of advanced CCA.

Future Treatment Considerations

With the rising incidence of CCA, the modest benefit with chemotherapy, and the lack of standard regimens beyond frontline, finding more effective and personalized treatment options is an area of great unmet need. The early efficacy signal seen with targeting FGFR2 and IDH mutations should encourage development of future trials focusing on optimizing the targeted approach through stronger high affinity molecules or in combination with other synergistic molecular agents or immune modulators. In addition to FGFR1-3 fusions or amplifications and IDH mutations, other promising targets in CCA include ERBB2 amplification; BRAF and KRAS mutations; CDKN2A/B loss; ARID1A loss; MET amplifications; and rare fusions including ROS1, ALK, and NTRK1. Targeting those molecular pathways is being explored in pipeline and ongoing biomarker-driven clinical trials, either as dedicated CCA trials or as part of various basket trials. The results from those trials will ultimately define the extent of application of those novel agents in different treatment settings in this patient population.

On the other hand, efforts are needed to further improve on results from targeting key signaling pathways using single-agent multi-TKI therapy like regorafenib. Optimizing this approach with combination regimens is a next-step move to achieve better survival outcome for this patient population. This approach will likely address the majority of patients with advanced CCA as the population with druggable targets is still considered an orphan group.

When considering the rapidly advancing field of immunotherapy, finding subgroups beyond the MMR-deficient/MSI-high population that would respond to checkpoint blockade is still an open and immature research field. Relative to CCA, the other potential subgroups that might benefit from fostering anti-tumor immunity include patients with chronic infection- or inflammation-driven CCA-like bacterial pyogenic cholangitis, viral hepatitis, ulcerative colitis, and sclerosing cholangitis. This subgroup of patients, given the nature of their background disease, would likely have high neoantigen burden and resultant inflamed tumor microenvironment that would predict for favorable response to immune modulators. Thus far, those patients have been excluded from most, if not all, immunotherapy trials. Extrapolating from the positive safety and efficacy results of checkpoint inhibitors in viral hepatitis induced hepatocellular carcinoma, including those patients in carefully designed future immunotherapy trials would likely be an enlightening move. Another consideration would be the prospective evaluation of multiple immune biomarkers through baseline and on treatment biopsies. Despite challenges, this would greatly add granularity to this field and support the ultimate goal of finding effective targeted immunotherapy for this intractable disease.

Disclosure

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