Molecular Targets and Therapies for Ampullary Cancer

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ABSTRACT

Ampullary carcinomas are rare but increasing in incidence. Ampullary cancers have molecular alterations that guide choice of therapy, particularly in nonresectable cases. These alterations can be more common by subtype (intestinal, pancreaticobiliary, or mixed), and next-generation sequencing is recommended for all patients who cannot undergo surgery. In this article, we review the approach to tissue acquisition and consideration for molecular testing. Common molecular targets of interest in ampullary cancer are also discussed in this review, including HER2/ERBB2, HER3, tumor mutation burden, microsatellite instability, KRAS, and germline BRCA and ATM mutations, along with emerging and rarer alterations.

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Ampullary cancer arises from the ampulla of Vater at the confluence of the distal bile duct, pancreatic duct, and duodenum. There are emerging molecularly targeted treatments that can be considered. This is important as ampullary cancers vary by intestinal and pancreatobiliary subtypes, which confer selected alterations that can guide treatment.

Anatomy and Pathology of Ampullary Cancer

Periampullary carcinoma describes neoplasms arising from the head of the pancreas, distal common bile duct (CBD), and duodenum. This is distinguished from ampullary carcinoma, which defines tumors centered in the region of the ampulla of Vater. The ampulla, named after the 18th century physician Dr. Abraham Vater, was first described in 1543. It is formed by the convergence of the CBD and main pancreatic duct (MPD) which then forms the major papilla. The ampullary region encompasses the distal segments of the MPD and CBD, while the periampullary region encompasses the ampulla of Vater, distal CBD, and the adjacent duodenum.

Most ampullary cancers consist of adenocarcinomas with 3 histologic subtypes: intestinal, pancreaticobiliary, or mixed subtype, based on epithelial origin (Figure 1). The intestinal form originates from the intestinal epithelium, whereas the pancreaticobiliary subtype comes from the distal pancreatic duct and CBD. Frequencies of the ampullary cancer subtypes are variable, but several reports show the intestinal type is present in approximately 30% to 50% of patients, pancreaticobiliary in approximately 30% to 50%, and mixed subtype in 5% to 10%, although this mixed subtype has been reported in up to 40% of patients.

Immunohistochemical (IHC) markers can distinguish between the different types. CK7 can distinguish pancreatobiliary adenocarcinomas from nonmucinous adenocarcinomas. MUC1+/CK7+ immunohistochemical markers are associated with pancreatic ductal carcinomas, ampullary carcinoma of pancreaticobiliary origin, and cholangiocarcinoma. In contrast, MUC2+/CDX2+/CD20+ is associated with intestinal-type ampullary adenocarcinoma.

Differentiating histologic subtypes has biologic and prognostic relevance for ampullary adenocarcinomas. Pancreatobiliary-type ampullary cancers are associated

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with a more aggressive phenotype compared with intestinal-type tumors.\textsuperscript{4,7,11,12} Patients with an intestinal phenotype and no lymph node involvement had an excellent prognosis, with a 5-year survival of 88.4% and median survival of 172.8 months.\textsuperscript{8} Patients with a pancreaticobiliary phenotype with lymph node metastases had the poorest prognosis, with a 5-year survival rate of 20% and median survival of 7.4 months.\textsuperscript{8} Patients with mixed subtype histology tumors have improved prognosis closer to the intestinal subtype in most of the studies where it is reported.\textsuperscript{7} However, this prognosis finding is not universal, with some studies reporting that histologic subtype did not predict outcome beyond other clinical findings, such as stage or nodal involvement.\textsuperscript{10}

Recurrence is common in ampullary cancer, at 43.5% after curative intention surgery.\textsuperscript{13} Recurrence patterns include liver (23%), locoregional sites (19%), peritoneum (7%), and bone (5%).\textsuperscript{14,15} Microscopic venous and lymphatic vessel invasion is correlated with an increase in distant metastases and lymphomatous metastases.\textsuperscript{14} Although pancreatic invasion and tumor size predicted locoregional occurrence, lymph node involvement was the sole predictor for liver metastases.\textsuperscript{14}

**Epidemiology of Ampullary Cancer**

Ampullary cancer accounts for only 0.2% of gastrointestinal cancers, but incidence has been increasing since 1970 at a rate of 0.5% to 0.9% per year.\textsuperscript{12,16–19} It remains uncertain how improved imaging and procedural modalities have contributed to this increase. Multiple meta-analyses of ampullary, hepatic, pancreatic, and biliary cancers demonstrate that most patients are male and aged >60 years, with an increasing incidence over the previous 3 decades.\textsuperscript{12,19} Black race, higher stage and grade, and nonsurgical treatment are associated with worsened clinical outcomes.\textsuperscript{12,16,19}

**Genetic Risk Factors**

Several genetic mutations are considered risk factors for ampullary cancer. Patients with ampullary cancer can harbor pathogenic germline alterations in \textit{BRCA2}, \textit{ATM}, \textit{RAD50}, and \textit{MUTYH}.\textsuperscript{20} Familial adenomatous polyposis (FAP; \textit{APC} gene), Peutz-Jeghers syndrome (PJS; \textit{STK11} gene), and hereditary nonpolyposis colorectal cancer (HNPCC; \textit{MLH1}, \textit{PMS2}, \textit{MSH2}, \textit{MSH6} genes) are also associated with ampullary cancers (Figure 2).\textsuperscript{21,22} Patients with FAP and PJS tend to be diagnosed before the third decade of life, whereas those with HNPCC tend to be diagnosed after the third

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**Figure 1.** Subtypes of ampullary carcinoma. Pancreatobiliary subtype arising within the ampulla (A) and composed of small glands with cuboidal cells exhibiting minimal pleomorphism in a desmoplastic stroma (B) (hematoxylin eosin [H&E], original magnification $\times 20$). The intestinal subtype arises from an exophytic mass in the periampullary region (C) and is composed of columnar cells with elongated, pseudostratified nuclei with scattered goblet cells (D) (H&E, original magnification $\times 200$). (Images courtesy of Kristina Matkowskyj, MD, PhD, Madison, Wisconsin.)
decade of life. Given the nature of FAP, patients are more likely to receive surveillance compared with the general population, and thus see improved outcomes with early diagnosis and surgical intervention.23 Of those with PJS, there is a greater risk of developing ampullary cancer after the sixth decade of life, at 11.1%, with the cumulative risk increasing by an order of magnitude from age 40 to 70 years from 2.4% to 25.6%.23 Patients with neurofibromatosis type 1 (NF1) with mutations in the NFI gene also have an association with ampullary tumors.24 Most of these were somatostatinomas, but adenocarcinomas also have an association.24

**Tissue Acquisition**

For patients with advanced disease who are otherwise not candidates for pancreaticoduodenectomy, multiple approaches can be used to obtain tissue samples.25 When liver metastases are present, liver biopsy is generally preferred because it allows staging and can often be obtained with core needle biopsy with sufficient tissue for comprehensive next-generation sequencing (NGS). For localized disease, although core biopsy has traditionally been characterized as the gold standard technique to maximize diagnostic yield in ampullary cancer, other less-invasive approaches likely also have utility (Figure 3). Endoscopic ultrasound (EUS) with fine-needle biopsy (FNB) has been shown to be an effective biopsy method in ampullary tumors, with overall diagnostic accuracy near 90%.25,26 One study examining the use of ampullary and pancreatic carcinoma samples obtained by EUS with FNB for molecular profiling found a 100% concordance rate with matched surgical pathology specimens in the identification of 13 pathogenic mutations. The overall concordance rate was 83% due to the detection of multiple genetic mutations in FNB samples that were not identified in surgical specimens.27

Brush cytology represents a less-invasive tissue sampling option. Previous studies have demonstrated that brush biopsies have high diagnostic specificity and can have applications in identifying pathogenic mutations in pancreatic and biliary neoplasms.28-30 Further research is needed to determine the true sensitivity of brush biopsy cytology for detection of pathogenic mutations in ampullary cancer.

**Considerations for Type of NGS Panel**

Standard pathologic evaluation should be prioritized, including evaluation for expression of mismatch repair proteins and HER2. The remaining tissue block can then be considered for
Due to the infiltrative nature of ampullary cancer, including excessive desmoplastic stroma, core needle biopsy specimens have notoriously contained limited tumor content. To overcome this limitation, techniques of microdissection can be integrated, with series reporting approximately 70% of EUS core biopsies evaluable for NGS profiling. In-house targeted panels developed for colorectal cancer (ie, KRAS, BRAF) are not comprehensive enough for ampullary carcinomas given the breadth of possible molecular targets present in this diverse cancer.

To understand the appropriate NGS testing platform, it is important to consider the benefits and limitations of testing. The 2 commonly used NGS technologies include PCR or hybrid capture techniques. Most recently, a multiplex PCR-based testing platform using StrataNGS (Strata Oncology) reported improved testing capabilities, specifically for limited tissue specimens. This type of testing can be prioritized in cases of limited available blocks with as little as 2 mm² of tissue. Although hybrid capture testing is widely available through commercial vendors (eg, Tempus xT or FoundationOne CDx), they often require >20% tumor content with larger tissue blocks.

Liquid tumor biopsy, which uses blood samples containing circulating tumor DNA (ctDNA) or cell-free DNA, is a form of noninvasive tissue sampling. Molecular profiling of liquid biopsies has shown high levels of concordance with tissue biopsy in certain gastrointestinal malignancies, although limitations such as reduced gene fusion sensitivity have been noted. Sparse data exist to define sensitivity and specificity for detecting gene mutations of interest in ampullary cancers.

It is important to acknowledge the limitations of different testing platforms. Although ctDNA assays lack sensitivity in rare fusion events, they have excellent sensitivity in characterizing resistance mechanisms with variants. DNA-based hybrid capture sequencing has limited capabilities in identifying gene fusions, specifically due to the large variance of partner genes in a fusion event. NGS platforms that integrate mRNA amplicon–based testing provide optimized identification of rare gene fusions; however, this comes at the cost of complex primer design and bioinformatics analyses. Taken together, tissue-based NGS, if feasible, remains a standard of care, with an emerging role for ctDNA when adequate tissue sampling is not possible.

**Precision Targets: An Emerging Landscape**

**HER2**

HER2 (ERBB2) functions as a transmembrane protein in the growth signaling across cancer types. It functions as a homodimer (HER2 only) or heterodimer (HER2 with HER3).
Targetable genomic alterations in HER2/3 have been reported more commonly in extrahepatic cholangiocarcinoma (~15%) versus intrahepatic cholangiocarcinoma (~7%). Although HER2 amplification is exceedingly rare in pancreatic cancer (<1%), it is an important subgroup of ampullary cancer. The largest retrospective series of ampullary cancer has reported the frequency of HER2 amplification as high as 13%, although this has been highly varied across historical cohorts.

Importantly, HER2 has been successfully targeted in prospective clinical trials. The phase II MyPathway basket trial evaluated open-label trastuzumab and pertuzumab for metastatic HER2-amplified solid organ malignancy defined as IHC 3+, in situ hybridization ratio >2.0, or NGS copy number >6.0. The combination was active in ampullary cancers, including partial response observed in 40% (2/5) and an extended duration of disease control. These results are consistent with early reports from the TAPUR basket trial across biliary tract cancer, although this comparison is limited by the fact that the TAPUR trial had only 3 patients with ampullary cancer. When clinical trials cannot be considered, the combination of trastuzumab and pertuzumab has the most established safety for HER2-directed therapy.

Additionally, methods for targeting HER2 are ongoing. The phase II HERB trial investigated the use of trastuzumab deruxtecan in patients with HER2-positive (including low expression) advanced biliary tract cancer achieving a 30% overall response rate (ORR). The subgroup analysis of 3 subjects with ampullary cancer was not included in initial reporting. For activating HER2 mutations, the phase II open-label SUMMIT trial eligibility allowed ampullary cancer. Although responses were seen for cholangiocarcinoma, disease control was reported for a single patient with ampullary cancer (1/4).

Ongoing trials specific to ampullary cancer include the phase II basket of tucatinib and trastuzumab (ClinicalTrials.gov identifier: NCT04579380). Additionally, multiple early-phase strategies of targeting HER2 are in development using tyrosine kinase inhibitors, novel antibody-based therapies, and immunotherapeutic strategies. Importantly, many early-phase trials allow inclusion of low HER2 expression by IHC (1+), for which clinical trial enrollment is recommended currently.

**KRAS and EGFR Mutations**

KRAS mutations are present in 30% to 40% of ampullary cancers. The presence of a KRAS mutation predicts worse progression-free survival and overall survival among patients with ampullary cancer. KRAS G12D mutation is the most common mutation found in ampullary cancers. The pancreateobiliary subtype of ampullary cancer appears to be more likely to harbor a KRAS mutation than the intestinal subtype (61% vs 29%, respectively). A randomized phase II study, called BINGO, investigated the addition of cetuximab (an EGFR monoclonal antibody) to gemcitabine and cisplatin for first-line chemotherapy treatment of advanced biliary tract cancers, including ampullary cancer. Although this was a negative trial, it is notable that only one patient with ampullary cancer was enrolled, and KRAS status was not part of the inclusion criteria. Multiple other trials of EGFR-targeting agents in biliary tract cancer included so few patients with ampullary cancer as to be uninformative. A phase II study of panitumumab in patients with KRAS wild-type small bowel adenocarcinoma, including ampullary cancer, was negative. This study included only one patient with ampullary cancer, of the pancreaticobiliary subtype, and that patient did not have a response to panitumumab. As such, despite the abundance of data showing that EGFR-targeting agents, such as panitumumab and cetuximab, are effective in KRAS wild-type colorectal cancers, the NCCN Clinical Practice Guidelines in Oncology (NCCN Guidelines) for Ampullary Adenocarcinoma do not currently recommend use of anti-EGFR therapy.

**PD-L1/PD-1 and TMB**

Identifying patients whose cancers have a high tumor mutational burden (TMB) is important because this provides another potential target of treatment with immunotherapy. In 45 patients with ampullary cancer, 2 were found to have microsatellite instability and a high TMB of >15 nonsynonymous mutations/megabase (mut/Mb). Based on results from the KEYNOTE-158 trial, patients with previously treated, unresectable, or metastatic solid tumors were treated with pembrolizumab, and those with TMB-high (≥10 mut/Mb) tumors were found to have an ORR of 29% (30/102 patients) and median duration of response (DoR) that was not reached. As a result of these data, pembrolizumab has been approved for patients with TMB-high, advanced solid tumors, and we would recommend testing all patients with advanced ampullary cancers for TMB and considering pembrolizumab in patients with TMB-high disease, in alignment with the NCCN Guidelines for Ampullary Adenocarcinoma.

According to one report of patients with ampullary adenocarcinoma, PD-L1 expression by IHC was seen in 26.9% (7/26) patients; 85.7% (6/7) of these patients had intestinal-subtype ampullary cancers. Although there is a paucity of data surrounding the role of immunotherapy in PD-L1-expressed ampullary cancers due to the rarity of the disease, one case report demonstrated benefit of off-label pembrolizumab in a patient with microsatellite-stable ampullary carcinoma with PD-L1 expression of 35% who had prolonged disease stability of 12 months while on pembrolizumab. At this time, the role of immunotherapy in PD-L1-expressed ampullary cancers is yet to be defined, aside from those with a high TMB or microsatellite instability.
**BRCA Mutations**

In ampullary cancer, pathogenic germline variations in *BRCA* have been described with rates of 7% to 14%. Even higher frequencies were reported in a study in which whole-exome sequencing of 37 patients with ampullary carcinoma revealed that 23 (62%) had mutations in *BRCA1* and 19 (51%) had mutations in *BRCA2*. These data are important because pathogenic mutations in *BRCA1*2 in other malignancies have implications for treatment, particularly for the use of platinum chemotherapy as well as PARP inhibitors.

In a study of patients with a *BRCA* mutation and pancreatic adenocarcinoma, overall survival was improved for those treated with platinum-based chemotherapy (22 vs 9 months; *P* < .039). Similar findings have been seen in other studies of platinum regimens in patients with pancreas cancer and *BRCA1*2 or *PALB2* mutations. Furthermore, in a single-institution study of patients with ampullary cancer, 7% (3/44) were determined to have a pathogenic germline mutation in *BRCA2*. All of these patients received platinum-based chemotherapy, and 2 had a partial response on imaging. Given that treatment of the pancreaticobiliary subtype of ampullary cancers is largely extrapolated from treatment of pancreas cancer, this underscores the importance that patients with pathogenic mutations in *BRCA1*2 or *PALB2* are treated with platinum-based chemotherapy.

*BRCA* mutations also predict sensitivity to PARP inhibitors. A phase II study of olaparib was conducted in patients with metastatic pancreas cancer and germline *BRCA1*2 mutations and found a tumor response rate of 21.7% (5/23). Subsequently, the POLO trial demonstrated that olaparib was an effective maintenance therapy in patients with metastatic pancreas cancer with a germline *BRCA* mutation whose disease had not progressed on first-line platinum-based chemotherapy. This study found that progression-free survival was improved with olaparib compared with placebo (7.4 vs 3.8 months; *P* = .004). Because treatment of the metastatic pancreaticobiliary subtype of ampullary cancers mirrors that of pancreas cancer, it would be reasonable to consider maintenance olaparib in patients with advanced ampullary cancer and a *BRCA1*2 mutation.

**ATM Mutations**

In a cohort of patients with ampullary cancer, 7% (3/44) were found to have pathogenic germline mutations in *ATM*. Although guidelines currently suggest a first-line platinum-based regimen only for advanced pancreas cancer with either *BRCA* or *PALB2* mutation, given that *ATM*-mutated cells are predicted to be sensitive to platinum drugs, platinum-based chemotherapy may be considered for patients with ampullary cancer and an *ATM* mutation.

**Other Potentially Druggable Targets**

The NCCN Guidelines for Ampullary Adenocarcinoma note rarer molecular alterations, such as *NTRK* and *BRAF* V600E, which may be used to guide treatment and are generally available on most NGS panels. *NTRK* fusion is treated with larotrectinib or entrectinib. A pooled analysis of phase I and II studies of larotrectinib in any solid tumor with *TRK* fusion–positive tumors included 8 patients with colon cancer, 2 with pancreas cancer, and 2 with biliary tract cancer, and had an ORR of 79%. There have been other basket trials targeting the *BRAF* V600E mutation. Other targetable alterations that have few data specific to ampullary cancer include *ALK, NRG1, ROS1, FGFR* fusions, *IDH1*, and *RET*. Identification of these alterations in ampullary cancer should prompt a discussion of clinical trials or registries as the preferred treatment approach.

**Emerging Targets**

*MDM2* functions as a negative regulator of the tumor suppressor p53. Recent early-phase trial data have suggested the role of *MDM2* amplification as a precision target in biliary tract cancer using BI 907288, including a single-agent response in ampullary cancer. The pooled analysis included additional cases of biliary tract cancer in combination with ezabenlimab (anti-LAG-3), with 5 of 8 experiencing partial response (62.5%). The upcoming Brightline-2 clinical trial (ClinicalTrials.gov identifier: NCT05512377) is currently enrolling to further characterize this emerging target.

**Conclusions**

The understanding of ampullary cancer has been improved largely due to dedicated investigation of both histologic and molecular subtypes. For unresectable disease, mapping the histologic origin provides a guide for predicting effective systemic therapy. Multidisciplinary care should review adequate tissue sampling for comprehensive NGS. The design of hepatobiliary and pancreatic cancer basket trials should integrate ampullary cancer across study designs to characterize the actionability in this important cancer type.

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