Gastroenterology Research and Practice

Carcinoma of Pancreatobiliary Origin

Lead Guest Editor: Darina Kohoutova
Guest Editors: Jan Bures, Sameer Zar, and Ondrej Urban

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Editorial

Carcinoma of Pancreatobiliary Origin

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Cancers of the pancreas and biliary tree remain one of the most dreadful oncological diagnoses with a very poor prognosis. Our special issue focused on the management of precancerous conditions and efforts to find new biomarkers which would enable precise and early diagnosis of these deadly cancers. We also focused on the role of diagnostic and therapeutic endoscopy, treatment of established carcinomas, and effective palliation.

Pancreatic cysts including mucinous ones and solid-cystic pseudopapillary tumours belong to precancerous lesions of a pancreatic adenocarcinoma. Despite all the progress in this field, including publishing the recent guidelines [1, 2], our clinical decision-making remains limited and further research, which will stratify the risk, is needed. Precursors to cholangiocarcinoma have been defined; nevertheless, these are rarely diagnosed, and precise characteristics and features of their progression into cancer are yet to be assessed [3, 4]. Management of indeterminate biliary stenoses is difficult in the clinical practice and evaluation of bile acids in the liver bile might help to differentiate between benign and malignant biliary stenoses [5].

Endoscopy plays an important role in the diagnosis of extrahepatic cholangiocarcinoma and pancreatic tumours. Endoscopic ultrasound (with fine-needle aspiration or fine-needle biopsy) and endoscopic retrograde cholangiopancreatography are essential methods for obtaining the tissue diagnosis. Cholangioscopy is being used for direct visualization of biliary intraductal processes and to aquire suspicious/malignant tissue when classical methods (including cytology and biopsies) have failed [6, 7].

Tailored surgical and oncological treatment should be available to every patient if this is clinically appropriate. Still, only 20% of patients with pancreatic ductal adenocarcinoma have resectable or borderline resectable pancreatic cancer at diagnosis. Neoadjuvant treatment is being evaluated in this group of patients [8]. Chemotherapy regimens have shown incremental survival gains in those where surgery is not an option, but more progress in this area is needed [9]. The critical situation in the cholangiocarcinoma group of patients remains similar.

Tailored palliation plays a crucial role in pancreatic cancer and cholangiocarcinoma, taking into account, that these cancers are usually diagnosed in late stages. Effective biliary drainage belongs to the cornerstones of palliative interventions, yet drainage of especially hilar cholangiocarcinoma can be challenging. Drainage of nonatrophic segments should be planned in an optimal scenario with a magnetic resonance cholangiography, and appropriate stents should be used (plastic or uncovered metal stents) [10].

The guest editors will appreciate if the readers benefit from reading this special issue which provides an overview of the most up-to-date knowledge from the world-leading experts.

Conflicts of Interest

There is no conflict of interest for any of the Guest Editors.

Darina Kohoutová
Jan Bureš
Sameer Zar
Ondřej Urban

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Review Article

Open and Minimal Approaches to Pancreatic Adenocarcinoma

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Surgical options and approaches to pancreatic cancer are changing in the current era. Neoadjuvant treatment strategies for pancreatic cancer combined with the increased use of minimal access surgical techniques mean that the modern pancreatic surgeon requires mastering a number of surgical approaches with to optimally manage patients. Whilst traditional open surgery remains the most frequent approach for surgery, the specific steps during surgery may need to be modified in light of the aforementioned neoadjuvant treatments. Robotic and laparoscopic approaches to pancreatic resection are feasible, but these surgical methods remain in their infancy. In this review article, we summarise the current surgical approaches to pancreatic cancer and how these are adapted to the minimal access setting with discussion of the patient outcome data.

1. Introduction

Pancreatic adenocarcinoma (PDAC) is one of the leading causes of death with 432,242 deaths per year and 458,918 new cases recorded per year according to GLOBOCAN 2018 [1]. Despite significant progress in the detection and management of PDAC, the 5-year survival is still reported at a disappointing 9% [2]. Surgical resection is the only treatment option that offers the prospect of long-term remission; however, only approximately 20% of those patients presenting PDAC are eligible for resection [3]. Surgery for PDAC has evolved since Allen Whipple reported the first surgical series in 1935 [4] with recent studies reporting an improved 5-year survival of 30% [5]. Despite this progress in improving patient outcome following surgery for PDAC, the modern pancreatic surgeon is faced with a number of new challenges that can involve modifications to the traditional surgical approach. These include the advent of neoadjuvant regimens to PDAC such as those in the current ESPAC-5 trial [6]. In addition, surgical advancements in minimal access and robotic surgery have added further techniques to the pancreatic surgeon's armamentarium. In this review, we summarise the current surgical approaches available for the pancreatic surgeon when planning surgery for patients with PDAC.

2. Current Era of Pancreatic Surgery for PDAC

Approximately 65% of PDAC is located in the pancreatic head with 15% in the body or tail and multifocal disease accounting for the remaining cases [5]. Indeed, an inferior survival is noted in patients with PDAC located in the body and tail relative to the pancreatic head [6], and as discussed below, this may be accounted for by the extent of lymphadenectomy performed in distal pancreatectomy. Therefore, after appropriate preoperative staging and investigations, patients with PDAC are generally eligible for one of three operations: Whipple/pylorus preserving pancreaticoduodenectomy (PPPD), total pancreatectomy with or without splenectomy, or distal pancreatectomy with or without splenectomy (DP). PPPD in contrast to the classical Whipple procedure retains the distal stomach and pylorus with the aim of decreasing the postoperative incidence of delayed gastric emptying, marginal ulcerations, and bile reflux gastritis, but the data to support this surgical approach is lacking [7]. In the modern era, all surgical approaches to the pancreas can be performed via the open, laparoscopic, or robotic routes. For instance, PDAC limited to the pancreatic head with no vascular involvement would be suitable of Whipple/PPPD whilst multifocal disease would be suitable for total pancreatectomy

with splenectomy. There are now guidelines available on many aspects pertaining to pancreatic surgery for PDAC including the definition of standard lymphadenectomy, consensus statements of borderline resectable PDAC, and extended pancreatic resections, and the reader is referred here (https://www.ihpba.org/183_Guidelines-.html) for a further detailed information.

These international standardized nomenclatures for pancreatic surgery have allowed some degree of harmonisation in surgical approaches to PDAC and in time will also allow surgical data globally to be interpreted appropriately [8]. This is particularly important in an era of increased use of preoperative chemotherapy and/or chemoradiotherapy for PDAC in the presence of locally advanced disease (see below). The use of the neoadjuvant regimens means that the modern pancreatic surgeon must be prepared to adapt surgical approaches to achieve oncological pancreatic resections. To highlight this currently, patients with early-stage PDAC and no vascular involvement can be offered robotic pancreatic PPPD/Whipple (RPD) [9], whilst those surgeons further along the learning curve with laparoscopic approaches can offer laparoscopic PPPD/Whipple (LPD) with concomitant vascular resection [10] whilst patients receiving neoadjuvant chemoradiation will generally have open pancreatic resection [11].

As eluded to above, vascular involvement of either the superior mesenteric vein (SMV) and/or artery add an extra layer of complexity to surgical resection for PDAC. These patients are referred to as having "borderline resectable pancreatic cancer" (see below). Generally, these patients have limited vascular involvement with predominantly SMV involvement. A recent systematic review concluded that the portal vein and/or SMV resection combined with pancreatectomy is a safe and feasible, enabling an increased number of patients to undergo curative resection and improving patient survival [12]. However, in contrast, a recent meta-analysis suggests that patients undergoing vein resection as part of pancreatic resection have a higher R1 rate, lower survival, and the surgery was deemed not cost-effective [13], although a UK nationwide study has demonstrated that pancreatic resection combined with vein resection is superior to surgical bypass [14]. However, these data must be interpreted with caution; as surgical units comprising both hepatobiliary/transplant units, there is an increased likelihood of performing vascular resection due to the availability of cadaveric vein for portal vein/SMV reconstruction [15]. Whether cadaveric vein is used or another suitable conduit, the literature does support the use of vascular resection, especially venous resection, in PDAC to improve patient survival especially when neoadjuvant regimens have been utilized [16].

In summary, although international guidelines are available for the management of patients with PDAC, the optimal surgical approach in an era of increasing availability of surgical approaches and evolving neoadjuvant regimes means that constant reevaluation is required to ensure that patients receive optimal management. In the following section, we discuss the current surgical strategies available for the management of patient with PDAC.

3. Open Pancreatic Surgery with No Vascular Involvement

All patients with PDAC should have appropriate preoperative work-up with cross-sectional imaging and multidisciplinary team discussion prior to surgery. At present, most pancreatic surgeries are performed by surgeons specialising in hepatobiliary and pancreatic surgery at centralized tertiary referral centers, although data demonstrates that there is a marked global variation in the specialty of surgeons carrying out pancreatic surgery [17]. As discussed above, conventional Whipple or PPPD is considered for PDAC limited to the pancreatic head. In those patients with tumours confined to the pancreatic neck, a central pancreatectomy can be considered and if such tumours are extending toward the body/neck, then subtotal pancreatectomy can be performed in order to preserve pancreatic function [18].

A standard protocol for open pancreatic resection has not yet been internationally agreed. Therefore, in many instances, open pancreatic resection is performed based upon surgeon preference and patient factors. The generic oncological principles of resection, however, remain those of safe dissection, and appropriate and diligent lymphadenectomy to obtain an R0 resection. Surgeons may elect to perform Whipple/PPPD via a midline laparotomy or rooftop incision. The falciform ligament is divided and mobilised ensuring that it remains intact (see later). For patients in whom vascular resection is contemplated, the authors suggest a midline incision to enable the Cattell-Braasch manoeuvre to be performed (Figure 1). A thorough examination of the peritoneal cavity and liver is performed to exclude metastatic disease which would preclude resection. This may involve intraoperative ultrasound with some evidence to suggest that it may select out a small proportion of patients with liver metastasis [19]. After inspection of the peritoneal cavity, the surgeon may elect to perform a Cattel-Braasch manoeuvre and then adopt an "artery-first approach" (discussed below) or alternatively, the SMV may be approached by opening the gastrocolic ligament and dissecting it free from the inferior pancreatic border. In patients with disease limited to the pancreatic head, the Cattell-Braasch manoeuvre, which involves dissection along the right-sided white line of Toldt and then across the small bowel mesenteric root, allows the colon and small bowel to be retracted into the left upper quadrant and facilitates exposure of the SMV as it passes over the third part of the duodenum.

The complete extended Kocherization of the duodenum allows skeletonization of the inferior vena cava (IVC) and left renal vein. By using the superior border of the left renal vein as an anatomical landmark, the root of the SMA can be dissected free and neurectomy around the SMA can then be performed [20]. The Cattel-Braasch manoeuvre can be extended to the ligament of Treitz and with careful dissection the ligament can be fully mobilised and peritoneal cavity entered lateral to the ligament of Treitz. In the absence of neoadjuvant treatment, previous pancreatitis, or previous upper abdominal surgery, the Cattel-Braasch manoeuvre is achieved with minimal blood loss and economy of effort. As eluded to above, the Cattel-Braasch is recommended when the "artery-first

Figure 1: The Cattell-Braasch manoeuvre. The right colon, small bowel mesentery root, and duodenum (Kocherization) are mobilised from right to the left quadrant to allow exposure of the retroperitoneal structures and major vasculature. With completion of the Cattel-Braasch manoeuvre, the root of the SMA can be dissected free and SMA isolated from the tumour. (Taken from https://media.springernature.com/original/springer-static/image/art:10.1007/s11605-015-29581/MediaObjects/11605_2015_2958_Fig2_HTML.gif.)

approach" is being considered for resection [20]. The approach is discussed in detail below. In the conventional surgical approach, following Kocherization of the duodenum and skeletonization of the IVC and left renal vein, again the ligament of Treitz can be fully mobilised. The gastrocolic ligament can be opened widely with an energy device preserving the gastric arcade. The SMV can be identified by tracing the middle colic vein to the SMV at the lower pancreatic border. The retropancreatic tunnel can be developed at the inferior border of the pancreas. It is the author's view that if resistance is encountered here to suggest a portal vein/SMV involvement, then a Cattel-Braasch manoeuvre be performed at this point to aid vein reconstruction should it be required (see below).

Following this, attention turns to dissection of the hepatoduodenal ligament. If the gallbladder is present, a cholecystectomy is performed with ligation and division of the cystic artery. The dissection is commenced at the base of segment 4 of the liver, and the peritoneum opened here. The left, right, and hepatic arteries are skeletonised with ligation of the right gastric artery. Portal lymphadenectomy is completed, and the gastoduodenal artery (GDA) is dissected free. The GDA is clamped to ensure adequate flow in the common hepatic artery and exclude retrograde flow to the hepatic vasculature via the SMA because of coeliac artery stenosis. If adequate flow is present in the hepatic artery, then the GDA is ligated and divided. The common hepatic artery lymph node can be resected en bloc at this point. In general, coeliac and SMA lymphadenectomy are only performed if there is clear evidence of lymphadenopathy. This clearly has an impact upon patient morbidity and survival after pancreatic surgery. Of note, extended lymphadenectomy offers no survival benefit to patients but increases surgical complications [21]. In our unit, CT-PET is performed on all patients considered for pancreatic surgery and may be able to inform on the utility of this dissection [22]. During the dissection of hepatoduodenal ligament, care must be taken to avoid injury to accessory vessels and preoperative imaging in the form triple-phase CT

Figure 2: Preoperative CT triple phase may identify the anomalous anatomy shown here where the right hepatic artery originates from the SMA (red sloop) and left hepatic artery from the left gastric artery (blue sloop). The above patient had received neoadjuvant chemoradiation, and therefore, normal anatomical planes are not present and preoperative imaging can assist in safe dissection.

may assist the surgeon in this phase of the operation [23] (Figure 2). At this point, the retropancreatic tunnel can be completed and a nylon tape passed behind the pancreas and in front of the portal vein/SMV.

The stomach (classical Whipple) or duodenums (PPPD) are now divided with surgical stapling devices followed by the stapling and division of the jejunum. If the duodenum and pylorus have been compromised by a tumour and/or inflammation, then a classical Whipple is performed. The jejunal mesentery is divided using an appropriate energy device ensuring no injury occurs to the inferior mesenteric vessels. The jejunum can then be delivered under the superior mesenteric vessels and into the surgical field. Once this has been achieved, the pancreatic neck can be divided with either a knife or diathermy. This now allows the uncinate process of the pancreas to be dissected free from the SMV using

Figure 3: Postoperative CT demonstrating a single layer pancreaticojejunostomy using a dunking technique performed over a pancreatic duct stent.

ligatures and/or clips to control small venous tributaries. The inferior (IPDA) and superior (SPDA) pancreaticoduodenal arteries are also controlled and divided during this phase of resection. The remaining retroportal lymphadenectomy is completed with the skeletonisation of the lateral border of the SMA. This latter dissection has already been performed if the posterior artery-first approach has been utilised. The end result at the conclusion of the resection will appear similar to Figure 2.

Following pancreatic resection, the reconstructive phase of surgery commences and begins with pancreatic resection. There is little clinical evidence to support pancreatico-jejunostomy (PJ) over pancreatico-gastrotomy (PG) reconstruction, but a recent meta-analysis clearly demonstrated that most surgeons' preference is to perform a P-J anastomosis [24]. This area is controversial as the PJ anastomosis can be performed using a variety of methods. The PANasta Trial is attempting to clarify this area although it is only evaluating duct-to-mucosa PJ anastomosis techniques (Cattell and Blumgart techniques) and not other methods of pancreatic anastomosis such as the dunking method that is the preferred method for, at least, the authors on this manuscript [25]. There is emerging evidence that pancreatic duct diameter combined with gland texture can predict the incidence of postoperative pancreatic fistula (POPF) in addition to the PJ anastomosis technique used [26]. In addition, the authors use a pancreatic stenting during reconstruction routinely with a reported benefit in reduced pancreatic fistula incidence [27] (Figure 3).

Following this, end-to-side, single-layer, interrupted, or single-layer continuous hepaticojejunostomy with or without stenting is performed using the same jejunal loop. The decision as to the mode of reconstruction is usually dependent upon the bile duct caliber. It is the author's preference to perform a double-layer, continuous, hand-sewn antecolic gastrojejunostomy or duodenojejunostomy, with a nasogastric tube placed in the stomach and a feeding nasojejunal tube in the efferent jejunal limb of the anastomosis for postoperative nutrition. The authors advocate encircling the earlier mobilised falciform ligament around the pancreatic anastomosis to reduce the risk of GDA pseudoaneurysm. The postoperative management of pancreatic resections is outside the scope of this manuscript, but the reader is referred to recent excellent review article [28].

In a recent single center study, Picozzi et al. reported a 5-year survival of patients with resected PDAC of 32% and overall survival of 34% [29]. Whilst these results are encouraging, they still far short of the survival rates for other resected cancers. As noted in the above study, 50% of the patients developed systemic disease after resection for PDAC [29]. To address this, many groups are assessing the role of neoadjuvant chemotherapy in setting of resectable PDAC such as the NorPACT-1 Trial [30] with recent meta-analysis demonstrating that multiagent neoadjuvant chemotherapy is associated with improved survival in patients undergoing pancreatic resection [31]. Although as suggested by Heinrich et al., despite supporting evidence of the treatment effect neoadjuvant therapy, it has not proven superior to surgery in randomised trials for resectable PDAC with most available data based upon small phase II trials [32]. To add further weight to this latter point, Zhan et al. recent meta-analysis of both resectable, borderline resectable, and locally advanced PDAC suggested that whilst it may offer benefit to the patients in the latter two groups, it should be used with caution in patient with resectable PDAC as it was not shown to be beneficial [33]. Much of this discordant surgical data may in part be due to the type of chemotherapy used as Unno et al. demonstrate that a combination of gemcitabine and S1 in resectable PDAC significantly improved overall survival (36.7 months versus 26.6 months (upfront surgery) [34]. This area of PDAC management is likely to remain a fertile area of research especially as more sophisticated chemotherapy agents are developed and the results of the NorPACT-1 may demonstrate that neoadjuvant chemotherapy is beneficial in resectable PDAC in a randomised trial setting. The results of such trials are eagerly awaited.

4. Artery-First Approach to Pancreatic Resection

The artery-first technique for pancreatic resection has been excellently summarised by Sanjay et al. [35]. Most pancreatic surgeons are trained or prefer the approach originally described by Marzano et al. and is referred to as the "posterior approach" [36] (Figure 4).

Following Cattell-Braasch manoeuvre that has been detailed above, the root of the SMA is dissected and exposed where it passes in front of the left renal vein in front of the abdominal aorta. The perineural and perivascular tissue can be carefully divided dissecting away from the abdominal aorta, posterior to the pancreatic head aiming towards the duodenum. As this dissection continues, tissue between the SMA and uncinated process are progressively divided to expose the lateral border of the portal vein/SMV. This allows a relatively easy access to the IPDA and SPDA for ligation. Data demonstrate that this dissection enables assessment of vascular involvement and also allow safe dissection of accessory/replaced right hepatic artery (Figure 2) whilst also reducing blood loss during surgery

Figure 4: The posterior artery-first approach for the pancreatic cancer. The pancreatic resection has been completed. The blue sloop is encircling the SMA and the green sloop the portal vein. All the retropancreatic tissues have been divided prior to pancreatic neck division. Gerota's fascia is apparent, and the yellow sloop is displacing the common hepatic artery. This patient had also received neoadjuvant chemoradiation.

[37–39]. Aside from this approach, the medial uncinate approach, inferior infracolic approach, the left posterior approach, inferior supracolic approach, and superior approach have also been described [35].

The decision to perform total pancreatectomy may have been made preoperatively because of the presence of multifocal disease or, in some instance, be made intraoperatively because of vascular involvement thereby performed to ensure oncological clearance (see below). Additionally, positive resection margins on frozen sections sent during surgery may necessitate total pancreatectomy. The operative approach for total pancreatectomy is as described above. Dependent upon surgeon preference, an artery-first approach can be utilised or a more conventional approach be adopted. We recommend posterior artery-first approach when performing a total pancreatectomy as this firstly allows skeletonisation of the SMA and without pancreatic neck division, as in a Whipple/PPPD, allows division for the retropancreatic tissue and mobilisation of the uncinate process ensuring that the correct plane posterior to the pancreatic body and tail is entered. Following the division of this tissue, the tortuous splenic artery is dissected free and controlled with a sloop until division, followed by isolation of the splenic vein. Short gastric vessels can be controlled with a suitable energy device and the stomach reflected cranially. By adopting the artery-first approach, the SMA now lies outside the dissection plane and adrenal gland along with Gerota's fascia is used as the posterior extent of resection of the left side of the abdomen. At this point, the splenic artery, splenic vein, and the inferior mesenteric vein can be ligated. The spleen is included in the resection for oncological reasons, and thus, the splenorenal ligament is divided and drawn medially to meet the plane developed by the dissection posterior to the pancreatic body and tail. The duodenum/stomach and jejunum are mobilised and transected as described above followed by en bloc resection of the pancreas, duodenum, spleen, and the peripancreatic lymph nodes. Total pancreatectomy eliminates the need for a pancreatic reconstruction anastomosis but causes iatrogenic diabetes mellitus, which maybe brittle, and exocrine insufficiency [40]. However, in selected cases, total pancreatectomy can ensure R0 resections with comparable patient outcome to Whipple/PPPD [41].

5. Pancreatic Resection in the Presence of Vascular Involvement

As eluded to above, pancreatic surgery is evolving with surgical approaches being modified in patients who have received neoadjuvant treatment [42–44]. These preoperative regimens are principally being used for patients with borderline resectable or locally advanced PDAC [45], although the use of these strategies is attracting growing attention in patients with resectable disease, too [42], as discussed above [30]. There are multiple definitions of borderline resectable and locally advanced PDAC from many learned global societies (Table 1).

Surgery after neoadjuvant regimens for PDAC presents challenges to the pancreatic surgeon. Preoperative radiation especially causes localised inflammation that changes the surgical field by inducing adhesions particularly between the tumour and adjacent organs such the colon, stomach, and IVC. A particular concern for the surgeon is adherence between the tumour and the portal vein/SMV. Clearly, if this cannot be dissected freely, then vein resection needs to be considered. The radiation field will also encompass the porta hepatitis which results in fibrosis with again the loss of normal tissue planes as demonstrated in Figures 2 and 4. Whilst possible via a rooftop incision, a midline incision with Cattel-Braasch manoeuvre as described above is considered the optimal approach in patients where vein resection is likely to be needed. The posterior artery-first approach described above can be difficult to achieve due to inflammation and/or fibrosis present at the root of the mesentery. There is merit in pursuing this approach as if the SMA can be dissected freely and mobilised away from the tumour, then resectability of the tumour has been proved and a vein resection can be carried out then if needed. Whether this approach is adopted or not, the pancreas must be dissected freely from the splenic vein to the left of the portal vein/SMV junction to facilitate safe division of the pancreas to the left of the pancreatic neck. Prior to pancreatic division, adequate venous clearance is required to enable safe clamping of the veins prior to vein resection. The portal vein is dissected free up to its bifurcation into the left and right portal veins and the SMV dissected free to its first order branches, although if a safe plane can be cleared above the SMV branches, this maybe satisfactory for vein clamping. Once this has been achieved, the patient is heparinized with an unfractionated heparin bolus. The already slooped portal vein, splenic vein, and SMV are all clamped with appropriate vascular clamps. The pancreas is divided, followed by division of the SMV and portal and splenic vein (if required), and then the surgical specimen is removed. The advantage of having previously mobilised the mesentery using the Cattell-Braasch manoeuvre in this scenario is that if a complete vein resection is required, a defect of up to 4 cm can be reconstructed using an end-to-end venous anastomosis

Table 1: Different international classifications of locally advanced and borderline resectable PDAC (taken by JA Pietryga and DE Morgan (2015), Journal of Gastrointestinal Oncology, vol. 6, no. 4).

Anatomy	NCCN 2014	AHPBA/SSAT/SSO	MD Anderson Cancer Center	ISGPS	ACTO
Superior mesenteric vein/portal vein	Involvement with distortion/narrowing and/or occlusion amenable to reconstruction	Abutment encasement or short-segment occlusion amenable to reconstruction	Short-segment occlusion amenable to reconstruction	Involvement with distortion/narrowing and/or occlusion amenable to reconstruction	Tumor-vessel interface ≥180° and/or occlusion amenable to reconstruction
Superior mesenteric artery	Abutment (≤180°)	Abutment (≤180°)	Abutment (≤180°)	Abutment (≤180°)	Tumor-vessel interface <180°
Common hepatic artery	Abutment or short-segment encasement	Abutment or short-segment encasement	Short-segment encasement/abutment	Abutment or short-segment encasement	Short-segment tumor-vessel interface (any degree) amenable to reconstruction
Celiac artery	No abutment or encasement	No abutment/encasement	No abutment or encasement	No abutment or encasement	Tumor-vessel interface <180°

NCCN: National Comprehensive Cancer Network; AHPBA/SSAT/SSO: American Hepato-Pancreato-Biliary Association/Society for Surgery of the Alimentary Tract/Society of Surgical Oncology; ISGPS: International Study Group of Pancreatic Surgery; ACTO: Alliance for Clinical Trials in Oncology.

Figure 5: Surgical methods for vein reconstruction after vein resection for PDAC. (Taken from Glebova et al., J Vasc Surg 622: 424-443).

thus precluding the need for interposition venous graft (Figure 5(d)), meaning that pancreatic resection with full-vein reconstruction can be safely performed outside transplantation units [46].

In most patients with venous involvement, a wedge of the portal vein or SMV can be performed if a small portion of the pancreatic head mass is inseparable from a vein by applying a Satinsky vascular clamp to the appropriate area of the vein and closuring the defect with fine Prolene sutures (Figure 5(b)). For segmental resections of the portal vein or the SMV longer than 4 cm, end-to-end anastomosis can be performed with the use of a vascular conduit for reconstruction (Figure 5). Appropriate conduits include autologous vein (left renal vein, great saphenous vein, or internal jugular vein (Figure 5(d)), cryopreserved cadaveric vein or artificial gortex grafts (Figure 5(f)), although vein grafts have an increased risk of late venous thrombosis [47–49]. The anastomosis is usually preformed using a continuous single layer 6/0 Prolene suture with an appropriate growth factor. The remainder of the operation including reconstruction is completed as detailed above.

The first randomised controlled trial using neoadjuvant regimens for borderline resectable PDAC has demonstrated that patients in the neoadjuvant arm derive such a significant survival benefit that the trial was closed early [50]. In this trial, a resection rate of 63% was reported following neoadjuvant chemoradiation, although vascular reconstruction rates were not reported. Although in a recent study from John Hopkins Institute, the need for vein resection was reported as 49% in patients undergoing pancreatic resection following neoadjuvant treatment [51]. In addition, Dhir et al.'s recent meta-analysis of 5520 patients with resected PDAC demonstrated neoadjuvant treatment increases in R0 resection rates and has equivalent survival in the resectable versus borderline resectable PDAC cohorts after surgery [52]. Although arterial resection and reconstruction has been reported in patients with PDAC, this is currently not favoured because of the high risk of patient mortality [53]. In summary, the literature suggests that pancreatic resection with or without vein resection in locally advanced and borderline resectable PDAC following neoadjuvant treatment appears safe and derives survival benefit for the patient.

6. Minimally Invasive Pancreatic Resections for PDAC

Minimally invasive pancreatic surgery for PDAC (either laparoscopic or robotic) aims to give the same oncological outcomes as open surgery but negate the need for a large abdominal incision. Whilst surgeons have a longer experience with laparoscopic pancreatic resection, robotic platforms are gaining momentum as a viable option for patients requiring pancreatic resections. Robotic platforms, in addition, offer technical advances that aid in the performance complex surgical procedures such as Whipple/PPPD. Indeed, with the use of such facets as 3-D viewing and EndoWrist

Figure 6: A 12 mm port is placed two fingerbreadths below and to the right of the umbilicus, two robotic ports are placed in the right upper quadrant, a port is placed in the right lower quadrant, a port in the left lower quadrant, and a port in the anterior axillary line on the left side of the abdomen.

technology, encouraging early results have been reported with robotic Whipple/PPPD [11]. The procedure of robotic pancreaticoduodenectomy (RPD) remains in its infancy, and a standard stepwise approach to this surgery is not well described in the literature. Similarly, laparoscopic pancreaticoduodenectomy (LPD) is performed globally but a standard approach remains to be agreed internationally. In general, for either RPD or LPD, the patient is placed in a supine position with reverse Trendelenburg positioning. For LPD, between 5 and 6 trocars can be used, but for RPD 4, robotic arms can be used on the platform with two assistant ports [54]. The placement for robotic PPPD is demonstrated in Figure 6.

For both RPD and LPD, the peritoneal cavity is generally accessed using an open (Hassan) technique as indicated in Figure 6. The abdominal cavity is insufflated with CO2 gas, and as in open surgery, the presence of metastatic disease is excluded. The remaining working ports are then inserted. Variations on the port placement arrangement demonstrated in Figure 6 are common. As for many upper abdominal surgical procedures, a liver retractor is placed and the liver retracted in a cranial direction. Although intuitively the approach to minimal access is markedly different to open surgery, once the liver is retracted, the ligament of Treitz is identified and a marker suture is placed 70-80 cm distal to the duodenal-jejunal flexure. This is a critical step as once pancreatic resection is completed, it may become extremely difficult to assess the optimal position for the gastrojejunostomy, and this suture aids in this decision-making process. The patient is now manoeuvred into a steep head-up position with the right side up in order to allow the small bowel to migrate to the lower abdominal cavity. This is akin to the position of patients undergoing bariatric surgery. If a RPD is being performed, then the robotic platform is docked and remaining instruments are docked to the robot arms.

In contrast to the open approach described above in LPD/RPD, the greater omentum is opened below the gastroepiploic pedicle to gain access to the lesser sac and hence the pancreas. The greater curvature omentum is mobilised by employing a minimal access energy device. The transverse colon and hepatic flexure can now be mobilised, and the colonic mesentery is dissected off Gerota's fascia allowing the surgeon access to the duodenum and pancreatic head. Once this point in the dissection has been reached, Kocherization can be performed with skeletonisation of the IVC and left renal vein. Akin to the open approach, the ligament of Treitz can be fully mobilised. The duodenum and jejunum can be divided with a suitable stapling device, and the jejunum can be transposed to the right side of the abdomen. Clearly, this is a committal step, and hence, most experience with the minimal access approach involves PDAC that does not compromise the major vasculature or pancreatic neck. A cholecystectomy can be performed and the common bile duct divided. The hilar lymphadenectomy can be completed with attendant care to the hepatic artery. It remains to be demonstrated that these approaches provide similar lymphadenectomy to open approach. The next phase of the dissection is similar to the open approach with GDA division and completion of the retropancreatic tunnel that is fashioned from the inferior pancreatic border to the superior aspect. A nylon tape can be passed and an assistant port used to retract the pancreas towards the abdominal wall with the pancreas divided with diathermy. In an analogous manner to open surgery, the uncinate process, venous tributaries, and IPDA/SPDA are controlled with a combination of energy devices and metallic clips. The surgical specimen can now be placed in a sterile bag.

The proximal jejunum is transferred to the supracolic compartment lateral to the middle colic vessels through a mesocolic defect. As with the open approach, there are a number of methods for minimal access reconstruction of the pancreatic remnant with or without a stent. Most minimal access pancreatic surgeons perform the hepaticojejunostomy using self-locking V-Loc sutures. The gastrojejunostomy can be fashioned using sutured or stapled methods. Abdominal drainage is as per open surgery.

Although not based upon randomised data, retrospective series suggests that there is an advantage to performing minimal access pancreatic surgery in the form of RPD [55]. It is reported that once a surgeon has completed the learning phase of RPD, total operative time, in-patient stay, and estimated blood loss can be lower than those in open surgery and at least equivalent to LPD [56]. In addition, the rates of POPF and other serious complications following RPD were reported at lower frequencies [56]. In studies that have compared RPD to open Whipple/PPPD in a prospective manner, RPD was reported to have a longer operative time but faster return-to-functional recovery [57]. Importantly, the two groups had similar reported rates of surgical morbidities and mortality, R0 resection rates, and overall and disease-free survival. Furthermore, recent analysis of RPD versus LPD demonstrated that R0 resection rates of 100% with acceptable postoperative complications [58].

Laparoscopic and robotic total pancreatectomy remains in their infancy, although there are an increasing numbers of single surgeon reports. This is likely because many of the as-yet unresolved challenges with open pancreatic surgery, such as the optimal mode of pancreatic reconstruction, are not addressed by the minimal access approach. Indeed, minimally invasive pancreatic resection for borderline resectable disease is not yet recommended and open surgery remains the standard approach here.

7. Minimally Invasive PPPD with Vascular Involvement

There are very few reports of successful minimal access resection of PDAC in the presence of vascular involvement. As with open surgery in patients with vascular involvement, the preferred approach would be an artery-first approach to prove resectability. However, without tactile feedback, in the case of robotic surgery and potentially limited views with laparoscopic surgery, such cases are technically demanding and hence not commonly performed. In such patients, most surgeons will opt to perform open surgery. The exception to this is if venous involvement is limited and resection can be achieved with primary vein repair, but again, if this feature was evident on preoperative imaging or noted intraoperatively, most surgeons would adopt an open approach.

8. Distal Pancreatectomy and Splenectomy (DPS)

For PDAC that is located in the pancreatic body and tail, minimally invasive DPS is now accepted as the standard of care [59]. A number of factors have contributed to the surgical community adopting this practice. Principally, the lack of a pancreatic anastomosis means that the procedure is associated with reduced levels of morbidity and mortality and additionally, the learning curve for the procedure is of lesser magnitude than the operations discussed above [60]. Whilst the laparoscopic DPS is practiced by most surgeons when indicated, robotic DPS is being increasingly performed but further oncological data is required before it rivals laparoscopic DPS as the standard of care [61].

Minimal access DPS can be performed through a variety of closely aligned methods. Generally, whether performed by the laparoscopic or robotic approach, 4 to 5 port trocars are placed in a semicircular fashion centered on a periumbilical optical port. Similar to minimal access Whipple surgery, a liver retractor is used to reflect the left lobe cranially. The gastrocolic ligament is opened with an energy device, while preserving the gastroepiploic vessels. Once this is achieved, the liver retractor is repositioned to retract the stomach and left lobe of the liver cranially allowing access to the pancreatic tail and spleen. Although intraoperative imaging can be used to locate the tumour, lesions in the pancreatic body or tail are usually macroscopically visible. In the conventional approach, the inferior border of the pancreas is defined with adequate mobilisation such that the splenic vein can be visualised allowing the dissection to continue posterior to it. With an adequate plane developed, the superior border of the pancreas is defined with isolation and control of the splenic artery. At this point, it is safe to pass a nylon tape around the pancreatic body/tail. Fine vessels entering the posterior pancreas can usually be controlled with an appropriate energy device. The splenic artery and vein can be divided after application of surgical clips or Hem-o-loc® clips. The pancreas can then be divided with a stapling device, the spleen is then mobilised in a similar manner to that described above for total pancreatectomy with splenectomy, and the DPS is resected en bloc.

Despite this well-described surgical approach, the patient survival after DPS for PDAC is poor [62]. To improve on these disappointing patient outcomes, the radical antegrade modular pancreaticosplenectomy (RAMPS) was developed by Strasberg et al. The premise of this procedure being that the DPS described above does not provide an adequate or radical lymphadenectomy. Thus, although the initial approach is similar between DPS and RAMPS, once the surgeon enters the plane posterior to the pancreas in the RAMPS, the plane is carried deeper to include Gerota's fascia and the adjacent lymph nodes ensuring a more radical lymphadenectomy. The RAMPS can then entail resection of the left adrenal gland (posterior RAMPS) or not (anterior RAMPS) [63]. A key feature of the RAMPS procedure is the additional lymphadenectomy along the coeliac trunk. There is limited data as to the oncological outcomes for patients following RAMPS for PDAC, the anatomical logical of the approach are undoubtedly sound. Indeed, a recent meta-analysis demonstrated that minimally invasive DPS had comparable 5-year survival to open surgery with significantly lower positive margin rates, shorter in-patient hospital stay, less blood loss, and lower patient morbidity and mortality [64]. As discussed above, the data on RAMPS is limited by analysis of retrospective data which demonstrate that there is no increase in POPF, complications, and mortality but a significantly improved R0 rate and number of lymph nodes resected [65]. Although as Cao et al. state further, trial data are needed before confirmation of the survival benefits of RAMPS which can be assessed although the authors state that based upon their meta-analysis, RAMPS is an oncologically superior procedure [66]. Finally, the use of robotic DPS/RAMPS for PDAC may allow further advances in the radicality of dissection and regional lymphadenectomy although robust data on this is required [67].

9. Summary

Many surgical approaches are available when planning resection of PDAC. In general, when vascular resection and reconstruction are required, the open approach appears most feasible whilst in those patients with early disease, the minimal access/robotic approach appear to offer improved patient metrics without apparent comprise on oncological outcomes.

Conflicts of Interest

The authors have no conflict of interests to declare regarding the publication of this manuscript.

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Review Article

Endoscopic Perspective in Cholangiocarcinoma Diagnostic Process

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Cholangiocarcinoma is a malignancy arising from the epithelial lining of the intrahepatic or extrahepatic biliary tract. Timely diagnosis is challenging due to its silent clinical course. As reliable laboratory markers are lacking, diagnostic imaging plays a pivotal role. While cross-sectional imaging studies are usually conclusive for intrahepatic lesions, endoscopy plays an essential role in cases of extrahepatic tumors. Rational utilization of different diagnostic methods based on available evidence is needed. This article focuses on the diagnostic role of advanced biliary endoscopy, including endoscopic retrograde cholangiopancreatography, cholangioscopy, endoscopic ultrasonography, and intraductal sonography.

1. Introduction

A timely diagnosis of cholangiocarcinoma remains challenging due to its silent clinical course. As reliable laboratory markers are lacking, diagnostic imaging plays a pivotal role. While cross-sectional imaging studies are usually conclusive for intrahepatic lesions, endoscopy plays an essential role in cases of extrahepatic tumors. Nevertheless, tissue specimen is usually necessary in both locations. This article addresses cholangiocarcinoma from the perspective of endoscopic imaging and tissue sampling.

2. Epidemiology

CCA is a malignancy arising from the epithelial lining of the intrahepatic or extrahepatic biliary tract, excluding the gallbladder, cystic duct, and ampulla of Vater. It accounts for 2% of all human malignancies and is the second most common primary hepatic malignancy following hepatocellular carcinoma.

The highest global incidence of CCA is in north-east Thailand where infestation by the liver fluke *Opisthorchis viverrini* is endemic. In this geographic area, age-standardized incidence rates of approximately 100 per 100,000 individuals among men and 50 per 100,000 individuals among women have been reported, while it ranges between 0.5 and 2.0 per 100,000 individuals in the Western world [1, 2].

As the incidence of CCA increases with age, the median age at diagnosis is 65-68 years. Sex- and race-specific disparities have also been noted. In the USA, males and specifically Asians are affected more frequently than females and non-Hispanic whites [3].

Worldwide time trends in mortality for CCA are changing. Multiple studies have reported that the incidence of ICCA has increased by up to tenfold in high-income countries, while the incidence of ECCA has decreased at a similar rate around the turn of the 20th century. Nevertheless, it has also been suggested that this shift may have been influenced by implementing different classification systems [4, 5].

3. Prognosis and Treatment

The prognosis of biliary malignancies is dismal with the overall five-year survival as low as 10%. Radical surgery remains the only option for curative therapy offering a median disease-free survival of 12-36 months. However, due to the silent clinical course, two-thirds of CCAs are diagnosed at an inoperable stage. The natural course without

chemotherapy or radiotherapy leads to overall survival of 3.9 months only. If palliative chemotherapy is used, median survival can be prolonged up to 12-15 months [6, 7]. After resection, negative margin (R0 resection) is the most important variable associated with long-term survival [8]. Recently, an excellent five-year survival of 65% in patients with "very early" perihilar cholangiocarcinoma was demonstrated after neoadjuvant chemoradiation followed by liver transplantation [9].

4. Etiopathogenesis

While the etiopathogenesis remains unknown, the development of biliary cancer is linked to a wide spectrum of conditions causing biliary inflammation, cholestasis, and inflammation of the liver. Different precancerous conditions and recommendations for the follow-up of patients with increased risk are described in detail elsewhere. Despite increasing knowledge within this field, the diagnosis of CCA at an early stage remains a challenge since the majority of cases are sporadic, affecting patients without the presence of any known risk factor [10].

5. Diagnostic Approach

The clinical presentation of CCA depends on tumor location. The typical triad of ECCA consists of symptoms of biliary obstruction, right upper quadrant pain, and weight loss. However, patients with jaundice have mostly advanced disease already. Therefore, making a diagnosis of ECCA in a nonjaundiced person remains a crucial issue. It has been shown by Sugiyama et al. that abnormalities in hepatic function and tumor marker tests combined with transabdominal ultrasonography (US) can be used for early diagnosis of ECCA in patients without jaundice, with resulting resectability of 83% and survival of 50% at 5 years [11].

A reliable laboratory test for CCA is still lacking. Extrahepatic cholestasis is reflected in elevated conjugated bilirubin, alkaline phosphatase, and gamma-glutamyltransferase levels. As for "tumor markers," it is widely accepted that testing for the carbohydrate antigen (CA) 19-9 is useful in diagnostics, as a prognostic factor and indicator of tumor resectability. However, the levels of CA 19-9 can also increase in other hepatobiliary conditions, including cholangitis. On the other hand, about 4-14% of the population with Lewis-negative phenotype are not able to secrete CA 19-9 even when malignant tumor is present [12].

Due to the unspecific clinical presentation and the absence of accurate laboratory tests, the diagnosis of CCA depends mainly on imaging with or without tissue sampling. For ICCA, cross-sectional imaging methods are mostly conclusive. On the other hand, the role of advanced pancreatobiliary endoscopy is essential in the cases of ECCA, as will be discussed further in this text.

6. Classifications

Most classifications of CCA were developed to guide surgical and oncological treatment. Nonetheless, they can also be used for tailoring palliative treatment including endoscopic or percutaneous drainage and locoregional therapy.

Figure 1: Classification of cholangiocarcinoma according to the anatomical location.

As with most other tumors, CCA can be staged according to the tumor, node, metastasis (TNM) system [13]. Yet, the TNM system is unable to provide the information necessary for an assessment of surgical resectability in the case of ECCA. Therefore, additional staging systems based on anatomical, pathological, and surgical characteristics are also used [14].

According to the anatomical location, CCA can be divided into ICCA and ECCA with the anatomical border at the level of second-order bile ducts. As for ECCA, it can be subdivided into PCCA and DCCA, two entities anatomically separated by the insertion of the cystic duct into the common bile duct. From all CCAs, the proportion of PCCA, DCCA, and ICCA is 50-60%, 20%, and 20%, respectively [15] (Figure 1).

PCCA, usually referred to as a Klatskin tumor, can be further described according to the *Bismuth and Corlette* classification as types I-IV: type I involves the common hepatic duct below the confluence of the right and left hepatic ducts, type II involves the confluence, type IIIa involves the confluence while extending into the right hepatic duct, type IIIb involves the confluence while extending into the left hepatic duct, and type IV involves the confluence while extending into both the right and left hepatic ducts, or it is multifocal [16, 17] (Figure 2).

As for macroscopic morphology, three types of CCA have been described by the Liver Cancer Study Group of Japan including (1) periductal-infiltrating, (2) intraductal-papillary, and (3) exophytic or mass-forming types. The American Joint Committee for Cancer also recognizes mixed periductal-infiltrating and intraductal-growing types. The periductal infiltrative type is the most common, representing 70% of cases [18] (Figure 3).

It has been shown that the type of tumor spread correlates with the morphology growth pattern. While mucosal extension is predominantly seen with intraductal-papillary and mass-forming (nodular) tumors, submucosal extension is mainly seen with periductal-infiltrating types. Generally, the extension occurs more frequently to the hepatic side, where it also tends to reach further [19, 20].

Figure 2: Classification of perihilar carcinoma according to Bismuth and Corlette.

Figure 3: Classification of cholangiocarcinoma carcinoma according to the macroscopic morphology.

Histologically, 90% of CCAs are adenocarcinomas that are well, moderately, or poorly differentiated. They consist of either cylindrical mucin-producing glands or cuboidal non-mucin-producing cells. All types of CCAs are associated with the rapid proliferation of tumor-associated stroma cells that contribute to desmoplasia.

Recently, a new staging system integrating important anatomic, pathologic, and surgical features of PCCA has been suggested by a group of international experts. Attempting to reflect a unique complexity of this tumor, it employs eight characteristics for staging: tumor extent, tumor size, tumor growth type, vascular involvement, hepatic lobar atrophy, underlying hepatic disease, lymph node metastases, and distant metastases [21].

7. Cross-Sectional Imaging

Cross-sectional imaging involves any technique that produces an image in the form of a plane through the body with the structures cut across.

7.1. Transabdominal Ultrasonography. Transabdominal ultrasonography (US) is frequently used as a first-line imaging test in jaundiced patients. While ICCA presents as a hypoechoic mass, the direct visualization of ECCA on US is usually impossible. In one study, the sensitivities of US in demonstrating hilar tumor, middle bile duct tumor, and distal bile duct tumor were 86%, 59%, and 33%, respectively [22]. US has nevertheless proven to be useful in detecting biliary tract dilation, level of obstruction, and the presence of gallstones.

7.2. Computed Tomography/Magnetic Resonance Imaging (PET/CT). Computed tomography (CT) is performed in the vast majority of CCAs. This is not the place to discuss the role of CT in the diagnosis of ICCA. The performance of a CT scan as a diagnostic tool for PCCA was evaluated in a meta-analysis of 16 studies demonstrating an accuracy of 86% for the ductal extent of the tumor. Sensitivity and specificity were 89% and 92% for the evaluation of portal vein involvement, 83% and 93% for hepatic artery involvement, and 61% and 88% for lymph node involvement, respectively [23]. As for the PCCA resectability assessment, another meta-analysis calculated the pooled sensitivities of CT, MRI, and PET/CT to be 95%, 94%, and 91%, respectively, with corresponding specificities of 69%, 71%, and 81%. As concluded by the authors, CT is the most frequently used modality. The diagnostic performance of MRI proved to be generally comparable with CT, while PET/CT appears to be the best method in detecting lymph node and distant metastasis [24].

8. The Role of Endoscopy

8.1. Endoscopic Retrograde Cholangiopancreatography (ERCP). Since its introduction in 1968, ERCP has been widely used for imaging of the biliary tree and is still considered the gold standard of biliary imaging. Nevertheless, ERCP remains an invasive procedure with considerable risks. As reported by Cotton et al., unavoidable overall, severe, and lethal complication rates of 4%, 0.7%, and 0.06%, respectively, must be expected even in the hands of experts [25].

The most important ERCP-related complication is pancreatitis occurring in 2.6% of patients followed by ascending cholangitis. Although several preventive measures have proven effective in post-ERCP pancreatitis prophylaxis, including the administration of certain drugs, guidewire cannulation technique, vigorous intravenous hydration, and pancreatic duct stenting, the exclusion of patients in whom ERCP is unnecessary remains a crucial point [26].

In expert centers, ERCP has been replaced by less-invasive diagnostic modalities such as CT, MRCP, and EUS at the position of the first-line imaging method. It has been shown that MRCP in combination with MRI has a comparable diagnostic accuracy with direct (ERCP or percutaneous transhepatic) cholangiography combined with CT in the detection and staging of ECCA [27]. However, ERCP is still being widely utilized for transpapillary tissue sampling. Moreover, the availability of MRI and EUS is not universal, and the position of ERCP in the diagnostic algorithm for

Figure 4: Endoscopic retrograde biopsy of biliary stricture.

ECCA may, therefore, vary according to the level of local expertise [28].

In ERCP, CCA presents as a stricture or a filling defect with or without upstream dilation of the biliary tract. Malignant etiology is suggested in a long (≥10 mm), asymmetric, and irregular stricture opposed to a short, regular, and symmetric stricture, which is typical for benign disease. As shown by Park et al., using these criteria, the diagnostic sensitivity and specificity for ERCP were 74% and 70%, respectively [29].

When necessary, material for histopathology examination can be obtained by one of 3 different approaches during ERCP: (1) bile aspiration, (2) brush cytology, and (3) endobiliary biopsy. While the specificity of diagnosing malignancy approaches 100% uniformly for each method, sensitivity varies widely and generally remains unsatisfactorily low. For instance, the sensitivity of cytology examination of aspirated bile ranges between 6 and 24% [30, 31].

Endoscopic brush cytology may be considered a standard method that is safe and easy to perform. Its diagnostic performance has been evaluated in many studies with the sensitivity for CCA in the range of 23-80%. In a meta-analysis of 16 studies including 1556 patients, Burnett et al. calculated its sensitivity as 42%. Two to five brush passes through the stricture have been suggested in various studies [28, 32].

As for endoluminal forceps biopsy, the sensitivity for CCA is in the range of 52-81%. Compared to brushing, forceps biopsy is technically more demanding and may require sphincterotomy. Biopsy can also be difficult or impossible in a narrow duct. The number of biopsy bites in studies ranges between 1 and 6. As shown by Tamada et al., three biopsy bites are sufficient to obtain sensitivity of 100% in patients with the papillary type CCA, while multiple biopsies are necessary for the infiltrating type [33–37] (Figure 4).

It makes sense that different sampling methods could be combined. In a meta-analysis of 9 studies, Navaneethan et al. found the pooled sensitivity of endoscopic brush cytology, intraductal biopsy, and combination of both to be 45%, 48%, and 59%, respectively, with corresponding specificity of 99%, 99%, and 100%. The authors concluded that both brushing and biopsy are comparable with limited sensitivity, which can only modestly be increased by the combination of both [38].

Several factors contribute to frequent false-negative results of CCA tissue analysis. Among them, general difficulties in tissue sampling, the desmoplastic paucicellular character of CCA, and background inflammatory changes are the most prevalent. In a study devoted to endoscopic brush cytology, Logrono et al. proved that tissue sampling error was the cause of false-negative findings in 67%, followed by technical error in 17% and interpretive error in the remaining 17% [39].

Different technical solutions have been investigated in order to increase successful tissue sampling. Among them, using a larger cytology brush (3 mm × 5 cm) increased cellularity but did not improve the cancer detection rate when compared to the standard brush. The combination of biliary dilation, endoscopic needle aspiration, and subsequent brushing cytology showed higher sensitivity (85%) than brushing alone (57%). A cytological evaluation of postbrushing biliary lavage fluid increased sensitivity by 24%. A dedicated basket for tissue grasp provided better sensitivity than brushing (80 vs. 40%). Moreover, combining brush cytology with CA 19-9 assessment brought better diagnostic accuracy in patients with primary sclerosing cholangitis [40–44].

To improve the sensitivity of routine cytology, advanced cytological methods can be used. Among them, fluorescence in situ hybridization (FISH) is a test employing fluorescently labelled DNA probes to detect gains or losses of chromosomal regions. As shown by Fritcher et al., a combination of FISH probes 1q21, 7p12, 8q24, and 9p21 identified pancreatobiliary malignancies with sensitivity of 93% and specificity of 100%. The typical finding was polysomy, indicating a presence of five or more cells with gains detected for two or more probes. In other studies, FISH proved to increase sensitivity from 20% to 43% as compared to routine cytology. In cases with negative brush cytology and forceps biopsy, FISH could predict malignancy in 62% of patients with an indeterminate biliary stricture [45, 46]. Next-generation DNA sequencing (NGS), another ancillary cytological method, has also turned out to be promising. In a study combining NGS with cytology, NGS increased sensitivity to 85% as compared to 67% for cytology alone [47]. Triple modality testing combining brush cytology, FISH, and biopsy showed overall sensitivity of 82% and specificity 100% [48].

To summarize the above-mentioned, while positive ERCP-guided tissue sampling can be regarded as diagnostically conclusive, a negative finding should be interpreted with caution. The term "indeterminate stricture" is used to indicate biliary strictures remaining of a likely malignant etiology despite negative pathology results. In such cases, surgical exploration must be considered in order not to miss the opportunity of curative surgery for a potential malignancy. With that being said, 5-30% of surgically treated lesions were reported benign on final histopathology according to different studies [49]. Currently, novel endoscopic modalities, including cholangioscopy, can be used to avoid unnecessary surgery.

8.2. Cholangioscopy. The history of cholangioscopy dates back to the 1970s when two different endoscopic approaches to the biliary tract were described: (1) the "mother-baby"

Figure 5: Cholangioscopy (SpyGlass) view of the intraductal-type cholangiocarcinoma.

Figure 6: Cholangioscopy (SpyGlass) view of the periductal-type cholangiocarcinoma.

technique using a small endoscope that was passed through the working channel of a duodenoscope and (2) peroral direct cholangioscopy using a pediatric or dedicated forward viewing endoscope with an additional bending function that were advanced through the mouth [50–52].

As shown mainly by Japanese endoscopists, cholangioscopy increases sensitivity to 96-100% for the diagnosis of malignancy when combined with ERCP [53–56]. Nonetheless, both these techniques had important limitations that prevented their widespread usage. The former "mother-baby" endoscopy required two operators and the "babyscope" was fragile. The peroral cholangioscope was difficult to manipulate, especially within nondilated ducts, plus the dedicated video cholangioscope did not become commercially available in the West [57–61].

The current technological standard for the "mother-baby" approach is represented by a digital single-operator cholangioscope (DSOC, SpyGlass DS; Boston Scientific, Inc., Natick, Massachusetts, USA) commercially available since 2015. SpyGlass comprises a disposable 10.5 Fr scope with an integrated digital sensor and portable processor. The scope allows four-way tip deflection and enables suction, irrigation, and passage of miniaturized biopsy forceps (SpyBite; Boston Scientific, Inc.) or any of several other accessories [62].

DSOC enables direct visualization of the biliary tract and intraluminal biopsy. Although no formal consensus on visual diagnostic criteria has been established, the presence of either nodular or papillary masses, irregular surface, tortuous dilated vessels, and fragile mucosa are considered features typical of neoplasia. For benign lesions, a flat surface, fine network of vessels, regular granular appearance, nonfragile mucosa, convergence of folds, and presence of pseudodiverticula are deemed diagnostic. The sensitivity and specificity of visual diagnosis were reported in the range of 90-100% and 76-96%, respectively [63–67] (Figures 5 and 6).

As for SpyBite biopsies, a sensitivity of 85-86% and specificity of 100% can be expected. Moreover, as shown by Varadarajulu et al., biopsy sensitivity can be increased up to 94% by using rapid on-site examinations of touch imprint cytology [68].

It has been suggested by Sugiyama et al. that the evaluation of ECCA extension may influence surgical strategy. Yet, as shown by Itoi et al., the proximal tumor margin can be visualized only in a limited number of patients and submucosal tumor extension is not possible to estimate at all. In one study comparing DSOC and ERCP, DSOC provided no additional information regarding the local spread of the disease in three surgically treated cases. Among these cases, an intraoperative frozen section revealed a spread of malignant cells beyond the predicted tumor margin in one of them, while the remaining two corresponded in preoperative and intraoperative findings. Thus, the results of local staging with DSOC should be interpreted cautiously [67, 69, 70].

As for the complications of the method, a retrospective study comparing ERCP with and without cholangioscopy showed increased morbidity when cholangioscopy was performed. Of note, the risk of cholangitis increased five times [71]. It was suggested that the higher risk of cholangitis is caused mainly by an increase in intraductal pressure due to intermittent water irrigation during the procedure. In a recent meta-analysis of 49 studies including 2193 patients who underwent either diagnostic or therapeutic peroral cholangioscopy, Korrapati et al. reported an overall and a serious adverse event rate of 7% and 1%, respectively. To prevent cholangitis, antibiotic prophylaxis and adequate biliary drainage are necessary [72].

Despite excellent operating characteristics, the position of DSOC in the diagnostic algorithm of uncertain biliary strictures must be further evaluated in regard to other modalities. Because of its costs, complexity, and procedure-related morbidity, DSOC should be considered mainly in the case of indeterminate biliary strictures in which previous ERCP tissue sampling was not conclusive. Even so, DSOC is becoming widely available and may become an option at hand during index ERCP in the future, particularly in cases with proximal strictures. Although such an approach could theoretically shorten the diagnostic process, its impact on safety remains to be investigated.

8.3. Endoscopic Ultrasound. Endoscopic ultrasound (EUS) was introduced in clinical practice in the 1980s. Since then,

Figure 7: Linear endosonography view of a distal cholangiocarcinoma.

the position of EUS in diagnosing and staging pancreatobiliary diseases, including CCA, has been firmly established. During the EUS procedure, CCA can be readily visualized in both cross-sectional and longitudinal views. If a mass is present, it usually appears hypoechoic or, less frequently, heterogenous (Figure 7). The upstream dilatation of the biliary tract can also be estimated. In addition to tumor depiction, EUS allows for the identification of the hilum, celiac axis, and para-aortic lymph nodes facilitating the staging process.

As for malignant biliary stricture detection, EUS without fine needle aspiration (FNA) was found to provide sensitivity of 78% and specificity of 84% [73]. Another study proved EUS to be superior in biliary cancer detection when compared to CT and MRI/MRCP (94%, 30%, and 42%, respectively), with a statistical significant difference. In the same study, EUS detected 100% of distal and 83% of proximal CCAs while demonstrating sensitivity of 53% and specificity of 97% in the assessment of unresectability [74].

The development of linear echoendoscopes enabled EUS-FNA which further improved the diagnostic capability of EUS. Across several studies, EUS-FNA sensitivity and specificity for differentiating ECCA from benign biliary lesions range between 43-89% and 79-100%, respectively. Moreover, sensitivity of 45% could be achieved even in the case of ECCA with no definite mass on cross-sectional imaging. Similar to EUS alone, the operating characteristics of EUS-FNA proved better for distal as compared to proximal lesions. One study has demonstrated sensitivity of 81% for distal and 59% for proximal lesions with the overall sensitivity being 73%.

Based on these results, EUS-FNA can be readily recommended for tissue sampling in cases with inconclusive results from ERCP brushing. A meta-analysis of patients with negative brush cytology has revealed the sensitivity and specificity of EUS-FNA to be 59% and 100%, respectively, for the diagnosis of ECCA [75–78].

The possibility of using EUS-FNA as a safer alternative to ERCP was also investigated. For instance, Onda et al. performed EUS-FNA as the first-line method in patients with suspected ECCA based on CT or other imaging modalities with sensitivity of 89% and accuracy of 87% [79]. In a recent meta-analysis, De Moura et al. calculated the mean sensitivities of ERCP and EUS-FNA for the tissue diagnosis of a malignant biliary stricture to be 49% and 75%, while specificities were 96% and 100% [80].

However, not all literature is in agreement with the superiority of EUS over ERCP in the assessment of malignant biliary strictures. For example, although the study by Rosch et al. proved EUS to be more sensitive in the diagnosis of pancreatic tumors, ERCP was a better diagnostic modality for CCA [75]. In a study by Weilert et al., ERCP brushing had the same sensitivity (79%) as compared to EUS-FNA [81].

Finally, same-session EUS-FNA and ERCP-based tissue sampling was superior to EUS-FNA alone for both pancreatic and biliary lesions [82].

It is important to note that the negative predictive values of EUS-FNA for malignancy were relatively low in most of the studies, ranging from 29% to 67%. Therefore, similarly to ERCP, a negative EUS-FNA may not exclude the malignant etiology of biliary strictures.

The short-term endoscopic risks of EUS-FNA are low, including acute pancreatitis (0.3-2%), bleeding (1%), perforation (0.4%), and infection (0.3%) [83].

Amid late complications, tumor cell seeding after transperitoneal FNA must be considered in operable patients. As reported by Heimbach et al., peritoneal metastasis occurred in 83% of patients who underwent a percutaneous or transluminal FNA biopsy of the primary hilar tumor mass as opposed to 8% in cases where no biopsy was performed [84]. As a result, the Mayo Clinic transplantation protocol excludes patients who have undergone a biopsy of the primary tumor from neoadjuvant therapy and liver transplantation. So far, there have been no reports of tumor seeding in those with distal CCA. In theory, the puncture route for a distal biliary lesion is usually nontransperitoneal and the needle tract is removed during the Whipple resection. Furthermore, a retrospective study found a significantly lower incidence of peritoneal carcinomatosis in patients with pancreatic adenocarcinoma after EUS-FNA as compared to percutaneous procedures (2.2% in the EUS group vs. 16.3% in the percutaneous group) [85].

In conclusion, EUS-FNA performs well in the detection and staging process of ECCA, although the relatively low negative predictive value is a fact that must be taken into consideration. Still, its nearly 100% positive predictive value is the utmost power when it comes to indicating aggressive treatment including surgery. Due to the concern of tumor seeding, the EUS-FNA of proximal lesions should be indicated with caution [86].

8.4. Intraductal Sonography (IDUS). IDUS uses high-frequency (12-20 MHz) catheter-based probes of 2 mm diameter introduced into the biliary tract over a guidewire through the working channel of a duodenoscope. In most cases, preceding sphincterotomy is not necessary. IDUS is safe and technically easy to learn, but image interpretation remains challenging. It is used both for CCA detection and local staging. Lymph node assessment with this high-frequency ultrasound is not possible due to a penetration depth of only 20 mm.

IDUS distinguishes three layers of the normal bile duct wall: (1) inner hyperechoic corresponding to mucosa, (2) middle hypoechoic of smooth muscle fibers with fibroelastic tissue, and (3) outer hyperechoic corresponding to connective tissue.

Several diagnostic features of malignant strictures were described such as the disruption of the normal sonographic pattern, presence of a hypoechoic infiltrating lesion with irregular margins, and a tumor invasion into surrounding tissues. Malignancy is extremely likely if IDUS detects a tumor invasion into the hepatic artery, portal vein, or pancreatic parenchyma. Secondly, findings considered typical for a benign bile duct stricture include the preservation of the normal wall pattern, a homogeneous echo pattern, smooth margins, hyperechoic lesions, and absence of a mass lesion. The accuracy of IDUS in differentiating benign from malignant strictures ranges from 76 to 98% in the literature [87–89].

Other diagnostic features such as interrupted wall structure, the presence of a sessile tumor intraductally or outside the bile duct, or tumor size greater than 10 mm were suggested by Tamada et al. The likelihood of malignancy was as high as 97% when two or three of these features were present. To the contrary, an IDUS examination with no positive feature correlated with negative findings at the final diagnosis [90].

Even though IDUS has no capability for tissue sampling, it proved to be more accurate in distinguishing benign and malignant strictures than ERCP with transpapillary biopsy. In a retrospective study, IDUS was more specific (92% vs. 42%) and similarly sensitive (89% vs. 83%) as ERCP with biopsy. Compared to EUS, IDUS had higher sensitivity (91% vs. 75%) and specificity (80 vs. 75%) [91, 92].

IDUS has demonstrated to be a useful tool in predicting positive biopsy. When an intraductal sessile tumor could be visualized, the sensitivity of the biopsy was 92%. Moreover, as previously suggested, IDUS-directed bile duct sampling was found to be more sensitive than ERCP-guided sampling (87% vs. 67%) [28, 93].

As for the assessment of longitudinal tumor spread, IDUS proved better accuracy than ERCP (84% vs. 47%). In a study investigating bile duct wall thickness using IDUS in patients who had not undergone biliary drainage, in 95% of cases, the biliary wall of the common hepatic duct was not thicker than 1.8 mm unless the patients were diagnosed with primary sclerosing cholangitis or had longitudinal cancer extension along the bile duct [94]. A different study reported the mean length of longitudinal extension beyond the estimated measure by cholangiography to be about 6-10 mm for submucosal and 10-20 mm for mucosal spread [95].

The depth of tumor invasion can also be assessed by IDUS. When the outside hyperechoic layer is interrupted, a serosa invasion is suggested with an accuracy of 86%-93%. A vascular invasion is suspected if a high-echoic ("interface") echo in between the tumor and vessel wall disappears. While diagnostic accuracy for portal vein and right hepatic artery invasion assessment was 86-100% and 92-100%, respectively, the visualization of the left and proper hepatic artery was poor. Therefore, CT and IDUS should be regarded as complementary staging methods [96–98].

8.5. Confocal Laser Endomicroscopy. Probe-based confocal laser microscopy is a method providing real-time, 400-fold-magnified imaging of the mucosa. The probe is introduced into the biliary tract through a catheter or the working channel of a cholangioscope. The intravenous injection of 10% fluorescein sodium is mandatory. A high diagnostic performance in malignant biliary strictures with accuracy of 79-82% has been reported. Nevertheless, this technology is costly and interpretation of images challenging [99–101]. Of note, in cases with prior biliary stenting, accuracy decreased to 45% in one study. Therefore, to decrease the rate of misclassification of benign stricture as malignant, usage of Paris (instead of Miami) Classification was recommended in this clinical scenario [102, 103].

9. Patients with PSC

The diagnosis of CCA in patients with primary sclerosing cholangitis (PSC) is cumbersome since background inflammation can obscure the clinical and morphological manifestation of the disease. According to the ESGE/EASL guidelines, ERCP with tissue sampling should be considered in patients with worsening symptoms, a rapid increase of cholestatic enzyme levels, or a new dominant stricture or progression of existing dominant strictures at MRCP. The diagnostic performance of DSOC was shown to be similar to non-PSC patients [104].

10. Conclusion

A rational utilization of cross-sectional imaging and endoscopic procedures in patients with clinical and laboratory suspicions of CCA is mandatory. The availability of advanced endoscopic methods, such as cholangioscopy and EUS, is increasing and makes the diagnostic algorithm easier. The limitation of tissue sampling resides in its low negative predictive value for malignancy. Despite the considerable progress in this field, further clinical research is needed.

Abbreviations

CCA: Cholangiocarcinoma
ECCA: Extrahepatic cholangiocarcinoma
ICCA: Intrahepatic cholangiocarcinoma
PCCA: Proximal (perihilar) extrahepatic cholangiocarcinoma
DCCA: Distal extrahepatic cholangiocarcinoma
ERCP: Endoscopic retrograde cholangiopancreatography
EUS: Endoscopic ultrasound
EUS-FNA: Endoscopic ultrasound (-guided) fine needle aspiration
IDUS: Intraductal ultrasonography.

Conflicts of Interest

The authors declare that there is no conflict of interest regarding the publication of this paper.

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Research Article

Investigation of 23 Bile Acids in Liver Bile in Benign and Malignant Biliary Stenosis: A Pilot Study

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Differential diagnosis between benign and malignant biliary stenosis can be difficult in clinical practice. Histology of biopsy specimens is often indeterminate. Laboratory markers (serum bilirubin > 75 μmol/L, carbohydrate antigen 19-9 > 400 U/mL) and the length of stenosis (>15 mm) can be helpful but are not specific enough. The aim of this study was to investigate bile acids in liver bile of patients with benign and malignant biliary stenosis and controls without stenosis. A total of 73 patients entered the study: 7 subjects with benign biliary stenosis (6 men, 1 woman; 68 ± 13 years old), 21 with malignant biliary stenosis (15 men, 6 women; 72 ± 14 years old), and 45 patients without biliary stenosis (22 men, 23 women; 70 ± 13 years old); out of those, 25 subjects have and 20 do not have choledocholithiasis. Twenty-three different bile acids were investigated by high-performance liquid chromatography/mass spectrometry. Serum total bilirubin was significantly higher in patients with malignant biliary stenosis compared with nonstenotic controls ($p = 0.005$). Significant relationship ($r > 0.7$) was found between several pairs of bile acids. Significantly lower bile acid concentrations in malignant biliary stenosis compared to controls without stenosis were found for GLCA ($p = 0.032$), GUDCA ($p = 0.032$), GCDCA ($p = 0.006$), GDCA ($p = 0.031$), GHCA ($p = 0.005$), TUDCA ($p = 0.044$), and TDCA ($p = 0.036$). Significant difference in cholic acid was found between benign and malignant stenosis ($p = 0.022$). Analysis of bile acids might be helpful in the differential diagnosis of malignant and benign biliary stenosis. More patients need to be enrolled in further studies so that the real diagnostic yield of bile acids can be determined.

1. Introduction

Differential diagnosis of benign and malignant biliary stenosis can be difficult in the clinical practice. Histology of biopsy specimens is often indeterminate. Laboratory markers (serum bilirubin > 75 μmol/L, carbohydrate antigen 19-9 > 400 U/mL) and the length of stenosis (>15 mm) can be helpful but are not specific enough [1]. One study of altered bile composition in benign biliary strictures (after liver transplantation) has been published [2]. Few other studies evaluated bile acids in patients with cancer in the biliary tree [3–5]. Other authors looked at the biomarkers which can help in differentiation between benign and malignant biliary stenosis in other body fluids for the sample matrix [6–8]. The biological basis of benign and malignant biliary stenosis differs significantly. One can assume that different compositions and/or concentrations of bile acids can impact on these processes. The aim of our study was to evaluate 23 different bile acids in liver bile of consecutive patients to analyze whether patients with malignant biliary stenosis have a different concentration of bile acids from the concentration of bile acids of patients with benign stenosis or without

stenosis. This observation could have relevance in the differential diagnosis.

2. Materials and Methods

2.1. Patients. A total of 73 patients entered the study: 7 subjects with benign biliary stenosis (6 men, 1 woman; 68 ± 13 years old), 21 with malignant biliary stenosis (15 men, 6 women; 72 ± 14 years old), and 45 patients without biliary stenosis (22 men, 23 women; 70 ± 13 years old); out of those, 25 subjects have and 20 do not have choledocholithiasis. Seven patients received long-term treatment with peroral UDCA (daily dose 500-1500 mg; median 500, IQR 500-875).

2.2. Sample Collection and Storage. Serum total bilirubin and C-reactive protein were investigated less than 48 hours before ERCP (endoscopic retrograde cholangiopancreatography). Samples of hepatic bile were collected from the common hepatic duct endoscopically (prior to contrast media use) and frozen at -80°C immediately until the analysis was carried out.

2.3. Chromatography and Mass Spectrometry

2.3.1. Chromatography. The separation of 23 endogenous bile acids was done using UPLC system Acquity I-Class. Briefly, the separation was carried out on an Acquity BEH C18 column (50 × 2.1 mm with 1.7 μm particle size) (Waters, Milford, USA), protected with a disposable 0.2 μm in-line filter (Waters, Milford, USA). The separation was performed in a gradient elution mode with a flow rate of 0.35 mL min^{-1} and with the following composition of mobile phases: Solvent A consisted of ammonium acetate (0.5 mM) and acetic acid (0.001% *v*/*v*) in water. Solvent B was composed of methanol : acetonitrile (75 : 25, *v*/*v*) mixture containing 0.5 mM ammonium acetate and 0.001% (*v*/*v*) of acetic acid. The gradient program was as follows: 0-0.2 min, 40% of solvent B; 0.2-7.0 min, 40-70% of solvent B; 7.0-8.0 min, 70-90% of solvent B; 8.0-8.5 min, 90 - 95% of solvent B; and 9.0-11 min, 40% of solvent B.

The column was held at 45°C during the analysis. Samples were kept at 10°C in a light-tight autosampler unit. Two microlitres of sample was injected into the column. Retention times of all 23 bile acids were verified with authentic standards, and quantification was done against the 8-point calibration curve covering range 5-2000 nmol L^{-1}.

2.3.2. Mass Spectrometry. A Xevo-TQ/XS mass spectrometer (Waters, Milford, USA) coupled with the Acquity I-Class UPLC was used for detection of compounds. The spectrometer was operated with an ESI interface in a negative ion mode. Optimized settings of an ion source were as follows: capillary voltage, 2.5 kV; cone voltage, 50 V; source temperature, 150°C, desolvation temperature, 600°C; desolvation gas, 1000 L/h; cone gas, 150 L/h; and nebuliser, 6 L/h.

Compounds were monitored using the multiple reaction monitoring (MRM) mode of operation. Since nonconjugated bile acids generally do not offer CID (collision-induced dissociation) fragments of sufficient intensity for quantification, the so-called pseudo-MRM was used for their quantification. The following MRM transitions were used: 375 ⟶ 375 (nonconjugated, monohydroxy BA), 391 ⟶ 391 (nonconjugated, dihydroxy BA), 407 ⟶ 407 (nonconjugated, trihydroxy BA), 432 ⟶ 74 (glycine-conjugated, monohydroxy BA), 448 ⟶ 74 (glycine-conjugated, dihydroxy BA), 464 ⟶ 74 (glycine-conjugated, trihydroxy BA), 482 ⟶ 80 (taurine-conjugated, monohydroxy BA), 498 ⟶ 80 (taurine-conjugated, dihydroxy BA), and 514 ⟶ 80 (taurine-conjugated, trihydroxy BA). Energies involved in CID were as follows: nonconjugated bile acids, CE = 10 eV; glycine-conjugated bile acids, CE = 30 eV; and taurine-conjugated bile acids, CE = 60 eV. MassLynx and TargetLynx software was used for LC/MS data acquisition and evaluation (version 4.2, Waters, Milford, USA).

2.3.3. Sample Preparation for LC/MS Analysis. Due to high concentration of bile acids in bile, the samples were diluted prior to analysis. Two dilution factors, 1000x and 10000x, were used to cover a broad range of concentrations, including major and minor bile acids in the samples.

A sample preparation corresponding to the 1000x dilution factor was as follows: Ten microlitres of bile in the 1.5 mL Eppendorf tubes was diluted with 90 μL of 0.1% HCOOH in 50% (*v*/*v*) acetonitrile. In the next step, 900 μL of acetonitrile solution containing 1% (*v*/*v*) of formic acid and internal standard IS-CAD_5 ($c = 5\,\mu$M) was added to the same tube.

The samples with the dilution factor 10000x were prepared as follows: Ten microlitres of bile in the 1.5 mL Eppendorf tubes was diluted with 990 μL of 0.1% (*v*/*v*) formic acid in 50% (*v*/*v*) acetonitrile. One hundred microlitres of this solution was taken and pipetted to a clean 1.5 mL Eppendorf, to which 900 μL of cold acetonitrile solution containing 1% (*v*/*v*) of formic acid and internal standard IS-CAD_5 ($c = 5\,\mu$M) was further added.

The samples, with the dilution factor 1000x or 10000x, prepared in the previous step were vortexed for 1 minute, left for 10 minutes at -20°C to achieve sample deproteination, and spun at 14.000 × *g* (5 min, room temperature). One hundred microlitres of a supernatant was taken and mixed in a clean 1.5 mL Eppendorf tube with 900 μL of 0.1% (*v*/*v*) formic acid in 50% (*v*/*v*) acetonitrile. Samples were vortexed, and 250 μL is transferred to the AcroPrep filter plate (96 wells, wwPTFE membrane, pore size 0.20 μm, Pall Corporation, USA). The filtrate was collected to a 96-well sample collection plate (700 μL, round well, Waters, Milford, USA), sealed with a sealing mat (Cap-mat 96-well 7 mm round plug preslit silicone/PTFE, Waters, Milford, USA), and put into an autosampler for analysis.

2.4. Statistical Analysis. The obtained data were tested statistically by means of descriptive statistics. Data with normal distribution were further analyzed by the parametric unpaired *t*-test, and data with nonnormal distribution were tested by the nonparametric Mann-Whitney test. The Pearson test was used for correlation analysis (SigmaStat software, version 3.1, Jandel Corp., Erkrath, Germany).

2.5. Ethics. The study was approved by the Joint University Ethics Committee (Protocol No. 201712S06P). All procedures were in accordance with the ethical standards of the institutional research committee and with the 1964 Helsinki declaration and its later amendments.

All patients signed the written consent. For all data obtained, all personal identification information was deleted in compliance with the laws for the protection of confidentiality of the Czech Republic.

3. Results

Serum total bilirubin was significantly higher in patients with malignant biliary stenosis compared with nonstenotic controls ($p = 0.005$; see Table 1). Significant relationship ($r > 0.7$) was found between several pairs of bile acids (GLCA-GDCA, GLCA-TLCA, GCDCA-GDCA, GCDCA-GCA, CDCA-CA, GUDCA-TUDCA, GDCA-TDCA, TCDCA-TCA, and TMCA-THCA). Significantly lower bile acid concentrations in malignant biliary stenosis compared to controls without stenosis were found for GLCA ($p = 0.032$), GUDCA ($p = 0.032$), GCDCA ($p = 0.006$), GDCA ($p = 0.031$), GHCA ($p = 0.005$), TUDCA ($p = 0.044$), and TDCA ($p = 0.036$). Significant difference between benign and malignant stenosis was found for cholic acid (CA) ($p = 0.022$, Figure 1). Other results did not reach statistical significance. Concentrations of serum C-reactive protein and 15 bile acids in primary bile in the investigated groups are listed in Table 1. The remaining 8 bile acids were not detectable or their concentrations were <2.5 μmol/L. Total bile acid concentrations are listed in Table 1. They were higher in malignant stenosis compared to benign stenosis ($p = 0.027$) and controls without stenosis ($p < 0.001$).

Bile UDCA was detectable in 14/73 persons (19%). Out of those, 7 patients were not treated and 7 subjects were treated with peroral UDCA (daily dose 500-1500 mg; median 500, IQR 500-875). Bile UDCA was lower in the nontreated group (40.0 ± 44.7 vs. 186.2 ± 182.9 μmol/L), yet this difference did not reach statistical significance ($p = 0.128$, type 2 error beta 0.632). Bile UDCA did not correlate tightly enough either with bile GUDCA ($r = 0.513$, $p < 0.001$) or with bile TUDCA ($r = 0.388$, $p < 0.001$). Correlation between bile GUDCA and bile TUDCA was significant ($r = 0.835$, $p < 0.001$). Seven patients treated with peroral UDCA had significantly higher concentrations of bile GUDCA ($p < 0.001$) and bile TUDCA ($p < 0.001$) compared to those without treatment with peroral UDCA. Treatment with peroral UDCA did not influence significantly the concentration of other bile acids in primary liver bile.

4. Discussion

Our current pilot case-control study has shown a new original insight. Bile acid concentrations were significantly lower in malignant biliary stenosis compared to controls without stenosis. There was a significant difference of cholic acid between benign and malignant stenosis. The number of analyzed bile acids is unique. Such a detailed analysis might enable precise mapping of different pathological situations.

One study of altered bile composition in benign biliary strictures (after liver transplantation) was published [2]. Bile salts, phospholipids, and cholesterol were significantly lower in 14/111 patients (13%) who developed nonanastomotic biliary strictures after liver transplantation. Patients who developed biliary stenosis were characterized by a reduced biliary secretion of bile salts and phospholipids and a decreased biliary phospholipid/bile salt ratio. Four bile salts were investigated (cholate, deoxycholate, chenodeoxycholate, and ursodeoxycholate): there were no significant differences in the absolute amounts of the various bile salts between patients who later developed and those who have not developed nonanastomotic biliary strictures [2].

Few other studies analyzed bile acids in patients with cancer of the biliary tree [3–5]. Lankisch et al. [4] published a bile proteomic analysis enabling differentiation of cholangiocarcinoma from primary sclerosing cholangitis and choledocholithiasis. Subsequently, a US patent was submitted [9]. Voigtländer et al. from the same research group [10] published a paper on early diagnosis of cholangiocarcinoma. In this study, combined bile and urine proteome analysis was first established on 87 patients with known diagnosis and then applied to a group of 45 patients without established diagnosis at the time of sample analysis. Thus, validation of the diagnostic method was carried out in a prospective study cohort [10]. Park et al. [3] analyzed five bile acids (cholic, deoxycholic, chenodeoxycholic, lithocholic, and ursodeoxycholic acid) in the biliary tract cancer group, bile duct stone group, and controls. Total bile acid concentration was lower in the cancer group than in the biliary stone and control groups. This result was similar when disease localisation was limited to the bile duct or gallbladder. Although the presence of bile duct obstruction explains some of the differences in total concentration and composition of bile acids, there are other contributing mechanisms. Park et al. stated that alteration of bile acid transport might decrease bile acid excretion and can lead to accumulation of carcinogenic bile acids in the bile duct epithelium [3].

A key question that must be addressed is what is the cause and what is the consequence. Bile salts are potentially cytotoxic [11, 12] and antiapoptotic and might cause cholangiocyte injury and induce periductal fibrosis [13]. Bile acids due to their signalling properties have differential effects on cholangiocyte intracellular regulation, and they can initiate inverse effects on cholangiocyte secretion, proliferation, and survival; e.g., taurocholic and taurolithocholic acids can stimulate, whilst ursodeoxycholic acid may reduce the proliferative effect of other bile acids [2, 14–16]. In an experimental study in rats, intrahepatic accumulation of bile acids did not induce carcinogenesis directly but facilitated a cocarcinogenic effect due to stimulation of bile duct proliferation, enhanced inflammation (increased expression of interleukin-6), and reduction in farnesoid X receptor-dependent chemoprotection (downregulation of FXR) [17].

Although the pharmacokinetics of UDCA have been studied since its approval for its use in primary biliary cholangitis, limited work has been published on modelling the metabolism of UDCA and its two major conjugates, a glycine conjugate, GUDCA, and a taurine conjugate, TUDCA [18].

Table 1: Serum bilirubin and C-reactive protein and bile acids in liver bile in benign and malignant biliary stenosis and controls without stenosis.

Parameter	Benign stenosis ($n = 7$) Mean ± Std.Dev. C.I. of mean Median IQR	Malignant stenosis ($n = 21$) Mean ± Std.Dev. C.I. of mean Median IQR	Controls without stenosis ($n = 45$) Mean ± Std.Dev. C.I. of mean Median IQR
Serum bilirubin (μmol/L)	39.4 ± 47.9 44.3 15.0 11.0–49.0	174,667 ± 178,556 81.3 138.0 14.5–310.0	37.4 ± 42.9 12.9 15.0 11.0–49.0
C-reactive protein (mg/L)	15.1 ± 27.8 25.7 2.5 2.0–13.3	40.7 ± 50.8 28.1 10.0 5.5–57.5	31.8 ± 42.6 14.2 11.0 2.8–55.0
CDCA (μmol/L)	203.4 ± 305.4 282.4 43.8 4.5–269.7	18.3 ± 25.1 11.4 2.9 0–27.9	68.7 ± 157.9 47.4 16.6 0–29.5
GLCA (μmol/L)	73.1 ± 56.9 52.6 73.0 27.2–105.8	38.3 ± 62.8 28.6 12.4 0–44.7	169.6 ± 272.6 81.9 32.1 4.1–260.1
GUDCA (μmol/L)	3003.2 ± 4860.6 4495.4 192.7 77.4–7507.2	740.5 ± 1847.6 841.0 123.7 2.6–319.9	2160.1 ± 5088.9 1528.9 259.2 38.0–1247.0
GCDCA (μmol/L)	6209.0 ± 4347.8 4021.1 6132.0 3027.3–10097.8	3425.5 ± 3684.1 1677.0 2642.0 308.7–5746.8	6739.6 ± 5380.0 1616.3 4995.0 2404.9–9474.5
GDCA (μmol/L)	2476.4 ± 2803.8 2593.1 1300.7 844.1–2995.0	1096.8 ± 1845.7 840.1 381.0 8.9–1497.8	3244.3 ± 4605.7 1383.7 1110.5 135.9–4421.3
GCA (μmol/L)	8196.7 ± 6767.3 6258.7 6929.0 2361.8–13126.0	4639.1 ± 4891.7 2226.7 3335.0 709.1–6549.5	5818.5 ± 4335.7 1302.6 5354.0 2794.8–7259.0
GHCA (μmol/L)	22.8 ± 15.9 14.7 30.1 7.7–36.3	14.1 ± 17.6 8.2 8.0 2.7–14.6	43.8 ± 80.2 24.1 16.4 10.3–33.8
TLCA (μmol/L)	30.7 ± 35.2 32.5 22.3 2.5–56.9	19.4 ± 40.3 18.4 5.2 0–16.8	52.8 ± 75.0 22.5 19.3 2.1–77.8

Table 1: Continued.

Parameter	Benign stenosis ($n = 7$) Mean ± Std.Dev. C.I. of mean Median IQR	Malignant stenosis ($n = 21$) Mean ± Std.Dev. C.I. of mean Median IQR	Controls without stenosis ($n = 45$) Mean ± Std.Dev. C.I. of mean Median IQR
TUDCA (μmol/L)	218.1 ± 454.6 420.5 46.8 9.5–115.3	72.9 ± 135.6 61.7 19.2 0–58.8	228.6 ± 474.5 142.6 58.9 9.5–152.8
TCDCA (μmol/L)	1837.6 ± 2285.3 2113.5 1118.5 275.2–2301.4	1674.9 ± 1617.8 736.4 1734.2 282.3–2319.5	2324.9 ± 1758.2 528.2 1689.3 1113.4–3290.7
TDCA (μmol/L)	603.8 ± 834.8 772.0 278.8 30.1–1100.8	368.4 ± 655.1 298.2 118.3 7.1–321.6	786.4 ± 1136.1 341.3 377.5 79.8–930.7
TMCA (μmol/L)	7.5 ± 9.5 8.8 4.5 0–16.1	7.2 ± 13.3 6.0 3.4 0–8.7	12.9 ± 22.5 6.8 5.9 0–13.5
THCA (μmol/L)	11.1 ± 16.3 15.1 3.8 0.9–14.8	28.3 ± 38.3 17.4 11.1 3.1–35.0	39.2 ± 71.5 21.5 12.1 4.0–37.2
TCA (μmol/L)	2183.6 ± 3050.8 2821.6 1634.8 366.1–1906.2	2316.5 ± 2234.9 1017.3 1658.5 652.5–3343.5	2443.0 ± 2141.9 643.5 1923.1 1031.2–3048.0
CA (μmol/L)	242.5 ± 280.7 259.6 97.8 5.8–465.9	16.6 ± 32.2 14.6 0 0–20.4	50.2 ± 145.5 43.7 2.6 0–17.7
Total bile acids (mmol/L)	37.7 ± 23.6 21.8 26.7 20.5–50.0	68.4 ± 31.8 14.5 65.8 43.0–87.2	24.2 ± 15.9 4.8 21.9 11.2–33.0

In our study, seven patients treated with peroral UDCA had significantly higher concentrations of bile GUDCA and bile TUDCA.

Our current project is a pilot study, and our data must be interpreted with caution. We are not able to provide any final explanation for the difference found. Nevertheless, we can hypothesize that long-standing low concentrations of cholic acid can contribute to biliary mucosal instability as one of initial steps in tumorous biology. Unlike us, Jusakul et al. [5] investigated the bile acid profile in gallbladder bile from patients who underwent liver resection for cholangiocarcinoma, hepatocellular carcinoma, and benign biliary diseases. They found markedly elevated cholic and chenodeoxycholic acid in cholangiocarcinoma with high total serum bilirubin. The authors revealed a different pattern of bile acid concentration in cancer patients compared to patients with benign biliary diseases. Accumulation of certain bile acids may be involved in carcinogenesis according to these authors [5].

We are aware of possible limits of this pilot study. The number of patients was limited, especially those with benign stenosis. The control group was rather heterogenic, with or without previous cholecystectomy, some patients underwent

Figure 1: Bile cholic acid in benign ($n = 7$) and malignant ($n = 21$) biliary stenosis and controls without stenosis with (control A; $n = 25$) or without (control B; $n = 20$) choledocholithiasis (mean + Std.Dev.). Significant difference between benign and malignant stenosis was found ($p = 0.022$).

repeated ERCPs with or without papillotomy, common bile duct stones were of different aetiology, and not all bile samples were sterile. Patients with malignant stenosis and subjects without stenosis and without choledocholithiasis have both low levels of cholic acid. This observation does not rule out the possible utility of concentration of cholic acid in differential diagnosis between benign and malignant stenosis.

5. Conclusions

Analysis of bile acids might be helpful in the differential diagnosis of malignant and benign biliary stenosis. More patients must be enrolled in the future studies so that the real diagnostic yield of bile acids can be determined.

Abbreviations

CA: Cholic acid
CDCA: Chenodeoxycholic acid
CE: Collision energy
C.I.: Confidence interval
CID: Collision-induced dissociation
DCA: Deoxycholic acid
ERCP: Endoscopic retrograde cholangiopancreatography
HCA: Hyocholic acid
HDCA: Hyodeoxycholic acid
LCA: Lithocholic acid
IQR: Interquartile range
IS-CAD$_5$: Internal standard-D$_5$-cholic acid
MCA: Muricholic acid
GCA: Glycocholic acid
GCDCA: Glycochenodeoxycholic acid
GDCA: Glycodeoxycholic acid
GHCA: Glycohyocholic acid
GHDCA: Glycohyodeoxycholic acid
GLCA: Glycolithocholic acid
GUDCA: Glycoursodeoxycholic acid
Std. Dev.: Standard deviation
TCA: Taurocholic acid
TCDCA: Taurochenodeoxycholic acid
TDCA: Taurodeoxycholic acid
THCA: Taurohyocholic acid
THDCA: Taurohyodeoxycholic acid
TLCA: Taurolithocholic acid
TMCA: Tauromuricholic acid
TUDCA: Tauroursodeoxycholic acid
UDCA: Ursodeoxycholic acid.

Data Availability

The study data used to support the findings of this study have not been made available because of the General Data Protection Regulation (Regulation 2016/679 of the European Parliament and of the Council of 27 April 2016 on the protection of natural persons with regard to the processing of personal data and on the free movement of such data and repealing Directive 95/46/EC).

Disclosure

Preliminary results of the study were presented as a poster at the European Gastroenterology Congress (UEG Week, Barcelona 2019, Oct 19-23, available from doi:10.1177/2050640619854671).

Conflicts of Interest

The authors declare that they have no conflict of interest.

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Review Article

Contemporary Tailored Oncology Treatment of Biliary Tract Cancers

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Biliary tract cancers (BTCs) are poor prognosis malignancies with limited treatment options. Capecitabine has recently emerged as an effective agent in the adjuvant setting; however, treatment of advanced disease is still limited to first-line cisplatin and gemcitabine chemotherapy. Recent global efforts in genomic profiling and molecular subtyping of BTCs have uncovered a wealth of genomic aberrations which may carry prognostic significance and/or predict response to treatment, and several targeted agents have shown promising results in clinical trials. As such, the uptake of comprehensive genomic profiling for patients with BTCs and the expansion of basket trials to include these patients are growing. This review describes the currently approved systemic therapies for BTCs and provides insight into the emerging targeted and immunotherapeutic agents, as well as conventional chemotherapeutic regimes, currently being investigated in clinical trials.

1. Introduction

Biliary tract cancers (BTCs), encompassing cholangiocarcinoma (CCA) and gallbladder cancer (GBC), are relatively rare cancers with wide geographical diversity [1]. Cholangiocarcinomas are generally divided into intrahepatic (ICC) and extrahepatic tumours (ECC) with the latter further was separated into perihilar cholangiocarcinoma (PCC) and distal cholangiocarcinoma (DCC) [2]. The highest rates of CCA are seen in countries such as China and Thailand where liver fluke infection is prevalent [3, 4]. However, the incidence of CCA in most Western countries is also slowly rising [5], probably as a consequence of improved imaging techniques and diagnosis in addition to the increasing burden of chronic diseases such as fatty liver disease and viral hepatitis [6, 7]. On the other hand, the highest incidence of GBC is in Chile where gallstone disease is the primary risk factor [8].

Unfortunately, BTCs carry an extremely poor prognosis with an overall 5-year survival in the region of 5-15% [9]. The majority of patients present with unresectable or advanced disease at diagnosis [10]; thus, systemic therapy is their only treatment option. In addition to the diverse aetiological origins of BTCs, it is well recognised that the BTC subtypes also differ in their tumour biology [11–13] and clinical presentation [2]. For example, patients with ECC are more likely to present with obstructive jaundice, and thus, their disease may be diagnosed at an earlier stage. For those who do present with resectable disease, surgery may be curative; however, these patients represent a small minority and relapse rates are high [14]. Furthermore, the patterns of relapse usually preclude further curative resection [9, 15]. There is, therefore, a huge unmet need for more effective therapies for the treatment of BTCs.

This review describes the currently approved systemic therapies for BTCs and, following recent advances in the molecular profiling of these rare tumours, provides insight into some of the promising new agents under investigation in clinical trials, with the goal of improving patient outcomes.

2. Systemic Adjuvant Treatment for Resected Biliary Tract Cancers

The first randomised controlled trial to assess the benefit of adjuvant chemotherapy in resected BTCs was run in Japan and included patients with resected carcinoma of the pancreas and ampulla of Vater [16]. The group found that the

5-year survival rate of patients with gallbladder cancer who received postoperative mitomycin C and 5-flurouracil ($n = 69$) was significantly better compared to that of patients who received surgery alone ($n = 43$) in the per-protocol analysis but not the intention-to-treat analysis (26.6% vs. 14.4%) ($p = 0.0367$). However, no significant benefit was seen in any other tumour types and the improved survival of patients with gallbladder cancer who received chemotherapy was confined to those who had "noncurative" resections [16]. A subsequent meta-analysis of data from ten nonrandomised retrospective studies, including a total of 3191 patients with gallbladder cancer, supported a survival benefit for postoperative chemotherapy overall (HR = 0.42); however, the benefit of all adjuvant treatments (including radiotherapy and chemoradiotherapy) was limited to patients with node- and margin-positive diseases only [17]. Furthermore, the survival benefit of adjuvant treatment appeared to be restricted to patients from Asia [17]. A second meta-analysis, including all BTC subgroups, only identified a survival advantage for adjuvant chemotherapy in patients with resected node-positive disease; however, the trials included were also mostly nonrandomised, retrospective studies [18]. The lack of available robust and prospective data supporting the use of adjuvant chemotherapy in resected BTCs meant that worldwide practice varied.

The multicentre phase III PRODIGE-12 study, which randomly allocated 196 patients with resected BTCs to either combination gemcitabine and oxaliplatin (GEMOX) chemotherapy or surveillance, reported in 2017 [19]. Patient stratification factors included tumour location split into ICC, ECC, or GBC; R0 versus R1 resection; and lymph node involvement or not. The trial was negative for the coprimary endpoint of relapse-free survival (RFS) with a median of 20.4 months in the GEMOX arm compared to 18.5 months in the surveillance arm (HR 0.88; 95% CI 0.62-1.25; $p = 0.48$). The authors attribute the likely reason for this lack of difference in RFS to an ambitious hazard ratio which was set at 0.6. Furthermore, in the preplanned subgroup analysis looking specifically at outcomes in the GBC cohort, there were significantly worse RFS (HR 2.56) and median overall survival (OS) (HR 3.39) in patients with GBC who received GEMOX ($n = 17$) compared to surveillance ($n = 21$).

The BILCAP study also reported in 2017. In this larger phase III U.K. study, 447 patients with resected BTCs were randomised to either capecitabine for 24 weeks or observation [20]. Most commonly, patients with DCC followed by ECC or PCC were recruited; in contrast, only 8% of the patients in the PRODIGE-12 study had PCC. BILCAP did not reach statistical significance for the primary endpoint of OS in the intention-to-treat population; however, a significant OS difference was observed in the per-protocol population (only 17 patients less than the intention-to-treat population) with a median OS of 53 months in the capecitabine arm compared to 36 months in the observation arm (HR 0.75). The RFS in the per-protocol analysis was also significantly longer in the interventional group compared to surveillance (HR 0.71). Of interest, in preplanned subgroup analyses, there was a significant benefit of capecitabine in men and those with poorly differentiated disease. There was also a trend towards benefit in lymph node-positive patients; however, this was just outside the level of statistical significance. Treatment was well-tolerated, and there were no significant differences in the quality of life between the two study arms. The updated ASCO guidelines now recommend 6 months of adjuvant capecitabine for all patients with resected biliary tract cancer [21] whereas the NCCN continues to advocate for individualised decisions and recommend varying strategies dependent upon the BTC subtype, lymph node involvement, and resection margin status [22].

The authors of the BILCAP study highlight that because BTC is a rare disease, and of course resectable BTC even more so, the study needed 10 years to fully accrue. PRODIGE-12 required fewer patients, but recruitment still took 5 years. In the meantime, it has become clearer that not all biliary tumours are born equal, and while males or those with poorly differentiated tumours or lymph node involvement may benefit the most from adjuvant chemotherapy, other patients such as those with GBC who had the worst outcomes in PRODIGE-12 may require a different treatment strategy altogether. Additionally, it is also important to bear in mind that PRODIGE-12 and BILCAP only recruited from centres in Europe whereas some of the highest incidences of BTCs are seen in Asia. Given the diverse aetiologies of these tumours, we should be mindful of the applicability of BILCAP worldwide. The BCAT study was another phase III study which assessed the benefit of single-agent gemcitabine over observation in patients with resected BTCs recruited from 48 Japanese centres [23]. BCAT restricted inclusion to patients with PCC or DCC. Again, adjuvant gemcitabine failed to show a significant advantage in improving OS compared to placebo [23]. Table 1 summarises the completed positive and negative trials investigating adjuvant therapies following resection of BTCs.

The ongoing randomised ACTICCA-1 study recently changed its control arm from surveillance to capecitabine following the results of BILCAP. It will determine whether there is added clinical benefit of intensification of chemotherapy with cisplatin plus gemcitabine in the adjuvant setting compared to single-agent capecitabine. Given the results of PRODIGE-12, BILCAP, and BCAT thus far, it seems that future study design should also concentrate on determining which specific characteristics, global populations, or BTC subtypes would most meaningfully benefit from adjuvant chemotherapy and its intensification or not. Table 2 summarises some ongoing clinical trials investigating adjuvant therapies following resection of BTCs.

3. Systemic Treatment of Advanced Disease

3.1. Chemotherapy

3.1.1. First-Line Chemotherapy. The ABC-02 study, conducted across 37 U.K. centres, established cisplatin and gemcitabine as the standard of care regimen for the first-line treatment of advanced BTCs in 2010 [24]. In this randomised phase III study, 410 patients with advanced BTCs were allocated to receive either single-agent gemcitabine or the cisplatin/gemcitabine doublet and there was a confirmed

Table 1: Completed clinical trials investigating adjuvant therapies following resection of biliary tract cancers.

Trial	Study arms	Phase	Tumour site	Resection margins	Nodal status	Overall survival (months)	Disease recurrence
Positive trials							
BILCAP [20]	Capecitabine × 8 vs. observation (1 : 1)	III Open-label $n = 447$	ICC: $n = 84$ (19%) PCC: $n = 128$ (29%) DCC: $n = 156$ (35%) GBC: $n = 79$ (18%)	R0: $n = 279$ (62%) R1: $n = 168$ (38%)	N0: $n = 236$ (53%) N1: $n = 210$ (47%)	*ITT:* 51·1 vs. 36·4 HR 0·81 [95% CI 0·63–1·04]; $p = 0 \cdot 097$ *PPA:* 53 vs. 36 HR 0·75 [95% CI 0·58–0·97]; $p = 0 \cdot 028$	*Median RFS (months) ITT:* 24·4 vs. 17·5 HR 0.75 [95% CI 0·58–0·98]; $p = 0 \cdot 033$ *PPA:* 25.9 vs. 17.4 HR 0·70 [95% CI 0·54–0·9]; $p = 0 \cdot 0093$
Negative trials							
Takada et al. [16]	Mitomycin C and 5-flurouracil vs. surgery alone	III Open-label $n = 508$	Pancreas: $n = 173$ (34%) Bile duct: $n = 139$ (27%) GBC: $n = 140$ (28%) Ampulla of Vater: $n = 56$ (11%)	Curative: $n = 256$ Noncurative: $n = 180$	Not reported	*ITT (GBC cohort only):* 16.4 vs. 14.1 ($p = 0.28$) *PPA of 5-year survival rate (%) in GBC cohort:* 26.0% vs. 14.4%, ($p = 0.0367^*$)	*5-year DFS rate (%) in GBC cohort:* 20.3% vs. 11.6%; $p = 0.0254^*$
PRODIGE 12-ACCORD 18 [19]	GEMOX × 12 vs. observation	III Open-label $n = 194$	ICC: $n = 86$ (44%) PCC: $n = 15$ (8%) DCC: $n = 55$ (28%) GBC: $n = 38$ (20%)	R0: $n = 169$ (87%) R1: $n = 25$ (13%)	N0: $n = 97$ (50%) N1: $n = 69$ (36%) N2: $n = 2$ (1%) Nx: $n = 26$ (13%)	75.8 vs. 50.8 HR 1.08 [95% CI 0.70-1.66]; $p = 0.074$	*Median RFS (months):* 30.4 vs. 18.5 HR 0.88 [95% CI 0.62-1.25]; $p = 0.48$
BCAT [23]	Gemcitabine × 6 vs. observation (1 : 1)	III Open-label $n = 225$	PCC: $n = 102$ (45%) DCC: $n = 123$ (55%)	R0: $n = 204$ (91%) R1: $n = 25$ (11%)	N0: $n = 147$ (65%) N1: $n = 78$ (35%)	62.3 vs. 63.8 HR 1.01 [95% CI 0.70–1.45]; $p = 0.964$	*Median RFS (months):* 36.0 vs. 36.9 HR 0.93 [95% CI 0.66–1.32]; $p = 0.693$
ESPAC-3 [84]	Fluorouracil/folinic acid vs. gemcitabine vs. observation (1 : 1 : 1)	III Open-label $n = 428$	Ampullary: $n = 297$ (69%) Bile duct: $n = 96$ (22%) Other: $n = 35$ (8%)	R0: $n = 360$ (84%) R1: $n = 68$ (16%)	N0: $n = 177$ (41%) N1: $n = 251$ (59%)	38.9 vs. 45.7 vs. 35.2 HR for FU vs. observation: 0.95 [95% CI 0.71-1.28]; $p = 0.74$ HR for gemcitabine vs. observation: 0.77 [95% CI 0.57-1.05]; $p = 0.10$	*Median RFS (months):* 23.0 vs. 29.1 vs. 19.5 HR for FU vs. observation: 0.69 [95% CI 0.51-0.95]; $p = 0.02$ HR for gemcitabine vs. observation: 0.68 [95% CI 0.50-0.95]; $p = 0.02$

OS: overall survival; RFS: relapse-free survival; ICC: intrahepatic cholangiocarcinoma; PCC: perihilar cholangiocarcinoma; DCC: distal cholangiocarcinoma; GBC: gallbladder carcinoma; ITT: intention-to-treat; PPA: per protocol analysis; HR: hazard ratio; 95% CI: 95% confidence interval; GEMOX: gemcitabine+oxaliplatin. *This subgroup analysis was statistically significant, and therefore, there is a positive finding in the study.

Table 2: On-going clinical trials investigating adjuvant therapies following resection of biliary tract cancers.

Agent [trial]	Mechanism of action	Population	Phase	Treatment arms	Planned recruitment	Primary endpoint	Clinical trial identifier
GEM/CIS [ACTICCA-1]	Cytotoxic	Resected localised biliary tract cancer, following complete macroscopic resection	III Open-label	GEM/CIS × 8 vs. capecitabine × 8 (1 : 1)	Recruiting Target $n = 781$	DFS	NCT02170090
GEM/CAP [AdBTC-1]	Cytotoxic	Resected localised biliary tract cancer, following complete macroscopic resection	III Open-label	GEMCAP × 8 vs. capecitabine × 8	Recruiting Target $n = 460$	DFS	NCT03779035
S-1 [JCOG1202, ASCOT]	Cytotoxic	Resected localised biliary tract cancer, following complete macroscopic resection	III Open-label	S1 × 4 vs. observation	Recruiting Target $n = 440$	OS	UMIN000011688
Apatinib	VEGFR2	Resected biliary tract cancer	II Open-label	Apatinib plus capecitabine vs. capecitabine	Not yet recruiting Target $n = 40$	PFS	NCT03609489
Nivolumab+cabrilizumab	PD1; CSF1	Confirmed biliary tract cancer	II Single-arm	Nivolumab+cabrilizumab	Not yet recruiting Target $n = 16$	Drug-related toxicity	NCT03768531

GEM/CIS: gemcitabine+cisplatin; GEMCAP: gemcitabine+capecitabine; DFS: disease-free survival; OS: overall survival; VEGFR2: vascular endothelial growth factor receptor 2; PFS: progression-free survival; PD1: programmed death 1; CSF1: colony-stimulating factor 1.

significant OS advantage of cisplatin/gemcitabine over the single agent (11.7 vs. 8.1 months; HR 0.64) (95% CI 0.52–0.80; $p < 0.001$) [24]. A corresponding randomised study in Japanese patients confirmed benefit in this population [25]. However, a subsequent meta-analysis of these two studies suggested that patients with poor performance status may not derive benefit from the doublet [26], and so, current guidelines permit the use of single-agent gemcitabine in these patients [2]. Cisplatin may also be substituted by oxaliplatin in cases of renal impairment [2].

Since the pivotal results of ABC-02, a number of phase II and III studies have assessed the potentially added benefit of combining various targeted agents, e.g., erlotinib [27], panitumumab [28–30], cetuximab [31, 32], bevacizumab [33], and cediranib [34], with doublet chemotherapy. However, in most cases, these were unselected populations and results were either negative or not convincing enough to alter clinical practice. A retrospective analysis of 42 patients with advanced BTCs treated with FOLFIRINOX chemotherapy in the first line suggested that the triplet regimen was efficacious without increased toxicity [35]. The prospective phase II/III AMEBICA study will investigate whether intensification of chemotherapy with FOLFIRINOX will further improve outcomes compared to gemcitabine/cisplatin in the first line advanced setting [36] (Table 3).

3.1.2. Second-Line Chemotherapy. Due to the aggressive nature of advanced BTC and problems with recurrent biliary obstruction; it has been historically difficult to robustly assess further treatment in trials after progression on cisplatin/gemcitabine. There had been some evidence to suggest possible benefit from second-line 5-FU chemotherapy in fit patients [37, 38], but the results of the U.K.-led phase III randomised ABC-06 study have since confirmed an overall survival benefit of FOLFOX (5-FU and oxaliplatin) chemotherapy plus best supportive care (BSC) compared to BSC alone [39] (6.2 vs. 5.3 months; HR 0.69) (95% CI 0.50–0.97; $p = 0.031$). Following these results, FOLFOX plus BSC may well become the established second-line regimen for fit patients with advanced BTC.

3.2. Genomic Profiling of Biliary Tract Cancers. Recent genomic sequencing data from across the world have shown that BTCs display a diverse mutational landscape [11–13]. Almost half of patients with BTCs have been shown to harbour at least one driver mutation which may represent a therapeutic opportunity and/or a prognostic biomarker [13]. Given that studies of targeted agents in unselected populations have not shown significant benefit, these genomic data represent a novel approach for trials of targeted therapies in biomarker-enriched populations.

Javle et al. were the first group to correlate genomic mutational patterns, using the FoundationOne platform, with clinical outcomes [11]. They found that the most commonly aberrant genes varied depending on BTC subtype—TP53 (27%) in ICC, KRAS (42%) in ECC, and ERBB2 (16%) in GBC, and that FGFR mutations, mostly detected in ICC, were associated with a good prognosis [11]. Subsequently, by performing integrative clustering analysis of mutation, copy number, gene expression, and epigenetic data on tissue from nearly 500 CCAs, four different and distinct molecular subtypes of CCA have emerged [12]. Cluster 1 are mostly fluke-positive tumours enriched with TP53 and ARID1A gene alterations, ERBB2 amplification, and CpG island hypermethylation, whereas Cluster 4 are mostly fluke-negative ICC enriched with FGFR alterations and CpG shore hypermethylation [12]. Interestingly, this group also showed that the molecular rather than anatomic subtype of CCA has much more of a bearing on prognosis, with Cluster 4 tumours in the better prognosis category [12]. This is in keeping with findings from Javle et al. who showed that FGFR mutations were associated with improved OS [11]. A prospective analysis using the MSK-IMPACT platform also identified distinct molecular patterns between ICC and ECC [13].

These genetic alterations and distinct molecular subtypes present potential therapeutic targets which could be exploited by targeted agents and support the implementation of a platform for genomic profiling to be available to all patients with advanced BTCs. The MOSCATO-1 trial was a large-scale prospective study which performed genomic analyses on over 1000 tumour samples and matched 199 patients to a targeted therapy based on a genetic alteration, 18 of whom had advanced BTC and had been treated with at least one prior line of systemic treatment [40]. In these 18 who received a matched targeted therapy, the overall response rate was 33% and progression-free survival (PFS) was 5.2 months [41]. Furthermore, the median OS of those who received a targeted treatment was 17 months, which far surpasses the median OS in the second-line setting so far reported [37]. A much higher rate of potentially actionable mutations was also seen in BTCs compared to other tumour types analysed in the MOSCATO-1 study [41].

Given that sequencing of tissue samples can be limited by low tumour content, liquid biopsy is also being harnessed for genomic profiling in BTC. Circulating tumour DNA (ctDNA) analysis using the Guardant 360 assay on 138 patient samples detected at least 1 genomic alteration in 89% of patients—most commonly, TP53, KRAS, and FGFR2 [42], although concordance with tissue-based alterations in BTC has yet to be proven. A nationwide ctDNA genomic screening platform using the Guardant 360 assay is currently recruiting in Japan and enrolling patients into clinical trials based on targetable genomic alterations [43].

3.3. Emerging Therapeutic Targets

3.3.1. FGFR Fusions. FGFR gene fusions are present in approximately 15% of patients with mostly noninfectious ICC [44], the most common fusion partners being BICC1 and KIAA1217 [13]. They result in the activation of canonical downstream signalling and have been associated with improved survival [11]. Infigratinib (BGJ398) is a potent oral FGFR1-3 kinase inhibitor which was first tested in patients with CCA and FGFR aberrations in a phase II study [45]. Sixty-one patients with advanced CCA and FGFR aberrations (mostly FGFR fusions, $n = 48$) were treated with the agent in the second- or later-line setting, and results demonstrated an ORR of 14.8% and DCR of 75.4%. Interestingly, all

Table 3: On-going clinical trials investigating therapies for advanced biliary tract cancers.

Agent [trial]	Mechanism of action	Population	Phase	Treatment arms	Planned recruitment	Primary endpoint	Clinical trial identifier
Intensified cytotoxic therapies							
Nab-paclitaxel	Cytotoxic (antimicrotubule)	2nd line Unresectable advanced or metastatic biliary tract and gall bladder carcinoma	III Open-label	GEM/CIS+nab-paclitaxel vs. GEM/CIS	Recruiting, target $n = 268$	OS	NCT03768414
mFOLFIRINOX [AMEBICA]	Combination chemotherapy	2nd line Unresectable advanced or metastatic biliary tract and gall bladder carcinoma	II/III Open-label	mFOLFIRINOX vs. GEM/CIS	Recruiting, target $n = 316$	II: % alive at 6 months without radiological progression III: OS	NCT02591030
Agents targeting FGFR aberrations							
Ponatinib	Multitargeted TKI (including FGFR2)	Advanced biliary tract cancer harbouring FGFR2 fusion or amplification, 2nd line or more	II Single-arm	Ponatinib	Active, not recruiting; $n = 12$	Clinical benefit rate	NCT02265341
Erdafitinib	Pan-FGFR TKI	Advanced solid tumours, including cholangiocarcinoma, with FGFR2 abnormalities	II Single-arm	Erdafitinib	Active, recruiting, target $n = 55$	ORR	NCT02699606
Derazantinib (ARQ087) [FIDES-01]	Multikinase inhibitor	Advanced/inoperable intrahepatic cholangiocarcinoma with FGFR2 fusion, 2nd line or more	II Single-arm	Derazantinib	Active, recruiting, target $n = 100$	ORR	NCT03230318
TAS-120 [FOENIX101]	Pan-FGFR inhibitor	Advanced solid tumours, including intrahepatic/ extrahepatic cholangiocarcinoma, with FGFR2 gene alteration, 2nd line or more	II Single-arm	TAS-120	Recruiting, target $n = 371$	ORR	NCT02052778
Infigratinib (BJG 398) [PROOF]	FGFR1-3 kinase inhibitor	FGFR2 mutated (fusion/translocations) advanced cholangiocarcinoma, 1st line	III Open-label	Infigratinib vs. GEM/CIS	Active, recruiting, target $n = 350$	PFS	NCT03773302

Table 3: Continued.

Agent [trial]	Mechanism of action	Population	Phase	Treatment arms	Planned recruitment	Primary endpoint	Clinical trial identifier
Pemigatinib (INCB054828) [FIGHT302]	Pan-FGFR TKI	FGFR2 rearranged advanced/irresectable cholangiocarcinoma	III Open-label	Pemigatinib vs. GEM/CIS	Active, recruiting, target $n = 432$	PFS	NCT03656536
Agents targeting IDH1/IDH2 mutations							
BAY1436032	IDH1 inhibitor	IDH-1 mutant advanced solid tumours	I	BAY1436032	Active, not recruiting, $n = 81$	MTD, no. of pts with AEs, established dose for phase II	NCT02746081
Dasatinib	Multitargeted TKI	IDH-1 mutant advanced intrahepatic cholangiocarcinoma	II	Dasatinib	Completed (results awaited), $n = 8$	ORR	NCT02428855
Olaparib	PARP inhibitor	IDH-1/IDH-2 mutant advanced solid tumours	II Single-arm	Olaparib	Recruiting, target $n = 145$	ORR	NCT03212274
Immunotherapy							
Pembrolizumab [ABC-09]	Anti-PD1	2nd line Unresectable or metastatic biliary tract and gall bladder carcinoma	II Single-arm	GEM/CIS+ pembrolizumab	Not yet recruiting, target $n = 50$	6-month PFS	NCT03260712
Durvalumab Tremelimumab	Anti-PD-L1 Anti-CTLA4	2nd line Unresectable or metastatic biliary tract and gall bladder carcinoma	II Single-arm	Durvalumab+ tremelimumab+ GEM/CIS	Recruiting, target $n = 31$	ORR	NCT03046862
Nivolumab Ipilimumab	Anti-PD1 Anti-CTLA4	2nd line Unresectable advanced or metastatic biliary tract and gall bladder carcinoma	II Open-label	Nivolumab/ ipilimumab vs. GEM/CIS+ nivolumab	Recruiting, target $n = 64$	PFS	NCT03101566
Nivolumab	Anti-PD1	2nd line Unresectable or metastatic biliary tract and gall bladder carcinoma	II Single-arm	Nivolumab	Active, not recruiting, $n = 52$	ORR at 8 weeks	NCT02829918
Pembrolizumab	Anti-PD1	2nd line or more, advanced irresectable biliary tract cancer	Phase II Single-arm	Pembrolizumab+ CAPOX	Recruiting, target $n = 19$	5-month PFS	NCT03111732

Table 3: Continued.

Agent [trial]	Mechanism of action	Population	Phase	Treatment arms	Planned recruitment	Primary endpoint	Clinical trial identifier
Ipilimumab+nivolumab [CHECKMATE848]	Anti-PD1 Anti-CTLA4	Advanced or metastatic TMB-H solid tumours	Phase II Open-label	Ipilimumab+ nivolumab vs. nivolumab	Recruiting, target $n = 159$	ORR	NCT03668119
Nivolumab Ipilimumab	Anti-PD1 Anti-CTLA4	Rare tumours (2nd line or more)	II Single-arm	Nivolumab+ ipilimumab	Recruiting, target $n = 120$	Clinical benefit rate	NCT02923934
Nivolumab Ipilimumab [DART]	Anti-PD1 Anti-CTLA4	Advanced GI tumours (2nd line or more)	II Single-arm	Nivolumab+ ipilimumab	Recruiting, target $n = 707$	ORR	NCT02834013
Durvalumab [TOPAZ-1]	Anti-PD-L1	2nd line Unresectable or metastatic biliary tract and gall bladder carcinoma	III Double-blind	GEM/CIS+ durvalumab vs. GEM/CIS+ placebo	Recruiting, target $n = 474$	OS	NCT03875235
Autologous tumour-infiltrating lymphocytes+ pembrolizmab	TIL Anti-PD1	Metastatic cancer including hepatobiliary (refractory to standard therapy)	II Multiarm	(i) CD enriched TIL (ii) Unselected TIL (iii) Unselected TIL+ pembrolizumab prior to cells (iv) Unselected TIL+ pembrolizumab at disease progression	Recruiting, target $n = 332$	ORR	NCT01174121
Modified autologous cytokine-induced killer cells	Cytokine-induced killer cells	Cholangiocarcinoma	I/II Single-arm	Cytokine-induced killer cells	Unknown, target $n = 13$	MRI scan for monitoring of tumour size and CIK cell homing	NCT01868490
Agents targeting BRAF mutations							
HM95573	RAF inhibitor	Solid tumours with RAS/RAF mutation, 2nd line or more	I	HM95573	Recruiting, target $n = 100$	ORR	NCT03118817
Vemurafenib+HL-085	BRAF+MAPK inhibition	BRAF V600E mutant advanced solid tumours	I	Vemurafenib+ HL-085	Recruiting, target $n = 39$	Incidence of AEs	NCT03781219
Vemurafenib	BRAF inhibitor	Tumours with BRAF mutation, 2nd line or more	II Single-arm	Vemurafenib	Recruiting, target $n = 500$	ORR	NCT02304809
Encorafenib+MEK162	BRAF inhibitor+ MEK inhibitor	BRAF V600E mutant advanced solid tumours	II Single-arm	Encorafenib+ MEK162	Recruiting, target $n = 179$	DCR/ORR	NCT01543698
Dabrafenib+trametinib [ROAR]	BRAF inhibitor+ MEK inhibitor	Rare tumours with BRAF V600E mutation, 2nd line or more	II Open-label	Dabrafenib+ trametinib	Completed, $n = 206$ (BTC, $n = 35$)	ORR	NCT02034110

Table 3: Continued.

Agent [trial]	Mechanism of action	Population	Phase	Treatment arms	Planned recruitment	Primary endpoint	Clinical trial identifier
Binimetinib+encorafenib [BEAVER]	BRAF inhibitor+ MEK inhibitor	Advanced solid tumours with BRAF MT (non-V600E), 2nd line or more	II Single-arm	Binimetinib+ encorafenib	Not yet recruiting, target $n = 26$	ORR	NCT03839342
Agents targeting TRK aberrations							
Larotrectinib (BAY2757556) [NAVIGATE]	TRK inhibitor	Advanced solid tumours harbouring NTRK fusion, 2nd line or more	II Single-arm	Larotrectinib	Recruiting, target $n = 320$	ORR	NCT02576431
Entrectinib (RXDX-101) [STARTRK-2]	NTRK1/2/3, ROS1, ALK	Advanced solid tumours that harbour NTRK1/2/3, ROS1, or ALK gene fusion	II Open-label	Entrectinib (RXDX-101)	Recruiting, target $n = 300$	ORR	NCT02568267
Agents targeting BAP1 aberrations							
Niraparib [UF-STO-ETI-001]	PARP inhibitor	Advanced solid malignancies with BAP1/DDR defects	II Nonrandomised, open-label	Niraparib	Recruiting, target $n = 57$	ORR	NCT03207347
Agents targeting HER2 aberrations							
DS-8201a [HERB]	HER2 inhibitor	HER2-positive biliary tract cancer	II Non-randomised	DS-8201a	Recruiting, target $n = 32$	ORR	JMA-IIA00423

GEM/CIS: gemcitabine+cisplatin; OS: overall survival; mFOLFIRINOX: modified FOLFIRINOX; FGFR: fibroblast growth factor receptor; TKI: tyrosine kinase inhibitor; ORR: overall response rate; PFS: progression-free survival; IDH: isocitrate dehydrogenase; MTD: maximum tolerated dose; AEs: adverse events; PARP: poly(ADP ribose) polymerase; PD1: programmed death 1; PDL1: programmed death ligand 1; CTLA4: cytotoxic T lymphocyte-associated protein 4; TMB-H: tumour mutation burden high; MAPK: mitogen-activated protein kinase; DCR: disease control rate; TRK: tropomyosin receptor kinase; ALK: anaplastic lymphoma kinase; BAP1: BRCA1-associated protein 1; DDR: DNA damage repair; PD: progressive disease.

patients who experienced a radiological response had an FGFR2 fusion suggesting that BGJ398 was particularly sensitive to this oncogenic driver. Furthermore, the side effect profile including hyperphosphataemia, fatigue, and stomatitis was manageable. Preliminary results of a phase II study of erdafitinib, another potent oral pan-FGFR tyrosine kinase inhibitor, tested in Asian patients with CCAs and FGFR alterations also indicate an ORR (CR and PR) of 45.5% in 11 evaluable patients which is encouraging [46]. Side effects from erdafitinib were similar to those from BGJ398, most commonly, hyperphosphataemia, stomatitis, dry skin, and nail disorders [45, 46]. Hyperphosphataemia appears to be a class effect on FGF23 which is involved in phosphate metabolism [47]. INCB054828, another pan-FGFR, is currently being investigated in a phase III trial against gemcitabine and cisplatin after demonstrating promising early activity in patients with CCA (Table 3). ARQ087 (derazantinib), a nonselective multikinase inhibitor which includes the FGFR as a target, has also recently entered phase III testing in pretreated patients after phase II data from 29 patients with ICC, and FGFR fusions treated with derazantinib revealed a median PFS of 5.7 months (95% CI: 4.04–9.2 months) and an ORR of 20.7% [48].

As frequently seen with kinase inhibitors, however, secondary acquired resistance eventually ensues. For the BGJ398 drug, the mechanism of acquired resistance has been identified as the development of a polyclonal point mutation in the FGFR2 kinase domain from serial analysis of tissue and cell-free circulating DNA (cfDNA) from patients on treatment and at disease progression [49]. Another postulated escape mechanism included the PI3K/PTEN pathway [49]. TAS-120 is a highly selective covalent pan-FGFR inhibitor which is active against FGFR2 resistance mutations. Following promising clinical activity in FGFR aberrant ICC in an early-phase study [50], the phase II FOENIX-101 study of TAS-120 in patients with ICC harbouring FGFR2 gene rearrangements after progression on first-line treatment is currently recruiting (Table 3). Going forward, there may also be a rationale to combine FGFR2 inhibition with agents that target the PI3K/PTEN pathway.

3.3.2. IDH Mutations. IDH1 and IDH2 mutations are present in approximately 20% of mainly noninfectious ICC [12, 13, 51, 52]. They result in the accumulation of 2-hydroxyglutarate (2-HG) which drives tumourigenesis and can be measured in tumour and blood [53]. Ivosidenib (AG-120) is a first in class oral, selective, reversible IDH1 inhibitor which first showed efficacy in patients with advanced IDH-1-mutated CCA ($n = 73$) in a phase I study of solid tumours with IDH1 mutations [54]. 6% of patients had a partial response to treatment, and the other 56% had stable disease. There were no dose-limiting toxicities, and the main side effects, including fatigue and nausea, were manageable. The randomised phase III placebo-controlled ClarIDHy trial results have since demonstrated a PFS advantage in patients with IDH1-mutated CCA who have failed prior treatment who took ivosidenib 500 mg four times a day over placebo; 2.7 months compared to 1.4 months (HR 0.37; 95% CI 0.25–0.54; $p < 0.001$) [55]. The disease control rate (stable disease plus partial response) was also superior in the ivosidenib arm at 53% compared to the placebo arm at 28%; there was a trend towards OS benefit, and the main side effects associated with ivosidenib including mostly gastrointestinal toxicities such as nausea and diarrhoea and fatigue were manageable. The ClarIDHy trial results are the first phase III data to show benefit from IDH1 inhibition in patients with IDH1-mutated CCA. IDH1-mutated ICC cells have been shown to be exquisitely sensitive to dasatinib [56], a multitargeted TKI currently approved to treat certain leukaemias, and results of a phase II trial in patients with IDH-mutant advanced ICC are awaited (Table 3). BAY1436032 is another recently developed drug which is being investigated in an IDH1 mutation basket study with a CCA cohort (Table 3). Given that 2-HG can hamper homologous recombination and has demonstrated sensitivity to PARP inhibition in preclinical models [57], another IDH1 mutation basket study with a CCA cohort is also assessing whether the PARP inhibitor olaparib can affect ORR (Table 3).

3.3.3. DNA Damage Repair Mechanisms and BAP1 Mutations. The DNA damage repair (DDR) pathways are essential for maintaining genomic integrity by promoting DNA repair, cell cycle arrest, and apoptosis. Somatic or germline alterations to the DDR genes not only have been linked to carcinogenesis but also represent increased sensitivity to DNA-damaging agents which can be exploited in cancer therapy. The prevalence of mutations in DDR genes is generally low in BTCs; however, mutations in BAP1, which is a tumour suppressor gene involved in DNA double-strand break repair associated with noninfectious CCA [12], have emerged as a potential target [58]. A phase II basket trial is currently investigating the clinical benefit of the PARP inhibitor (niraparib) in patients with BAP1 mutations and other DDR-deficient cancers including CCA (Table 3).

3.3.4. Mismatch Repair Deficiency, Microsatellite Instability, and Tumour Mutational Burden. It is widely recognised that tumours which exhibit deficient mismatch repair (dMMR) expression and an unstable microsatellite (MSI) phenotype, via either germline or somatic mutations, have favourable responses to checkpoint inhibiting immunotherapy. As such, the PD1 inhibitor pembrolizumab was given the first tumour-agnostic approval by the FDA in 2017 for the treatment of any dMMR or MSI-high tumour [59]. While the rate of dMMR/MSI in CCA is only in the region of 2.5% [60], upregulated immune-related pathways including PD1 have been reported in one of the molecular subtypes of BTC (Cluster 3) [12] which may also confer benefit from treatment with anti-PD1 therapy. Indeed, in the KEYNOTE-028 basket study of pembrolizumab in biomarker-selected patients, 17% of patients with PDL1-positive tumours in the BTC cohort achieved a partial response [61]. KEYNOTE-016 and KEYNOTE-158 also assessed the benefit of pembrolizumab in dMMR/MSI-high tumours and achieved an ORR of 53% and 37% in the BTC cohorts, respectively [60, 62]; responses were also durable with a 2-year OS of 64% (95% CI: 53–79) in KEYNOTE-016 [60]. In contrast, the ORR for unselected BTC patients in KEYNOTE-158 ($n = 104$) was

only 5.8% [63]. These results support testing for dMMR/MSI in all patients with advanced BTC.

High tumour mutational burden (TMB) has also been shown to predict response to immune checkpoint inhibition due to increased neoantigen presentation [64]. In a retrospective analysis of patients with solid tumours (mostly lung and melanoma), 42% of those with high TMB, defined as over >20 mutations/megabase (Mb), had an objective response to checkpoint inhibiting therapy compared to only 2/46 with low TMB [65]. CHECKMATE-848 is a currently recruiting randomised study of combination checkpoint inhibition with nivolumab, anti-PD1, and ipilimumab, anti-CTLA4, or nivolumab alone in patients with solid tumours, including BTC, with high TMB defined as >15 mutations/Mb (Table 3). It will determine whether the combination strategy leads to increased clinical benefit as seen in other immunogenically "hot" malignancies such as melanoma and, for patient benefit, crossover is allowed. Given the promising responses to immunotherapy thus far, there is, of course, a strong rationale for combining checkpoint inhibition with chemotherapy and the randomised phase III TOPAZ-1 study will assess whether gemcitabine and capecitabine with durvalumab, anti-PDL1, will improve OS compared to placebo in 1st line unselected patients with BTC (Table 3).

The role of adoptive immunotherapy, whereby a patient's own tumour-infiltrating lymphocytes are harvested and then infused back into them to boost the immune response, is also currently being investigated in a number of clinical trials in patients with biliary tract cancers (Table 3). In one case report, a patient with lymph node-positive disease and portal vein invasion at surgery was treated with CD3-activated T cells and dendritic cells in the adjuvant setting and was still alive more than 3 and a half years later [66]. Another patient with a heavy burden of metastatic disease was treated with CD4+ T cells which recognised an erbb2 epitope on the cancer cell and experienced a durable response to treatment which was recapitulated on disease progression [67].

3.4. Other Potential Targets

3.4.1. ERBB2 (HER2) Aberrations. ERBB2/HER2 aberrations have been detected in 3.9-8.5% of most commonly fluke-positive tumours CCAs and 16% of GBC and have been associated with poorer prognosis [11, 12]. There are several HER2-directed agents with well-defined safety profiles already approved to treat a number of malignancies such as breast and gastric cancer; however, thus far, the only indication of a signal in patients with BTCs harbouring HER2 aberrations treated with HER2-directed therapy has been in retrospective series [68], and efficacy is yet to be confirmed in prospective trials. The HERB trial is a currently recruiting phase II trial of the HER2 inhibitor, DS-8201a, in patients with HER2-positive (1+ by IHC and positive by ISH) biliary tract cancer in Japan (Table 3).

3.4.2. RAS/MAPK Pathway. KRAS is a key oncogenic driver in many malignancies and has been proven notoriously difficult to target due to the number of different proteins it interacts with both directly and indirectly. Most developed agents therefore target downstream proteins in the signalling pathway such as BRAF or MEK. MEK inhibitors have had limited activity as single agents in BTCs [69]. There has however been efficacy reported with the MEK inhibitor selumetinib in combination with cisplatin/gemcitabine chemotherapy in a phase Ib study, and the side effects were tolerable [70]. There are also a number of BRAF and MEK inhibitor combination studies currently ongoing for patients with BRAF-V600E-mutated advanced solid tumours (Table 3). The preliminary results of the biliary tract cohort with BRAF-V600E mutations from the ROAR trial show encouraging efficacy with dabrafenib (BRAF inhibitor) and trametinib (MEK inhibitor). In 32 evaluable patients in the BTC cohort, the ORR was 41% and the median OS reached 11.3 months (95% CI, 7.3–17.6) [71]. As MEK inhibitors have been shown to increase immune recognition of tumour cells and promote T cell survival and accumulation [72], there is also rationale to combine them with immunotherapeutic agents; however, a recent phase III trial assessing the combination of cobimetinib (MEK inhibitor) and atezolizumab (PDL1-inhibitor) against the standard of care in patients with microsatellite stable (MSS) colorectal cancer did not improve OS [73].

3.4.3. PI3K/AKT/mTOR. Aberrations in the PI3K/AKT/mTOR pathway, such as PI3K mutations, PI3KCA amplifications, phosphorylated AKT (p-AKT), and p-mTOR overexpression, have been detected in BTCs and are associated with poorer prognosis [74]. The loss of expression of PTEN, a tumour suppressor gene involved in the regulation of the PI3K/AKT/mTOR pathway, has also been found in 4.1-51.8% of GBC [75, 76]. Thus far, early-phase clinical studies of an AKT inhibitor (MK-2206) [77], an mTOR inhibitor (everolimus) [78], and a PI3K inhibitor (buparlisib) together with FOLFOX [79] have shown limited tumour responses. Postulated reasons for these disappointing results include the lack of robust molecular stratification in these initial studies, likely resistance mechanisms related to the use of single targeted agents, and the small patient population suitable for clinical trial entry [74].

3.4.4. NTRK Fusions. The FDA has recently granted a second tumour-agnostic approval to larotrectinib, a neurotrophic receptor tyrosine kinase (NTRK) inhibitor, for patients with solid malignancies and a proven NTRK gene fusion without a known acquired resistance mutation [80]. The accelerated approval was based on efficacy data from 55 patients from the NAVIGATE trial with 12 different pretreated solid malignancies harbouring NTRK fusions, 22% of whom demonstrated a complete response and 53% a partial response to treatment with 73% of patients experiencing a maintained response for more than 6 months [81]. However, while NTRK fusions have been characterised in patients with ICC in Asia [82], NTRK fusions were not identified in a pooled cohort of 106 Caucasian patients [83]. The NAVIGATE trial is currently still recruiting as is a basket study assessing the benefit of entrectinib, another NTRK inhibitor, in patients with advanced solid tumours harbouring NTRK1/2/3 or ROS1 or ALK gene fusions (Table 3).

4. Conclusion

Recently, a role for empirical capecitabine chemotherapy in the adjuvant setting has been defined. However, in the advanced setting, there has been a concerted move towards adoption of a truly personalised approach to treatment by selecting appropriate targeted therapies based on particular molecular aberrations specific to an individual patient's tumour. This has been possible though global advances in genomic profiling and molecular subtyping of BTCs which have broadened our understanding of their hugely complex molecular landscape and the potential "druggable" targets which could be exploited. The evolution of histology-independent basket trials, where patients can be enrolled into studies based on a specific molecular aberration rather than tumour type, has also been vital in order to assess the potential benefit of these targeted therapies in rare cancers such as BTCs. So far, therapies targeting FGFR2 fusions and IDH mutations have gone the farthest in trials with the most promising results; however, a deeper understanding of potential resistance mechanisms and the complex crosstalk between molecular pathways is growing and combination strategies targeting more than one pathway are being proposed. In order to benefit from tailored therapy, genomic testing for all patients with BTC should be considered and liquid biopsy may be the most convenient way to implement this.

Conflicts of Interest

Fiona Turkes and Juliet Carmichael have no conflict of interest to disclose. David Cunningham receives research funding from AstraZeneca, Celgene, MedImmune, Bayer, 4SC, Clovis, Eli Lilly, Janssen, and Merck. Naureen Starling has received research funding from AstraZeneca, Bristol-Myers Squibb, and Pfizer; travel and accommodation from AstraZeneca, Bristol-Myers Squibb, Eli Lilly, Merck, and Roche; and honoraria from AstraZeneca, Eli Lilly, Merck, and Servier. Naureen Starling is on the advisory board for Pfizer, AstraZeneca, and Servier.

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Review Article

Precursors to Cholangiocarcinoma

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Cancers of the biliary tract include intra- and extrahepatic cholangiocarcinomas and gallbladder cancer. Biliary tract cancers are diseases with unfavorable prognoses. In recent years, several lesions have been described as precursors that precede biliary cancers. They include flat and microscopic lesions known as biliary intraepithelial neoplasia, macroscopic and tumor-forming intraductal papillary biliary neoplasia, intraductal tubular neoplasia, and mucinous cystic neoplasm of the bile duct. These conditions are rarely diagnosed, while their natural history and progression to cancer have yet to be adequately characterized. This review examines the epidemiology, pathology, molecular biology, diagnosis, and therapy of these various precursors. Further research is required if we are to better understand this evolving field and improve the prevention and early detection of bile duct cancer.

1. Introduction

The biliary tree consists of extrahepatic biliary ducts that enter the duodenum in the ampulla, extending proximally into intrahepatic biliary ducts. The gallbladder enters extrahepatic bile ducts via the cystic duct. The biliary mucosa is made up of a single layer of a columnar epithelium. Small oval nuclei are located at the base of cells below the eosinophilic cytoplasm. The mucosa is flat, except when present in the gallbladder where it forms villi. Located alongside the epithelium, mucous glands are drained into the lumen.

Biliary tract cancers can originate in any part of the biliary system. Representing the second most common hepatobiliary malignancy, biliary tract cancers are classified based on location: intrahepatic cholangiocarcinoma, extrahepatic cholangiocarcinoma (perihilar and distal), and gallbladder carcinoma. Of these cancers, gallbladder cancer is the most commonly observed. However, the incidence of biliary tract cancer (mainly intrahepatic) is increasing.

While gallbladder cancer is more common in women, the other biliary cancers occur predominantly in males. The geographical diversity of risk factors is responsible for major differences in the world incidence of biliary cancer. Chronic inflammation due to primary sclerosing cholangitis, hepatocolithiasis, biliary-enteric drainage, and metabolic chronic liver disease, as well as chronic biliary tract infections, including *Opisthorchis viverrini*, *Clonorchis sinensis*, HBV, and HCV, are the most significant risk factors. The highest rates are found in Asia (intrahepatic) and South America (gallbladder) [1–3].

Hepatobiliary flukes, *Opisthorchis viverrini*, and *Clonorchis sinensis*, endemic to Asia, are food-borne parasites that infect the bile duct. Patients become infected by eating raw or undercooked fish, the second intermediate host of the parasite. Their presence in the bile ducts induces chronic inflammation. Parasitic infection can be proven in the majority of ICC cases in endemic areas, with a relative risk of developing cholangiocarcinoma among carriers reported between 5 and 30 [1–3].

Primary sclerosing cholangitis results in chronic inflammation of intrahepatic and/or extrahepatic bile ducts and is the main risk factor of cholangiocarcinoma in the Western population. The lifetime prevalence of cholangiocarcinoma in PCS patients is between 5 and 15% with a yearly risk of 0.5-1.5% [4].

Choledochal cysts are rare (1/13 000 in Asia to 1/150 000 in the USA) congenital bile duct cystic dilations characterized by the presence of acutely or chronically inflamed mucosa. These cysts may be, at least in part, caused by biliary stasis

and pancreatic secretion reflux from the often-present anomalous pancreaticobiliary junction [2]. The risk of developing cholangiocarcinoma, which increases with advancing age, has been estimated at around 10-15% [5]. Cancer occurs earlier than in sporadic cases and may also develop in noncystic portions of the bile duct even after cyst removal.

Gallstones are found in 70-90% of gallbladder cancers. The relative risk of developing cancer in patients with cholecystolithiasis may be as high as 23% according to some studies [6]. Nevertheless, only a small proportion of patients with gallstones develop cancer during their lifetimes. About 7% of patients with hepaticolithiasis develop cholangiocarcinoma [7].

Gallbladder polyps are elevations of the gallbladder wall that project into the lumen. According to a recent systematic review by Elmasry et al., around 70% of all polyps are pseudopolyps [8]. Nonmalignant pseudopolyps include cholesterol polyps, adenomyomatous polyps, and inflammatory polyps, whereas true polyps typically present as adenomas or carcinomas. The adenoma-carcinoma sequence in the gallbladder is not as well understood as that found in the colon. The management of gallbladder polyps is contentious. According to a recent guideline, cholecystectomy is recommended in those with polyps greater than 10 mm. Management of smaller polyps depends on patient and polyp characteristics [9].

Biliary tract cancers are highly aggressive tumors. Most patients with biliary tract cancers present at a late stage, by which time the disease is likely to have microscopically or macroscopically spread. Surgical resection, the only curative modality, can be offered to less than a third of patients, with overall 5-year survival at around 10% [2].

Due to advances in neoplasia research, precursor lesions of various cancers have gained recognition. Studies of precursors have enhanced our understanding of the origins of associated diseases and of certain developmental stages, including molecular processes that inform phenotypic changes. Furthermore, biochemical, molecular, and imaging diagnoses of precursors are being used to identify either patients at risk of developing cancer or those in the early stages of the disease as a means of providing adequate therapies and improving survival. For instance, in the pancreas, the following conditions are now acknowledged as precursors of cancer: pancreatic intraepithelial neoplasia (PanIN), intraductal papillary mucinous neoplasia, and mucinous cystic neoplasia. The latter two, which can be identified using imaging methods, are currently the subject of extensive research, with recommendations being formulated for successful management [10, 11]. All three precursors have counterparts in the biliary tract [1].

2. Precancerous Lesions

Based on the majority of the current literature, four types of biliary precancerous lesion are recognised. The two main precursors of biliary carcinoma are biliary intraepithelial neoplasia (BilIN) and intraductal papillary neoplasm of the bile duct (IPNB) [1, 12]. To these, the World Health Organization (WHO) 2010 classification has added mucinous cystic neoplasm (MCN) [13], with the most recent entry being intraductal tubular/tubulopapillary neoplasm (ITN) [14].

3. Biliary Intraepithelial Neoplasia

Biliary intraepithelial neoplasia (BilIN) is a microscopic flat or micropapillary lesion of the dysplastic epithelium [15]. BilIN and its grades represent the most frequent dysplasia-carcinoma sequence through which intra- and extrahepatic cholangiocarcinomas develop [1].

Since it is a microscopic disease, BilIN produces no symptoms and cannot be detected using current imaging methods. It is most likely found in specimens of invasive biliary tract cancer or in gallbladders resected for cholecystolithiasis. As such, its real incidence cannot be precisely determined. However, it is likely comparable to invasive cholangiocarcinoma [16]. Matthaei et al. examined resection margins of 55 biliary tree adenocarcinomas, including gallbladder cancers. BilIN was detected in the resection margins of 29 patients (53%). In the majority of cases, it was identified as low-grade (BilIN-1, 14 out of 29, 48%), with occurrence most frequent in extrahepatic carcinomas (6 out of 8, 75%). Notably, patients with positive resection margins for cancer (R1 resection) exhibited significantly shorter overall survival than those with negative resection margins (R0), irrespective of the presence of BilIN or its grade [17]. Lewis et al. examined 100 consecutive PSC liver explants, including 30 with cholangiocarcinoma, for the presence of biliary dysplasia. Cancer patients exhibited greater prevalence for all types of BilIN (83% vs. 36%, $p < 0.0001$) and high-grade dysplasia (BilIN-2 or 3, 60% vs. 11% ,$p < 0.0001$) [18]. Wu et al. investigated the presence of BilIN in large intrahepatic and hilar ducts of 244 explanted livers from patients with EtOH cirrhosis and HCV cirrhosis alongside noncirrhotic controls. Patients with EtOH and HCV cirrhosis were much more likely to harbor BilIN compared with noncirrhotics (97%, 92%, and 55%, $p < 0.001$). BilIN-3 was present in about 5% of all cases of EtOH and HCV cirrhosis but absent in noncirrhotic controls. Three out of 8 patients (36%) with BilIN-3 also had cholangiocarcinoma in another part of the liver [19].

On gross examination, BilIN can either appear normal or display subtle granularity or thickened mucosa [16]. BilIN may be totally flat or form micropapillary or pseudopapillary projections into the lumen. Microscopically, it is characterized by columnar epithelial cells with multilayering of more or less abnormal nuclei [1]. Similar to pancreatic intraepithelial neoplasia, it can be classified into three grades depending on the extent of cytological or structural abnormalities (Table 1 and Figure 1). The original criteria for each grade were established using livers of patients with hepatolithiasis [15]. However, following an international study by 17 pathologists, which revealed suboptimal interobserver agreement in respect of 30 noninvasive neoplastic biliary lesions (kappa value of 0.45 overall and 0.16 for BilIN-2); these criteria were subsequently revised [20].

In one study, analysis of microdissected BilIN for the *KRAS* and *GNAS* mutations and p53 expression of BilIN and cholangiocarcinoma revealed similarities to pancreatic intraepithelial neoplasia [10]. Existing *KRAS* mutations were observed in early BilIN-1 lesions (25%), increasing slightly during progression to BilIN-3 (30%). *GNAS* mutations were not identified in any lesions. Occurring as a late event,

Table 1: Classification and characterization of biliary intraepithelial neoplasia (based on Ainechi S, *Arch Pathol Lab Med* 2016).

	BilIN-1	BilIN-2	BilIN-3
Cells	Polarity maintained Increase in basally placed nuclei	Loss of polarity Nuclei reaching the luminal surface	Marked loss of polarity Nuclei reaching the luminal surface
Nuclei	Mild atypia	Moderate atypia	Severe atypia
Architecture	Flat	Flat or micropapillary	Likely micropapillary
Mitosis	Rare	Rare	Likely
Dysplasia	Low-grade	Intermediate	High-grade/carcinoma in situ

Figure 1: Histology image of biliary cancer precursors. (a) Biliary intraepithelial neoplasia grade 1 (BilIN-1)—bile duct with tall columnar cells with mild nuclear abnormalities. (b) Biliary intraepithelial neoplasia grade 2 (BilIN-2)—bile duct with columnar cells with moderate nuclear abnormalities and focal pseudopapillary or micropapillary architecture of the epithelium. (c) Biliary intraepithelial neoplasia grade 3 (BilIN-3)—bile duct with columnar cells with severe nuclear abnormalities and pseudopapillary and cribriform architecture of the epithelial layer. Haematoxylin-eosin staining (courtesy of Dr. Jana Maluskova).

overexpression of p53 has been found only in invasive carcinoma (intrahepatic—18%, extrahepatic—38%, and gallbladder—62%) [21].

Variable mucin and epithelial markers have been described for BilIN lesions. Typically, BilIN lesions are characterized by MUC1 and MUC2 negativity. However, increased expression of MUC1 occurs as BilIN-1 progresses to BilIN-3 [22]. This is in agreement with tubular adenocarcinoma, typically associated with BilIN, being positive for MUC1 [23]. MUC2 positivity is rarely found, suggesting an intestinal phenotype [24].

4. Intraductal Papillary Neoplasia of the Bile Duct

Intraductal papillary neoplasia of the bile duct (IPNB) is a macroscopic lesion that can present in either single or multiple form. Situated along the biliary tree, it is characterized by intraductal growth of the dysplastic epithelium with mucin hypersecretion [25, 26]. Formerly known as biliary papillomas, biliary papillomatosis, and papillary adenomas, its current term is now recognised in the World Health Organization classification from 2010 [13]. It shares similarities with its pancreatic counterpart, intraductal papillary mucinous neoplasms (IPMN) of the pancreas, and can be found anywhere on the bile tree, including the gallbladder (intracholecystic papillary neoplasm) [1].

IPNB is a rare disease that accounts for about 10-15% of bile duct tumors. It is typically found in patients between 60 and 70 years of age. It is more common in Asia (Taiwan, Korea, and Japan) where it is associated with common risk factors such as hepaticolithiasis and fluke. Interestingly, while IPNB predominates among males in Western countries, it either predominates among females or is equally distributed between genders in Asia [25–27].

Macroscopically, IPNB manifests as a single or multiple yellowish papillary mass. Filling the bile duct, it can be accompanied by a cystic dilatation of the affected segment, more

Table 2: Classification and characterization of intraductal papillary neoplasms of the bile duct (based on Wan XS, *WJG* 2013).

	Pancreatobiliary	Intestinal	Gastric	Oncocytic
Histology	Columnar cells with eosinophilic cytoplasm and round nuclei	Stratified columnar cells with goblet cells	Columnar cells resembling gastric foveolae	Cells with abundant eosinophilic cytoplasm
MUC1	+	−	−	Focal +
MUC2	−	+	−	Focal +
MUC5AC	+	+	+	+
CK7	+	+	+	+
CK20	+	+	+	+

(a) (b)

Figure 2: Histology image of biliary cancer precursors. (a) Intraductal papillary neoplasia of the bile duct (IPNB)—bile duct with papillary architecture of the epithelium and cytological nuclear abnormalities. (b) Biliary mucinous cystic neoplasia (BMCN)—cyst with the epithelium of the mucinous type and variable grade of nuclear abnormalities. Haematoxylin-eosin staining (courtesy of Dr. Jana Maluskova).

commonly in extrahepatic ducts. Mucin secretion in ducts can be macroscopically visible in some patients [1, 3, 12].

IPNB follows an adenoma-carcinoma sequence [28]. It can be classified as low-grade or high-grade based on the degree of dysplasia but can also manifest in an invasive cancerous form. Invasive carcinomas are found in about 40-80% of IPNBs [3, 28].

Like IPMN of the pancreas, IPNB is classified into four types based on histological and immunohistochemical features [27, 29] (Table 2 and Figure 2). While the pancreatobiliary type is the most prevalent lesion in Western countries, the intestinal type is most common in Asia. When IPMN becomes invasive, it typically develops into tubular adenocarcinoma, with the exception of the intestinal type, which usually progresses into mucinous adenocarcinoma [1]. It remains unclear, however, whether these histology subtypes have any prognostic relevance. In a retrospective analysis of 97 patients with IPNB, the frequency of invasive carcinoma in the pancreatobiliary type was significantly higher than in gastric and intestinal types (72.7 vs. 26.7 and 32.6%, $p < 0.001$) [30]. Accordingly, survival was significantly worse in subjects with the pancreaticobiliary type compared to those with gastric and intestinal types ($p = 0.035$). However, although these observations are similar to findings for pancreatic IPMN, they have not been confirmed by other IPNB studies [1, 28].

The progression of dysplasia is accompanied by the accumulation of genetic alterations. *KRAS* alterations occur early and precede increased expression of TP53 and loss of SMAD4. Surprisingly, *GNAS* mutations, which are linked to the molecular pathogenesis of pancreatic IPMN [31], seem to be much less important [28]. Notably, all *GNAS*-mutated IPNBs are of the intestinal subtype, similar to pancreatic IPMN [28, 31].

IPNB can be asymptomatic and found incidentally. Typical clinical manifestations include abdominal pain, most commonly in the right upper quadrant, obstructive jaundice, and cholangitis. Weight loss and anemia are signs of malignancy [3, 12].

Laboratory investigation may reveal signs of biliary obstruction. The role of tumor markers is unclear. In one study, elevation of CA 19-9 occurred in 40% of patients with IPNB [32]. In another study, CA 19-9 elevation occurred in 35% of benign lesions and 61% of malignant lesions; however, this difference was not statistically significant [33].

Imaging results often show IPNB in the form of an intraductal mass and/or a dilatation of the surrounding bile duct. Kim et al. have proposed the following anatomical classification [30]: type 1—a grossly visible intraductal mass with diffuse dilation; type 2—presence of a diffuse dilatation without any intraductal mass; type 3—a localized dilatation around an intraductal papillary mass; type 4—a mild ductal dilatation filled with cast-like lesions; and type 5—a focal, stricture-like lesion accompanied by a proximal duct dilatation (Figure 3). Abdominal ultrasound can show a bile duct dilatation or a low-echoic mass in the bile duct. Endoscopic ultrasound or intraductal ultrasound can show a duct dilatation or hypoechoic mass and also evaluate signs of invasion and lymph node metastasis. CT and MR scans can detect bile duct dilatations and, generally, tumors greater than 1 cm. Cholangiography, either with MR, ERC, or PTC, can further delineate bile duct anatomy and the extent of the disease.

Figure 3: Anatomical classification of intraductal papillary neoplasms of the bile duct (figure based on Wan XS, WJG 2013).

Cholangioscopy is used for visual diagnosis, targeted biopsies, evaluating the extent of the disease, and ruling out additional synchronous lesions [3, 32]. Nevertheless, accurate preoperative diagnosis is difficult due to the rarity of the disease and the nonspecific nature of most of its clinical manifestations and imaging findings.

Surgery is the treatment of choice for those without distant spread. Preoperative staging is needed before locally advanced diseases, lymph nodes, or other distant metastases can be ruled out. Surgical treatment follows the same rules as applicable for cholangiocarcinoma and may require liver and pancreas resection [34]. Liver transplantation has been reported in individual cases [35]. In cases where surgery cannot be performed, palliative systemic therapy or local treatments may be considered [36].

Survival of patients resected for IPNB is understood to be better than of those resected for conventional cholangiocarcinoma [37]. In a study of 58 patients with IPNB, 5-year survival after curative resection was 81%, while mean survival was 60 months compared to 36 months in patients treated conservatively [32]. In a recent study by Kim et al., which documented outcomes of 112 patients resected for IPNB, positive resection margins (75.9% vs. 25.7%, $p = 0.004$) and lymph node metastasis (75.3% vs. 30.0%, $p = 0.091$) were associated with poor 5-year overall survival rates. Interestingly, histological subtypes had no effect on survival [38].

5. Intraductal Tubular/Tubulopapillary Neoplasia

Small case series have reported rare types of noninvasive intraductal bile duct tumor: intraductal tubular [14] and tubulopapillary neoplasms [39]. They are characterized by tubular architecture, sparse formation of papillary elements, and minimal or no mucin production [39]. In the first report, 10 patients with tubular neoplasia were examined, all presenting with obstructive jaundice and abdominal pain. Eight patients underwent surgery, and their tumors were between 0.6 and 8 cm in size. Eight cases were intrahepatic, with the remaining two extrahepatic (hilar and distal). An extraductal invasive component was present in 7 out of 9 cases [14].

In the second report, 20 cases of intraductal tubulopapillary neoplasia were compiled from various parts of the world. The mean age of patients was 62 years. Tumor location was intrahepatic in 70% and extrahepatic in 30%, with a mean tumor size of 7 cm. Necrosis was present in the vast majority of cases, with tumors staining positive for MUC1 and MUC6 and negative for MUC2. In 80% of the patients, an invasive carcinoma was present. *KRAS* mutations were present only in 6%, whereas *CDKN2A*/*p16* alterations were found in 44% of tumors. Despite the presence of an invasive component in most patients, 5-year survival reached 90% [39].

First described by Yamaguchi et al. [40], its pancreatic counterpart is defined as an intraductal, grossly visible,

(a) (b) (c)

Figure 4: Imaging findings in a patient with biliary mucinous cystic neoplasm. (a) CT image of a large BMCN in the right liver lobe with a hypodense solid component. (b) Ultrasound image of the same BMCN with a hypoechoic solid component by the wall. (c) ERCP image of the same patient showing s smooth stricture of the hepatic duct caused by extrinsic compression of the cyst.

tubule-forming epithelial neoplasm with high-grade dysplasia and ductal differentiation without overt production of mucin (2010 WHO classification) [13].

6. Mucinous Cystic Neoplasia

Biliary mucinous cystic neoplasms (BMCN) are rare tumors characterized, like their counterparts in the pancreas, by the presence of ovarian-type stroma and the absence of communication with the biliary tree (Figure 2). They predominantly occur in females [1].

Little is known about these tumors, and because they so rarely occur, identification is difficult. Formerly referred to as bile duct/biliary cystadenoma and cystadenocarcinoma, MCN can develop in extrahepatic biliary ducts. However, as most of them arise intrahepatically, they are also called cystic neoplasms of the liver (MCN-L). They are classified based on the grade of dysplasia (low-grade, intermediate-grade, high-grade, or in association with invasive carcinoma) [1, 12].

Albeit designated as mucinous neoplasms, the neoplastic epithelium may not produce mucus [41]. Representing only up to 5% of cystic liver lesions, they rarely occur and, when they do, typically in middle-aged women. Their origin is the subject of debate, but their most likely source is the peribiliary glands. Grossly, the neoplasms appear as a single or septated/multicystic lesion between 1 and 40 cm in size (Figure 4). Multilocular structures are more frequent. The presence of mural nodules points to malignant change. They can form a clear fluid or a thick mucus or contain blood or pus. The reported frequency of invasive carcinoma in MCN varies from 2 to 38.5% [41]. The risk of developing invasive cancer in a patient with benign MCN is estimated at around 20% [42].

Patients with MCN may be asymptomatic or present with symptoms due to a slowly growing mass, such as abdominal pain/discomfort, nausea, vomiting, obstructive jaundice, or cholangitis. Abdominal ultrasound, CT, or MRI will typically show a large cyst that must be distinguished from other more common cystic liver lesions [41]. The value of cystic fluid analysis is controversial. In one study, mucinous cysts had a higher median level of CA 19-9 compared to simple cysts (364.8 vs. 21.4 U/ml). However, no significant difference in CEA concentrations was observed (6.8 vs. 4.2 mg/l) [43]. These findings have not been confirmed in other studies [44].

Due to the high risk of disease recurrence after incomplete resection, complete R0 resection surgery is the method of choice for treating MCN. Resection or enucleation is

recommended, since other interventions such as marsupialisation, aspiration, enteral anastomosis, and partial resection are associated with high complication rates [41]. Liver transplantation is another option, but early recurrence has been reported. Although dependent on the presence of invasive cancer and the completeness of the resection, the general prognosis for complete resection of a benign cyst is excellent. The 5-year survival of resected malignant MCN is 65-70%, which is better than that reported for hepatocellular carcinoma, cholangiocarcinoma, and intraductal papillary tumors [42].

7. Conclusions

Several precancerous lesions that precede biliary carcinoma have been reported. Even though their exact incidence is unknown, they are rarely found. Biliary intraepithelial neoplasia is an asymptomatic microscopic lesion of the dysplastic epithelium that cannot be detected by imaging. Intraductal papillary mucinous neoplasms, which can be divided into four different histological types, communicate with the biliary tree and pose a high risk of malignancy. Mucinous cystic neoplasms, which occur in middle-aged women, do not communicate with bile ducts and pose a lower risk of progression to cancer. Intraductal tubular or tubulopapillary neoplasms are rare entities similar to intraductal papillary neoplasms. However, they exhibit tubular growth and lack mucin production.

These precursors are characterized by the expression of specific mucins and follow the dysplasia-carcinoma sequence and its progressive accumulation of genetic alterations. They all have counterparts in the pancreas. However, pancreatic precursors are more frequent and better characterized, enabling more accurate preoperative diagnosis. This also means many precursors can be kept under surveillance, avoiding the need for surgery. On the contrary, not only are biliary precancerous lesions rare and difficult to diagnose, their natural history and progression to cancer are still poorly understood. As imaging methods and knowledge of the biology of biliary premalignant lesions improve, so may their detection and characterization along with the selection of appropriate treatment strategies.

Conflicts of Interest

There is no conflict of interest regarding the publication of this paper.

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Review Article

Endoscopic Stenting in Hilar Cholangiocarcinoma: When, How, and How Much to Drain?

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Hilar cholangiocarcinoma (HCCA) involves a complex anatomical region where bile ducts, arteries, and veins create a complex network. HCCA can lead to biliary strictures at the main hepatic confluence, involving the right and left radicles. Endoscopic drainage of jaundiced patients with HCCA is challenging and carries a high risk of infective complications. HCCA needs a careful multidisciplinary evaluation to assess the indication and purposes (preoperative/palliative) of the biliary drainage. Biliary drainage in HCCA needs to be planned by magnetic resonance cholangiography in order to study the biliary anatomy and perform a target drainage of the intrahepatic ducts above the malignant hilar stricture; all the opacified intrahepatic ducts above the hilar stricture must be drained to reduce septic complications. Drainage of >50% of the liver volume is important to obtain bilirubin reduction and less complications, but atrophic liver segments (identified by CT scan) do not require drainage due to the increased risk of cholangitis. When preoperative biliary drainage is planned, plastic stents must be inserted. Self-expandable metal stents are indicated for palliative purposes and should be placed only when a complete liver drainage is possible; only uncovered metal stents are indicated to drain malignant hilar strictures to avoid side-branch occlusion.

1. Introduction

Endoscopic drainage of hilar cholangiocarcinoma (HCCA) is a technically demanding procedure due to the tumor location which can obstruct several intrahepatic radicles at the main hepatic confluence. Endoscopic and percutaneous biliary drainages are the available techniques to treat jaundice secondary to HCCA. ERCP provides internal drainage by insertion of multiple plastic or metal stents, with a better effect on the quality of life compared to percutaneous drains [1, 2]. Both techniques resulted in effective jaundice resolution, and recent guidelines propose their use to be modulated according to the local expertise [3]. EUS-guided transgastric drainage is another available technique to drain HCCA but data and results are still limited.

Infectious complications are the "Achilles' heel" of both the endoscopic and percutaneous drainages of HCCA due to the contamination of the intrahepatic ducts above the complex malignant hilar stricture (MHS). Suboptimal drainage of intrahepatic ducts is the main reason for the high rate of cholangitis secondary to MHS drainage [4].

Magnetic resonance cholangiography (MRC) can provide a detailed "road-map" to perform optimal biliary drainage of HCCA, thus reducing the rate of infective complications avoiding the scenario of "opacified and undrained biliary ducts."

2. Anatomical Considerations

When approaching MHS secondary to HCCA, the normal anatomy of the intrahepatic ducts should be considered to obtain effective drainage.

The left hepatic duct is usually 3 cm long before dividing into the ducts for segments 2, 3, and 4, while the right hepatic duct is about 1 cm in length and divides early into the two sectorial ducts (anterior for segments 5 and 8, posterior for segments 6 and 7) [5] (Figure 1). Thus, as HCCA advances, right lobe sectors are involved and obstructed earlier compared to the left. The two right sectorial ducts (anterior and

Figure 1: Bile duct anatomy. The left hepatic duct is 3 cm long. The right hepatic duct (dark green) is 1 cm long before dividing into the right anterior (red) and right posterior (yellow) sectorial ducts.

posterior), when separated, require 2 stents to obtain complete drainage of the right liver lobe. Segment 1 has its own biliary drainage into both hepatic ducts and its biliary ducts are rarely injected during cholangiography.

3. Classification of Malignant Hilar Biliary Strictures

The degree of involvement of the intrahepatic ducts in HCCA is commonly classified according to the Bismuth classification [6, 7]. This is a surgical classification which should drive the operative strategy (tumor local excision with or without extended liver resections). Nevertheless, the Bismuth classification is widely used by radiologists and endoscopists because it describes the extent of the tumor into the intrahepatic ducts, which can have a practical impact on drainage strategy.

Type I: only the proximal common hepatic duct is involved, without reaching the main hepatic confluence; it is a nonhilar stricture, 1 stent/PTBD can drain the whole liver.

Type II: the main hepatic confluence is separated; 2 stents/PTBDs are needed to obtain complete liver drainage (Figure 2).

Types IIIa and IIIb: the main hepatic confluence and the right (anterior/posterior) or left (IV/II and III segments) secondary branches are obstructed; 3 stents/PTBDs can drain all the intrahepatic ducts (Figure 3).

Type IV: reflects an advanced disease with involvement of both the primary and secondary right and left biliary confluences; theoretically, 4 stents/PTBDs are needed.

A schematic approach to stenting in MHS according to Bismuth classification is summarized in Figure 4.

4. Biliary Drainage in Malignant Hilar Strictures

4.1. Unilateral/Bilateral. Published studies simplify MHS drainage into two strategies: *unilateral* and *bilateral* depending on the number of inserted stents (one = unilateral, two = bilateral) [8, 9]. These definitions apply to the Bismuth type II strictures (Figure 2) where the right and left biliary systems are separated at the main hepatic confluence; insertion of one stent is enough to obtain *unilateral* (right or left liver lobe) biliary drainage, while two stents are needed to achieve *bilateral* drainage including the whole liver.

Figure 2: Bismuth type II hilar stricture: the main hepatic confluence is separated; 2 stents are needed to drain the whole liver.

Figure 3: Bismuth type IIIa hilar stricture: the main hepatic confluence and the right secondary are involved; 3 stents are needed to drain the whole liver.

When the MHS is more complex (i.e., Bismuth IIIa), one stent can obtain partial liver drainage (left, right anterior or right or posterior) (Figure 5(a)), and also two stents are not enough to drain the whole liver (Figure 5(b)): three stents are needed to obtain complete liver drainage (Figure 5(c)).

4.2. Complete/Incomplete. Due to MHS complexity, draining all the intrahepatic ducts is not always technically feasible. The terms *complete* (all intrahepatic ducts) and *incomplete* (one or some intrahepatic duct) biliary drainage reflect the amount of liver parenchyma that is successfully drained. These definitions seem more appropriate than unilateral and bilateral, which cannot be applied to all types of MHS.

4.3. How Much to Drain? Historically, it was assumed that drainage of about 25-30% (i.e., the left biliary system alone) of the liver parenchyma could be enough to obtain relief of obstructive jaundice [10, 11].

(a) (b) (c)

Figure 4: Schematic approach to complete endoscopic drainage in Bismuth type I ((a) one stent), II ((b) two stents), and IIIa ((c) three stents) malignant hilar strictures.

(a) (b) (c)

Figure 5: Bismuth type IIIa hilar stricture. One (a) or 2 (b) stents obtain an incomplete liver drainage; 3 stents are needed for a complete liver drainage (c). RA: right anterior; RP: right posterior.

Hintze et al. [12] introduced magnetic resonance cholangiography (MRC) as a diagnostic tool to identify the best liver segment to drain during ERCP in 35 nonresectable Klatskin tumors (13 Bismuth type III and 22 type IV). Cannulation of the MRC-selected duct to drain was achieved under fluoroscopic control with a catheter and guidewire, and then contrast was injected proximally to the stricture avoiding opacification of other ducts. A single 10 Fr plastic stent was inserted in the opacified duct with jaundice resolution in 86% of the cases. The rate of cholangitis during the first 30 days was 6%. This single arm study described promising results when performing *incomplete* drainage of MHS but was limited by the absence of a control group.

A French study [13] analyzed, by CT scan, liver volumetry of the three main liver segments (left, right anterior, and right posterior) in 107 patients that underwent endoscopic stenting of MHS (Bismuth type ≥ 2); drained liver volume was classified into 3 groups: less than 30%, 30% to 50%, and more than 50%. These authors concluded that stenting more than 50% of the liver volume is an independent factor significantly contributing to a greater decrease in bilirubin level, a lower incidence of early cholangitis, and longer survival. Similar results were reported in other two studies [14, 15].

When approaching MHS, recent guidelines [3] recommend to *drain* $\geq$ 50%*of the liver volume* and to avoid the opacification of biliary ducts that will not be drained.

4.4. Which Liver Parenchyma Should be Drained? Evaluation of the "quality" of the liver parenchyma before draining HCCA is another important issue. In the setting of HCCA, monolateral portal vein thrombosis and subsequent segmental liver atrophy can occur. CT scan/MRI is essential to study liver parenchymal atrophy.

Drainage of an atrophic liver segment should be avoided because clinical success and survival are not increased [16], and the risk of cholangitis is even significantly higher [13].

5. Endoscopic Stenting in Hilar Cholangiocarcinoma

5.1. When Is Stenting Needed? Jaundice is the typical clinical manifestation of HCCA. Immediate biliary drainage should be avoided because stents or percutaneous drains impair tumor staging by cross-sectional imaging modalities (CT scan, MRI/MRC), due to image artifacts [3, 17]. MRC is also important to plan the biliary drainage strategy, and

(a) (b) (c) (d)

Figure 6: Bismuth type IIIA hilar stricture. Selective cannulation with antegrade opacification of the left (a), right posterior (b), and right anterior (c) biliary ducts obtaining a complete liver drainage (d). RA: right anterior; RP: right posterior.

(a) (b)

Figure 7: Opacified and undrained left hepatic duct in hilar cholangiocarcinoma (a). Percutaneous drainage obtained a complete liver drainage (b).

therefore can be considered a prerequisite before any attempt to biliary drainage [3, 12].

The aim of biliary drainage in HCCA should be decided in the setting of a multidisciplinary team [3]. Surgery is the only curative option for HCCA and improper biliary drainage, leading to infective complications, can transform a potentially resectable patient into a nonoperable one.

If the patient is a candidate to left hepatectomy, preoperative biliary drainage is not indicated [18, 19], while it is required when estimated future liver remnant volume is <30% and portal vein embolization is needed to obtain hypertrophy of the remnant liver after surgery [20, 21].

Patients that are not a candidates to surgery receive jaundice palliation by stent insertion and histological characterization for further radiochemotherapy. ERCP remains the method of choice to perform brush cytology and/or intraductal biopsy. Recent advances in cholangioscopy made direct inspection with target biopsy of MHS [22, 23] reproducible and widely available in clinical practice. As far as EUS is concerned, the experience with staging and sampling of MHS is still limited [3].

5.2. Plastic Stents. Plastic stenting is commonly the first-line drainage method for jaundice relief in HCCA. Some characteristics of plastic stents (PS) should be considered:

(i) Removability

(ii) The insertion does not impair the subsequent therapeutic plan

(iii) Other PS can be added in case of ineffective biliary drainage

(iv) PS size can be adapted to the common bile duct diameter which is not dilated in HCCA

PS are indicated for preoperative biliary drainage and when no final decision about curative/palliative treatment has been taken.

Insertion of multiple plastic stents in HCCA should be performed by (1) selective cannulation with a guidewire of the intrahepatic ducts above the hilar stricture; (2) antegrade contrast injection to define the anatomy and perform a selective drainage of the biliary duct; (3) balloon dilatation (4-6 mm) of the stricture, if needed; (4) plastic stent insertion with drainage of the opacified duct, thus avoiding the scenario of "injected and undrained ducts"

(a) (b)

Figure 8: Bismuth type IIIa hilar stricture (a). Three uncovered self-expandable metal stents are inserted side-by-side for palliative purposes (b).

(a) (b)

Figure 9: Complex hilar stricture. A single uncovered self-expandable metal stent has been inserted into the right anterior duct obstructing the other intrahepatic ducts (a), leading to cholangitis. Left and right posterior ducts are drained by plastic stents through the meshes (b).

(Figure 6); plastic stents' length needs to be tailored case by case, but 12 cm long stents are more frequently used. In the case of opacified and undrained biliary ducts, percutaneous drainage should be performed as soon as possible [3] (Figure 7). To reduce the risk of cholangitis, antegrade injection of air, instead of contrast, was proposed, but the risk of infection does not seem significantly reduced [3].

Japanese authors proposed preoperative biliary drainage of the future remnant liver by nasobiliary drains with reintegration of the same bile by mouth or through a nasogastric tube; results of this kind of treatment seem promising but further data are expected [24].

5.3. Uncovered Self-Expandable Metal Stents (U-SEMS). Among the various types of SEMS (uncovered, partially covered, fully covered), only U-SEMS are recommended in MHS because the drainage of the side branches is possible through the uncovered meshes [3, 25]. U-SEMS are not removable, and their presence at the hepatic hilum can preclude surgery; for these reasons, U-SEMS are indicated only for *palliative* purposes in HCCA [3].

Insertion of multiple U-SEMS "side-by-side" is easier when plastic stents, obtaining complete biliary drainage, have been previously placed; placement of U-SEMS in HCCA should be performed only when all the intrahepatic ducts have been properly cannulated (Figure 8); usually 8 or 10 (rarely 12) cm long U-SEMS are needed to drain MHS.

The importance of complete biliary drainage in HCCA is underlined by the difficulties in draining intrahepatic ducts

through the meshes of an improperly placed U-SEMS (Figure 9).

As previously discussed, incomplete biliary drainage in HCCA can be considered in the presence of segmental atrophy.

U-SEMS are more expensive than plastic, but their patency is significantly longer thus reducing the need for further hospitalization due to cholangitis and stent malfunction [9, 26].

U-SEMS in HCCA can be inserted "side-by-side" or "stent-in-stent"; the latter configuration is complex, placement of more than 2 stents is rarely reported, and retreatment is challenging and not always possible [27, 28]. For these reasons, "side-by-side" multiple U-SEMS is the preferred configuration for HCCA palliation because retreatments are easy and successful, especially if the U-SEMS are placed transpapillary [29]. The possibility to retreat malfunctioning multiple "side-by-side" SEMS seem important because survival resulted significantly higher in patients who received retreatments, according to a large series on 134 patients [29].

U-SEMS can occlude because of sludge deposition or tissue ingrowth; sludge can be removed with a Fogarty balloon, while ingrowth can be resolved by insertion of a second U-SEMS or a plastic stent according to life expectancy.

SEMS patency can be increased by adding photodynamic therapy or radiofrequency ablation before stent insertion [30, 31]. These techniques seem promising but are not widely diffuse; data are limited and cost-effective analysis is lacking.

6. Conclusions

Endoscopic drainage of HCCA is challenging and its approach should be performed in referral centers where a multidisciplinary team (radiologist, interventional radiologist, surgeon, endoscopist, pathologist) [3] is available.

The first step is staging HCCA and assessing its surgical resectability; at this point, the aim of the biliary drainage can be established.

(i) *Preoperative biliary drainage* is indicated in case of extended right hepatectomy performing complete drainage of the future remnant liver before portal vein embolization; in case of left hepatectomy, biliary drainage is not routinely indicated, if necessary, the future remnant liver should be drained. Preoperative drainage is performed with *plastic* stents only

(ii) *Palliative biliary drainage* can be done by insertion of multiple plastic stents or U-SEMS

Among SEMS design, only *uncovered* stents are indicated for HCCA palliation to avoid occlusion of side branches. It is suggested to place multiple U-SEMS side-by-side and transpapillary to facilitate retreatments.

Complete drainage of all the intrahepatic is desired to reduce infective complications; atrophic liver parenchyma does not need biliary drainage. Opacified biliary ducts above MHS must be drained to reduce infective complications.

Conflicts of Interest

Andrea Tringali was a consultant for Boston Scientific Corp. Ivo Boškoski is a consultant for Apollo Endosurgery. Guido Costamagna has received grant/research support from Olympus Japan; is a member of advisory committees or review panels for Cook, Inc., Boston Scientific Corp., and Taewoong Medical, Inc.; and has been a speaker and teacher for Boston Scientific, Corp. and Given Imaging.

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Research Article

Factors Associated with the Technical Success of Bilateral Endoscopic Metallic Stenting with Partial Stent-In-Stent Placement in Patients with Malignant Hilar Biliary Obstruction

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Background. Bilateral biliary drainage decreases the risk of cholangitis, but bilateral endoscopic metallic stenting is technically challenging. *Aim*. We retrospectively evaluated the factors associated with successful bilateral self-expanding metal stent (SEMS) placement using the partial stent-in-stent (PSIS) method for malignant hilar biliary obstruction and also assessed the safety and efficacy of this technique. *Methods*. From April 2010 to February 2016, 47 consecutive patients (mean age, 73.0 ± 8.6 years; 32 males and 15 females) underwent PSIS placement for malignant hilar biliary obstruction in our hospital. The technical success of PSIS, clinical response, and complications were investigated. Factors associated with the technical success of PSIS were assessed. Using a propensity score-matched analysis, we compared the procedure time, clinical response, complications, stent patency, and survival time in 17 matched patients treated with bilateral SEMS placement using a SEMS delivery system of <6.0 or ≥6.0 Fr. *Results*. The technical success rate was 77%. The clinical response rate was 91%, and the complication rate was 26%. Regarding complications, pancreatitis occurred in 5 patients (11%), cholangitis in 6 (13%), and cholecystitis in 1 (2%). A multiple logistic regression analysis identified the use of a SEMS with a delivery system < 6.0 Fr as a factor associated with technical success ($P = 0.033$; odds ratio, 10.769; 95% confidence interval, 1.205-96.212). In the 17 matched patients assigned according to the SEMS delivery system size, the procedure time was significantly shorter in those with a delivery system size < 6.0 Fr than in those with ≥6.0 Fr ($P < 0.01$). There were no significant differences in the clinical response, complication rate, stent patency, or survival time between the two groups. *Conclusion*. Using a delivery system < 6.0 Fr in size helped improve the technical success and reduced the procedure time for the placement of a SEMS by the PSIS method.

1. Introduction

Whether unilateral or bilateral self-expandable metallic stent (SEMS) placement is preferable in patients with malignant hilar biliary obstruction (MHBO) is controversial. Many endoscopists have attempted bilateral SEMS placement because bilateral SEMS placement is more physiological than unilateral placement, thereby preventing obstructive cholangitis [1–4]. Furthermore, evidence suggests that bilateral endoscopic drainage has a longer stent patency than unilateral drainage [2, 5, 6]. In a recent study, Lee et al. reported that bilateral SEMSs had not only merits with regard to stent patency but also low reintervention rates [7].

Bilateral endoscopic metallic stenting can be achieved by the partial stent-in-stent (PSIS) method and the side-by-side (SBS) method. The stent patency and survival time were similar between the two methods [8]. In Japan, many endoscopists have attempted the PSIS method, showing a technical success rate of 81.8%-100% [8, 9], but they have often

Table 1: SEMSs used in this study.

	Diameter of delivery system (Fr)	Type	$n = 47$	
			Diameter of SEMS (8 mm)	Diameter of SEMS (10 mm)
WallFlex Biliary	8	Braided	1	2
Niti-S large cell D-type stent	8	Braided	0	1
X-suit NIR	7.5	Laser-cut	0	10
Zeo stent plus	7.2	Laser-cut	2	8
Bonastent	7	Braided	0	1
Zilver 635	6	Laser-cut	0	4
BileRush Selective	5.7	Laser-cut	0	18

encountered difficulty inserting the second SEMS through the mesh of the first stent with this method.

No study has yet evaluated the factors associated with successful bilateral SEMS placement via the PSIS method. We therefore retrospectively evaluated the factors associated with successful bilateral SEMS placement using this method for MHBO and assessed the safety and efficacy of this technique.

2. Materials and Methods

2.1. Patients. From April 2010 to February 2016, SEMS placement with the PSIS method for MHBO was attempted in 54 consecutive patients in our hospital. Patients were excluded from this study if they had been previously treated with SEMS placement ($n = 5$), received SEMS placement by the SBS method ($n = 1$), or previously received gastric surgery with gastrointestinal reconstruction ($n = 1$). After these exclusions, 47 patients (mean age, 73.0 ± 8.6 years; 32 males and 15 females) were included in the analysis.

Diagnoses of malignancy were histologically confirmed by tissue samples. If tissue samples were not available, then the diagnoses of malignancy were confirmed by the combination of clinical, laboratory, and radiologic findings. In the study, patients were considered to have unresectable tumors if they had radiological findings of massive horizontal tumor extension of the bile duct and/or metastasis, an insufficient predicted remnant liver volume, or a poor general condition.

All patients provided their written informed consent, and this study protocol was approved by the ethics committee of our hospital (approval number 27-195) and conducted in accordance with the Declaration of Helsinki.

2.2. Endoscopic Procedures. We usually perform the PSIS method for patients with MHBO. In the present study, endoscopic procedures were performed under conscious sedation with diazepam, midazolam, and pentazocine; the doses of which depended on the patient's age and general condition. Prophylactic treatment with broad-spectrum antibiotics and nafamostat mesilate was initiated after the procedure. A TJF240, JF260V (Olympus Medical Systems, Tokyo, Japan), was used for endoscopic retrograde cholangiopancreatography (ERCP) in this study. After biliary cannulation, the location and length of the stricture were evaluated by cholangiography. Two guidewires (Visiglide2 (Olympus Medical Systems) and/or Jagwire (Boston Scientific Corp., Natick, MA, USA)) were advanced one each into the left hepatic duct and anterior or posterior branch of the right hepatic duct through the stricture. If the guidewires did not pass through the sites of biliary stenoses, then a hydrophilic guidewire (Radifocus (Terumo, Tokyo, Japan) or Navipro (Boston Scientific Corp.)) was used to negotiate through the sites of stenoses. The first stent was inserted and placed over a guidewire via the working channel of the scope. The guidewire utilized for the deployment of the first stent was then inserted into the contralateral side via the open mesh of the first stent based on information regarding the configuration of the second guidewire under fluoroscopic guidance. After successful insertion of the first guidewire into the opposite side, the second guidewire was retrieved. Finally, the second metal stent was deployed over the guidewire into the contralateral hepatic duct. A dilation catheter (7, 8, 10 Fr, Gadelius Medical, Tokyo, Japan) and/or balloon 6 or 8 mm in diameter (Hurricane RX; Boston Scientific) was used for the dilation of both the mesh and inner lumen of the first stent if the first guidewire and second SEMS were difficult to insert into the opposite side via the first stent. Unilateral stent placement was performed if the initially planned PSIS placement failed.

Fourteen endoscopists performed the procedure in this study. The trainee usually started the endoscopic procedure; however, the experts (S. H, S. T, and H. T) replaced the trainee when the trainee was unable to complete the procedure. The kind of SEMS to be placed was left to the discretion of the endoscopist and institution. The SEMSs used in this study were WallStent ($n = 3$, Boston Scientific Japan K.K., Tokyo, Japan), Niti-S large cell D-type ($n = 1$, Century Medical, Tokyo, Japan), X-suit NIR ($n = 10$, Olympus, Tokyo, Japan), Zeo stent ($n = 10$, Zeon Medical, Tokyo, Japan), Bonastent M-hilar ($n = 1$, Medico's Hirata Inc., Tokyo, Japan), Zilver 635 ($n = 4$, Cook Medical Endoscopy, Winston-Salem, NC, USA), and BileRush Selective ($n = 18$, Piolax Medical Devices, Inc., Yokohama, Japan) (Table 1). All SEMSs were uncovered type. In all patients who received SEMS placement with a delivery system < 6.0 Fr, BileRush Selective of 10 mm in the stent diameter was used for the bilateral stenosis of the hilar biliary tree.

2.3. Outcome Measurements. The technical success rate for the PSIS method, clinical response rate for the method, and

Figure 1: Flowchart of the study population selection and matching by propensity score. MHBO: malignant hilar biliary obstruction; SEMS: self-expandable metallic stent; PSIS: partial stent-in-stent; EST: endoscopic sphincterotomy.

complications were investigated. Factors associated with the technical success of PSIS were assessed. In patients treated with bilateral SEMS placement, the procedure time, clinical response, and complications were compared based on the SEMS delivery system size (<6.0 vs. ≥6.0 Fr).

Technical success was defined as the success of the planned placement of a bilateral SEMS for the biliary stenosis by the PSIS method. A clinical response was defined as a reduction in the serum total bilirubin level to normal (<1.2 mg/dL) or less than half of the pretreatment level for 2 weeks. The total procedure time of ERCP and stenting time, which was defined as the time from cannulation of the initial SEMS to the complete placement of all SEMS, were calculated by referencing the medical record of the procedure. A diagnosis of post-ERCP pancreatitis was defined according to the consensus criteria [10]. Cholangitis was defined as a condition accompanied by abdominal pain and a fever (body temperature > 38°C) with an elevated serum level of hepatobiliary enzymes from 24 h after the procedure.

2.4. The Propensity Score-Matched Analysis. The patients who underwent bilateral endoscopic metallic stenting were divided into two groups by the delivery system size ((i) thin delivery system group: diameter of the delivery systems for both stents < 6.0 Fr and (ii) thick delivery system group: diameter of the delivery systems for either stent ≥ 6.0 Fr). These two groups were matched in a 1 : 1 ratio (<6.0 Fr, $n = 17$; ≥6.0 Fr, $n = 17$) by a propensity score-matched analysis with adjusting for 6 covariates (age, sex, cause of MHBO, drainage before SEMS placement, endoscopic sphincterotomy (EST) before SEMS placement, and Bismuth classification) to minimize inherent bias.

2.5. Statistical Analyses. Student's t-test and the Mann-Whitney U test were used for continuous data comparisons. Fisher's exact test and Pearson's χ^2 test were used for comparisons of categorical data, as appropriate. A multivariate analysis was performed as a multiple logistic regression analysis of factors with P values < 0.10 according to a univariate analysis. Propensity scores were estimated using logistic regression. The stent patency and survival time were calculated by Kaplan–Meier curves and log-rank test. A P value of <0.05 was considered to be significant in all analyses. For the statistical analyses, the SPSS software program (ver. 22; IBM Corp., Armonk, NY, USA) was used.

3. Results and Discussion

3.1. Results. A flow chart of this study is shown in Figure 1. Overall, 47 patients were included in this study. The characteristics of all patients are shown in Table 2. The mean age was 73.0 ± 8.6 years, and there were 32 men (68%). The cause

Table 2: Clinical characteristics of the 47 patients with MHBO.

	$n = 47$	
Age, mean ± SD (years)	73.0 ± 8.6	
Male/female, n (%)	32/15	(68/32)
The cause of MHBO, bile duct cancer/others, n (%)	35/12	(74/26)
Drainage before SEMS placement, +/-, n (%)	34/13	(72/28)
Existence of EST before SEMS placement, +/-, n (%)	15/32	(32/68)
Bismuth classification, II/III, IV, n (%)	12/35	(26/74)
Chemotherapy or radiation before SEMS placement, +/-, n (%)	10/37	(21/79)

MHBO: malignant hilar biliary obstruction; SEMS: self-expandable metallic stent; EST: endoscopic sphincterotomy.

of MHBO was bile duct cancer in 35 (74%), gallbladder cancer in 5 (11%), hepatocellular carcinoma in 4 (9%), metastatic disease in 2 (4%) (primary origin: colorectal cancer and pancreatic cancer), and biliary invasion of gastric cancer in 1 (2%). Drainage before SEMS placement was performed in 34 (72%). EST before SEMS placement was performed in 15 (32%). The Bismuth classification was type I in 0, type II in 12 (26%), type III in 14 (30%), and type IV in 21 (44%). Chemotherapy or radiation was performed before SEMS placement in 10 (21%).

3.1.1. Technical Success and the Factors Affecting the Success of Bilateral SEMS Placement by the PSIS Method. The technical success rate for the PSIS method was 77%. In 11 patients (23%), bilateral SEMS placement was unsuccessful. In nine of these patients, the double guidewires could not pass through the MHBO, and in the remaining two, the second SEMS could not pass through the mesh of the first SEMS.

The clinical response rate for the method was 91%, and the complication rate after stenting was 26%. Regarding complications, pancreatitis occurred in 5 patients (11%), cholangitis occurred in 6 patients (13%), and cholecystitis occurred in 1 patient (2%).

Among the 36 patients who underwent bilateral endoscopic metallic stenting, dilation of the first SEMS was performed in 27 patients (75%). The mesh of the first SEMS was dilated in 9 patients, and the lumen of the first SEMS was dilated in 2 patients. Both of them were dilated in 16 patients. In contrast, all patients who underwent unilateral SEMS placement received dilation of both the mesh and the lumen of the SEMS.

The factors affecting the success of bilateral SEMS placement by the PSIS method are shown in Table 3. There were no significant differences in the patient age, sex, cause of MHBO, existence of biliary drainage before SEMS placement, existence of EST before SEMS placement, Bismuth classification, SEMS type, or use of dilation devices between bilateral and unilateral SEMS placement. In the univariate analysis, the use of a SEMS delivery system < 6.0 Fr in diameter was the only significant factor influencing the technical success ($P = 0.023$). A multivariate analysis confirmed that the use of a SEMS delivery system < 6.0 Fr in diameter was a factor associated with the technical success ($P = 0.033$; odds ratio, 10.769; 95% confidence interval, 1.205-96.212).

3.1.2. Clinical Outcomes of Bilateral Placement of SEMS with the Delivery System Size < 6.0 Fr in Diameter. The patients who underwent bilateral SEMS placement by the PSIS method were divided into two groups based on the delivery system size (thin vs. thick delivery system group). The characteristics of these two groups after propensity score matching are shown in Table 4. The characteristics were similar between the two groups. However, the total ERCP time and stenting time were significantly shorter in the thin delivery system group than in the thick delivery system group ($P = 0.009$ and $P = 0.009$, respectively). There were no significant differences in the rate of using dilation devices (balloon and/or dilation catheter), clinical response, or the complication rate between the two groups (Table 5).

The median time of stent patency was 234 and 211 days in the thin and thick delivery system groups, respectively ($P = 0.462$, Figure 2). The 90-day and 6-month stent patency rates were also similar between the 2 groups (93% vs. 82% ($P = 0.350$) and 53% vs. 53% ($P = 0.982$)). Twenty-nine patients (thin delivery system group, $n = 13$; thick delivery system group, $n = 16$) died during the follow-up. The cause of death was tumor progression in 29 patients. Kaplan–Meier curves revealed no significant differences in the stent patency or survival time between the 2 groups (383 days vs. 341 days, $P = 0.979$, Figure 2).

3.2. Discussion. We showed here that the use of a SEMS delivery system < 6.0 Fr in diameter contributed to the successful placement of SEMSs using the PSIS method. Bilateral endoscopic metallic stenting is technically challenging. Kogure et al. [11, 12], Lee et al. [13], and Hwang et al. [14] reported high technical success rates with large-cell stents using PSIS deployment. However, failure to insert the second SEMS through the mesh can be caused by insufficient expansion of the first SEMS due to low radial force [15]. Consequently, a large mesh size and strong radial force of the first SEMS are considered factors supporting the success of bilateral SEMS placement by the PSIS method. While many kinds of SEMSs have been used with the PSIS method, which ones are the most suitable has been unclear.

The most difficult part of the PSIS method involves advancing the second stent through the wire mesh of the initial stent. For the introduction of the second stent, the PSIS method for MHBO is often required, not only to negotiate through the site of biliary stenosis but also to dilate the site of biliary stenosis and the mesh of the initial stent [16–18]. Because the thin delivery system could be placed smoothly through the mesh of the initial stent without dilating the wire mesh, this system helped in shortening the procedure time. Kawakubo et al. reported that a laser-cut-type SEMS with a large mesh and thin delivery system was preferable for the PSIS procedure [19]. Our result supported the findings of that study because SEMSs with a thin delivery system (5.7 Fr; BileRush Selective) have a laser-cut-type configuration and

Table 3: The factors for technical success of PSIS.

	Bilateral SEMS placement $n = 36$	Unilateral SEMS placement $n = 11$	Univariate analysis P value	Multivariate analysis P value	OR (95% CI)
Age, mean ± SD (years)	73.3 ± 9.0	72.3 ± 8.0	0.741		
Male	26	6	0.229		
Bile duct cancer	28	7	0.285		
Drainage before SEMS placement	10	3	0.647		
EST before SEMS placement	13	2	0.232		
Bismuth classification, type III, IV	28	7	0.285		
Laser-cut-type SEMS	33	9	0.332		
Use of dilation devices	27	11	0.069		
Delivery system size of placed SEMS < 6.0 Fr	17	1	0.023	0.033	10.769 (1.205-96.212)

MHBO: malignant hilar biliary obstruction; SEMS: self-expandable metallic stent; EST: endoscopic sphincterotomy; OR: odds ratio; CI: confidence interval; PSIS: partial stent-in-stent.

Table 4: Clinical characteristics of patients with bilateral SEMS placement.

	All participants, $n = 36$ Used delivery system of SEMS			Matched pairs, $n = 34$ Used delivery system of SEMS		
	<6.0 Fr $n = 17$	≥6.0 Fr $n = 19$	P value	<6.0 Fr $n = 17$	≥6.0 Fr $n = 17$	P value
Age, mean ± SD	71.7 ± 8.8	74.7 ± 9.2	0.31	71.7 ± 8.8	74.2 ± 9.6	0.427
Male/female, n (%)	13/4 (76/24)	13/6 (68/32)	0.436	13/4 (76/24)	11/6 (65/35)	0.452
The cause of MHBO, bile duct cancer/others, n (%)	14/3 (82/18)	14/5 (82/18)	0.414	14/3 (82/18)	12/5 (71/29)	0.344
Drainage before SEMS placement, +/-, n (%)	6/11 (35/65)	4/15 (21/79)	0.281	6/11 (35/65)	4/13 (24/76)	0.452
Existence of EST before SEMS placement, +/-, n (%)	9/8 (53/47)	4/15 (21/79)	0.047	9/8 (53/47)	4/13 (24/76)	0.151
Bismuth classification, II/III, IV, n (%)	5/12 (29/71)	3/16 (16/84)	0.281	5/12 (29/71)	3/14 (18/82)	0.344

MHBO: malignant hilar biliary obstruction; SEMS: self-expandable metallic stent; EST: endoscopic sphincterotomy.

Table 5: Outcomes of the two groups.

	All participants, $n = 36$ Used delivery system of SEMS			Matched pairs, $n = 34$ Used delivery system of SEMS		
	<6.0 Fr $n = 17$	≥6.0 Fr $n = 19$	P value	<6.0 Fr $n = 17$	≥6.0 Fr $n = 17$	P value
Procedure time, min (range)						
Total procedure time of ERCP	54.3 (20-132)	81.0 (35-177)	0.004	54.3 (20-132)	86.7 (35-149)	0.009
Stenting time	12.8 (3-23)	22.0 (7-124)	0.003	12.8 (3-23)	26.6 (7-84)	0.009
Use of dilation devices, n (%)	14 (82)	13 (68)	0.283	14 (82)	11 (65)	0.219
Clinical response, effective, n (%)	15 (88)	19 (100)	0.216	15 (88)	17 (100)	0.242
Complication, n (%)	4 (24)	5 (26)	0.577	4 (24)	4 (24)	0.656

SEMS: self-expandable metallic stent; ERCP: endoscopic retrograde cholangiopancreatography.

large stent mesh. These characteristics of the SEMS contributed to the technical success and shorter procedure time of the present study.

The clinical response, complication rates, and stent patency were similar between the two delivery system groups in this study. Sofuni et al. reported that a procedure time of ≥30 minutes is a risk factor of post-ERCP pancreatitis [20], so reducing the procedure time of ERCP is desired. In addition, there were no significant differences in the stent patency or survival time between the two groups. Given these findings, a thin stent delivery system appears to have merit for patients with MHBO.

Several limitations associated with the present study warrant mention. First, this study was retrospective with an uncontrolled design and was conducted at a single center. The sample size was also quite small. Second, the choice of SEMS was not standardized in this study and was left to each ERCP operator. The cut-off value of 6 or 7 Fr for the diameter

Figure 2: Kaplan–Meier curves revealed no significant difference in the stent patency ((a) $P = 0.462$) and survival time between the 2 groups ((b) $P = 0.979$). The thin delivery system group used a SEMS delivery system of <6.0 Fr, and the thick delivery system group used a SEMS delivery system of ≥6.0 Fr.

of the delivery system for the stent that was used did not significantly affect the success of bilateral SEMS placement by the PSIS method ($P = 0.138$ and $P = 0.341$). The result may be due to the small number cases in which a 6 or 7 Fr delivery system was used. However, the use of a thin delivery system is considered to be effective since it allows the site of biliary stenosis to be easily passed through. In addition, Kim et al. reported that the outcomes were better for techniques performed during the late period than in the early period because the new devices required technical experience [21]. Given that the SEMS with a thin delivery system was not available from April 2010 to January 2015, we cannot exclude the possibility of technical bias with regard to technical immaturity, although early cases of ERCP performed before March 2010 were excluded. A prospective, randomized controlled trial of SEMS placement by the PSIS method for MHBO should be conducted.

4. Conclusions

The use of a delivery system < 6.0 Fr in diameter helps improve the technical success and reduce the procedure time of ERCP.

Abbreviations

ERCP: Endoscopic retrograde cholangiopancreatography
EST: Endoscopic sphincterotomy
SEMS: Self-expanding metallic stent
MHBO: Malignant hilar biliary obstruction
PSIS: Partial stent-in-stent
SBS: Side-by-side.

Data Availability

The data used to support the findings of this study are included within the article.

Disclosure

The study was presented as the previous draft in UEG Week 2016 Poster Presentations.

Conflicts of Interest

The authors declare that there is no conflict of interest regarding the publication of this paper.

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Review Article

Pancreatic Cysts: Diagnostic Role of EUS-Guided Microforceps Biopsy and Confocal Laser Endomicroscopy

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Frequent use of high-quality cross-sectional imaging has led to a significant rise in diagnosis of pancreatic cystic lesions (PCLs). Despite the fact that enormous effort has been put into the research of PCLs within the last two decades and multiple guidelines have been developed, our clinical decision-making especially in regard to mucinous lesions remains limited. Currently, clinical assessment, cross-sectional imaging and EUS with fluid analysis (if appropriate) belong to the standard care in patients with PCLs. For differentiation of mucinous from nonmucinous cysts, the sensitivity of cytological investigation and CEA in the cyst fluid is 42% and 52-79%, respectively. Due to the limited accuracy, further diagnostic tools are warranted. Two EUS-guided approaches have been introduced recently. Through-the-(19-gauge EUS) needle Moray microforceps have been developed, and several studies have acknowledged their contribution to the correct diagnosis as they help to overcome limited cellularity of the EUS-guided cyst fluid aspiration and traditional cytology. Confocal laser endomicroscopy offers real-time images and seems to be a promising method for the diagnosis and differential diagnosis of pancreatic PCLs. Example images of the needle-based confocal laser endomicroscopy criteria for the diagnosis of PCLs have been suggested recently. Before both, Moray microforceps and confocal laser endomicroscopy can be widely accepted, further studies are necessary to determine the real diagnostic yield and the clinical efficacy.

1. Introduction

Frequent use of high-quality cross-sectional imaging has led to a significant rise in diagnosis of pancreatic cystic lesions (PCLs). The recent meta-analysis has confirmed pooled prevalence of 8% in asymptomatic individuals [1]. Incidence of PCLs increases with age and reaches 37% in patients aged >80 years [2]. It has been acknowledged that individuals with PCLs have a significantly higher overall risk of pancreatic cancer [3]; nevertheless, clinicians face a challenge how to optimize management of individuals with a PCL, when currently insufficient diagnostic tools are taken into account [4]. Patients should not be overtreated with surgery and on the contrary, individuals with a malignant PCL should not be kept under surveillance inappropriately [5].

The aim of our paper is to review classification of pancreatic cysts and to discuss the role of the most recent EUS- (endoscopic ultrasound-) guided diagnostic options for PCLs.

2. Classification of PCLs and Current Knowledge

Pancreatic cystic lesions are divided into mucinous lesions, including mucinous cystic neoplasm (MCN) and intraductal papillary mucinous neoplasm (IPMN) and nonmucinous

Table 1: IPMN: intraductal papillary mucinous neoplasm; MCN: mucinous cystic neoplasm; SCN: serous cystic neoplasm; SPN: solid pseudopapillary neoplasm; p: predominantly; HOP: head of pancreas.

	IPMN	MCN	SCN	Pancreatic pseudocyst	SPN
Male	60-70%	5%	10%	75%	10%
Age (years)	60-70	40-50	70	Around 50	30
Localization	p HOP	p body and tail	p tail	Any localisation	Any localisation
Communication with PD	Yes	No	No	Yes	No
Cytology	Mucinous cells	Mucinous cells		Inflammatory cells	
CEA in cyst	High	High	Low	Low	Low
Mucin in cyst	Yes	Yes	No	No	No
Amylase in cyst	High	Low	Low	High	Low

lesions which include serous cystic neoplasm (SCN), pseudocyst, cystic neuroendocrine tumour, solid pseudopapillary tumour, and cystic pancreatic ductal adenocarcinoma [6–8]. Basic characteristics of PCLs are summarized in Table 1. Mucinous cystic lesions belong, together with pancreatic intraepithelial neoplasia, to the precursor lesions for pancreatic adenocarcinoma [9].

Patients with symptomatic PCLs can present with jaundice, recent onset of type 3 diabetes, (recurrent) pancreatitis, anorexia, weight loss, abdominal/back pain, nausea, and/or vomiting [7, 8]. Clinical assessment, cross-sectional imaging and EUS with fluid analysis, if appropriate (cytology, CEA), play the major role in current standard care in patients with a PCL. MRI/MRCP has been proven to be superior to CT in identifying communication between a PCL and the pancreatic ductal system and the presence of a mural nodule and in identifying if a patient has single or multiple PCLs [10–13]. In a recent meta-analysis, cytological investigation of the cyst fluid had 42% sensitivity and 99% specificity for differentiation of mucinous from the nonmucinous PCLs [14]. Cyst fluid CEA $\geq$ 192 ng/mL can differentiate mucinous from nonmucinous cyst with a sensitivity of 52-78% and specificity of 63-91%. Cytology and/or cyst fluid CEA level is not helpful in differentiation between MCN and IPMN [10, 15]. Despite the fact that the evidence is weak, antibiotic prophylaxis prior to an EUS-guided FNA of PCLs and 3-5 days after keeps being used routinely. Guarner-Argente et al. [16] and recently Facciorusso et al. [17] have not observed reduction of risk of infection after antibiotic prophylaxis. In view of this, further prospective studies are warranted with the aim to abandon routine periprocedural use of antibiotics.

2.1. Intraductal Papillary Mucinous Neoplasia (IPMN). IPMNs are classified into the main duct (Figures 1 and 2), mixed type, and branch duct neoplasias according to the communication with and involvement of the main and/or branch pancreatic ductal system [9]. Majority of IPMNs are solitary and are localized in the pancreatic head, yet 20-40% are multifocal [18]. Typically, males (in 60-70%) of age 60-70 years would be diagnosed with an IPMN. The absence of capsule, communication with the pancreatic duct, presence of mucin, and cystic fluid high in CEA and amylase belong to the main features of

Figure 1: Main duct intraductal papillary mucinous neoplasia (asterisk: dilatation of the main pancreatic duct).

Figure 2: Main duct intraductal papillary mucinous neoplasia, FNA performed (arrow pointing at the FNA needle).

IPMNs [19]. Based on histological characteristics and immunohistochemical reactivity for mucins (MUC), IPMNs are classified into gastric (less aggressive phenotype, usually originating in branch ducts), intestinal, pancreatobiliary, and oncocytic (more aggressive phenotypes, usually originating in the main pancreatic duct) types [9, 18, 20, 21]. Invasive carcinomas related to IPMN can be either colloid or tubular (conventional), and there is a clear evidence that the colloid carcinomas have a better

prognosis than the tubular carcinomas [22–24]. The most typical mutations observed in IPMNs are those in oncogenes KRAS and GNAS and in tumour suppressor gene RNF43 [6]. Due to the risk of malignant transformation, patients with main duct IPMNs and mixed type IPMNs should be considered for surgery. According to the recent European guidelines, the absolute indications for surgery are tumour-related jaundice, solid mass, presence of an enhancing mural nodule (≥5 mm), positive cytology for malignant/high grade dysplasia, and/or main pancreatic duct dilatation ≥ 10 mm [10].

2.2. Mucinous Cystic Neoplasm (MCN). MCNs are usually solitary large unilocular cysts predominantly found in the body or the tail of the pancreas in 40-50-year-old females (Figure 3). Cysts are characterized by the absence of communication with the pancreatic ductal system [19]. Peripheral "eggshell" calcification is seen in less than 20% of MCNs; nevertheless, such a finding is specific for a mucinous cystic neoplasm and is highly predictive of malignancy [25, 26]. Further typical features are the presence of ovarian-like stroma (with expression of hormone receptors) and mucin-producing epithelium. Cystic fluid is high in CEA and low in amylase [6, 25]. Mutation in KRAS oncogene is the most commonly found mutation; GNAS mutation is not observed in MCN—on contrary to an IPMN [27]. As MCN is usually discovered in younger patients in the body or the tail of the pancreas and has clearly a malignant potential, majority of centres will recommend surgery [25]. The recent European guidelines suggest resection for all patients with a MCN ≥ 40 mm in size or who are symptomatic or have risk factors (such as a mural nodule), irrespective of the size [10].

2.3. Serous Cystic Neoplasm (SCN). SCN are benign cystic lesions predominantly found in the tail of the pancreas in females aged around 70 years (Figures 4 and 5). Imaging usually shows microcystic "honeycomb-like" lesion with central scar and central calcification with no communication with the pancreatic duct. The absence of mucin in the cyst, low CEA, and low amylase in the cyst fluid are characteristics of SCN [19, 25]. Mutations in VHL gene are typical for serous cystic lesions [28]. Surgical treatment should be proposed only if the diagnosis remains uncertain after a complete workup, if significant and related symptoms are present (jaundice, pancreatitis, and gastric outlet obstruction), or if exceptionally, a concern regarding malignancy arises [25, 29].

2.4. Pseudocyst. Pancreatic pseudocysts can be present anywhere in the pancreas and are more frequently found in males, usually in association with chronic pancreatitis (Figures 6 and 7). CEA is low, no mucin, and no molecular markers related to malignancy can be detected [6, 25]. Communication with the pancreatic duct is usual; therefore, the content is rich in amylase. Amylase <250 U/L (4.2 μkat/L) may exclude the presence of a pseudocyst with a sensitivity 44% and specificity 98% [30].

2.5. Solid Pseudopapillary Tumour (SPT). SPTs, rare lesions, are typically identified in young females and express progesterone and estrogen receptors (Figure 8). They can be found anywhere in the pancreas and usually consist of mixed solid-cystic lesions. There is no communication with the pancreatic duct; the cyst fluid is low in CEA and amylase [6, 19, 25]. SPTs belong to slowly growing tumours with low malignant potential and infrequent metastases [31]. Surgical resection is warranted [25].

Figure 3: Mucinous cystic neoplasm. CEA: 242 ng/mL. Cytology benign.

Figure 4: Serous cystic neoplasm with vascular septa.

Figure 5: Serous cystic neoplasm with micro- and macrocysts.

Enormous effort has been put into the research regarding pancreatic cystic lesions within the last two decades; nevertheless, diagnostic accuracy, as shown above, is rather

Figure 6: Pancreatic pseudocyst (FNA had been performed before the AXIOS stent was inserted; arrow pointing at the FNA needle).

Figure 7: Large pancreatic pseudocyst (143 × 105 mm).

Figure 8: Solid-cystic pseudopapillary tumour.

poor. Multiple guidelines have been developed (including Sendai [32], Fukuoka [22], revised Fukuoka [33], American Gastroenterological Association (AGA) [34], and European guidelines [10]); still, our current clinical decision-making especially in regard to mucinous lesions remains limited. Further diagnostic tools are warranted. We offer an update on two EUS-guided methods, and we discuss their role in the PCL diagnosis.

3. EUS-Guided Microforceps Biopsy

In an attempt to improve the diagnostic yield of PCLs, through-the-needle direct intracystic biopsy and pancreatic cystoscopy were first performed in 2010 [35]. Biopsy forceps and a SpyGlass fiber optic probe were passed through the 19-gauge EUS needle in two patients with a PCL in the head of the pancreas. Diagnosis of a mucinous lesion was established in both cases. One patient developed severe acute pancreatitis one month after the biopsies, which was rather not associated with the procedure [35]. Another report came in 2015, when Barresi et al. documented a contribution of miniforceps biopsy to the diagnosis of a mucinous tumour in the body of the pancreas in a 46-year-old woman. Biopsies were taken from the wall of the cyst [36]. Subsequently, novel through-the-needle Moray microforceps have been developed, and Pham et al. reported a first successful biopsy of an intracystic nodule leading to a diagnosis of a mucinous cyst [37]. Further, authors have confirmed in individual cases that Moray microforceps can be useful in determination of the nature of the PCLs and can contribute to their management and risk stratification [38–41]. Moray microforceps are 230 cm in length with a jaw opening width of 4.3 mm and a sheath of 0.8 mm in diameter that easily passes through a 19-gauge EUS-FNA needle [38]. The role of Moray microforceps in the preoperative diagnosis has been acknowledged subsequently [42–44].

A first larger study, retrospective in design, which involved 27 patients with PCLs, was published in 2018: 14 patients with cysts located in the pancreatic head and/or uncinate process and 13 patients with cysts located in the body and/or tail of the pancreas were enrolled. Moray microforceps were passed through the 19-gauge needle under the EUS guidance, and 3-4 subsequent samples were taken from the cyst wall and placed into formalin. After completion of biopsies, cyst fluid was aspirated and sent for cytology and CEA level analysis. Microforceps biopsies were technically successful in all 27 cases and provided a pathology diagnosis in 24 of 27 cases. No periprocedural adverse event was recorded (including bleeding, infection, perforation, and pancreatitis). Overall, microforceps biopsy results changed the diagnosis in 7 patients; nevertheless, cytology provided a diagnosis of a mucinous cyst in 4/27, and these have not been detected by microforceps biopsies. The authors therefore concluded that Moray forceps could be a useful adjunctive tool, which would be complementary to existing EUS-FNA sampling protocols for PCLs [45]. Also in 2018, Basar et al. published data on 42 patients from a multicentre study: they confirmed that Moray microforceps biopsy was far superior to cytology in providing a specific cyst diagnosis [46]. A similar conclusion came from Zhang et al.: pancreatic cyst fluid analysis and microforceps biopsy have comparable results in distinguishing between mucinous and nonmucinous cysts and for detecting high-risk cysts;

nevertheless, similar to the study performed by Basar et al. [46], microforceps biopsy has been superior for diagnosing specific cyst subtypes [47]. Another most recent study published on microforceps biopsy by Kovacevich et al. has shown promising results, nevertheless, three adverse events (11%) have been recorded [48].

In conclusion, dedicated through-the-needle Moray microforceps allow biopsy of the PCL wall or a mural nodule. This helps to overcome the limited cellularity of the EUS-guided cyst fluid aspiration and traditional cytology. Further, it can provide guidance when at best modest accuracy of CEA is taken into account. Yet, the precise role of microforceps biopsy remains to be defined by large prospective studies before routine clinical implementation is recommended.

4. Confocal Microscopy

The principle of confocal laser scanning microscopy is not new; it was invented as early as 1957 [49]. Subsequent use in gastroenterology started in the mid-1990s [50]. The first generation of dedicated endoscopes enabled the introduction of confocal laser endomicroscopy (CLE) in the late nineties. A confocal endomicroscope was miniaturised to a size that made it possible to be integrated in the distal end of a high-resolution videoendoscope. A lot of research work was done afterwards, both experimental and clinical [51]. CLE classification was suggested for Barrett's oesophagus and colorectal neoplasia [51, 52]. Our group studied experimental pharmacokinetics, and organ distribution of fluorescein determined the optimum time interval for diagnostic scanning (5-10 minutes after the fluorescein administration) and found high concentration in all organs of the gastrointestinal tract (including the pancreas), necessary for optimal confocal laser imaging [53]. Recently, single miniaturised CLE probes have become commercially available. These probes can be introduced through a working channel of a conventional videoendoscope into the lumen of the gastrointestinal tract or through a 19-gauge needle for fine-needle-based CLE. They enable observation of the inner wall of pancreatic cystic lesions during an endoscopic ultrasound-guided fine-needle aspiration [6, 51, 54–58].

Since 2010, more than fifty papers have been published, including ESGE [54, 59] and ASGE technology reviews [51] and a meta-analysis [60]; however, only few clinical studies have been accomplished so far [61–68]. Konda et al. [61] published their first experience with a prototype confocal laser probe. Eighteen patients (with 16 cysts and 2 mass lesions) were investigated in this multicentre feasibility study at a tertiary setting. CLE was technically feasible (in 17 of 18 cases) using a 19-gauge needle under EUS guidance. There were no device malfunctions; two cases were complicated with acute pancreatitis. The diagnosis was confirmed with histology or positive cytology in 10 out of 18 patients [61]. The INSPECT study investigated 66 patients (images were available for 65) in USA, Germany, and France. The authors aimed to define criteria for differentiation of mucinous and nonmucinous cystic lesions. An epithelial villous structure on confocal images was associated with mucinous cysts significantly [62]. Eighteen patients with indeterminate pancreatic duct strictures were investigated prior to surgery in another study [63]. Real-time CLE images were obtained during ERCP. Cytology or histopathology in 15 of 16 cases showed similar results to CLE interpretation. Agreement between cytology or histopathology and CLE was high ($\kappa = 0.8$). Pancreatic CLE changed management in four patients [63]. The DETECT study combined EUS-guided through-the-needle direct visualisation (SpyGlass fiber optic probe) and probe-based CLE inserted through a 19-gauge needle. Thirty patients with pancreatic cystic neoplasms were enrolled. The combination of cystoscopy and CLE of pancreatic cysts might have strong concordance with the clinical diagnosis of pancreatic cystic neoplasms (sensitivity 87, specificity 77, and positive and negative predictive values 100%) [64]. Napoleon et al. [65] investigated 31 patients with pancreatic cysts and identified criteria for the diagnosis of serous cystadenoma [65]. Twenty patients with pancreatic cystic neoplasms were investigated within a 16-month period in another study [66]. The procedure and confocal image acquisition were successful in 90%. The sensitivity, specificity, and diagnostic accuracy were 66, 100, and 80%. No complications were recorded [66]. The pancreatic cyst epithelial wall can be visualised successfully by CLE also in ex vivo surgical specimens [67]. Krishna et al. [68] investigated ten patients for the reproducibility of the in vivo endoscopic ultrasound-guided needle-based CLE image patterns in an ex vivo setting. Both in vivo (preoperative) and ex vivo confocal laser imaging of the surgically resected pancreatic cystic lesions correlated with surgical histopathology [68].

Example images of the needle-based CLE criteria for the diagnosis of PCLs have been suggested: (a) serous cystadenomas with the "superficial vascular network" criterion; (b) intraductal papillary mucinous neoplasms with the "papillae" criterion; (c) mucinous cystadenomas with the "epithelial border" criterion; (d) neuroendocrine neoplasms with the "dark aggregates of cells surrounded by gray areas of fibrosis and vessels" criterion; and (e) pseudocysts with the "field of bright, gray, or black particles" criterion [56, 65, 66] (Figure 9). For the near future, it will be mandatory to set a validated interpretation system of the CLE of pancreatic cystic lesions, to establish a unified training programme and to create a close standardized cooperation of gastroenterologists/endoscopists and pathologists. There is conflicting data on the reproducibility and accuracy of needle-based CLE in the available literature. In a multicentre US study, interobserver agreement of needle-based CLE recordings of 15 patients was low; the mean accuracy of observers was only 46%. Interobserver agreement for the final diagnosis was poor ($\kappa = 0.13$) [69]. According to another project [70], interobserver agreement, intraobserver reliability, and diagnostic accuracy were high in differentiation of mucinous versus nonmucinous pancreatic cystic lesions. In a study with 29 consecutive patients (between 2013 and 2016), the overall sensitivity, specificity, and accuracy for the diagnosis of mucinous lesions were 95%, 94%, and 95%, respectively. The interobserver agreement and intraobserver reliability were high ($\kappa = 0.81$ and $\kappa = 0.86$, respectively). Similar results

Figure 9: Confocal laser endomicroscopy for evaluation of pancreatic cystic lesions. Adopted from Napoleon et al. [65], Napoleon et al. [56], and Kadayifci et al. [66]. Drawing: Hana Kotlandova.

were achieved for recognizing the characteristic image pattern of serous cystadenomas ($\kappa = 0.83$ and $\kappa = 0.85$). The overall specificity, sensitivity, and accuracy for the diagnosis of serous cystadenomas were 99%, 98%, and 98%, respectively [70].

Safety of needle-based CLE refers either to EUS-guided puncture of the cyst lesions or to side effects of i.v. fluorescein administration. Acute pancreatitis can complicate up to 4% of procedures; bleeding associated with a needle puncture is another possible complication [65]. A series of 2272 patients with CLE revealed no serious adverse complications of i.v. fluorescein administration. Mild adverse events (in 1.4%) included nausea, vomiting, transient hypotension, diffuse rash, and mild epigastric pain [71].

Financial aspects must be also considered. Needle-based CLE of pancreatic cyst lesions increases the cost of the diagnostic process significantly [51]. On the other hand, needle-based CLE used with EUS-based fine-needle aspiration and/or biopsy might improve diagnostic accuracy, helping to reduce unnecessary surgery and patient follow-up, thus resulting in significant economic benefits by reducing the incidence of misdiagnosis owing to improved diagnostic accuracy [72]. Before the technology can be widely accepted, further studies are necessary to determine the real clinical efficacy and to evaluate the cost-effectiveness [51].

CLE seems to be a promising method for the diagnosis and differential diagnosis of pancreatic cystic lesions. Nevertheless, the common use is limited by its high cost and need of specific operator training (as a standardized institutional programme). That is why CLE is not easily available yet. Further studies are needed to evaluate the real diagnostic yield, cost-effectiveness, and health care economic analyses prior to implementation into a routine clinical practice.

5. Conclusions

Based on the current clinical practice, diagnostic accuracy for pancreatic cystic lesions is modest at best. Further diagnostic tools are warranted. Two EUS-guided methods have been introduced recently: Moray microforceps and CLE. They seem to be promising in regard to diagnostic yield; nevertheless, further studies are warranted to determine clinical efficacy and to evaluate the cost-effectiveness.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

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Review Article

Up-to-Date Tailored Systemic Treatment in Pancreatic Ductal Adenocarcinoma

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Despite intensive research efforts, pancreatic ductal adenocarcinoma is still regarded as an aggressive and life-limiting malignancy. Combination chemotherapy regimens that underpin the current treatment approach in the advanced setting have led to incremental survival gains in recent years but have failed to confer patients with a median overall survival that exceeds 12 months from diagnosis. Research has since focussed on understanding the role and interplay between various components of the desmoplastic stroma and tumour microenvironment, in addition to developing targeted therapies based on molecular features to improve the prognosis associated with this malignancy. This review will summarise the available systemic treatment options and discuss potential methods to refine the resolution of patient selection to enhance responses to currently available therapies. Furthermore, it will explore newer approaches anticipated to come to the fore of future clinical practice, such as agents targeting the DNA damage response and tumour microenvironment as well as immunotherapy-based combinations.

1. Introduction

Pancreatic ductal adenocarcinoma (PDAC) is the 14th most common malignancy worldwide, with a global incidence of 458,918 cases in 2018 [1]. In comparison, 432,232 deaths were attributed to PDAC, making it the seventh most common cause of cancer-related death worldwide [1].

At present, surgical resection is the sole curative treatment modality but is relevant to only 15% of patients presenting with a new diagnosis of PDAC who have resectable disease. Even so, current data indicates that patients who undergo curative resection followed by adjuvant chemotherapy have a limited prognosis consisting of a median overall survival (OS) ranging from 28 to 54 months [2–4] and that patients in the United Kingdom have a five-year survival rate of 3.3%, regardless of stage at diagnosis [5]. Treatment within the palliative paradigm consists of chemotherapy, with the most intensive combination achieving a modest median OS of 11.1 months [6].

The poor prognosis associated with PDAC is a culmination of vague symptomatology leading to late presentation, a complex mutational landscape, and a dense desmoplastic stroma with an immunosuppressive tumour microenvironment (TME) that collectively pose challenges in developing and delivering effective systemic treatments. By 2030, PDAC is projected to become the second leading cause of cancer deaths in America, second only to lung cancer [7]. This forecasted statistic reflects the stagnation of progress in PDAC management relative to other cancers despite intense research efforts over recent years and highlights the urgent need for novel approaches that can provide patients with clinically meaningful benefit. Here, we will outline the current standard-of-care in the advanced disease setting and discuss emerging treatment strategies approaching clinical practice.

2. Current Approach to Systemic Therapy

For thirty years, the cornerstone of systemic treatment for metastatic PDAC consisted of 5-fluorouracil (5-FU). This was surpassed in 1997, when patients randomised to receive gemcitabine monotherapy demonstrated an improvement in clinical benefit response, a composite measure consisting of pain, performance status, and weight, in comparison to

Table 1: Summary of first- and second-line trials in metastatic pancreatic ductal adenocarcinoma.

Reference	Treatment arms	N	Primary endpoint	Response rate (%)	Median PFS (months)	Median OS (months)
		First-line therapy				
Burris et al. [8]	Gemcitabine vs. 5-FU	126	CBR	23.8 4.8 $p = 0.0022$	2.1 1.2 $p = 0.0002$	5.65 4.41 $p = 0.0025$
Cunningham et al. [14]	Gemcitabine-capecitabine vs. gemcitabine	533	OS	19.1 12.3 $p = 0.034$	5.3 3.8 HR 0.78 $p = 0.004$	7.1 6.2 HR 0.86 $p = 0.08$
PRODIGE4/ACCORD 11 [6]	FOLFIRINOX vs. gemcitabine	342	OS	31.6 9.4 $p < 0.001$	6.4 3.3 HR 0.47 $p < 0.001$	11.1 6.8 HR 0.57 $p < 0.001$
MPACT [15]	Gemcitabine/nab-paclitaxel vs. gemcitabine	861	OS	23 7 $p < 0.001$	5.5 3.7 HR 0.69 $p < 0.001$	8.5 6.7 HR 0.72 $p < 0.001$
		Second-line therapy				
CONKO-003 [18]	OFF vs. FF	168	OS	NR	2.9 2.0 HR 0.68 $p = 0.019$	5.9 3.3 HR 0.66 $p = 0.010$
PANCREOX [19]	mFOLFOX6 vs. 5-FU+leucovorin	108	PFS	13.2 8.5 $p = 0.361$	3.1 2.9 HR 1.00 $p = 0.99$	6.1 9.9 HR 1.78 $p = 0.024$
NAPOLI-1 [21]	Nal-IRI+5-FU/LV vs. 5-FU/LV	417	OS	17 1 $p < 0.000$	3.1 1.5 HR 0.57 $p = 0.0001$	6.2 4.2 HR 0.75 $p = 0.039$
	Nal-IRI vs. 5-FU/LV			6 1 $p = 0.02$	2.7 1.6 HR 0.81 $p = 0.81$	4.9 4.2 HR 1.07 $p = 0.568$

Abbreviations: CBR: clinical benefit response; FF: leucovorin+fluorouracil; HR: hazard ratio; FOLFIRINOX: 5-fluorouracil, irinotecan+oxaliplatin; mFOLFOX6 (modified FOLFOX6): 5-fluorouracil, leucovorin, and oxaliplatin; OFF: oxaliplatin+leucovorin+fluorouracil; LV: leucovorin; Nal-IRI: nanoliposomal irinotecan; OS: overall survival; PFS: progression-free survival; 5-FU: 5-fluorouracil.

patients treated with 5-FU (23.8% vs. 4.8%, $p = 0.002$) [8] (Table 1). Additionally, the gemcitabine group demonstrated a small survival advantage over patients who received 5-FU (median OS 5.65 vs. 4.41 months, $p = 0.002$).

Clinical trials examining the efficacy of gemcitabine-containing doublet regimens with a second chemotherapeutic agent or targeted therapies were largely negative [9–12]. There are notable exceptions. Although a phase III randomised trial comparing gemcitabine and erlotinib against gemcitabine alone showed a statistically significant improvement in both progression-free survival (PFS) and OS in the combination arm, this failed to translate clinically as it amounted to an absolute difference of 6 and 10 days, respectively [13]. Interestingly, a subset of patients with grade ≥ 2 skin rash obtained a more significant survival benefit compared to patients with milder or no skin toxicity (10.5 months vs. 5.8 months vs. 5.3 months, HR 0.74, $p = 0.037$). Nevertheless, this combination is rarely utilised in clinical practice as its potential benefit is limited to a small proportion of patients. A randomised controlled trial examining the efficacy of gemcitabine and capecitabine against gemcitabine monotherapy demonstrated statistically significant improved response rates (19.1% vs. 12.4%, $p = 0.34$) and median PFS (5.3 vs. 3.8 months, HR 0.78, $p = 0.004$), with a

trend towards improved median OS (7.1 vs. 6.2 months, HR 0.86, $p = 0.08$) [14]. A meta-analysis of these results amalgamated with data from two further studies detected a survival benefit associated with gemcitabine and capecitabine over monotherapy (HR 0.86; 95% CI, 0.75 to 0.98; $p = 0.02$), providing support for the use of this regimen in treatment-naïve advanced PDAC patients [14].

Two further chemotherapy combinations provide treatment options in the first-line setting for advanced PDAC in patients fit for combination therapy. The triplet combination of 5-FU, irinotecan, and oxaliplatin (FOLFIRINOX) was shown to improve median OS to 11.1 months, in comparison to 6.8 months in patients receiving gemcitabine (HR 0.57, $p < 0.001$), with a parallel improvement in quality of life at 6 months as assessed by the EORTC QLQ-C30 questionnaire (HR 0.47, $p < 0.001$) [6]. Separately, gemcitabine and nanoparticle albumin-bound paclitaxel (nab-paclitaxel) also extended median OS to 8.5 months, in comparison to 6.7 months with gemcitabine monotherapy (HR 0.72, $p < 0.0001$) in patients with metastatic disease (Table 1) [15].

At face value, FOLFIRINOX may be perceived as the superior regimen due to its longer median OS, albeit with a less tolerable safety profile. However, a direct comparison of outcomes between both trials cannot be made due to variations in study methodologies and patient characteristics. A systematic review and meta-analysis of 16 retrospective studies involving 3,813 patients comparing the effectiveness of FOLFIRINOX against gemcitabine/nab-paclitaxel showed that both regimens resulted in similar OS, PFS, overall response rates, and risks of death and disease progression with distinct toxicity profiles [16]. These findings reinforce the notion that both combinations are reasonable upfront treatment options for patients with a good performance status, and the choice of treatment should ultimately be individualised accordingly to patient's comorbidities and preferences. In patients with poor performance status, gemcitabine monotherapy can still be considered in patients treated with palliative intent.

Approximately 40% of patients eventually receive second-line systemic therapy [17] following gemcitabine-based first-line therapy. There is currently no prospective trial data to determine the optimal sequencing of FOLFIRINOX and gemcitabine/nab-paclitaxel, and these remain as treatment options in patients requiring second-line therapy provided they remain fit for these regimens. Oxaliplatin-based chemotherapy has been considered as standard-of-care in this setting after oxaliplatin, leucovorin, and 5-FU (OFF) demonstrated superior median OS in the CONKO-003 trial when compared to leucovorin and 5-FU (FF) (5.9 vs. 3.3 months, HR 0.66, $p = 0.001$) [18]. These findings were challenged by results from the PANCREOX trial, which showed that survival outcomes were inferior in patients randomised to receive modified FOLFOX6 (mFOLFOX6; 5-FU, leucovorin, and oxaliplatin) in comparison to patients in the 5-FU-leucovorin arm [19]. A higher incidence of grade ≥ 3 adverse events and adverse events leading to treatment discontinuation was observed in the mFOLFOX6 arm compared to the 5-FU-leucovorin arm (63% and 20.4% vs. 11% and 1.9%, respectively). Furthermore, a higher proportion of patients in the 5-FU-leucovorin arm received postprogression therapy in comparison to patients assigned to receive mFOLFOX6 (25% vs. 6.8%), possibly due to the poorer tolerability associated with the former combination. As the median PFS of both arms were similar (HR 1.00, $p = 0.36$), the discrepancy in OS could be attributed to the differences in postprogression therapy uptake [19]. More recently, long-term data from the NAPOLI-1 trial [20] has since confirmed that nanoliposomal irinotecan and 5-FU-leucovorin is associated with superior median OS when assessed against 5-FU-leucovorin [21], providing a further option for patients with gemcitabine-refractory disease who remain well enough for additional treatment (Table 1). Although therapeutic options for second-line therapy are available, the risk benefit of pursuing further systemic treatment should be carefully considered in view of the significant symptomatic burden and rapid clinical deterioration commonly seen in these patients at this juncture.

3. Improving Patient Selection to Maximise Treatment Efficacy

In contrast with other malignancies, targeted therapies do not feature in the treatment armamentarium due to a lack of a targetable driver mutation in this disease. Although over 90% of PDAC cases harbour activating *KRAS* mutations [22], thus providing an attractive therapeutic target, KRAS is deemed "undruggable" due to its relatively smooth 3D structure [23]. Efforts to target downstream components of the RAS pathway, such as mitogen/extracellular signal-regulated kinase (MEK), have also been unsuccessful in the clinical setting [24–26]. The epidermal growth factor receptor (EGFR) is overexpressed in 80% [27] of PDACs, which collectively with KRAS mutations may explain the lack of efficacy from anti-EGFR-targeted therapies [13, 28]. Given that there has been a paucity of novel therapeutic agents coming to the fore of PDAC management, research activity has also focussed on devising methods to refine patient selection for treatment based on individual patient molecular profiles. Several molecular characterisations for PDAC exist [29–33] using tissue from both patients with early and advanced disease, and current classifications have identified molecular features with prognostic and responsive associations. For example, the basal subtype is associated with chemoresistance and poor prognosis, whereas the classical subtype is associated with a more favourable outlook [29]. The COMPASS trial was designed as a prospective study to assess the feasibility of comprehensive real-time whole genome sequencing and RNA sequencing of advanced PDAC to identify predictive mutational and translational features to inform treatment selection [34]. In addition to demonstrating that prospective genomic profiling was feasible with a median turnaround time of 35 days, the investigators detected better objective responses to first-line chemotherapy in patients with *GATA6*-amplified classical PDAC RNA subtype in comparison to basal-like patients ($p = 0.004$), with the best PFS observed in patients who received modified FOLFIRINOX [34]. While this finding provides some prospective evidence in a nonrandomised cohort that the characterisation of RNA subtypes via molecular profiling may allow upfront

identification of chemosensitivity to potentially guide treatment selection, it requires further assessment in a controlled trial to fully ascertain its role in the clinic.

Another promising method to enhance treatment selection is through the use of patient-derived organoids (PDOs) to facilitate *in vitro* drug sensitivity testing [35]. PDOs are cultured from LRG5$^+$ stem cells that can be isolated from a number of organs and propagated as epithelial cyst-like structures *in vitro* with genomic, transcriptomic, and phenotypic properties resembling those of the original tissues [36]. Vlachogiannis et al. successfully developed PDOs from metastatic lesions of gastrointestinal primaries obtained at baseline and posttreatment time points [36]. Concordance of immunohistochemical markers and genetic aberrations such as *ERBB2* and *FGFR2* amplifications between PDOs and parental tissue was observed, in addition to a 96% overlap in mutational spectrums. Furthermore, it was demonstrated that PDOs could predict response to both targeted therapies and chemotherapy and mirrored the development of drug resistance in tandem with clinical outcomes. Similar findings have been reproduced in 66 PDAC PDOs cultured from both tissue obtained from surgical resections and fine needle biopsies [37]. Both these studies highlight that PDOs are robust preclinical models that can provide accurate representation of tumour histopathology and molecular heterogeneity by predicting treatment efficacy *in vitro* and are posed to be an invaluable tool in future drug development with possible clinical utility in prospective therapeutic selection.

Large-scale efforts to accrue molecular profiles representative of the patient population are essential to better our understanding of this complex disease and identify newer, rational therapeutic options. An example is the PRECISION-Panc platform in the United Kingdom, which serves as a portal protocol for patients with PDAC that offers patients full molecular characterisation of tumour tissue using upfront genomic sequencing with subsequent access to appropriate clinical trials alongside an integrated preclinical development programme [38]. An example of a clinical trial affiliated with the PRECISION-Panc platform is PRIMUS-001, which aims at comparing the efficacy of a novel platinum-containing regimen FOLFOX-A (5-FU, oxaliplatin, and nab-paclitaxel) against gemcitabine and nab-paclitaxel, with an incorporated translational research objective to further identify and characterise biomarkers of response to DNA-damaging agents. By allowing bidirectional translation between the laboratory and the clinic, platforms such as PRECISION-Panc represent an important opportunity to obtain and analyse multidimensional datasets which can enable identification of novel pathophysiology and treatment approaches to inform the direction of future research within the field.

4. Selected New Treatment Approaches on the Horizon

4.1. Targeting DNA Damage Repair Signalling. Endogenous and exogenous stressors such as reactive oxygen species and cytotoxic agents continuously produce DNA damage. This triggers a coordinated response orchestrated by an intricate network of DNA damage response (DDR) proteins to maintain genomic integrity and sustain cell viability. Consequently, defective DDR is associated with susceptibility to cancers including PDAC, ovarian, breast, and prostate cancers [39, 40]. The prevalence of a deleterious germline mutation in *BRCA1* and *BRCA2*, which encode for mediators of homologous recombination (HR), is estimated at 5% in PDAC patients [41], while aberrations in *PALB2* [42], *ATM* [43], *ATR* [44], *RAD51* [45], and *CHK1/2* [44] genes can also confer a HR-deficient or BRCA-like phenotype. Additionally, approximately 14% of PDACs have large numbers of structural variation events, resulting in genomic instability and DDR deficiency [31].

Impaired DDR signalling has classically been targeted by DNA-damaging agents such as platinums and alkylating agents. Agents interfering with DNA repair, most notably polyADP-ribose polymerase (PARP) inhibitors, are currently being assessed in PDAC. Clinical evaluation of PARP inhibitors in combination with chemotherapy (NCT01585805) is underway. Recently, results from the POLO study demonstrated that patients with germline BRCA-mutated metastatic PDAC who achieve disease control following a minimum of 16 weeks of platinum-based first-line chemotherapy reported a statistically significant improvement in PFS (7.4 months vs. 3.8 months, HR 0.53, 95% CI 0.35 to 0.82; $p = 0.004$) when randomised to receive olaparib over placebo. Although an OS benefit was not detected, these results provide a basis for the potential clinical application of a new class of therapeutics in a novel clinical setting within pancreatic cancer management [46]. Beyond BRCA mutant patients, HR-deficient patients represent another population who could derive benefit from agents that target DNA damage repair signalling.

4.2. Understanding the Desmoplastic Stroma and Immune Microenvironment in Pancreatic Ductal Adenocarcinoma. PDACs are encased by a desmoplastic reaction, which forms a fibrous layer of tissue surrounding malignant cells in both primary and metastatic lesions [47]. This consists of a diverse network of cellular components, including cancer-associated fibroblasts (CAFs), endothelial cells, immune cells, and extracellular matrix. The majority of these cells are CAFs, which secrete extracellular matrix proteins such as collagen, proteoglycans, fibronectin, matrix metalloproteases, and glycosaminoglycans [48]. While the resultant stroma is thought to be integral in promoting cancer growth and metastasis in addition to forming a physical barrier that facilitates a hypoxic environment and limits drug penetration [49], there is an increasing body of evidence to suggest that CAFs may restrain rather than promote PDAC tumour growth [50, 51]. Recent observations demonstrating significant intra- and intertumoural heterogeneity in patient-derived CAF primary cultures have provided insight into the complexity of CAFs and its role in the desmoplastic stroma [52] and support the hypothesis that CAFs play a context-dependent role in PDAC tumorigenesis and progression.

Although PDAC has a relatively low mutational load with a median somatic mutational prevalence of 1 mutation/-megabase [53], the majority of PDACs express candidate neoantigens required to generate an antitumour response

[54]. Additionally, it has been shown using *in silico* neoantigen prediction that tumours with the highest number of neoantigens alongside the most abundant CD8+ T-cell infiltrates are associated with the longest survival [55]. The presence of dendritic cells, a form of antigen-presenting cell required to stimulate a T-cell response, is infrequent in PDAC and tends to be immature even when present, compromising tumour antigen recognition and T-cell activation [56]. While PDAC tumour samples also demonstrate robust presence of tumour-infiltrating lymphocytes, active immune suppression mechanisms mediated by the presence of T regulatory cells, myeloid-derived suppressor cells (MDSCs), tumour-associated macrophages (TAMs), and inhibitory cytokines such as TGF-β, IL-10, and nitric oxide synthase mitigate against effective T-cell responses [54]. In light of these immunosuppressive mechanisms, PDAC is regarded as an immune-quiescent tumour.

The TME permits cross talk between malignant cells, its stromal components, and immune cells, creating a dynamic milieu which evolves throughout the course of the disease. Clearly, understanding the role of the desmoplastic stroma and its immune microenvironment is a crucial step in developing new therapeutic targets in PDAC, improve drug delivery, and broaden the clinical application of immunotherapy in this disease.

4.3. Targeting Components of the Desmoplastic Stroma. Interest was initially focussed on targeting the Hedgehog pathway, a key driver of the desmoplastic process. Its promise was first highlighted when the combination of saridegib, a Hedgehog inhibitor, and gemcitabine induced a transient increase in intratumoural vascular density and intratumoural levels of gemcitabine, resulting in improved survival in a patient-derived xenograft murine model [57]. These preclinical observations failed to translate into clinical benefit in early phase clinical studies. Further research has since shown that stromal constituents driven by the Hedgehog pathway act to restrain rather that promote tumorigenesis in PDAC [51, 58], highlighting the diverse roles of individual stromal components which subsequently pose challenges to successful drug development.

Focal adhesion kinases (FAKs) are nonreceptor tyrosine kinases that regulate cell signalling within the TME. They are frequently overexpressed in several advanced-stage solid tumours, including PDAC, and are important regulators of the fibrotic and immunosuppressive TME in PDAC when hyperactivated [59]. FAK inhibition has demonstrated antiproliferative therapy *in vitro* [60] and extended response to gemcitabine and nab-paclitaxel in patient-derived xenograft models [59]. These preclinical observations collectively form the basis of clinical trials incorporating FAK inhibition with trametinib (NCT02428270), gemcitabine (NCT02546531), and pembrolizumab (NCT02758587) (Tables 2 and 3). Preclinical models of solid tumours lacking neurofibromatosis type 2 (*NF2*) tumour suppressor gene product, merlin, exhibit exquisite sensitivity to FAK inhibition monotherapy [61], a finding that has also been recapitulated in early phase clinical trials [62]. Merlin deficiency is noted in over 40% of PDAC and is associated with adverse prognostic factors such as higher T stage, increased lymph node disease, and poorly differentiated histology [63]. The integration of predictive biomarkers of response similar to merlin loss could form the rationale for future biomarker-selected trials.

Another TME component that holds potential as a therapeutic target in PDAC is hyaluronan (HA), a glycosaminoglycan found in the stromal matrix. Structurally, HA has multiple anionic repeats that attract cations, resulting in osmotic swelling [64]. Tumours with high levels of HA have higher intratumoural interstitial gel fluid pressure which acts as a barrier to perfusion and reduces drug penetration to cancer cells [65]. When tested in preclinical models, pegylated recombinant human hyaluronidase (PEGPH20) was shown to degrade HA with subsequent normalisation of interstitial fluid pressures and reexpansion of the microvasculature within PDAC tumours [66]. Phase II evaluation has shown that PEGPH20 in combination with gemcitabine and nab-paclitaxel is particularly efficacious in patients with HA-high tumours, defined as extracellular matrix HA staining $\geq 50\%$ of tumour surface at any intensity [67], and a phase III trial is ongoing in this subgroup of patients (NCT02715804). Interestingly, PEGPH20 given concurrently with FOLFIRINOX in an unselected group of patients resulted in detrimental OS outcomes and was associated with significantly higher rates of grade ≥ 3 toxicity [68]. These results highlight the importance of considered selection of individual combination therapy components as the interest in combinatorial strategies intensifies and becomes an increasingly common treatment approach in this tumour type. Efforts to exploit various targets within the desmoplastic stroma such as angiogenesis, hypoxic environment with the TME, and the Wnt-β-catenin pathway as therapeutic targets have also resulted in negative results to date (Table 2).

4.4. Extending the Impact of Immunotherapy in Pancreatic Ductal Adenocarcinoma. Although immune checkpoint inhibition has transformed the disease trajectory of tumour types historically associated with poor outcomes such as melanoma and non-small-cell lung cancer, these results have not been reproduced by both anti-CTLA-4 and anti-PD-L1 agents as monotherapy [69, 70] or in combination in biomarker-unselected populations with PDAC [71]. Immune checkpoint inhibition (ICPi) is only currently indicated in patients with mismatch repair- (MMR-) deficient disease, for which pembrolizumab received tumour-agnostic approval after it was shown to induce responses across multiple tumour types with this phenotype, including PDAC [72, 73]. The prevalence of MMR deficiency in PDAC is estimated between 1 and 2% [74], limiting the benefit of immune checkpoint inhibition to this small proportion of patients.

To that end, various combinatorial strategies aimed at priming the immune response in preparation for ICPi therapy have been explored in PDAC. For instance, chemotherapies facilitate dendritic cell recruitment and activation [75] in addition to tumour-specific antigen release [76], and studies evaluating the combination of anti-CTLA4 and anti-PD1/PD-L1 antibodies alongside chemotherapies are ongoing (Table 3).

Other combinations include agents targeting the desmoplastic stroma, such as PEGPH20 and FAK discussed

Table 2: Selected clinical trials targeting desmoplastic stromal components in metastatic pancreatic ductal adenocarcinoma.

Target	Reference/trial number	Intervention	Phase	Status	*N*	Endpoints	Results
Hedgehog	Catenacci et al. [102] NCT01064622	Vismodegib+gemcitabine	Ib/II	Completed	Phase 1b: 7 Phase II: 106	PFS, OS, ORR, safety	(i) Phase Ib: no safety issues identified (ii) Phase II: no significant difference in PFS, OS, and ORR
	Ko et al. [103] NCT01383538	IPI-926+FOLFIRINOX	Ib	Completed	15	MTD, PFS, ORR, CA19-9 decline, safety	(i) MTD reached at 1 dose level below standard FOLFIRINOX (ii) trAEs: LFT abnormalities, neuropathy, nausea, vomiting, and diarrhoea (iii) ORR 67.7% (iv) CA19-9 decline in 66.7% when elevated at baseline
	Richards et al. [104]* NCT01130142	IPI-926+gemcitabine	Ib	Completed	16	Safety, MTD, OS, PK, PFS, TTP, ORR	(i) MTD IPI-926 160 mg OD (ii) No IPI-926-related serious AEs (ii) Median PFS >7 months
FAK	Aung et al. [105]* NCT02428270	GSK002256098+trametinib	II	Ongoing	16	ORR, safety, PFS	(i) PD as best tumour response in 10/11 and SD in 1/11 evaluable patients (ii) Median PFS 1.6 months (95% CI 1.5-1.8) (iii) Median OS 3.6 months (95% CI 2.7-not reached) (iv) No grade ≥ 3 trAEs
	NCT02546531 [106]	Defactinib+gemcitabine	I	Ongoing	N/A	RP2D, ORR, PFS, OS	N/A
Wnt-β-catenin	Ko et al. [107]* NCT01764477	PRI-724+gemcitabine	Ib	Completed	20	MTD, PK, pharmacodynamics, ORR	(i) MTD not reached (ii) Grade ≥ 3 trAEs: abdominal pain, neutropenia, anaemia, fatigue, and elevated ALP (iii) No DLTs (iv) SD as best response in 8 patients (40%) (v) Median PFS = 2.0 months
	Dotan et al. [108]* NCT02050178	Ipafricept (IPA)+ gemcitabine/nab-paclitaxel	Ib	Completed	26	Safety, MTD, PK	(i) IPA-related AEs in ≥20% of patients: fatigue, nausea, vomiting, anorexia, and pyrexia (ii) IPA-related grade ≥ 3 AEs: AST elevation, nausea, maculopapular rash, vomiting, and WBC decrease (iii) No DLTs or fragility fractures (iv) PR in 9/26 evaluable patients (34.6%) (v) SD in 12/26 patients (46.2%) (vi) Study closed due to termination of the programme by sponsor (a) Median PFS 5.9 months (95% CI 3.4-18.4); median OS 9.7 months (95% CI: 7.0-14) at data cut-off

Table 2: Continued.

Target	Reference/trial number	Intervention	Phase	Status	*N*	Endpoints	Results
PEGPH20	Hingorani et al. [67] NCT01839487	PEGPH20+ gemcitabine/nab-paclitaxel	II	Completed	279	PFS, ORR, OS, safety, PK	(i) Overall median PFS 6.0 months (PAG) vs. 5.3 months (AG) (HR 0.73; 95% CI, 0.53 to 1.00) (ii) In HA-high subgroup: (a) Median PFS 9.2 months (PAG) vs. 5.2 months (AG) (HR 0.51; 95% CI, 0.26 to 1.00; $p = 0.048$) (b) Median OS 11.5 vs. 8.5 months (HR 0.96; 95% CI, 0.57 to 1.61) (c) ORR 46% (PAG) vs. 34% (AG) (iii) No significant difference in TE events with LMWH prophylaxis (iv) Grade 3/4 trAEs: muscle spasms, neutropenia, myalgia
	NCT02715804 [109]	PEGPH20+ gemcitabine/nab-paclitaxel	III	Ongoing	N/A	OS, PFS, ORR, DOR, safety	N/A
	Ramanathan et al. [68] NCT01959139	PEGPH20+ mFOLFIRINOX	II	Completed	114	OS, PFS, ORR, safety	(i) HR 2.07 in favour of control arm at interim futility analysis (ii) Median OS mFOLFIRINOX vs. combination: 14.4 months (95% CI, 10.1 to 15.7 months) vs. 7.7 months (95% CI, 4.6 to 9.3 months) (iii) Grade 3/4 trAEs significantly higher in combination arm (OR 2.7; 95% CI, 1.1 to 7.1)
Hypoxia	Borad et al. [110] NCT01144455	TH302+gemcitabine	II	Completed	214	PFS, OS, ORR, change in CA19-9, PS, and pain score	(i) Median PFS combination vs. gemcitabine: 5.6 vs. 3.6 months (HR 0.61; 95% CI, 0.43 to 0.87; $p = 0.005$) (ii) TH302-related AEs: skin and mucosal toxicities and myelosuppression; unrelated to treatment discontinuation
	Van Custem et al. [111]* NCT01746979	TH302+gemcitabine	III	Completed	693	OS, PFS	(i) Median OS: 8.7 months (TH302+gemcitabine) vs. 7.6 months (gemcitabine+placebo) (HR = 0.84, 95% CI 0.71-1.01, $p = 0.059$) (ii) Median PFS: 5.5 months (TH302+gemcitabine) vs. 3.7 months (gemcitabine+placebo) (HR = 0.77, 95% CI: 0.65-0.92, $p = 0.004$) (iii) Similar safety profile between both arms

Table 2: Continued.

Target	Reference/trial number	Intervention	Phase	Status	*N*	Endpoints	Results
Angiogenesis	Kindler et al. [112]	Bevacizumab+gemcitabine	III	Completed	602	OS, PFS, ORR, safety	(i) No significant difference in median OS and PFS (ii) Grade 3/4 hypertension and proteinuria significantly higher in combination arm
	NCT03316599 [113]	INC280+AV-299+ gemcitabine/nab-paclitaxel	I	Ongoing	N/A	MTD, ORR, PFS, safety	N/A

References marked with * denote results derived from published abstracts. Abbreviations: AE: adverse event; AG: gemcitabine/nab-paclitaxel; CBR: clinical benefit response; CI: confidence interval; DLT: dose-limiting toxicities; DOR: duration of response; HA-high: hyaluronan-high; HR: hazard ratio; MTD: maximum tolerated dose; OR: odds ratio; ORR: overall response rate; OS: overall survival; PAG: PEGPH20 +gemcitabine/nab-paclitaxel; PD: progressive disease; PFS: progression-free survival; PK: pharmacokinetics; PR: partial response; RP2D: recommended phase 2 dose; SD: stable disease; trAEs: treatment-related adverse events; TTP: time to tumour progression.

Table 3: Selected immunotherapy combination studies in metastatic pancreatic ductal adenocarcinoma.

Combination	Reference	Intervention	Phase	Status	*N*	Endpoints	Results
Chemotherapy + ICPi	Renouf et al. [114]* NCT02879318	Durvalumab+tremelimumab+ gemcitabine/nab-paclitaxel vs. gemcitabine/nab-paclitaxel	II	Ongoing	11	OS, PFS, ORR, safety	(i) Data from safety run-in: (a) Grade ≥ 3 AEs: fatigue, myelosuppression, hyponatraemia, hypoalbuminaemia, deranged lipase, colitis (b) PR in 8/11 patients (73%) (c) Median DOR 7.4 months (d) Median PFS 7.9 months (95% CI 3.5-9.2 months)
	O'Reilly et al. [71]* NCT02558894	Durvalumab (D) vs. durvalumab+ tremelimumab (D+T)	II	Completed	65	ORR, PFS, OS, BOR, DCR, PK (durvalumab+ tremelimumab), presence of ADAs	(i) ORR 3.1% (D+T) vs. 0% (D) (ii) Median PFS 1.5 months (both arms) (iii) Median OS 3.1 (D+T) vs. 3.6 months (D) (iv) Grade ≥ 3 trAEs in D+T arm: diarrhoea, fatigue. Treatment discontinued in 3 patients
	Wainberg et al. [115]* NCT02309177	Nivolumab+gemcitabine/nab-paclitaxel	I	Completed	50	DLT, safety, PFS, OS, DCR, ORR, DOR	(i) Hepatitis as DLT, related to gemcitabine/nab-paclitaxel (ii) Grade ≥ 3 teAEs in 48/50 patients (96%) including anaemia, neutropenia, gastrointestinal events, hepatotoxicity, peripheral neuropathy, thrombocytopenia, and colitis (iii) 1 grade 5 teAE (respiratory failure) (iv) Median PFS 5.5 months (v) Median OS 9.9 months
PARPi+ICPi	NCT03851614	Olaparib+durvalumab	II	Ongoing	N/A	Genomic and immune biomarker changes, ORR, CBR, PFS, OS, safety	N/A
Desmoplastic stromal agent+ICPi	NCT02758587	Defactinib+pembrolizumab	I/II	Ongoing	N/A	Safety, ORR, DOR, PFS, change in FAK Y397 phosphorylation and immune cell infiltrate	N/A
	Wang-Gillam et al. [116]* NCT02546531	Defactinib+pembrolizumab+ gemcitabine	I/II	Ongoing	17	RP2D, safety, ORR, PFS, OS	(i) Results from dose escalation cohort: (a) No DLTs (b) Level 5 dose as RP2D (defactinib 400 mg, pembrolizumab 200 mg, gemcitabine 1,000 mg/m^2) (c) Common trAEs: fatigue, nausea, myalgia, vomiting, anorexia, pruritus, fever (d) SD in 7/13 evaluable patients (54%)

Table 3: Continued.

Combination	Reference	Intervention	Phase	Status	*N*	Endpoints	Results
	Desai et al. [117]* NCT03193190	Atezolizumab+PEGPH20	Ib/II	Ongoing	N/A	ORR, safety, PFS, OS, DOR, PKs	N/A
	NCT03481920	Avelumab+PEGPH20	I	Ongoing	N/A	ORR, safety, OS, PFS, change in CA19-9	N/A
	Hidalgo et al. [79]* NCT02826486	Pembrolizumab+BL-8040	II	Ongoing	37	ORR, OS, PFS, DOR	(i) 29 evaluable patients (ii) PR in 1 patient (3.5%); SD in 10 patients (34.5%) (iii) Overall median OS 3.4 months; 7.5 months in second-line subgroup
Bruton's tyrosine kinase+ICPi	Overman et al. [118]* NCT02362048	Pembrolizumab+ acalabrutinib vs. acalabrutinib	II	Ongoing	58	Safety	(i) No DLTs (ii) Grade ≥ 3 AEs in combination arm: dehydration, anaemia, hypotension (iii) Grade ≥ 3 AEs in monotherapy arm: anaemia, abdominal pain (iv) SD in 4/21 evaluable patients in monotherapy arm (v) PR in 3/23 and SD in 5/23 evaluable patients in combination arm
Myeloid inhibitor+ICPi	Wainberg et al. [119]* NCT02526017	Cabiralizumab+nivolumab	I	Ongoing	205	Safety, ORR, RD, PK, immunogenicity, biomarker analysis	(i) Grade ≥ 3 trAEs due to cabiralizumab in 43% of patients (ii) Treatment discontinuation due to AEs in 13% (iii) Most common grade ≥ 3 AEs: elevated CPK and AST. Reversible without sequalae (iv) 31 evaluable pretreated patients (a) 3 PR; 1 SD (b) ORR 10%
	NCT02777710	Pexidartinib+durvalumab	I	Ongoing	N/A	DLT, ORR, DOR, PFS, safety, PK	N/A
CXCR2 inhibitor+ICPi	NCT02583477	AZD5069+durvalumab	Ib/II	Completed	N/A	DLT, ORR, DOR, DCR, PFS, presence of ADAs, PK, safety	No results available
Oncolytic virus+ICPi	Mahalingam et al. [99]* NCT02620423	Pelareorep+pembrolizumab+ chemotherapy (5-FU/leucovorin, gemcitabine, or irinotecan)	I	Completed	11	Safety, DLTs, ORR, PFS, immune markers	(i) Grade ≥ 3 teAEs in 8 patients (ii) PR in 1/5 and SD in 2/5 evaluable patients (iii) On-treatment biopsy showed reovirus infection and immune infiltrates

Table 3: Continued.

Combination	Reference	Intervention	Phase	Status	*N*	Endpoints	Results
Vaccines+ICPi	Le et al. [120]* NCT02243371	GVAX/Cy+CRS-207±nivolumab	II	Ongoing	N/A	OS, safety, PFS, TTP, ORR, tumour marker kinetics	N/A
	NCT03190265	CRS-207 (±GVAX/Cy)+ nivolumab+ipilimumab	II	Ongoing	N/A	ORR, safety, OS, PFS, DOR, TTP, tumour marker kinetics	N/A

References marked with * denote results derived from published abstracts. Abbreviations: ADA: antidrug antibodies; AE: adverse event; AST: aspartate transaminase; BOR: best objective response; CBR: clinical benefit rate; CI: confidence interval; CPK: creatinine phosphokinase; DLT: dose-limiting toxicities; DCR: disease control rate; DOR: duration of response; ICPi: immune checkpoint inhibitor; ORR: overall response rate; OS: overall survival; PARPi: polyADP-ribose polymerase inhibitor; PK: pharmacokinetics; PR: partial response; PFS: progression-free survival; RP2D: recommended phase 2 dose; SD: stable disease; teAEs: treatment-emergent adverse events; trAEs: treatment-related adverse events; TTP: time to tumour progression.

previously. The C-X-C motif chemokine receptor type 4 (CXCR4)/stromal-derived factor-1 (CXCL12) is another candidate for combination with ICPi. Cancer stem cells with strong CXCR4 expression on the invasive front have been found to be a driver of metastatic behaviour and are prerequisite for the development of liver metastases [77]. CXCL12 secreted by CAFs has been shown to mediate immunosuppression, and its inhibition with AMD3100 induced rapid T-cell accumulation and synergistic activity with an anti-PD-L1 therapy in a PDAC mouse model [78]. Clinically, a phase II trial is underway with early results reporting that BL-8040 with pembrolizumab achieved a median OS of 7.5 months in patients receiving this as second-line therapy [79] (NCT02826486, Table 3).

As myeloid cells such as TAMs and MDSCs are important mediators of immune evasion, these have been identified as potential therapeutic targets in PDAC in the hope of overcoming its innate immunologically resistant phenotype. For example, C-C chemokine receptor type 2 (CCR2) and colony-stimulating factor-1 receptor (CSF1R) are involved in the recruitment and differentiation of TAMs within the PDAC TME. CSF1R or CCR2 inhibition has been shown to reduce the numbers of pancreatic tumour initiating cells and improve chemotherapy efficacy [80], and CSF1R inhibition upregulates PD-L1 and CTLA-4 checkpoint molecules in response to ICPi [81]. CSF1R inhibitors are currently being evaluated in combination with immunotherapy and chemotherapy (NCT02526017, NCT02777710) (Table 3).

Newer immune targets such as the CXCR2 axis are also being evaluated with ICPi. The primary role of the CXCR2 axis is to regulate neutrophil migration to the site of inflammation [82], and CXCR2 signalling at the tumour border is a poor prognostic indicator in human PDAC [83]. CXCR2 inhibition has been found to augment T-cell entry and increase sensitivity to ICPi therapy when used in combination in a mouse model [84], providing a rationale to pursue clinical testing (NCT02583477) (Table 3).

Another target thought to have immunomodulatory capabilities in PDAC is Bruton's tyrosine kinase (BTK), a Tec family nonreceptor tyrosine kinase that is required for B-cell receptor signalling. The BTK inhibitor, ibrutinib, has also been shown to exert antifibrotic effects on the desmoplastic stroma through inhibition of mast cell activity [85], as well as amplify cytotoxic T-cell activity with subsequent enhancement of responsiveness to chemotherapy in PDAC murine models [86]. Despite this encouraging preclinical data, a phase III trial randomising patients between gemcitabine/nab-paclitaxel in combination with ibrutinib or gemcitabine/nab-paclitaxel with placebo as first-line treatment of metastatic PDAC failed to meet its primary endpoints of PFS and OS (NCT02436668) [87] . Acalabrutinib is currently being assessed in combination with pembrolizumab at the phase 2 level, with preliminary results indicating an acceptable safety profile and encouraging antitumour activity in a pretreated PDAC patient population (NCT0236048) (Table 3).

Various types of vaccine therapies are also in active development. Personalised peptides designed to prevent progressive tolerance to cancer-related antigens given concurrently with gemcitabine have also shown promise [88] but require further investigation to establish its efficacy and development into a deliverable treatment modality. Another vaccine-based approach is whole-cell tumour vaccines, which enable multiple antigens to be targeted simultaneously to elicit a more robust T-cell response. Amongst the most studied is GVAX, which is genetically engineered to secrete GM-CSF, a cytokine that mobilises leucocytes to the TME and generates large immunoglobulin (Ig) G and IGM responses [89]. GVAX is thought to be an ideal primer for checkpoint inhibition, as it increases immunogenicity by inducing T-cell infiltration and formation of tertiary lymphoid aggregates [90], as well as upregulates the PD-1/PD-L1 pathway in PDAC patients [91]. Based on preclinical data suggesting that sequential administration of two vaccines to firstly "prime" the immune system then "boost" the immune response achieves synergistic enhancement of T-cell induction, the combination of GVAX and CRS-207, a live-attenuated *Listeria monocytogenes* vaccine expressing mesothelin, was evaluated at the phase II level. These results demonstrated extended survival of PDAC patients with minimal toxicity with this combination [92]. However, administration of CRS-207/GVAX or CRS-207 alone did not amount to a survival benefit over single-agent chemotherapy in a pretreated metastatic PDAC cohort (NCT02004262) [93]. Ongoing evaluation of its clinical potential in combination with ICPi therapy is currently being investigated (Table 3).

Additionally, the modulation of the gut microbiome in PDAC is an emerging field. The cancerous pancreas has been found to harbour distinct and more abundant gut microbiota in comparison to normal pancreatic tissue in both mice and humans [94]. Bacterial ablation using antibiotic therapy has been shown to be protective against oncogenesis, reversed intratumoural immune tolerance, and increased susceptibility to immune checkpoint blockade [94]. On this basis, the effects of metronidazole and ciprofloxacin given with pembrolizumab on immune activation in pancreatic cancer tissue in patients with surgically resectable PDAC will be investigated in an upcoming pilot study (NCT03891979).

A further immunotherapeutic approach is the application of tumour-oncolytic viruses (TOVs) to selectively infect, replicate in, and lyse tumour cells to unleash virions that can subsequently infect adjacent tumour cells, a process that potentiates an inflammatory response through immunogenic cell death [95]. Cell lysis also releases pathogen- and damage-associated molecular pattern molecules which activate the innate immune response, as well as viral- and tumour-associated antigens which stimulate the adaptive immune response [95]. Adenoviruses have been most extensively evaluated thus far. Given alone, ONYX-015, an adenovirus that selectively replicates and lyses in cells with p53 abnormalities, failed to induce objective responses in a phase I dose escalation study following intratumoural injection or exhibit evidence of viral replication [96] but resulted 2 partial responses and disease control in 8 patients when administered alongside gemcitabine in a cohort of 21 patients [97]. Phase II data investigating pelareorep, a reovirus, in combination with gemcitabine induced a partial response in one patient ($n = 29$) and PD-L1 upregulation following treatment

[98], suggesting an immunomodulatory effect in PDAC and providing a rationale for pursuing combination therapy with immune checkpoint blockade. Pelareorep in combination with pembrolizumab and single-agent chemotherapy has since resulted in disease control in three of five evaluable patients in relapsed metastatic PDAC patients with a manageable safety profile (NCT02620423) (Table 3) [99].

Adoptive T-cell transfer techniques are also being investigated in the context of PDAC. Chimeric antigen receptor T-cell (CAR-T) therapy, which uses T-cells engineered to recognise a specific tumour antigen, appears to be the most effective method available. CAR-T therapy targeted to MUC1, a neoantigen expression in a variety of cancers including PDAC, has been shown to demonstrate target-specific cytotoxicity and improved survival in xenograft models of PDAC [100], with a phase I/II clinical study currently recruiting (NCT02587689). Other targets under clinical evaluation are mesothelin and carcinoembryonic antigen (NCT02465983, NCT02349724), the former having preliminary data suggesting activity in patients with PDAC [101].

5. Conclusion

The systemic treatment of advanced PDAC remains challenging. Although clinical trials have led to incremental gains in a survival benefit in recent years, significant improvements in patient outcomes have remained elusive for decades. Developing methods to improve the resolution of patient selection is crucial to exploit the benefit derived from existing therapies while we wait for newer therapies that target stromal elements or the TME to be fully evaluated and approved. Simultaneously, further translational and clinical work will be essential to advance our understanding of novel therapeutic targets and methods of manipulating the immune microenvironment to ensure the successful translation of rational precision therapeutics into the clinic. Finally, combinatorial strategies supported by sound biological rationale hold potential to fulfil the persistent unmet need posed by this aggressive malignancy.

Conflicts of Interest

Caroline Fong and Emma Burke have no conflicting interests to declare, commercial, intellectual, or otherwise.

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