Biliary Cancer: Gateway to Comprehensive Molecular Profiling

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Abstract: Cholangiocarcinoma is a rare malignancy with a poor prognosis. The majority of tumors present at an advanced stage, and relapse often occurs after surgery conducted with curative intent. In both of these cases, standard treatment is a combination of cisplatin and gemcitabine. The use of folinic acid, 5-fluorouracil, and oxaliplatin (FOLFOX) in second-line treatment improves survival, but outcomes remain dismal. Studies have shown that cholangiocarcinoma possesses a wide spectrum of genetic aberrations. Clinical trials evaluating targeted therapies in patients with *FGFR2* fusions, *IDH1* mutations, and *BRAF* mutations have yielded very promising results, and the agents were generally well tolerated. Several *FGFR2* fusion–targeted agents have achieved response rates between 20.7% and 35.5%, with disease stability rates ranging between 76% and 82%. Agents targeting *FGFR2* fusions also have produced median progression-free survival (PFS) ranging from 5.7 to 6.9 months and median overall survival (OS) ranging from 12.5 to 21.1 months. Ivosidenib in patients with an *IDH1/2* mutation has produced a response rate of 2% and a disease stability rate of 51%, with median PFS of 2.7 months and median OS of 10.8 months. In patients with a *BRAF* mutation, a combination of dabrafenib and trametinib led to an overall response rate of 51% and disease stability in another 40% of patients. Median PFS and OS were 9 and 14 months, respectively. Patients should be encouraged to participate in clinical trials.

Keywords
BRAF, cholangiocarcinoma, *FGFR2* fusions, *IDH1/2* mutation, molecular profiling, targeted therapy

Introduction

Cholangiocarcinomas are a heterogeneous group of cancers that arise from the epithelial cells of intrahepatic and extrahepatic bile ducts.[1] Anatomically, they are divided into intrahepatic, peri-hilar, and distal cholangiocarcinomas, with relative frequencies of 10%, 50%, and 40%, respectively.[2] Cholangiocarcinomas are relatively rare, accounting for 3% of all gastrointestinal malignancies and with an overall annual incidence of less than 2 per 100,000 adults.[3] Interestingly, the incidence of intrahepatic cholangiocarcinoma in the United States rose by 128% between 1973 and 2012, whereas the incidence of

extrahepatic cholangiocarcinoma remained stable.[4] This finding could in part be explained by the increasing recognition of carcinoma of unknown origin as intrahepatic cholangiocarcinoma owing to improvements in molecular diagnostic and histopathologic techniques. The majority of cases of cholangiocarcinoma (70%) are reported to be sporadic, but risk factors exist that vary geographically. Parasitic infection with either of the liver flukes *Opisthorchis viverrini* and *Clonorchis sinensis* increases the risk for cholangiocarcinoma 5-fold and is one of the most important causative factors in eastern Asia. In the western hemisphere, however, cholangiocarcinoma is most strongly associated with primary sclerosing cholangitis, cirrhosis, cholelithiasis, and lifestyle-related factors such as obesity, diabetes, alcohol use, and tobacco use.[5]

In almost two-thirds of people with cholangiocarcinoma (65%), nonresectable disease is diagnosed, and in a substantial number of patients, a relapse occurs after surgery with curative intent.[6-8] Overall survival (OS) remains dismal, with a 5-year survival rate for all-stage disease of approximately 8% for intrahepatic cholangiocarcinoma and of 10% for extrahepatic cholangiocarcinoma, according to the Surveillance, Epidemiology, and End Results (SEER) Program database (2009-2015).[9] According to the 2019 American Society of Clinical Oncology (ASCO) clinical practice guidelines, the standard of care for patients with resected disease is adjuvant capecitabine for 6 months and a consideration of chemoradiation for those with positive surgical margins or extrahepatic cholangiocarcinoma.[10] In advanced, unresectable cases, the current standard of care is a combination of cisplatin and gemcitabine according to the phase 3 ABC-02 and BT-22 trials.[11,12] The median OS was similar in the 2 trials (11.2 vs 11.7 months), although a considerably higher percentage of patients in the BT-22 trial received second-line chemotherapy (75% vs 15%).[13] However, the standard-of-care first-line chemotherapy may change in response to the results of multiple phase 3 frontline studies that are ongoing. For example, a phase 2 trial comparing patients with advanced biliary tract cancers vs historical controls showed an improvement in median progression-free survival (PFS; 11.8 months vs 8 months in the ABC-02 trial) and in OS (19.2 vs 11.7 months in the ABC-02 trial) with the addition of nab-paclitaxel (Abraxane, Celgene) to gemcitabine and cisplatin.[14] A randomized phase 3 trial, S1815 from SWOG, is currently under way to confirm the efficacy noted in the phase 2 trial (NCT03768414).

In the second-line setting, folinic acid, 5-fluorouracil (5-FU), and oxaliplatin (FOLFOX) showed an improvement in OS compared with best supportive care in the recently reported, randomized phase 3 ABC-06 trial (n=162). Patients randomly assigned to the FOLFOX arm had a median OS of 6.2 months, which was longer than the 5.3 months seen in patients receiving best supportive care (hazard ratio [HR], 0.69; *P*=.031). The rates of grade 3 and 4 toxicities were higher in the treatment arm as well (59% vs 39%). These data establish FOLFOX as the current second-line chemotherapy regimen, but a median OS of 6.2 months highlights the poor prognosis in these patients.[15] Moreover, patients whose disease has progressed on first-line chemotherapy often have a rapidly worsening performance status that can preclude further cytotoxic therapy. Given the poor response to chemotherapy and associated cumulative cytotoxicities, especially in the second-line setting, there is an unmet need to develop new strategies to improve survival in a high-mortality cancer such as cholangiocarcinoma.

Genetic Landscape of Cholangiocarcinoma

As we have seen in multiple tumor types during the past decade, the paradigm for cancer treatment has shifted dramatically toward precision oncology, and biliary tract cancers are no exception. Numerous studies in biliary tract cancers have shown a spectrum of heterogeneous molecular alterations. The molecular profiling of biliary tract carcinomas has drastically improved our understanding of the underlying pathophysiology and opened avenues for targeted therapeutic intervention. The relative frequencies of common genetic mutations in intrahepatic and extrahepatic cholangiocarcinomas are shown in the Table.[16] Intrahepatic and extrahepatic cholangiocarcinomas are not only anatomically distinct but also molecularly very different, which underscores their unique pathophysiology. The most common and clinically significant genetic aberrations in intrahepatic cholangiocarcinoma are fibroblast growth factor receptor 2 (*FGFR2*) fusions and isocitrate dehydrogenase 1/2 (*IDH1/2*) mutations, whereas the most significant genetic aberrations in extrahepatic cholangiocarcinoma are tumor protein 53 (*TP53*) mutations, *KRAS* mutations, and human epidermal growth receptor 2 (*HER2*) amplifications.

In the era of targeted therapy, cholangiocarcinoma is of special interest because its mutational spectrum seems to possess a high proportion of druggable alterations. In the prospective MOSCATO-01 trial evaluating the clinical benefit of high-throughput genomic analysis in hard-to-treat cancers, 43 of 1035 patients had advanced biliary tract cancer (4%). The median prior number of therapies in this cohort was 2 (range, 1-5), and the majority of patients had intrahepatic cholangiocarcinoma. Of the 34 patients for whom a molecular analysis was eventually obtained (79%), 23 (68%) had druggable molecular aberrations. A total of 18 patients (53%) either were enrolled on an appropriate clinical trial or received a matched targeted agent; these patients had

an impressive overall response rate of 33% and a disease control rate of 88%. The median OS in this group was longer in the patients who received a targeted agent than in those were not offered a targeted therapy, at 17 months (range, 15 to not reached) vs 5 months (range, 4 to not reached).[17] It was encouraging to see that more than half of the patients with a molecular evaluation had druggable mutations, a percentage higher than that for the overall cohort of patients in this trial (199/844, or 24%) and for those in similar trials of targeted therapies, such as the SAFIR-01 trial in breast cancer (55/407, or 13%) and the SHIVA trial (195/496, or 39%).[18,19] In another series of 28 patients with intrahepatic cholangiocarcinoma, two-thirds had targetable mutations.[20] In addition to exciting therapeutic implications, genetic aberrations in cholangiocarcinoma have helped in formulating a prognosis for what are considered to be a heterogeneous group of cancers. For example, in one retrospective series, mutations in *TP53*, *KRAS*, *CDKN2A/B*, and the mitogen-activated protein kinase (MAPK)/extracellular signal-regulated kinase (ERK) pathway were associated with significantly worse OS, whereas *FGFR2* fusions were associated with better OS.[21]

Fibroblast Growth Factor Receptor Alterations

The FGFR family consists of 4 transmembrane receptors and is responsible for ligand-dependent activation of the downstream MAPK, signal transducer and activator of transcription (STAT), and phosphoinositide 3-kinase/protein kinase B (PI3K/AKT) pathways.[22] Aberrant expression of FGFR has been implicated in various solid cancers, with exciting therapeutic implications.[23] Among cholangiocarcinomas, it is found almost exclusively in the intrahepatic subtype, at a frequency of 10% to 16%.[24,25] In addition, an interesting predilection for *FGFR* fusions has been noted in patients with cholangiocarcinoma who are of younger age, are female, or have undergone surgical resection with curative intent.[26] *FGFR* fusion–positive cholangiocarcinoma correlates with a relatively indolent course, evidenced by a median PFS of 14 months in patients with this alteration vs 3.1 months in patients with *FGFR*–wild-type disease.[27] In another retrospective analysis, OS was longer in patients with *FGFR* fusions than in those without *FGFR* fusions (37 vs 20 months; $P<.001$), a difference that remained significant after 36 patients treated with FGFR inhibitors were excluded.[28]

Several FGFR-targeting inhibitors have been evaluated in cholangiocarcinoma, with promising results. Infigratinib is an adenosine triphosphate (ATP)–competitive oral tyrosine kinase inhibitor selective for FGFR1-3 that was evaluated in a phase 2 clinical trial at a dose of 125 mg daily for 21 days in 28-day cycles. A total of 71 patients were enrolled, with a median age of 53 years. Patients had received a median of 2 prior therapies. At a median follow-up of 8.4 months, the overall response rate was 31% (95% CI, 20.5%-43.1%), with a median PFS of 6.8 months (95% CI, 5.3-7.6) and a median OS of 12.5 months (95% CI, 9.9-16.6). Two-thirds of the patients (66.2%) experienced grade 3 or 4 adverse events; the most commonly reported side effects were hyperphosphatemia (73.2%), fatigue (49.3%), stomatitis (45.1%), alopecia (38%), and constipation (35.2%).[29]

Derazantinib is another pan-FGFR inhibitor. It was evaluated in a multicenter phase 1/2 study in patients (n=29) harboring *FGFR2* fusions in the second-line setting. Disease control was achieved in the majority of the patients (82.8%), with an overall response rate of 20.7%. The median PFS was 5.7 months (95% CI, 4.04-9.2

Table. Common Genetic Aberrations in Intrahepatic and Extrahepatic Cholangiocarcinoma

Genetic Aberration(s)	Intrahepatic Cholangiocarcinoma, %	Extrahepatic Cholangiocarcinoma, %
TP53 mutation	2.5-44	40
FGFR1-3 fusion, amplification, and mutation	11-45	–
KRAS mutation	11-25	8-42
IDH1/2 mutation	23-28	–
ARID1A mutation	15-36	12
CDKN2A/B loss	6-30	17
SMAD4 mutation	4-17	21
EGFR overexpression	11-27	5-9
MCL1 mutation	21	–
HER2 amplification	–	11-17
MLL3 mutation	15	–
BAP1 mutation	13	–
HER3 mutation	7	–
CDK6 mutation	6	–
PIK3CA mutation	–	7
BRAF V600E mutation	3-7.1	3

–, not known.

months). Grade 3 or 4 adverse events occurred in just over one-quarter of the patients (27.6%). The most common all-grade toxicities were asthenia/fatigue (69%), eye toxicity (41.4%), and hyperphosphatemia (75.9%).[30]

Recently, pemigatinib (Pemazyre, Incyte), an FGFR inhibitor that targets FGFR1-3, was evaluated in a multicenter phase 2 trial that included 107 patients with *FGFR2* fusions/rearrangements, 20 patients with other *FGFR* alterations, and 18 patients without any *FGFR* alterations. Pemigatinib was given daily at a dose of 13.5 mg every 2 weeks with 1 week off until disease progression or unacceptable toxicities. Of the patients with *FGFR2* fusions/alterations, more than one-third (35.5%) had an objective response at a median follow-up of 17.8 months (interquartile range [IQR], 11.6-21.3). A complete response was noted in 3 patients (2.8%), and disease remained stable in another 46.7% of the patients. The median duration of response was 7.5 months (95% CI, 5.7-14.5). The median PFS in patients with *FGFR2* fusions or rearrangements was 6.9 months (6.2-9.6), whereas the median OS was 21.1 (95% CI, 14.8 to not reached). A response was not achieved in any of the patients who had other *FGFR* alterations or no *FGFR* alterations, with disease stability rates of 40% and 22.2%, respectively. Almost two-thirds of the patients (64%) had grade 3 or higher adverse events, with hyperphosphatemia the most common all-grade adverse event (60%), followed by alopecia (46%), dysgeusia (38%), diarrhea (34%), and fatigue (31%). Common grade 3 or 4 adverse events included hyperphosphatemia (12%), arthralgia (6%), stomatitis (5%), hyponatremia (5%), abdominal pain (5%), and fatigue (5%).[31] Pemigatinib was granted accelerated approval for the treatment of adults with previously treated, unresectable, locally advanced or metastatic cholangiocarcinoma with *FGFR2* fusions/rearrangements.[32]

Futibatinib (TAS-120) is a small-molecule inhibitor of FGFR1-4 that showed encouraging results in a phase 2 trial of patients with relapsed cholangiocarcinoma (n=103) who had *FGFR* fusions. Interim data reported for 67% of patients showed an overall response rate of 34.3% and a disease control of 76.1%. Grade 3 or 4 toxicities were reported in 73.1%. The most common all-grade toxicities included hyperphosphatemia (79.1%), diarrhea (37.3%), and dry mouth (32.8%).[33]

Multicenter randomized phase 3 clinical trials are currently looking at futibatinib, infigratinib, and pemigatinib vs a combination of gemcitabine and cisplatin as first-line treatment in patients with unresectable disease who have *FGFR2* fusions (NCT04093362, NCT03773302, and NCT03656536).[34]

Despite the impressive results we have discussed, resistance to FGFR2-directed treatment is a significant problem. Various implicated mechanisms include gatekeeper mutations in the FGFR kinase domain, activation of parallel membrane receptor signaling pathways, and activation of drug efflux transporter (ATP-binding protein G2).[35] Future directions to overcome this problem may include selecting anti-FGFR2 agents on the basis of resistance patterns and utilizing combination therapies to inhibit parallel pathways for cellular proliferation.

Isocitrate Dehydrogenase

The *IDH1* and *IDH2* genes code for the enzyme isocitrate dehydrogenase in the cytoplasm and mitochondria, respectively, and are responsible for catalyzing the conversion of isocitrate to α-ketoglutarate. Genetic aberrations in the *IDH1* and *IDH2* genes lead to neomorphic enzyme activity that transforms α-ketoglutarate to 2-hydroxyglutarate, which is implicated in tumorigenesis via cell signaling, epigenetic regulation, and extracellular matrix maturation.[36-39] *IDH1*/2 mutations have been described in various malignancies, such as acute myeloid leukemia, glioblastoma, chondrosarcoma, and cholangiocarcinoma.[40] In cholangiocarcinoma, they are found almost exclusively in the intrahepatic subtype in about 15% to 20% of patients.[27]

The prognostic significance of *IDH1/2* genetic aberrations is less clear.[41] For example, in a retrospective analysis, the 3-year survival rate was 33% in patients with *IDH1/2* mutations vs 81% in patients with wild-type *IDH*. This difference was significant even after adjustment for the patients' stage of disease and age.[42] On the other hand, multiple analyses have shown a trend toward longer disease-free survival and OS in patients with *IDH* mutations,[41,43] and yet another analysis reported no association between *IDH* mutation and prognosis.[44]

Ivosidenib (AG-120), which is a potent, targeted inhibitor of mutated *IDH1* that is administered orally, was evaluated in the multicenter randomized double-blind placebo-controlled phase 3 ClarIDHy study. This study enrolled patients with advanced cholangiocarcinoma that had progressed on up to 2 previous lines of therapy. A total of 185 patients with a median age of 61 years (range, 33-80) were randomized to either ivosidenib (n=124) or placebo (n=61). A partial response was seen in 3 patients (2%) in the treatment group, with 51% of patients having stable disease. By contrast, no patients in the placebo group had a response, and 28% had stable disease. After patients were followed for a median of 6.9 months (IQR, 2.8-10.9), the median PFS was 2.7 months (IQR, 1.6-4.2) in the ivosidenib group *vs* 1.4 months (IQR, 1.4-1.6) in the placebo group ($P<.0001$). The difference in median OS according to the intention-to-treat analysis was statistically not significant, at 10.8 months (95% CI, 7.7-17.6)

vs 9.7 months (95% CI, 4.2-12.1; HR, 0.69; *P*=.060). Importantly, 35 patients from the placebo group crossed over to the treatment group after progression. When adjusted for crossover, the median OS in the placebo group was 6 months (HR, 0.46; *P*=.0008). Serious adverse events were reported in 30% of patients in the ivosidenib arm, but only 2% were deemed treatment-related; these included grade 4 hyperbilirubinemia, grade 3 cholestatic jaundice, grade 2 QT prolongation on electrocardiogram, and grade 3 pleural effusion. Common all-grade toxicities in the treatment arm included nausea (35%), diarrhea (31%), fatigue (26%), cough (21%), abdominal pain (21%), vomiting (19%), and ascites (20%).[45] These data are consistent with phase 1 data for ivosidenib in 73 patients with a median age of 60 years (range, 32-81 years), which showed an overall response rate of 5% and disease stability in 56%. Median PFS and OS were 3.8 months and 13.8 months, respectively.[46] Mature OS data are pending and will likely affect the potential approval of this drug by the US Food and Drug Administration.

BRAF V600E

The *BRAF* V600E mutation leads to aberrant activation of the MAPK pathway, which regulates cellular proliferation and survival. The *BRAF* V600E mutation has been reported in various malignancies and is of established therapeutic significance in melanoma, non–small cell lung cancer, colorectal cancer, anaplastic thyroid cancer, and other forms of cancer. It is found in approximately 5% to 7% of biliary tract carcinomas.[47,48] The presence of a *BRAF* mutation has prognostic significance because these patients have a more advanced tumor stage at the time of resection, are more likely to have lymph node positivity, and have a shorter OS.[49]

A phase 2 basket study of single-agent vemurafenib (Zelboraf, Genentech/Daiichi Sankyo) in nonmelanoma cancers with the *BRAF* V600E mutation included 8 patients with cholangiocarcinoma. A partial response was noted in 1 patient, stable disease in 4 patients, and disease progression in 3 patients.[50] Experience in melanoma has shown that dual BRAF and MEK inhibition reduces the risk for death and results in fewer occurrences of cutaneous squamous cell carcinoma and other skin-related conditions compared with BRAF inhibitor monotherapy.[51] ROAR was a phase 2 multicenter basket trial evaluating a combination of the BRAF inhibitor dabrafenib (Tafinlar, Novartis) and the MEK inhibitor trametinib (Mekinist, Novartis) in patients who had cancers with the *BRAF* V600E mutation. The study enrolled 43 patients with advanced biliary tract cancer that had progressed on frontline chemotherapy. After a median follow-up of 10 months (IQR, 6-15), the investigator-assessed overall response rate was 51%. A total of 40% of patient had stable disease and 7% had progressive disease. The median duration of response was 9 months (95% CI, 6-14). Investigator-assessed median PFS was 9 months (95% CI, 5-10), whereas median OS was 14 months (95% CI, 10-33) in a heavily pretreated patient population. Serious adverse events occurred in 40% of the patients, but only 21% were deemed treatment-related. The most common all-grade adverse events were pyrexia (60%), nausea (42%), vomiting (33%), and fatigue (33%).[52]

Human Epidermal Growth Factor Receptor

The human epidermal growth factor receptor (HER) family, which consists of HER1 (epidermal growth factor receptor [EGFR]), HER2, HER3, and HER4, is of therapeutic and prognostic significance in breast and gastroesophageal adenocarcinoma.[53,54] The HER family receptors consist of an extracellular ligand-binding domain, a transmembrane lipophilic component, an intracellular tyrosine kinase domain, and an intracellular C terminus.[55] Ligand binding leads to receptor homo-/heterodimerization, which induces activation of the intracellular tyrosine kinase domain, in turn eventually causing the activation of downstream signaling cascades, including the MAPK and PI3K/ATK pathways.[56] In a meta-analysis of 27 clinical trials, the HER2 overexpression rate was 19.9% in extrahepatic biliary tract cancer and 4.8% in intrahepatic cholangiocarcinoma.[57] The prognostic significance of HER2 overexpression is less clear.[5861]

Targeting *HER2* amplification is of therapeutic importance in cholangiocarcinoma. The MyPathway study was a phase 2 multicenter basket clinical trial of patients who had *HER2*-amplified cancers treated with pertuzumab (Perjeta, Genentech) and trastuzumab, including 11 patients with biliary cancer. After a median follow-up of 4.2 months (range, 2-12), 4 patients had a partial response and 3 patients had stable disease. Median PFS was longer in those with *HER2*-amplified disease (n=8) than in those with *HER2*-mutated disease (n=3), at 4.2 months (range, 1.2-5.4) vs 2.8 months (range, 1.4-2.8).[62] Neratinib (Nerlynx, Puma Biotechnology), which is an oral, irreversible tyrosine kinase inhibitor of EGFR, HER2, and HER4, was tested in the SUMMIT basket trial. The number of patients with biliary tract carcinoma was 21 (intrahepatic cholangiocarcinoma in 4, extrahepatic cholangiocarcinoma in 5, gallbladder cancer in 9, and ampullary cancer in 2). The median number of prior therapies was 2 (range, 0-7). The overall response rate was 10%, and the rate of stable disease at 16 weeks was 20%. The median PFS was reported to be 1.8 months (range, 0.9-3.7). In the 2 patients who responded, the duration of response was 3.7 months in the patient with

cholangiocarcinoma and 3 months in the patient with gallbladder cancer. A total of 70% of the patients had at least one grade 3 or 4 adverse event. The most common all-grade toxicities were vomiting (55%), diarrhea (50%), nausea (40%), and fatigue (40%).[63] Updated data were recently reported from the phase 2 TreeTopp clinical trial evaluating varlitinib, a reversible, small-molecule pan-HER inhibitor, in advanced biliary tract cancer. Unselected patients (n=127) were randomly assigned to receive either varlitinib plus capecitabine (n=64) or placebo plus capecitabine (n=63). The overall response rate was numerically higher in the treatment group than in the placebo group (9.4% vs 4.8%; *P*=.42), but the difference was not statistically significant. Similarly, median PFS was identical in the 2 groups (2.8 months), and no statistically significant difference occurred in the median OS (7.5 vs 7.8 months; *P*=.66). Although the study was not powered for subgroup analysis, improvement in median PFS was noted in the patients in the treatment arm who had gallbladder carcinoma (2.9 vs 1.6 months). Grade 3 or 4 toxicities were reported in 66% of the patients in the treatment arm vs 59% of those in the placebo arm. The incidence of all-grade hyperbilirubinemia, diarrhea, and fatigue was higher in the treatment arm.[64]

Epidermal Growth Factor Receptor

The EGFR signaling pathway plays a significant role in the pathogenesis and progression of various malignancies, including biliary tract cancers.[65-67] EGFR overexpression occurs in 27.4% of patients with intrahepatic cholangiocarcinoma and 19.2% of those with extrahepatic cholangiocarcinoma, and it is independently correlated in extrahepatic disease with tumor stage, lymph node involvement, perineural involvement, and lymphovascular involvement.[60] Blockade of EGFR tyrosine kinase activity in prospective clinical trials reduced the proliferation of cholangiocarcinoma cells in vitro; however, targeting EGFR through either tyrosine kinase inhibitors (eg, gefitinib [Iressa, AstraZeneca] and erlotinib) or monoclonal antibodies (ie, cetuximab [Erbitux, Lilly] and panitumumab [Vectibix, Amgen]), given alone or in combination with chemotherapy, has yielded only modest results without any significant improvement in median PFS or OS.[68-71]

Vascular Endothelial Growth Factor

Vascular endothelial growth factor (VEGF) overexpression, which is implicated in various malignancies, occurs when a certain family of molecules attach to VEGF ligands, leading to angiogenesis, tumor growth, and tumor propagation. Tumor biopsy specimens from 236 patients with cholangiocarcinoma showed VEGF overexpression in 53.8% of the intrahepatic cholangiocarcinomas and 59.2% of the extrahepatic cholangiocarcinomas.[60] VEGF overexpression is known to be associated with intrahepatic metastasis in intrahepatic cholangiocarcinoma and lymph node involvement.[60,72] A phase 2 trial of dual VEGF and EGFR inhibition with erlotinib and bevacizumab (n=53) showed a 12% partial response rate and a 51% rate of stable disease. The median time to progression and the OS were 4.4 and 9.9 months, respectively. This combination was associated with elevated toxicity rates, with at least one-third of patients experiencing a grade 3 or 4 adverse event.[73] Dual VEGF and MEK inhibition with pazopanib (Votrient, Novartis) and trametinib failed to achieve a statistically significant improvement in 4-month PFS over the prespecified null-hypothesis 4-month PFS.[74] A phase 2 trial of single-agent regorafenib (Stivarga, Bayer HealthCare) in 43 patients showed a partial response in 11% and stable disease in 44%. The median PFS was 15.6 weeks and the median OS was 31.8 weeks, and 40% patients had grade 3 or 4 adverse events.[75]

Neurotrophic Receptor Tyrosine Kinase Gene Fusion

Neurotrophic tyrosine kinase (NTRK) inhibitors have received a tumor-agnostic approval for the treatment of advanced solid cancers that harbor the *NTRK* gene fusion with a known acquired resistance mutation. In a phase 1/2 trial of larotrectinib (Vitrakvi, Loxo), the response rate in 55 patients with *NTRK* fusion–positive disease was 75%. The rate of treatment-related grade 3 or 4 adverse events was less than 5%. This study enrolled only 2 patients with cholangiocarcinoma. The rate of *NTRK* fusion mutations in cholangiocarcinoma is quite low, with one series reporting a rate of 0.75%.[76]

Microsatellite Instability/Programmed Death Ligand 1

Immune checkpoint inhibitors can be used as second-line treatment in patients with advanced biliary tract cancers whose tumors are deficient in mismatch repair proteins (dMMR), are high in microsatellite instability (MSI-H), or express programmed death ligand 1 (PD-L1). The phase 2 KEYNOTE-158 study included 22 patients with dMMR/MSI-H cholangiocarcinoma. The overall response rate was 41%, and the duration of response ranged from 4.1 to 24.9 months. The median PFS was 4.2 months, and the median OS was 24.3 months.[77] Unfortunately, only a fraction of advanced biliary tract cancers are dMMR (1%-1.3%).[78] In one phase 2 trial, nivolumab (Opdivo, Bristol-Myers Squibb) was evaluated in 46 patients with advanced biliary tract cancer in the relapsed/refractory setting. The investigator-assessed objective response rate

was 22%, and the disease control rate was 59%. These numbers were higher than those on central independent review, which reported a response rate of 11% and a disease control rate of 50%. The discrepancy was attributed to new and enlarging lymphadenopathy secondary to treatment with a checkpoint inhibitor. At a median follow-up of 12.4 months, the median PFS was 3.68 months, and the median OS was 14.2 months. Interestingly, of the 10 patients who had a response, none had dMMR disease, whereas 9 patients had PD-L1 expression. Of note, 18 of 42 patients had PD-L1 expression (PD-L1 cutoff: >1% of tumor cells).[79]

Conclusion

In summary, the cholangiocarcinomas are a heterogeneous group of cancers with distinct etiologies and important molecular differences. Extensive molecular profiling studies have revealed a wide spectrum of potentially targetable genetic aberrations, which has led to an exciting era of precision oncology in this disease. Positive results from trials evaluating targeted therapies against *FGFR2* alterations, *IDH1/2* mutations, and the *BRAF* V600E mutation have broadened our therapeutic armamentarium for patients whose disease has progressed on first-line chemotherapy. Genetic profiling should be standard in all advanced cholangiocarcinomas, and patients should be encouraged to enroll in clinical trials so that the rapid pace of drug development in this field can continue.

Disclosures

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