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Contents

Gastric and Small Bowel Tumors

L. Mark Knab and Anthony Yang

Abstract

The incidence of gastric adenocarcinoma has decreased in the United States over the past 70 years although it continues to have a poor prognosis. While radical resection was initially the primary treatment for adenocarcinoma of the stomach, systemic chemotherapy and radiation have been shown to play a role in prolonging survival in most patient populations. This chapter explores the evidence that guides treatment for gastric cancer today. It also discusses the treatment for gastrointestinal stromal tumors (GIST), and small bowel tumors. In addition to systemic therapies, this chapter explores the surgical management of gastric and small bowel tumors including the extent of the gastric lymph node dissection.

Keywords

Gastric adenocarcinoma · Gastrointestinal stromal tumors (GIST) · Small bowel tumors · Lymph node dissection

1 Gastric Adenocarcinoma

The incidence of gastric cancer in the United States (US) has decreased substantially over the past several decades. While it was once a leading cause of cancer-related death in the United States, it now ranks 13th among major causes. Gastric cancer remains the second leading cause of cancer-related death worldwide and its incidence varies dramatically with geography. It occurs with the highest

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rates in East Asia and the lowest rates in North America. Part of this discrepancy is believed to be due to the decreased use of salted, pickled, and smoked foods and increased use of refrigeration in developed countries.

The decrease in gastric cancer rates in the US unfortunately has not correlated with an improvement in 5-year survival. The 5-year survival rate for all races with gastric cancer from the period of 2001–2007 was 27 % [1]. Unlike countries with screening policies in place for gastric cancer, most of the patients that present in the US are in advanced stage. A study from the National Cancer Data Base (NCDB) demonstrated that 55 % of patients with gastric cancer presented with locally advanced or metastatic disease [2]. The 5-year survival rates have been shown to be directly related to stage upon presentation. The survival rate for stage IA disease was 78 %, for stage IB it was 58 %, for stage II, 34 %, for stage IIIA, 20 %, for stage IIIB, 8 %, and for stage IV it was 7 %.

1.1 Classification

Gastric adenocarcinoma can be divided into two histologic subtypes: intestinal (or glandular) and diffuse [3]. The intestinal subtype has been associated with atrophic gastritis and diets high in nitrates [4]. It is also associated with elderly patients and generally arises in the distal stomach. The diffuse subtype most commonly occurs in the cardia of the stomach and in younger patients. It has no identifiable precursor, and cancer cells infiltrate the tissue diffusely. This results in a thickened stomach without a discrete mass or ulceration which is typically seen in the intestinal subtype.

1.2 Risk Factors

Multiple epidemiologic studies worldwide have demonstrated a strong association between gastric cancer and H. pylori infection [5]. Serologic studies have shown that individuals who are infected with H. pylori are three to six times more likely to develop gastric cancer compared with those who do not have H. pylori [6]. The exact mechanism is unclear given that a large proportion of the entire world's population is thought to be infected with H. pylori, yet only a small fraction will eventually develop gastric adenocarcinoma. H. pylori infection results in a chronic state of inflammation which can potentiate environmental and/or genetic factors that can result in cancer [7]. There is also a genetic predisposition for gastric cancer, and individuals with first-degree relatives that have gastric cancer have a higher risk of developing the disease [8].

It is thought that certain environmental factors exert a teratogenic effect on the stomach and increase the risk of gastric cancer. Gastric specimens from operations and autopsies have been associated with areas of atrophic gastritis and intestinal metaplasia. It is thought that environmental factors such as nitrates and nitrose compounds found in smoked, pickled, and salted foods potentiate the effect of chronic gastritis, leading to metaplasia and eventually carcinoma [9].

1.3 Clinical Evaluation

In the United States, due to the low incidence of gastric adenocarcinoma, it is not cost effective to use screening programs. Because of this, the majority of cases are diagnosed after they are locally advanced or metastatic. Early stage gastric cancer generally does not result in symptoms and it is often only after the cancer has progressed that patients will present with symptoms such as fatigue, weight loss, early satiety, vomiting, and hematemesis. Physical findings such as jaundice, ascites, or a palpable mass are generally indicative of incurable disease. Other physical findings consistent with advanced disease include periumbilical metastases (Sister Mary Joseph nodule), supraclavicular lymphadenopathy (Virchow node), and a palpable mass in the rectovesical or rectouterine space from dropped metastases (Blumer shelf).

The primary diagnostic tool is now upper gastrointestinal endoscopy which allows for direct visualization of the stomach and biopsies to confirm the diagnosis. It is recommended that at least four biopsies be taken of the area in suspicion to prevent false negative results [10]. Imaging studies should be obtained for staging purposes, including a CT chest, abdomen, and pelvis. The overall accuracy of CT in staging tumors is about 70 % for advanced tumors and 44 % for early lesions [11]. The sensitivity of CT for detecting N1 and N2 disease is 24–43 %, and the specificity approaches 100 % [12]. While CT needs to be done for metastatic workup, locoregional staging is ideally performed with endoscopic ultrasound (EUS). EUS is able to assess the depth of tumor invasion through the gastric wall (T stage) as well as evaluate regional nodes (N stage). The overall accuracy of EUS for evaluating the extent of tumor infiltration ranges from 67 to 92 % [13].

1.4 Staging

There are two main systems in place for staging gastric cancer. There is the Japanese classification which is an elaborate staging scheme based on the anatomic location of the tumor and including specific lymph node stations. The second staging system developed by the American Joint Committee on Cancer (AJCC) and the International Union Against Cancer (UICC) is widely used in Western countries, and uses the tumor-node-metastasis (TNM) system commonly used in other cancer types. This system stages tumors according to depth of tumor invasion into the gastric wall (Figs. 1 and 2), lymph node involvement, and the presence of distant metastases (Tables 1 and 2).

1.5 Surgical Management

Management of gastric adenocarcinoma has shifted over the years from surgery alone to multimodality therapy including surgery in combination with chemotherapy or chemoradiotherapy. NCCN guidelines recommend surgery alone or

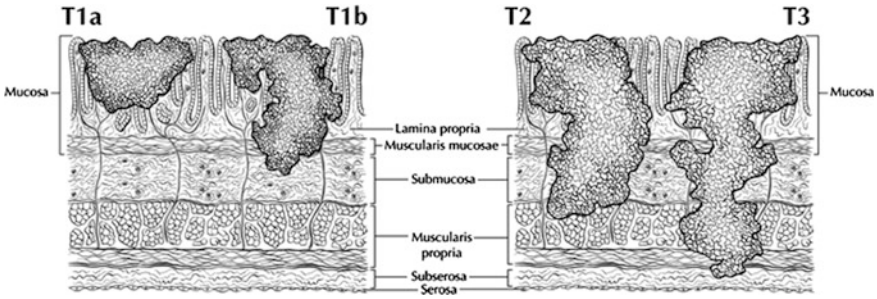

Fig. 1 American Joint Committee on Cancer staging T1–T3 diagram. T1a is defined as tumor that invades the lamina propria and T1b invades the submucosa. T2 is defined as a tumor that invades the muscularis propria, and T3 extends into the subserosal tissue

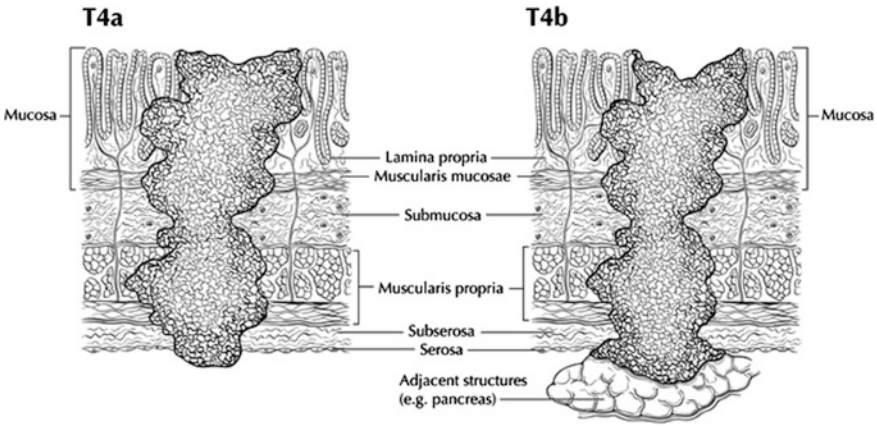

Fig. 2 American Joint Committee on Cancer staging T4 diagram. T4a is defined as a tumor that invades the serosa without invasion of adjacent structures, while T4b is a tumor that invades adjacent structures

endoscopic mucosal resection (EMR) for Tis or T1a lesions. Surgery alone is recommended for T1b tumors, and for T2 tumors and greater, surgery with chemotherapy or chemoradiotherapy is recommended.

Surgical resection remains the mainstay of potentially curative therapy for gastric adenocarcinoma. Surgical cure requires excision of the tumor with clear gross and microscopic margins. An R0, or margin negative resection, requires wide local excision of the primary tumor with en bloc resection of involved adjacent organs, lymphatics, and blood vessels.

The type of gastric resection for gastric adenocarcinoma will vary depending on the location of the tumor. For tumors originating from the distal esophagus, an esophagectomy is the procedure of choice. For tumors of the cardia, a total gastrectomy is preferred to an esophagogastrectomy as long as an R0 resection can be

Table 1 American Joint Committee on Cancer TNM Clinical Classification of Gastric Carcinoma, 7th Edition

TX	Primary tumor cannot be assessed
T0	No evidence of primary tumor
Tis	Carcinoma in situ: intraepithelial tumor without invasion of the lamina propria
T1	Tumor invades lamina propria, muscularis mucosae, or submucosa
Tla	Tumor invades lamina propria or muscularis mucosae
T1b	Tumor invades submucosa
T2	Tumor invades muscularis propria
T3	Tumor penetrates subserosal connective tissue without invasion of visceral peritoneum or adjacent structures. T3 tumors also include those extending into the gastrocolic or gastrohepatic ligaments, or into the greater or lesser omentum, without perforation of the visceral peritoneum covering these structures
T4	Tumor invades serosa (visceral peritoneum) or adjacent structures
T4a	Tumor invades serosa (visceral peritoneum)
T4b	Tumor invades adjacent structures such as spleen, transverse colon, liver, diaphragm, pancreas, abdominal wall, adrenal gland, kidney, small intestine, and retroperitoneum

Table 2 American Joint Committee on Cancer Staging of Gastric Carcinoma, 7th Edition

Stage 0	Tis	N0	M0
Stage IA	Tl	N0	M0
Stage IB	T2	N0	M0
	Tl	N1	M0
Stage IIA	T3	N0	M0
	T2	N1	M0
	Tl	N2	M0
Stage IIB	T4a	N0	M0
	T3	N1	M0
	T2	N2	M0
	Tl	N3	M0
Stage IIIA	T4a	N1	M0
	T3	N2	M0
	T2	N3	M0
Stage IIIB	T4b	N0 or N1	M0
	T4a	N2	M0
	T3	N3	M0
Stage IIIC	T4b	N2 or N3	M0
	T4a	N3	M0
Stage IV	Any T	Any N	M1

performed. Lesions in the proximal stomach and fundus can be resected with a total gastrectomy and a Roux-en-Y esophagojejunostomy. For lesions in the antrum of the stomach, a subtotal gastrectomy with a Billroth II reconstruction can be performed.

1.6 Lymph Node Dissection

Extent of lymph node dissection has been an area of controversy in gastric adenocarcinoma for many years. Some surgeons believe that cancer metastasizes through a stepwise progression, and an extensive lymphadenectomy is necessary to improve survival and/or cure the patient. Other physicians argue that extensive lymphadenectomies only add perioperative morbidity and mortality and do not improve survival. Asian countries have been performing extended lymphadenectomies routinely for many years with promising survival data, although Western countries have not been able to reproduce those results. The debate is all the more relevant in the United States where the majority of patients present with advanced disease [14].

Much of the controversy surrounding lymphadenectomies started in the 1980s when Japanese studies reported superior survival rates matched stage for stage, compared to the United States. This was theorized to be secondary to the more extensive lymphadenectomy performed in Japan compared to the United States [15]. This was the impetus for future randomized control trials in gastric cancer.

A United Kingdom study randomized 400 patients to either a D1 or a D2 lymph node dissection (see Fig. 3 for lymph node stations) [16]. Those patients with tumors in the upper or middle third of the stomach underwent a distal pancreaticosplenectomy to obtain retropancreatic and splenic hilar nodes. While the 5-year survival rates were not statistically significant between the two groups, on multivariate analyses it was noted that those patients in the D2 group that did not undergo the distal pancreaticosplenectomy had an increased survival compared with the D1 group. A trial in the Netherlands randomized 380 gastric cancer patients to a D1 lymphadenectomy and 331 patients to a D2 lymphadenectomy [17]. Similar to

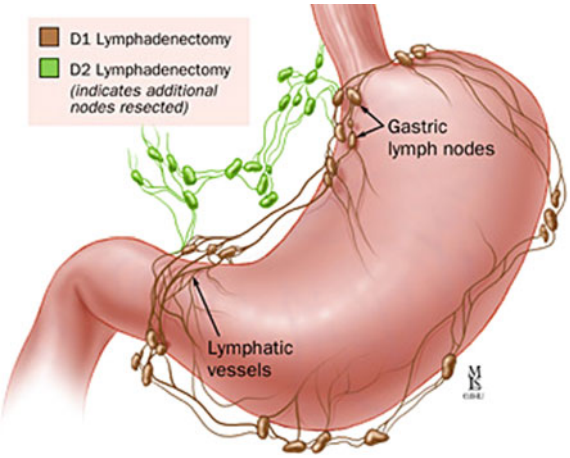

Fig. 3 Gastric Lymph Node Stations. A D1 lymphadenectomy includes lymph nodes along the greater and lesser curve of the stomach. A D2 lymphadenectomy includes D1 lymph nodes in addition to lymph nodes along the common hepatic, splenic, left gastric, and left hepatoduodenal arteries

the United Kingdom study, there was not a significant difference in survival between the two groups, even when followed out to 11 years [18]. There was a significant increase in postoperative complications in the D2 group compared with the D1 group (43 % vs. 25 %, respectively) as well as mortality (10 % vs. 4 %, respectively). The data from these two studies suggest that a pancreaticosplenectomy performed to harvest lymph nodes seems to only add morbidity and mortality while not improving survival.

One concern raised about the prior two studies was the variation in surgical technique and lack of standardization of surgeon experience. A Taiwanese study accounted for this by performing the study at a single institution with three surgeons, each of whom had completed at least 25 D3 lymph node dissections prior to the study. Patients with gastric cancer were randomized to a D1 lymph node dissection (defined as resection of perigastric lymph nodes along the lesser and greater curves of the stomach) or a D3 lymph node dissection (defined as resection of additional lymph nodes surrounding the splenic, common hepatic, left gastric arteries, nodes in the hepatoduodenal ligament, and retropancreatic lymph nodes). There was an overall 5-year survival benefit with the D3 group of 60 % compared with the D1 group of 54 % [19]. A Japanese study evaluated a more aggressive lymph node dissection and randomized patients to a D2 dissection or a para-aortic lymph node dissection (PAND). There was no significant difference in 5-year survival between the two groups with a trend toward an increase in complications in the PAND group [20].

Multiple studies have shown that the number of positive lymph nodes is a significant predictor of survival [21, 22]. Current AJCC guidelines stipulate that at least 15 lymph nodes are needed for pathologic examination to obtain adequate staging [23].

1.7 Endoscopic Mucosal Resection

Early gastric cancer (EGC) is defined as cancer that is confined to the mucosa or submucosa. Traditionally surgical resection was the only curative option available for patients with EGC although studies demonstrated that only 3 % of patients would have nodal metastases [24]. The advent of endoscopic mucosal resection (EMR) about 25 years ago has changed the treatment options for EGC. Several studies have demonstrated high survival and cure rates. A Japanese study included 131 patients with EGC (defined in this study as a tumor less than 2 cm and no ulcerative change) who underwent EMR [25]. The overall 5-year and 10-year survival rates were 84 and 64 %, respectively. A German study included 39 patients with EGC (defined here as tumor less than 3 cm, no invasion of lymph or vessels) and showed a 97 % remission with one treatment, and recurrent lesions in 29 % which were all successfully treated with a second treatment [26].

According to the NCCN guidelines, EMR can be considered for tumors less than 2 cm in diameter, without lymphovascular invasion, with clear deep and lateral margins, and without invasion beyond the submucosa [27]. It is important to note that for EMR to be successful, routine follow-up and surveillance are critical.

1.8 Neoadjuvant Therapy

Several large randomized trials have shifted the standard of care for gastric cancer from surgery alone to surgical resection in addition to perioperative chemotherapy or chemoradiation therapy. The advantages cited for neoadjuvant therapy include the possibility of "downstaging" a tumor in those patients who present with borderline or locally advanced tumors. Neoadjuvant therapy also has the potential to spare patients with aggressive tumor biology the morbidity of a large operation if their disease advances during a trial of neoadjuvant therapy. The NCCN guidelines recommend neoadjuvant therapy for T2 tumors and larger.

The guidelines for neoadjuvant therapy are based on several large trails. The largest trial is the United Kingdom Medical Research Council Adjuvant Gastric Infusion Chemotherapy MAGIC trial which randomized 250 patients to perioperative chemotherapy and surgery, and 253 patients to surgery alone [28]. The patients included in the trial consisted of those with resectable adenocarcinoma of the stomach (74 %), gastroesophageal junction (GEJ) (11 %), and lower esophagus (15 %). The chemotherapy included three cycles of cisplatin, epirubicin, and 5-FU given pre- and postoperatively. Both treatment groups had similar complication and 30-day mortality rates. The perioperative chemotherapy group demonstrated a significantly improved 5-year survival rate compared to the surgery alone group (36 % vs. 23 %, respectively). Limitations of the study include the mix of cancer locations as well as the fact that only 42 % of the chemotherapy group were well enough to receive the postoperative chemotherapy treatment.

A similar multicenter French trial (FNCLCC/FFCD) included patients with resectable distal esophagus, GEJ, and gastric adenocarcinoma [29]. Patients were randomized to perioperative chemotherapy (n = 113) or surgery alone (n = 111). The chemotherapy regimen used was cisplatin and 5-FU. Two to three cycles were given preoperatively, and 3–4 cycles were given postoperatively. The 5-year survival of the perioperative chemotherapy group was significantly higher than the surgery alone group (38 % vs. 24 %, respectively). In addition, the perioperative chemotherapy group was significantly more likely to achieve an R0 resection compared to the surgery alone group (84 % vs. 73 %, respectively). When comparing this trial to the MAGIC trial, it is important to note the difference in cancer locations. The MAGIC trial had a higher percentage of patients with gastric cancer compared to this trial (74 % vs. 25 %, respectively). Similar to the MAGIC trial, of those patients in this study that received at least one cycle of preoperative chemotherapy, only one half received any postoperative chemotherapy.

1.9 Adjuvant Therapy

Given the fact that over 80 % of patients that die from gastric cancer have local recurrence at some point, the utility of radiation therapy has been studied [30]. The Adjuvant Chemoradiotherapy in Stomach Tumors (ARTIST) trial compared adjuvant chemoradiation with adjuvant chemotherapy alone [31]. The trial included 458

patients with completely resected gastric cancer (including a D2 lymph node dissection) randomly assigned to six courses of capecitabine and cisplatin or two courses of capecitabine and cisplatin followed by 45 Gy of radiotherapy with concurrent capecitabine, followed by two courses of capecitabine and cisplatin. While the addition of radiotherapy did not reduce local recurrence rates, it was found to improve disease-free survival in those patients with positive lymph nodes. Those with nodal disease that received chemoradiotherapy had a 3-year disease-free survival of 76 % compared to 72 % for those that received chemotherapy alone.

The largest trial thus far is the INT 0116 trial which has the added advantage of using contemporary radiotherapy techniques. This trial randomized 556 patients with potentially curative esophagogastric junction or gastric cancer to observation alone versus adjuvant chemoradiotherapy [32]. The chemoradiotherapy included one cycle of 5-FU and leucovorin for 5 days with subsequent radiation therapy with 5-FU and leucovorin, followed by two more cycles of chemotherapy one month after the radiotherapy. The majority of the patients had T3 or T4 tumors and 85 % of the patients had nodal metastases. The 3-year overall survival rate was significantly improved in the combined modality group compared to the observation group (50 % vs. 41 %, respectively). Median survival was also significantly improved in the chemoradiotherapy group compared to the observation group (36 months vs. 27 months, respectively). The main criticism of this study is that only 10 % of the patients underwent a D2 lymphadenectomy. The majority, 54 %, underwent a D0 resection and 38 % had a D1 resection. This most likely resulted in falsely elevated surgical failure rates as well as falsely elevated adjuvant benefit.

The Japanese S-1 trial demonstrated the benefit of adjuvant chemotherapy for patients with stage II or III gastric cancer who had undergone potentially curative surgery, including a D2 lymphadenectomy [33]. S-1 is an oral fluoropyrimidine that is made of three agents: ftorafur (tegafur), oteracil, and gimeracil. In this Japanese ACTS-GC trial, 1059 patients were randomized to surgery alone or S-1 therapy daily for 4 weeks repeated every 6 weeks for one year. The overall 5-year survival was significantly improved in the S-1 group compared to the surgery alone group (72 % vs. 61 %, respectively). One year of postoperative S-1 treatment is now the standard of care for East Asian patients with gastric cancer. Unfortunately, the efficacy of S-1 has not been demonstrated in non-Japanese patient populations.

As a result of the above randomized trials, it is clear that surgery alone is insufficient treatment for local advanced gastric cancer. Consensus groups such as the NCCN recommend perioperative chemotherapy or postoperative chemoradiotherapy for locally advanced gastric adenocarcinoma, and preoperative chemoradiotherapy for localized GEJ adenocarcinoma.

2 Gastrointestinal Stromal Tumor

Gastrointestinal stromal tumors (GIST) are the most common sarcoma of the GI tract, although overall relatively rare [34]. The annual incidence in the United States is about 4000–6000 new cases per year. The most common site in the GI tract for

GISTs is the stomach (60–70 %), followed by the small intestine (25 %), the rectum (5 %), and the esophagus (2 %) [35]. GISTs were initially classified as a leiomyosarcoma due to their appearance on light microscopy although with the advent of immunohistochemistry (IHC), they were found to have both smooth muscle and neural cell elements. The cell of origin of GISTs is thought to be an intestinal pacemaker cell, or a precursor of the interstitial cells of Cajal [36]. Diagnosis of a GIST tumor is confirmed with IHC staining for the CD117 antigen which is part of the KIT transmembrane tyrosine kinase receptor. More than 95 % of GISTs in adults overexpress KIT and about two-thirds also express CD34 [37]. The histology of GISTs usually fall into three categories: spindle cell type, epithelioid type, or mixed type.

2.1 Clinical Evaluation

The median age at diagnosis is 63 years [37]. The tumors can grow to be very large and patients may present with mass-related symptoms such as early satiety, bloating, and abdominal pain. One study demonstrated three major presentations: GI bleeding (44 %), abdominal mass (47 %), and abdominal pain (21 %) [38]. Despite the above symptoms, often these tumors are discovered incidentally on imaging or endoscopy.

Once a GIST is suspected, a CT of the chest, abdomen, and pelvis should be performed with contrast. Occasionally magnetic resonance imaging (MRI) is useful depending on the anatomic location of the tumor. Imaging is used to determine the extent of the tumor as well as evaluate the presence of distant metastases. Occasionally, endoscopy can aid in surgical planning although it is rarely diagnostic because the tumors are submucosal and usually do not involve the mucosa [27]. Surgical consultation is recommended to determine if the tumor is resectable with an acceptable morbidity. Generally biopsy of the tumor should be avoided to prevent tumor rupture or intraabdominal dissemination. Biopsy can be considered if tissue is needed to confirm a diagnosis prior to neoadjuvant therapy for those unresectable or metastatic lesions.

2.2 Staging

The biologic behavior of GISTs and prognosis is best predicted by the size and mitotic rate of the tumor. GISTs are notoriously difficult to predict in terms of the biologic behavior of individual cases. Low-risk gastric GISTs are those tumors less than 2 cm with less than 5 mitoses per 50 high-powered fields (HPF) (metastases rate or tumor-related mortality is 0 %). High-risk tumors are those greater than 10 cm in diameter and have greater than 10 mitoses per HPF (metastases rate or tumor-related mortality is 86 %) [27].

2.3 Surgical Management

The surgical management of GISTs involves tumor resection with grossly negative margins, without violating the capsule. Formal gastric resections are rarely necessary unless the tumor is near the pylorus or GEJ. Extended lymph node dissections are also not necessary because GISTs generally do not spread through the lymphatics.

2.4 Nonsurgical Management

If a GIST is locally advanced and not technically feasible to resect, or if there are distant metastases, neoadjuvant therapy with imatinib mesylate can be initiated. Imatinib is a small molecule tyrosine kinase inhibitor, and was initially used for the treatment of chronic myelocytic leukemia, although it has been approved for the treatment of KIT-positive GISTs since 2002. Most GISTs express the KIT tyrosine kinase receptor which can be inhibited by imatinib. Patients with borderline resectable disease should be treated with imatinib and reimaged periodically to determine if surgical resection is technically possible. Even those patients with distant metastases can benefit from surgical resection of the primary tumor if they exhibit some response to imatinib.

The benefit of imatinib compared to surgery alone was demonstrated in the American College of Surgeons Oncology Group (ACOSOG) Z9001 phase III, double-blinded, multicenter trial [39]. It randomized 713 patients with completely resected gastrointestinal GISTs (at least 3 cm in diameter and KIT positive) to one year of adjuvant imatinib vs placebo. The trial was stopped early due to evidence that imatinib was superior to placebo. The recurrence-free survival at one year was 98 % for the imatinib group versus 83 % in the placebo group. Subsequent analysis showed that imatinib was more effective in those patients with high-risk GISTs.

The Scandinavian Sarcoma Group (SSG) XVIII trial evaluated a longer duration of imatinib treatment in 400 patients with high-risk GIST [40]. The trial compared 36 months to 12 months in those patients with at least one of the following: tumor diameter greater than 10 cm, mitotic count greater than 10 per 50 HPF, tumor diameter great than 5 cm with a mitotic rate greater than 5 per HPF, or tumor rupture. About half of the patients had gastric GISTs. The prolonged treatment group demonstrated significantly improved 5-year recurrence-free survival (66 % vs. 48 %) as well as overall survival (92 % vs. 82 %). Due to these results, the standard treatment for high-risk GIST includes 36 months of imatinib. Within 6–12 months of discontinuing the imatinib, recurrence rates were similar between the two treatment groups which raises the possibility that treatment longer than 36 months may be beneficial.

3 Small Bowel Tumors

Malignant tumors of the small bowel account for less than 5 % of all GI tract malignancies, and are very rare. The incidence of small bowel cancer in the United States is about 6000 annually [41]. Traditionally adenocarcinoma of the small

bowel was reported to be the most common tumor, although carcinoid tumors, lymphomas, sarcomas, and GISTs are increasing in frequency [42]. The majority of small bowel adenocarcinomas occur in the duodenum (46–55 %) and about 13 % occur in the ileum [42–46].

3.1 Clinical Evaluation

Patients with small bowel tumors can present with a variety of symptoms including nausea, vomiting, GI bleeding, weight loss, and abdominal pain. Tumors can also cause small bowel obstructions or intussusception. Occasionally duodenal tumors are discovered during endoscopy. Tumors elsewhere in the small bowel can be found with CT imaging or small bowel follow-through. Wireless capsule endoscopy can also be used [47].

3.2 Surgical Management

Surgical resection is the treatment of choice for small bowel adenocarcinoma [48]. Periampullary lesions often require a pancreaticoduodenectomy. Distal duodenal lesions can often be removed with a sleeve resection and a duodenojejunosotmy. As long as resection margins are negative, more aggressive resections are not necessary. A study from Mayo Clinic evaluated 68 patients with duodenal adenocarcinoma, and the overall 5-year survival rates of those that underwent a pancreaticoduodenectomy versus a sleeve resection were similar [49]. Lesions distal in the small bowel should be removed with a segmental resection including a wide margin of mesenteric lymph nodes. Involved organs should be resected en bloc as technically feasible [50].

Small bowel adenocarcinoma is often diagnosed late, and only about 65–76 % of patients are potentially resectable at the time of diagnosis and about half of these have nodal disease [45, 51]. A series that used the SEER database looked at 1991 patients with small bowel adenocarcinoma and demonstrated the 5-year survival by stage: stage I, 85 %, stage II, 69 %, stage III, 50 % [52]. Predictors of improved overall survival include complete R0 resection, location in the jejunum, low AJCC tumor stage, and low-grade tumors [45, 51, 53, 54]. There is a paucity of data regarding the use of adjuvant therapy for small bowel adenocarcinoma. Often treatment modalities used in colon cancer are used for small bowel adenocarcinoma.

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Pathologic Features of Esophageal and Gastric Malignancies

Eduard Matkovic, Michael Schwalbe and Kristina A. Matkowskyj

Abstract

Esophageal and gastric carcinomas affect millions of individuals worldwide, placing a considerable burden on society. Unfortunately, preventative medicine falls short as screening methods for the upper gastrointestinal tract lack the ability to detect early onset disease. The overwhelming majority of cases present after symptoms appear when individuals have advanced disease with a poor prognosis. Further complicating matters, the anatomic location of these neoplasms engenders rapid tumor progression, which repeatedly thwarts successful surgical treatment. This chapter will focus on the pathological features of malignant neoplasms of the esophagus and stomach.

Keywords

Esophageal squamous cell carcinoma · Esophageal adenocarcinoma · Adenosquamous carcinoma · Adenoid cystic carcinoma · Gastric adenocarcinoma · Helicobacter pylori

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1 Esophagus

Esophageal cancer affects more than 450,000 people worldwide and currently ranks sixth among cancer-related mortality [45]. The prognosis is poor as many patients present with locally incurable or metastatic disease. The incidences rates vary dramatically worldwide, which can be attributed to demographic and socioeconomic factors. Although the vast majority of esophageal neoplasms arise from the epithelial layer and include squamous cell carcinoma (SCC) and adenocarcinoma (AC), a subset of neuroendocrine and soft tissue tumors can also occur in the esophagus (Table 1). Several tasks are presented to the surgical pathologist when dealing with esophageal carcinoma: rendering a diagnosis, classifying the histological type, and assessing prognostic factors. Therefore, we will focus on these topics as we discuss various esophageal neoplasms.

1.1 Squamous Cell Carcinoma

Worldwide, squamous cell carcinoma (SCC) is the most common variant of esophageal carcinoma. The highest incidence rates are seen in underdeveloped settings, particularly in parts of Iran, China, and Africa. In contrast, western countries have seen a considerable decrease in squamous lesions, with an accompanying increase in adenocarcinoma [13]. This demographic discrepancy is not completely understood, and possibly attributed to varying etiological factors as no single causative agent has been identified. Males are affected more commonly than females, and incidence peaks in the sixth decade [6]. The pathogenesis remains incompletely defined, but thought to be a

Table 1 Classification of esophageal tumors

Epithelial tumors	Premalignant Squamous Glandular	Malignant Squamous cell carcinoma Adenocarcinoma Adenoid cystic carcinoma Adenosquamous carcinoma Basaloid squamous cell carcinoma Mucoepidermoid carcinoma Verrucous (squamous) carcinoma Spindle cell (squamous) carcinoma Undifferentiated carcinoma
Mesenchymal tumors	Benign Granular cell tumor Hemangioma Leiomyoma Lipoma	Malignant Kaposi sarcoma Gastrointestinal stromal tumor Leiomyosarcoma Melanoma Rhabdomyosarcoma Synovial sarcoma
Neuroendocrine tumors	Neuroendocrine tumors (NET)	Neuroendocrine carcinoma Mixed adenoneuroendocrine carcinoma
Lymphoma		

multistep process stemming from precancerous changes in the squamous epithelium. It was once believed that esophagitis was a precursor for SCC as prospective studies in high-risk areas have documented a dysplasia-to-carcinoma progression [47, 59]. Risk factors include tobacco, alcohol, poverty, dietary N-nitroso compounds, lack of dietary fruits and vegetables, and poor nutritional status. A history of smoking and alcohol use account for the majority of cases in Europe and North America, however the importance of these factors is substantially different in developing nations. The current literature remains inconclusive on whether human papilloma virus (HPV) is a prominent carcinogen in esophageal SCC [36].

1.1.1 Clinical Features

The most common symptoms of advanced lesions are dysphagia and weight loss. Pain occurs in the epigastrium or retrosternal area. Superficial lesions have vague and nonspecific symptoms, sometimes associated with a tingling sensation or persistent cough. The majority of SCCs are located in the lower two-thirds of the esophagus, followed by the upper segment. The lesion presents as either a depression or elevation of the mucosa.

1.1.2 Gross Pathology

Superficial lesions more frequently appear as plaques or small ulcers, while advanced lesions are often deep ulcerations or fungating masses. However, features do overlap and depth of invasion is not always clearly discernible on endoscopy. Misinterpreting an infiltrative luminal narrowing for a benign stricture is a common pitfall. Alternatively, benign esophagitis presents as an extensive, diffusely ulcerated, flat lesion similar to superficial-type SCC. Imaging techniques like three-dimensional CT, endoscopic ultrasound, or 18-fluoro-2deoxyglucose-positron emission tomography (PET) can effectively demonstrate certain staging parameters. While preoperative imaging and endoscopy are nearly diagnostic, a biopsy is required for confirmation and accurate histological classification, no matter how high the index of clinical suspicion.

1.1.3 Microscopic Pathology

Dysplasia evolves through a spectrum, where changes begin at the base of the epithelium (low-grade) and progress to the surface (high-grade). The cytological features include large, dark staining nuclei with coarse chromatin (hyperchromasia), variation in nuclear size and shape (pleomorphism), loss of epithelial order, and mitotic activity above the basal layer. Atypical cells trailing off into the lamina propria or deep aberrant keratinization are potentially worrisome signs of invasion. Squamous cell carcinoma is composed of polygonal cells with abundant eosinophilic (pink) cytoplasm, intercellular bridges (tight junctions between neighboring cells), and variable amounts of keratinization. The nucleus is large, dark, and contains a prominent nucleolus. Tumor grading evaluates cellular differentiation, the degree of atypia, and mitotic activity. Generally, tumor grading is a means to predict the tumors biological behavior. A low-grade SCC has a lesser degree of atypia as the cells are well-differentiated (Grade 1), thus resembling native squamous epithelium (Fig. 1a). High-grade SCC (Grade 3) has severe atypia and is

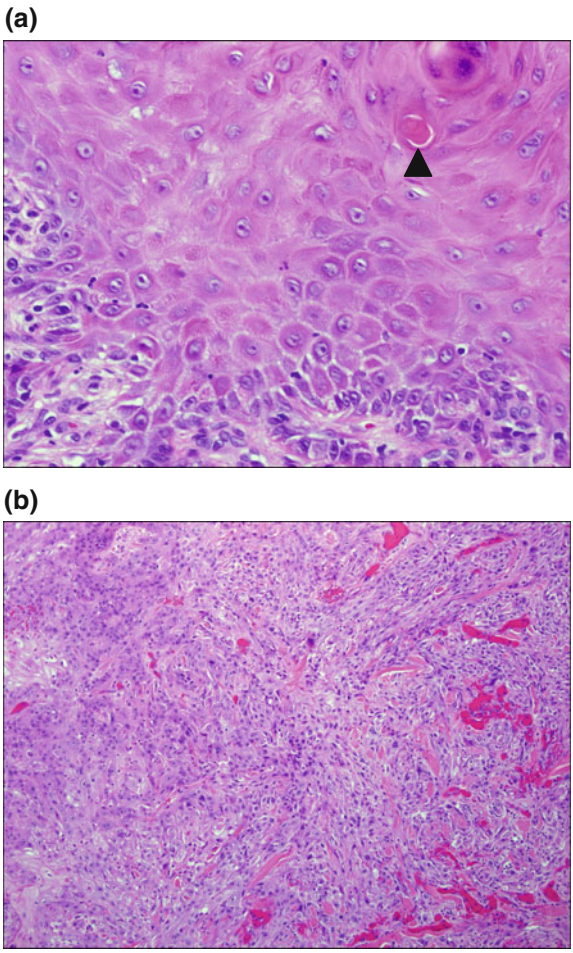

Fig. 1 Squamous cell carcinoma (SCC). **a** In well-differentiated SCC (Grade 1), the tumor cells have abundant pink cytoplasm and keratin formation is evident (*arrowhead*). **b** In poorly-differentiated SCC, the tumor cells are more difficult to appreciate as being squamous in origin and invade as single cells or small clusters of cells

inherently aggressive (Fig. 1b). The frequency of lymphatic and blood vessel invasion increases with increasing depth of invasion [52]. Immunohistochemistry is used judiciously, as most diagnoses can be made solely on histological grounds, however, squamous cell carcinomas are typically positive for cytokeratin 5/6, p63, and p40 by immunohistochemistry. There are currently three histological variants of SCC recognized:

Verrucous Carcinoma: Verrucous carcinoma grossly has a distinctive wart-like appearance (Fig. 2). It is considered a low-grade tumor with pushing borders (bulbous growth of neoplastic cells which push normal tissue aside). The behavior is defined by slow growth with local spread and infrequent metastases. If not properly excised, recurrence tends to be local. Endoscopic correlation is essential as superficial biopsies can underdiagnose the low-grade histology [37].

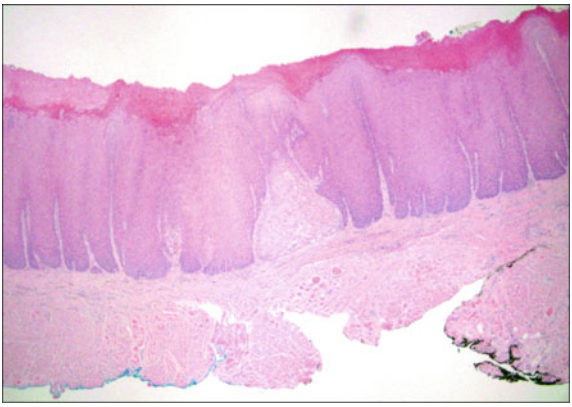

Fig. 2 Verrucous carcinoma. This low-power photomicrograph reveals well-differentiated hyperplastic squamous epithelium with orderly maturation, hyperkeratinization, and broad "finger-like" projections with a typical downward, pushing border characteristic of this entity

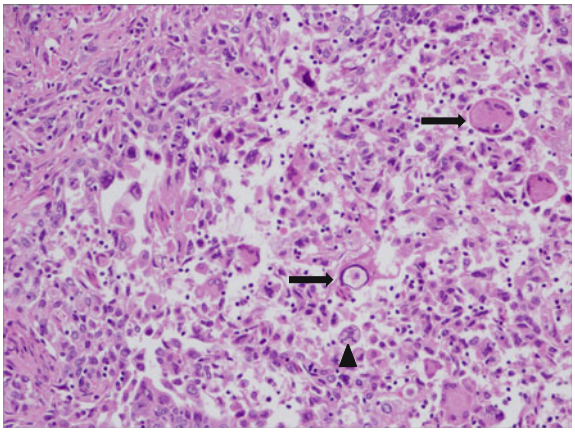

Fig. 3 Spindle cell (squamous) carcinoma. The tumor cells have lost their epithelioid morphology and appear more spindled with bizarre, pleomorphic nuclei (*arrows*) and atypical mitoses (*arrowhead*)

Spindle Cell (Squamous) Carcinoma: The key diagnostic feature is biphasic morphology; well- to moderately differentiated squamous cells admixed with sarcoma-like spindle cells (Fig. 3). The spindle cell component is usually high grade with increased pleomorphism. Overall the prognosis is favorable because the tumor tends to grow outward in a polypoid fashion [48]. Immunohistochemical staining for pancytokeratin can be used to highlight the epithelial differentiation of the neoplastic cells.

Basaloid Squamous Cell Carcinoma: Basaloid squamous cell carcinoma is distinctive for its proximal location. Characterized by large, rounded nests of small blue cells with peripheral palisading and central necrosis (Fig. 4). These tumors tend to be deeply invasive with widespread metastasis at the time of diagnosis. Patients demonstrate poor cancer-related and disease-free survival rates [51].

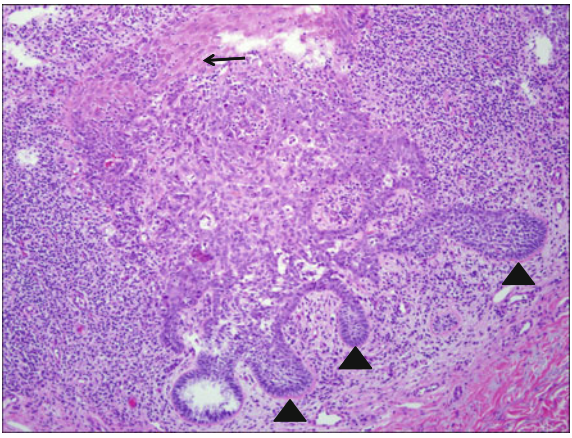

Fig. 4 Basaloid squamous cell carcinoma. Both squamous and basaloid components are present within this tumor. Basaloid cells are *blue* cells with peripheral palisading row of elongated nuclei parallel to one another) (*arrowhead*). The squamous cell component reveals a densely *pink* cytoplasm (*arrow*)

1.1.4 Prognostication

The TNM system used by the American Joint Commission on Cancer (AJCC) is the most widely accepted staging system [14, 56, 61]. The extent of tumor spread is determined by specific staging parameters (TNM classification) and the type of

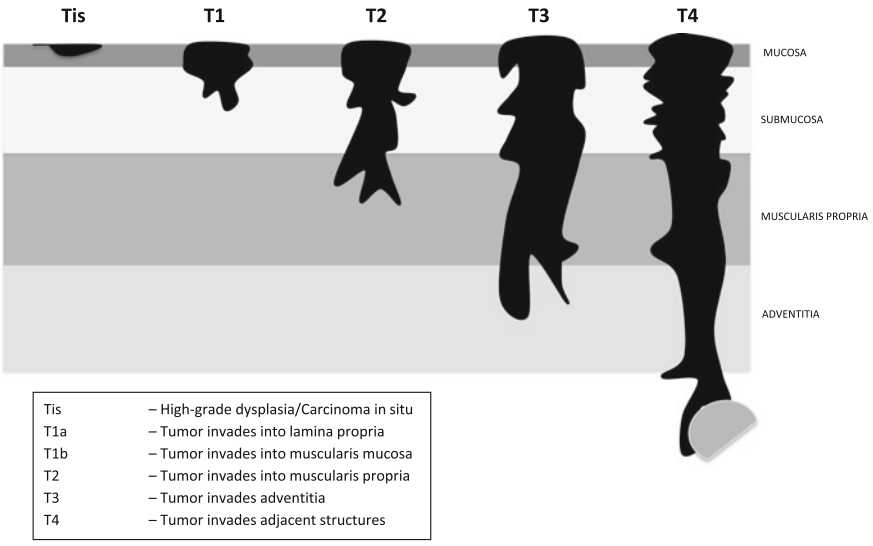

Fig. 5 Tumor staging based on depth of invasion. The single most important parameter, in terms of prognostics, is depth of invasion. Lymphatics originate within the lamina propria and invasion into the lamina propria or beyond results in a T1 stage or higher. Invasion of tumor cells beyond the adventitia with involvement of other structures is a T4 stage

treatment a patient receives is dependent on the extent of disease. The best predictor of outcome and treatment response is the depth of invasion (T-stage), and therefore demands an accurate microscopic measurement. Like all segments of the gastrointestinal tract, the esophagus can be divided into four distinct histological layers: mucosa, submucosa, muscularis propria, and serosa (Fig. 5). The mucosa consists of a protective stratified squamous epithelium and is contained by a basement membrane. Any dysplastic process contained within the mucosa is defined as high-grade dysplasia or carcinoma in situ (Tis). Any neoplastic process invading beyond the basement membrane of the mucosa into deeper layers will be upstaged from Tis into T1 through T4. The reason for this is because the lymphatic system originates in the lamina propria, where neoplastic cells have the potential to metastasize [52, 58]. At the time of diagnosis, most patients present with advanced lesions invading into the muscularis propria, heralding a grim prognosis. The 5-year survival rate amidst tumors restricted to the esophageal wall is roughly 50 %, while penetration into or beyond the adventitia is associated with a worse outcome. Roughly 60 % of patients demonstrate lymph node involvement as the frequency of lymph node involvement is related to depth of invasion (40 % in submucosal extension compared to 5 % for intramucosal lesions) [1]. In addition to traditional features such as invasion and lymph node involvement, tumor grading is implemented to help clinically stratify patients and further predict outcome. Although controversy exists as to whether tumor grading significantly influences survival, over the last decade the American Joint Committee on Cancer (AJCC) has incorporated histology as a parameter for clinical staging of esophageal carcinoma [14, 39].

1.1.5 Molecular Pathology

The loss of several tumor suppressor genes is associated with SCC, of which mutation in the TP53 gene is an early event sometimes detectable in high-grade dysplasia [39]. Other molecular factors include: alterations in p16/INK4a [64], amplification of cyclin D1 [26], and inactivation of CDKN2A [53]. TP53 is mutated in 35–80 % of SCCs [38], and its nuclear accumulation has shown to be a negative prognostic indicator [54].

1.2 Esophageal Adenocarcinoma

Adenocarcinoma (AC) differs from squamous cell carcinoma based on histology, but also on various epidemiological characteristics. For the past three decades, the occurrence of adenocarcinoma has increased dramatically [7, 46]. This trend has been particularly dominant in Western countries, like the United States and United Kingdom, where rates have exceeded that of squamous cell carcinoma. Epidemiological factors of adenocarcinoma overlap with Barrett esophagus (BE), as the incidence of BE has increased in tandem with the increasing rates of AC [62].

At the gastroesophageal junction, complications of chronic gastroesophageal reflux disease (GERD) result in the development of intestinal metaplasia. That is, after repeat bouts of injury, the healing process transforms squamous epithelium

into a mucin filled, columnar glandular-type epithelium with goblet cells (Barrett esophagus). Over time, the columnar epithelium can progress in a stepwise fashion through dysplasia and eventually develop into invasive carcinoma. Although familial association has been reported, population-based studies have shown rapid changes in incidence rates in different populations over short periods of time, likely indicating limited hereditary influence [33]. Barrett esophagus is the single most important risk factor in developing esophageal adenocarcinoma [29]. Obesity appears to be associated with increased risk and smoking appears to have a negative impact.

1.2.1 Clinical Features

Clinical symptoms of esophageal adenocarcinoma are generally associated with dysphasia, weight loss, and abdominal pain. In contrast to squamous cell carcinoma, AC invariably arises distally and therefore tumor location is not incorporated into the staging. Since esophageal adenocarcinoma can involve the gastroesophageal junction (GEJ) and carcinoma of proximal stomach can invade the distal esophagus, distinguishing between these two entities is challenging [57]. Accurate anatomical localization is imperative for proper classification and staging [27]. Tumors with an epicenter at or above the gastroesophageal junction (GEJ) or within the proximal 5 cm of stomach with extension into GEJ are staged and classified as esophageal tumors [55]. Endoscopists should attempt to identify the most proximal gastric folds or the columnar (Barrett) epithelium to establish the GEJ. Large tumors can obliterate evidence of adjacent Barrett mucosa, concealing the esophageal origin.

1.2.2 Gross Pathology

Grossly, adenocarcinoma looks similar to squamous cell carcinoma, and can present as either flat, irregular plaques (Fig. 6a), polypoid lesions (Fig. 6b), or an ulcerated, fungating mass (Fig. 6c). At early stages, macroscopic findings are often subtle, with the salmon pink mucosa of Barrett esophagus (columnar epithelium with goblet cells) located adjacent to the tumor. High-grade dysplasia is often not visibly apparent and requires tissue sampling [15]. Even invasion is difficult to identify grossly; often being flat and occasionally arising independently from Barrett mucosa. Determining invasion on endoscopy (without the assistance of ultrasound) can be unreliable.

1.2.3 Microscopic Pathology

In general, when dysplasia develops in Barrett esophagus, glands acquire a dark blue hue as cells loose mucin, become more crowded, and develop enlarged, hyperchromatic nuclei. These features should extend from the base of the glands up to the luminal surface. Unique in low-grade dysplasia, the crypt architecture is preserved with minimal distortion, containing cells with elongated, “pencil-shaped” nuclei limited to the basal portion of the cytoplasm. In high-grade dysplasia, cytological atypia progresses with increased nuclear pleomorphism, high nuclear-to-cytoplasmic ratio, frequent mitoses, loss of nuclear polarity and increased glandular complexity (back-to-back glands, cribriform/papillary patterns) (Fig. 7).

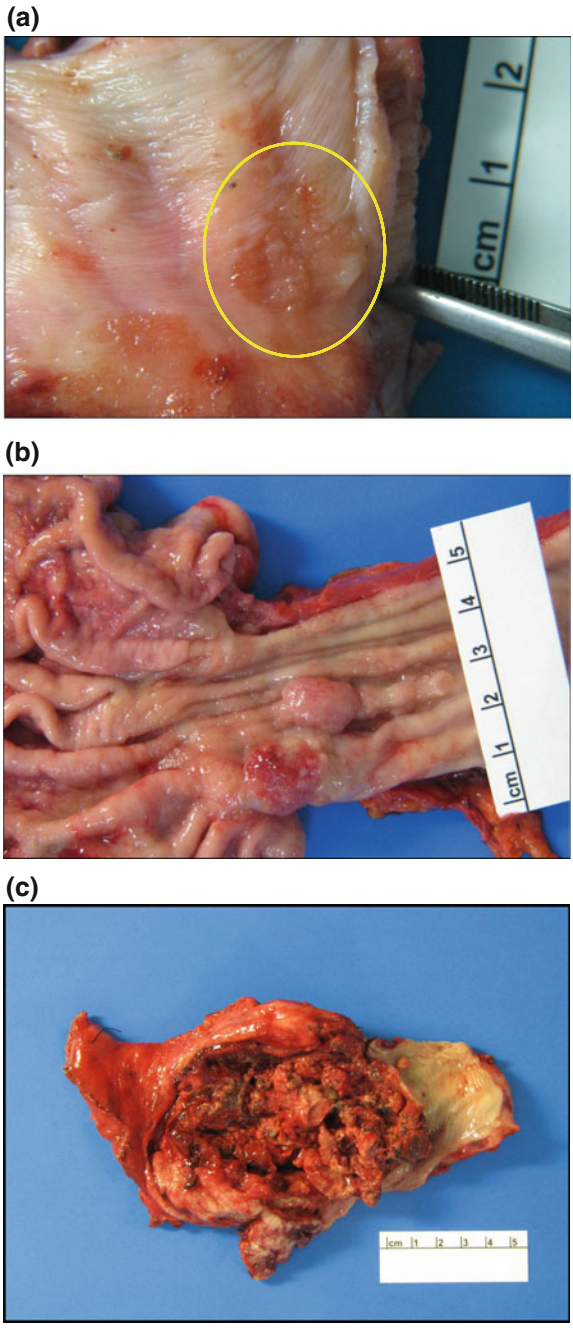

Fig. 6 Macroscopic appearance of esophageal adenocarcinomas. **a** Superficial lesions appear as plaque-like areas (*yellow circle*) with punctate ulceration and erythematous mucosa. In more advanced lesions, the tumor can appear as a polypoid mass (**b**) or fungating lesion (**c**)

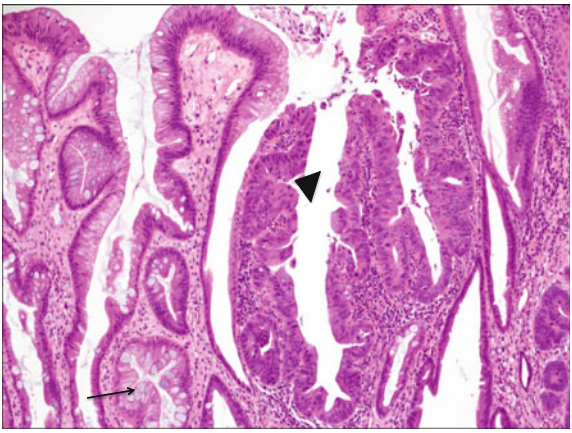

Fig. 7 Barrett esophagus. **a** The presence of mucinous, columnar epithelium with readily identified goblet cells (*arrow*) within the squamous mucosa of the esophagus is diagnostic of Barrett esophagus (BE). **b** Within the area of BE, there is increased hyperchromasia, loss of mucin, increased nuclear-to-cytoplasmic ration and loss of polarity, characteristic of high-grade dysplasia (*arrowhead*)

With invasion, the border of the basement membrane appears ragged with single cells streaming off into the lamina propria. Tumor grading (well, moderately, or poorly-differentiated) classifies the tumor according to proportion of glandular formation. Well-differentiated tumors are composed of irregular glands with cuboidal–columnar epithelium (Fig. 8a). The nuclei contain coarse chromatin with prominent nucleoli. In moderately-differentiated carcinomas, there will be less glandular structures admixed with more complex architecture, composed of irregular cell clusters, nuclear stratification, and cribriform pattern (Fig. 8b). Glandular structures are scarcely visible in poorly-differentiated carcinomas, defined by bizarre pleomorphic cells arranged in sheets or scattered cells within a desmoplastic stroma (Fig. 8c). In small biopsies of well-differentiated tumors, the invasion is not striking and poses difficulties in diagnosis. If present, desmoplasia can help separate high-grade dysplasia from invasive carcinoma. Post-neoadjuvant therapy specimens may demonstrate extensive treatment effect, which consists of highly degenerated neoplastic cells within pools of acellular mucin (Fig. 9).

1.2.4 Prognostication

Although adenocarcinoma and squamous cell carcinoma differ in a number of features, including location, predisposing factors, and tumor biology, they share the same poor prognosis. Tumor stage is the most important parameter in determining survival [58]. Patients with tumors limited to the mucosa have a superior 5-year survival rate (85 %) than patients with muscularis propria involvement [49]. Unfortunately, by the time symptoms appear, most tumors have already reached advanced stages, with invasion into the esophageal wall noted in 60–80 % of cases and nodal involvement in up to 30 % of cases [19]. Neoadjuvant therapy is often

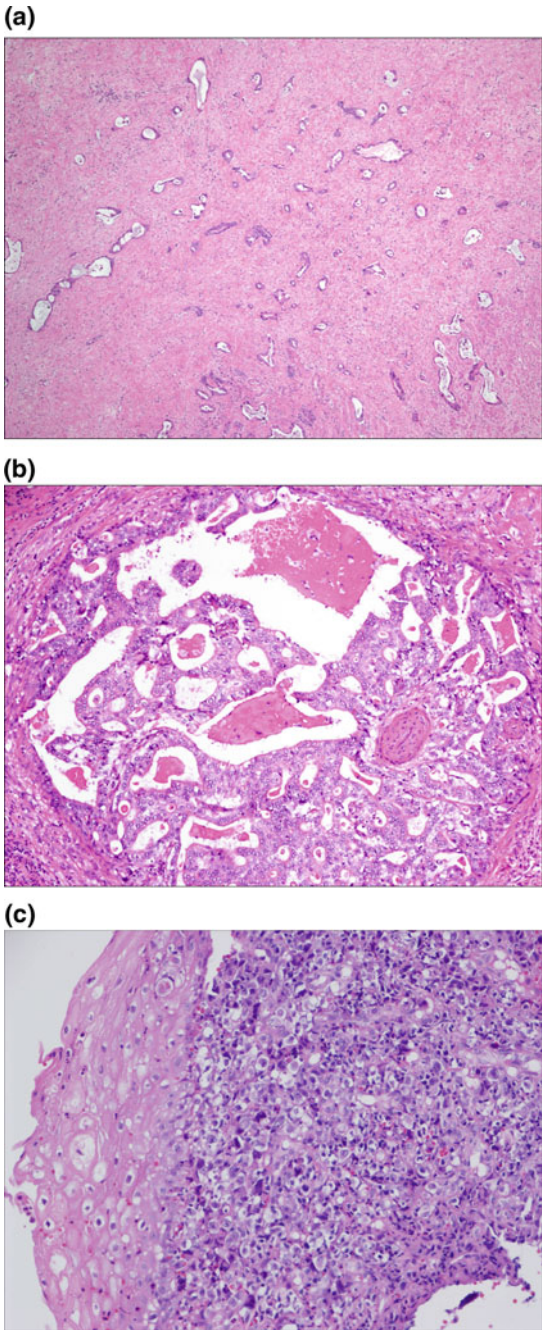

Fig. 8 Morphologic spectrum of esophageal adenocarcinoma. **a** Infiltrating, well-differentiated adenocarcinoma demonstrates glands (tubular structures) within the muscularis propria, **b** "punched-out," colander-like holes in a cribriform pattern in a moderately-differentiated tumor, and **c** solid sheet of single, poorly-differentiated tumor cells undermining the squamous mucosa

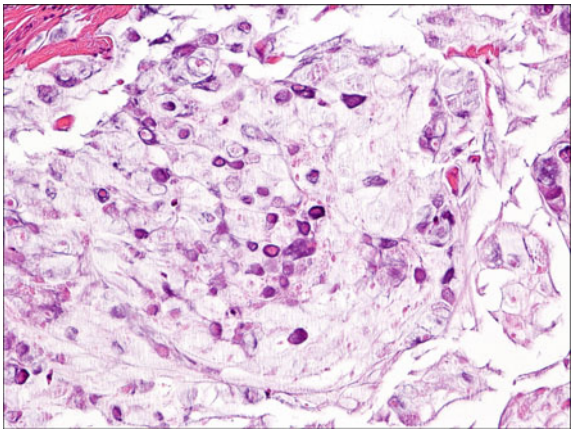

Fig. 9 Treatment effect. Following neoadjuvant treatment, there can be abundant collections of acellular mucin containing areas of calification and rare, markedly degenerated, non-viable tumor cells

provided for advanced-stage tumors and the extent of residual carcinoma found in the resection specimen after therapy is a predictor of patient outcome [63]. Complete regression (0 % residual tumor remaining) reflects a positive tumor response to pre-operative treatment, and is recognized as a positive prognostic factor [3]. Modification of the AJCC TNM staging system resulted in separating squamous cell carcinoma from adenocarcinoma and both incorporate histological grade as a separate prognostic category [14]. Histological grading for AC carries a different weight in determining prognostic groups such that a poorly-differentiated (Grade 3) adenocarcinoma will be placed under the same prognostic grouping as a well- or moderately-differentiated squamous cell carcinoma (Table 2).

Table 2 The prognostic grouping of esophageal carcinomas

	Group	T	N	M	Grade	Location
Adenocarcinoma	IA	1	0	0	1–2	
	IB	1	0	0	3	
		2	0	0	1–2	
	IIA	2	0	0	3	
	IIB	3	0	0	Any	
		1–2	1	0	Any	
Squamous cell carcinoma	IA	1	0	0	1	Any
	IB	1	0	0	2, 3	Any
		2, 3	0	0	1	Lower
	IIA	2	0	0	1	Upper/middle
		2, 3	0	0	2, 3	Lower
	IIB	2–3	0	0	2,3	Upper/middle
		1–2	1	0	Any	Any

1.2.5 Molecular Pathology

Multiple genetic alterations involving tumor suppressor genes, oncogenes, and growth factor receptors play a role in cancer progression. Mutations or overexpression of TP53 occur as dysplasia progresses from low-grade to high-grade dysplasia and onto carcinoma [18, 25]. Allelic loss or epigenetic silencing by promoter methylation of cyclin dependent kinase inhibitor (CDKN2A/p16) has been demonstrated to be an early event in tumorigenesis [40]. Additional genetic changes include amplification of *c*-ERB-B2 [32] and cyclin D1 [2], and upregulation of COX2 [8] have been identified. Increased epithelial NF-κB expression insinuates that inflammation is a likely contributing factor [24]. Several molecular markers are associated with a negative clinical outcome, including DNA aneuploidy, TP53 mutations, ERB-B2 amplification, and expression of COX2 or NF-κB [23, 32].

1.3 Other Esophageal Carcinomas

1.3.1 Adenosquamous Carcinoma

These tumors contain mixed element of adenocarcinoma and squamous cell carcinoma, which are clearly demarcated within the lesion (Fig. 10). The histological mixture is diagnostically insignificant and prognosis is similar to typical squamous cell carcinoma.

1.3.2 Mucoepidermoid Carcinoma

A rare tumor derived from submucosal glands, displaying more intimately admixed elements of squamous cells, mucus secreting cells, and intermediate cells, which morphologically falls in between the other two cells types (Fig. 11).

1.3.3 Adenoid Cystic Carcinoma

Also an infrequent variant, believed to arise like mucoepidermoid carcinoma, from esophageal glands. The tumor is composed of small, bland, myoepithelial cells with

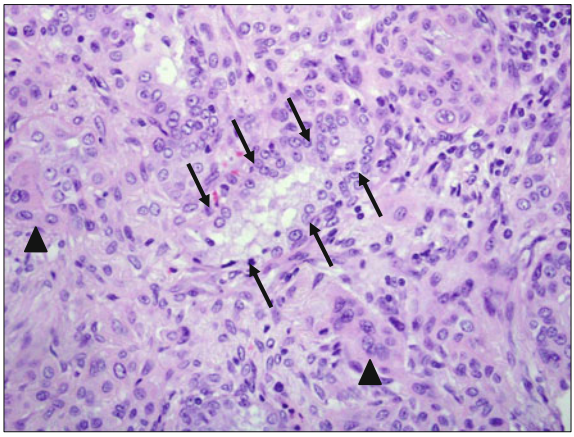

Fig. 10 Adenosquamous carcinoma. This tumor contains tumor cells with abundant pink cytoplasm characteristic of squamous cell carcinoma (*arrowheads*), along with areas that have the gland formation seen in adenocarcinoma (*arrows*)

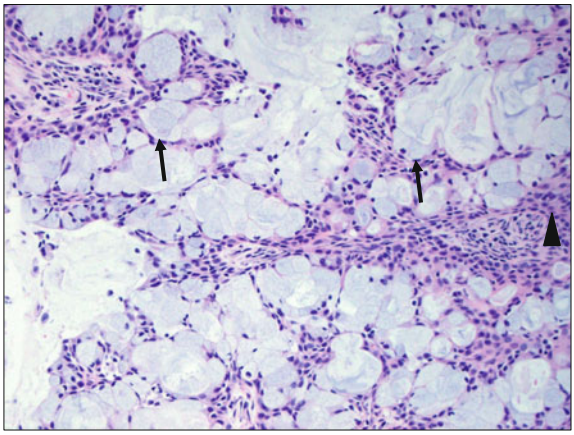

Fig. 11 Mucoepidermoid carcinoma. The tumor consists of cords or clusters of mucous (*arrows*), squamous cells (*arrowhead*), and intermediate cells, and often lacks high-grade atypia

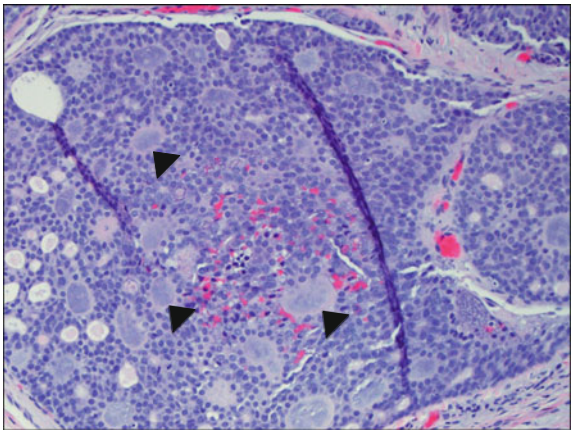

Fig. 12 Adenoid cystic carcinoma. The tumor is composed of small, bland cells with scant cytoplasm and the characteristic "punched-out," cribriform pattern with mucus and basement membrane material within the center (*arrowheads*)

scant cytoplasm, dark compact angulated nuclei surrounding pseudoglandular spaces containing excess basement membrane material and mucin (Fig. 12). The principle diagnostic challenge is to distinguish this tumor from basaloid squamous cell carcinoma. Both adenoid cystic and mucoepidermoid carcinoma have a favorable prognosis compared to squamous carcinomas [41].

2 Stomach

There are numerous benign and malignant primary neoplasms that occur in the stomach arising from a multitude of precursor cells. Overwhelmingly, the majority of malignant tumors in the stomach are of epithelial origin (carcinomas), but

various other tumors of lymphoid, mesenchymal, and neuroendocrine differentiation may also arise in this location. As a group, primary gastric malignancies comprise a wide spectrum of morphology, histopathology, clinical behavior, and genetic makeup; an evolving understanding of the pathophysiology and genetic underpinnings of these lesions is necessary to guide new treatments and most effectively manage patients with these conditions.

3 Gastric Adenocarcinoma

Gastric adenocarcinoma is the fourth most common malignancy worldwide, with an incidence that varies widely with regard to geography. Although it was the leading cause of cancer-related mortality worldwide until the 1990s, primary gastric adenocarcinoma has become relatively rarer in many western countries with a rate that continues to decrease [4]. This decline has been attributed to decreased prevalence of risk factors, such as *Helicobacter pylori* infection and tobacco use [16]. However, there are still regions in which gastric adenocarcinoma is significantly more prevalent, such as Eastern Asia, South America, and Eastern Europe. There is also a correlation between geographic incidence and the location of the tumor in relation to gastric anatomy (i.e., centered at the gastric cardia vs. antrum/pyloris). Generally, the incidence of gastric tumors occurring in the cardia is higher in "low-incidence" regions such as North America, while the opposite is true of antral/pyloric tumors [28].

3.1 Pathogenesis of Gastric Carcinoma

The pathogenesis of gastric adenocarcinoma is closely related to several environmental risk factors. One notable risk factor is infection by the Gram-negative spirochete *Helicobacter pylori.* International epidemiologic studies indicate that the incidence of gastric cancer is proportional to the *H. pylori* infection rate within a given country, that falling rates of *H. pylori* infection coincide with a drop in the incidence of gastric adenocarcinoma [28], and infection with *H. pylori* confers at least a sixfold higher risk of gastric carcinoma than that of the general population [21]. It is well known that chronic inflammation serves an etiological role in many cancers [43], and the chronic gastritis that results from *H. pylori* infection has been cited as precursor to gastric adenocarcinoma. Additional proposed pathways of carcinogenesis by *H. pylori* include interference with apoptosis and the cell cycle, alteration of intercellular signaling, disruption of intercellular adhesive processes, and activation of proliferative intracellular signaling pathways, among others [42]. In addition to infection, other risk factors for gastric adenocarcinoma include smoking, prior gastric surgery, dietary factors (high salt intake, diets low in fruits and vegetables, high nitrite intake), ionizing radiation, and pernicious anemia/autoimmune gastritis [17]. Genetic and hereditary factors are also implicated in some types of gastric carcinoma; generally speaking, solid tumors with tubular architecture (intestinal-type carcinomas) are more related to environmental

risk factors while poorly circumscribed, widely infiltrative (diffuse type) carcinomas are more likely to be associated with genetic derangements [22]. The classification and molecular features of gastric carcinomas are discussed later in this chapter.

3.2 Gross Pathology

Gastric adenocarcinomas exhibit a wide spectrum of macroscopic morphology, ranging from grossly exophytic lesions to subtle widely infiltrative lesions. Gastric carcinomas may be macroscopically separated into early and advanced carcinomas. Early gastric carcinomas are tumors of any size and lymph node status which invade no deeper than the submucosa. Most early carcinomas range from 2 to 5 cm in greatest dimension [22]. The Paris system, proposed in 2002, designates early carcinomas as type 0 and further subcategorizes these lesions into types 0-I (polypoid), type 0-II (superficial), and type 0-III (excavated) according to their macroscopic architecture. These categories are further subclassified as shown in Table 3. Advanced carcinomas—those that invade deeper than the submucosa- are categorized into types I-IV according to macroscopic architectural features (the Borrmann Pathologic Classification of Gastric Cancer). Type I cancers (polypoid) are exophytic tumors which are dome-shaped and are usually attached to the surrounding stomach mucosa via a wide base. Polypoid tumors may range from having a relatively smooth overlying mucosa to more complex architecture featuring lobulation or irregular excrescences. Type II (ulcerated, circumscribed) carcinomas have a well-demarcated border which may be elevated or rolled, and there is often some degree of central ulceration. This type may feature irregular mounds and projections of the mucosa and is sometimes described macroscopically as fungiform/fungating. Type III (ulcerated, infiltrative) tumors are similar to type II in that central ulceration is a prominent feature, but these cancers demonstrate poor demarcation macroscopically and it is often difficult to grossly identify the extent of the lesion (Fig. 13a and b). Finally, type IV (infiltrative, non-ulcerative) tumors are

Table 3 Paris and Borrmann endoscopic classifications for gastric adenocarcinoma

Early carcinomas (Paris)	Type 0 0-I: polypoid 0-Ip: pedunculated 0-Is: sessile 0-II: superficial 0-IIa: elevated 0-IIb: flat 0-IIc: depressed 0-III: excavated
Advanced carcinomas (Borrmann)	Type I: polypoid
	Type II: ulcerated, circumscribed
	Type III: ulcerated, infiltrative
	Type IV: infiltrative, non-ulcerative

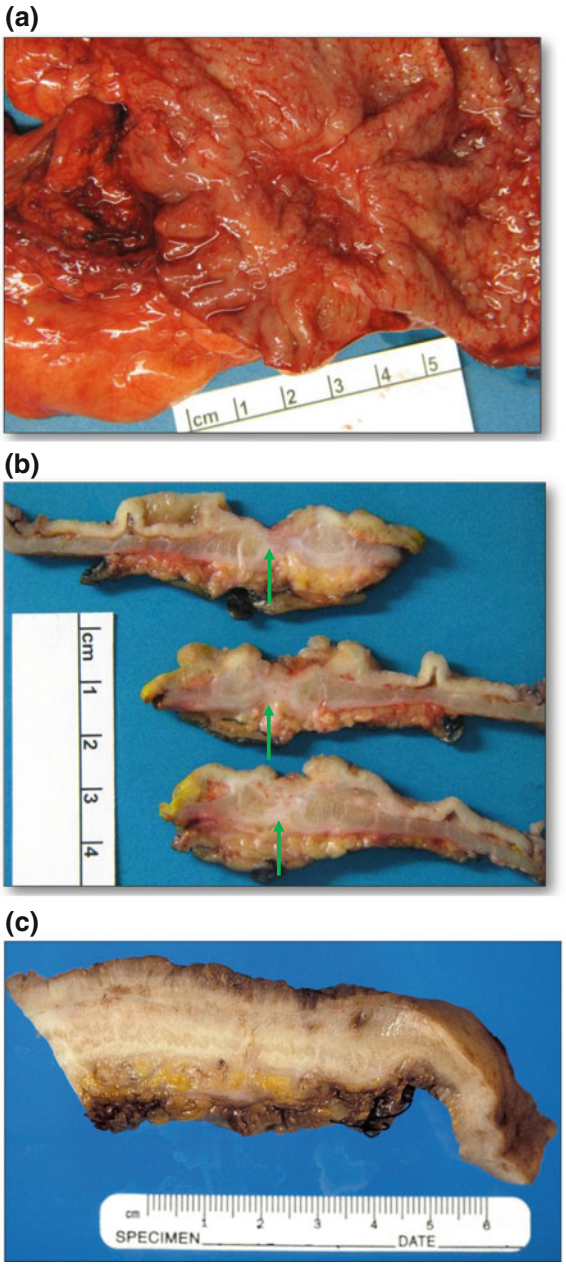

Fig. 13 Macroscopic appearance of gastric adenocarcinoma. **a** Tumor within the antrum exhibits central ulceration and heaped-up, poorly defined mucosal borders. **b** Sectioning of the tumor reveals infiltration into the muscularis propria but without extension to the inked serosal surface *(green arrows)*. **c** There is diffuse thickening of the gastric wall by infiltrative tumor without obvious disruption of the mucosal surface, grossly consistent with linitis plastica

generally flat lesions with extensive infiltration and very little discernible demarcation; the term linitis plastica is applied when a gastric carcinoma exhibits a type IV pattern of growth and involves the majority of the gastric wall (Fig. 13c) [44].

Although seemingly straightforward, there has been some ambiguity in the distinction between cancers arising from the esophagus and gastroesophageal junction (GEJ) and those arising from the gastric cardia. This may be difficult to determine histologically due to overlapping microscopic features of the two entities, and there has even been some disagreement as to whether the gastric cardia is anatomically native mucosa or a metaplastic/reactive process to esophageal reflux or other insults [12]. The 7th AJCC Cancer Staging Manual addresses these difficulties by defining esophageal carcinoma as any tumor arising 5 cm or less from- and also involving—the gastroesophageal junction; tumors not fulfilling these criteria should be staged as authentic gastric carcinomas [60].

3.2.1 Microscopic Pathology

Intestinal-type carcinomas are usually preceded by a well-characterized progression of premalignant lesions (as opposed to diffuse-type carcinomas, which usually lack coincident precursor lesions) [10]. This process may be instigated by infection with *H. pylori*, which incites chronic gastritis characterized by infiltration of the lamina propria by chronic inflammation (plasma cells, lymphocytes) with the addition of neutrophilic infiltration in active cases (Fig. 14a and b). The next step, intestinal metaplasia, occurs when the gastric foveolar and glandular epithelium is replaced by intestinal-type epithelium, which is histologically characterized by columnar cells with small, oval, basally oriented nuclei and voluminous, basophilic (blue) apical cytoplasm. This is in stark contrast to the polymorphic cell population of fundic gland epithelium of the gastric body and the pale mucinous epithelium found in the antrum. Goblet cells (pale columnar cells with a large apical mucin droplet) are interspersed throughout intestinal-type epithelium (Fig. 15a and b); they are readily visualized on low power microscopy and offer a conspicuous indication that gastric epithelium has undergone intestinal metaplasia [11, 21]. Once intestinal metaplasia is established, dysplasia may then occur within the metaplastic epithelium which is subclassified into low-grade or high-grade dysplasia depending on the severity of both cytologic and architectural atypia. Cytologically, dysplasia of gastrointestinal epithelium is characterized by optically dark nuclei (hyperchromasia) which usually exhibit a coarsely granular chromatin pattern. In low-grade lesions, dysplastic nuclei may become enlarged, elongated ("pencillate"), and pseudostratified. At low power, these changes impart an overall darkness or "blueness" to the dysplastic epithelium. High-grade dysplastic nuclei feature exaggerated and variable enlargement/irregularity of the nuclear envelope (nuclear pleomorphism) with a higher ratio of the size of the nucleus to that of the cytoplasm (nucleocytoplasmic/N:C ratio). High-grade dysplasia exhibits even more striking architectural atypia, with "back-to-back" (cribriform) gland formation and a generally disorganized appearance when compared to benign glandular epithelium.

As alluded to previously, gastric adenocarcinomas have historically been categorized into two main histologic groups per the Lauren classification [34]:

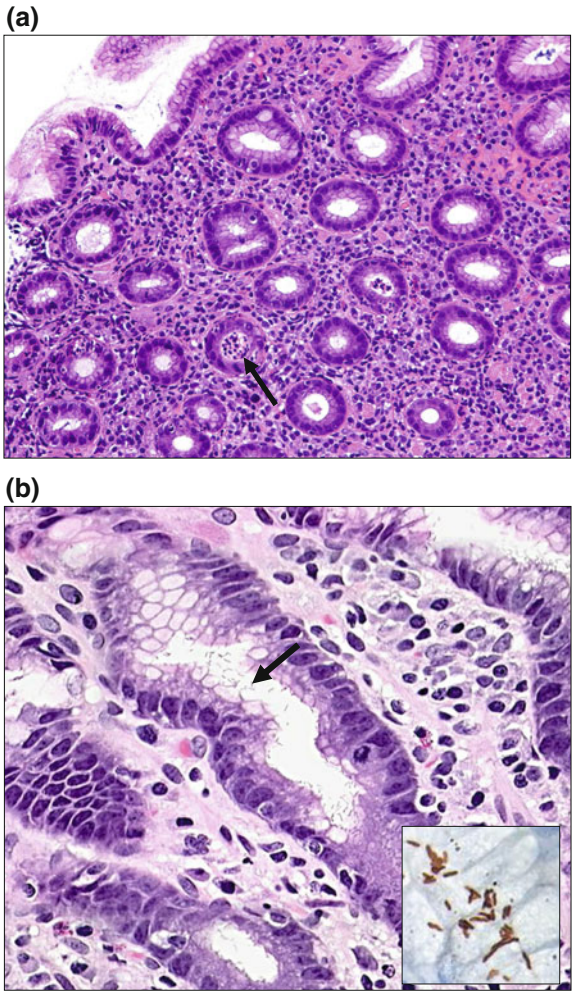

Fig. 14 *Helicobacter pylori* gastritis. **a** There is dense infiltration of the lamina propria by chronic inflammation, primarily plasma cells and scattered lymphocytes. There is also an acute gastritis present as demonstrated by neutrophils present within the gastric pits (*arrow*). **b** At higher power, seagull-shaped spirochetes are noted within the lumen of gastric pits (*arrow*) and these *Helicobacter pylori* organisms stain positively by immunohistochemistry (*inset*)

intestinal-type and diffuse-type. Tumors can be of a mixed type or even classified as indeterminate to encompass unusual morphologies which do not fit well into the aforementioned categories. Approximately 54 % of gastric carcinomas are of the intestinal type, 32 % are diffuse type, and 15 % are indeterminate [22]. Intestinal-type carcinomas, as the name suggests, bear striking resemblance to carcinomas arising more distally from intestinal epithelium. Histologically, they are composed of irregular glandular and/or cord-like arrangements of cells which permeate the surrounding stroma in an infiltrative pattern (Fig. 16a). Well-differentiated adenocarcinomas tend to recapitulate glandular architecture in the majority of the tumor, while poorly-differentiated lesions generally display a solid (sheet-like) pattern of growth with high mitotic rates and highly atypical and pleomorphic nuclei (Fig. 16b, c). The stroma surrounding these invasive tumors

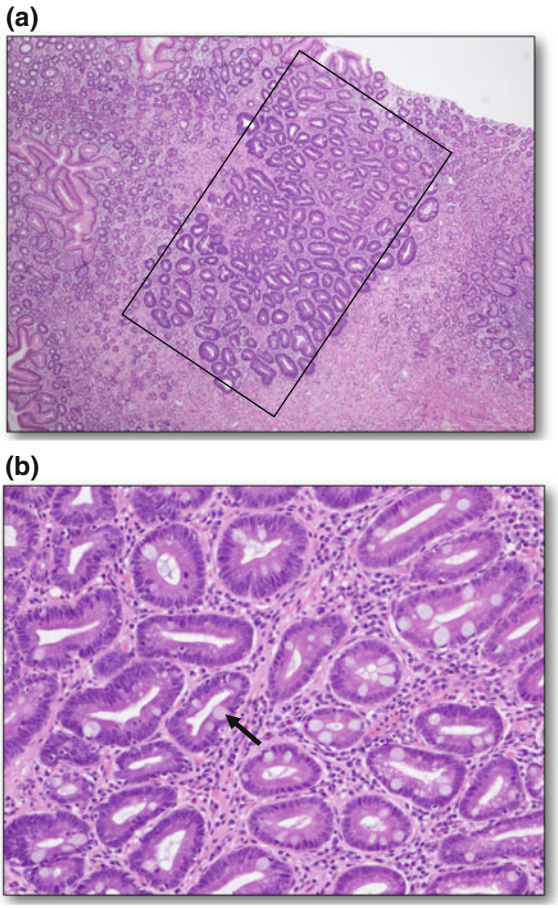

Fig. 15 Gastric mucosa with intestinal metaplasia. **a** Focus of intestinal metaplasia (*black box*) seen in a case of nearby adenocarcinoma. At low power, the metaplastic glands have a darker staining quality than the adjacent native glandular epithelium (far *left* and *right* portion of image). **b** High-power magnification demonstrates bland, basally oriented nuclei and interspersed pale, goblet cells (*arrow*), virtually indistinguishable from normal intestinal epithelium

often displays gray-blue coloration (in contrast to the eosinophilic or pink normal stromal collagen) with numerous spindle-shaped fibroblasts which architecturally contour around the invading tumor. Known as desmoplasia, this phenomenon is a fibroblastic reaction to the infiltrating tumor and can be a helpful histologic clue for the low-power identification of invasive glands (Fig. 16a).

In contrast, diffuse-type gastric carcinomas are composed of discohesive cells which widely infiltrate the gastric stroma either singly or in small clusters. There is no glandular architecture, but lace-like cords of neoplastic cells may occasionally be seen. Signet ring cell-type gastric carcinomas are a subtype of diffuse cancers which contain at least 50 % signet ring cells which are histologically defined by a large, gray-blue intracytoplasmic mucin droplet which peripherally displaces the cell's nucleus and deforms it into a crescent shape (Fig. 17). These tumors are very poorly demarcated and may extensively permeate the gastric wall. Cytokeratin immunohistochemistry can be helpful in detecting inconspicuous signet ring cells

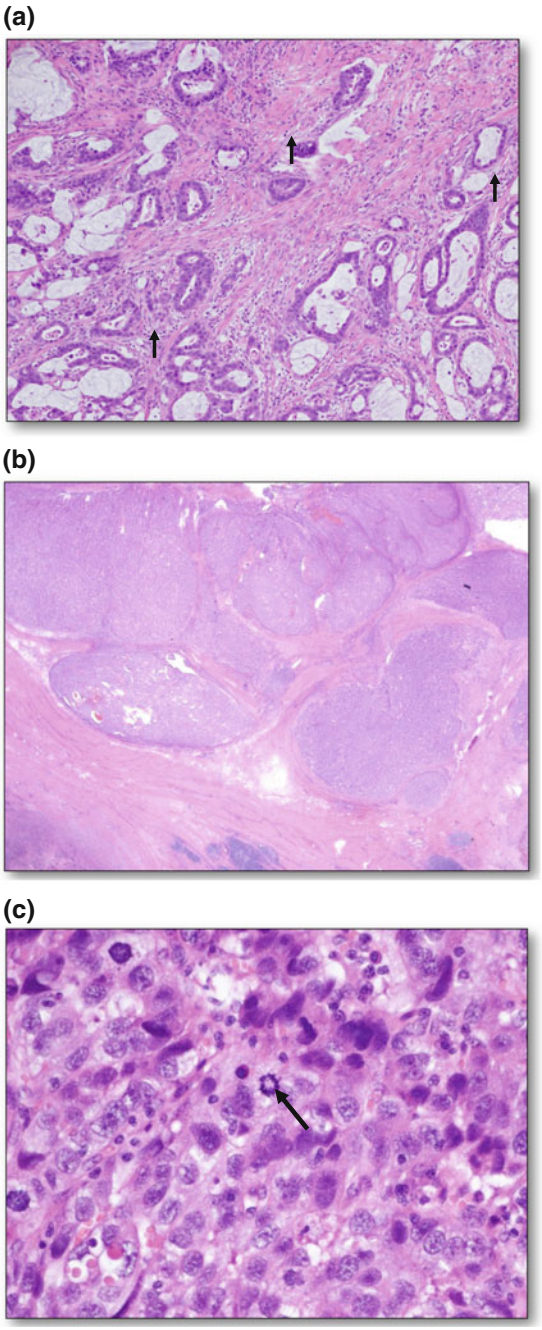

Fig. 16 Intestinal-type carcinoma. **a** This well-differentiated intestinal-type carcinoma demonstrates irregular, infiltrative glands with hyperchromatic nuclei with surrounding *gray-blue*, desmoplastic stroma (*arrows*). **b** This poorly-differentiated intestinal-type adenocarcinoma demonstrates a lobular, sheet-like growth at low power, while at high-power **c** the tumor cells are highly pleomorphic and exhibit an atypical ringed mitosis (*arrow*)

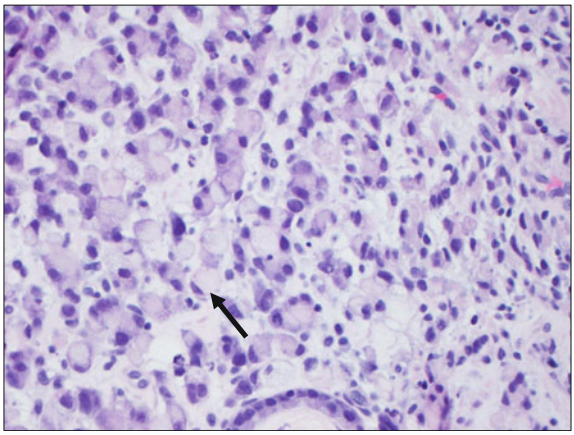

Fig. 17 Diffuse-type carcinoma. These discohesive, malignant cells have a prominent cytoplasmic mucin vacuole (*arrow*) which displace and compress the nucleus to the periphery. The nuclei are pleomorphic and exhibit significant atypia

infiltrating the lamina propria, as early stage lesions may appear quite innocuous at first glance on light microscopy. In cases of macroscopic involvement of most or all of the stomach by cancer, the stomach may assume a thickened and stiff morphology which lends itself to the term "linitis plastica" (leather bottle stomach, Fig. 13c). Linitis plastica most commonly occurs in the setting of diffuse-type carcinoma; however, intestinal-type carcinomas have rarely been shown to produce this macroscopic characteristic as well [20].

In addition to the two broad aforementioned histologic categories, the 2010 WHO Classification of Tumors of the Digestive System introduced an alternative and more specific classification system which addresses tumors with features that might not have otherwise fit well into strictly intestinal versus diffuse types [35]:

Tubular adenocarcinoma: These tumors are characterized by branching glands which range from compressed and slit like to large and dilated. The epithelium ranges from columnar to cuboidal and the cytoplasm may range tinctorially from dark to clear. Desmoplasia may be present, and these tumors range from well- to poorly differentiated architecturally. This type is roughly analogous to the intestinal-type carcinoma of the Lauren classification.

Papillary adenocarcinoma: Papillary carcinomas are generally well-differentiated and predominantly feature exophytic growth and villus-like architecture, with finger-like projections that are lined by neoplastic cuboidal or columnar epithelium and contain a fibrovascular core.

Mucinous adenocarcinoma: The primary feature of these tumors is glandular-type epithelium with exuberant production of mucin, and is analogous to mucinous carcinoma seen in other organs such as the breast and colon. Strips or clusters of neoplastic cuboidal or columnar cells may detach and float within the mucin pools. The mucin itself stains light blue and has a "wispy" character on hematoxylin and eosin (H&E) stained slides. Largely acellular mucin lakes may be seen dissecting throughout the stroma. In order to qualify as a mucinous

carcinoma, at least 50 % of the tumor must consist of extracellular mucin. Occasionally, signet ring-type cells may be seen, but should not be the predominant cell type to qualify for this category.

Poorly cohesive carcinoma, including signet ring cell carcinoma: Analogous to diffuse carcinoma of the Lauren classification system, poorly cohesive carcinomas feature poorly circumscribed tumors with tumor cells which infiltrate singly or in small clusters. Signet ring cell-type carcinomas are a subtype of this group; other tumors include those featuring lymphoid, histiocytoid (resembling macrophages), eosinophilic, or bizarre cell morphology so long as the overall architectural pattern is that of discohesive growth and poor circumscription.

3.3 Molecular Pathology

The majority of sporadic gastric carcinomas (85 %) show an accumulation of various structural and numerical chromosomal changes including translocations, sequence amplifications, gains or losses of chromosomes, etc. Gains of 3q, 7q, 13q, 17q, and 20q as well as losses of 4q, 5q, 6p, 9p, 17p, and 18q are frequently detected by comparative genomic hybridization [21]. Numerous genetic and epigenetic events have been also been described, and carcinogenesis sequence paradigms have been attempted in gastric cancer similarly to those that are widely accepted in colorectal cancer. Generally, gastric carcinogenesis involves the accumulation of mutations of tumor suppressor genes (e.g., *TP53)*, activation of telomerase, aberrations of adhesion molecules and cell cycle regulators, and epigenetic factors such as CpG island methylation and silencing of mismatch repair genes (MLH1, MSH2, MSH6, and PMS2) via promoter hypermethylation [65]. As with some breast carcinomas, amplification of human epidermal growth factor 2 (HER2) is now a recognized driver of tumorigenesis in 7–34 % of gastric carcinomas. Tumors with amplification of HER2 are susceptible to targeted therapy with trastuzumab; for this reason, assessment of HER2 status by immunohistochemistry or FISH is recommended for all gastric cancers at initial diagnosis [50].

In the case of diffuse gastric cancers, a genetic anomaly of note is mutation of the gene encoding the intercellular adhesion protein E-cadherin (*CDH1).* E-cadherin is a calcium-dependent transmembrane protein that connects to the actin cytoskeleton of the cell, helping to maintain normal cellular morphology and cell-to-cell attachment. Loss of *CDH1* is reflected morphologically by the total loss of cell cohesion that is microscopically characteristic of diffuse carcinomas. Germline mutations in *CDH1* have been implicated in familial clusters of diffuse gastric cancers, an entity now known as hereditary diffuse gastric cancer (HDGC). Progression to carcinoma occurs in a "two-hit" fashion, in which a susceptible individual (carrier of the *CDH1* germline mutation) incurs a sporadic mutation or methylation of the second allele. Approximately 1–3 % of diffuse cancers occur in a hereditary fashion, and the estimated lifetime risk of diffuse gastric cancer in susceptible individuals is 67 % in men and 83 % in women [21]. These tumors

generally present at an early age and behave more aggressively than sporadic tumors; due to this grave prognosis, genetic testing is indicated in the following situations: (1) families with two or more cases of diffuse cancer with at least one diagnosed earlier than 50 years of age; (2) families with three or greater cases of diffuse cancer diagnosed at any age; (3) any patient diagnosed with diffuse cancer before age 35; (4) patients with concurrent diagnoses of diffuse gastric cancer and lobular breast carcinoma (due to the role of *CDH1* loss in the pathogenesis of lobular carcinoma); and (5) families with one case of diffuse gastric carcinoma and at least one other case of either lobular breast carcinoma or signet cell carcinoma of the colon [9].

3.4 Prognostic Factors

As with many cancers, one of the most important prognostic factors in gastric adenocarcinoma is tumor stage at resection. Other general predictors of favorable prognosis are negative margin status at resection, benign lymph node status, female gender, high socioeconomic status, Hispanic race, intestinal-type histology, tumors originating in the fundus/body/antrum (vs. gastric cardia), age younger than 70 years, absence of venous or lymphatic invasion, carcinoembryonic antigen (CEA) less than 10 ng/mL, and CA19-9 less than 37 μg/mL [30, 31]. Microsatellite instability, present in approximately 15 % of gastric cancers, has also been shown to confer a favorable prognosis [5].

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Pathologic Features of Miscellaneous Foregut Malignancies

Eduard Matkovic, Michael Schwalbe and Kristina A. Matkowskyj

Abstract

In addition to tumors arising from the primary mucosal epithelium, the foregut is host to a variety of non-epithelial precursor cells which may give rise to neoplasms of neuroendocrine, mesenchymal, and hematolymphoid lineages. Many of these lesions also occur outside of the gastrointestinal tract, such as the extranodal lymphomas and many of the sarcomas, and in many cases share the features of their non-alimentary counterparts. This heterogeneous collection of malignancies features a wide spectrum of clinical presentations, morphologic and histopathologic features, genetic underpinnings, and treatment considerations. Although encountered less frequently than primary carcinomas, it is important to correctly recognize and classify these lesions to effectively manage and prognosticate the patients in which they occur. In this chapter, we focus on the clinical, morphologic, and genetic features of the primary esophageal and gastric neoplasms of neuroendocrine, mesenchymal, and lymphoid origin.

Keywords

Neuroendocrine tumor · Mixed adenoneuroendocrine carcinoma · Gastrointestinal stromal tumor · Synovial sarcoma · Leiomyosarcoma · Rhabdomyosarcoma · Melanoma · Lymphoma

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1 Neuroendocrine Neoplasms

Neuroendocrine neoplasms are tumors that arise from cells of endocrine cell system. Neoplasms with neuroendocrine differentiation include well-differentiated neuroendocrine tumors (NET), poorly differentiated neuroendocrine carcinomas (NEC), and mixed adenoneuroendocrine carcinomas (MANEC). One of the major problems in management of neuroendocrine neoplasms of the digestive system is the lack of universally accepted standards, both for nomenclature and for staging [29]. Recently, NET and NEC have been classified according to grade, which is determined based on the degree of cellular differentiation (morphology) and proliferative activity [33]. Well-differentiated neoplasms have less proliferative activity, characterized by low mitotic counts and a low Ki67 proliferative index by immunohistochemical staining (Ki67 highlights cells in the active phases of the cell cycle). Conversely, poorly differentiated neoplasms have a higher proliferative activity (Table 1). MANEC is a neoplasm with both neuroendocrine and malignant glandular components. Arbitrarily, two major diagnostic criteria must be met: (1) at least 30 % of each component should be identified and (2) the histologic features of the neuroendocrine component reveals a well-differentiated organoid or diffuse growth pattern [37]. Reasons that have hampered a unified classification system include difficulties understanding the tumors unpredictable behavior, complexities in the clinical-pathological correlation, and the widespread use of the term "carcinoid," with its incorrect benign connotation. In order to bridge the classification gap, the WHO [5] combined two complementary classification tools—a grading and a site-specific staging system.

1.1 Clinical Features

Esophageal neuroendocrine neoplasms are typically located in the distal third of the esophagus, and gastric NET are all located in the mucosa at the body-fundus or body-antrum border. Esophageal neuroendocrine neoplasms are exceptionally rare and account for less than 1 % of malignant esophageal neoplasms [15], with the majority being neuroendocrine carcinomas [16]. It is most commonly presents in males (6:1 male-to-female ratio) with a wide age range, between 40 and 90 years [21].

Table 1 WHO Nomenclature and classification of neuroendocrine neoplasms

Classification	Mitotic rate	Ki67 proliferative index (%)
Well-differentiated neuroendocrine tumor (NET), Grade 1	<2 mitosis per 10 HPF[a]	<2
Well-differentiated neuroendocrine tumor (NET), Grade 2	2–20 mitosis per 10 HPF[a]	3–20
Poorly-differentiated neuroendocrine carcinomas (NEC), Grade 3	>20 mitosis per 10 HPF[a]	>20

[a]HPF = high-power field (or 40× objective)

In the esophagus, symptoms are similar as with other esophageal cancers, dysphagia, pain, or blood in stool/hematemesis. Rare reports of paraneoplastic syndrome have been described in the literature, which include inappropriate secretion of antidiuretic hormone (SIADH) and hypercalcemia [9]. The tumor is of neuroendocrine differentiation and believed to arise from a multipotential cell or a Merkel cell in the squamous epithelium. Patients with esophageal neuroendocrine neoplasms often have a history of smoking and frequently report co-existing Barrett's esophagus [34].

With superior diagnostic technology, the time trend has revealed an increase incidence (11 %) of gastric NET as compared to the past (2–3 %), and the majority of these tumors are nonfunctioning enterochromaffin-like cell neuroendocrine tumors. The cause of neuroendocrine neoplasms is relatively better understood in the stomach than in NETs found in the esophagus. Gastrin has a trophic effect on enterochromaffin-like cells found in the stomach. Long-standing hypergastrinemia, resulting from either unregulated release by a gastrinoma or secondary to achlorhydria (due to chronic *H. pylori* infection or autoimmune chronic atrophic gastritis), is consistently accompanied by enterochromaffin-like cell hyperplasia. Consistent trophic stimulation in unison with chronic inflammation can potentially advance neuroendocrine hyperplasia further into neoplasia. The clinical presentation for gastric neuroendocrine neoplasms varies according to etiology. Patients can present with a chronic gastritis with a distinctive achlorhydria and hypergastrinemia. Autoimmune gastritis will less frequently present with pernicious anemia. The type of neuropeptide secreted from the neoplastic cell determines the physiological response. Gastrin secreting tumors, as in Zollinger–Ellison syndrome (ZES), result in parietal hyperplasia and hyperacidity, which leads to symptoms of peptic ulcer disease. Carcinoid syndrome occurs in cases with extensive liver metastasis that cause the release of histamine and 5-hydroxytryptophan, resulting in diarrhea, flushing, and restrictive cardiomyopathy.

In general, well-differentiated NETs are mostly indolent with rare lymph node spread, and have an excellent prognosis. Poorly differentiated NEC are invariably high grade, and have an aggressive nature with a short overall survival. Prognosis is largely dependent on stage and tumor type. Due to the rarity of MANEC, the clinical behavior of these tumors is still unclear. Both components are malignant and should be evaluated accordingly. Clinical judgment should focus on the more aggressive cell type [37]. Due to the rarity of esophageal NETs there is no proposed TNM staging classification (or even treatment protocol); consequently, the TNM staging system for esophageal carcinomas is currently employed for practical purposes.

1.2 Pathologic Features

Well-differentiated NETs are characterized grossly by small polypoid lesions, which can ulcerate and even hemorrhage with increasing size. Cytologically, these neoplasms retain similarities to normal gastrointestinal endocrine cells, characterized by small blue cells with scant granular cytoplasm and the classic speckled chromatin, often described in the literature as having a "salt and pepper" appearance.

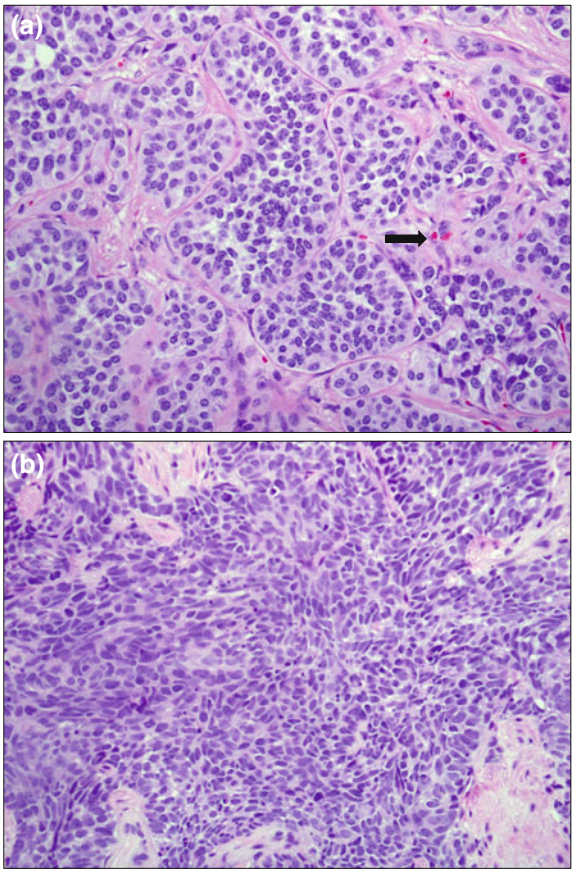

Fig. 1 Neuroendocrine tumor (NET). **a** Well-differentiated neuroendocrine tumor, Grade 1, reveals nests of monotonous cells with characteristic "salt and pepper" chromatin and delicate vascular stroma surrounding the nests (*arrow*). **b** Poorly-differentiated neuroendocrine carcinoma, Grade 3, with small, blue cells containing scant cytoplasm, indistinct cellular borders, focal nuclear contours that appear to mold to the shape of the adjacent cell, scattered mitotic figures and karyorrhectic debris

These neoplasms stain positive for classic neuroendocrine markers chromogranin and synaptophysin. Morphologically, the cells are arranged in nests surrounded by a delicate fibrovascular stroma (Fig. 1a). In contrast, poorly differentiated neuroendocrine carcinoma is composed of either a small cell or large cell component, which shows hyperchromatic nuclei with prominent nuclear molding and smeared chromatin (due to crush artifact), arranged in various patterns like solid sheets, cords or nests with mitosis and tumor cell necrosis being common (Fig. 1b).

1.3 Molecular Pathology

Information on genetic susceptibility is scant. However, a few abnormalities are associated with gastric NETs, the most relevant being the dominantly inherited MEN1 gene on chromosome 11q13 [2]. Patients with MEN1-associated ZES have a higher risk of developing gastric neuroendocrine neoplasms than in patients with sporadic ZES, despite equally elevated long-standing levels of gastrin [19].

2 Mesenchymal Tumors of Foregut

2.1 Gastrointestinal Stromal Tumor (GIST)

Gastrointestinal stromal tumors comprise the bulk of mesenchymal tumors of the gastrointestinal tract and occupy a spectrum of clinical behavior and histologic appearance that can range from benign to overtly malignant. They may occur at any location along the alimentary canal, but most commonly arise in the stomach (60–70 %) and the small intestine and more rarely occurring in the esophagus, rectum, appendix, gallbladder, pancreas, mesentery, and retroperitoneum. GISTs may occur across a wide age range, but the majority affect older patients with a median age of 63 years [26]. Malignant clinical behavior is seen in approximately 30 % of GISTs; local recurrence, peritoneal spread, and liver metastases comprises a common course for the disease [7, 24, 25]. Generally accepted prognostic factors for GISTs include patient age, tumor site, mitotic rate, nuclear atypia, tumor cellularity, tumor size, necrosis, and invasive growth pattern (Table 2) [6, 13, 24, 25, 36].

Prior to their immunohistochemical and molecular characterization, GISTs were historically labeled as various other tumors of smooth muscle or neurogenic origin, namely leiomyomas, leiomyosarcomas, and schwannomas. It was not until the early 1990s that the GIST moniker became accepted with the observation that these tumors were largely CD34 positive by immunohistochemistry. It is now widely recognized that GISTs arise from the interstitial cells of Cajal or their stem-cell precursors, which are specialized "pacemaker" cells found within the myenteric plexus of the GI tract. "Interstitial cell of Cajal-like" cells have also been identified in the omentum, which may account for the occurrence of primary GISTs outside of the gastrointestinal tract proper.

Table 2 Risk assessment for primary gastrointestinal stromal tumors

Tumor features	Risk of metastasis or tumor-related death (%)				
Mitotic rate	Size	Gastric	Duodenum	Jejunum/Ileum	Rectum
≤5 per 50 high-power fields (HPF)	≤2 cm	0	0	0	0
	>2 to ≤5 cm	1.9	8.3	4.3	8.5
	>5 to ≤10 cm	3.6	Insufficient data	24	Insufficient data
	>10 cm	10	34	52	57
>5 per 50 HPF	≤2 cm	0*	Insufficient data	High*	54
	>2 to ≤5 cm	16	50	73	52
	>5 to ≤10 cm	55	Insufficient data	85	Insufficient data
	>10 cm	86	86	90	71

An asterisk (*) denotes a small number of cases

2.1.1 Gross Pathology

Macroscopically, most GISTs appear as spherical or ovoid nodules with variable lobulation arising within the wall of the GI tract. They may also grow as polypoid lesions with or without ulceration, attached to the gut wall by either a wide base or narrow pedicle (Fig. 2a). They may also present on the serosal surface as small nodular tumors to large hemorrhagic masses. The cut surface is solid and pink, yellow, tan, or gray in color with variably cystic or necrotic components. Benign lesions are generally small and firm, while malignant tumors are usually larger with a softer and "fleshier" consistency [26].

2.1.2 Microscopic Pathology

Histologically, GISTs demonstrate a wide array of morphology, sometimes with features reminiscent of smooth muscle or neurogenic differentiation. Broadly, GISTs are histologically classified into spindle cell type (most common), epithelioid type (20–25 %), and mixed type [22, 23]. Spindle-type GISTs are comprised of whorls and fascicles of spindle-shaped cells with blunted nuclei (Fig. 2b). The cytoplasm is usually pale pink to eosinophilic and fibrillary, and tumors with features of smooth muscle differentiation may display intracytoplasmic vacuoles which abut the nucleus

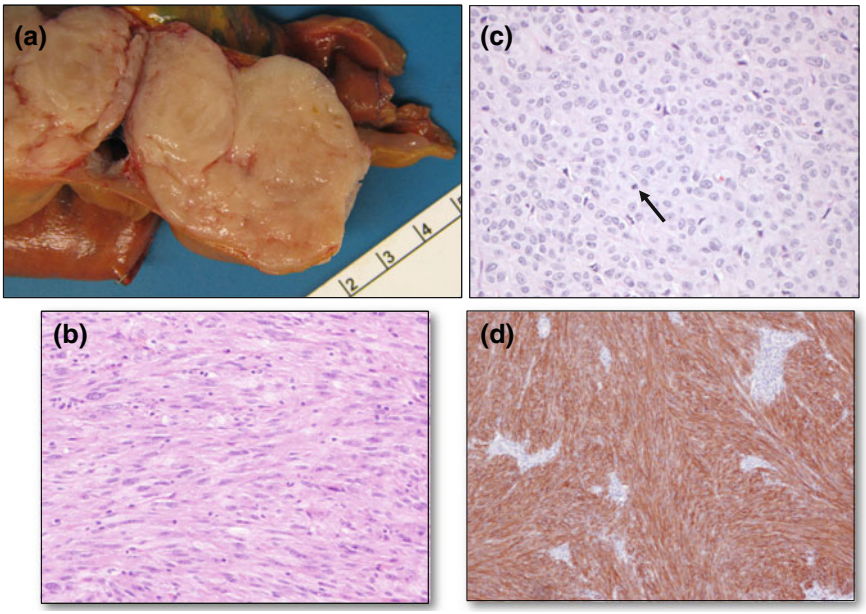

Fig. 2 Gastrointestinal stromal tumor (GIST). **a** This tumor was attached to the mucosa by a thin pedicle and bisecting the tumor reveals a lobulated, tan-yellow, fleshy cut surface. **b** The tumor is composed of spindle cells with elongated nuclei, open chromatin pattern, focal perinuclear clearing and mild nuclear pleomorphism. **c** Epithelioid GISTs have round to polygonal cells with well-defined cell borders and centrally located nuclei. A rare mitotic figure is seen (*arrow*). **d** Immunohistochemistry for CD117 (c-kit) is strongly and diffusely positive in the cytoplasm of the tumor cells

on either or both ends. Nuclear palisading may be seen, in which nuclei align in a side-by-side fashion as is commonly seen in schwannomas. The appearance of the stroma can be quite variable, ranging from collagenous and sclerotic to myxoid and edematous. The epithelioid variant cytologically resembles an epithelial tumor with cells that are polygonal or round with well-defined cell borders and a somewhat centrally placed, round nucleus (Fig. 2c). Pleomorphism and mitotic rate can vary widely, and should be taken into consideration with other features when assessing malignant potential. Immunohistochemically, GISTs are classically positive for CD117 (c-kit) (Fig. 2d), although this marker is non-specific. Recently, an immunohistochemical stain for the protein product of the gene *DOG1* ("discovered on GIST") has been shown to be fairly sensitive and specific for GIST. Other immunohistochemical markers that may be positive include CD34, smooth muscle actin, desmin, S100, and cytokeratins 8 and 18 [24–26, 28].

2.1.3 Molecular Pathology

Recently, oncogenic mutations in the genes for the tyrosine kinase receptors KIT and PDGFRA have been characterized in the majority of GISTs. The presence of these driver mutations has led to the use of tyrosine kinase inhibitors as adjuvant treatment to surgery in many patients [7, 24, 25]. However, the majority (85 %) of pediatric GISTs and 15 % of GISTs occurring in adults are negative for either of these mutations. In some of these cases, oncogenesis may be attributed to inactivation of the succinate dehydrogenase (SDH) complex, which is a hetero-oligomeric enzyme complex of the citric acid cycle composed of subunits A-D [18]. Carney–Stratakis syndrome, which is a clinical dyad of GIST and paragangliomas, is genetically characterized by autosomal dominant germline mutations in SDHB, -C, or -D. This is not to be confused with the Carney Triad that includes GIST, paraganglioma, and pulmonary chondroma [35]. Mutations in SDH are also found in 12 % of patients with no family history of hereditary GIST or paraganglioma and wild-type *KIT* and *PDGFRA* genes [18].

2.2 Synovial Sarcoma

Synovial sarcomas are malignant mesenchymal tumors that frequently occur in association with tendons and bursae of large joints, particularly the knee, in young and middle-aged adults. However, a handful of cases of synovial sarcomas arising in the upper GI tract have been reported. Originally described as "synovial endothelioma" by Lejars and Rubens-Duval in 1910 [12], it has since been determined that synovial sarcoma is related to native synovium in name only, and demonstrates markedly different immunohistochemical and ultrastructural features than its namesake [27]. It is generally regarded as a high-grade sarcoma, and 5- and 10-year survival rates range from 24 to 68 % and 11–56 %, respectively. Prognostically, several features predictive of positive outcome have been identified, including young age (<40 years), small tumor size (<4 cm), exuberant tumoral calcification, peripheral location, and increased intratumoral mast cells. Negative

prognostic indicators include SYT-SSX1 translocation [t(X;18)], poorly differentiated histology, necrosis, high proliferative index, and vascular invasion [4, 20].

2.2.1 Pathologic Features

Synovial sarcomas are typically infiltrative masses with a soft, friable, brown to gray cut surface [12]. In the GI tract, synovial sarcoma may present as a plaque-like mucosal mass that can progress to transmural involvement [20]. Histologically, they may appear as a monophasic proliferation of elongated to blunt spindle cells arranged in a haphazard or storiform pattern (Fig. 3a). The nuclei are often bland and may overlap; mitoses range from inconspicuous in low-grade lesions to prominent in more high-grade specimens. Curiously, a biphasic type has also been characterized in which a portion of the neoplastic cells assume an epithelioid arrangement and line cystic and gland-like spaces (Fig. 3b). Two main variants of the X;18 translocation (termed SYT-SSX1 and SYT-SSX2) have been shown to correlate with histologic appearance; SYT-SSX1-positive tumors are frequently biphasic in appearance, while monophasic tumors are typically SYT-SSX2-positive [4]. Immunohistochemically,

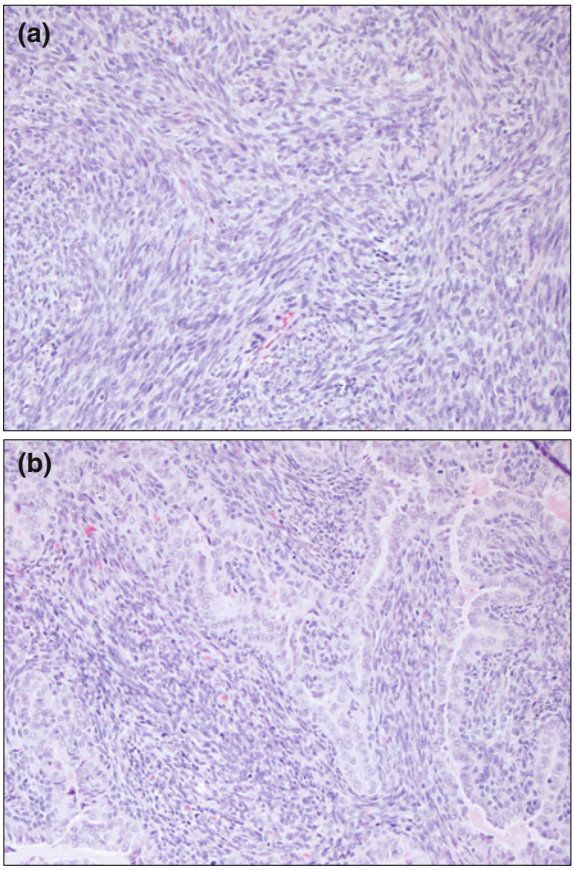

Fig. 3 Synovial sarcoma. **a** Low-grade tumors are composed of bland, elongated spindle cells with tapered nuclei growing in fascicles and whorls. **b** Biphasic synovial sarcomas are characterized by a conventional spindle cell component and a population of epithelioid cells that form gland-like and cystic spaces within the tumor

synovial sarcomas express epithelial membrane antigen (EMA) and cytokeratins and are negative for CD34, separating them from GISTs which are the primary differential consideration in this location [12, 20]. Identification of SYT-SSX translocation by FISH, karyotyping, and other molecular techniques can serve both a diagnostic and prognostic function in high-grade synovial sarcomas [4].

2.3 Leiomyosarcoma

Leiomyosarcoma is a malignant smooth muscle neoplasm usually seen in elderly patients, but can occur at any age. Although rare, it is the most commonly identified sarcoma of the esophagus. Clinical presentation is that of an enlarging, polypoid mass resulting in dysphagia. The morphological features are similar to those occurring in other areas of the body, with histological resemblance to smooth muscle (fascicles of spindle cells with cigar shaped nuclei). Leiomyosarcoma needs to be differentiated from the benign leiomyoma. Nuclear atypia, increased mitotic activity, and necrosis should sway the pathologist toward a malignant process. Like all smooth muscle tumors, immunohistochemistry should show positivity for smooth muscle actin (SMA) and desmin. Prognosis of leiomyosarcoma of the esophagus has been disheartening.

2.4 Rhabdomyosarcoma

Rhabdomyosarcoma is an exceedingly rare neoplasm in this location, as only 15 cases have been reported in the literature [3]. There are three variants—embryonal, alveolar, and pleomorphic—and most of the documented case where of embryonal type and have occurred in the distal esophagus of older adults [22, 23]. The presence of pleomorphic malignant cells with intracytoplasmic cross striations is a characteristic feature of pleomorphic rhabdomyosarcoma [3]. The embryonal variant consists of irregular spindle cells seen in a myxoid stroma. The alveolar variant is characterized by monomorphic, hyperchromatic, round cells which cling to fibrovascular septa. Immunohistochemical positivity for myogenin and/or MyoD1 nuclear reactivity, and expression of desmin and muscle specific actin (MSA) is diagnostic of rhabdomyosarcoma [14]. Associated cytogenetic characteristics for alveolar rhabdomyosarcoma involves the PAX3 or PAX7-FKHR fusion involving chromosome 13 [32]. Risk stratification for rhabdomyosarcoma is based on histology, patient age, tumor stage, and site of origin.

3 Melanoma

The squamous epithelium of the esophagus contains melanocytes, which can give rise to malignant melanoma, an uncommon entity found in mid or lower esophagus. The malignancy principally shows predilection in men over the age of 50 years [8].

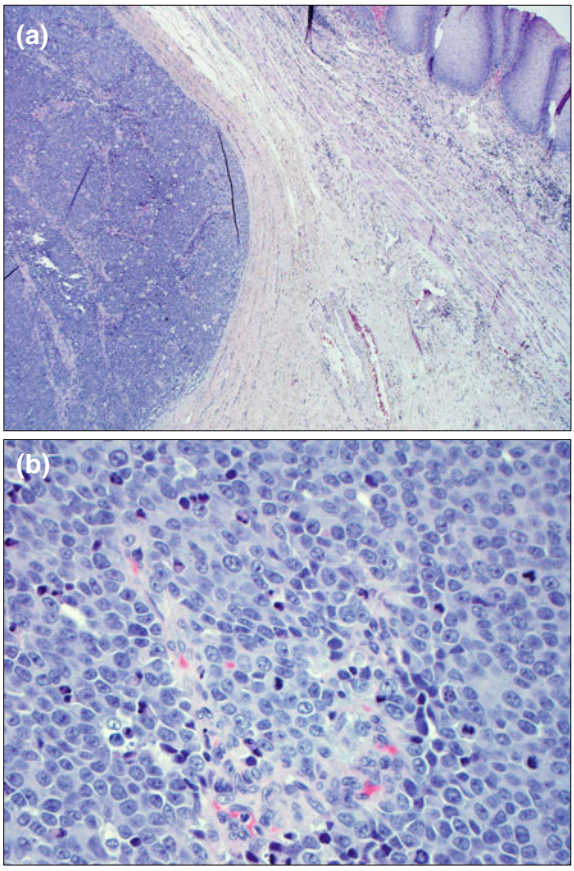

Fig. 4 Esophageal melanoma. **a** Low-power view reveals a basophilic (*blue*) tumor with extension into the submucosa. **b** The neoplastic cells reveals epithelioid features with marked nuclear pleomorphism, prominent nucleoli, abundant cytoplasm, and numerous mitotic figures

Grossly the tumor presents as a pigmented mass protruding into the lumen. Microscopically, the tumor shows wide variation in morphology, most common variants include epithelioid or spindle cells composed in sheets or nests (Fig. 4a, b). The adjacent epithelium reveals an in situ component confirming the tumor is in fact a primary malignancy. Melanocytic markers including HMB-45, MART-1, and S-100 can be diagnostically helpful in difficult cases. These tumors are highly aggressive with poor prognosis as the majority of patients die within 2 years of diagnosis [10, 17].

4 Lymphoma

Approximately 30–40 % of extra-nodal lymphomas involve the gastrointestinal tract, with the stomach being the most common site of involvement (50–75 %) [30]. Esophageal involvement is rare. Primary gastric lymphomas are primarily of the non-Hodgkin type; as a category these tumors are the second most common

primary gastric malignancy following adenocarcinoma [11]. Gastric lymphomas are almost exclusively of B-cell origin, with 30–60 % being marginal zone lymphomas of mucosa-associated lymphoid tissue (commonly referred to as MALT lymphomas) [30]. The remainder is comprised largely of diffuse large B-cell lymphomas (DLBCL) as well as rare cases follicular lymphoma, mantle cell lymphoma, and peripheral T-cell lymphoma [11]. In the case of MALT-type lymphomas, infection with *H. pylori* is a well-characterized risk factor; adequate treatment of the infection results in complete regression of lymphoma in some cases [31]. It is suggested in the literature that a portion of gastric DLBCLs may evolve from low-grade MALT lymphomas [38], evidenced by the concurrent presence of low-grade MALT lesions in some cases. Furthermore, some studies have shown that DLBCLs with a MALT lymphoma component may also regress following eradication of *H. pylori* [11]. In cases in which complete regression is not achieved with antimicrobial therapy, conventional chemotherapy regimens are warranted. Surgery is generally reserved for a select subset of patients [38].

4.1 Gross Pathology

Gastric lymphomas display a heterogenous appearance endoscopically. Grossly, the lesions may be undetectable or exhibit subtle findings such as small nodules, superficial erosions, petechial hemorrhage, or mucosal thickening. Alternatively, they may present endoscopically with obviously malignant features, such as large ulcerations and irregular or exophytic masses [38]. Lymphomas demonstrate a cut surface that is gray to yellow with a soft consistency that is characteristically compared to "fish flesh."

4.2 Microscopic Pathology

Microscopically, MALT lymphomas are generally comprised of sheets or nodules of small- to medium-sized neoplastic lymphocytes with irregular nuclei (Fig. 5a). The cytoplasm is generally pale and may be slightly voluminous; MALT lymphomas with exaggerated cytoplasm often impart a "monocytoid" appearance to the infiltrate. They may be found involving or seemingly extending from the mantle zones of preexistent reactive lymphoid follicles, with subsequent effacement of follicles and the native gastric mucosal elements in advanced lesions. The signature feature of MALT lymphoma is the so-called "lymphoepithelial lesion," in which clusters of neoplastic lymphocytes infiltrate native epithelial structures such as glands and crypts. In these areas, the epithelium shows degenerative changes such as cellular swelling, eosinophilia, and apoptosis.

Immunohistochemically, there are no unique markers to MALT lymphomas, which usually display pan-B molecules (CD19, CD20, CD79a) and marginal zone antigens (CD21, CD35). They are usually negative for CD5, CD10, and cyclin D1 [1]. Diffuse large B-cell lymphomas, in contrast, feature predominantly large and

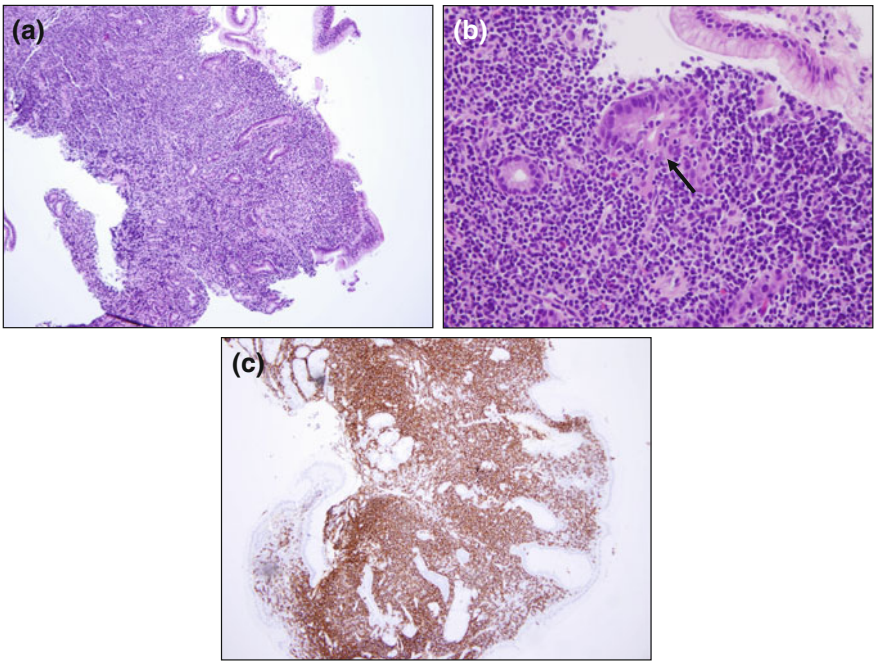

Fig. 5 Mucosa-associated lymphoid tissue (MALT) lymphoma. **a** There is marked expansion of the gastric lamina propria by a homogeneous population of small, bland-appearing lymphocytes. **b** The native gastric epithelium becomes infiltrated by neoplastic lymphoctyes (*arrow*), demonstrating the characteristic "lymphoepithelial lesion" of MALT lymphoma. **c** Immunohistochemistry for CD20 confirms the B-cell lineage of the neoplastic lymphocytes

atypical-appearing lymphocytes which demonstrate sheet-like effacement of the mucosa. In addition to expression of pan-B-cell antigens, CD10, BCL-6, and MUM-1 may be variably expressed and are useful in differentiating the subtype of DLBCL and determining prognosis [30].

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Management Controversies and Treatment Strategies for Borderline Resectable Pancreatic Cancer

Mark S. Talamonti

Abstract

The management of borderline resectable cancer requires multi-disciplinary care including state-of-the-art radiographic imaging, combination treatment planning with medical oncology and radiation oncology, and technical surgical expertise combining gastrointestinal and vascular surgery.

Keywords

Borderline resectable pancreatic cancer · Portal vein resection · Reconstruction · Pancreaticoduodenectomy · Gemcitabine · FOLFURINOX

1 Introduction

Adenocarcinoma of the pancreas continues to be a daunting clinical challenge, with approximately 42,000 deaths per year in the United States [31]. It is a disease characterized by its late presentation, rapid demise thereafter, and until recently, relatively ineffective systemic therapies. Despite this grim prognosis, appreciable progress has been made in the identification of patients with localized disease who may be candidates for potentially curative resections and in the understanding of the technical nuances and efficacy of aggressive surgical procedures. Currently, the

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overall 5-year survival rate is 15–25 % for patients who undergo resection and receive adjuvant chemotherapy with or without chemoradiation therapy [29, 32].

Borderline resectable pancreatic head cancer represents a relatively new classification for patients with intermediate tumors between those that are well-localized with no radiographic evidence of significant mesenteric vascular involvement and those considered to have locally advanced and technically unresectable disease based on the inability to safely perform a vascular resection and reconstruction of the vital blood vessels [36]. Traditional clinical and radiographic classifications of pancreatic head tumors have consisted of localized cancer with no evidence of metastatic disease and no evidence of mesenteric venous or arterial involvement (Fig. 1), locally advanced disease in which there is no evidence of metastatic disease but the extent of vascular involvement was thought to preclude a safe and complete resection of local disease, and finally, patients with clear evidence of peritoneal or visceral metastases. With advances in 3-dimensional imaging and improved operative techniques resulting in decreased morbidity and mortality of superior mesenteric/ portal venous resections (SMV/PV) and limited arterial resections, the term "borderline resectable pancreatic cancer" has evolved. Surgical resection of these tumors is likely to require major vascular resection and reconstruction and oftentimes these vascular resection margins will demonstrate microscopic extension to within a millimeter of the transection (R1 resections). Whether the patient with a borderline resectable tumor should undergo a surgery-first approach, followed by adjuvant therapies, versus a neoadjuvant course of combined modality therapy preceding an attempt at surgical resection is currently one of the most controversial topics in pancreatic surgery [14]. Because of the high likelihood of a margin-positive resection and the potential for early tumor recurrence, neoadjuvant strategies employing chemotherapy with and without radiation therapy have been used in an attempt to "down-stage" tumors to margin-negative resections and to biologically select those patients who develop progressive disease while on neoadjuvant therapy and can then be spared the morbidity of surgery. Furthermore, large randomized clinical trials in the United States and Europe have demonstrated survival benefits for multi-modality therapy compared to surgery alone for resected cancers [20–22]. While not specifically designed to address the issue of treatment sequencing for borderline resectable tumors, combined modality therapy for resectable pancreatic cancer has become the current standard of care. The theoretical advantages of a neoadjuvant approach to these high-risk borderline tumors are rational and logical; however, the potential increase in operative complications and the delay in surgical resection and subsequent adjuvant treatments are legitimate concerns and have served to heighten the current controversy. This chapter describes the current radiographic definition of borderline resectable pancreatic head cancer, outlines the potential benefits of a neoadjuvant strategy for these tumors, reviews the results of existing series and trials employing neoadjuvant therapy relative to postoperative therapy, and describes some of the technical challenges and considerations when vascular resection and reconstruction are done at the time of surgery.

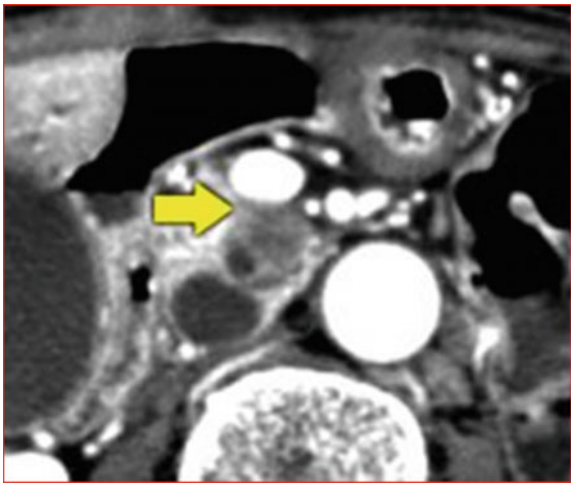

Fig. 1 Localized pancreatic cancer with no evidence of significant mesenteric venous abutment or distortion and normal fat planes between the tumor and superior mesenteric artery

2 Radiographic Staging and Definition of Borderline Resectable Pancreatic Head Cancer

From a surgical prospective, the first objective in the management of suspected or confirmed pancreatic cancer is to determine the potential for resection. Routine exploratory laparotomy for the purpose of operatively determining resectability has been diminished by modern three-dimensional radiographic imaging along with effective and sustainable nonoperative methods of palliation. Contrast-enhanced computed tomography (CT) accurately predicts resectability in 80–90 % of patients [26, 34, 37] Careful correlation between preoperative CT findings and surgical results has better defined CT criteria for resectability. The critical aspects that need to be evaluated in a thorough radiographic assessment are: the presence or absence of peritoneal or hepatic metastases; the patency of the superior mesenteric vein (SMV) and portal vein and the relationship of these vessels and their tributaries to the tumor; the relationship of the tumor to the superior mesenteric artery, celiac axis, hepatic artery, and gastroduodenal artery; and the presence of any aberrant vascular anatomy [5]. Unequivocal radiographic findings contraindicating resection include distant metastases, major venous thrombosis of the portal vein or superior mesenteric vein extending for several centimeters, and circumferential encasement of the superior mesenteric, celiac, or proximal hepatic arteries [5] (Fig. 2).

It is important to emphasize the borderline resectable classification is a radiographic determination using precise 3-dimensional imaging and applying defined criteria to categorize the extent of mesenteric and portal venous and arterial involvement. Early studies on neoadjuvant therapy for resectable cancers were flawed by the use of suboptimal scanning technology and limited vascular imaging. More recent studies are limited by the lack of a universally accepted definition of borderline resectable cancer. Two major definitions have been proposed. The MD

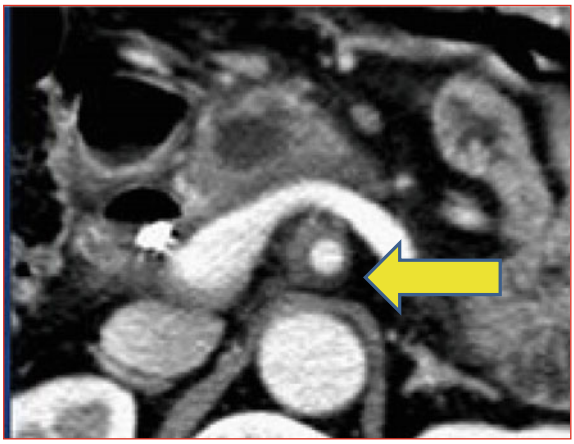

Fig. 2 Locally advanced pancreatic cancer with circumferential encasement of the superior mesenteric artery

Anderson anatomic definition differs from that proposed by the American Hepato-Pancreatico-Biliary Association (AHPBA), Society of Surgical Oncology (SSO), and Society for Surgery of the Alimentary Tract (SSAT) in terms of mesenteric vein involvement [5, 36]. Minimal abutment without distortion of the SMV or PV is considered potentially resectable in the MD Anderson criteria while the AHPBA/SSO/SSAT definition considers any vein involvement that may require even a small vein resection as borderline resectable. The International Study Group of Pancreatic Surgery (ISGPS) recently published a consensus statement to address these differences on the definition and subsequent treatment recommendations for borderline resectable cancers [4]. In an attempt to reconcile these subtle but important differences and to underscore the importance of accurate assessment of arterial involvement, the ISGPS has recommended the adoption of the following definitions when reporting institutional series outcomes and when designing future clinical trials.

- Determination of borderline resectability should be done using a specialized pancreatic protocol and a multidetector CT with high-resolution, multiplanar reconstructions.
- The radiographic findings supporting the designation of a borderline tumor in the head of the pancreas are: venous distortion of the SMV/portal venous axis (Fig. 3) even including short-segment venous occlusion with proximal and distal sufficient vessel length allowing safe reconstruction (Fig. 4); encasement of the gastroduodenal artery up to the hepatic artery, with either short-segment encasement or direct abutment of the hepatic artery without extension to the celiac axis; and tumor abutment of the SMA but with no greater than 180° of the vessel wall circumference (Fig. 5).

Ongoing clinical trials now use these definitions and patients are deemed borderline resectable and included in the study group only after central review of the CT scans.

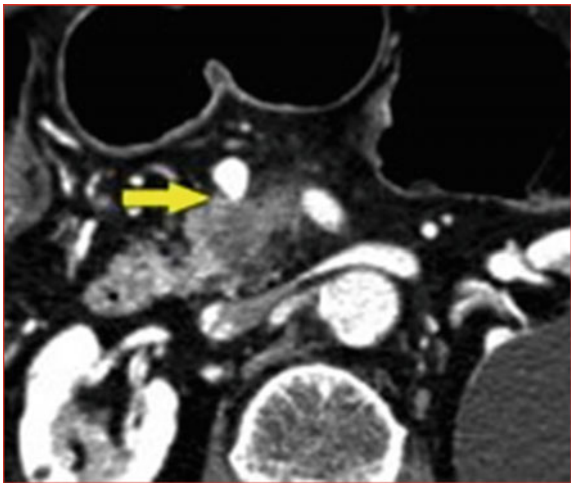

Fig. 3 Borderline resectable pancreatic cancer with mesenteric venous distortion and "tear-drop sign" on the posterior margin of the superior mesenteric vein with minimal abutment of the superior mesenteric artery

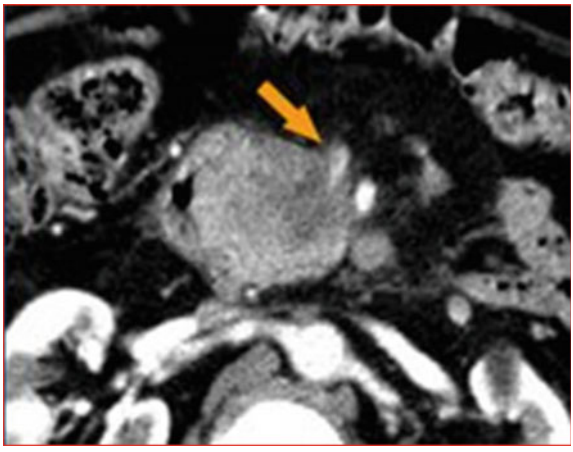

Fig. 4 Borderline resectable pancreatic cancer with short-segment superior mesenteric vein occlusion and 90° involvement of the superior mesenteric artery

With the development of neoadjuvant protocols for borderline resectable pancreatic cancer, preoperative tissue diagnosis is required to differentiate adenocarcinoma from neuroendocrine tumors and chronic pancreatitis. Endoluminal ultrasonography (EUS) is currently the procedure of choice for obtaining a tissue biopsy prior to the commencement of treatments. Furthermore, there is a theoretical oncologic advantage over percutaneous biopsy because the needle tract is contained in the eventual surgical specimen. The major limitation of EUS is the dependence on a skilled and experienced gastroenterologist to perform the procedure [30]. Endoscopic retrograde cholangiopancreatography (ERCP) has an important role in the management of patients with borderline resectable pancreatic cancers. Placement of a biliary stent preoperatively for decompression of biliary obstruction is required before initiation of preoperative therapy. In general, stents placed in

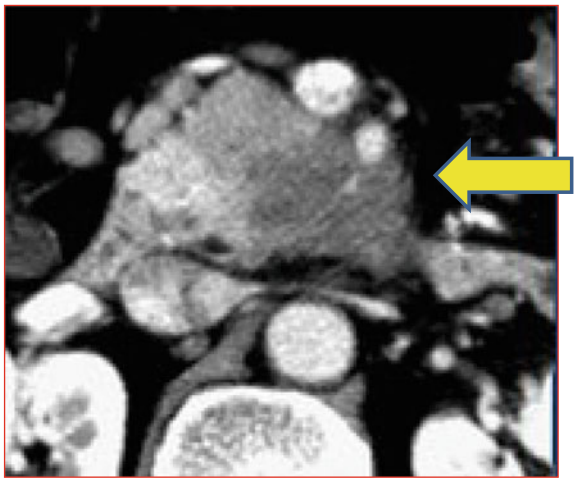

Fig. 5 Borderline resectable pancreatic cancer with sparing of the superior mesenteric vein but with 180° encasement of the superior mesenteric artery

patients with borderline resectable cancers should be short, covered metal stents to maintain patency during therapy and avoid treatment interruptions due to stent-related infections and occlusions. Occlusion of the cystic duct confluence should be avoided when possible to avoid subsequent cholecystitis [36].

3 Rationale and Current Evidence for Neoadjuvant Therapy for Borderline Resectable Disease

Clinical trials examining combined modality therapy for resected pancreatic cancer were justified given the high risk of systemic and locoregional recurrence following surgery alone. Early clinical trials of adjuvant therapy for resected patients were limited by their small size, lack of standardized patient entry criteria, and less than rigorous quality controls. Over the past decade, several adjuvant therapy trials were completed and have established a survival benefit for combined therapy versus surgery alone. The ESPAC-1 study, while criticized for its enrollment criteria, analytical design, and radiation therapy techniques, concluded that adjuvant chemotherapy with 5-FU administered for 6 months offered an overall survival advantage over no post-surgical therapy. Excluding patients who received any radiation, the patients receiving adjuvant chemotherapy alone had a median survival of 21.6 months compared to 16.9 months for the observation group [21]. An important modern adjuvant therapy study, noteworthy for its rigorous trial design, is the CONKO-001 trial, which compared six cycles of gemcitabine to observation alone after surgery in 354 patients [22]. Disease-free survival and overall survival were 6.9 and 20.5 months for the observation arm and 13.4 months ($p < 0.001$) and 24.2 ($p < 0.06$) months for the treatment arm. After the initial publication of this trial, gemcitabine became an accepted and standard recommendation for adjuvant therapy. The Radiation Therapy Oncology Group (RTOG) 97.04 study examined

the role of adjuvant chemotherapy combined with modern radiation planning and stringent treatment quality controls. A five-year update of the trial reported by Regine et al. [28] demonstrated a trend toward improved survival ($p = 0.08$) for those patients receiving adjuvant gemcitabine versus conventional 5-FU. More importantly, and more relevant to borderline tumors, the local failure rates were 25 % for the gemcitabine arm and 30 % for the 5-FU arm; both markedly improved from earlier studies using lower doses of radiation and without contemporary 3-dimensional image planning, and data used to justify the inclusion of radiation therapy in most series examining neoadjuvant therapy for borderline tumors at high risk for local recurrence. Finally, Johns Hopkins University and Mayo Clinic recently reported large series of patients who had undergone surgical resection for pancreatic cancer and received postoperative 5-FU-based chemoradiation with a median dose of 50.4 Gy [7, 10, 11]. Both series found chemoradiation associated with improved survival and increased locoregional control compared to surgery alone. While these adjuvant trials and large institutional series do not specifically address borderline resectable cancers, collectively they provide justification to employ combined modality therapy for these high-risk tumors and serve as high quality standards for comparison purposes.

There are several theoretical and potential advantages of neoadjuvant therapy for borderline resectable disease [1]. These include the potential to decrease tumor volume such that borderline resectable disease may become more easily resectable and to sterilize the peripheral extent of tumor infiltration, resulting in fewer R1 resections and reducing locoregional recurrences. Patients who receive neoadjuvant therapy may be more likely to complete the full course of treatments since 20–30 % of patients undergoing resection may not complete adjuvant treatments due to postoperative morbidity and frailty [7, 10]. Lastly, and perhaps most importantly, patients who exhibit disease progression during neoadjuvant therapy self-select themselves as poor responders who are least likely to gain benefit from resection and may forgo the morbidity of pancreatic resection.

Despite these biologic considerations and clinical justifications, to date, there are no sufficiently powered randomized clinical trials that demonstrate significant improvements in local control rates or disease-free survival and overall survival rates for neoadjuvant therapy for resectable or borderline resectable cancers compared to a surgery-first approach. In most reports, patients were not randomized to a surgery-first versus neoadjuvant strategy, thereby interjecting obvious selection bias in any group comparisons. The determination of borderline resectable status was often done retrospectively using poorly defined or inconsistent criteria but clearly including patients ranging from localized disease to minimal venous distortion to those with significant arterial abutment and partial encasement. Neoadjuvant treatments were variable and not controlled for the use of radiation. Early studies employed 5-FU-based protocols while more recent investigations either added or replaced 5-FU with gemcitabine [1]. Surgical techniques for vascular resection and reconstruction and the dissection of the critical uncinate margin on the right lateral wall of the superior mesenteric artery are not reported or not standardized. And the currently recommended guidelines for standard pathology handling of the

specimens and critical margin determinations were not performed or poorly documented [13].

Prospective trials analyzing only patients with borderline resectable pancreas cancer are rare and limited by extremely small patient numbers, inconsistent inclusion criteria, and short follow-up times. The safest conclusions from these trials are that neoadjuvant therapy was relatively safe and associated with acceptable resection rates. Follow-up times are short, survival data are inconsistently reported, and conclusions regarding the effects on median survival and overall survival cannot be consistently determined. Marti et al. reported a phase I/II trial of induction gemcitabine and cisplatin followed by concurrent radiation in borderline resectable disease with 4 of 26 patients (15 %) undergoing resection [18]. Median survival was 13 months. A randomized phase II trial comparing two different gemcitabine-based protocols in borderline resectable disease was terminated early due to poor accrual, but toxicities were considered acceptable and 5 of 21 patients (24 %) underwent resection [16]. Kim et al. reported a recent multi-institutional phase II trial using full-dose gemcitabine, oxaliplatin, and radiation, and included 39 patients with borderline resectable disease. The overall resection rate was 63 % and the R0 resection rate was 53 % in the borderline resectable group [15].

Single institution reports represent by far the largest number of reports examining neoadjuvant therapy for borderline resectable cancers. In the largest, 84 patients with anatomically borderline resectable tumors were treated at MD Anderson with 5-FU- or gemcitabine-based chemoradiation (Group A), typically preceded by systemic chemotherapy prior to planned resection [14]. Of this group, 38 % underwent resection and 97 % of these had R0 resections. The median survival of all patients was 21:40 months for resected patients and 15 months for patients who did not undergo resection. The authors emphasized that two additional subsets of patients exist; those with questionable metastatic disease (Group B) and those who display either a suboptimal performance status or have prohibitive medical comorbidities and who are not initially surgical candidates (Group C). All 160 patients received either chemotherapy, chemoradiation or both. This primarily consisted of either 50.4 Gy or 30 Gy of EBRT with radiosensitizing doses of 5-FU, paclitaxel, gemcitabine, or capecitabine. Resection rates following neoadjuvant therapy were 38, 50 and 38 % in groups A, B, and C, respectively. Furthermore, resected patients in groups A, B and C exhibited 40/29/39 month median survivals compared with 15/12/13 month median survivals for unresected patients. This important study certainly does not demonstrate an obvious benefit of one treatment regimen versus another, but does clearly make two salient points. First, patients with stringently defined borderline resectable disease who respond to neoadjuvant therapy and undergo definitive surgical therapy have a significant survival advantage compared to patients with unresected disease. Second, and perhaps more provocatively, there are further subsets of patients, not encompassed by traditional AJCC criteria, who may equally benefit from a trial of neoadjuvant chemo- or chemoradiation therapy.

Patel et al. prospectively examined 17 patients with borderline disease treated with gemcitabine-docetaxel-capecitabine induction chemotherapy and 5-FU-based chemoradiation. Resections were successful in 64 and 89 % had an R0 resection [24]. Stokes et al. prospectively examined 40 borderline resectable cases treated with capecitabine and concurrent radiation. The resection rate was 40 %, the R0 resection rate was 88 % and the reported median survival was 23 months [33]. McClain et al. reported 26 borderline resectable patients treated at the University of Cincinnati who completed neoadjuvant chemotherapy (gemcitabine) and then underwent exploration. Surgical resections were completed in 12 patients (46 %) with 67 % R0 resections and a median survival in the resected patients of 23.3 months [19].

Systematic reviews and meta-analyses of neoadjuvant therapy for pancreatic cancer have been performed but recommendations from these reviews are limited by the inconsistencies of the individual studies and the variability of the treatment schemes. Assifi et al. reviewed a total of 14 phase II clinical trials including 536 patients with resectable and/or borderline resectable disease [2]. After treatment, resectability was 65.8 % (95 % CI, 55.4–75.6 %) in patients with localized, resectable tumors compared with 31.6 % in patients with borderline disease (95 % CI, 14.0–52.5 %). A partial response was observed in patients with borderline/unresectable tumors; 31.8 % (95 % CI, 24.2–39.8 %) compared to 9.5 % (95 % CI, 2.9–19.4 %) in the resectable group ($p = 0.003$). Progressive disease was seen in 17.0 % (95 % CI, 11.9–22.7) of patients with resectable tumors versus 21.8 % (95 % CI, 10.1–36.5 %) in the borderline group ($p = 0.006$). Median survival in resected patients was 23 months for the resectable group and 22 months for borderline patients [2]. Laurence et al. reviewed prospective trials and retrospective series with a focus on complication rates, surgical morbidity and survival [17]. The meta-analysis found that patients with "unresectable" (criteria not well-described) pancreatic cancer who underwent neoadjuvant chemoradiotherapy achieved similar survival outcomes to patients with resectable disease, even though only 40 % were ultimately resected. Neoadjuvant chemoradiotherapy was not associated with a statistically significant increase in the rate of pancreatic fistula formation or total complications. Patients receiving neoadjuvant chemoradiotherapy were less likely to have a positive resection margin, although there was an increased risk of perioperative death.

It is apparent that optimal management of borderline resectable disease is less clearly defined than that of resectable disease, as evidenced by both relatively fewer studies addressing the issue as well the wider variability in treatment regimens and outcomes. Most importantly, the lack of consistency in defining study participants makes interpretation challenging. Certainly, as treatment strategies become more disease-stage specific, efficacious, tolerable, and consistent, better data will exist to guide ongoing management. Many high-volume centers will now accept radiographic findings of a borderline resectable tumor as an indication for a neoadjuvant treatment strategy based on the possible survival benefits versus a primary surgical approach for these patients. There does not yet exists consensus agreement as to the optimal treatment schema for neoadjuvant therapy in borderline resectable disease.

Most investigators would now agree that single-agent 5-FU or single-agent gemcitabine are insufficient in biologic activity to warrant further clinical trials. Most centers use similar regimens as for locally advanced/unresectable disease. The combination of 5-flurouracil (5-FU), leucovorin, irinotecan, and oxaliplatin (a regimen referred to as FOLFIRINOX), the combination of gemcitabine, docetaxel, and capecitabine (a regimen referred to as GTX), and gemcitabine with nab-paclitaxel are possible chemotherapy regimens that are typically followed by continuous infusion 5-FU, capecitabine, or gemcitabine-based chemoradiation to 45 to 54 Gy in 1.8 to 2.5 Gy fractions or 36 Gy in 2.4 Gy fractions [9, 25]. Memorial-Sloan Kettering Cancer Center recently reported a single-arm trial of neoadjuvant gemcitabine and oxaliplatin without radiation for patients with localized pancreatic cancers. While not intended to address patients with borderline resectable tumors, that combination of drugs has shown activity in metastatic cancer and locally advanced tumors. It may therefore be an active combination in patients with borderline resectable tumors. There were 38 patients who received four cycles of neoadjuvant gemcitabine 1000 mg/m2 intravenously over 100 min and oxaliplatin 80 mg/m2 intravenously over 2 h, every 2 weeks. Patients whose tumors remained resectable at restaging proceeded to operation and subsequently received five cycles of adjuvant gemcitabine (1000 mg/m2 intravenously over 30 min days 1, 8, and 15 every 4 weeks). The primary endpoint was 18-month overall survival and secondary endpoints included radiological, tumor marker and pathological response to neoadjuvant therapy, time to recurrence, patterns of failure, and feasibility of obtaining preoperative core biopsies. Thirty-five of 38 patients (92 %) completed neoadjuvant therapy. Twenty-seven patients underwent tumor resection (resectability rate 71 %) of which 26 initiated adjuvant therapy for a total of 23 patients (60.5 %) who completed all planned therapy. The 18-month survival was 63 % (24 patients alive). The median overall survival for all 38 patients was 27.2 months (95 % confidence interval: 17–NA) and the median disease-specific survival was 30.6 months (95 % confidence interval: 19–NA) [23].

More specifically, for borderline tumors and locally advanced cancers, the group at Massachusetts General recently reported encouraging results using neoadjuvant FOLFURINOX relative to patients undergoing primary surgical therapy. A total of 188 patients underwent pancreatic resection for ductal adenocarcinoma. Of these 188 patients, 40 received neoadjuvant FOLFIRINOX with or without chemoradiation for locally advanced disease or borderline resectable cancers, and 87 received no neoadjuvant therapy. The patients who received no neoadjuvant therapy were determined to have resectable cancers on preoperative imaging and declined participation or did not qualify for other neoadjuvant protocols. A total of 47 patients underwent neoadjuvant treatment with FOLFIRINOX followed by surgical exploration for attempted resection. The patients received a median of eight complete cycles (range 1–24), and only three patients (6.3 %) developed severe treatment-related toxicity resulting in disruption of therapy. Chemoradiation with 50.4 Gy and 5-FU (fluorouracil) was administered to 24 patients who had no evidence of progressive disease after FOLFIRINOX and before surgical exploration. FOLFIRINOX resulted in a significant decrease in tumor size, yet 19

patients were still classified as locally advanced and 9 as borderline. Despite post-FOLFIRINOX imaging suggesting continued unresectability, 92 % had an R0 resection. When compared with no neoadjuvant therapy, FOLFIRINOX resulted in significantly longer operative times (393 vs. 300 min) and blood loss (600 vs. 400 mLs), but significantly lower operative morbidity (36 vs. 63 %) and no post-operative pancreatic fistulas. Length of stay (6 vs. 7 days), readmissions (20 vs. 30 %), and mortality were equivalent (1 vs. 0 %). On final pathology, the FOLFIRINOX group had a significant decrease in lymph node positivity (35 vs. 79 %) and perineural invasion (72 vs. 95 %). Median follow-up was 11 months. Progression was documented in 38 % of patients receiving neoadjuvant FOLFIRINOX, compared with 49 % of patients receiving no neoadjuvant therapy. Distant disease was the most common first site of progression for both cohorts. Median overall survival for the entire cohort was 34 months. Patients who received FOLFIRINOX and underwent surgical resection had a significant increase in overall survival compared with the group of patients with clearly resectable tumors who received no neoadjuvant therapy (18 vs. 34 m, $P = 0.008$) [8].

In the United States, Katz et al. have recently completed a multi-institutional feasibility trial with FOLFIRINOX followed by a capecitabine-based chemoradiotherapy protocol (Alliance trial A0201102). Results are in press at this time. A comparable study has been initiated by high-volume German centers.

4 Surgical Management of Borderline Resectable Pancreatic Cancer

The fundamental objectives of pancreatic cancer surgery are total extirpation of the primary tumor with microscopically clear resection margins, complete regional lymphadenectomy of appropriate peri-pancreatic nodal groups, and reconstruction of the gastrointestinal tract so as to facilitate early and sustained return of normal physiologic function. Surgeons operating on patients with borderline resectable cancers should anticipate portal and mesenteric venous involvement and extension of the cancer to arterial margins. Thus, an already formidable surgical procedure becomes even more technically demanding and potentially more complicated with the near obligatory addition of vascular resection and reconstruction. Only surgeons experienced with advanced techniques of vascular resection and reconstruction should therefore undertake these operations.

The critical need for a complete margin-negative resection (R0) is evident when examining survival data. Most surgical series suggest similar survival rates for margin-positive resections compared to nonoperative local-regional therapies in patients with locally advanced disease. Unlike potentially resectable cancers, attempts at surgical resection for borderline resectable cancers are likely to be compromised by positive margins because of the proximity of the primary tumor to major vascular structures. Therefore, a margin-negative resection is likely to only be accomplished by the addition of a major vascular resection and reconstruction. Most of the evidence evaluating the safety and efficacy of vascular resection in

these cases comes from single institution retrospective studies. These studies have shown mixed results in terms of both complications and survival. Some have demonstrated increased perioperative complication rates and decreased survival with major vascular resection, while others have reported similar short- and long-term outcomes with and without vascular resection [3]. The two largest series to date on pancreaticoduodenectomy with vascular resection are a multi-institutional retrospective British study of high-volume centers and a National Surgical Quality Improvement Program (NSQIP) study. The British study examined 230 vascular resections of T3 pancreatic adenocarcinoma. The authors found similar overall complication rates between compared groups, although there were significantly increased rates of delayed gastric emptying and postoperative blood transfusion in the vascular resection group compared to patients who underwent pancreaticoduodenectomy without vascular resection. Median overall survival was equivalent between the two groups (approximately 18 months) [27]. The NSQIP study examined 281 vascular resections for pancreatic cancer. Those authors found significantly increased perioperative mortality and increased overall 30-day morbidity rates for the patients who underwent vascular resection compared to patients who underwent a standard pancreatic resection alone. No staging or long-term survival data was available in the study [6]. Both studies found similar postoperative hospital lengths of stay for the two groups.

Kantor and Baker used a case-matched cohort analysis to compare patients undergoing pancreaticoduodenectomy with vascular resection for pancreatic adenocarcinoma to a pathologic stage and age-matched cohort of patients undergoing pancreaticoduodenectomy without vascular resection at a single high-volume site. They applied a system for grading postoperative complications that included all 90-day readmissions to fully evaluate perioperative morbidity in addition to short- and long-term recurrence and survival outcomes between groups. The study demonstrated that major vascular resection was associated with an increased rate of severe adverse postoperative outcomes, readmissions, total length of stay, and hospital costs. Although the rates of R0 resection and overall recurrence were equivalent between groups, there was a significantly shorter time to recurrence in the vascular resection group as well as a decreased overall survival. The need for major vascular resection was the only independent predictor of mortality on multivariate Cox regression survival analysis [12].

4.1 Technical Considerations

A comprehensive description of surgical techniques used to perform these vascular resections is beyond the scope of this chapter; however, several recent publications have detailed operative approaches for safe resection combined with sustained patency of the reconstructed vessels. These authors all emphasize several critical considerations at the time of surgery. To minimize the possibility of microscopically positive cancer cells being left on the superior mesenteric artery (SMA), a periadventitial dissection along the right lateral aspect of the SMA must be

performed for all patients with pancreatic ductal adenocarcinoma. Of the different resection margins, it is the posteromedial resection margin, limited by the right aspect of the proximal SMA that usually presents the major technical challenge. An "SMA-first" technique may add to the safety of venous resection. Early dissection of the SMA results in the tumor being attached only to the involved veins, so clamping of the portomesenteric confluence may be easier and shorter. Katz et al. [13] have outlined the general principles of this approach. The critical dissection of the SMA margin begins at the inferior border of the uncinate process and extends cephalad to where the SMA diverges from the aorta. The proximal SMA typically lies directly posterior to the superior mesenteric vein and portal vein (SMV-PV) confluence. To access this tissue safely, the SMV-PV confluence must be completely mobilized. For primary tumors that do not involve the SMV or PV, the confluence can be retracted to the patient's left, after dissection of the surgical specimen from the vein to expose the underlying artery. When the pancreatic tumor involves the venous confluence, however, division of the pancreatic neck superficial to the vein and leftward mobilization of the SMV-PV are both impossible. In this circumstance, the splenic vein is divided and the SMV-PV is retracted to the patient's right [13]. The dissection is begun by sharply accessing the periadventitial plane of the SMA caudal to the tumor and proceeding cephalad along the vessel. The inferior mesenteric vein and first jejunal branch of the SMV can each be freely ligated as they are encountered if they course anterior to the artery, but otherwise are typically left in situ. The splenic vein must be identified and carefully dissected from the posterior aspect of the pancreas to protect it as the pancreatic neck or proximal body is divided. After division of the pancreas, the left aspect of the SMV-PV confluence is completely exposed and the splenic vein is safely ligated. The periadventitial dissection of the SMA then proceeds cephalad toward the aorta. All fat, fibrous tissue, lymphatics, and nerves are swept to the patient's right, and the inferior pancreaticoduodenal artery or arteries are individually ligated. The relatively avascular deep retroperitoneal tissues are then divided, leaving the specimen attached by the portal vein and SMV only (Fig. 6). Division of these veins frees the specimen. Venous reconstruction can then be performed using primary end-to-end anastomosis if there is no tension at the suture line. To avoid the potential for short-term bleeding and long-term thrombosis with an anastomosis not amenable to primary repair, interposition grafts using the internal jugular vein, the left renal vein, or the superficial femoral vein have all been described. A ligated splenic vein need not be reconstructed if the inferior mesenteric vein enters into it well away from the resection site. If the inferior mesenteric vein is sacrificed then drainage of the splenic vein is necessary to avoid thrombosis, hypersplenism, and vascular congestion of the foregut. This can be accomplished with reimplantation of the splenic vein stump into the interposition conduit or with the creation of a distal spleno-renal shunt [13].

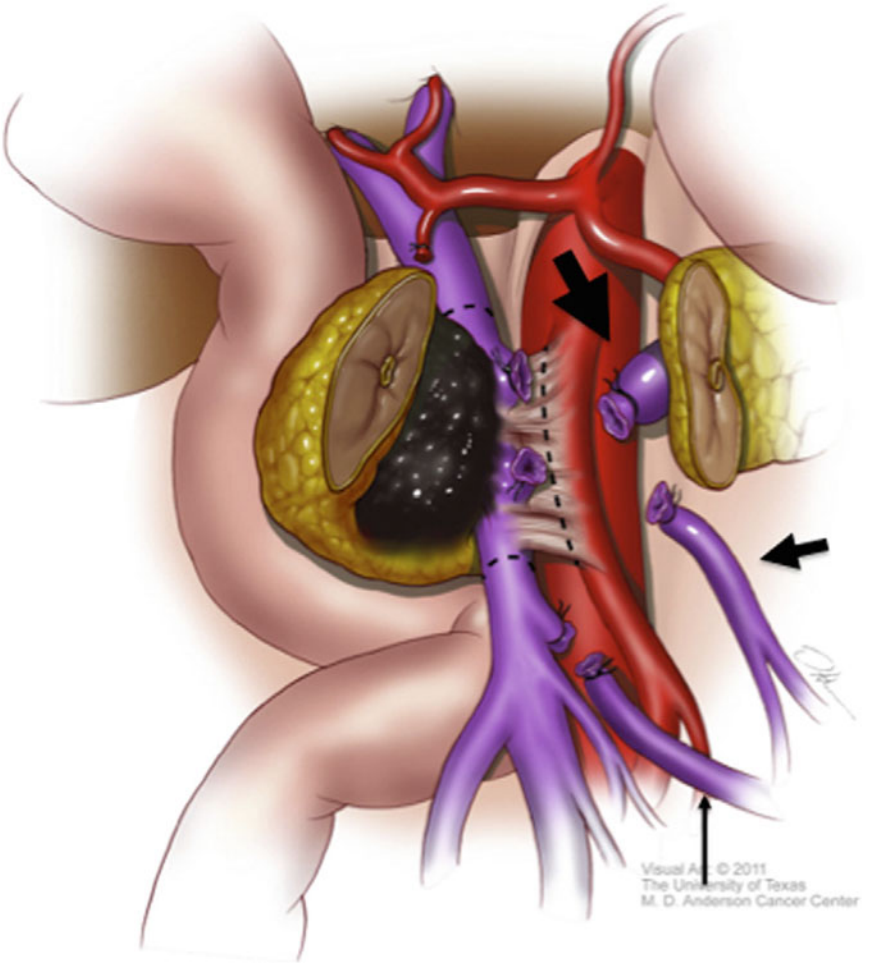

Fig. 6 "SMA first" dissection of the superior mesenteric artery in the periadventitial plane and from a caudal to cranial direction with right lateral traction of the tumor en bloc with a resectable segment of attached superior mesenteric vein. Reprinted with permission from [13]

5 Conclusion

There have been two expert consensus statements on borderline resectable pancreas cancer crafted in the last five years and the most recent NCCN guidelines for pancreatic cancer are now published (Callery, Chang et al. [4, 5, 35]. All acknowledge the limitations of the currently available data. All unequivocally emphasize the need for high-quality radiographic imaging to accurately categorize these cancers and clearly delineated and stringently applied definitions of vascular

involvement for appropriate treatment recommendations. The NCCN guidelines recommend that patients with borderline resectable cancer should be treated first with chemotherapy, and then consolidated with chemoradiation and surgery if appropriate. The basis for this recommendation was not data-driven but rather on the authors' perspectives that patients with pancreatic cancer should be selected for a surgery-first approach based on the likelihood of obtaining margin-negative resections. Patients with borderline resectable tumors are at high risk for margin-positive resections that are associated with poorer outcomes in most surgical series. The use of neoadjuvant therapy in borderline cases was recommended because of the potential to increase R0 resections [35].

Future clinical trials will undoubtedly address the use of neoadjuvant treatment strategies for patients with borderline resectable cancers. Specific aims of these trials will be to determine the optimal chemotherapy regimens, the selective use of radiation therapy, and the development of precision medicine driven by genomic analyses to identify molecular subtypes of pancreatic cancer. Surgical techniques will continue to evolve with the incorporation of minimally invasive surgery into the operative management of these formidable oncologic resections. It will be imperative to define quality outcomes for these interventions and the hopefully improved patient benefits that result from these investigations. Surgeons will be at the forefront of these advances as we will be uniquely positioned to evaluate the safety, efficacy, and costs of multi-modality treatment for patients with borderline resectable pancreatic cancer.

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Pathologic Features of Primary Pancreatic Malignancies

Ashley M. Cunningham, Patrick S. Rush and Kristina A. Matkowskyj

Abstract

This chapter explores the pathologic features of benign and malignant lesions of the pancreas. As pathologic classifications evolve, particularly for cystic lesions and neuroendocrine tumors, it is important for physicians who treat patients with pancreatic neoplasms to fully evaluate these pathologic classifications.

Keywords

Solid pseudopapillary neoplasm · Mucinous cystic neoplasm · Pancreatic intraepithelial neoplasia · Intraductal papillary mucinous neoplasm · Pancreatic ductal adenocarcinoma · Pancreatic neuroendocrine tumor · Acinar cell carcinoma · Pancreatoblastoma

1 Premalignant Lesions

1.1 Solid Pseudopapillary Neoplasm

Solid pseudopapillary neoplasm (SPPN) is a low-grade malignant neoplasm that occurs preferentially in young women (mean age at diagnosis is 30-years old) [15].

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Clinical presentation is nonspecific including abdominal pain, weight loss, and nausea. Majority of solid pseudopapillary neoplasms are incidental findings on routine imaging [17]. Rarely these neoplasms can undergo malignant transformation and metastasize to the peritoneal cavity or liver; however, the overall prognosis is excellent after surgical resection with cure rates of 85–95 % [17] (Table 1).

1.1.1 Molecular Pathology and Associated Syndromes

Activating mutations of the APC/β-catenin pathway including point mutations in exon 3 of the beta-catenin gene *CTNN31* are common and lead to nuclear expression of beta-catenin by immunohistochemistry [17].

1.1.2 Pathologic Features

Grossly, solid pseudopapillary neoplasms are well-demarcated solitary masses often separated from the surrounding pancreatic parenchyma by a fibrous capsule (Fig. 1a). Hemorrhage and cystic degeneration are common. The histologic appearance of SPPN is distinct with solid areas admixed with areas of discohesive cells which fall away from stromal septae giving the tumor its characteristic papillary appearance (Fig. 1b). The neoplastic cells have round to oval nuclei and eosinophilic cytoplasm. Cytoplasmic or extracellular eosinophilic globules are variably present and stain positively with periodic acid–Schiff–diastase (PAS-D) [16].

Immunohistochemical profile of SPPN is characterized by positive staining with alpha-1-antitrypsin in individual cells or small cell clusters, diffuse positivity with neuron specific enolase, progesterone receptor, and CD10. Nuclear beta-catenin staining is also characteristic although nonspecific [1].

Table 1 Classification of primary tumors of the pancreas

Benign	Borderline	Malignant
Serous cystadenoma Acinar cell cystadenoma	Solid Pseudopapillary Neoplasm Mucinous cystic neoplasm (MCN) Intraductal papillary Mucinous neoplasm (IPMN)	Ductal adenocarcinoma • Colloid (mucinous) • Signet ring cell • Undifferentiated (anaplastic) • Adenosquamous • Medullary • Hepatoid • Undifferentiated carcinoma with osteoclast-like giant cells Pancreatic neuroendocrine tumor Acinar cell carcinoma Mixed acinar-ductal carcinoma Mixed acinar-neuroendocrine carcinoma Pancreatoblastoma

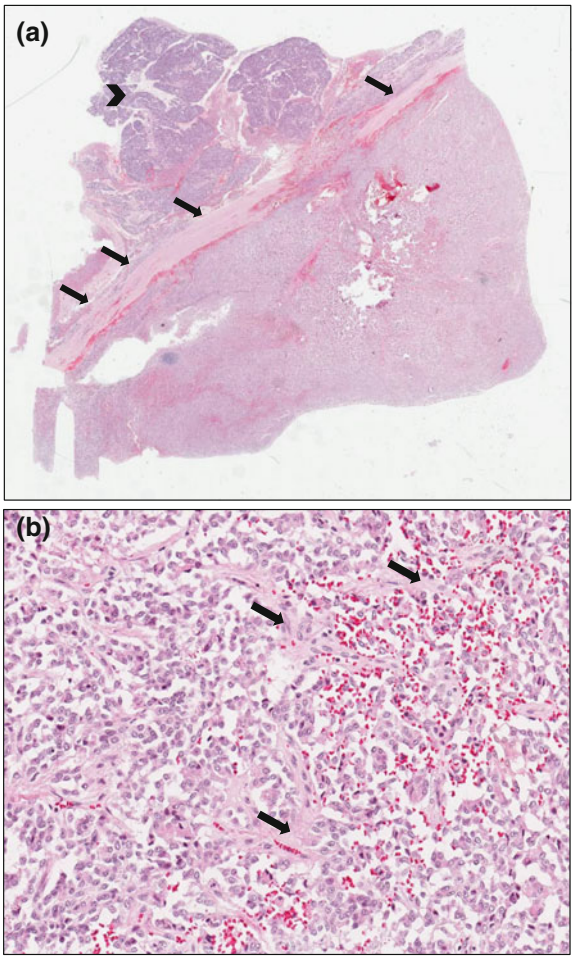

Fig. 1 Solid pseudopapillary neoplasm (SPPN). **a** Low power image shows a well-circumscribed neoplastic proliferation separated from the uninvolved pancreatic parenchyma (*arrowhead*) by a fibrous capsule (*arrows*) (**a**). Discohesive tumor cells clinging to stromal bands (*arrows*) giving the neoplasm its characteristic papillary appearance (**b**)

1.2 Mucinous Cystic Neoplasm

Mucinous cystic neoplasms (MCNs) are rare epithelial neoplasms of the pancreas. MCNs occur most commonly in women between 40 and 50-years old. The majority of these neoplasms are identified incidentally, arising in the body and tail of the pancreas, and typically have no connection to the main pancreatic duct or its branches [33]. MCN is considered a premalignant neoplasm with up to one third of these neoplasms having an associated invasive pancreatic adenocarcinoma identified after surgical resection [4]. This association with invasive carcinoma is why the majority of MCNs are treated with surgical resection. If there is no invasive carcinoma present at the time of resection surgery is curative. MCN with an associated invasive carcinoma has a 5-year survival of 50 % [4]. If an invasive ductal adenocarcinoma is present, the tumor should be staged similar to other invasive pancreatic ductal adenocarcinomas.

1.2.1 Molecular Pathology and Associated Syndromes

Molecular alterations identified in MCN are similar to those identified in invasive ductal adenocarcinomas, highlighting the premalignant nature of these neoplasms. *KRAS* mutations are the most common and their frequency increases with the degree of dysplasia identified in the MCN. Aberrant hypermethylation of *CDKN2A* has also been identified in MCN with low- or intermediate-grade dysplasia [33].

1.2.2 Gross Pathology

Grossly, MCN is a solitary well-circumscribed mass that is often separated from the adjacent pancreatic parenchyma by a fibrous pseudo-capsule, which may contain calcifications. MCN can be unilocular or multilocular, with thin-walled cysts containing thick viscous mucinous material. The cyst walls are smooth; however, in cases of MCN with an associated invasive carcinoma, irregular thickening of the cyst wall, mural nodules, or papillary projections may be identified [4]. There is usually no communication between the cystic mass and the pancreatic duct; the pancreatic duct and its branches appear normal in the majority of cases.

1.2.3 Microscopic Pathology

MCN is composed of cystic spaces lined by mucinous epithelium (Fig. 2a) with underlying cellular stroma consisting of spindle cells that stain positively for estrogen and progesterone receptors by immunohistochemistry (Fig. 2b). The stroma in MCN resembles that of the ovary, which is a distinct feature of MCN that is helpful for differentiating MCN from other pancreatic neoplasms with mucinous epithelium [33]. MCN are classified according to the degree of epithelial dysplasia into MCN with low- or intermediate-grade dysplasia, MCN with high-grade dysplasia, and MCN with an associated invasive carcinoma [4]. Low-grade dysplasia is characterized by mucinous epithelium with round basally oriented nuclei and absent mitotic figures. Intermediate-grade dysplasia has some architectural atypia characterized by papillary projections or invaginations, mild overlapping or crowding of the nuclei, and occasional scattered mitotic figures. High-grade dysplasia is typified by a loss of nuclear polarity where the nuclei no longer sit along the basal aspect of the cell, and the nuclei appear pseudostratified. Nuclear atypia (pleomorphism), prominent nucleoli, and frequent or atypical mitotic figures are also characteristic of high-grade dysplasia [33].

1.3 Pancreatic Intraepithelial Neoplasia

Pancreatic intraepithelial neoplasia (PanIN) is the most common precursor lesion to invasive pancreatic carcinoma and represents an epithelial proliferation with variable nuclear and architectural atypia involving the pancreatic duct [7]. PanIN is subclassified into three categories according to the degree of dysplasia: PanIN-1A/B, PanIN-2 and PanIN-3. PanIN-1 lesions are frequently found in elderly patients, including resections for benign masses/conditions. The presence of

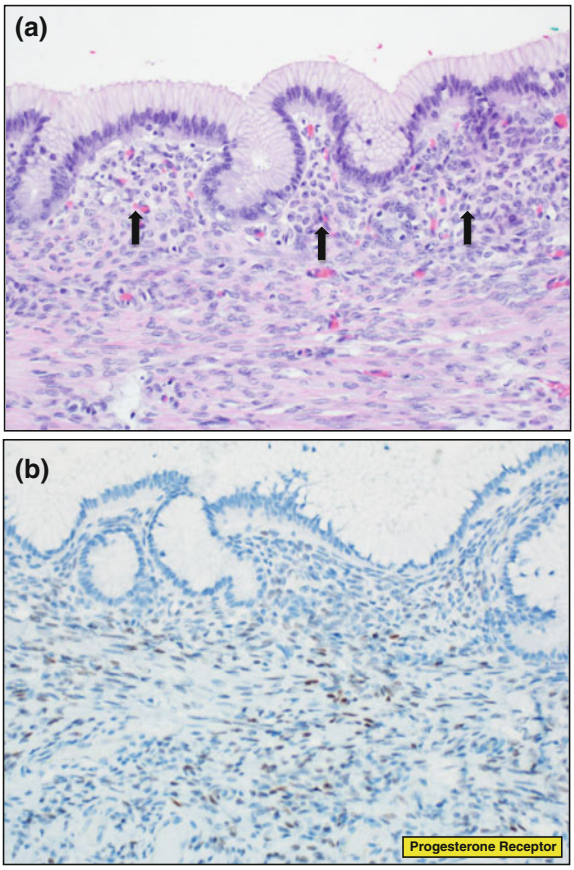

Fig. 2 Mucinous cystic neoplasm (MCN). **a** Cystic space lined by columnar mucinous epithelium. The stroma underlying the mucinous epithelium is composed of closely packed spindle-shaped cells (*arrows*) that resemble the stroma of the ovary (ovarian-type stroma), which is characteristic of MCN. **b** Many of the stromal cells are positive for progesterone receptor by immunohistochemistry, confirming the diagnosis of MCN

PanIN-2 and PanIN-3, however, are more commonly found in pancreatic parenchyma with concurrent invasive pancreatic adenocarcinoma [15].

1.3.1 Molecular Pathology and Associated Syndromes

Historically PanIN-1A and PanIN-1B were considered benign hyperplastic lesions; however, identification of clonal *KRAS* mutations (a driver mutation in the progression to invasive pancreatic adenocarcinoma) supports their designation as a neoplasm with thepotential to progress to invasive carcinoma [15]. PanIN lesions often harbor molecular alterations commonly seen invasive pancreatic ductal adenocarcinoma including activating KRAS mutations, telomerase shortening, and inactivation of *CDKN2A, TP53* and *SMAD4* [7]. These shared molecular changes support the multistep tumor progression model of pancreatic cancer.

1.3.2 Pathologic Features

PanIN lesions are not grossly identifiable nor are they detected by current imaging modalities. This is a characteristic feature of these lesions especially when trying to

differentiate them from other noninvasive intraductal proliferations, such as intraductal papillary mucinous neoplasm (IPMN). An arbitrary size cutoff of less than 1 cm is commonly used to separate PanIN lesions from IPMN [2]. The microscopic features of PanIN lesions depend on the degree of dysplasia. PanIN-1A is composed of small cystic spaces lined by columnar mucinous epithelium with round basally oriented nuclei. Nuclear atypia and mitotic figures are typically absent. PanIN-1B is cytologically similar to PanIN-1A, however, there is architectural atypia in these lesions characterized by papillary projections of the epithelium (Fig. 3a). The columnar mucinous epithelium of PanIN-2 shows nuclear stratification and the nuclei themselves appear elongated and hyperchromatic. Papillary architecture can sometimes be seen in PanIN-2. PanIN-3 shows marked nuclear atypia with loss of polarity of the cells, hyperchromatic pleomorphic nuclei, and branching papillae or irregular cellular tufts (Fig. 3b).

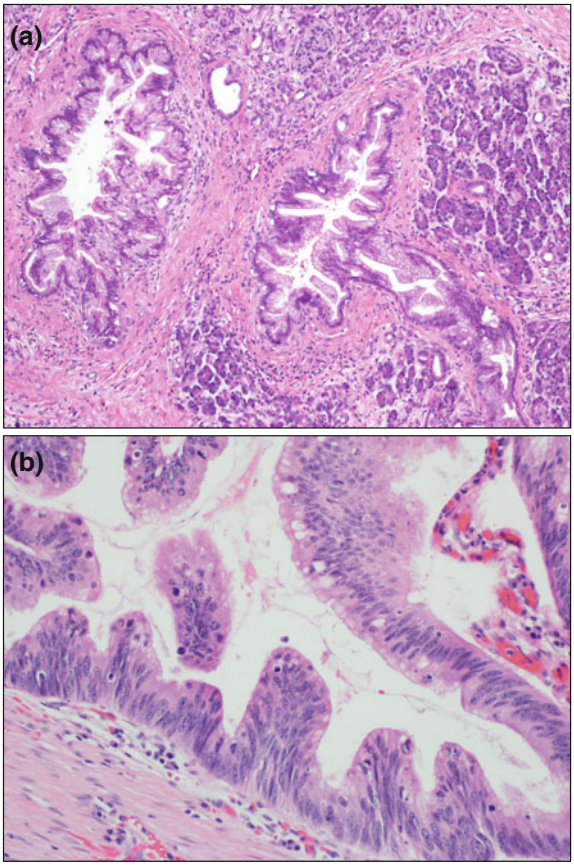

Fig. 3 Pancreatic intraepithelial neoplasia (PanIN). **a** Pancreas with a cystically dilated duct lined by columnar epithelium with small, round, basally oriented nuclei and abundant cytoplasmic mucin. There is a delicate papillary architecture which distinguishes PanIN-1B from that of PanIN-1A. **b** PanIN-3 demonstrates a complete loss of nuclear polarity, prominent nucleoli, and numerous mitotic figures extending toward the luminal surface

1.3.3 Intraductal Papillary Mucinous Neoplasm

Intraductal papillary mucinous neoplasm (IPMN) is an epithelial derived mucin-producing neoplasm characterized by its relationship to the pancreatic duct and its branches. It can arise anywhere along the pancreatic duct and even extend to involve the ampulla of Vater or common bile duct [2]. IPMNs are a heterogeneous group of premalignant neoplasms with variable degrees of dysplasia (low-, intermediate- and high-grade) to IPMN with associated invasive ductal adenocarcinoma. There are two main classifications of IPMNs based on their location, main duct type IPMN and branch duct type IPMN. These classifications are not only anatomic designations but also have prognostic implications. Main duct type IPMN tends to have a higher incidence of being involved by high-grade dysplasia or invasive ductal adenocarcinoma compared to branch duct type IPMN. These slow-growing neoplasms are typically found incidentally during routine imaging, however, some patients may present with symptoms of epigastric pain, jaundice, weight loss, pancreatitis, and diabetes [30]. The true incidence of these neoplasms is uncertain; however, an increasing number of small asymptomatic IPMNs are being discovered due to advanced imaging techniques [14]. IPMN can occur at any age but the prevalence increases with age and the majority are found in elderly patients [2]. While the etiology of IPMN remains unclear, associations with smoking, Puetz–Jegher syndrome and familial adenomatous polyposis syndrome have been identified.

Due to the risk of progression to or concurrent presence of invasive adenocarcinoma, the International Association of Pancreatology recommends surgical excision for all main duct-IPMN, symptomatic patients or branch duct-IPMN that are larger than 3 cm or have associated mural nodules [2]. After surgical resection, the prognosis of the majority of IPMN is good with a 5-year survival of 75 % [15] if no invasive adenocarcinoma is identified. Even with an associated invasive adenocarcinoma, the prognosis of the carcinoma arising in the setting of IPMN is better than conventional pancreatic ductal adenocarcinoma [2].

1.3.4 Molecular Pathology and Associated Syndromes

A variety of molecular defects involving oncogenes (*KRAS, BRAF*, *ERBB2*) and tumor suppressor genes (*CDKN2A, TP53, SMAD4*) have been described. *KRAS* mutations are seen in 30–80 % of IPMN and the frequency is positively correlated with the degree of dysplasia [2]. While the molecular profile of IPMN mimics conventional pancreatic ductal adenocarcinoma, retention of the tumor suppressor gene *DPC4*, which is typically lost in most ductal adenocarcinomas is usually retained in IPMN [15]. These subtle molecular differences may be clues to the differences in prognosis observed in invasive carcinoma arising from IPMN and invasive ductal adenocarcinoma not associated with IPMN.

1.3.5 Gross Pathology

The gross appearance of IPMN varies depending on its association with the pancreatic duct, however, to distinguish IPMN from another pancreatic precursor lesion, pancreatic intraepithelial neoplasia (PanIN), an arbitrary minimum size

cutoff of at least 1 cm is used to designate most IPMNs [2]. Main duct type IPMN (MD-IPMN) is primarily located in the head of the pancreas and intraluminal mucin production causes obstruction leading to diffuse dilation of the pancreatic duct (Fig. 4a). While not seen in the majority of cases, mucin extravasation from the ampulla of Vater is a pathognomonic finding in MD-IPMN. Branch duct type IPMN (BD-IPMN) is primarily located in the uncinate process and forms a multicystic lesion with thin septae. IPMN with associated invasive carcinoma will have features of invasive growth including an irregularly thickened or fibrotic duct wall. IPMN can be multicentric in 40 % of cases [2], and associated invasive carcinoma may be adjacent to or located separate from the main IPMN mass, so thorough inspection and sampling of the entire cystic lesion is important. Fistulous tracts between the pancreas and surrounding organs (e.g., duodenum, stomach) can rarely develop and should not be interpreted as a sign of invasive carcinoma without supporting histologic evidence [2].

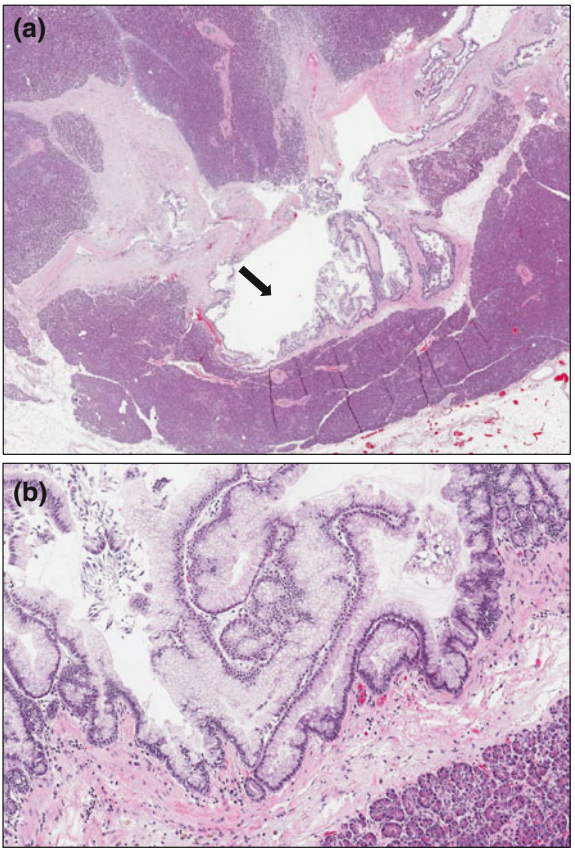

Fig. 4 Intraductal papillary mucinous neoplasm (IPMN). **a** Cystically dilated pancreatic duct measuring greater than 1 cm (*arrow*) with proliferation of mucinous epithelium. **b** The lining epithelium demonstrates a papillary growth pattern along a fibrovascular stalk. The neoplastic cells have basally oriented, uniform, round nuclei, and pale apical mucin droplets characteristic of gastric foveolar epithelium

1.3.6 Microscopic Pathology

IPMN are subclassified by the type of mucinous epithelium, the degree of dysplasia and the presence or absence of invasive adenocarcinoma. The gastric subtype recapitulates gastric foveolar epithelium including tall columnar cells with basally oriented nuclei and pale apical mucin vacuoles (Fig. 4b). Intestinal-type IPMN has a papillary architecture lined by pseudostratified columnar cells with elongated cigar-shaped nuclei and basophilic cytoplasm and variable numbers of goblet cells interspersed between the columnar cells. Pancreaticobiliary-type IPMN is much less common than gastric and intestinal-types. These lesions are characterized by thin delicate branching papillae lined by cuboidal cells with dark round nuclei with prominent nucleoli. The last subtype is oncocytic type IPMN which has a highly complex architecture with branching papillae and fused glands giving a cribriform appearance. The epithelium in oncocytic type IPMN is hyperplastic composed of multiple layers of cuboidal to columnar cells with abundant eosinophilic cytoplasm and uniform round eccentrically located nuclei. In this setting, it is often referred to as intraductal oncocytic papillary neoplasm (IOPN).

In addition to indicating the type of epithelium lining the IPMN, the degree of dysplasia must be reported and includes IPMN with low-grade dysplasia, intermediate-grade dysplasia, or high-grade dysplasia. The final comment should indicate whether it is associated with an invasive carcinoma [2]. Low-grade dysplasia is characterized by uniform cells with basally located nuclei and simple papillary architecture. Intermediate-grade dysplasia is characterized by mild-to-moderate nuclear atypia, nuclear pseudostratification, and branching papillae. High-grade dysplasia is characterized by complex architectural patterns such as cribriform (compressed back-to-back glands) and often striking nuclear atypia [15]. The degree of dysplasia corresponds with the epithelial subtype in most cases. The intestinal, pancreaticobiliary and oncocytic type IPMNs more often have intermediate-to high-grade dysplasia and are more likely to be MD-IPMN. While gastric-type IPMN usually has low to intermediate-grade dysplasia and are often BD-IPMN.

The immunohistochemical profile of IPMN also varies depending on the histologic subcategory. The majority of IPMN stain diffusely positive with ductal epithelial markers such as cytokeratin 7 and 19, CA19-9, and CEA. Gastric type IPMN is positive for MUC5AC, while intestinal-type IPMN is positive for MUC2 and CDX2. Pancreaticobiliary-type IPMN is often positive for MUC1, and oncocytic type IPMN is positive for MUC1, MUC2, and MUC6 [2].

2 Malignant Lesions

2.1 Pancreatic Ductal Adenocarcinoma

Pancreatic cancer accounts for 3 % of all malignant neoplasms in the United States, with an average lifetime risk of 1.5 % in both men and women. Yet it is responsible for 7 % of all cancer deaths; as it is one of the most fatal of all human cancers [3]. Pancreatic neoplasms are delineated via a classification system that is based on the

line of cellular differentiation that comprises the tumor. Despite the fact that cells of the pancreatic ducts comprise a low total volume of non-diseased pancreatic mass, tumors that recapitulate this cell type are far and beyond the most common of all pancreatic tumors. Ductal adenocarcinoma accounts for 85–90 % of all pancreatic neoplasms and as such is synonymous with the colloquial phrasing of "pancreatic cancer" [15, 29]. Ductal adenocarcinoma of the pancreas itself is divided into several subtypes differentiated by their morphologic appearance, each of which portends a varied clinical response and prognosis. However, it should be understood that all variants carry an extremely dismal prognosis. Ductal adenocarcinoma of the pancreas is considered a lethal disease despite the histologic variant. Ductal adenocarcinoma holds one of the worst survival rates of any human cancer as the fourth leading cause of cancer mortality, with only 7.2 % of patients surviving 5 years, a median survival rate under 6 months [6, 28]. However, individual survival and prognosis is based on stage and 5-year survival will range from 27.1 % with localized T1 disease to 2.4 % for patients with distant metastasis at the time of diagnosis [6, 28]. These tumors occur more commonly in an older population, age 65–74, with a median age at diagnosis of 71. It affects men and women approximately equally, with a slight male predilation of 1.3:1, and an overall incidence of 12.4 per 100,000 [28].

The underlying cause of ductal adenocarcinoma of the pancreas as a whole is complex, and not entirely understood. However, the cause is likely multifactorial with multiple differing starting points and lines of development arising both sporadically and in familial settings [15]. That being said, there are known risk factors for the development of disease. Smoking, high dietary fat intake, hereditary chronic pancreatitis, tropical calcifying pancreatitis, and individuals with blood group A or B are all considered to be risk factors for the development of ductal carcinoma [15]. Pancreatic ductal adenocarcinoma can arise from any of the so-called "precursor" lesions, pancreatic intraepithelial neoplasia (PanIN), intraductal papillary mucinous neoplasia (IPMN), and mucinous cystic neoplasm (MCN) [8, 10, 12].

2.1.1 Gross Pathology

Most patients do not develop clinical symptoms until late in the disease process. When symptoms do appear they paint a picture of obstruction; pain radiating to the back, clay-colored stool, dyspepsia, early satiety, painless jaundice, and diabetes mellitus. In fact, diabetes mellitus is one of the most commonly seen associations, affecting 70 % of patients; new onset diabetes sometimes being the first presenting symptom [9]. The gross features of the lesion are typically in keeping with such clinical findings, often involving surrounding structures, particularly the duodenum and common bile duct [15]. Two-thirds of cases occur in the head of the pancreas, and in the tail or body the remainder of the time [26]. When tumors do occur in the tail of the pancreas, it is common for them to directly involve surrounding organs, such as the stomach and spleen by direct extension [15]. Adenocarcinomas may be difficult to define grossly [19]. Grossly the tumor is a poorly delineated, infiltrative, nonencapsulated, firm, white to yellow mass [26] (Fig. 5). Therefore, foci of tumor are often difficult to discern from regions of chronic pancreatitis grossly [19]. The

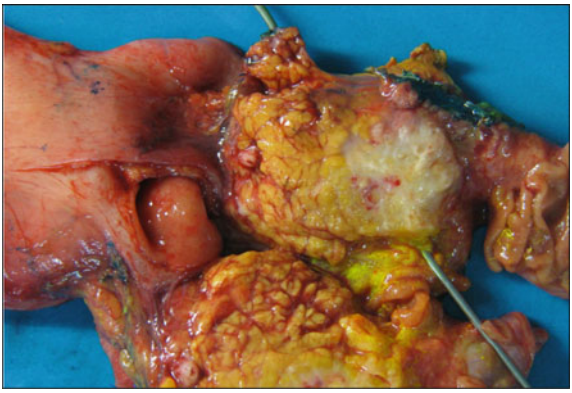

Fig. 5 Pancreatic ductal adenocarcinoma. The cut surface of the pancreas demonstrates an ill-defined, white-tan-stellate lesion within the normal yellow, lobulated pancreatic parenchyma

majority of ductal carcinomas are solid in nature (75 %), however, cystic degeneration and necrosis may be present in large lesions centrally, which is important from a sampling perspective particularly in regards to fine needle aspiration (FNA).

Pancreatic ducts may be dilated often containing necrotic tumor emboli. If the tail of the pancreas is uninvolved it is common for the ductal dilation to continue to this region, surrounded by atrophic pancreas. It is important to note that while the body and tail may be free of a large infiltrative tumor, they can harbor a precursor lesion and should be evaluated thoroughly [26]. In up to 80 % of cases, the disease burden is so great that the tumor may be deemed unresectable at the time of initial diagnosis [15]. Tumors which occur in close proximity to the ampulla tend to be smaller in size, but may ulcerate into the duodenum; giving rise to clinical symptoms earlier in the disease process [9, 19].

2.1.2 Molecular Pathology and Associated Syndromes

The progression of ductal adenocarcinomas has been determined to begin with somatic mutations in *KRAS*, *p16/CDKN2A*, *TP53* and *SMAD4/DPC4*, typically occurring in PanIN or IMPN first [23]. Once a series of somatic mutations accumulates in these lesions there is a progression to invasive carcinoma [27, 32]. The induction and progression of mutations in these precursor lesions are thought to be different, which is in keeping with their differing clinical progression [27]. The acquisition of pathogenic mutations is thought to begin in most cases with activating mutations of the *KRAS* gene, one of the members of the RAS family of GTPases, an important component in a number of signal transduction pathways [32]. However, mutations in the *KRAS* gene have been shown to be plastic and there is no agreement on the dependence of *KRAS* as an activating mutation in all pancreatic ductal carcinoma cases [5].

In rare circumstances, genetic susceptibility plays a more identifiable role than in sporadic pancreatic ductal carcinoma. One of the most significant risk factors for the development of ductal adenocarcinoma is the presence of chronic injury; one of

these injury pathways is in the autosomal dominant disorder of hereditary pancreatitis. Hereditary pancreatitis is due to gain of function mutation in the *PRSS1* gene, resulting in an aberrant cationic trypsinogen, causing decades of inflammation and chronic injury. Nonetheless, only a small percentage of patients with hereditary pancreatitis will develop carcinoma [32]. Additionally, about 10 % of cases of ductal adenocarcinoma will be truly familial, although the cause for this is unfortunately unknown in 80 % of cases. It is thought that at least a portion of these hereditary carcinomas can be ascribed to other known syndromes with increased cancer risk, such as Peutz–Jeghers syndrome, hereditary nonpolyposis colorectal cancer (HNPCC), hereditary breast cancer syndrome, familial atypical multiple mole melanoma (FAMMM) syndrome, among others [15]. Currently recognized susceptibility genes include mismatch repair genes (*MLH1, PMS2, MLH6, MSH2*) as well as *BRCA1*, *BRCA2*, *ATM*, *SPINK1*, *PRSS1*, *PALB2*, *STK11* [23].

2.1.3 Microscopic Pathology

The treatment and prognosis of ductal adenocarcinoma differ starkly from those of chronic pancreatitis. Yet, the histopathologic discrimination of these vastly different diseases can be extremely difficult in some cases. The differentiation between reactive glands and those of an invasive neoplasm or in situ neoplasia in a reactive background, while difficult, can be distinguished, in most cases, with careful evaluation and strict adherence to well-defined morphologic criteria. Conventional ductal adenocarcinoma is composed of a proliferation of small tubular and glandular structures which are lined by cuboidal cells in the setting of a desmoplastic stroma. From low power, the individual glands may not look concerning in the well-differentiated tumor. However, what should be concerning from low power is the lack of retention of a normal lobular architecture. In chronic pancreatitis, the lobular arrangement of atrophic acini, islets of Langerhans and clusters of smaller ductules surrounding a larger duct should be maintained (Fig. 6). In ductal adenocarcinoma, the normal low power appearance is lost and is instead replaced by haphazardly distributed ductular elements of varying shapes and sizes (Fig. 7a). A more rigorous examination of the cytology of the glandular cells reveals loss of polarity (the nucleus no longer remains at the basal aspect of the cell), nuclear pleomorphism, prominent nucleoli, and mitotic activity. Observation of high-grade cytology alone is not sufficient for the diagnosis of ductal adenocarcinoma. Signs of invasion, such as around a nerve (perineural invasion), must also be observed to make a diagnosis of ductal adenocarcinoma over high-grade PanIN. The presence of a desmoplastic stroma and irregular angulated duct contours are signs of invasive disease. When attention is drawn to blood vessels and nerve fibers, perineural invasion will be observed in 90 % of cases, and vascular invasion in about half of cases [26] (Fig. 7b). This can be an extremely helpful feature in determining the invasive nature of the tumor.

There are distinct histologic variants of pancreatic ductal carcinoma, and in fact, quite a few are recognized by the WHO as they seem to retain a gallimaufry of clinical or prognostic features compared to conventional ductal adenocarcinoma. The WHO accepted variants are adenosquamous carcinoma, colloid carcinoma

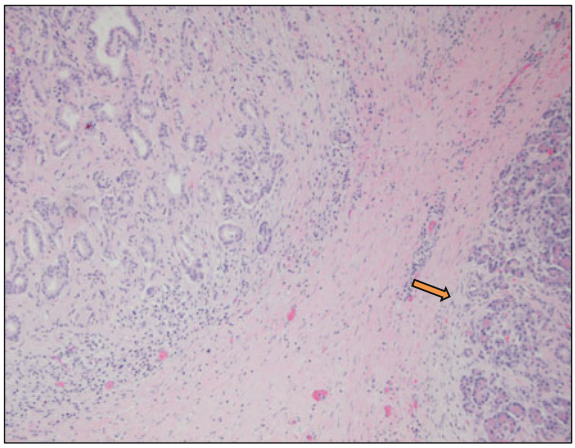

Fig. 6 Chronic pancreatitis. Residual pancreatic parenchyma is present in the lower, right corner of the image (*arrow*). In the upper left portion of the image, the parenchyma is replaced by extensive fibrosis, characteristic of chronic pancreatitis. Within this area, the lobular architecture is maintained with atrophic acini noted around ducts

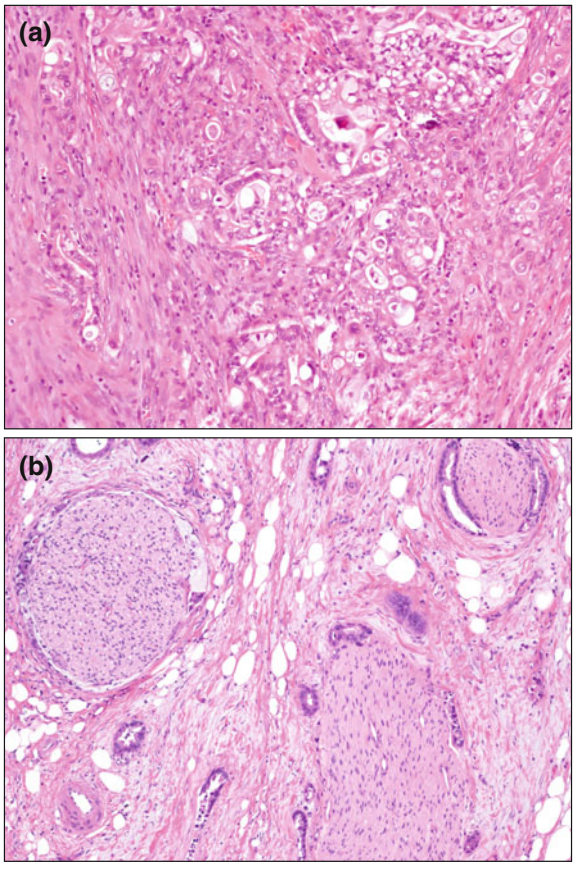

Fig. 7 Pancreatic ductal adenocarcinoma. **a** The maintenance of lobular architecture is lost in invasive carcinoma. The glands are angulated, show significant nuclear pleomorphism, begin to fuse and form a cribriform pattern with areas of desmoplasia (gray spindly appearing stroma). **b** Large nerves in the area of the pancreas are encircled by malignant glands and is consistent with extensive perineural invasion

(mucinous carcinoma), hepatoid carcinoma, medullary carcinoma, signet ring cell carcinoma, carcinomas with mixed differentiation, undifferentiated (anaplastic), and undifferentiated carcinoma with osteoclast-like giant cells. Other patterns of growth do exist, such as vacuolated, foamy gland, and large duct pattern. While awareness of these patterns is important so that they can be recognized as adenocarcinoma, the WHO does not include these variants in their classification of ductal adenocarcinoma [9, 15].

Adenosquamous carcinoma:
As its name suggests, adenosquamous carcinoma is a malignant neoplasm composed of cells that have both squamous and ductal differentiation (Fig. 8). Squamous metaplasia does occur in the pancreas in the setting of chronic injury. In order for the lesion to qualify as an "adenosquamous" carcinoma, the squamous component must comprise at least 30 % of the total sampled tumor volume. These are extremely uncommon tumors and therefore when squamous differentiation is encountered careful consideration should be given to the possibility of a metastatic tumor first. Adenosquamous carcinoma portends an even worse prognosis than conventional ductal adenocarcinoma [13].

Colloid carcinoma (Mucinous carcinoma):
Malignant epithelial cells suspended in expansive extracellular mucin pools (Fig. 9) characterize this infiltrating epithelial adenocarcinoma histologically [12]. The majority of these lesions have arisen from or are in association with IPMN. These lesions have a much better prognosis, as they have been reported to have a 57 % 5-year survival [11]. Clinically, these tumors have been associated with recurrent superficial migratory thrombophlebitis, eponymously named Trousseau

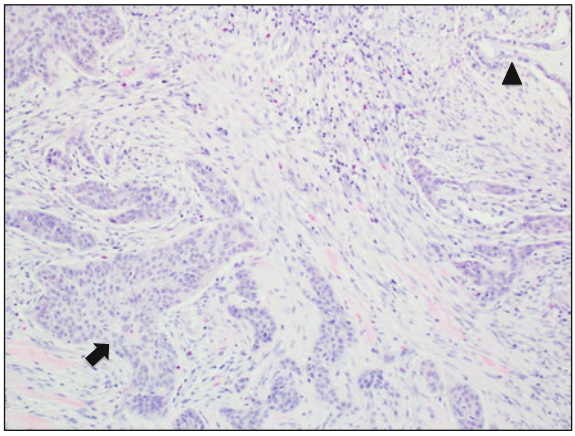

Fig. 8 Adenosquamous variant of pancreatic ductal adenocarcinoma. **a** Both adenocarcinoma (*arrowhead*) and nests of squamous cell carcinoma (*arrow*) are present within the same tumor. The squamous carcinoma cells have eosinophilic cytoplasm, nuclear pleomorphism, and scattered mitotic figures

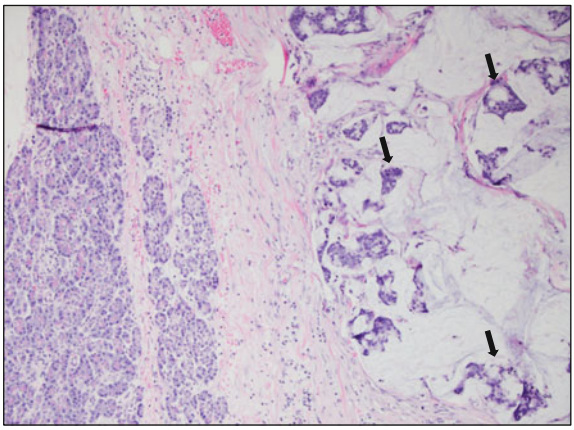

Fig. 9 Colloid carcinoma (mucinous carcinoma) variant of pancreatic ductal adenocarcinoma. Extracellular mucin pools containing neoplastic epithelial cells (*arrows*) are shown adjacent to uninvolved pancreatic parenchyma

sign of malignancy (Trousseau's syndrome) after Armand Trousseau who first described the syndrome [31]. Coincidently, Trousseau developed and identified this clinical finding in himself late in life before succumbing to pancreatic cancer at age 65.

Medullary carcinoma:

Medullary carcinoma is a poorly differentiated carcinoma with an absence of gland formation, a syncytial growth pattern with a "pushing" border and infiltrating an peritumoral lymphocytes (Fig. 10a, b) [9]. There is a proposed association with familial versions of pancreatic carcinoma in these patients. The tumors are commonly microsatellite instable as their histopathologic pattern (presence of high-grade histology, mucinous differentiation, intratumoral/peritumoral lymphocytes, and pushing border) might suggest [12]. These tumors classically fair better than conventional ductal carcinoma.

Signet ring cell:

This variant is also said to be extremely uncommon and has a very poor prognosis. Histologically, it is defined as non-cohesive round cells with intracytoplasmic mucin (Fig. 11).

Undifferentiated carcinoma with osteoclast-like giant cells:

Rare tumor composed of round to spindle-shaped, pleomorphic cells and abundant non-neoplastic multinucleated giant cells containing up to 20 nuclei (Fig. 12). In most cases, there is an associated focus of pancreatic ductal adenocarcinoma present. The neoplastic spindle cells express vimentin and patchy expression of keratin and p53, while the giant cells are positive for macrophage markers such as CD68 and LCA. Prognosis is poor with a mean survival of only 12 months [12]

Hepatoid carcinoma:

This particular variant is extremely uncommon, and only a handful of cases have been reported. The tumor cells are composed of large eosinophilic polygonal cells, reminding one of the polygonal-shaped cells of the liver [9].

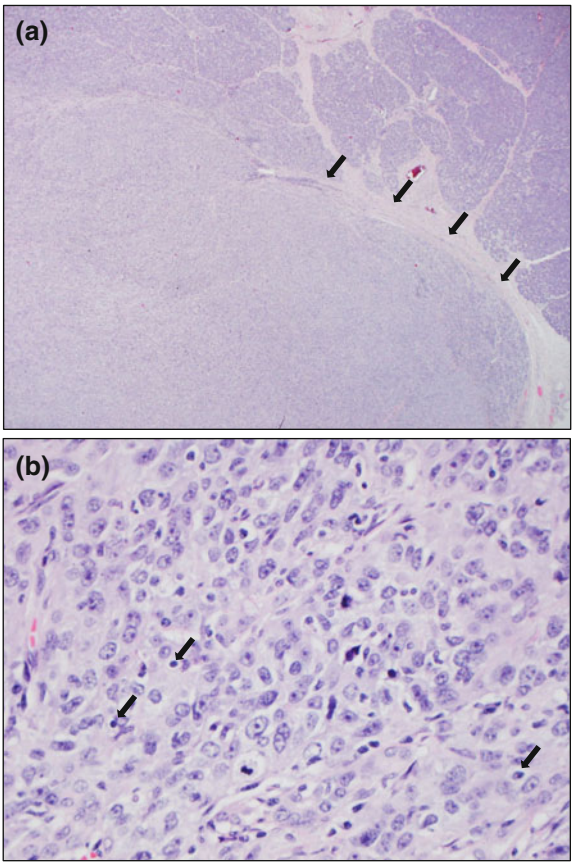

Fig. 10 Medullary carcinoma variant of pancreatic ductal adenocarcinoma. **a** At low magnification, the characteristic "pushing" border (*arrows*) between medullary carcinoma and the uninvolved pancreatic parenchyma can be appreciated. **b** A syncytial growth pattern of epithelioid cells with prominent, central, nucleoli along with scattered mitotic figures and occasional infiltrating lymphocytes (*arrows*) are typical of medullary carcinoma

2.2 Neuroendocrine Tumors

Pancreatic neuroendocrine tumors (NETs) are uncommon neoplasms, representing a spectrum of tumors. They comprise only 1–2 % of pancreatic tumors [21], with an incidence of 0.3–0.4 per 100,000 in the United States [34]. The majority of NETs in the pancreas are sporadic and occur at any age, but are most common in the fourth–sixth decade. A small number of cases are associated with hereditary syndromes, such as multiple endocrine neoplasia type 1 (MEN1), neurofibromatosis, tuberous sclerosis, and von Hippel–Lindau syndrome (VHL). In hereditary cases alterations in the genes *MEN1*, *VHL*, *TSC*, and *NF* are thought to be the primary driver mutations. Pancreatic NETs have a wide spectrum of clinical presentation, which

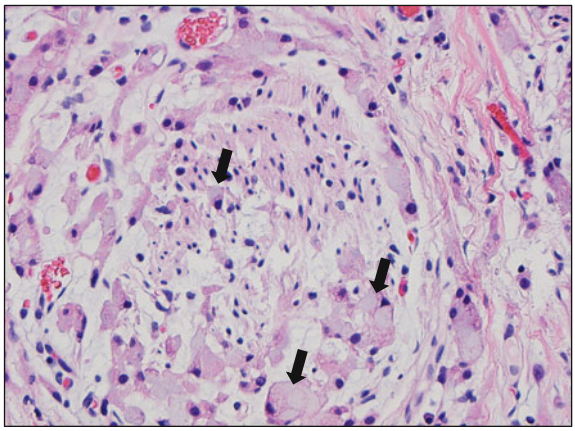

Fig. 11 Signet ring cell carcinoma variant of pancreatic ductal adenocarcinoma. In the center of the image is a nerve infiltrated by neoplastic cells containing a large mucin droplet (*arrows*), which displace the nucleus to the periphery of the cells. These are the characteristic signet ring cells which give the neoplasm its name

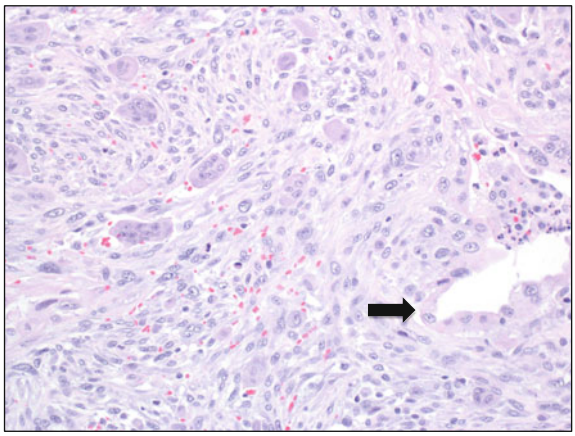

Fig. 12 Undifferentiated carcinoma with osteoclast-like giant cells variant of pancreatic ductal adenocarcinoma. The tumor is composed of round to spindle-shaped, pleomorphic cells and non-neoplastic multinucleated giant cells. A focus of pancreatic ductal adenocarcinoma is also present (*arrow*)

corresponds to the biochemical functionality of the cells comprising the mass. NETs can be separated based on the substances they secrete, resulting in the following classifications: Insulinoma, gastrinoma, glucagonoma, VIPoma, and somatostatinoma. Other subtypes do exist, however, they are vanishingly rare. Half or more of pancreatic NETs are classified as nonfunctional, meaning they do not produce clinical symptomology; however, these tumors generally do produce some

Table 2 WHO 2010 classification for neuroendocrine neoplasms

Classification	Definition	Mitosis	Ki-67 index (%)
Well-differentiated neuroendocrine tumor, Grade 1	Well-differentiated, little change from the cytology of the native neuroendocrine cells	<2 mitosis/10 HPFs	<2 %
Well-differentiated neuroendocrine tumor, Grade 2		2–20 mitoses/10 HPFs	3–20 %
Poorly-differentiated neuroendocrine carcinoma, Grade 3	Poorly-differentiated, with either pleomorphic large cells with prominent nucleoli or abnormal small cells with hyperchromatic nuclei and occasional crush artifact	>20 mitoses/10 HPFs	>20 %
Mixed adenoneuroendocrine carcinoma (MANEC)	At least 30 % glandular components and 30 % neuroendocrine components		

HPF= high power field (40X objective)

small amount of peptide at a cellular level. Despite this fact, cellular markers are not commonly used to discern the predominant peptide secreted by NETs. These neoplasms do harbor malignant potential. This potential is weighed by evaluating the tumors characteristics by the WHO classification of neuroendocrine neoplasms, which stratifies tumors into Well-differentiated NET Grade 1 (G1), Well-differentiated NET Grade 2 (G2), poorly-differentiated neuroendocrine carcinoma Grade 3 (G3), and mixed adenoneuroendocrine carcinoma (MANEC). This classification system is based on a combination of cell morphology and Ki67 proliferation index (Table 2) [25].

2.3 Pathologic Features

NETs are fleshy, homogeneous, red to tan in color. The lesions are often unencapsulated and contain variable amounts of cystic degeneration, calcification, or bone. Well-differentiated NETs have characteristic nested arrangements of the neoplastic cells that form acini, rosettes, ribbons, festoons, or trabeculae intermixed with a delicate vasculature (Fig. 13a). The cells are uniform with round to oval nuclei and have coarsely stippled "salt-and-pepper" chromatin pattern (Fig. 13b). Poorly differentiated neuroendocrine carcinomas usually demonstrate marked cellular atypia, frequent necrosis and a high proliferative rate. They have a more sheet-like or diffuse growth pattern, irregular nuclei, and less cytoplasmic granularity. Immunohistochemistry for markers of neuroendocrine differentiation (chromogranin-A and synaptophysin) are often performed to confirm the diagnosis (Fig. 13c). The mitotic rate and/or Ki67 proliferative index (whichever is higher) is used to grade the neoplasm (Fig. 13c).

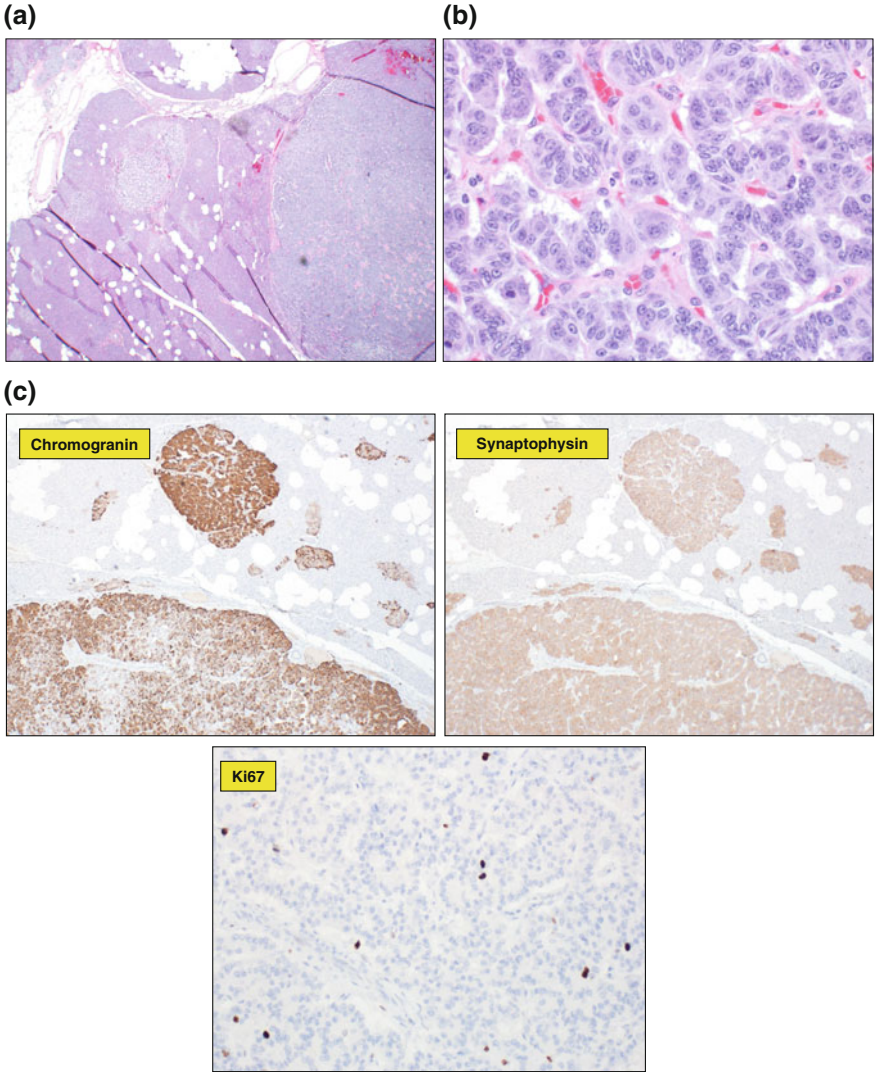

Fig. 13 Pancreatic neuroendocrine tumor (NET). **a** Two discrete nodules of neuroendocrine tumor are present within the pancreatic parenchyma. **b** The neoplastic cells have a trabecular growth pattern and the characteristic "salt and pepper" nuclear chromatin. **c** Immunohistochemical staining with chromogranin and synaptophysin (markers of neuroendocrine differentiation) highlight the neoplastic cells and staining with Ki67 highlights a proliferative index of less than 2 %, consistent with a Grade 1 neuroendocrine tumor

2.4 Acinar Cell Carcinoma

Acinar cell carcinoma is a rare epithelial neoplasm with differentiation toward the acini that make up the exocrine pancreas. Acinar cell carcinoma accounts for 1–2 %

of adult pancreatic tumors and roughly 15 % of pancreatic tumors in children (typically less than 15-years old) [18]. There is a slight male predominance. Clinical presentation is usually nonspecific secondary to mass effect and includes abdominal pain, nausea, and vomiting. Rarely patients present with peripheral eosinophilia, polyarthralgia, and subcutaneous fat necrosis secondary to elevated serum lipase which is produced by the neoplastic cells. This is referred to as hypersecretion syndrome or Schmidt's triad and is seen in less than 10 % of cases [20]. This group of neoplasms is a heterogeneous group, of which 25 % are considered mixed tumors having components of conventional invasive ductal adenocarcinoma and/or neuroendocrine carcinoma at the time of diagnosis [16]. While the majority of acinar cell neoplasms are malignant, rarely small benign cystic lesions with similar acinar cell differentiation are identified. In general, acinar cell carcinoma is not graded and is staged in a similar manner as invasive ductal adenocarcinomas.

While acinar cell carcinomas are highly aggressive with nearly 50 % presenting with metastatic disease at the time of diagnosis, the prognosis is still slightly better than invasive ductal adenocarcinoma with a 5-year survival of 25–50 % [16]. It has been reported that younger age and the ability to obtain negative resection margins portend a better prognosis, however, stage of disease at diagnosis remains the most reliable prognostic factor.

2.5 Molecular Pathology and Associated Syndromes

The molecular profile of acinar cell carcinoma is characterized by alterations in the APC/β-catenin pathway. Abnormalities commonly identified in conventional invasive ductal adenocarcinoma such as *KRAS, DPC4*, and *TP53* are usually absent in acinar cell carcinoma [18].

2.6 Pathologic Features

Grossly, acinar cell carcinomas are large solid well-circumscribed tumors with a yellow or brown cut surface often with areas of hemorrhage or necrosis [16]. When large cystic spaces are identified, a diagnosis of acinar cell cystadenocarcinoma may be entertained. Microscopically, acinar cell carcinoma is a cellular lesion, which typically forms nodules of tumor cells surrounded by fibrous septae (Fig. 14a). There are two major histological patterns, acinar and solid. The acinar pattern is composed of small glandular spaces (acini) lined by cells with abundant granular eosinophilic cytoplasm and round uniform nuclei with a single prominent nucleolus (Fig. 14b). The solid variant is composed of similar cells, although the cytoplasm tends to appear more amphophilic (blue), but lacks the glandular structures of the acinar pattern. Less commonly papillary and trabecular patterns have also been described. Mitotic figures are frequently seen scattered throughout the tumor.

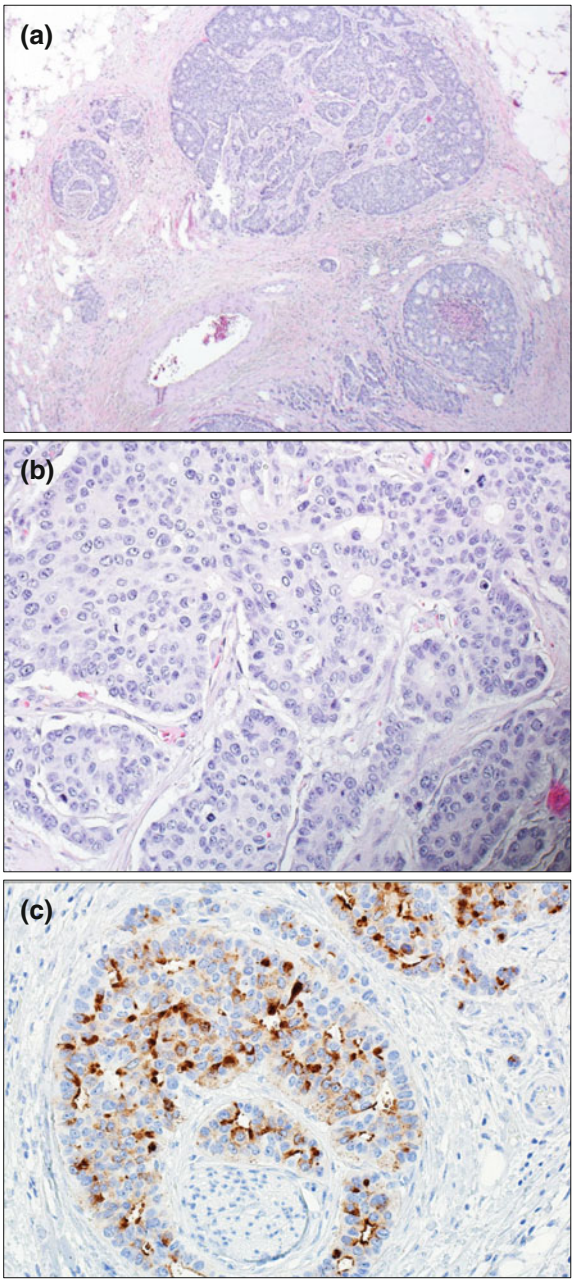

Fig. 14 Acinar cell carcinoma. **a** The neoplastic cells grow in a nested pattern separated by bands of fibrous stroma. **b** The tumor is composed of cells with round to oval nuclei with prominent nucleoli and frequent mitotic figures. **c** Immunohistochemical staining with trypsin shows cytoplasmic positivity in neoplastic cells, confirming the diagnosis

Immunohistochemical staining for exocrine pancreatic enzymes including trypsin, chymotrypsin, lipase, amylase and carboxyl ester lipase is a characteristic feature of acinar cell carcinoma [18]. Trypsin and chymotrypsin are very sensitive markers for detecting acinar differentiation [20] in these neoplasms (Fig. 14c).

2.7 Pancreatoblastoma

Pancreatoblastoma is a rare neoplasm that accounts for 25 % of pediatric pancreatic neoplasms [22]. Rare cases have also been described in adults and appear to have the same clinical and histologic features as pancreatoblastomas in children [24]. There is a male predominance and some cases have been associated with Beckwidth–Weideman Syndrome. Most pancreatoblastomas are found incidentally although a palpable abdominal mass is commonly identified on physical exam [22]. Elevated serum alpha-fetoprotein can also be seen in a subset of patients. Pancreatoblastomas are malignant neoplasms with an aggressive clinical course and frequent metastases. Overall survival is approximately 50 % [22]. Staging is similar to that of conventional ductal adenocarcinomas of the pancreas.

2.8 Molecular Pathology and Associated Syndromes

Molecular profile of pancreatoblastomas is similar to acinar cell carcinomas including alterations in the APC/β-catenin pathway. This is reflective of the acinar cell differentiation of pancreatoblastomas; however, endocrine and ductal differentiation are also seen [22].

2.9 Pathologic Features

Grossly, pancreatoblastoma is a large (mean 11 cm) circumscribed lobulated mass with a fleshy cut surface commonly with areas of necrosis [22]. Pancreatoblastoma can occur anywhere in the pancreas. Histologically, pancreatoblastoma is composed of cellular sheets of polygonal cells with prominent nucleoli that alternate with areas of acinar (gland-forming) differentiation. Squamoid nests composed of plump round cells or whorled spindle cells are the characteristic finding in pancreatoblastoma [24]. The nuclei within the squamoid nests may appear cleared out due to an accumulation of biotin [22].

The immunohistochemical pattern is consistent with the multidirectional differentiation of these neoplasms including acinar differentiation and the expression of pancreatic exocrine enzymes (trypsin and chymotrypsin), focal neuroendocrine differentiation (focal positivity for synaptophysin and chromogranin), and ductal differentiation (focal positivity for CEA) [24].

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Gall Bladder Cancer

Audrey E. Ertel, David Bentrem and Daniel E. Abbott

Abstract

Gallbladder carcinoma (GBC) is the most common biliary epithelial malignancy, with an estimated 10,910 new cases and 3700 deaths per year (Siegel et al. in CA Cancer J Clin 65:5–29, 2015 [1]). This disease's insidious nature and typically late presentation place it among the most lethal of invasive neoplasms. Gallbladder cancer spreads early by lymphatic or hematogenous metastasis and by direct invasion into the liver. While surgery may well be curative at early stages, both surgical and nonsurgical treatments remain largely unsuccessful in patients with more advanced disease.

Keywords

Gall bladder cancer · Cholangiocarcinoma · Gall bladder polyps · Porcelain gall bladder

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1 Introduction

While GBC is the most common malignancy of the biliary system, it is a relatively rare neoplasm. The incidence steadily increases with age, reaching a maximum in the seventh decade of life. GBC predominantly affects women (75–85 %) and is more commonly found in Native, Alaskan, or Hispanic Americans [2, 3]. There is in addition an association between the presence of gallstones and GBC, though this relationship is not well understood. Most patients with GBC have gallstones [4]. The high-risk ethnic groups noted above develop GBC at an even higher proportional rate than they incur gallstones, while other patients develop GBC in the absence of cholelithiasis [2, 5, 6]. Interestingly, size of gallstones is also a risk factor; patients with stones >3 cm are 10 times more likely to develop GBC compared with patients with subcentimeter-sized gallstones. Summarily, we must acknowledge a lack of understanding of the pathophysiology in GBC development. While cholelithiasis and its associated repeated inflammation may contribute, this relative risk and the influence of environmental toxins (carcinogens in tobacco, diet, etc.) or hormones remain unclear. Additionally, body mass index (BMI) has been associated with an increased risk of gallbladder cancer however this relationship remains incompletely defined [7].

Lack of effective screening and early symptomotology contribute to the relatively delayed presentation of the disease. Symptoms of invasive gallbladder carcinoma are notoriously vague and nonspecific. Right upper quadrant pain primarily associated with benign biliary disease may be the first and only presenting symptom. Alternatively, a smaller percentage of GBC patients may present with obstructive jaundice secondary to local disease progression and obstruction of biliary outflow tracts [8]. Other physical signs are limited. Recent incisions and biopsy sites as well as distant nodal basins should be examined (Fig. 1). However, the vast majority of patients are diagnosed with malignancy after cholecystectomy. Approximately 1 % of cholecystectomy specimens contain evidence of invasive disease [9], and this rate is significantly higher in patients >70 years.

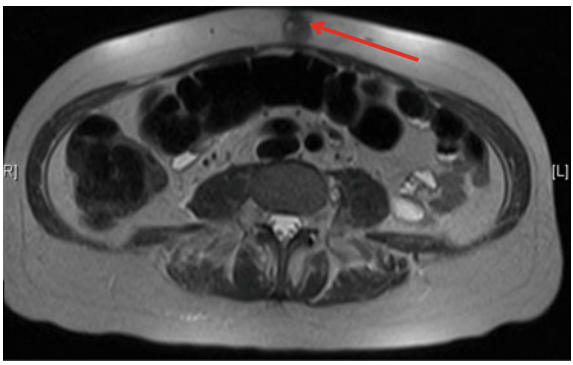

Fig. 1 Magnetic resonance imaging revealing Sister Mary Joseph nodule, a distant site of disease from the patient's primary gallbladder carcinoma

Broadly, surgical therapy is indicated for localized disease, that is, pathology limited to the gallbladder, liver bed, and regional lymph nodes (Fig. 2a, b). Operative strategies differ, however, depending on depth of invasion of the primary

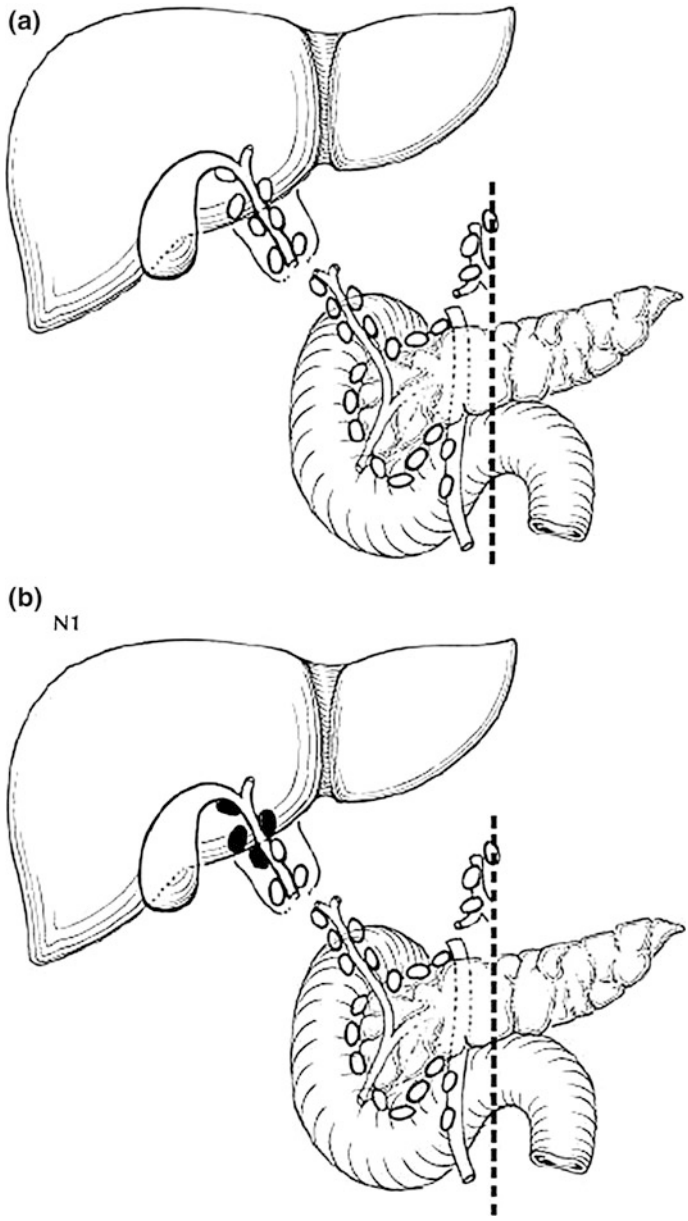

Fig. 2 **a** Regional lymph nodes of the gallbladder. **b** N1 disease is defined as metastasis to regional lymph nodes [57]

lesion and the presence of lymphadenopathy concerning for metastasis. Generally, T1a lesions, defined as tumors limited to the gallbladder mucosa without extension to muscular layers of the wall, can be sufficiently treated with cholecystectomy alone without further radical surgical resection. Patients whose disease extends into the muscular layers of the gallbladder and beyond, however, require a more aggressive surgical approach. For T1b, T2, or T3 lesions with (or without) associated regional adenopathy, *en bloc* resection with regional lymphadenopathy is appropriate. Extension of disease proximally to the bile duct may require bile duct excision with reconstruction for biliary drainage. For patients with resected T2 lesions, 30 % have been found to have nodal disease and 16 % metastatic disease. With resected T3 lesions, 58 % were found to have nodal disease and 42 % peritoneal metastases [10]. This leads to a low likelihood of complete resection (27 %) for patients with clinically staged T3 tumors.

With respect to extent of disease related to hepatic parenchyma, critical attention must be given to invasion of the hepatic arteries and ducts. Notably, invasion of the right hepatic artery or duct is not a contraindication to aggressive surgical therapy; while these findings may necessitate a right (or even extended right) hepatectomy, adequate reconstruction can be accomplished. Disease involving the left hepatic artery, left hepatic duct, or the confluence of the portal vascular or biliary structures is indicative of more extensive disease less likely controlled by surgery (Fig. 3a). Similarly if a tumor progresses distally toward the duodenum and pancreas with (perhaps) invasion of one of those structures, a Whipple procedure may be considered but long-term disease control is doubtful (Fig. 3b).

1.1 Polyps

Gallbladder wall polyps are not uncommon, and polypoid lesions of the gallbladder are frequently noted on abdominal ultrasonography. The clinical relevance of this finding, however, is not entirely clear. The surgeon's suspicion of growth as well as the evolution of dysplasia and eventual invasive carcinoma serves as the basis for recommending close surveillance, or even resection, for certain polypoid lesions. Concerning features include solitary, large, immobile, or changing lesions.

Traditionally, gallbladder polyps >1 cm in size were thought to be at higher risk for containing GBC foci (Table 1). Myers et al. recommended resection for polypoid lesions >10 mm, in patients 50 years of age or older, associated with gallstones, and increasing in size [11]. A larger series from Japan concurred, finding that 8 of 50 polyps revealed in cholecystectomy specimens were malignant [12]. In this series, 88 % of malignancies were identified in lesions >10 mm, and 75 % of invasive tumors were found in patients older than age 60. These results were corroborated in yet another study, confirming that malignant risk is elevated in elderly patients and in those lesions >10 mm in size [13]. However, one large retrospective review from Memorial Sloan-Kettering Cancer Center (MSKCC) detailed 417 patients found to have a polypoid lesion of the gallbladder [14]. Of these, 94 % were ≤10 mm, and on repeat ultrasonography only 6 % of 143 lesions

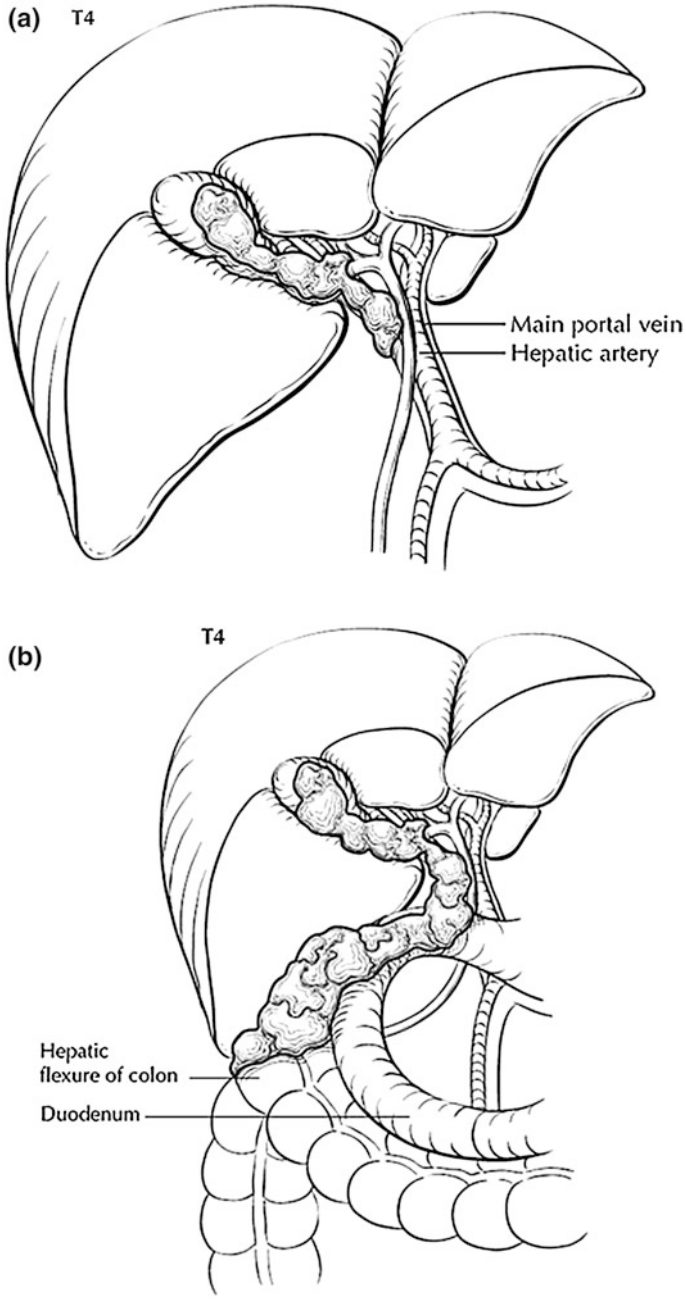

Fig. 3 **a** Tumor invading main portal vein or hepatic artery. **b** Tumor invading extrahepatic organs [57]

Table 1 Series of gallbladder (GB) calcifications and polyps

References	*N*	Entity studied (calcification or polyp)	Size, lesion characteristics	Association or incidence of GBC	Conclusion
Kim et al. [51]	9 (of 3159 inspected)	Calcifications	Pathologically proven porcelain gall bladder	No porcelain GB had GBC; no patients with GBC had calcification	No association between porcelain GB and GBC
Ito et al. [14]	417	Polyps	94 % of polyps ≤10 mm, 7 % >10 mm	No cases of invasive GBC in 80 surgical specimens	Risk of invasive cancer in GB polyp is extremely low
Park et al. [15]	1558	Polyps	2.1 % of polyps were neoplastic	19 adenomas, 2 low-grade dysplasia, 4 high-grade dysplasia, 4 early GBC and 4 advabced GBC	Polyp size ≥10 mm failed to predict nearly 50 % of neoplastic polyps. Smaller polyps must be carefully observed
Stephen and Berger [17]	150 GBC (of 25,900 inspected)	Calcifications	Selective mucosal $n = 27$, intramural $n = 17$	7 % of selective mucosal with GBC, none with intramural	Pattern of calcification determines cancer risk
Towfigh et al. [18]	103 (of 10,741 inspected)	Calcifications ("porcelain" GB)	Partial calcification of wall ($n = 15$) and GBC ($n = 88$)	No porcelain GB had GBC; no patients with GBC had calcification	Porcelain GB/calcifications not associated with GBC
Terzi et al. [13]	100	Polyps	74 % benign (20 % adenomas, 15 % adenomatous hyperplasia)	26 % rate of malignancy	Risk factors for GBC are age >60, size >10 mm, coexistence of gallstones
Mainprize et al. [52]	38	Polyps	11/34 patients with polyps pathologically proven; 7/11 benign cholesterol polyps	All neoplastic disease ($n = 4$) arose in polyps >10 mm	Should perform cholecystectomy for polyps >10 mm
Kubota et al. [53]	72	Polyps	47/72 cholesterol polyps, GBC in 16/72	88 % of GBC in polyps >18 mm, 56 % were sessile	Polyps <18 mm potentially early stage, >18 mm likely advanced
Koga et al. [12]	32 (of 411 resected specimens)	Polyps	Primarily examined age, size and type (most were cholesterol polyps)	88 % of malignancy in polyps >10 mm, 75 % in patients >60 year	Risk factors for GBC are age >60, size >10 mm

GBC Gallbladder carcinoma

demonstrated growth. Furthermore, in 80 patients who underwent cholecystectomy, only 10 % of polyps contained adenomatous tissue, one 14-mm polyp had a carcinoma-in situ focus, and no polyp demonstrated invasive carcinoma. This large, most recent series demonstrated that the optimal treatment for gallbladder polyps, and, specifically, what risk factors correlate with malignant degeneration, are poorly defined. Thus, presence of gallbladder polyps alone is less clearly an indication for cholecystectomy. Lastly, a Korean study by Park et al. determined the optimal size to predict neoplastic change within gallbladder polyps to be $\geq$8 mm with a sensitivity and specificity of 64 and 86 % as compared to 55 and 94 % when using the cutoff of 10 mm [15].

1.2 Calcifications

Calcifications of the gallbladder (leading to the oft-used term "porcelain gallbladder") have been viewed as a risk factor for developing GBC (Table 1). Polk, in 1966, was one of the original proponents of this hypothesis and pointed to two pieces of evidence to substantiate it. Firstly, work by Etala that revealed 16 cases of gallbladder carcinoma within 26 porcelain gallbladders was highly suggestive of calcification as a sign of malignant progression. Furthermore, in his review of 100 patients identified with porcelain gallbladder, 22 GBAs were revealed [16].

More recent literature more specifically defined gallbladder calcifications. A report by Stephen and Berger explicitly classified gallbladder calcifications as intramural (n = 17) or selective mucosal (n = 27). They reported that of gallbladder specimens of the intramural type, no cases of adenocarcinoma were identified, whereas selective mucosal calcifications heralded a 7 % malignancy rate [17]. In a much larger review of over 10,000 patients, Towfigh et al. [18] reported that of 88 patients with GBC, none exhibited wall calcifications. Although evidence-based guidelines are lacking, many still view calcifications as an indication for resection. A recent review of the literature by Schnelldorfer revealed that, although not as high as once thought, the risk for GBC in the presence of calcifications remains—quoting a 6 % increased risk in those patients with GB calcifications versus 1 % in those patients without [19].

2 Preoperative Planning

2.1 Diagnosis

There is no single clinical physical exam finding, laboratory value, or imaging modality that can unequivocally diagnose early GBC, and rarely are any of these approaches given consideration preoperatively. As noted above, the usual clinical scenario is that of incidentally found GBC at the time of cholecystectomy or during routine pathologic examination. Mass lesions, focal thickening, or mucosal calcifications should raise suspicion of an underlying malignancy.

2.2 Staging

Cancers of the gallbladder are staged according to their depth of penetration and extent of spread (Fig. 4). Staging for GBC consists of imaging modalities as well as pathologic specimens when available from prior surgical resections. These cancers frequently spread to the liver, which is involved in ≤70 % of patients at the time of surgical evaluation.

Tumor size (T)—depth of invasion
TX—Primary tumor cannot be assessed
T0—No evidence of primary tumor
Tis—Carcinoma *in situ*
T1—Tumor invades lamina propria or muscular layer
T1a—Tumor invades lamina propria
T1b—Tumor invades muscular layer
T2—Tumor invades perimuscular connective tissue; no extension beyond serosa or into liver
T3—Tumor perforates the serosa (visceral peritoneum) and/or directly invades the liver and/or one other adjacent organ or structure, such as the stomach, duodenum, colon, pancreas, omentum, or extrahepatic bile ducts
T4—Tumor invades main portal vein or hepatic artery or invades two or more extrahepatic organs or structures

Regional Lymph Nodes (N)
NX—Regional lymph nodes cannot be assessed
N0—No regional lymph node metastasis
N1—Metastases to nodes along the cystic duct, common bile duct, hepatic artery, and/or portal vein
N2—Metastases to periaortic, pericaval, superior mesentery artery and/or celiac artery lymph nodes

Distant Metastasis (M)
M0—No distant metastasis (no pathologic M0; use clinical M to complete stage group)
M1—Distant metastasis

Staging (I-IV)

Stage 0	Tis	N0	M0
Stage I	T1	N0	M0
Stage II	T2	N0	M0
Stage IIIA	T3	N0	M0
Stage IIIB	T1-3	N1	M0
Stage IVA	T4	N0-1	M0
Stage IVB	Any T	N2	M0
	Any T	Any N	M1
Stage unknown			

Fig. 4 2010 American Joint Committee on Cancer tumor-node-metastasis (TNM) staging for gallbladder carcinoma [58]

For GBC discovered by the pathologist postoperatively, subsequent staging depends on pathologic tumor (T) stage. Extent of locoregional disease is directly correlated with the T stage of GBC, with more invasive lesions exhibiting higher rates of lymph node and distant metastases [10, 20]. The wall of the gallbladder has a mucosa, a smooth muscle layer, perimuscular connective tissue, and serosa. Along the attachment to the liver, no serosa exists, and the perimuscular connective tissue is continuous with the connective tissue of the liver. T stage classification depends on the depth of tumor penetration into the wall of the gallbladder, on the presence of tumor invasion into the liver, hepatic artery, or portal vein, and on adjacent organ involvement. T1a lesions, those that have not breached the muscular layers, require no further staging or treatment. Accurate staging for resectable tumors requires that all hilar lymph nodes be removed and analyzed. The hilar nodes include the lymph nodes along the common bile duct, hepatic artery, portal vein, and cystic duct. Nodal spread beyond the hepatoduodenal ligament (celiac, retropancreatic, aortocaval) generally represents metastatic disease precluding a curative operation.

For any tumor that has invaded the muscular layer or beyond, additional postoperative imaging is mandatory for clinical staging. This should include high-resolution imaging of the chest, abdomen, and pelvis, specifically evaluating regional lymphadenopathy or any evidence of distant metastases. Additionally, because even sophisticated imaging may understage disease and underestimate the propensity to seed the peritoneal surfaces after the prior operation, staging laparoscopy should be strongly considered. A minority of patients may present not with a tissue diagnosis, but with either (A) an incidentally found mass discovered while imaging the patient for other reasons, or (B) jaundice. Under these circumstances, in addition to the imaging studies described above, one should obtain a complete blood count (CBC), liver function tests, consider adding Ca 19-9 and carcinoembryonic antigen (CEA) serum markers, and again strongly consider staging laparoscopy, depending on results of the aforementioned tests.

2.3 Imaging

Ultrasonography is the most frequently utilized imaging modality for biliary pathology. Perhaps the only caveat of this approach is the user-dependent nature of both acquiring and analyzing the imaging. For GBC, ultrasound has a touted 70–90 % sensitivity in detecting GBC, but this does not hold true for all stages [21, 22]. A number of sonographic findings are consistent with GBC, though many of them are quite nonspecific; these include gallbladder wall thickening, calcifications, any mass or soft tissue density interrupting or protruding through the mucosa, and any architectural ambiguity at the gallbladder–liver intersection. Furthermore, ultrasound may be helpful in determining extent of locoregional disease progression to portal structures including the hepatic artery or portal lymph nodes [23]. Endoscopic ultrasound is an increasingly utilized adjunct in this regard, with the added benefit of a fine needle aspiration tissue biopsy of suspicious regional abnormalities.

Computed tomography (CT) may define advanced (distant) disease and provide specific information about extent of local disease (Fig. 5a, b). Most importantly, CT will help delineate T3 and T4 lesions but is not to be relied upon for discrimination of smaller lesions due to the lack of sensitivity in differentiating mucosal and submucosal planes. CT can also be particularly useful for assessing regional lymphadenopathy, predominantly nodes measuring >1 cm. Magnetic resonance is yet one further diagnostic modality that may be utilized if a preoperative diagnosis of GBC is being considered. Though the most expensive tool available, magnetic resonance imaging (MRI) may be extremely useful in outlining invasion of carcinoma into surrounding soft tissues. Specifically, MRI can help distinguish extent of invasion into surrounding liver parenchyma, presence of tumor around and into adjacent biliary structures that may or may not manifest as biliary narrowing, and

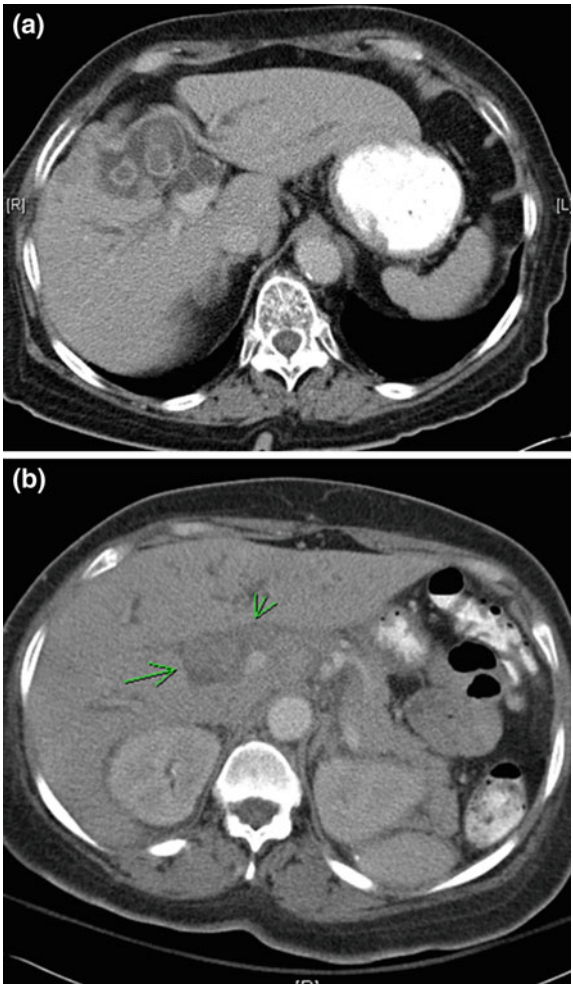

Fig. 5 **a**, **b** Computed tomography imaging of locally advanced gallbladder carcinoma

obstruction or dilation, and also help define extrahepatic disease. When used as an adjunct to MRI, magnetic resonance cholangiopancreatography (MRCP) may better clarify extent of biliary pathology and help guide operative management with respect to not only whether resection is appropriate, but also what precise liver segments must be excised.

Fluorodeoxyglucose positron emission tomography (PET) has evolved to become an important test in the management of GBC. It is capable of confirming lymphatic metastases in high risk patients. In a recent series of 126 patients with biliary malignancy, 24 % of PET scans influenced therapy [24]. For T3 or T4 GBC, we now consider PET an important staging adjunct in patient selection for radical surgery.

2.4 Neoadjuvant Therapy

There is a paucity of data about the role of neoadjuvant therapy for GBC. In fact, due to low response rates with adjuvant therapy, current standards require aggressive surgical resection for any disease that may be amenable to excision with negative margins. The only sizable series addressing this topic details 23 patients given continuous 5-fluorouracil (5-FU) and 45 Gy of external beam radiation therapy (EBRT) prior to surgical resection. Eight patients required delayed cholecystectomy due to adverse reactions, and there was no survival benefit demonstrated in this cohort compared to 18 patients who were not given neoadjuvant therapy. Thus, the authors' conclusions were that neoadjuvant chemoradiation for GBC was not helpful, and in fact may be deleterious [25]. Advances in adjuvant chemotherapy, namely the administration of gemcitabine and cisplatin, have provided guarded optimism, a recent study out of India consisting of 37 pateints, demonstrated an overall response rate of 67.5 % following neoadjuvant chemotherapy using a gemcitabine-platinum based regimen. R0 resection rate was 46 % with improvement in median overall survival and progression free survival of 13.4 and 8.1 months [26]. Although encouraging, the rarity of this tumor and the general lack of regression in response to chemoradiation mean that, practically, neoadjuvant therapy for GBC should be considered selectively.

3 Surgical Technique

When performing a cholecystectomy for presumed gallstone disease and intraoperative concern for GBC is raised, the surgeon should convert to an open operation (if begun laparoscopically) and explore the right upper quadrant for evidence of disease; any suspicious findings should prompt pathologic review. If GBC is confirmed and appears confined to the gallbladder, an extended cholecystectomy with *en bloc* hepatic resection and regional lymphadenectomy is indicated. If the surgeon's comfort with advanced hepatobiliary procedures is in question, closing

the wound is most appropriate, with definitive resection reserved for a more experienced practitioner [27].

The surgical approach to GBC resection has varied historically. The spectrum has ranged from simple cholecystectomy to cholecystectomy with either (A) wedge resection of adjacent liver parenchyma, (B) formal segment IVb-V liver resection, or even (C) complete right/extended right hepatectomy [28]. When a major hepatectomy is not required by tumor location, determining the optimal extent of the hepatectomy may be difficult, as it has not been well studied [29]. While some investigators have described laparoscopic cholecystectomy (LC) for GBC, there is risk of spillage, tumor dissemination, and port-site recurrence [30, 31]. If there is preoperative suspicion of GBC, the surgeon should plan an open operation. As noted above, simple cholecystectomy is adequate for T1a lesions. For resectable T1b lesions and larger, the standard has become a segment IVb-V liver resection (with, of course, cholecystectomy if the gallbladder has yet to be removed) with regional lymphadenectomy. Additionally, this may include resection and reconstruction of the extrahepatic bile ducts (Fig. 6).

Preoperative medical optimization, with particular emphasis placed on coronary and pulmonary disease, as well as preparation of blood products that may be required, is imperative. At operation, the patient is laid supine on the operating room table with appropriate preinduction measures taken. Communication with the anesthesiologist is critical. Data have shown that low central venous pressure maintained during parenchymal transection can lead to significantly lower blood loss [32]. Likewise, mild Trendelenburg positioning may guard against air

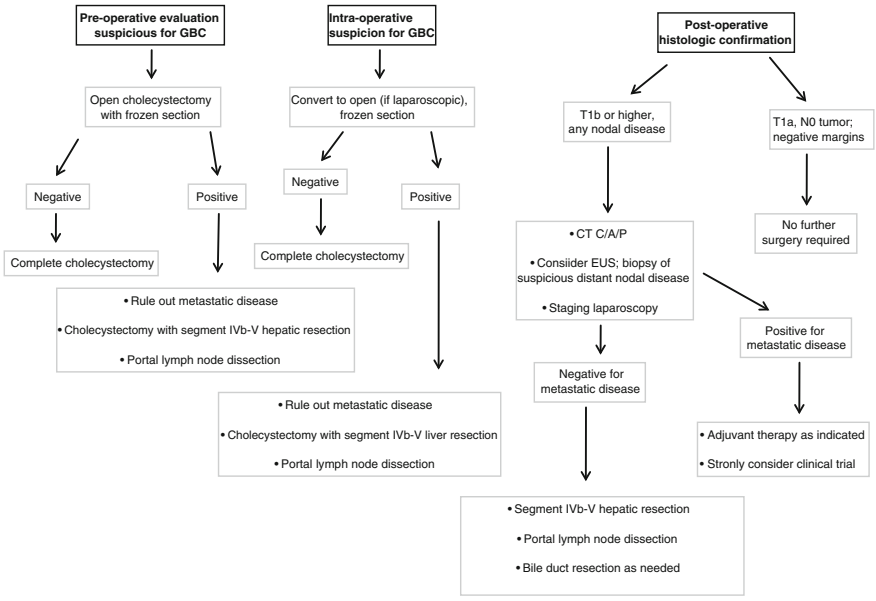

Fig. 6 Management algorithm for gallbladder carcinoma

embolism during division of the liver. Epidural anesthesia may also be utilized to decrease postoperative narcotic requirements as well as decrease the incidence of postoperative pneumonia. Lastly, preoperative preparation should include mobilization of ultrasonography equipment for intraoperative use. A right subcostal (Kocher) incision should provide adequate exposure to the critical structures of the right upper quadrant, with extension in the midline or to a chevron incision if required.

Once the abdomen is entered, the falciform ligament is divided and the bowel excluded from the operative field. Inspection of the abdomen should be performed to evaluate for metastatic disease. A Kocher maneuver will provide access to distant lymph nodes for evaluation, namely celiac, para-aortic, and retropancreatic. Disease in these nodes represents advanced disease. The portal structures from the duodenum to the hilum of the liver are dissected and inspected and nodal tissue is removed. Arterial or venous involvement in the hepatoduodenal ligament should result in termination of the procedure. If tumor is adherent to the right-sided vascular inflow structures, an extended right hepatectomy is mandated for complete resection. Similarly, disease in left-sided vascular or biliary structures is less likely controlled by surgery. In the absence of these findings of more distant disease, the extrahepatic bile ducts should then be evaluated. For disease extending up or down the common hepatic or bile ducts, an extensive resection of these structures will be required. Reconstruction options include either Roux-limb or loop hepaticojejunostomy or choledochojejunostomy, depending on extent of ductal resection. Of note, when these large biliary structures are divided, special care should be used to eliminate bile spillage out of concern for tumor dissemination; this includes prompt (and permanent) closure of the common bile duct stump and temporary ligation or clipping of the remaining biliary duct.

For the more extensive hepatic resection (*en bloc* with the gallbladder if it remains in situ), encircling the portal structures and hepatic veins will provide appropriate inflow and outflow control should substantial bleeding be encountered. To begin, score the liver to the right of the ligamentum teres, the medial aspect of the dissection. This line of transaction can be carried through the parenchyma posteriorly, which will include ligating a branch of the left portal vein supplying segment IVb. Intraoperative ultrasound will be especially useful, as identification of major vascular structures will minimize unnecessary hemorrhage. As the dissection proceeds laterally, the middle hepatic vein will be encountered and must be divided, as this structure serves as the outflow for both segments IV and V. The lateral and superior aspects of segment V are identified and parenchymal transaction is continued from superficial to deep into the liver. The remaining large vascular structure encountered to complete segment V resection will be an anterior branch of the right portal vein, which should be ligated and divided. The remaining parenchymal division will include smaller arterial and biliary radicals. Hemostasis, with or

without the use of topical agents, should complete the resection. A large randomized trial has demonstrated that drain placement following hepatic resection may actually lead to increased morbidity, and is unnecessary [33].

4 Postoperative Management

Immediately postoperatively, maintaining central venous pressure and close monitoring for symptoms and signs of hemorrhage are important. Coagulopathy from either underlying liver disease or malnutrition should be corrected if the international normalized ratio (INR) reaches 1.8 or higher. Hepatic regeneration may require substantial phosphorous for ATP production, and close attention should be paid to this and other electrolytes. In the absence of a pronounced ileus, a diet can be introduced and advanced early.

4.1 Follow-up

Postoperative follow-up should include biannual 3-dimensional imaging of the chest, abdomen, and pelvis for at least two years, though this recommendation is not based on high-quality evidence.

4.2 Chemotherapy

Generally, chemotherapy is indicated for patients with evidence of disease, which is to say, patients with positive margins or known metastases. The question of utilizing chemotherapy for node-positive disease ostensibly resected is still unresolved. Some practitioners may provide adjuvant chemotherapy for pathologic stage II or higher disease. Traditionally, chemotherapy has been 5-FU based. In a study by Takada et al. [34], postoperative 5-FU/mitomycin-C treatment of patients with resected stage II-IV GBC demonstrated a 5-year survival of 26 %, versus 14 % in patients who were not given chemotherapy (Table 2). More recent trials have included the administration of a variety of agents. Chatni et al. described 65 patients with unresectable or metastatic GBC receiving 5-FU and cisplatinum, which was reportedly well tolerated. Five patients demonstrated a complete response, with the other patients responding variably—median survival was only 5.7 months overall [35].

Special attention should be paid to the increasing use of gemcitabine as an adjunctive therapy. There are in vitro data demonstrating that gemcitabine, in conjunction with oxaliplatin, exhibits synergistic effects in G0/1 arrest in four GBC cell lines [36]. In clinical trials, the use of gemcitabine has been used in variable dosing regimens (800–1000 mg/m^2) and with other agents, including pemetrexed, 5-FU/leucovorin, and capecitabine [37–39]. Median survival in these studies ranged from 6.6 to 16.0 months. Caution should be used in interpreting some of these data, however, as some included other biliary tract malignancies.

Table 2 Trials of chemotherapy (CTx) and radiotherapy (RTx) for gallbladder carcinoma

References	*N*	CTx, RTx, or both	Type of CTx	Type of RTx	Control arm	Survival	Study caveat
Valle et al. [59]	86–410	Chemotherapy	Cisplatin (25 mg/m^2) then gemcitabine (1000 mg/m^2)	–	Gemcitabine (1000 mg/m^2) alone	11.7 months in study arm versus 8.1 months in control arm	Included all biliary cancers
Gold et al. [41]	73 (25 in study arm)	CTx/RTx	5-FU	50.4 Gy (median dose)	Surgery alone	4.8 versus 4.2 years (median survival)	Retrospective review
Chatni et al. [35]	65	Chemotherapy	5-FU, cisplatinum	–	None	5.7 months (median survival)	Case series, no control group
Alberts et al. [36]	58	Chemotherapy	Pemetrexed, gemcitabine (800 mg/m^2)	–	None	6.6 months (median survival)	Case series, no control group
Alberts et al. [38]	42	Chemotherapy	Gemcitabine (1000 mg/m^2), 5-FU, leucovorin	–	None	9.7 months (median survival)	Case series, included all biliary tract disease
Cho et al. [39]	24	Chemotherapy	Gemcitabine (1000 mg/m^2), capecitabine	–	None	16 months (median survival)	Unresectable patients
Takada et al. [34]	112	Chemotherapy	Mitomycin-C, 5-FU	–	Surgery alone	26 % versus 14.4 % (5 year survival)	Subset of larger study group
Kresl et al. [42]	21	CTx/RTx	5-FU	54 Gy (median dose)	None	65 % versus 0 % (5 year survival for stage I-III versus IV, respectively)	Retrospective review, no control group

5-FU 5-fluorouracil

4.3 Radiation Therapy

The role of radiation therapy as adjuvant therapy for GBA is even more poorly defined. This is true for two specific reasons: (1) most recurrent disease is found at distant sites, and (2) external beam radiation is often given for symptomatic recurrence, and the extent of even regional recurrence may not become symptomatic until disease progression is very advanced [40]. There are data to suggest that, on a limited basis and in select patient cohorts, the addition of radiation to chemotherapy following surgical resection provides a small survival benefit [41]. A series of 21 patients from a high volume center concluded that the addition of EBRT to 5-FU following R0 resection, with no evidence of residual disease, exhibited a 5-year survival (all stages of disease included) of 64 %, superior to many published reports. Five-year survival was 0 % in patients with evidence of residual disease. Additionally, these authors reported 100 % 5-year local control rates with high dose EBRT (>54 Gy) [42]. These studies do not provide definitive evidence for standard use. Alternatively, some will propose reserving radiation therapy for symptomatic recurrence that simply needs palliation. Clearly, randomized study is still needed to define the benefit of adjuvant radiation for GBC.

5 Complications

5.1 Bile Duct Injury/Leak

Perhaps the most feared complication of biliary surgery is unintentional injury of bile ducts that are not involved in resection and, thus, will be critical to biliary drainage from remaining liver segments. Attention should be paid especially when energy devices are used in proximity to essential draining ducts. Simple bile leaks from liver parenchyma will almost always be self-limiting, with adequate internal and/or external decompression (endoscsopic stent placement with or without sphincterotomy and percutaneous drainage of the biloma). Furthermore, if a common bile duct resection has been performed and the reconstruction has, by definition, eliminated the downstream sphincter, this should aid resolution.

5.2 Port-Site Disease

Decades of operative experience and the evolution of laparoscopic cholecystectomy have led to the oft-described and universally concerning phenomenon of port-site disease. Evidence for this entity and suggested treatment are born of case series and reports. A Japanese study has published a report of 28 laparoscopic cholecystectomies performed for GBC; 3 patients developed port-site recurrences [43]. Another large series of 37 patients with undiagnosed GBC who underwent LC demonstrated a 14 % port-site recurrence rate [44]. Additionally, this and other studies found an increased incidence of disseminated disease when the gallbladder was perforated

during LC. With this in mind, the practice of port-site excision at the time of radical resection seems justified, adding minimal morbidity while perhaps eliminating a devastating process.

6 Results

Survival is closely correlated to stage, with 5-year survival ranging from 60 % to 1 %, for stage 0—IV disease, respectively, in a retrospective review of 2500 patients (Fig. 7) [45]. Patients with less invasive disease will exhibit better long-term disease-free and overall survival, and are more likely to benefit from aggressive surgical therapy [10, 29]. In fact, even patients with carcinoma in regional lymph nodes may enjoy a 45–60 % 5-year survival with *en bloc* resection and regional lymphadenectomy [29, 46, 47]. Duffy et al. reported median survival for all patients in a 10-year study from MSKCC at 10.3 months; substratification revealed median survival of 12.0 and 5.8 months for stages 1a-III and IV, respectively [48]. For tumors confined to the gallbladder mucosa, simple cholecystectomy was associated with up to 90–100 % disease-specific survival [49]. For tumors invading the muscular and serosal layers of the gallbladder, results have varied by extent of disease and extent of resection (Table 3) [29, 50]. The treatment of more extensive tumors invading into the liver and adjacent organs has been more controversial. These tumors tend to be more invasive and require more extensive

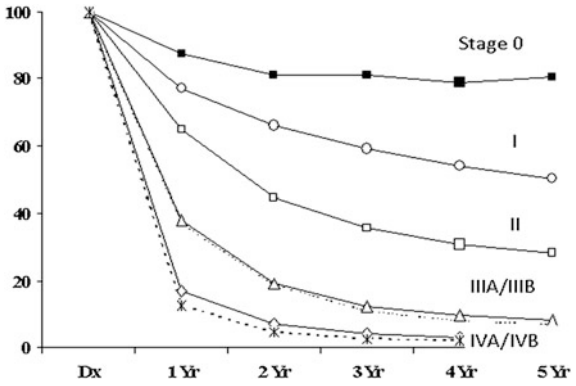

Fig. 7 Five-year survival based on disease stage [45]

Table 3 Reports of survival following surgery for advanced gallbladder carcinoma

References	Subject size (*N*)	Survival (median, mo)	Survival (1/3/5 year, %)
Kohya and Miyazaki [54]	29	13	50/17/17
Coburn et al. [55]	71	19	60/42/20
Reddy et al. [56]	12	38	58/50/15
Fong et al. [10]	36	17	71/27/21

resections, which previously have not resulted in improved long-term survival with the exception of node-negative tumors [50]. Additional prognostic factors include histologic grade and lymphatic/vascular invasion.

7 Conclusions

GBC remains a dire disease. A fortunate few will be diagnosed at an early stage and cured by aggressive surgical therapy, whereas the majority of patients will be relegated to palliative therapy. Here we have detailed the appropriate workup, indications (and contraindications) for surgery, as well as an accepted operative approach. Resection can (and should) be accomplished with minimal mortality and acceptable morbidity so that a select cohort of patients can enjoy extended survival. Future improvements in outcome will rely on primarily nonsurgical therapeutics and improved screening/diagnostic mechanisms.

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Diagnosis and Management of Intrahepatic and Extrahepatic Cholangiocarcinoma

Jason Ho and Steven A. Curley

Abstract

Cholangiocarcinomas (CC) are rare tumors which usually present late and are often difficult to diagnose and treat. CCs are categorized as intrahepatic, hilar, or extrahepatic. Epidemiologic studies suggest that the incidence of intrahepatic CCs may be increasing worldwide. In this chapter, we review the risk factors, clinical presentation, and management of cholangiocarcinoma.

Keywords

Cholangiocarcinoma · Bile duct cancer · Gallbladder cancer

1 Introduction

Cholangiocarcinomas (CC) are rare tumors which usually present late and are often difficult to diagnose and treat. CCs are categorized as intrahepatic, hilar, or extrahepatic. Epidemiologic studies suggest that the incidence of intrahepatic CCs may be increasing worldwide. Several risk factors for CC have been identified, including primary sclerosing cholangitis, liver fluke infestation, hepatolithiasis, viral hepatitis, and congenital abnormalities of the biliary tree. Diagnosis of CC requires thoughtful integration of clinical information, imaging studies, cytology and/or histology, and serum tumors markers. Although complete surgical resection is required for the chance of a cure, the majority of patients are unresectable at presentation due to poor performance status or comorbidity, locally advanced

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disease, extensive adenopathy, or metastases. Preliminary studies suggest that neoadjuvant therapy followed by liver transplantation may result in long-term survival in highly selected patients with localized, node-negative CC. In patients who are not surgical candidates, palliative biliary decompression (particularly when combined with photodynamic therapy) and definitive radiotherapy may be of benefit.

2 Classification

CCs are primarily categorized by anatomic location within the hepatobiliary system as intrahepatic, perihilar, and distal extrahepatic owing to differences in staging, treatment, and prognosis (Fig. 1a) [1, 2].

Intrahepatic disease arises from peripheral bile ducts within the liver parenchyma proximal to the secondary biliary radicles, whereas perihilar and distal extrahepatic CCs occur within the hepatic confluence and distal common bile duct, respectively (Fig. 1) [3]. Intrahepatic, perihilar, and distal extrahepatic tumors comprise approximately 10, 50, and 40 % of CCs, respectively. The Liver Cancer Group of Japan further stratifies CCs by macroscopic morphology, each with characteristic CT and MR imaging patterns [4, 5], to improve prognostic and growth-pattern predictions (Fig. 1b). Duplication of terminology exists in the literature, compiled as follows for clarity: mass-forming (exophytic, nodular); periductal infiltrating (infiltrating, sclerosing); and intraductal-growing (polypoid, papillary) [5–10].

For perihilar CCs, the Bismuth-Corlette classification system established in the 1960s provides further anatomic distinction, which is useful in preoperative surgical planning [11], as follows: type I, tumors involving the common hepatic duct below the confluence of the right and left hepatic ducts; type II, tumors involving the biliary confluence; type IIIa and IIIb, tumors involving the biliary confluence extending into the right or left hepatic duct, respectively; and type IV, tumors involving the confluence extending into both the right and left hepatic ducts or multifocal tumors (Fig. 1c) [3, 11, 12].

Histologically, the majority of CCs are well-differentiated adenocarcinomas, while other histologic variants (e.g., squamous and adenosquamous carcinomas, clear-cell adenocarcinomas, intestinal type adenocarcinomas, small-cell carcinomas, papillary adenocarcinomas, mucinous/signet-ring cell carcinomas, lymphoepithelioma-like carcinomas, and neuroendocrine type) make up fewer than 10 % of cases [9, 12, 13].

3 Epidemiology

Although CC is a relatively rare malignancy, it is the second most common primary hepatic tumor and accounts for almost 3 % of all gastrointestinal cancers worldwide [14, 15]. Excepting patients with primary sclerosing cholangitis (PSC), CC predominately affects the middle-aged and elderly, demonstrating steadily increasing

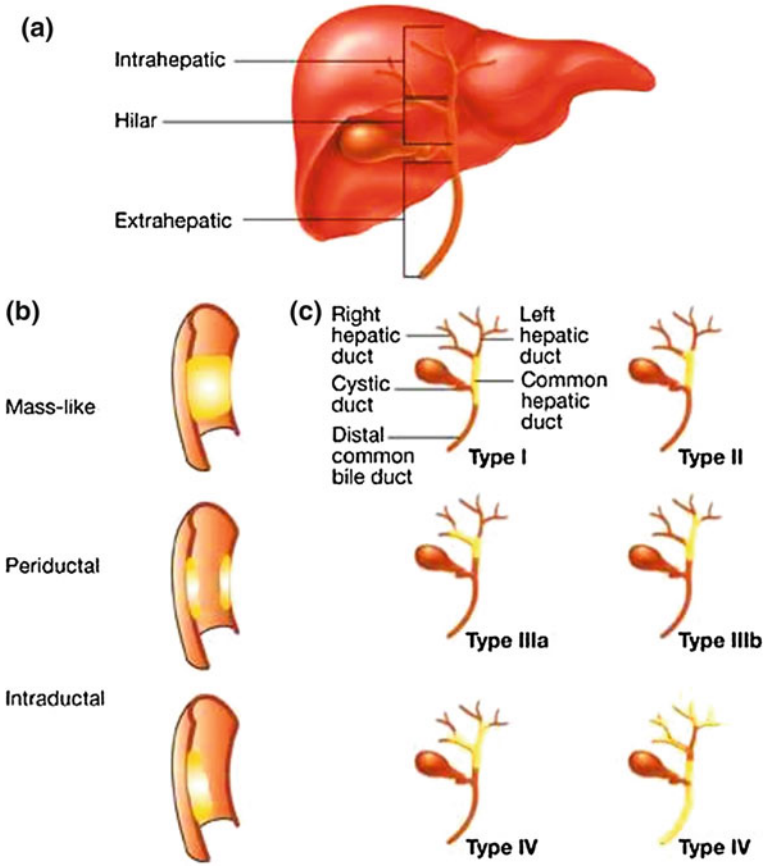

Fig. 1 Classification of CCs according to anatomic location and morphology. **a** CCs can be categorized as intrahepatic, hilar, or extrahepatic depending on their anatomic location with respect to the secondary biliary radicles and hepatic duct hilum. **b** Nonhilar lesions can be further classified as mass-forming, periductal, or intraductal based on their morphologic appearance. **c** Bismuth-Corlette classification of hilar lesions

incidence with advanced age (50+ years) [16–20]. Incidence rates for CC can vary substantially by geographical region, especially for the intrahepatic subtype, ranging from a high of 71.3 per 100,000 in the Khon Kaen province of Thailand to approximately 1 per 100,000 in the US and UK [20–22]. The substantially increased incidence and regional variability of intrahepatic CC in Asia is attributed to the prevalence of liver fluke infections and hepatitis B (see section on 'Risk Factors'). Within the U.S., demographic data shows a decreased risk of CC in women compared to men (ratio 0.6:1) and an increased risk in Asian/Pacific Islanders and Hispanic ethnicities (ratio of 1.5:1) [17, 19, 23].

Recently, the temporal trend of the incidence of CC has been the topic of considerable debate, where the previously reported significant increase in incidence in intrahepatic CC in the past decade has been questioned. From 1968 to 1998, a 15-fold increase in incidence of intrahepatic CC was observed in England and Wales [24, 25]; SEER data from 1983 to 1997 in the U.S. showed an average, sustained, and annual increase of 9.44 % in incidence of intrahepatic CC [16]. This trend was further corroborated in studies in other countries, including Australia, Scotland, Italy, France, Italy, Germany, Netherlands, and Japan [14, 21, 22, 26–31]. The number of patients encountered with intrahepatic CC at a tertiary referral center also increased [32]. In many studies, a concomitant decrease in extrahepatic CC was noted [14, 16, 21, 22, 24, 25, 28]. No etiologic cause was found to explain the increase in incidence, and some studies performed in Denmark [33], France [34], and Japan [18] found stable or decreasing incidences of intrahepatic CC. Further analysis revealed inconsistencies in the way the perihilar subtype was classified in registries, which is not recognized as its own category. Specifically, in ICD-O-1 (1973–1991) perihilar CCs could be classified as either intra- or extrahepatic; in ICD-O-2 (1992–2000), perihilar CCs were histologically cross-referenced as intrahepatic CCs. ICD-O-3 (2001-present) now classifies the perihilar subtype under extrahepatic disease (although this designation is not unanimously agreed upon [35]) [23, 36, 37]. Thus, it currently appears that the incidences of both intra- and extrahepatic CCs are relatively stable [18, 23, 38]. Due to changes and inconsistencies in registry classification of CC subtypes, prior data must be interpreted with caution and the importance of accurate disease classification cannot be understated.

4 Risk Factors

Although they account for a minority (30 %) of cases of CC [39, 40], several risk factors associated with chronic inflammation of the biliary epithelium have been identified.

4.1 Primary Sclerosing Cholangitis (PSC)

The relative risk of CC in the patients with PSC is 400–1500 corresponding to an annual incidence rate of 0.5–1.5 % and lifetime risk of 7–20 % [41–45]. Unlike sporadic cases, CCs in these patients tend to present at younger ages: between 30 and 50 years [46, 47], with rare pediatric cases reported in the literature [48]. Up to 50 % of CCs were diagnosed within the first year PSC is diagnosed [41, 42, 49, 50]. Although two-thirds of patients with PSC have associated inflammatory bowel disease, there is no apparent association between either the presence or severity of inflammatory bowel disease and risk of developing CC [43, 46, 51, 52].

4.2 Parasitic Infection

Two species of liver flukes, *Opisthorchis viverrini* and *Clonorchis sinensis*, are established causes of cholangiocarcinoma, and chronic infection with either results in a 4- to 6-fold increase in risk of the malignancy [53–55]. Both liver flukes are endemic in parts of Asia, albeit in different regions [53, 55, 56]. *O. viverrini* is endemic in Southeast Asia (Thailand, Laos, Cambodia, and Vietnam) with a prevalence of up to 70 % in the Khon Kaen province of Thailand, which has the highest rate of cholangiocarcinoma in the world [57]. *C. sinensis* predominately affects countries in East Asia (China, Taiwan, Korea, and Northern Vietnam, excluding Japan), with potentially 15 million persons infected and attributable to an estimated 4700 cases of cholangiocarcinoma [56]. Hosts become infected by ingesting undercooked fish harboring adult worms, which then migrate to the biliary system where they reside, mature, and reproduce. Carcinogenesis by liver flukes likely involves a pattern of chronic inflammation, immunologic disturbance, and direct release of mitogenic factors by the parasite [58, 59].

4.3 Hepatolithiasis

Although rare in the West, hepatolithiasis is endemic in portions of China, Japan, and Korea and is strongly associated with intrahepatic CC [60, 61]. Reportedly between 18 and 70 % of patients with CC in Japan and Taiwan (respectively) have evidence of intrahepatic biliary stones at the time of resection [62, 63]. Overall, it is estimated that up to 10 % of patients with hepatolithiasis will go on to develop CC [61]. As in PSC, it is believed that bile stasis and recurrent bouts of subclinical cholangitis in individuals with hepatolithiasis may contribute to carcinogenesis [3].

4.4 Viral Hepatitis and Cirrhosis

Meta-analyses summarizing a series of case-control and cohort studies performed in the last two decades have concluded a significant association between intrahepatic CC and the viral hepatitides B and C (OR 3.4) [64–69]. The association between intrahepatic CC and hepatitis B appears stronger in East Asia where hepatitis B is endemic, whereas the association between intrahepatic CC and hepatitis C is more pronounced in Western countries [67, 69, 70]. The association between extrahepatic CC is weak or nonexistent for hepatitis B or C, respectively [67]. In addition to chronic inflammation, the mechanism of tumorigenesis by the viral hepatitides may involve the mutagenic effect of viral oncoproteins in a manner similar to hepatocellular carcinoma [67]. Synergistic interaction between hepatitis B and cirrhosis further increases the risk of intrahepatic CC (OR 20) [71].

4.5 Other Risk Factors

Several chemical agents and environmental toxins have been associated with CC. Analyses of CC tissue have occasionally identified promutagenic DNA adducts, indicating potential exposure to DNA-damaging agents in these patients [72]. Exposure to Thorotrast, a colloidal, radiological contrast agent used in the early twentieth century and later banned in the 1960s, is associated with a 300-fold increased risk of developing CC, sometimes decades after exposure [14, 73]. Environmental toxins, such as dioxin and vinyl chloride, have also been postulated to cause CC [74, 75].

Although quite rare, choledochal cysts, anomalous pancreaticobiliary junction malformations, bile ducts adenomas, and biliary papillomatosis have been linked to CC [76]. Congenital, biliary tree abnormalities (e.g., choledochal cysts, Caroli's disease) may carry a 15 % risk of malignant degeneration after the second decade [77, 78]. Overall, it is estimated that patients with untreated cysts may harbor an overall incidence of CC of up to 28 % [70, 79, 80]. Although little is known about tumor pathogenesis in these patients, it is hypothesized that biliary stasis and reflux of pancreatic enzymes may lead to inflammation, bile acid activation, and deconjugation of carcinogens [81, 82]. Prior bilioenteric drainage procedures complicated by recurrent bouts of cholangitis may also lead to CCs [80]. Further, a number of possible risk factors such as inflammatory bowel disease [83], diabetes [84], obesity [85], alcohol, and smoking have been proposed but are inconsistent or mild in their contribution to CC risk [7, 38, 70].

5 Clinical Presentation

The clinical presentation of CC depends on tumor location. Lesions at the hilum or distal common bile duct usually present with signs and symptoms of biliary obstruction: painless jaundice, pruritus, pale stools, and dark urine. Weight loss and abdominal pain, when present, are usually manifestations of advanced, unresectable disease [86]. Distal periductal lesions can be difficult to differentiate from benign biliary structures, particularly in patients with PSC [3].

In contrast, intrahepatic CCs are usually asymptomatic. They are often diagnosed incidentally on imaging studies or during evaluations for liver enzyme abnormalities [87]. Radiologically, it is often difficult to differentiate between intrahepatic CCs and metastatic adenocarcinomas to the liver. When intrahepatic CCs do present with symptoms, these usually include abdominal pain as well as weakness, fatigue, or weight loss [86]. Few patients present with jaundice, and cholangitis is rare [1, 2]. In patients with PSC, CCs may present as worsening cholestasis and deteriorating performance status [42].

6 Diagnosis

6.1 Imaging Studies

In conjunction with other laboratory and pathology studies, the focus of radiologic evaluation is on establishing the diagnosis of CC and staging of disease. The primary modalities of determining tumor extent and surgical resectability are computed tomography (CT) and/or magnetic resonance imaging (MRI). Further visualization of disease either fluoroscopically or directly is performed by endoscopic or percutaneous techniques, which can also obtain tissue specimens. The role in the workup of CC of certain nonstandard diagnostic modalities such as positron-emission tomography, choledochoscopy, and intraductal ultrasound is discussed.

6.1.1 Transabdominal Ultrasonography

Transabdominal ultrasound is the initial diagnostic test of choice in evaluating patients with suspected liver disease or right upper quadrant abdominal pain with or without jaundice [88–90]. This modality is highly accurate in detecting the presence, extent, and location of obstruction within the biliary tree and is useful to rule out nonmalignant etiologies such as choledocholithiasis or Mirizzi syndrome. The location of the tumor can be inferred by the pattern of ductal dilatation: distal extrahepatic obstruction causes dilation of both intra- and extrahepatic bile ducts, whereas only intrahepatic ductal dilation is present in intrahepatic masses [91]. Ultrasonography alone is inadequate in determining extent of disease and resectability, but Doppler studies can assess patency of the hepatic and portal vessels. Lobar atrophy and portal venous involvement (72–83 % sensitivity and 93–100 % specificity) can also be detected and are of significant value in surgical decision-making [90]. Addition of ultrasound contrast agents may also increase diagnostic accuracy in differentiating intrahepatic CC from hepatocellular carcinoma (HCC) [92]; however, it was reported that this technique still had a greater rate of misdiagnosis compared to CT and MRI [93]. Ultimately, either multidetector computer tomography (MDCT) or MRI is required to determine tumor extent and resectability.

6.1.2 Multidetector Computed Tomography (MDCT)

The development of multidetector row helical computed tomography has brought substantial improvements to CT imaging, increasing spatial resolution, shortening scan time, and allowing for three-dimensional reconstructions to better assess the relationship of the biliary tree to the adjacent hilar/portal vasculature (Fig. 2) [90, 94, 95]. These improvements have made CT imaging competitive with MRI in characterizing biliary tumors [96]. Performed with triple-phase contrast (arterial, venous, portal), MDCT identifies CCs in virtually all patients, and the reported positive and negative predictive values to determine resectability are 92 and 85 %, respectively [94, 97]. Morphological subtype can be delineated by imaging patterns on MDCT, which are summarized in excellent reviews [5, 90, 98–100].

MDCT does have some limitations. In a series of studies, the accuracy range for MDCT in determining the extent of ductal spread (i.e. horizontal or longitudinal) was 68–92 % [100–105]; the accuracy for radial invasion (i.e. vertical), and involvement of the hepatic artery and portal vein were 100, 87, and 87 %, respectively [104, 105]. Due to the nature of imaging microscopic spread, in the cases in which radiologic imaging did not correlate with pathological findings, the imaging underestimated the extent of disease. Additionally, MDCT does not adequately identify lymph node involvement [103, 105]. Streak artifact and secondary inflammatory changes can further limit the diagnostic accuracy of CT in patients with biliary (particularly metal) stents.

6.1.3 Magnetic Resonance Imaging (MRI) and Magnetic Resonance Cholangiopancreatography (MRCP)

The extent of bile duct and vascular involvement, local lymphadenopathy, intrahepatic spread, and distant metastases and can all be assessed by MRI/MRCP, making it the imaging modality of choice at many major hepatobiliary centers (Fig. 3). MRI can detect intrahepatic spread and its intrinsically high tissue contrast helps detect hepatic parenchymal invasion and metastatic lesions [106]. CCs are hypointense on T1-weighted images and hyperintense on T2-weighted images [107]. The MRCP technique utilizes heavily T2-weighted sequences in order to enhance the signal intensity of biliary and pancreatic fluid [108]. Although MRCP does not allow for tissue sampling, it is noninvasive and its positive and negative predictive values for detecting the location and extent of biliary tree involvement are comparable to endoscopic retrograde cholangiopancreatography (ERCP) and percutaneous transhepatic cholangiopancreatography (PTC) [106, 109, 110]. MRCP can better visualize biliary ducts proximal to a lesion which may not adequately fill with ERCP, and its reported accuracy in determining extent of bile duct involvement ranges from 71 to 96 % [110–112]. In combination, MDCT + ERCP is equivalent to MRI + MRCP for evaluating tumor extent [113].

MRCP is particularly useful in patients with complete biliary obstruction that precludes safe guide-wire placement during ERCP and provides three-dimensional reconstructions of the biliary tree both above and below the stricture. Another advantage of MRCP in this situation is that the undrained bile ducts can be visualized without the injection of contrast, avoiding the potential risk of iatrogenic cholangitis [106].

Despite these advantages, studies suggest that MRI/MRCP still understages up to 20 % of patients with hilar CCs [114]. Compared to MDCT, MRI/MRCP has lower spatial resolution, requires a longer scan time, and is more sensitive to motion artifacts, limiting its utility in uncooperative patients [87]. Furthermore, MRI is particularly sensitive to blood flow anomalies around the hepatic duct and hilar liver parenchyma and can overstage patients with indwelling biliary stents [115].

6.1.4 Direct Cholangiography

Direct cholangiography involves the injection of radiographic contrast dye to visualize the biliary system, which can be performed via endoscopic retrograde

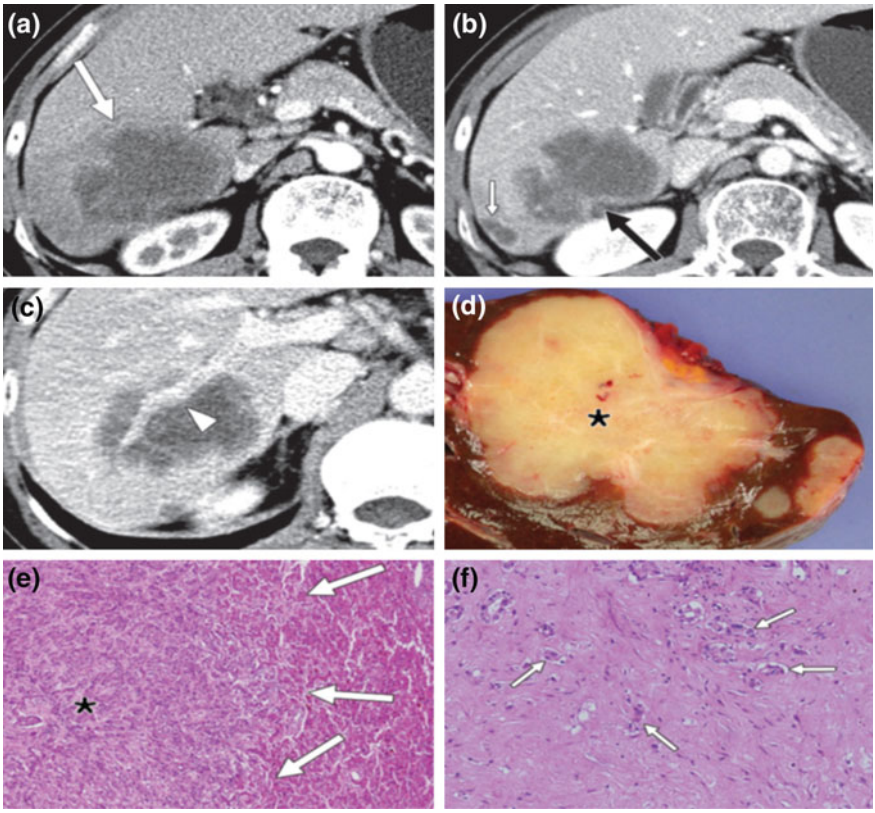

Fig. 2 MDCT images of a intrahepatic CC encasing the posterior branch of the right portal vein. **a** Arterial phase CT scan shows a PCC with ragged rim enhancement at the periphery (*white arrow*). **b** Axial portal venous phase CT scan shows gradual centripetal enhancement of the tumor with capsular retraction (*black arrow*). A satellite nodule is also seen (*white arrow*). **c** Three-minute delayed phase CT scan shows gradual centripetal enhancement with tumor encasement of the posterior branch of the right portal vein (*arrowhead*). Encasement of a portal or hepatic vein without formation of a grossly visible tumor thrombus is one of the distinguishing features of PCC as opposed to HCC. **d** Photograph of the gross specimen shows a homogeneous sclerotic mass with an irregular infiltrative margin and a central area of white scar tissue (*), that correlates well with microscopic findings of tumor cells more prominent at the periphery of the mass and fibrotic stroma in the center. **e** Photomicrograph (original magnification ×40; H-E stain) of the periphery of the tumor shows an indistinct tumor margin, in which tumor cells (*) are intermingled with normal hepatocytes in the adjacent liver (*white arrows*). **f** Photomicrograph (original magnification ×100; H-E stain) of the inner portion of the tumor showing a large amount of fibrous tissue among scattered tumor cells (*white arrows*)

cholangiopancreatography (ERCP) or via percutaneous transhepatic cholangiography (PTC) [91, 98]. While advances in MDCT and MRI have somewhat supplanted the need for direct cholangiography to establish a diagnosis of CC, this modality has the capability of obtaining tissue samples and performing therapeutic

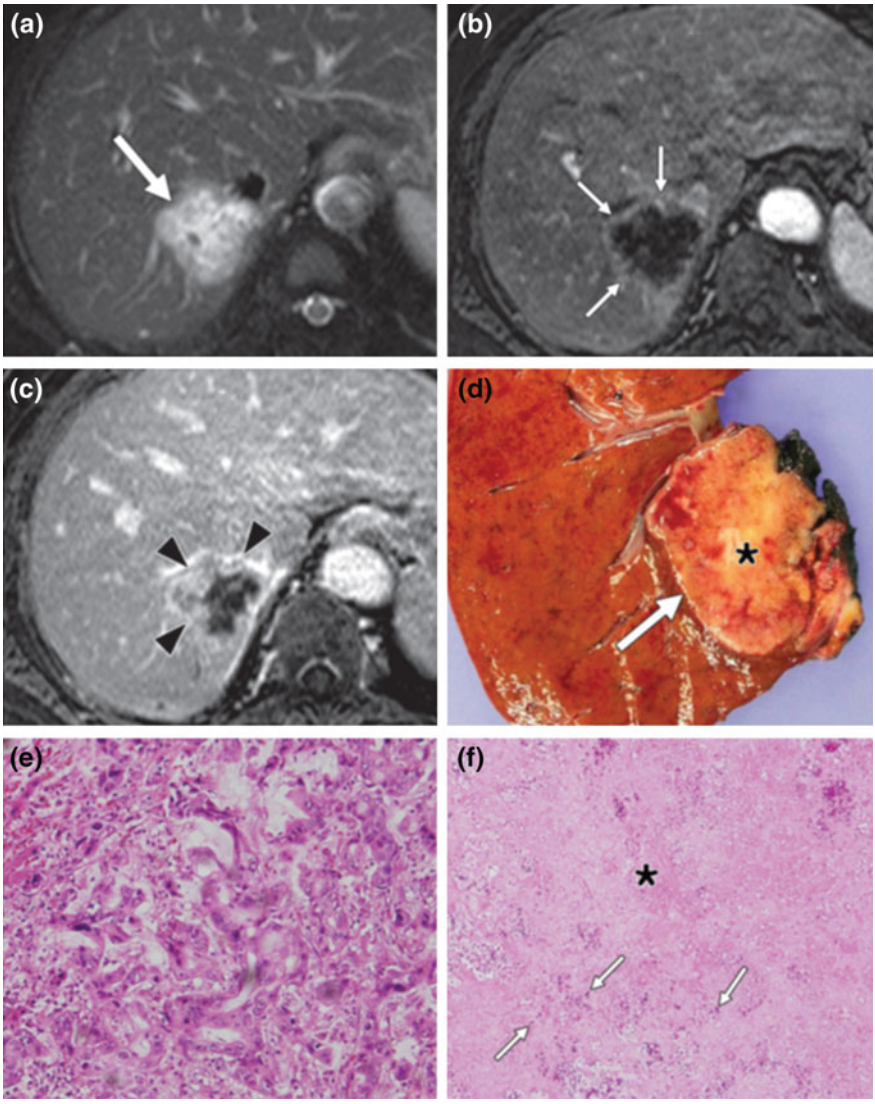

Fig. 3 Typical MR Imaging features, gross appearance, and histology of mass-forming PCC: **a** Axial fat-suppressed T2- weighted MR image shows a high-signal-intensity lobulated mass in the right hepatic lobe (*white arrow*). **b**, **c** Contrast-enhanced arterial phase **(b)** and equilibrium phase **(c)** T1-weighted MR images show irregular, ragged rim enhancement (*white arrows* in **b**) with gradual centripetal enhancement (*black arrowheads* in **c**). **d** Photograph of the gross specimen shows a mass with a relatively homogeneous appearance (*white arrow*), although the central area of the tumor is somewhat whitish (*). **e** Photomicrograph (original magnification, ×100; H-E stain) of the tumor periphery shows a moderately differentiated adenocarcinoma, a finding that is consistent with the peripheral rim enhancement seen in **b** and **c**. **f** Photomicrograph (original magnification, ×40; H-E stain) of the central portion of the tumor shows coagulative necrosis (*) with scanty tumor cells (*arrows*)

procedures. Determining the optimal approach for cholangiography—ERCP or PTC—depends on the location of the tumor and the degree of biliary obstruction. PTC may be required in patients with constricting, hilar lesions, whereas ERCP may be better suited for evaluating more accessible distal lesions. Both approaches allow for tissue sampling by brushings or biopsies, and biliary stenting for relief of jaundice can be performed at the time of the diagnostic evaluation. The main disadvantages of these invasive cholangiography techniques include limited availability/technical expertise, risk of technical failure, and procedural risks such as duodenal perforation, bile leaks, cholangitis, bleeding, and pancreatitis [116, 117].

6.1.5 Endoscopic Ultrasonography (EUS)

Approximately 50 % of patients with CC present with lymphadenopathy [86]. As noted above, the sensitivity and specificity of CT and MRI in detecting nodal metastases are limited. In contrast, endoscopic ultrasonography can be a useful staging adjunct by visualizing extent of tumor invasion, detecting local lymph node enlargement, and allowing fine needle aspiration (FNA) of the tumor mass or surrounding lymph nodes. Although EUS/FNA has a much greater sensitivity for detecting malignancy than ERCP with brushings (sensitivity of 94 % vs. 50 % [118]), it has the potential for tumor seeding. Due to this risk of seeding, patients who are being considered for curative resection and/or liver transplantation should not undergo EUS/FNA [119, 120].

6.1.6 Intraductal Ultrasound (IDUS)

IDUS involves the use of a miniature ultrasound probe introduced either transpapillary via ERCP or transhepatically via PTC to query the echogenicity of the biliary ducts intraluminally [121]. On IDUS imaging, a tumor diameter greater than 8 mm or evidence of disruption of bile duct wall structure is highly suspicious for malignancy. Absence of the echogenic layer between tumor and a nearby vessel is a sign of vascular invasion, and the accuracy of IDUS in assessing right hepatic artery or portal vein invasion is 93–100 % [122]. In a study by Noda et al., assessment of longitudinal spread of CC by IDUS of the proximal hepatic side and distal duodenal side had a sensitivity, specificity, and accuracy of 82, 70, 78 % and 85, 43, 70 %, respectively [123]. The low specificity of the duodenal tumor extent was attributed to obscure images due to inflammation and collapse of the distal bile ducts; the specificity of the extent of tumor on the duodenal side improved to 86 % when concomitant transpapillary biopsy was performed. Two Korean studies found that IDUS compared favorably to MDCT, MRCP, and ERCP in determining tumor extent [124, 125]. However, IDUS is not suitable for assessing lymph node involvement [121].

6.1.7 Choledochoscopy (Intraductal Endoscopy, Cholangioscopy)

Similar to IDUS, choledochoscopy is an auxiliary modality to either ERCP or PTC and is performed by passing a small caliber fiberoptic scope through a working

channel of a duodenoscope or transhepatic percutaneous catheter. This technique can be used to directly visualize luminal filling abnormalities noted on MRCP or direct cholangiography. Malignant biliary strictures can be identified by the presence of dilated, tortuous vessels, mucosal ulceration, polypoid or nodular masses, or villous mucosal morphology [126, 127]. Endoscopic choledochoscopy allows for passage across strictures to second- and third-order bile ducts, making it particularly useful in patients with PSC, for whom it can be used to locate and directly biopsy dominant strictures [128–130]. As with IDUS, the combination of endoscopic choledochoscopy with ERCP and tissue sampling has been reported to increase the sensitivity of detecting malignancy in patients with indeterminate biliary strictures from 58 to 100 %.

In the past few decades, choledochoscopy failed to gain mainstream use due to its steep learning curve, costly and fragile equipment, and the need for two simultaneous skilled endoscopists for operation. Recent technological advances in these devices have resulted in more robust equipment and require only a single endoscopist for operation. Therefore, this procedure has become more accessible and economically feasible for everyday practice [131]. Further studies are required to better define the role of choledochoscopy in the staging and management of CC.

6.1.8 Positron-Emission Tomography (PET)

In oncology, PET is based on the cellular uptake and metabolism of a radiotracer, 18F-fluorodeoxyglucose (FDG), which tends to be increased in malignancies compared to normal tissue [94, 132]. It can and is often performed in concert with a CT scan (PET/CT) to better correlate uptake measurements with anatomical landmarks. Most biliary tumors are FDG-avid and hence can be imaged by PET [133]. PET/CT is more sensitive in detecting intrahepatic CC compared to extrahepatic CC [133], as well as in detecting nodular subtype versus infiltrative tumors [134–136]. However, PET/CT is not superior to MDCT or MRI/MRCP for the diagnosis of CC nor does it determine tumor extent. Some studies suggest that PET/CT is more sensitive, specific, or accurate in determining lymph node involvement [137–140] although this benefit was not observed by others [141, 142]. For the detection of distal metastases, the range of cited sensitivity varies from 33 to 100 %, but researchers essentially unanimously agree that it is useful in finding occult disease that would be missed on conventional radiologic workup [133–135, 137, 139, 141–146]. Information provided by the addition of PET/CT in initial staging evaluation results in a change in disease management in 10–30 % of patients with CC [133, 135, 139, 141, 146–148]. Hence, PET/CT is a reasonable supplement to the diagnostic and staging evaluation of patients with an equivocal diagnosis of CC or those in whom distant metastatic disease is suspected.

6.2 Histology

Cytologic or histologic confirmation during endoscopic or transhepatic procedures is required to confirm the diagnosis of CC. As part of ERCP, brush cytology and

forceps biopsy are commonly used to obtain cellular/tissue samples. Both procedures are known for being essentially 100 % specific yet poorly sensitive (40–60 %) for detecting CC [149, 150]. Sensitivity is slightly improved to 60–70 % when the techniques are combined. Further improvement in detection has been demonstrated by incorporating cytological analysis with fluorescence in situ hybridization (FISH), which uses fluorescent DNA probes to detect chromosomal amplification that tend to be present in malignant cells. Ongoing studies suggest that use of FISH combined with routine cytology may increase the sensitivity without compromising the specificity for diagnosing CC [151–154].

Fine needle aspiration under endoscopic ultrasound (EUS-FNA) has been shown to have superb sensitivity and specificity for tissue diagnosis (94 % each) [118]; however, due to its potential for malignant seeding, it is not recommended for patients in which curative therapy is planned [155].

Although the identification and exploitation of molecular markers, such as k-ras and p53, have not yet translated into routine clinical practice, immunohistochemical markers (such as cancer antigen [CA] 19-9 and CA 50) and keratin staining with the anti-cytokeratin-type-1 monoclonal antibody are more characteristic of cells of biliary tract origin and may help distinguish between intrahepatic CCs and hepatocellular cancers [3]. In contrast, distinguishing between intrahepatic CCs and metastatic adenocarcinomas from other sites can be extremely difficult. The diagnosis must often be inferred from the clinical scenario, predisposing conditions, or the absence of extrahepatic primary tumors (e.g., breast, lung, gastric, colorectal, and pancreatic) on imaging studies [3].

6.3 Serum Markers

CA 19-9, although most known as a tumor marker for pancreatic cancer, is also commonly elevated in CC. Its sensitivity and specificity in CC ranges from 40–70 % to 50–80 %, respectively, depending on cut-off values [40]. However, elevations in CA 19-9 can also occur due to a variety of nonmalignant biliary conditions such as obstruction and cholangitis. Within patients with preexisting PSC, the sensitivity and specificity of CA 19-9 for the diagnosis of CC is improved in patients identified with allelic variants of fucosyltransferases 2 and 3 (FUT 2/3) [156]. CA 19-9 also serves as a prognostic marker: elevated levels of CA 19-9 pre- and postoperatively is associated with worse overall survival [157]. Other gastrointestinal tumor markers such as carcinoembryonic antigen (CEA) and CA-125 may also be elevated in CC. While nonspecific and not useful individually, the combination of these tumor markers and others has been shown to provide an acceptable sensitivity and specificity of greater than 90 % [158, 159]. Of note, some 7–10 % of the population lack the ability to produce CA 19-9, regardless of CC tumor burden [7, 157]. A host of other potential serum markers have been identified (e.g., MMP-7, CA-S27, CCA-CA, and IL-6) but require further validation before achieving clinical utility [160, 161].

6.4 Staging

Each of the anatomic subtypes of CC (intrahepatic, perihilar, and distal extrahepatic) have discrete classification schemes according to the 7th edition of the American Joint Commission on Cancer (AJCC)/Union Internationale Contre le Cancer (UICC), which was released in 2010 [162]. Notably, in the 6th edition, staging of intrahepatic CC was grouped with HCC under primary liver cancers, despite having well-acknowledged differences in etiology, histopathology, diagnosis, and management. The separation of intrahepatic CC into its own staging system has resulted in improved clinical relevance [163]. Another significant change in the 7th edition was splitting off the staging of perihilar CC from distal extrahepatic disease [162]. The ideal staging system would indicate resectability preoperatively and clearly delineate prognoses between stages, which is not met by current schemes [8].

6.4.1 Intrahepatic CC

The three staging systems for intrahepatic CC are: AJCC/UICC TNM, LCSGJ, and the National Cancer Center of Japan (NCCJ) staging systems. Each system assesses the nodal and metastatic status in a similarly straightforward presence/absence method. The major differences amongst these systems are in T-staging. The AJCC/UICC system characterizes tumor T-stage by vascular, ductal, and local invasion. The LCSGJ attributes increased T-stage by three discrete prognostic criteria: tumor size (threshold of 2 cm), number of tumors, and presence of portal vein, hepatic vein, or serosal invasion. The NCCJ system, which is specific for the mass-forming subtype of intrahepatic CC, delineates the T-stage by number of tumor and presence/absence of vascular invasion.

Among the T-stage factors, vascular invasion and number of tumors are consistent prognostic indicators, whereas the prognostic relevance of tumor size is controversial [164]. A large multicenter study found the tumor size to be a significant prognostic factor and included it into its survival nomogram [165]. Other studies reported inconsistent results regarding the prognostic relevance of tumor size [166, 167]. Blechacz et al. has enumerated deficiencies with each system: AJCC/UICC requires histologic evaluation and hence is not suitable to determine resectability preoperatively; the LCSGJ system is criticized for inclusion of serosal invasion, which has been shown to have no impact on survival [168]; and the survival correlation in NCCJ staging is also regarded as suboptimal [8]. Noticeably absent in the current staging systems is the lack of inclusion of morphologic subtype on T-staging, where the mass-forming and periductal infiltrating subtypes have been correlated with worse survival than intraductal subtype by Hwang et al. [167]. Correspondingly, the authors advocated for a new system that accounts for morphologic subtype. Another proposed staging system incorporating additional factors such as serum prealbumin, CA 19-9, and CEA, requires external validation [166].

6.4.2 Perihilar CC

Two staging systems exist: AJCC/UICC TNM and Memorial Sloan-Kettering Cancer Center (MSKCC) staging systems. The aforementioned Bismuth-Corlette classification system that describes the intraductal extent of tumor involvement, though not strictly speaking a staging system, is historically relevant in that it was one of the first systems to provide a framework for resectability for perihilar CC and hence is still useful as a "first estimate" for preoperative planning. However, it does not account for vascular and organ invasion, nor does it correlate with survival outcomes [8, 169]. The MSKCC system can be considered to be an extension of the Bismuth-Corlette system in which aspects of tumor involvement are more specifically defined (i.e., by including vascular involvement and hepatic lobar atrophy) to better assess surgical resectability of disease. As this system does not contain information on nodal or distant metastases, it too lacks prognostic value [8, 169, 170].

The T stages of the AJCC/UICC system account for various degrees of invasion relative to surrounding tissue and major vascular/ductal structures. Nodal status categorization differentiates between regional nodes (those along the cystic duct, common bile duct, hepatic artery, and portal vein) and distant nodes (including periaortic, pericaval, superior mesenteric, and celiac axis nodes). Similar to its staging system for intrahepatic CC, the AJCC/UICC perihilar CC staging system does not adequately assess for tumor resectability, and instead is primarily used after histologic evaluation has been performed [169]. Defining Bismuth type IV lesions as T4, hence stage IV and therefore deemed unresectable, has been criticized as not all Bismuth type IV lesions are in fact unresectable [171, 172]. Moreover, the designation of node-positive disease as stage III-B has been shown to have similar or worse survival than stage IV-A disease; thus, the reclassification of T and N stages has been suggested [171–173]. Two alternative and comprehensive staging systems have been proposed: DeOliveira et al. [169] devised a comprehensive if not complicated system that incorporates information useful to determine resectability; Chaiteerakij et al. [170] recommended a clinically based system that better discriminates prognoses amongst stages.

6.4.3 Distal Extrahepatic CC

The AJCC/IUCC TNM system is the sole staging system for distal extrahepatic CC. T classification is categorized by the degree of invasion in relation to bile ducts, adjacent organs (i.e. gallbladder, pancreas, and duodenum), and vascular structures (celiac axis and SMA). Nodal and distal metastasis is assessed in a binary manner. Lymph node involvement has been noted to be especially important in the prognosis for distal extrahepatic CC, although the current N classification scheme does not stratify by the number of involved nodes [8, 174]. Furthermore, one study found the depth of tumor invasion with thresholds at 5 and 12 mm to be a better predictor of outcome than the current AJCC/UICC system [175]. Nevertheless, the separation of perihilar CC from distal extrahepatic CC is considered a major step in the right direction in the staging of these diseases.

6.5 Treatment

Treatment for CC is determined by patients' performance status, the absence or presence of metastatic disease, the local extent of the tumor (including vascular and/or parenchymal involvement), and the availability and extent of surgical and endoscopic expertise.

6.6 Surgical Resection

Cure for CC requires a complete surgical resection with histologically negative margins. Evaluation by an experienced hepatobiliary surgeon is therefore recommended when CC is suspected, ideally before ERCP, PTC, or biliary stenting potentially complicates assessment of local tumor extent or results in inflammation or infection that can complicate subsequent surgery [86].

6.6.1 Assessment of Resectability

Careful preoperative staging is central to identifying appropriate candidates for attempted curative resection (Table 1). Patients must have good performance status and be medically and nutritionally fit, have localized disease amenable to resection with clear margins (i.e. R0 resection), and have no evidence of metastatic disease [176]. High-quality chest, abdomen, and pelvis imaging is required to rule out not only metastatic CC, but also to evaluate for an extrahepatic primary adenocarcinoma with porta hepatis metastasis causing biliary obstruction [81]. Staging laparoscopy may uncover occult metastatic disease in up to 30 % of cases of CC [177]. Tumors with bilateral extension to the second-order bile ducts (Bismuth-Corlette type IV), ipsilateral lobar atrophy (or extension to second-order bile ducts) with contralateral encasement of the lobar hepatic artery or portal vein branch, extensive regional lymphadenopathy (or N2 nodal metastases), and/or tumor involvement of the main hepatic artery or portal vein were historically considered unresectable [115]. However, advocate radical, *en bloc* resection with vascular reconstruction as needed to obtain tumor-free margins is becoming standard in many centers [178–181]. Other factors that may affect perioperative outcomes, such as competing comorbidities, liver dysfunction (and more specifically,

Table 1 Criteria for Unresectability [58]

Tumor-related criteria of unresectability
1. Biliary duct involvement to secondary radicles bilaterally
2. Main portal vein encasement or occlusion
3. Hepatic lobe atrophy with contralateral encasement or occlusion of portal vein
4. Hepatic lobe atrophy with contralateral involvement of secondary biliary radicles
5. Secondary biliary radicle involvement with contralateral portal vein branch encasement or occlusion

hyperbilirubinemia), and nutritional status, must also be carefully considered and addressed preoperatively [176, 182].

6.6.2 Preoperative Biliary Drainage

Preoperative biliary drainage (PBD) for obstructive jaundice is controversial. Obstructive jaundice is recognized to increase morbidity and mortality perioperatively; however, procedures for biliary drainage and relief of jaundice are not without complications that may delay definitive surgery [183–185]. A meta-analysis of randomized trials by Fang et al. showed that routine use of PBD is inappropriate [184]. A multidisciplinary team should carefully evaluate the decision to perform and the method of performing PBD. Current indications for PBD for all types of CC include: symptomatic jaundice, severe malnutrition, cholangitis, patients undergoing neoadjuvant therapy, or cases in which surgery may be delayed [186–188]. For perihilar CC in which proximal obstruction is common and major hepatic resection is anticipated, an additional consideration is selective drainage of future liver remnant in combination with portal vein embolization (PVE) to induce hypertrophy of the FLR (see next section) [187–189]. In this circumstance, it is recommended to unilaterally drain the remnant lobe (versus performing bilateral drainage) [188, 190]. There is no established serum level for which PBD should be performed, nor is there consensus on duration of drainage.

The three primary methods for performing PBD are percutaneous transhepatic (PTBD), endoscopic (EBD), and endosopic nasobiliary drainage (ENBD). Proximal and especially segmental obstructions are more easily accessed via PTBD [187, 191]. PTBD also has a lower complication rate than EBD [190, 192]. However, PTBD has been associated with catheter-tract cancer dissemination and peritoneal metastases [191–194], although a recent prospective study did not corroborate this finding [195]. As enterohepatic circulation is lost via external drainage with PTBD, reinfusion or replacement of bile fluids is recommended [188, 196].

ENBD is another external drainage modality that is less invasive than PTBD, although nasal discomfort and tube dislocations can occur with this technique. Furthermore, patient adaptation to the device may prolong hospitalization [187, 190, 191]. While not commonly used in Western centers, ENBD is the procedure of choice for PBD recommended by Kawakami et al. [194].

Compared to PTBD, EBD is also far less invasive. As an internal drainage modality, EBD restores enterohepatic circulation and is without external appliances that can cause patient discomfort. Tube/stent occlusion can occur up to 60 % of cases and results in significantly increased risk of cholangitis at a rate of 38.6 % versus 8.1 % compared to PTBD [192, 194]. However, in the absence of consistent and high-quality data comparing the various techniques of PBD, the method of PBD must be thoroughly evaluated with consideration to available expertise.

6.6.3 Portal Vein Embolization (PVE)

In cases of anticipated major hepatectomy for (typically hilar) CC, preoperative hepatic optimization with PVE reduces the risk of hepatic insufficiency and

postoperative morbidity and mortality by inducing compensatory hypertrophy of the nonembolized liver segments, i.e., future liver remnant (FLR) [197–200]. PVE has been demonstrated to be safe with a major complication rate as low as 1 % in experienced centers [201]. After adequate biliary drainage has been performed for biliary obstruction if present (see PBD above), embolization is achieved with agents such as gelfoam, fibrin glue, cyanoacrylate, or ethanol with or without embolization coils [197, 202]. Anticipated FLR in the range of 20–40 % is an indication for PVE (a precise cutoff is not agreed upon), which results in an increase in FLR volume of 9–14 % (relative volume increase of 33.8–43.8 %) in two to four weeks after injection [197, 198, 201, 202]. Measurement of FLR as a percent of total liver volume is calculated from CT scan; however, functional liver assessments such as indocyanine green clearance test may be a better predictor of operative outcome especially in diseased livers.

6.6.4 Neoadjuvant Therapy

The paucity of high-quality studies regarding the use of neoadjuvant therapies for CC precludes standard of care guidelines [203]. Patients with CC commonly present with obstructive jaundice and associated poor functional status that is a significant hindrance to neoadjuvant chemo- or radiation therapies [204]. While isolated reports of complete pathologic response after neoadjuvant therapy exist [205, 206], a retrospective study performed by Glazer et al. found that neoadjuvant therapy (majority being gemcitabine-based) delayed surgical resection by an average of 6.8 months and had a statistical trend toward worse mortality (HR 1.66, $p = 0.07$) [207]. Notably, neoadjuvant therapy consisting of external beam radiotherapy with radiosensitization, brachytherapy, and maintenance oral capecitabine is delivered to patients under the Mayo Clinic protocol for planned liver transplantation [208]. However, its efficacy independent of liver transplantation and generalizability to conventional surgical resection have not been characterized.

6.6.5 Surgical Considerations and Technique

Complete surgical resection with negative gross and microscopic margins is required for a chance of cure in patients with CC and is the most effective treatment available [209–211] Patients undergoing surgical resection of CC have a median survival of approximately 2- and 5-year survival rates of 25–40 % [209, 211–217]. Unfortunately, despite incremental improvements in diagnosis and treatment, long-term survival of CC has not significantly improved in the past two decades as demonstrated in a longitudinal single-center study in Japan by Furusawa et al. [212]. Nevertheless, it is regarded that margin-free R0 resection significantly impacts survival [211, 212, 218, 219]. Accordingly, the criteria for resectability have been broadened and surgical techniques have become progressively more aggressive to achieve R0 resection, especially at high-volume teaching hospitals [212–214, 219].

The type and extent of resection performed depends on the location of the tumor within the liver or biliary tree and the degree of local invasion. Peripheral, intrahepatic CCs are treated by either wedge or anatomic resection (major or extended

hepatectomy) with or without vascular resection and reconstruction [215, 220, 221]. Bismuth-Corlette class II, IIIA, or IIIB hilar CCs will often require extrahepatic bile duct resection with *en bloc* caudate lobectomy and right or left hepatectomy to ensure R0 resection, whereas more distal Bismuth-Corlette type I tumors may be amenable to extrahepatic bile duct resection alone [176, 181, 222]. Bismuth-Corlette type IV lesions, historically considered unresectable, are no longer a strict contraindication for surgical resection [223, 224]. Distal CCs are managed with standard pancreaticoduodenectomy with or without partial hepatectomy (hepatopancreatoduodenectomy) [211].

Assessment of resectability begins with a careful, intraoperative inspection of the liver, peritoneal surfaces, and viscera to rule of occult, metastatic disease. Selective laparoscopy to evaluate for occult intraperitoneal or noncontiguous hepatic metastatic disease may avoid unnecessary laparotomy in high-risk patients with long-standing percutaneous stents, large tumors, or markedly elevated serum CA 19-9 levels [176, 225, 226]. Next, a careful evaluation of the N1 (i.e., hilar, cholecystic, and/or pericholedochal) and N2 (i.e., peripancreatic, periduodenal, periportal, celiac and/or superior mesenteric artery) lymph nodes is performed. Particular attention is paid to the retropancreatic and paraaortic nodes N2 nodes, which are more likely to be involved in CC than the celiac or superior mesenteric nodes [227]. Surgical resection is aborted if the N2 nodes are histologically involved, but can proceed if only the N1 nodes are involved. The final step to determine resectability involves a systematic evaluation of the local extent of the CC. After cholecystectomy, the hilar plate is incised to allow direct palpation of the tumor and determine the extent of longitudinal bile duct involvement and radial extension into the adjacent vasculature and liver parenchyma. The right and left hepatic arteries are identified and the contralateral artery is carefully preserved. Tumor involvement of the portal vein trunk and bifurcation can be determined by transecting the common bile duct just proximal to the pancreas and carefully elevating it off the portal vein [228, 229]. Alternatively, the portal vein can also be evaluated by incising the hepatoduodenal ligament along its right lateral aspect, excising the right posterolateral portal nodes, and carefully elevating the bile duct to expose the main portal vein trunk up to its bifurcation. Main portal vein involvement precludes attempted resection. In contrast, limited involvement of the origin of the ipsilateral portal vein branch may be addressed with segmental portal vein resection and reconstruction, whereas more extensive involvement or encasement of the ipsilateral portal vein branch usually mandates ipsilateral hepatic lobectomy in order to achieve an R0 resection [115, 230].

Resection of any perihilar CC requires cholecystectomy, resection of the entire extrahepatic bile duct (proximal to the pancreas), complete portal lymphadenectomy, and bilioenteric anastomosis (usually a Roux-en-Y hepaticojejunostomy). Bismuth-Corlette class II, IIIA, and IIIB tumors may also require *en bloc* caudate lobectomy or right/left hepatectomy to achieve negative-margin resection. In the same way that CCs can extend longitudinally along major bile duct branches, Kawarada and colleagues recognized that perihilar CCs can extend into the caudate lobe via small branches that drain into the posterior aspects of the right and left

hepatic ducts near the confluence [231]. Subsequent investigators have reported caudate lobe involvement in 43–100 % of patients with perihilar CCs [232–234]. In a series of 46 patients with CC treated with radical resection, the overall 5-year survival rate was 17.5 %, and 25 % in patients treated with *en bloc* caudate lobectomy [235]. When caudate lobectomy is performed for perihilar CCs, only the perihilar portion of the caudate lobe may be resected and the remaining caudate lobe ducts are ligated (not anastomosed to the Roux-en-Y limb) and drained with closed suction postoperatively [115].

The overall resectability rate of patients with perihilar CC undergoing exploration is approximately 65 % [178, 210, 223]. The main reasons for unresectability are underestimation of local tumor extent on preoperative imaging, followed by N2 nodal involvement and occult metastatic disease.

6.6.6 Perioperative and Long-term Outcomes

It might be predicted that an increase in perioperative morbidity and mortality rates would result from progressive adoption of aggressive surgical resection techniques. However, a number of longitudinal studies from Japan showed a decrease in morbidity and mortality over the past two decades that was attributed to refinement of surgical technique and improved perioperative care [178, 212, 236]. For example, growing use of portal vein resection has permitted R0 resection on tumors involving the portal vein, which were previously considered unresectable, with no significant increase in morbidity and mortality [179, 180, 220, 237, 238]. Currently, morbidity and mortality rates range from 43 to 49 % and 1.7 to 11.8 %, respectively [236], and 5-year survival rates after surgical resection is 25–40 % [165, 209, 211–217]. Common complications following hepatectomy for perihilar CCs include hemorrhage, infection, hepatic failure, cardiorespiratory failure, and renal failure [115].

For all subtypes of CC, regional lymph node metastasis (HR 1.29–2.47), R1 or R2 margin status (HR 1.2–2.22), vascular invasion (HR 1.39–3.04), poor histologic differentiation (HR 2.14–3.68), and multifocality (HR 1.49–1.73) found during surgical resection are established as poor prognostic factors [209, 211, 212, 219, 236, 239–246]. Further prognostic stratification with nodal status and resection margins have been investigated: involvement of multiple nodes has worse survival than that of a single nodal metastasis [242], and negative resection margin widths of 1–4 mm and 5–9 mm were associated with decreased survival compared to a margin of ≥ 1 cm [242, 244]. Successful R0 resection occurs at a median rate of 70 % [218], whereas lymph node metastasis is found in 35–45 % of operative cases [239, 242, 245, 247]. Despite the negative impact of nodal metastases has for prognosis, the long-term survival benefit of extended lymph node dissection is uncertain [81, 227]. However, routine lymphadenectomy may be justified on the basis of improved staging of disease and is performed in most centers [217, 218].

6.7 Adjuvant Therapy

Worldwide utilization of adjuvant radio- and/or chemotherapy ranges from 59 to 68 % [248] Due to the relative rarity of CC, and an even smaller subset of surgically resectable patients, the literature regarding use of adjuvant therapy after resection for CC is limited, sometimes contradictory, and consists mostly of small, retrospective studies [204, 249, 250]. Correspondingly, there is insufficient data to support a specific radio- and/or chemotherapeutic regimen. However, a meta-analysis of 20 studies and 6712 patients evaluating the impact of adjuvant chemotherapy, radiotherapy, and chemoradiotherapy found a benefit of adjuvant therapy in patients with lymph node positive (OR 0.49) and R1 disease (OR 0.36) [251]. The finding that adjuvant therapy is more effective in high-risk node- or margin-positive patients was corroborated by another single institution study [252]. A multinational phase III trial investigating the combination of gemcitabine and cisplatin as adjuvant therapy for CC compared to observation is currently underway [253].

6.7.1 Adjuvant Chemotherapy

Perhaps the only randomized trial examining the role of any adjuvant therapy for CC used a combination of mitomycin C and 5-fluorouracil (5-FU) and found no benefit with a treatment versus control 5-year survival of 41.0 and 28.3 % ($p = 0.48$) [254]. In contrast, a retrospective study by Wirasorn et al. [255] found benefit of adjuvant chemotherapy with a median survival time increase from the no-treatment group of 13.4–21.6 months. Of the agents studied, the combination of gemcitabine and capecitabine had the greatest benefit with a median survival of 31.5 months. Two other retrospective studies found survival benefit with gemcitabine alone [256, 257]. However, it is interesting to note that gemcitabine alone was associated with a *worse* survival than no treatment in the aforementioned study by Wirasorn et al. [255], with a median survival 7.9 months. Gemcitabine alone was also found to lack benefit by Glazer et al. [207], further highlighting the inconsistences in these retrospective studies.

6.7.2 Adjuvant Radiation Therapy

There are no randomized controlled studies investigating the use of adjuvant radiation therapy alone for CC. Following a series of smaller retrospective studies demonstrating mixed results [249], two large retrospective studies based on the Surveillance, Epidemiology, and End Results database (SEER) showed no significant benefit of adjuvant radiation therapy on multivariate analysis in the treatment of extrahepatic CC [258, 259]. A subsequent meta-analysis of extrahepatic CC that excluded the SEER data reported an overall survival HR of 0.62 ($p < 0.001$) in favor of radiation therapy over no treatment controls [260].

6.7.3 Adjuvant Chemoradiotherapy

Like chemotherapy and radiation therapy, the data for adjuvant chemoradiotherapy is similarly sparse, and the heterogeneity of treatment makes study-to-study comparisons difficult [250]. Following a study that showed efficacy of chemoradiotherapy in extrahepatic CC and not intrahepatic or perihilar CC, most studies focus on extrahepatic disease [261]. One of the largest studies retrospectively drawing upon the American College of Surgeons National Cancer Database identified 8741 patients with resected extrahepatic CC and found survival benefit with chemoradiotherapy (overall HR 0.82) [262]. Additional benefit was found in node- and margin-positive patients with a HR 0.69 and 0.49, respectively, versus 0.88 for margin- and node-negative patients. The benefit of chemoradiotherapy in high-risk node and margin-positive patients has been noted in other smaller studies [263, 264].

6.8 Transplantation

Early attempts of liver transplantation for treatment of CC in the 1980s were met with disappointing results due to excessive tumor recurrence and poor overall survival [265, 266]. Sudan et al. [267] at the University of Nebraska subsequently proposed the use of neoadjuvant therapy prior to transplantation to control tumor growth and reduce the risk of recurrence as patients await organ availability for transplant. Results were promising, demonstrating 45 % long-term survival in transplanted patients who had otherwise unresectable disease (long-term survival including patients who died awaiting transplantation was 30 %, i.e. intention-to-treat analysis). The Mayo Clinic group adopted the approach of neoadjuvant treatment prior to transplantation and published an outstanding post-transplant 5-year survival of 82 % (58 % 5-year survival on intention-to-treat analysis) [268, 269]. The neoadjuvant regimen consisted of external beam, 5-FU-based chemoradiation (45 Gy over 5 weeks), brachytherapy with ^{192}Ir, and oral capecitabine. Enrollment criteria for transplantation included the presence of unresectable perihilar CC due to either tumor extent or underlying PSC, but only those who had stage I or II disease on staging laparotomy underwent transplantation. Notably, 16 out of 38 of the explanted livers showed no residual tumor on pathologic examination, despite having unequivocal pre-neoadjuvant cytologic diagnosis of CC in most of these cases. The study is criticized for its selection bias, specifically, that those in the transplant group were of stage I or II, had a higher proportion of PSC (>50 %), were younger, and otherwise met a separate set of criteria required for liver transplant [270]. Additionally, transplant recipients underwent neoadjuvant therapy whereas the surgical resection group did not [269].

A subsequent study in another center by Hong et al. [271] that included both intrahepatic and perihilar CC demonstrated improved 5-year recurrence-free survival in the transplant group versus the surgical resection group (33 and 0 %, respectively, $p = 0.05$). Use of neoadjuvant and adjuvant therapies in this study was heterogenous, although none of the surgically resected group received neoadjuvant

therapy. The observation that use of neoadjuvant therapy may improve the outcomes of liver transplantation as a treatment for CC was further suggested in a meta-analysis by Gu et al. [272].

Encouraged by the positive results of the Mayo Clinic protocol, 11 other liver transplant centers in the U.S. participated in a study to assess the suitability of the neoadjuvant therapy and liver transplantation in the treatment of perihilar CC that is unresectable or arising in the context of PSC [208]. The specific neoadjuvant therapy given was not identical in all centers; however, results showed that the 11 centers had similar overall and recurrence-free survival compared to the Mayo Clinic. Pooled 5-year recurrence-free survival of 65 % demonstrated the efficacy of this treatment strategy.

The manner in which PSC affects transplant outcomes in CC is uncertain. The aforementioned multicenter study by Murad et al. suggested PSC was associated with improved recurrence-free survival, whereas an outcome analysis in another center by Becker et al. did not find a survival difference attributable to concomitant diagnosis of PSC [208, 273]. However, patients with PSC tend to have poor hepatic reserve due to chronic progressive disease and hence do not tolerate extended hepatectomy that is often oncologically mandated for resection of CC. Therefore, liver transplantation serves to simultaneously treat both conditions in patients with PSC and coexisting CC [269].

In summary, the role of liver transplantation for the treatment of CC is evolving. Studies suggest a survival benefit in highly selected patients with CC in the setting of PSC or in patients with otherwise unresectable de novo perihilar CC [7]. Should liver transplantation be performed for treatment of CC, it should be done so with neoadjuvant therapy and in a center with appropriate multidisciplinary expertise and resources [269]. Organ availability and potential scarcity must be considered. Liver transplantation is *not* indicated in resectable de novo perihilar CC [274].

6.9 Palliation

The majority (80 %) of patients with CC present with unresectable disease, and a significant proportion of resected patients ultimately succumb to recurrent disease [275, 276]. The natural history of disease in these patients is rapid disease progression, worsening biliary obstruction, and early demise. Successful biliary decompression can be safely achieved by various means, as outlined below. The median survival of patients with unresectable CC is approximately 6 months with and 3 months without biliary drainage [277–279]. As such, palliative interventions can alleviate symptoms, improve quality of life, and potentially improve survival in a significant proportion of patients with unresectable or metastatic CC.

6.10 Surgical

Although endoscopic or percutaneous biliary decompression is preferable to elective surgical palliation in patients with unresectable CCs (because of their poor prognosis), surgical biliary drainage can be performed if a tumor is found to be unresectable at the time of exploration [280]. Surgical palliation can be achieved by Roux-en-Y hepaticojejunostomy in patients with distal CCs or segment III cholangiojejunostomy in patients with hilar lesions [281]. Gastrojejunostomy may also be indicated in patients with large portal masses or impending duodenal obstruction. Celiac plexus block can be performed in patients with severe, epigastric or back pain unresponsive to medical therapy [3, 176]. Although surgical biliary drainage is more durable, it is associated with greater morbidity and mortality, longer times to recovery, and higher costs (but comparable survival) compared to endoscopic or percutaneous biliary decompression [282].

6.11 Endoscopic (or Percutaneous) Biliary Drainage

As with surgical biliary drainage, the goals of palliative endoscopic biliary drainage are to relieve jaundice, prevent cholangitis and liver failure, and ultimately improve the quality of life [283]. Biliary stents are not without risk and have been associated with occlusion migration, cholecystitis, and tumor ingrowth and seeding [3]. In general, stent patency rates are lower for hilar (compared to distal tumors), and percutaneous insertion (compared to endoscopic insertion) is more likely to result in successful placement and biliary drainage [284, 285]. Percutaneous CT- or MRCP-guided placement of unilateral drainage of self-expanding metal stents in patients with hilar CCs may allow for better visualization of the biliary segments in question, reducing the need to inject contrast into multiple segments at ERCP (potentially decreasing the risk of cholangitis) [283, 286, 287].

The decision to place single or multiple biliary stents depends on the extent and location of the biliary obstruction and must balance the need to achieve biliary decompression with the potential risk of cholangitis. Given that only about 25 % of the liver needs to be drained to achieve adequate palliation, a single stent is usually sufficient in the absence of established cholangitis [283, 288]. Although single stents are usually adequate for distal and Bismuth-Corlette class I hilar CCs, there is persistent debate as to whether single or double stents should be inserted in patients with Bismuth-Corlette II–IV strictures. De Palma and colleagues randomized 157 consecutive patients with unresectable hilar obstruction (73 % of whom had a histological diagnosis of CC), to either unilateral or bilateral plastic stents [286]. Although median survival was similar in both groups (179 days), the rate of postprocedure cholangitis was lower in the unilaterally stented group (8.8 % vs. 16.6 %). The authors concluded that minimal injection of contrast and avoidance of overfilling of the remaining undrained ducts in the unilaterally stented group was responsible for the lower morbidity rate in this group.

Palliative endoscopic biliary drainage can be performed using plastic or metal stents. Plastic stents are more likely to occlude, usually need to be exchanged every 3 months, and can migrate distally when placed across the hilum. Metal stents are more expensive, but have a larger diameter and are more durable, often remaining patent for up to 6–12 months [289]. Because metal stents cannot be adjusted or removed, cytologic or histologic confirmation of malignancy should be obtained before placement. Although there is no survival difference when patients with CC are treated with plastic or metals stents, metal stents are more cost-effective in nonmetastatic patients who are expected to live beyond 3–6 months [86, 289]. Covered metal stents are less likely to become occluded in patients with unresectable distal lesions, but they can occlude biliary side branches or the cystic duct when placed across the hilum [290].

6.12 Radiation Therapy

External beam radiation (typically 40–60 Gy) may result in significant palliation, improved local control of disease, and occasional long-term survival in patients with locally advanced unresectable or recurrent perihilar CC [291–294]. In a retrospective study by Chen et al., patients with unresectable intrahepatic CC who underwent external beam radiation had an improved median survival of 9.5 months compared to 5.1 months in the untreated group ($p = 0.003$) [295]. Optimum radiation dose is uncertain; studies report no significant differences in outcome with varying dose levels (30–80 Gy) [294, 296]. The addition of intraluminal brachytherapy (using ^{192}Ir) to external beam radiation therapy may provide a further benefit to overall survival and rate of disease recurrence [297, 298], although this benefit was not supported by Isayama et al. [299]. Other, more recent, developing radiotherapy techniques such as stereotactic body radiotherapy and Yttrium-90 radioembolization show promise but require further investigation before their roles may be defined [300–303].

6.13 Chemotherapy

Glimelius et al. [304] published the first randomized trial investigating the use of systemic chemotherapy in the treatment of unresectable CC. Use of 5-FU/leucovorin with or without etoposide suggested a benefit in median survival of 6.5 months, an increase from that of 2.5 months for supportive care ($p = 0.1$). For the next decade, a multitude of small, nonrandomized retrospective studies were performed, but only a few other randomized trials or trials comparing chemotherapy to supportive care existed [305–307]. Hence it was difficult to assess and compare the efficacy of chemotherapeutic regimens for unresectable or metastatic CC. In 2007, Eckel and Schmid published a pooled analysis of 104 studies to determine the most active chemotherapeutic agents for the treatment of CC, for which gemcitabine in combination with platinum-based compounds emerged as the provisional

standard of chemotherapy in advanced biliary tract cancer (5-FU-based regimens also showed benefit) [308]. The superiority of gemcitabine/platinum-based doublet chemotherapy compared to gemcitabine alone was substantiated in the randomized ABC-02 trial involving 410 patients [309]. Compared to gemcitabine alone, gemcitabine and cisplatin doublet therapy was associated with improved median overall survival (11.7 vs. 8.1 months, HR 0.64, $p < 0.001$) and rate of tumor control (81.4 % vs. 71.8 %, $p = 0.049$) without a significant increase in adverse effects. Subsequently, gemcitabine/cisplatin was established as the standard first-line chemotherapy for locally advanced or metastatic CC [310–314]. Later studies found gemcitabine/oxaliplatin to be a reasonable alternative with a more favorable side effect profile [310]. Modification and addition of chemotherapeutic and biologic agents to gemcitabine/cisplatin have also been investigated. So far, addition of cetuximab (anti-EGFR antibody) and cediranib (VEGF inhibitor) to gemcitabine/cisplatin did not result in survival benefit [312, 315]. However, in unresectable k-ras wild-type CC tumors, the combination of gemcitabine, oxaliplatin, and panitumumab (another anti-EGFR antibody) showed encouraging efficacy with a median overall survival of 20.3 months in a phase II study [316]. Second-line therapy has not been firmly established, but it is reasonable to consider 5-FU-based regimens [311, 317].

6.14 Chemoradiation

Chemoradiation is considered a viable alternative treatment for locally advanced or metastatic CC, but is less well studied and published results are inconsistent. In a small series of ten patients, gemcitabine followed by 30 Gy SBRT demonstrated a median overall survival of 35.5 months [318]. Separately, cisplatin/5-FU with sequential gemcitabine and 30–55 Gy conformal radiation resulted in a median survival of 20.4 months [319]. However, in another study of 34 patients, chemoradiation also consisting of cisplatin/5-FU and 50 Gy conformal radiation was shown to be inferior to gemcitabine/cisplatin, with a median overall survival of 13.5 months versus 19.9 months, respectively [320]. Larger, prospective studies comparing chemoradiation to other treatment modalities will need to be performed to properly gauge its relative efficacy.

6.15 Transarterial Chemoembolization

Following positive results of its use in the treatment of HCC, transarterial chemoembolization (TACE) is establishing a role in the management of locally advanced unresectable intrahepatic CC [321, 322]. By selectively injecting cytotoxic agents into the tumor vasculature, the technique of TACE has the benefit of delivering chemotherapeutics at increased concentration to the tumor while concomitantly reducing whole-body exposure compared to systemic chemotherapy

[322]. Currently, various permutations of the following drugs are used: Cisplatin, oxaliplatin, mitomycin C, irinotecan, gemcitabine, doxorubicin, and 5-FU [323].

In 2005 Burger et al. [324] published the first report on the use of TACE in unresectable intrahepatic CC and demonstrated potential efficacy of the treatment with a median survival of 23 months. In another study, treatment with TACE was found to increase median survival from 3.3 to 12.2 months compared to supportive care [325]. A meta-analysis by Ray et al. showed that treatment of unresectable intrahepatic CC with TACE resulted in a median overall survival of 15.7 months, conferring approximately a 2- to 7-month survival benefit over systemic therapies [323]. Additionally, more than three-fourths of all patients exhibited complete/partial response or stable disease on postprocedural imaging. Improved prognosis was found in patients whose tumors exhibited radiologic evidence of response to TACE treatment [326, 327], and patient factors predictive of a favorable response to TACE were high tumor vascularity on imaging and Child-Pugh class A [327, 328].

6.16 Photodynamic Therapy

Photodynamic therapy involves intravenous systemic administration of photosensitizing agents that preferentially accumulate in cancer cells and are activated by specific wavelengths of light, leading to the release of oxygen free radicals and ischemic cell death [278]. Depth of tumor necrosis is approximately 4–6 mm, and the time to tumor progression (and need for repeat treatment) is around 6 months [329]. This technique has been shown to be effective in patients with pharyngeal, esophageal, lung, and stomach cancers, and recent studies suggest that it may be of benefit in patients with extrahepatic and hilar CC [278, 329]. In a recent randomized, controlled trial, patients treated with biliary stenting and photodynamic therapy had less cholestasis, better quality of life/performance status, increased duration of stent patency, and higher median survival times than patients treated with biliary stenting alone [330–332]. Photodynamic therapy is still not widely available, and further confirmatory studies are needed to determine its role in the treatment of patients with CC.

7 Conclusions

CCs are rare biliary tract tumors which usually present late and are difficult to diagnose and treat. The majority of patients with CC do not have any of the known or suspected risk factors for CC. Diagnosis of CC requires thoughtful integration of clinical information gathered from imaging, cytologic and/or histologic analysis, and serum studies. Although complete surgical resection is required for cure, the majority of patients are unresectable at presentation. Aggressive surgical techniques including major hepatectomy with or without portal vein resection expands tumor resectability and potentially improves the odds of R0 resection and long-term

outcomes. Local control and improved survival in unresectable disease are achievable with TACE (for intrahepatic CC), radiation, or chemoradiation therapies. Otherwise, advanced or metastatic CC may be treated with palliative biliary decompression and systemic chemotherapy such as combination gemcitabine and cisplatin. In selected metastasis-free patients, especially those with concomitant PSC, liver transplantation in experienced centers may provide durable survival.

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Hepatocellular Carcinoma: Surgical Management and Evolving Therapies

Olga Kantor and Marshall S. Baker

Abstract

HCC is the second leading cause of cancer death worldwide. The majority of cases arise within the background of liver cirrhosis and are most commonly related to chronic hepatitis B and C viral infection. Surgical resection, liver transplantation, and tumour ablation are potentially curative modalities in cases of localized, non-metastatic, hepatocellular carcinoma. Systemic sorafenib has been shown to be marginally effective in slow disease progression in patients whose cirrhosis is so severe that they are not candidates for liver directed therapy and in those with metastatic disease. Several large prospective and retrospective studies have demonstrated transplantation to provide better long term outcomes than resection in patients with small volume carcinoma. Other small retrospective series have demonstrated similar outcomes for patients with well matched tumour characteristics and compensated cirrhosis. There is not even level one evidence to guide the choice of modality to be used in individual cases and treatment algorithms vary widely among high volume centres. Newer and emerging techniques and approaches such as laparoscopic liver resection and living donor transplantation continue to evolve and impact choice of treatment in absence of well-controlled comparative trials. For locally advanced disease and in patients with significant cirrhosis, interventional technologies such as transarterial chemoembolization or transarterial radioembolization can provide disease control or result in tumour regression and hypertrophy in the future liver

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remnant and may allow interval resection or down-staging to liver transplantation. Improving transarterial, surgical, and transplant techniques continue to expand the surgical and interventional options for managing localized HCC and are driving a shift towards aggressive multimodality therapy in patients with localized hepatoma.

Keywords
Hepatocellular carcinoma · Liver resection · Liver transplantation · Multimodality treatment · Emerging therapies

1 Introduction

Primary hepatocellular malignancy comprises the fifth most common cancer in men and the eighth most common cancer in women worldwide. It is also the second leading cause of cancer death worldwide, with 746,000 deaths (9.1 % of all cancer deaths) in 2012 [1]. Eighty-three percent of all primary hepatic cancers are found in the less developed regions of Eastern Asia, Southeast Asia, Northern Africa, and Western Africa; however the incidence is increasing in developed countries. In the United States, primary hepatic malignancy has increased in incidence from 2.6 in 100,0000 people to 8.6 in 100,000 from 1975 to 2011 [1], and the incidence has also increased amongst younger people [2]. Hepatocellular carcinoma (HCC) comprises 80–90 % of primary liver tumors [3, 4]. Although there has been some improvement in prognosis over time with new treatment modalities, overall prognosis remains poor. Worldwide, there is a 4–13 % 2-year survival for small tumors and a median of four months from symptom onset to death in untreated cases [5–7]. In the United States, the 5-year overall survival (OS) rate was 18.5 % in 2007 compared to 3 % in 1975 [8].

In this chapter, we will review the etiology, staging, and surgical options for management of HCC. We will further discuss emerging interventional radiologic treatments used either alone or in combination with surgical methods.

2 Etiology

Eighty percent of cases of HCC arise within a background of liver cirrhosis [6]. Hepatitis B virus (HBV) is the most common etiology of cirrhosis-associated HCC worldwide, with a relative risk of 223:1 compared to noncarriers [9]. Chronic HBV accounts for 55 % of HCC globally and 89 % of cases in endemic regions [10]. The 3-year cumulative risk of HCC in HBV cirrhosis is 12.5 %, and HCC can also occur in non-cirrhotic chronic HBV carriers, with a 3.8 % 3-year risk [11]. Patients with increased viral load and active viral replication are independently associated with a higher HCC risk [12, 13], although there is still an increased risk in inactive HBV

carriers (HR 4.6 for HCC) compared to non-carrier controls [14]. Hepatitis C virus (HCV) is another common etiology of cirrhosis-associated HCC, with a 63-fold increased risk compared to population-based controls [15] and a fourfold increased risk in chronic HCV compared to cleared infection. Co-infection with HBV and HCV also increases cumulative lifetime risk by approximately 10 % [10, 16]. Although the presence of chronic HBV or HCV virus alone is associated with increased HCC incidence, about 90 % of cases occur within a background of viral-induced cirrhosis (88 % in HBV and 93 % in HCV) [17]. Non-viral causes of cirrhosis are also associated with an increased risk of HCC. Heavy alcohol use is associated with a 1.7 to 3.4-fold increased risk [18–20], and non-alcoholic steatohepatitis (NASH) is also associated with a significant risk [21]. NASH-cirrhosis has up to a 2.6 % yearly cumulative incidence of HCC. Additionally, alcohol consumption further significantly increases the risk of HCC in NASH-cirrhosis [22]. Cirrhosis from hereditary hemochromatosis increases HCC risk [23], as do alpha-1 antitrypsin deficiency [24] and primary biliary cirrhosis [25]—although the increase in risk is not limited to cirrhosis in these disease states. Exposure to aflatoxin [26] and tobacco [20] also are associated with increased risk of developing HCC. Studies suggest that metabolic syndrome and diabetes may be additional independent risk factors [27]. The inherited risk of HCC has not been well-defined, although some studies suggest family history may contribute to risk, especially in combination with other risk factors [28].

3 Prevention

Primary prevention of HCC has focused mainly on viral hepatitis in the form of HBV vaccination to prevent spread of viral hepatitis and therapies to treat progression of chronic HCV to cirrhosis. Recent development of antiviral agents have been especially efficacious in the treatment of HCV and are now standard of care for managing patients with HCV infection. Secondary prevention has focused on surveillance in patients at high risk. Screening with ultrasound and/or alpha-fetal protein (AFP) levels has proved to be cost-effective in HBV cirrhosis in patients that have a yearly HCC risk of 0.2 % or higher, or in non-HBV cirrhosis with an annual risk of at least 1.5 % [29]. Screening is recommended by clinical guidelines worldwide and may decrease HCC mortality up to 37 % [30], although the frequency of screening varies based on the prevalence of HCC [31]. National Cancer Center Network (NCCN) guidelines recommend screening in high-risk patients every 6–12 months with ultrasound and AFP [32]. European Association for the Study of the Liver-European Organisation for Research and Treatment (EASL-EORTC) guidelines recommend screening with ultrasound every 6 months [33], and Asian Oncology Summit (AOS) guidelines recommend ultrasound and AFP as often as every 3 months [34].

4 Staging

Treatment for HCC is generally based on tumor characteristics and staging. Many staging systems have been proposed, including the TNM, Okuda, Barcelona Clinic Liver Cancer (BCLC), Cancer of the Liver Italian Program (CLIP), Chinese University Prognostic Index, and Japan Integrated Staging systems [35]. The TNM system incorporates tumor number, tumor size, vascular invasion, and nodal and distant spread into a I–IV stage model [36]. The Okuda staging system incorporates the size of the tumor, presence of ascites, albumin, and bilirubin into I–III stage system [37]. The BCLC score is the most widely used, and incorporates the Okuda staging system in addition to performance status and liver functional status into an A–D classification. The CLIP score uses the Child-Pugh classification, tumor morphology, alpha-fetoprotein level, and presence of portal vein thrombosis to assign a prognostic score [38]. The Japanese Integrated Staging system further integrates the Child-Pugh classification with the TNM classification to assign a prognostic score [39]. The American Hepato-Pancreato-Biliary Association (AHPBA) consensus guidelines recommend the American Joint Committee on Cancer/International Cancer Control (AJCC/ICC) TNM staging system to predict outcomes in patients eligible for resection or transplantation, and the BCLC system in patients with advanced HCC who are not candidates for surgical treatment [40]. The prognostic value of these staging systems is outlined in Table 1.

5 Surgical Therapy

Resection, transplantation, and tumor ablation are potentially curative treatment modalities available to patients with HCC. Patients with localized tumors who are medically fit for surgery may be candidates for either surgical resection or transplantation. Patients with small unifocal tumors may be good candidates for ablation therapy. For patients that have more advanced disease or are not surgical candidates, disease control with transarterial chemoembolization (TACE), transarterial radioembolization (TARE), or systemic therapy with sorafenib may increase survival [41].

5.1 Patient Selection

Patient factors, tumor factors, and underlying liver function are all vital in the appropriate selection of patients for surgery. Patient functional status and non-liver comorbidities are important in determining their suitability for surgery. Tumor size, the number of tumors, and the presence of vascular invasion or extrahepatic spread play into both resection and transplant criteria. Classic transplant criteria (the Milan criteria) is limited to a single tumor ≤ 5 cm, or up to three tumors, each ≤ 3 cm [42]. In addition to tumor size and location, resection is additionally limited by the

Table 1 AJCC and BCLC staging systems

Staging	AJCC/UICC [36, 149]	Median OS	**Staging**	BCLC [150]	Median OS
Stage I	T1N0M0	58–87 months	**Stage A**: early	A1-3: single tumor	53 months
	T1: solitary tumor without vascular invasion			A4: 3 tumors <3 cm	
	N0: no regional lymph node metastasis				
	M0: no distant metastasis			*A1: no portal hypertension, normal bilirubin* *A2: portal hypertension, normal bilirubin* *A3: portal hypertension, abnormal bilirubin*	
Stage II	T2N0	37–51 months	**Stage B**: intermediate	Large multinodular tumor	16 months
	T2: solitary tumor with vascular invasion or multiple tumors, none more than 5 cm				
Stage III	IIIA: T3aN0M0	11–19 months	**Stage C**: advanced	Vascular invasion or extrahepatic spread	7 months
	IIIB: T3bN0M0				
	IIIC: T4N0M0				
	T3a: multiple tumors >5 cm				
	T3b: major vascular invasion				
	T4: direct invasion of adjacent organs				
Stage IV	IVA: any T, N1M0	5 months	**Stage D**: end-stage	Poor performance status, Child-Pugh C	3 months
	IVB: M1				
	N1: regional lymph node metastasis				
	M1: distant metastasis				
Fibrosis	*F0: none-moderate*				
	F1: severe fibrosis or cirrhosis				

AJCC/ICC American Joint Committee on Cancer/International Cancer Control
BCLC Barcelona Clinic Liver Cancer
OS Overall survival

residual future liver remnant (FLR). In patients with a non-cirrhotic liver, at least 20 % of the volume of the liver should be left in situ with appropriate vascular inflow and outflow and biliary drainage to prevent liver insufficiency postoperatively. In a cirrhotic liver, most evidence would indicate that a FLR of 40 % or more should be left in situ [41].

Methodology for assessing the degree of cirrhosis prior to resection and therefore estimating the FLR size continues to be an area of active investigation. The Child-Pugh classification of liver function is classically used to evaluate the severity of liver disease. The Child-Pugh system incorporates serum markers of liver dysfunction (total bilirubin, serum albumin, prothrombin time) and clinical factors (ascites, hepatic encephalopathy) to classify liver cirrhosis into class A (well-compensated), B (functional compromise), or C (decompensated) [43]. Increasing Child-Pugh class is significantly correlated with worse postoperative outcomes after liver resection [44, 45]. The Model for End-Stage Liver Disease (MELD) is a formula based on serum values of liver dysfunction and is commonly used for organ allocation in transplant [46]. Its adoption has significantly increased the number of liver transplants being done for HCC [47]. MELD and more traditional liver failure scoring systems do not always, however, accurately assess perioperative risk [48]. The presence of portal hypertension significantly increases the risk of postoperative liver failure and is usually a contraindication to resection [49]. Traditional scoring systems may fail to identify patients with substantial portal hypertension. Relative thrombocytopenia and transjugular portal gradient measurements are sensitive indicators of portal hypertension and should be used in patients with cirrhosis considering resection. Patients with a portal pressure gradient of 9 or greater or a platelet count of <100,000/mcL should not be considered for surgical resection [50, 51].

A combination of patient, tumor, and liver factors determines the type of surgical approach for which a patient is best suited. In a patient with a resectable tumor and adequate hepatic reserve, resection is indicated as first line treatment. In a patient with a tumor meeting Milan criteria and poor hepatic reserve, liver transplantation has the best prognosis. However, there is significant overlap for patients that may be good candidates for either resection or transplantation and much debate about the best approach in these individuals.

5.2 Liver Resection for HCC

FLR is one of the most important considerations when planning a resection for HCC. Volumetric analysis with CT is the standard way to predict hepatic reserve and accurately predicts liver failure and complications postoperatively based on FLV [52–54]. In normal livers with a projected FLR of <20 % or cirrhotic livers with a FLR of <40 %, portal vein embolization (PVE) is commonly used preoperatively to induce liver hypertrophy and increase the FLR prior to resection. A prospective study of PVE demonstrated a mean FLR increase of 44 % in normal livers and 35 % in cirrhotic liver [55]. Meta-analysis of 1088 patients undergoing

PVE found a 2.2 % overall morbidity rate with no mortality, with 85 % of patients going on to planned resection after PVE [56]. PVE has been successful in preventing liver failure compared to non-PVE in extended hepatectomy and may decrease perioperative mortality and length of inpatient stay [57, 58]. TARE is an emerging technology that may be used with increasing frequency to increase FLR prior to resection [59].

Improving techniques over the past several decades have made liver resection for HCC safer and significantly decreased perioperative mortality. In addition to the routine calculation of FLR and utilization of PVE preoperatively, intraoperative techniques such as intraoperative ultrasound, vascular occlusion, segmental resection, and minimally invasive surgery have led to improved perioperative outcomes. Intraoperative ultrasound to detect new nodules and delineate tumor anatomy has become routine in hepatic resection. New nodules may be detected by ultrasound in up to 30 % of resections [60] and may change the operative plan depending on their appearance [61]. Intermittent vascular occlusion with the Pringle maneuver decreases blood loss and improves postoperative liver function [62–64]. An anterior approach to right sided tumors and use of the liver hanging maneuver has also been associated with decreased blood loss and transfusion requirements [65–67], which is an independent prognostic factor of postoperative morbidity and long-term survival [68]. Although negative margins are generally considered adequate for resection [69], anatomic resection on a segmental or subsegmental level appears to have improved overall and disease-free survival (DFS) compared to nonanatomical resection in retrospective studies [65, 70]. Laparoscopic techniques have also been shown to be safe and oncologically effective, with 82–100 % margin-negative resection rates, and 5-year OS ranging from 50 to 75 % for HCC in a review of the literature [71]. In retrospective cohort studies of HCC in cirrhotic patients matched for tumor characteristics, laparoscopic resection was associated with significantly lower blood loss and intraoperative transfusions, less incidence of postoperative liver failure, and shorter hospital stay as well as similar long-term outcomes compared to open resection [72–74]. In addition, one prospective study found lower levels of circulating tumor cells after laparoscopic resection for HCC, although it is not clear if this is linked to outcomes [75].

Over the past two decades, there has overall been a significant decrease in perioperative morbidity and mortality with resection for HCC, as well as improved OS rates. Perioperative mortality rates have dropped from around 10 % to <5 % since the mid-1990s [76, 77], and are as low as 1 % in patients without underlying liver disease [78]. Perioperative morbidity ranges from 19 to 45 % in different series [79–81]. 5-year OS rates are reported between 33 and 52 % [36, 79, 82], but can be as high as 69 % in selected patients (small tumors, patients that meet Milan criteria) [80, 83]. 5-year DFS rates range from 20 to 48 % [79, 80, 82]. Independent predictors of early recurrence include larger tumor size >3 cm, multiple tumors, major vascular invasion, cirrhotic liver parenchyma, perioperative blood transfusion, and older age [84–86].

For large HCC tumors >10 cm, surgical resection is the only option for potentially curative resection. At experienced centers, perioperative mortality is

similar to that of resection of smaller HCC at 5 %, and margin-negative resection is possible about 80 % of the time [87, 88]. Overall and DFS is significantly worse in HCC >10 cm, with 5-year OS rates around 20–27 % and 5-year DFS around 15 % [87, 89]. Positive margins, satellite lesions, high AFP, and high intraoperative blood loss are predictors of worse outcomes [87–89]. For tumors with macrovascular invasion, surgical resection outcomes are poor but still superior to medical management, with 2 multicenter studies reporting 5-year OS rates of 21–24 % [90, 91].

Despite advances in surgical technique and safety, resection is an underutilized method in the management of HCC. A Surveillance, Epidemiology, and End-Results linked to Medicare (SEER-Medicare) analysis demonstrated that only 33 % of patients eligible for resection with solitary, unilobar HCC received any form of surgical treatment from 1998 to 2007 [92]. Another study using the National Inpatient Sample showed a decrease in liver resections and an increased trend towards liver transplantation for HCC between 1998 and 2008 in the United States [77].

5.3 Liver Transplantation for HCC

The Milan criteria (single tumor ≤ 5 cm or up to 3 tumors if each ≤ 3 cm, no evidence of gross invasion, no regional, or distant metastases) is the most common accepted transplant criteria for HCC and has been associated with 4-year survival rates of up to 75 % [42]. Indeed, several series of transplantation for HCC have shown that patients meeting Milan criteria have improved overall and disease-specific survival compared to those with tumors outside of Milan criteria [93]. While the Milan criteria is the most widely used system for patient selection for transplantation and has been incorporated into the United Network for Organ Sharing (UNOS) staging system [94], many large volume centers have been interested in expanding the criteria for transplant. The University of California at San Francisco (UCSF) criteria (single tumor ≤ 6.5 cm or up to 3 tumors if each ≤ 4.5 cm or sum of total tumors ≤ 8 cm) has been shown to have similar survival as the Milan criteria in single-institution studies [82, 95–98]. The Milan and UCSF criteria are further outlined in Table 2. For patients undergoing transplantation for HCC, perioperative mortality rates are generally ≤ 5 % [79, 98, 99]. Morbidity rates vary widely, from 20 to 72 % [81, 96]. 5-year OS rates have improved over time, with rates around 25 % in the early 1990s to rates about 60 % in the early 2000s in the United States [100]. Contemporary 5-year OS range from 58 to 75 % in patients meeting the Milan criteria [42, 98, 101] and 45–75 % in patients meeting the UCSF criteria [93, 95, 96]. 5-year DFS generally range from 50 to 70 % [79, 82, 101]. Vascular invasion and larger tumor size or tumor number have been associated with worse prognosis [99, 102], although slow tumor growth in large tumors exceeding the Milan or UCSF criteria have been associated with better survival in this subset of patients [103].

At Eastern centers, living donor liver transplant has had good success in HCC, with 5-year survival rates close to 65–80 % [104, 105]. Large tumors >5 cm and

Table 2 Transplant criteria for HCC

	Milan criteria [42, 94]	UCSF criteria [96]
Tumor size	Single tumor ≤ 5 cm	Single tumor ≤ 6.5 cm
Tumor number	Up to 3 tumors, each ≤ 3 cm	Up to 3 tumors, each ≤ 4.5 cm or sum of all tumors ≤ 8 cm
Vascular invasion	No vascular invasion	No vascular invasion
Extrahepatic spread	No regional or distant disease	No regional or distant disease
Recurrence	8 % at 4-years	21 % at 6-years
	20 % at 5-years	
Overall survival	75 % at 4-years	52 % at 5-years
	60 % at 5-years	

high AFP levels are associated with increased recurrence rates, which are generally similar to deceased donor liver transplant [104, 106–108].

5.4 Resection Versus Transplantation

In matched analyses comparing transplantation to resection in cirrhotic patients, OS is usually comparable but DFS is significantly increased with transplantation in tumors that meet Milan or UCSF criteria [82, 101, 109]. Two studies stratifying by MELD score found a survival benefit for resection in patients meeting Milan criteria with a MELD <10 compared to transplantation [82, 110]. Additionally, a cost analysis across three countries—the USA, Switzerland, and Singapore—found that in all three, liver resection was much more cost-effective, with savings compared to transplant ranging from $111,821–$156,300 per quality-adjusted life year gained [111].

5.5 Multimodality Strategies

Due to the shortage of donor livers available, bridge to transplantation with interventional procedures and transplantation as a salvage option after initial resection have been increasing in popularity as strategies in HCC treatment. In patients treated with preoperative TACE or ablation that had complete tumor necrosis on explant, living donor transplantation outcomes are excellent, with 10-year survival up to 90 % [112]. TACE and radiofrequency ablation (RFA) as a bridge in patients awaiting transplantation is considered safe [113, 114], and is often associated with significant tumor downstaging and improvement in long-term outcomes [115–119]. Although a newer technique, TARE with y90 has also shown to have up to 50 % tumor necrosis on liver explant and increased downstaging when compared to TACE [120, 121]. TARE has also been shown to produce compensatory hypertrophy in the remaining liver segment, which can facilitate potential future resection

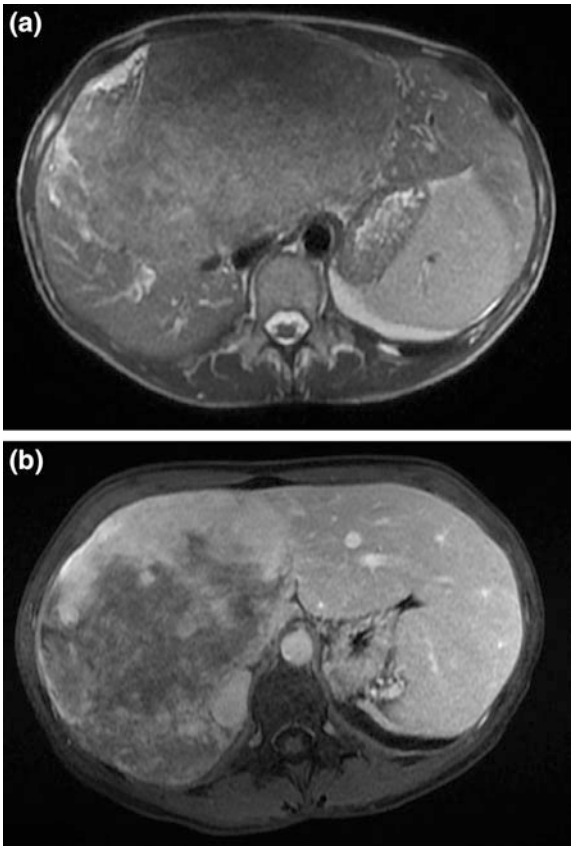

Fig. 1 40-year-old female presented with unresectable HCC, fibromellar subtype. **a** Large HCC tumor burden at presentation. **b** Status post three treatments of TARE with y90—post treatment decreased tumor burden and contralateral liver lobe hypertrophy. Patient went on to successful surgical resection with no evidence of disease recurrence at 2 years

with tumor downstaging [59] (Fig. 1). Studies of salvage transplantation for recurrence after hepatic resection for HCC show mixed results, with some findings of increased perioperative mortality and poorer survival compared to primary transplantation [122] while others demonstrate similar operative mortality, recurrence, and survival compared to primary transplantation [123]. Combined with improving survival rates after hepatic resection, the data generally suggests salvage transplantation is a reasonable option after resection for small HCC in patients with preserved liver function [124, 125].

6 Non-surgical Therapy

6.1 Potentially Curative

Tumor ablation is the only potentially curative treatment for HCC outside of surgical resection or transplantation, and can achieve good local control in tumors up to 3 cm [126]. The major options for ablation include microwave ablation, RFA,

percutaneous ethanol injection (PEI), and percutaneous acetic acid injection (PAI). RFA was found to be superior to both PEI and PAI in a randomized controlled trial in terms of recurrence and OS [127]. RFA for tumors ≤ 3 cm has shown to have equivalent 5-year OS and DFS rates as resection for HCC in many studies [128–130], and decreased efficacy compared to resection in others, although the rate of complications is consistently lower [131, 132]. In HCC >3 cm, resection has significantly better long-term outcomes [133].

6.2 Palliative

For patients with advanced stage HCC, palliative options include TACE or systemic therapy with Sorafenib. Randomized trials have demonstrated about a two-fold increased survival for TACE compared to symptomatic treatment in unresectable HCC [134, 135]. It is generally safe with low rates of major liver failure [136] and can have up to a 25 % 5-year OS rate [137]. Additionally, TARE is becoming an increasingly popular strategy for intermediate unresectable HCC in the absence of metastatic disease. Early data on TARE with y90 has shown 42–50 % response rates and low 30-day mortality with a time to progression of almost 8 months [138]. A meta-analysis comparing TARE to TACE found that TARE outperformed TACE with a longer time to progression and improved OS, although there was no significant difference in tumor response between the two approaches [139]. TARE is especially useful for multiple tumors or large locally advanced tumors and those with portal vein thrombosis, and is generally better tolerated than TACE with lower complication rates [140, 141]. Ongoing trials are also looking at the benefit of the combination of TARE and sorafenib for unresectable HCC [142]. Alone, systemic therapy with the oral agent Sorafenib is associated with delayed disease progression and increased survival, although only on the order of 2–3 months [143, 144]. Although not incorporated into standard therapy for HCC, there has been preliminary success with clinical trials of adjuvant interferon therapy [145, 146], acyclic retinoids [147], and PI-88 [148] after resection.

7 Conclusions

The optimal management of HCC continues to evolve. The past two decades have seen substantial improvements in outcomes with hepatic resection, improved selection of patients for transplantation, and minimally invasive techniques that have all played a crucial role in revolutionizing the treatment of early stage HCC. Treatment algorithms vary geographically depending on incidence, but there is a shifting focus towards surgical management even in more aggressive disease that has been met with overall success. The increasing utilization of multimodality therapy, especially with improving interventional radiological options, has also become more common and likely will play an integral role in future directions of therapy for HCC. While the ideal strategy is not yet known, especially in patients

that qualify for several types of treatment, the advances that have been made and are continuing to be made are providing a multitude of options for patients that historically had dismal outcomes. Ongoing and future studies will likely continue to change the scope of management of HCC for the better.

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Clinical Features of Metastatic Hepatic Malignancies

Ramiro Fernandez, Sam G. Pappas and David J. Bentrem

Abstract

The liver is a common site for gastrointestinal tumor metastases as it is the first major organ reached by blood draining the portal venous system. With the development of more effective chemotherapeutic agents which may eradicate residual microscopic disease in the liver and help reduce known tumor burden, partial hepatectomy to remove gross metastatic disease will likely become increasingly utilized in the future. This chapter discusses the presentation and clinical factors in liver directed surgical resection.

Keywords

Hepatectomy · Liver tumor · Secondary tumor · Liver resection

1 Introduction

The liver is a common site for gastrointestinal tumor metastases as it is the first major organ reached by blood draining the portal venous system. These metastases are characteristically asymptomatic, and are typically identified through radiographic or biochemical surveillance. When present, clinical symptoms include

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malaise, fever, weight loss, right upper quadrant pain, a palpable mass, and occasionally ascites or splenomegaly [1]. Jaundice is infrequently present and may be the result of biliary tract obstruction, or overwhelming hepatic tumor burden leading to loss of functional liver parenchyma. The majority of metastatic tumors to the liver originating from the GI tract are from colorectal carcinoma primary sites. According to the American Cancer Society, there were approximately 149,000 new colorectal cancers diagnosed in the United States in 2014 and roughly 20 % of patients present with liver metastases [2]. Liver metastases in the setting of colorectal cancer are defined by the American Joint Committee on Cancer staging criteria as stage IV disease. Historically, patients with metastatic disease were offered primary tumor resection and chemotherapy; however, advances in hepatic surgical techniques with decreasing morbidity associated with liver resection has led clinicians to pursue resection of isolated liver metastases. Major hepatobiliary centers report a perioperative mortality of <5 % for patients without cirrhosis making liver resection a viable option for selected patients [3–5]. With the development of more effective chemotherapeutic agents which may eradicate residual microscopic disease in the liver and help reduce known tumor burden, partial hepatectomy to remove gross metastatic disease will likely become increasingly utilized in the future. To this end, we have already begun to recognize the benefit of neoadjuvant therapy for the treatment of metastatic liver disease [6–13].

2 Natural History of Unresected Colorectal Liver Metastases

Colorectal cancer metastases to the liver are the prototypical disease treated by partial hepatectomy. Of all newly diagnosed cases of colorectal cancer, liver metastases will develop in one half of patients and in 30 % of these patients; the liver will be the only site of metastases. (See Fig. 1). If left untreated, the median time of survival is estimated between 6 and 12 months [14–20]. Even patients with limited metastatic burden have dismal prognosis with 5-year survival rates of 2–8 % when left untreated [21]. In order to evaluate prognostic variables in patients with unresected hepatic metastases, Stangl et al. performed a retrospective review of 484 patients with metastatic liver lesions from colorectal cancer who did not undergo partial hepatectomy. By multivariate analysis, they found that the percentage of liver volume replace by tumor was the greatest predictor of prognosis while grade of primary malignancy, presence of extrahepatic disease, mesenteric lymph node involvement, serum carcinoembryonic antigen level (CEA), and age were also independent predictors of survival [22].

3 Preoperative Evaluation

The goals of preoperative radiologic staging in patients with hepatic metastases are to accurately identify the location and extent of intrahepatic disease and exclude the presence of extrahepatic disease. Furthermore, the ideal imaging modality would

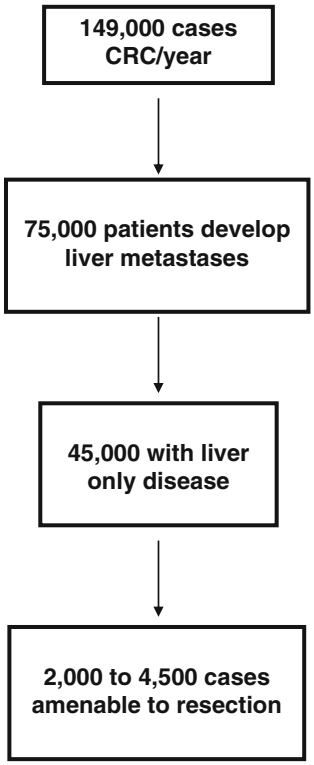

Fig. 1 The proportion of patients amenable to partial hepatectomy for metastatic colorectal cancer is small

identify the tumor's proximity to major vascular and biliary structures as the choice of surgical procedure is predicated on this information. Currently, the mainstay of preoperative staging for colorectal liver metastases is a triphasic CT scan (computed tomography) performed with intravenous contrast. In particular, CT scan has been shown to be especially useful for detecting the characteristic hypovascular liver tumors seen in metastatic colorectal cancer [23]. In order to rule out extrahepatic pulmonary metastases, a routine radiograph is sufficient since the incidence of lung metastases is very low [24, 25]. If a suspicious lesion is noted on chest x-ray, it may be further characterized by a CT scan of the chest with intravenous contrast. Moreover, a colonoscopy and bone scan may be performed to identify the primary tumor and rule out a synchronous cancer or bone metastases.

Two other commonly utilized imaging modalities for preoperative evaluation of metastatic liver lesions include transcutaneous ultrasound and magnetic resonance imaging (MRI). While ultrasound (US) is an inexpensive and widely available technology, its sensitivity is dependent on the experience of the ultrasound technologist. Furthermore, its use may be limited by overlying air-filled structures above the liver including bowel and lung parenchyma. Nevertheless, the

development of ultrasound contrast agents has improved the sensitivity and specificity for the detection of malignant versus benign liver lesions. Bernatik et al. evaluated contrast enhanced US versus helical CT and demonstrated that US showed 97 % of lesions seen on CT scan [26]. While more expensive than CT scan, MRI is highly accurate for the identification and characterization of liver lesions. The colorectal hepatic metastases are typically low-intensity on T1-weighted spin echo images and intermediate intensity on T2-weighted images [23]. Moreover, MRI is very accurate for delineation of vascular structures and it is possible to assess the biliary tree with magnetic resonance cholangiopancreatography (MRCP). To date, there is no randomized control trial comparing preoperative MRI vs. multi-detector CT scan to determine the ideal preoperative cross-sectional imaging modality.

Whole-body positron emission tomography (PET) after the administration of 5-fluorodeoxyglucose (FDG) has emerged as a useful adjunctive study in the assessment for occult metastatic disease. In a study by Fong et al., the authors evaluated 40 patients being considered for partial hepatectomy secondary to metastatic colorectal cancer to preoperative PET scanning [27]. They conclude that PET scanning directly altered management in 23 % of patients, spared a laparotomy in 15 % of patients, and was 85 % sensitive for the detection of hepatic metastases larger than 1 cm in size. While these results need to be corroborated by a larger prospective study, they support the current use of preoperative PET scanning to assess for occult metastatic disease. Of note, a study by Akhurst et al. examining the effect of recent chemotherapy on the sensitivity of PET scanning demonstrated a significantly decreased tumor FDG uptake in patients who were treated with preoperative chemotherapy so therefore, one should consider PET scan early in the preoperative assessment if the patient is being considered for neoadjuvant chemotherapy [28].

4 Indications for Surgery

The clinical criteria for determining suitability for resection of metastatic disease are continually evolving. In general, partial hepatectomy for metastatic colorectal cancer should be offered to patients who have disease isolated to the liver. Moreover, the surgeon must be able to resect all gross disease while maintaining adequate residual hepatic reserve. Debulking of hepatic metastases is not indicated. Neoadjuvant chemotherapy, however, may offer the chance to downstage hepatic tumor burden so that patients can be offered partial hepatectomy. The only absolute contraindications to partial hepatectomy based on preoperative evaluation are the inability to resect all intrahepatic disease or the presence of diffuse extrahepatic metastatic disease. Occasionally, extrahepatic metastases isolated to the lungs may not preclude the patient from surgical resection as various authors have demonstrated that resection of both lung and liver metastases may result in improved patient survival [29]. Furthermore, re-resection of hepatic metastases may be considered in select patients.

5 Partial Hepatectomy

Partial hepatectomy for metastatic disease requires a detailed understanding of hepatic anatomy. Segmental anatomic resection has been the gold standard for resection of liver metastases. While some have attempted hepatic wedge resection of colorectal liver metastases, Dematteo et al. [30] found that there was a high rate of positive surgical margins with hepatic wedge resection and that anatomic segmentectomy resulted in longer survival with a median of 53 months versus 38 months for wedge hepatectomy.

When performing partial hepatectomy, a number of techniques may be employed in an attempt to improve outcomes. One important anesthetic technique is the maintenance of a low central venous pressure (CVP) during partial hepatectomy. Melendez et al. [31] demonstrated that the technique of low CVP anesthesia during 496 consecutive major liver resections allowed for easy control of the hepatic veins before and during parenchymal transection, minimized blood loss, preserved renal function, and decreased perioperative mortality. Another technique which has been examined in an effort to improve outcomes is the use of laparoscopic liver resection. Fong et al. [32] reported that hand-assisted laparoscopic liver resection can be performed safely and is of particular advantage over traditional liver resection when the tumor is limited to the left lateral segment or at the edges of the liver. In a recent evaluation of 300 minimally invasive liver resections, Koffron et al. demonstrated that operative time, blood loss, transfusion requirement, length of stay, and overall operative complications were less in the minimally invasive cases compared to traditional open cases [33].

6 Adjuncts to Exploration at the Time of Surgery

Although most patients undergo extensive preoperative imaging prior to partial hepatectomy for hepatic metastases, there is still roughly a 20 % incidence of unresectable disease at the time of laparotomy. Approximately, half of these patients are unresectable due to extensive liver disease while the other half have occult extrahepatic metastases [1]. Both diagnostic laparoscopy and intraoperative ultrasound serve as valuable tools in helping identify occult metastases.

Utilization of diagnostic laparoscopy prior to laparotomy for patients with a high risk of hepatic metastases may help avoid unnecessary laparotomy. By performing laparoscopy, one may identify enlarged periportal lymph nodes or peritoneal disease that was not detected by preoperative imaging. In a study of 200 patients with potentially curable colorectal hepatic metastases, Mann et al. reported that laparoscopy identified 39 of 67 patients (58 %) with incurable disease. Others have reported that laparotomy can be avoided in up to 78 % of unresectable patients [34].

Intraoperative ultrasound (IOUS) is another useful technique to identify incurable hepatic metastases. Traditionally intraoperative ultrasound has been performed through open laparotomy in conjunction with bimanual palpation of the liver.

However, as laparoscopic technology has improved, many have begun utilizing laparoscopic ultrasound with a similar sensitivity to diagnose incurable hepatic disease. Laparoscopic ultrasound has been shown to identify incurable disease in 31–48 % of patients thereby sparing unnecessary laparotomy [1].

7 Results of Partial Hepatectomy for Metastatic Colorectal Cancer

7.1 Survival

Surgical resection of colorectal hepatic metastases in select patients can confer a significant long-term survival benefit. It is in fact the only therapy that may offer patients with metastatic liver tumors, the prospect of a cure. In order to assess the efficacy of surgical resection of metastatic colorectal cancer, one must compare the results of treatment to the natural history of untreated colorectal metastases. In a study by Sheele et al., the authors reported a median survival time of 30 months in those who underwent partial hepatectomy compared to 14.2 months for those who were deemed candidates for resection but did not chose to undergo surgery. In contrast, for 983 patients who were deemed unresectable, the median survival was 6.9 months with no 5-year survivors [35]. Wagner et al. reported a 25 % 5-year survival rate in 116 patients who underwent partial liver resection compared to only 2 % in those who did not undergo partial hepatectomy [36]. Fong et al. reported a 5-year survival rate of 37 % and a 10-year survival rate of 22 % in 1001 liver resections for colorectal metastases [1]. Similarly, Scheele et al. reported a 10 and 20 year survival rate of 23 and 18 %, respectively, in 434 liver resections performed between 1960 and 1992 [1, 35]. Most recently, Tomlison et al. have reported a 16.7 % actual survival rate in 612 patients with 10 year follow-up period. They note that those who survive 10 years appear to be cured of disease, whereas approximately one-third of actual 5-year survivors succumb to a cancer-related death [37].

Improvement in outcomes is influenced by increasing the number of patients who are eligible for partial hepatectomy. Pawlik et al. report that the indications for surgery have changed over the past 10 years. While traditional criteria, such as the number or metastases(3–4) the size of the lesion, and a mandatory 1-cm margin have dictated resectability, recent criteria have expanded to include any patient in whom all disease can be removed with a negative margin and who has adequate hepatic reserve [38]. The use of neoadjuvant chemotherapy has further increased the number of patients who may be offered partial hepatectomy. Another technique which has offered previously unresectable patients to undergo resection is portal vein embolization (PVE) which decreases blood supply to the tumor while inducing hypertrophy of the non-diseased liver. Azoulay et al. [39] compared 30 patients who underwent PVE prior to liver resection to 88 patients who did not undergo PVE and reported that PVE allows more patients with previously unresectable liver

tumors to benefit from resection with a long-term survival that is comparable to that after resection without PVE.

7.2 Morbidity

A critical step in providing meaningful 5-or 10-year survival rates following partial hepatectomy is the minimization of morbidity from the operation. In a recent systematic review of studies involving patients undergoing partial hepatectomy for colorectal metastases, the authors reported a median postoperative mortality rate (within 30 days of surgery) of 2.8 %. The most common major complication was bile leak with a mean cumulative incidence of 4 % while the most common minor complication was wound infection with a reported cumulative incidence of 5.4 %. The cumulative incidence of postoperative hepatic failure was low at 2.8 %; however, patients with this complication had a high postoperative mortality rate. In fact, hepatic failure was responsible for 18.4 % of fatal complications [40]. Postoperative hepatic failure has been associated with extensive liver resection of more than five anatomic segments and a number of patient factors, such as advanced age, obesity, and diabetes. In order to minimize risk of postoperative hepatic failure, preoperative CT scan may be used to assess functional hepatic reserve. Shoup et al. found that in patients with a large hepatic tumor burden, those who underwent partial hepatectomy with <25 % reserve had more than three times the risk of postoperative hepatic dysfunction than those patients with >25 % preoperatively assessed hepatic reserve [41].

7.3 Prognostic Variables

Numerous authors have evaluated the prognostic determinants of survival after partial hepatectomy for metastatic colorectal cancer. The factors which most consistently correlate with poor long-term prognosis are the presence of extrahepatic disease, positive liver resection margin, advanced primary tumor stage with regional lymph node involvement and the presence of synchronous liver metastases when the primary is first diagnosed [42–48]. It is important to note that patient age is not a contraindication to surgery. Zacharias et al. [49] demonstrated that both first and repeat hepatic resections for colorectal liver metastases can be performed safely in patients over 70 with a 5-year survival similar to that of younger patients.

In an effort to identify prognostic factors for patient survival following partial hepatectomy for colorectal cancer, Fong et al. retrospectively reviewed 1001 consecutive patients who underwent liver resection and proposed a clinical risk score (CRS) [1]. The CRS consist of five specific criteria:

- Nodal status of the primary tumor
- Disease-free interval from discovery of primary to discovery of liver metastases <12 months

- Number of tumors >1
- Preoperative carcinoembryonic antigen (CEA) level >200 ng/ml
- Size of largest tumor >5 cm

For each criterion that is present, the patient is assigned 1 point and the cumulative score is the individuals CRS. With a CRS of zero, the 5-year survival rate was 60 % while it was 14 % with a CRS of five. Calculation of an individual's CRS is useful in identifying which patients will benefit the most from partial hepatectomy.

8 Timing of Liver Resection

The optimal surgical treatment strategy for synchronous liver metastases diagnosed with a colorectal primary has not been well defined. Simultaneous liver and colon resection obviates the need for two laparotomies but some argue that such extensive surgery will result in an increased risk of major morbidity and mortality. In order to address this question, Reddy et al. compared their experience with 610 patients undergoing either simultaneous (135 pts) or staged (475 pts) resections for colorectal cancer with hepatic metastases. They reported that mortality was similar at 1.0 versus 0.5 % when comparing simultaneous colorectal resection and minor hepatectomy compared to staged resection. However, when the simultaneous resection included major hepatectomy, the mortality was 8.3 % compared to 1.4 % for staged resection. Furthermore, major morbidity in this group was 36.1 % compared to 15.1 % for the staged group. Therefore, the authors conclude that simultaneous colorectal and minor hepatic resections are safe and viable options for patients with synchronous liver metastases while caution should be exercised before performing simultaneous colorectal and major hepatic resections [50].

9 Follow-up After Resection

The most difficult challenge confronting the surgeon and patient considering liver resection for metastatic disease is the high rate of recurrence. This will present as disease isolated to the liver in roughly 28–50 % of patients which in some cases may be amenable to repeat hepatectomy [43, 51, 52]. While some authors have demonstrated significant 5-year survival rates with low morbidity in cases of repeat hepatectomy the surgery is often technically demanding due to adhesions and altered hepatic vascular and biliary anatomy [53, 54]. The reported operative morbidity of repeat resection, however, has been reported to be similar to the initial resection rates.

Currently, there is no conclusive evidence that close monitoring of patients after partial hepatectomy for metastatic colorectal cancer will improve survival. However, the National Comprehensive Cancer Network (NCCN) guidelines recommend

a similar approach for stage IV disease as for stage III disease with the exception of timing of CT scans. The panel recommends CT scan of the chest, abdomen, and pelvis every 3–6 months in the first 2 years following adjuvant treatment and then every 6–12 months for up to a total of 5 years. A serial physical is recommended every 3–6 months for 2 years and every 6–12 months for the following 3 years. A CEA level should be obtained every 3 months for the first 2 years and then every 6 months for the next 3 years. In the setting of a rising CEA, a PET scan may be obtained to evaluate for occult metastatic disease.

10 Chemotherapy for Advanced Colorectal Cancer

Conventional systemic therapy for colorectal metastasis to the liver has been based on the antimetabolite 5-fluorouracil (5-FU), which inhibits thymidylate synthase and leucovorin, a reduced folate that increases the affinity of 5-FU for thymidylate synthase. Response rates with this regimen range from 20 to 30 % although complete response is rare [55, 56]. Oral 5-FU prodrugs, such as capecitabine, have been shown to have a significantly higher response rate and a lower toxicity profile than conventional 5-FU/LV while resulting in an equivalent median survival [57, 58]. Two additional agents, irinotecan and oxaliplatin, were found to have activity against advanced colorectal cancer. Saltz et al. [56] demonstrated that the addition of irinotecan, an inhibitor of topoisomerase I, improved response rate to 39 % and the median survival to 14.8 months. The addition of oxaliplatin, a platinum-based drug which inhibits DNA replication by forming cross-linking adducts, to a regimen of infused 5-FU/LV increased response rates to 51 % and median survival to 16.2 months (without reaching statistical significance compared to infused 5-FU/LV) [59]. Goldberg et al. [60] compared oxaliplatin, infused 5-FU plus LV (FOLFOX) with irinotecan and bolus 5-FU (IFL) in a phase III multicenter trial. A response rate of 45 % and median survival time of 19.5 months were observed for FOLFOX which were significantly superior to those observed for IFL (31 % and 15 months). Most recently, a greater understanding of tumor biology has led to the development of molecular targeted therapeutics. The anti-VEGF antibody, bevacizumab, has been used to inhibit tumor angiogenesis and the anti-EGFR antibody, cetuximab, has been used in combination with irinotecan and 5-FU/LV to increase response rates [8].

The NCCN panel has recommended a chemotherapy treatment algorithm for advanced and metastatic colorectal cancer based on both the patient's ability to tolerate a specific regimen as well as whether the treatment is the initial therapy or a treatment for progression (see Fig. 2) [61]. For patients with advanced disease who are able to tolerate intensive therapy, a choice of FOLFOX, CapeOX, FOLFIRI, or 5FU/LV is recommended. In the setting of metastatic disease, bevacizumab should be added to the initial treatment regimen. The recommendation for therapy after first progression for patients on prior 5-FU/LV-based therapy includes irinotecan as single-agent therapy or in combination with cetuximab. For those patients who are unable to tolerate intensive therapy, capecitabine ± bevacizumab or infusional

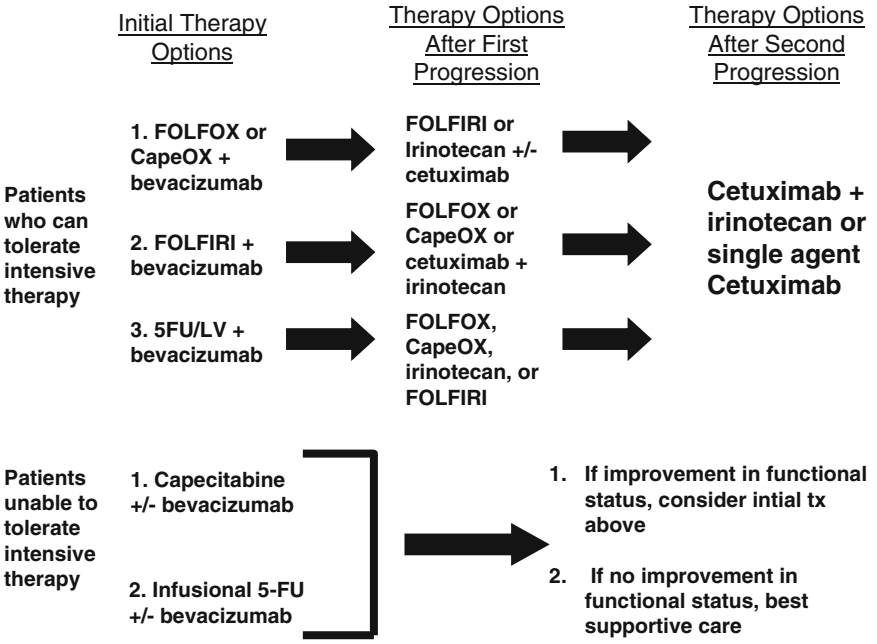

Fig. 2 Flow chart for unresectable advanced colorectal cancer adapted from the National Comprehensive Cancer Network (NCCN) guidelines for physicians

5-FU/LV ± bevacizumab is recommended. If there is a significant improvement in functional status, the patient may be reevaluated for a more intensive initial therapy regimen.

There have been recent advancements in the understanding and application of targeted therapies, specifically in anti-EGFR therapy. The receptor for EGFR is reported to be over expressed in up to 80 % of colorectal cancers making it an ideal target [62]. There are two currently available therapeutic agents, cetuximab and panitumumab, both monoclonal antibodies directed against EGFR. Cetuximab is a chimeric antibody whereas panitumumab is fully humanized. EGFR signaling goes through the *RAS/RAF* pathway and it is well established that mutations in exon 2 of *KRAS* render anti-EGFR therapy ineffective [63–65]. There is growing evidence that *KRAS* mutations outside of exon 2, i.e., exons 3 and 4, have a similar effect. Furthermore, other *RAS* mutations such as *NRAS* exons 2, 3, and 4 have shown to be predictive of lack of anti-EGFR response [66]. In an updated analysis of the PRIME trial, Douillard et al. found that patients with any *RAS* mutation had significantly decreased progression free survival (HR 1.31, 95 % CI 1.07-1.55) and overall survival (HR 1.21, CI 1.01-1.45) when treated with FOLFOX plus panitumumab versus FOLFOX alone [64]. Consequently, the NCCN panel has strongly recommended *KRAS/NRAS* genotyping in all patients with metastatic colorectal

Table 1 Results of partial hepatectomy for metastatic colorectal cancer

Author [reference #]	Number or patients	5-year survival (%)	Median survival (mo.)
Adson et al. [90]	141	25	24
Hughes et al. [46]	607	33	NA
Scheele et al. [48]	219	39	NA
Fong et al. [91]	456	38	42
Jamison et al. [92]	280	27	32
Jenkins et al. [93]	131	25	33
Bakalakos et al. [94]	301	29	23
Elias et al. [95]	151	28	NA
Rosen et al. [47]	280	25	NA
Choti et al. [96]	226	40	46

cancer. The panel further states that patients harboring any *RAS* mutations should not be treated with either cetuximab or panitumumab [61].

BRAF mutations affect the EGFR signaling pathway downstream of *RAS*. The most common *BRAF* mutation, V600E, has shown to be a poor prognostic factor, effectively doubling risk of mortality in a recent meta-analysis [67]. The data for *BRAF* mutations as predictive markers is not as conclusive but there is evidence that these patients do not have improved survival with anti-EGFR therapy [64, 68] Table 1.

11 Neoadjuvant Therapy Prior to Partial Hepatectomy

In order to offer patients the best chance of long-term survival, many clinicians have offered neoadjuvant chemotherapy to patients with colorectal hepatic metastases. In some cases, the intent is to downstage the hepatic burden of disease so the patient is a candidate for surgical resection while in other cases it is meant as an adjunctive measure to improve survival. Furthermore, many believe that a significant or complete response to neoadjuvant chemotherapy serves as a reliable indication of a good prognosis. A variety of neoadjuvant chemotherapy regimens have been reported including 5-Fluorouracil and folinic acid with or without oxaliplatin, ironitecan, cetuximab, or bevacizumab. The 5-year survival in patients undergoing partial hepatectomy with neoadjuvant chemotherapy ranges from 28 to 40 % while the median survival time ranges from 20 to 37 months (see Table 2). In a study by Adam et al., 701 patients with unresectable colorectal liver metastases were treated with neoadjuvant chemotherapy and 95 patients (13.5 %) were found to be resectable on reevaluation. The 5-year survival in this group of patients was 35 % which is comparable to the 5-year survival rate in patients who are initially respectable [6]. In essence, neoadjuvant chemotherapy may effectively downstage patients so that they become eligible for potentially curative resection. In another study, 131 patients who underwent neoadjuvant chemotherapy were retrospectively

Table 2 Results of neoadjuvant chemotherapy and partial hepatectomy for metastatic colorectal cancer

Author [reference #]	Chemotherapy regimen	Number of patients	Median survival (mo)	5-Year survival (%)
Adam et al. [6]	5FU/FA ± Oxaliplatin	87	NA	39
Pozzo et al. [12]	FOLFIRI	40	30.1	NA
Giacchetti et al. [9]	5FU/FA + Oxaliplatin	151	24	28
Tournigand et al. [13]	FOLFOX4	311	NA	32
Folprecht et al. [8]	5FU/FA + Ironotecan + Cetuximab	21	33	NA
Alberts et al. [7]	FOLFOX4	42	26	NA
Masi et al. [11]	FOLFOXIRI	74	36.8	NA
Hurwitz et al. [10]	IFL + Bevacizumab	402	20.3	NA

reviewed and grouped into three categories by response to chemotherapy. They found that patients who had an objective tumor response had a 5-year survival rate of 37 % while those who experienced tumor progression while on chemotherapy had a 5-year survival rate of only 8 % [69]. These results indicate that response to neoadjuvant chemotherapy helps to predict survival following partial hepatectomy for metastatic colorectal cancer.

12 Partial Hepatectomy for Other Metastatic Liver Tumors

12.1 Neuroendocrine Tumors

As liver resection has become an increasingly safe and effective procedure for metastatic colorectal cancer, there has been more aggressive use of partial hepatectomy for other metastatic liver tumors including neuroendocrine tumors (NET). These are rare tumors, representing less than 5 % of all gastrointestinal malignancies [70–72]. Despite their rarity, the burden of liver metastases in NET is not insignificant, with 46–93 % of patients developing liver metastases [73]. Unlike patients with colorectal hepatic metastases, patients with metastatic NET typically follow a protracted and variable time course from the onset of symptoms to death. Patient presentation ranges from small isolated liver metastases causing severe symptoms, to asymptomatic patients with near complete parenchymal replacement by NET metastases. In treating patients with metastatic NET, we must take into consideration the natural history of the primary tumor and the severity of presenting symptoms [74]. Approximately, 70 % of NETs with hepatic metastases are nonsecretory, and therefore, many patients instead present with vague symptoms of weight loss, abdominal pain, and abdominal mass [75]. For those with severe symptoms from secreted hormones, major advances have been made in medical therapies offering symptom relief. These treatments include H2-blockers, proton pump inhibitors, and somatostatin analogues; however, most patients develop resistance to

these treatments despite an initial favorable response [76, 77]. If left untreated, the 5-year survival of metastatic neuroendocrine tumors is roughly 40 % [77]. Furthermore, many of these patients will suffer symptoms resulting from their tumor burden. The rationale to resect neuroendocrine metastases is based on the relatively long tumor doubling time, the ineffectiveness of chemotherapy, and the need to reduce tumor-related symptoms. Surgery offers a chance for a cure and is associated with improved 5-year survival rates. In the largest study evaluating hepatic resection for metastatic neuroendocrine tumors, Sarmiento et al. report 5-and 10-year survival rates of 61 and 35 %, respectively, in 170 patients who underwent partial hepatectomy. Furthermore, symptom control was achieved in 104 of 108 patients [78]. Similarly, Yao et al. demonstrated actuarial 5-year survival of 70 % after hepatic resection for NET metastases in their series [79]. When compared to conservative management alone, Touzios et al. showed significantly improved survival following aggressive surgical resection or transarterial chemoembolization (TACE), with 5-year survival rates of over 70 and 50 %, respectively, versus 25 % in the conservatively managed group [80]. Despite improved 5-year survival and symptom relief in patients who undergo partial hepatectomy for metastatic neuroendocrine tumors, the rate of recurrence remains high at 84 % at 5 years from the time of surgery [78]. Even if the likelihood of long-term cure is small, cytoreductive surgery for symptomatic neuroendocrine tumors has proven safe and effective in providing long-term survival with fewer symptoms [74, 77]. When combined with adjuvant therapy (radiofrequency ablation, chemo/radioembolization), surgical debulking may be used to relieve symptoms in cases when an estimated 70–90 % of disease can be treated [81]. In patients where this is not possible, orthotopic liver transplant has been evaluated [82].

12.2 Non-neuroendocrine, Non-colorectal Hepatic Metastases

It is unusual to see non-neuroendocrine, non-colorectal metastases to the liver without evidence of extrahepatic disease, making liver resection of these metastases a rare event. Nonetheless, select patients have been offered partial hepatectomy as surgery offers the only hope for long-term cure or improved survival. In a series of 96 patients who underwent hepatic resection at Memorial Sloan-Kettering Cancer Center over a 15 year period, the overall actuarial 5-year survival was 37 % with a median survival of 32 months. The primary tumors included testicular, adrenal, ovarian, renal, uterine, cervical, sarcoma, melanoma, breast, gastric, and pancreas. They note that the subgroup of patients with genitourinary tumors, including nine patients with testicular and seven patients with ovarian, had a 5-year survival of 60 % [83]. Others have shown similar results. In a series of 95 patients, Earle et al. demonstrated a 5-year survival rate of 34.9 % after hepatectomy for non-colorectal metastases. Notably, those with non-gastrointestinal primaries had improved median survival compared to those with foregut primaries (pancreas, ampulla, stomach), 36 versus 20 months, respectively [84]. In another series of 64 consecutive patients

undergoing partial hepatectomy for non-colorectal, non-neuroendocrine hepatic metastases, the authors report that the most significant prognostic variable is a short-time interval from diagnosis of primary to tumor to hepatic metastases [85].

Hepatic metastases from breast cancer and melanoma are generally considered an ominous clinical finding. One study examined 21 patients who underwent partial hepatectomy for breast cancer and demonstrated a median survival of 26 months [86]. Another series of 13 patients undergoing hepatic resection for metastatic melanoma reported a median survival of 10 months, however, one patient survived 5 years [87]. One particular subgroup of melanoma for which hepatic resection has demonstrated significant improved survival is ocular melanoma. In a study evaluating 19 patient with metastatic ocular melanoma who underwent complete resection of all gross liver disease followed by intra-arterial chemotherapy, the authors reported a median survival of 22 months [88].

In 2006, The Society for Surgery of the Alimentary Tract published a report on the management of hepatic metastases on the following tumors: breast, melanoma, ovarian, sarcoma (GIST, gastrointestinal leiomyosarcoma) and gastric cancers. In selected patients, resection of hepatic metastases of these primary tumors is part of complete oncologic treatment [89].

13 Summary

Partial hepatectomy has become the standard therapy for select patients with hepatic metastases. While the prototypical and most common disease amenable to hepatic resection for metastatic disease is colorectal cancer, a number of patients with neuroendocrine and non-neuroendocrine, non-colorectal metastases have demonstrated improved survival with partial hepatectomy. Utilization of such techniques as staging laparoscopy and intraoperative ultrasound has helped guide surgical resection and occasionally avoided unnecessary laparotomy. For patients with colorectal cancer metastatic to the liver, the CRS can offer valuable prognostic information to patients. Advances in management strategies, such as preoperative portal vein embolization and neoadjuvant chemotherapy have led to significant downstaging of initially unresectable disease and patients who the undergo surgery have similar 5-year survival rates to those who were resectable at the time or presentation. Ultimately, hepatic resection for hepatic metastases offers the only chance for long-term improved survival and cure. As adjunctive diagnostic measures and chemotherapeutic options improve, we can expect to see further improvement in disease-free survival.

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Repeat Hepatectomy for Colorectal Liver Metastases

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Abstract

Surgical resection of hepatic metastatic disease from colorectal cancer offers the best survival advantage when compared to other treatment modalities as survival from unresected disease is rare. Even after adequate surgical excision of colorectal cancer, 20–40 % of patients will develop recurrent disease to the liver. This chapter discusses the management of patients with recurrent colorectal metastases to the liver after initial resection and offers strategies to optimize and guide their treatment with a multimodality approach.

Keywords

Repeat hepatectomy · Colorectal liver metastases · Hepatic resection · Recurrent hepatic metastases

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1 Introduction

With an incidence of approximately 132,700 new cases in 2015, colorectal carcinoma is the third most common malignancy of men and women in the United States [1]. Although the incidence and mortality from colorectal cancer has declined over the last twenty years, it is estimated that almost 50,000 deaths will occur as a result of colorectal cancer in 2015. Even after adequate surgical excision of colorectal cancer, 20–40 % of patients will develop recurrent disease to the liver [2–5]. In 30 % of individuals with metastatic colorectal cancer, the liver is the only site of recurrent disease. For this subset of patients, surgery remains a curative option [6, 7]. Surgical resection of hepatic metastatic disease from colorectal cancer offers the best survival advantage when compared to other treatment modalities as survival from unresected disease is rare [8–14]. Unfortunately, recurrence of metastatic disease is common as 50–80 % of patients will develop recurrent metastatic disease either in the liver or elsewhere, despite prior hepatic resection [7–22]. Patients with untreated recurrent metastatic disease, as expected, have a poor survival [13, 23]. Recurrent liver metastases occur in approximately 10–50 % of patients who previously underwent liver resections with curative intent. Approximately 15–40 % develop recurrent distant disease with or without the presence of concurrent hepatic metastases [12, 15, 18, 24–29]. Patients with recurrent hepatic metastases are no longer relegated to clinical trials or end-of-life care because aggressive treatment approaches have now become the norm with both advanced chemotherapeutic and surgical options leading to increased disease-free and overall survival rates. This chapter discusses the management of patients with recurrent colorectal metastases to the liver after initial resection and offers strategies to optimize and guide their treatment with a multimodality approach.

2 Primary Resection for Colorectal Liver Metastases

The liver is the most common site of metastases from colorectal cancer. Despite efforts in screening and early detection, 35 % of patients will present with synchronous liver metastases and an additional 20 % of patients will develop metachronous disease [4, 30, 31]. Surgical resection represents the only hope for long term survival and potential cure in patients with liver metastases secondary to colorectal cancer. Candidates for resection include those who are medically able to undergo a major hepatectomy. There is little evidence to suggest that age is an independent risk factor that increases operative mortality [32–37]. Contraindications to hepatic resection of metastatic disease include proximity to major biliary or vascular structures that may preclude an R0 resection, compromise the vascular inflow/outflow, or impact biliary drainage. The presence of extrahepatic disease or progression of hepatic disease while on chemotherapy represents two further contraindications [38]. Finally, resectability may be defined by the ability to spare at least two adjacent liver segments in order to preserve sufficient remnant liver

Table 1 Negative prognostic factors after initial liver resection for colorectal metastases in selected series

Author	Age	Node-positive primary	Poorly differentiated primary	Synchronous metastases	Size	Number metastases	Extrahepatic disease	CEA	R1 resection
Nordlinger	(+)	(+)		(+)	(+)	(+)		(+)	(+)
Fong	(−)	(+)		(+)	(+)	(+)	(+)	(+)	(+)
Scheele	(−)	(+)	(+)	(+)	(−)	(+)	(+)		
Kato		(−)	(+)	(−)	(−)	(−)	(+)	(+)	(+)
Wei	(+)	(−)	(−)	(−)	(+)	(+)	(−)	(−)	(+)
Wang	(+)	(+)	(+)	(−)					
Figueras				(+)		(+)	(+)		(+)
Rees	(−)	(+)	(+)	(+)	(+)	(+)	(+)	(+)	(+)

(+) Survival Disadvantage; (−) No difference in survival; *CEA* Carcinoembryonic Antigen

volume following resection (at least 20 % of the total estimate liver volume as measured by preoperative imaging) [20, 39]. In individuals who undergo metastatic resection, 5-year survival rates range between 30 and 58 % in most recent reports with less than 5 % mortality rate [4, 29, 40].

In a recent meta-analysis of 30 published series, more than 20 factors have been identified that affect outcomes after resection of colorectal liver metastasis [19]. Among these, several factors were consistent in each series that correlated with worse prognosis and more aggressive disease (Table 1). Positive resection margin represents one of the most important and consistent factors that predicts poor outcome after resection of liver metastases in terms of reduced survival rates and increased risk of intrahepatic recurrence [22, 41–43]. Risk factors for R1 resection margin include tumor size greater than 3 cm, use of wedge resection, greater than 3 hepatic lesions, bilobar disease, abnormal preoperative liver function tests, and intra-operative blood transfusion [42–44].

To assess risk and stratify patients, investigators have attempted to devise prognostic scoring systems and nomograms. Several authors have proposed unique scoring systems identifying several clinical criteria to aid in the prediction of recurrence and survival. Of these, the clinical risk score as proposed by Fong et al. in 1999 has been used most widely to predict recurrence and overall survival [22, 45]. In this model, one point is assigned to each of five clinical criteria: node-positive primary, less than 12 month disease-free interval, presence of multiple hepatic metastases, largest tumor greater than 5 cm, and CEA leval greater than 200 ng/mL. The 5-year actuarial survival rate for patients with 0 points was 60 %, whereas that for patients with 5 points was 14 %. Despite improved long term survival rates after surgical resection of colorectal liver metastases, recurrence is the leading cause of death in this patient population.

Consideration of neoadjuvant therapy prior to surgical intervention has also been considered. The European Organization for Research and Treatment of Cancer

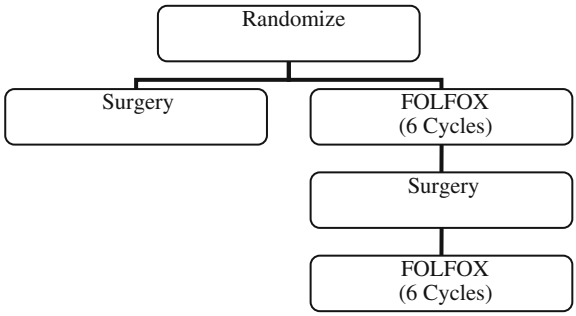

Fig. 1 Algorithm for perioperative FOLFOX therapy in resectable liver metastases (EORTC 40,983)

Intergroup trial 40983 (EORTC 40983) sought to ascertain the effect of perioperative systemic therapy on survival above and beyond surgery alone. EORTC 40983 stratified patients to 3 months of perioperative FOLFOX compared to surgery alone in patients with resectable liver metastases (Fig. 1). In the FOLFOX treatment group, 79 % of patients were able to complete the 6 cycles of chemotherapy. Of these, 7 % progressed while on chemotherapy of which 33 % were still able to be resected. Overall, there was a 25 % reduction in tumor diameter in the neoadjuvant arm and a 9.2 % increase in the rate of progression-free survival at three years in patients treated with perioperative FOLFOX compared to surgery alone [96]. These studies demonstrate that in those patients with recurrent disease, disease-free survival can be improved and can serve as an indicator of tumor biology.

3 Repeat Hepatectomy for Colorectal Liver Metastases

3.1 Introduction

Of patients who undergo initial curative resection of liver metastases due to colorectal carcinoma, 30–70 % will have recurrence of their disease, of which 33 % will be isolated to the liver [2, 24, 46, 47]. Bozzetti et al. described their follow-up experience after initial resection of colorectal liver metastases. With a median follow-up of 18 months, 62 % of their cohort developed distant recurrence after initial hepatectomy with 11 % developing simultaneous hepatic and distant recurrence, and 24 % developing disease limited to the liver [15]. Data on repeat hepatectomy for colorectal liver metastases is relatively sparse as only 10–30 % of patients who develop these isolated liver recurrences are candidates for surgery [28, 48, 49]. Patients with recurrent liver disease are at risk of harboring occult extrahepatic metastases thus it is imperative to determine which patients would benefit from repeat hepatic resection. Examining patterns of disease recurrence, determining associated risk factors, and understanding tumor biology are crucial prior to undertaking surgical explorations.

3.2 Indications for Repeat Hepatectomy

Indications for repeat hepatic resection in patients who develop recurrent colorectal liver metastases are largely similar to the indications required at the time of the first operation [46]. The patient should be medically able to undergo major hepatic resection, an R0 resection should be achieved, major vascular and biliary structures should be protected, and at least 20–30 % of liver parenchyma should be preserved in patients with normal liver function [27, 46, 50, 51]. Since recurrence rates after initial hepatectomy overall range from 30 to 70 %, an extensive metastatic workup should be completed prior to operative intervention.

It is our philosophy that patients with bulky hepatic disease—designated by large or numerous lesions, short disease-free intervals, or small-volume extrahepatic disease—should not initially be considered candidates for up front repeat hepatic resections. A subset of these patients may become candidates after undergoing treatment with neoadjuvant systemic therapy. Adam et al. demonstrated the feasibility of this strategy in patients presenting with first time recurrence [38]. One hundred forty patients with greater than 4 metastatic liver lesions underwent neoadjuvant chemotherapy consisting of 5-fluorouracil/folinic acid and were evaluated with serial imaging to detect their response to the regimen. A response was detected in 44 % of patients (group 1), defined as a 50 % or more reduction in tumor size. Thirty percent of patients had stabilization of disease (group 2) and 26 % had progression of their disease (group 3), defined as a 25 % increase in tumor size or the appearance of new lesions. One hundred eighteen patients ultimately underwent curative resection with an overall 5-year survival in all three groups of 28 %. Stratifying based on response to the neoadjuvant regimen, the overall 5-year survival was 37, 30, and 8 % in treatment groups 1, 2, and 3, respectively. Disease-free survival likewise demonstrated a benefit to those who had responsiveness to neoadjuvant chemotherapy. In this regard, disease-free survival at 5 years was 21, 17, and 3 % in groups 1, 2, and 3, respectively. Finally, hepatic recurrence was significantly less in those patients who had a response to chemotherapy versus those whom had a progression of disease (55 vs. 82 %, $p = 0.01$). These data support the use of neoadjuvant chemotherapy prior to hepatectomy in appropriate patients for the purpose of determining tumor biology and ultimately which patients are best served by this treatment algorithm. A similar strategy, therefore, should be utilized for repeat hepatic recurrence in patients who are at increased risk of harboring occult extrahepatic disease.

3.3 Preoperative Evaluation

Preoperative evaluation is critical to identify appropriate candidates for repeat hepatic resection for recurrent liver metastases from colorectal carcinoma. Patients should undergo a thorough medical evaluation to identify and minimize cardiopulmonary risk factors that would preclude a major hepatic resection. Common laboratory tests during the surveillance period after initial hepatectomy include liver

function tests and serum carcinoembryonic antigen (CEA) levels. CEA is routinely used in the preoperative workup and postoperative surveillance of patients treated for this disease. Numerous studies have demonstrated its superiority in comparison with liver function tests such as lactate dehydrogenase and alkaline phosphatase in the detection of liver metastatic disease [52–55].

Individuals with recurrent or suspected recurrent hepatic metastases as suggested by laboratory values, should undergo imaging to assess the location and extent of recurrent tumor burden within the liver. Historically, computed tomography (CT) has been used for preoperative imaging. CT is cost-effective when compared to magnetic resonance imaging (MRI) and is not user-dependent as is ultrasound evaluation of the liver parenchyma. Sensitivity and specificity of CT scan to detect hepatic metastases range from 52–84 to 85–95 %, respectively [56–62]. A meta-analysis by Wiering et al. demonstrated the sensitivity and specificity of CT imaging for detecting hepatic lesions to be 82.7 and 84.1 %, respectively [63]. Despite the reasonable sensitivity and specificity of CT scan in detecting recurrent hepatic disease, CT scan notoriously underestimates the presence of local recurrence when compared to 2-[^{18}F]-fluoro-2-deoxyglucose (FDG) positron emission tomography (PET) with a sensitivity and specificity range of 65–73 and 72–95 % for CT scan versus 90–100 % sensitivity and 92–100 % specificity for PET scan [58, 60, 61, 64, 65]. Even with the addition of colonoscopy to CT scan in the evaluation for local recurrence, PET scan still offer a higher degree of detection of locally recurrent disease [66]. The importance of these data is in identifying those patients with recurrent liver metastases who also have extrahepatic or locally recurrent disease that would preclude them from undergoing repeat resection. Of note, CT scan is often unable to detect patients with peritoneal metastases that is picked up by PET scan thus preventing this group of patients from undergoing a nontherapeutic laparotomy as well [67]. Recent studies suggest utilization of PET scan in the workup of recurrent disease from colorectal cancer that alters the management strategy in 19–47 % of patients, resulting in 12–60 % of patients being spared an unnecessary operation secondary to upstaging of their disease [59, 66, 68–70]. Typically, CT scans have a difficult time detecting tumor near the operative bed after a prior hepatectomy due to postoperative changes. With the combination of PET scan and CT scan, locally recurrent and liver metastatic disease can be detected with a higher level of accuracy. The combination of the two modalities provides desired anatomic imaging needed to plan surgical resection in addition to needed information on metabolic activity of suspicious areas. With this dual imaging approach, sensitivity for detection of any recurrent disease approaches 89 % with a specificity of 92 %, and the sensitivity and specificity of detecting hepatic recurrence ranges from 91–95 to 75–100 %, respectively. The overall accuracy of diagnosing hepatic recurrence has been documented as high as 97–99 % [71–73]. In particular, CT-PET scan has demonstrated a significantly improved ability to detect hepatic recurrence in the operative bed in patients who have had a prior hepatectomy. The specificity of detecting recurrent disease in the liver bed was 100 % in these instances compared to 50 % for CT scan alone [72].

More recently, the introduction of hepatobiliary contrast-enhanced agents such as Eovist for use with MRI has been shown to be a sensitive method for detecting hepatic metastases. Several studies have studied the efficacy of Eovist-enhanced MRI for detection of hepatic lesions. Ichikawa et al. demonstrated that Eovist enhanced MRI had higher sensitivity in lesion detection (67.5–79.5 %) than CT (61.1–73 %) ($p < 0.05$) and unenhanced MRI (46.5–59.1 %). Higher sensitivity was also noted with Eovist-enhanced MRI as compared to CT when examining smaller lesions; 38–55.4 versus 26.1–47.3 % for lesions <20 mm diameter and 71.1–87.3 versus 65.7–78.4 % for lesions 10–20 mm diameter [97]. Comparing Eovist-enhanced MRI with CT-PET, Donati et al. demonstrated a significant difference in lesion detection between the two modalities. The detection rate of liver lesions was significantly lower for CT-PET than for Eovist enhanced MRI; 64 versus 85 %, respectively ($p = 0.002$). For lesions less than 1 cm in diameter, the detection rate was similarly found to be significantly less for CT-PET than for Eovist enhanced MRI; 29 versus 71 %, respectively ($p = 0.013$) [98].

Combined imaging with MRI and hepatobiliary contrast-enhanced agents such as Eovist has therefore proved most useful for workup of patients as candidates for repeat hepatectomy for colorectal liver metastases. Using this modality appropriately stages individuals to either prevent them from undergoing a nontherapeutic operation or downstages their recurrent disease such that they may benefit from repeat hepatectomy.

3.4 Results After Repeat Hepatectomy

The data regarding initial hepatectomy for colorectal liver metastases is clear. A significant survival advantage is seen in those patients who have their disease resected as previously described. However, the main cause of death in these patients after undergoing hepatectomy for liver metastases is tumor recurrence. Thus, it is imperative to determine whether the survival advantage seen with initial hepatectomy for colorectal liver metastases remains with repeat hepatectomy, and if the benefits warrant taking on the operative morbidity and mortality associated with a repeat procedure. Numerous series have sought to evaluate the outcomes of individuals undergoing such repeat hepatectomy for recurrent colorectal metastases to the liver (Table 2). With a median follow-up of 29 months (range 13–59 months), 3 and 5-year survival rates for isolated hepatic recurrence closely mimic the survival rates seen after initial hepatectomy. The median survival after second hepatectomy is 38.9 ± 12.5 months (range 16–52 months) with a median 3-year and 5-year survival rate of 55 ± 12.2 months and 42.5 ± 6.6 months, respectively. In addition, operative mortality for second hepatectomy is similar to the initial hepatectomy with rates generally below 5 % (range 0–11 %), demonstrating that this is a safe and feasible option for patients with recurrent hepatic disease [27, 46, 74]. Although the morbidity of a second liver resection has been shown to be greater compared to the initial hepatectomy in certain series, presumably due to longer operative times and more tedious dissection, a statistically significant difference has

Table 2 Outcomes after repeat liver resection for recurrent colorectal liver metastases in selected series

Author	*N*	Median F/U (mo)	Morbidity (%)	Mortality (%)	3-year survival (%)	5-year survival (%)	Median survival (mo)	Recurrence (%)	HR (%)	HR + EHR (%)	EHR (%)
Pinson	10	25	60	0	–	–	25	60	17	67	17
Adam	64	27	20	0	60	41	46	–	–	–	–
Sa Cunha	40	31	42.5	2.5	55	31	31.8	67.5	44	33	22
Muratore	29	–	6.9	3.4	35.1	–	–	62.1	39	28	33
Petrowsky	126	58.8	28	1.6	–	–	37	66.7	36	67	33
Yamamoto	75	24	10.7	0	48.3	30.7	30.5	73	40	18	42
Fong	25	19	28	0	–	–	30.2	80	50	5	45
Chu	9	–	33.3	11.1	–	22	23	66	40	40	20
Nordlinger	116	20	24.7	0.9	33	16	19	66	51	34	15
Tuttle	23	–	22	0	–	16	39.9	70	19	44	37
Kin	15	16	20	0	42.4	21.2	16	53.3	–	–	–
Takahashi	22	23	18	0	49	–	23	63.6	57	29	14
Vaillant	16	33	37.5	0	57	30	33	68.8	55	18	27
Elias	28	–	–	–	37	–	39	–	–	–	–
Que	21	20	0	–	58	–	40.8	42.8	11	22	66
Stone	10	13.5	20	0	–	–	25	70	29	43	29
Lange	9	13	–	0	–	–	22	55.6	40	60	0
Huguet	8	24	–	0	–	–	31	71.4	60	0	20
Suzuki	26	–	27	0	62	32	31	69.2	17	83	0
Fernandez-Trigo	170	25	19	–	45	32	34	–	–	–	–
Griffith	9	21	–	11.1	–	–	21	33.3	33	0	66
Nishio	54	–	18.5	5.6	53	46	50.3	70.3	–	–	–
Shaw	66	32	18.3	0	68	44	52	–	–	–	–

mo months; *F/U* Follow-up; *HR* Hepatic recurrence; *EHR* Extrahepatic recurrence; Resection interval refers to the time interval between first and second liver resections for hepatic metastases

not been demonstrated with regards to hospital stay or survival [18, 51, 75]. Finally, the recurrence rates after a second liver resection are similar to those after initial hepatectomy. Recurrence rates after a second hepatectomy range from 33 to 80 % (median 64.8 ± 17 months) with a median time to second recurrence of 17.6 months. Most of these recurrences involve the liver with isolated recurrence in 19–94 % of cases and combination of liver and extrahepatic disease in 31–44 % [24, 27, 46, 76]. In the correct patient population, repeat hepatectomy can therefore be performed with low operative mortality and similar 3 and 5-year survival rates to those found after initial hepatectomy.

For selected patients, repeat hepatectomy in combination with ablative techniques may be indicated. This subset of patients, by definition, is likely to have more aggressive tumor biology and can be expected to present with higher rates of recurrence. Unfortunately, data is limited regarding the efficacy of repeat hepatectomy combined with RFA or other ablation techniques. The benefit of RFA as a standalone therapy, however, has been demonstrated for the treatment of repeat hepatic recurrences. Elias et al. evaluated their experience with RFA for repeat hepatic tumor recurrence after initial hepatectomy. Inclusion criteria in this study included having fewer than 5 recurrent lesions of maximal diameter less than 3.5 cm, lesions not amenable to percutaneous approach, location more than 1 cm from main bile ducts, and no extrahepatic disease. Sixty-two percent of patients were treated because of recurrent colorectal liver metastases. The 1 and 2-year overall survival rates in all treated patients was 88 and 55 %, respectively. In a retrospective comparison, this survival rate was similar to the repeat hepatectomy data of Elias et al. in which the 1 and 2-year overall survival rate was 84 and 60 %, respectively. Unfortunately, these groups of patients represented a heterogenous group that cannot be directly compared. In addition, the time to a second recurrence and thus need for repeat RFA was very short (6.1 months) with a 40 % recurrence rate at the RFA site itself necessitating repeat ablation [77, 78]. Both of these variables are far inferior to current literature on repeat hepatectomy. However, in individuals who are unable to tolerate a second liver resection, RFA alone may be a viable option. Further studies are needed in order to evaluate the benefit that RFA can add to repeat hepatectomy in order to limit tumor recurrence and improve survival.

4 Prognostic Factors After Repeat Hepatectomy

For certain individuals, the benefit of repeat hepatectomy for recurrent metastatic disease may be limited secondary to factors associated with their operative intervention, tumor location, or tumor biology. Multiple studies have delineated prognostic factors that are associated with decreased survival after the second hepatectomy (Table 3). In general, CEA levels greater than 30 ng/ml prior to repeat hepatectomy have been associated with a worse prognosis compared to levels less than 30 ng/ml [46, 50, 79]. This could be due to greater disease burden as higher preoperative CEA levels have been associated with more extensive hepatic disease

[52, 80, 81]. Takahashi et al. determined that a CEA level greater than 50 ng/ml before the initial hepatectomy was also associated with a decreased survival after repeat hepatectomy [82]. A disease free interval greater than 1 year between initial and repeat hepatectomy has been associated with a survival benefit. This is likely due to tumor biology as a decreased interval to tumor recurrence denotes a more aggressive tumor biology and higher likelihood for the presence of additional undetected recurrent disease [46, 51, 83]. The achievement of an R0 resection at the time of repeat hepatectomy has been shown to be a positive prognostic indicator for survival after repeat resection [46, 51, 74, 79, 84]. However, achieving margins greater than 1 cm have not necessarily made a difference on survival after repeat resection, despite the survival benefit seen after initial hepatectomy [85]. The presence of solitary, or in some cases less than 3, recurrent liver lesions was also associated with a survival benefit likely due to the ability to achieve an R0 resection and more favorable tumor biology [24, 46, 50, 84]. Nishio et al. and Petrowsky et al. demonstrated that increased size of recurrent liver metastases was associated with a worse survival after second hepatectomy. The presence of solitary initial metastatic disease and even the size of the initial metastases, however, showed no difference in survival after repeat hepatectomy. In addition, although some groups argue that there should be a limit to the number of metastases that warrant treatment, Fernandez-Trigo et al. demonstrated that the number of liver metastases at second hepatectomy did not impact overall survival [74]. Extrahepatic disease present at the time of repeat liver resection has generally incurred a decreased survival after re-resection. The location of the primary tumor, addition of adjuvant chemotherapy either after first or second hepatectomy, bilobar metastatic disease at first hepatectomy, and associated operative morbidity has shown no difference in survival after repeat resection. Delineating patients with the aforementioned prognostic factors would therefore assist in selecting patients appropriately as candidates for repeat interventions. These prognostic factors can also assist the physician in selecting patients for aggressive surveillance algorithms and consideration of perioperative systemic therapy.

5 Chemotherapy Strategy for Recurrent Disease

The vast majority of patients who undergo hepatectomy for colorectal metastases will recur with a disease-free survival of 15–35 % at 5 years [4, 16, 21]. In these individuals, recurrent disease is not necessarily isolated to the liver, but there is increased risk for extrahepatic recurrence as well. For this reason, consideration of neoadjuvant therapy prior to surgical intervention should be considered. By testing tumor biology prior to repeat surgical resection, patients may be stratified into groups. Those with favorable tumor biology would potentially receive maximal benefit from second-time hepatectomy versus those possessing unfavorable tumor biology. As discussed earlier, this strategy has proven beneficial in patients undergoing initial hepatectomy in the EORTC 40983 trial. Although these studies were not conducted in patients who had previously undergone a hepatectomy,

additional trials have examined the impact of systemic therapy on patients with previously treated metastatic colorectal cancer. Such studies can be used in determining appropriate treatment strategies for individuals who are candidates for repeat hepatectomy for recurrent disease. In the Eastern Cooperative Oncology Group Study E3200 (ECOG 3200), patients with recurrent metastatic colorectal cancer, previously treated with fluropyrimidine and irinotecan, were randomly assigned to one of three treatment groups: FOLFOX4 with Avastin, FOLFOX4 without Avastin, or Avastin alone. The corresponding median duration of survival were 12.9 months, 10.8 months, and 10.2 months ($p = 0.0011$). The corresponding median progression-free survival were 7.3 months, 4.7 months, and 2.7 months ($p < 0.0001$). Overall response rates were found to be 22.7, 8.6, and 3.3 %, respectively ($p < 0.0001$) [86]. An additional disease-free or survival advantage may therefore be seen in patients who undergo repeat hepatectomy with neoadjuvant FOLFOX ± biological agents, followed by surgery and adjuvant therapy.

Mutational analyses of primary or metastatic tumor samples may be needed in order to more accurately direct adjuvant or palliative treatment in patients with recurrent liver metastases. The use of cetuximab, a monoclonal antibody directed against the epidermal growth factor receptor (EGFR), in patients with stage IV colorectal cancer who had progressed on irinotecan-based therapy demonstrated benefit when combined with irinotecan compared to cetuximab use alone. Compared to treatment with cetuximab alone, there was an increase in response rates (22.9 vs. 10.8 %) and median time to disease progression (4.1 months vs. 1.5 months) in the cetuximab plus irinotecan arm [87]. There was no difference in response rates detected between the two groups when the percentage of EGFR-expressing cells or intensity of EGFR staining were evaluated. This may be secondary to the fact that overall EGFR expression is not as important as the amount of active, phosphorylated EGFR receptor, or that other pathways may be functioning. In fact, a cohort of patients who had previously failed irinotecan-based therapy for colorectal cancer and had EGFR negative tumors as designated by immunohistochemistry, likewise demonstrated a partial response rate of 25 % in the combined irinotecan/cetuximab group, similar to that seen by Cunningham et al. [88]. This demonstrates that EGFR status alone may not be predictive of a patient's responsiveness to cetuximab therapy for metastatic or progressive colorectal cancer. Additional markers may therefore be needed to ascertain tumor responsiveness.

Activation of EGFR is known to propagate many intracellular signaling cascades such as cellular proliferation, migration, and apoptotic pathways with perhaps the most important pathway being the Ras/Raf/MEK pathway [89]. The binding of ligand to EGFR activates k-ras resulting in the downstream activation of cellular processes. The k-ras gene encodes a protein that is involved in G-protein signaling and that is responsible for the GTPase activity needed to inactivate a G-protein complex. In situations where k-ras is mutated, the GTPase activity is lost, resulting in a constitutive activation of the G-protein. This constitutive activation allows persistent signaling for cellular proliferation, migration, and other oncogenic behavior. This persistent activity is likely the reason that cetuximab has not lived up to it full potential in clinical trials. Evaluation of individuals with either k-ras

Table 3 Negative prognostic factors after repeat liver resection for recurrent colorectal metastases in selected series

Author	*N*	CEA >30 ng/ml pre-repeat hepatectomy	>1 year interval between hepatectomies	Solitary recurrent metastases	Size recurrent metastases	Extrahepatic disease	R0 resection	Margi*n* >1 cm
Adam	64	(+)	(−)	(−) < 3 versus > 3	ND	(+)	(−)	
Sa Cunha	40		(-)	ND	ND	(+)	(−)	ND
Muratore	29	(+)	ND	(−)	ND	ND		
Petrowsky	126		ND	(−)	(+)		ND	ND
Yamamoto	75		ND	(−) < 3 versus > 3	ND	(+)	(−)	ND
Takahashi	22	ND	ND	ND	ND			
Suzuki	26		(−)	ND		ND		ND
Fernandez-Trigo	170		ND	ND		(+)	(−)	
Nishio	54	(+)	ND	ND	(+) >5 cm		(−)	

(+) Survival disadvantage; (−) Survival advantage; *ND* No difference in survival; *CEA* Carcinoembryonic antigen; Characteristics of extrahepatic disease, R0 resection, and Margin >1 cm refer to factors at the time of repeat hepatectomy

mutant primary or metastatic colorectal cancers and who failed irinotecan-based therapy and were subsequently started on cetuximab-based therapy, demonstrates that the presence of a mutant k-ras results in decreased responsiveness to cetuximab therapy, survival, and progression-free survival [90–94]. In addition, increased EGFR copy number has been associated with increased responsiveness to cetuximab therapy [93, 95]. Multiple escape pathways therefore exist in the pathogenesis of primary, metastatic, and recurrent colorectal carcinoma. Only with logical, combined, and targeted therapies can substantial improvements in current survival rates be achieved. Based on these data, if patients do not respond to first-line oxaliplatinum regimens and do not have k-ras mutations, they should be offered irinotecan regimens as second-line therapy.

6 Conclusion and Recommendations

Despite improvements in surgical technique, adjuvant chemotherapy, and post-surgical surveillance, recurrent colorectal liver metastases still account for a significant amount of morbidity and mortality and is the leading cause of death in patients treated for colorectal carcinoma. Since repeat resection has been shown to provide survival rates that mimic survival after initial hepatectomy with similar morbidity and mortality, it behooves the clinician to be able to expeditiously detect, workup, and treat recurrent metastatic disease so that each patient may be given the best hope to advance to surgical resection. For those individuals who have already undergone first time liver resection for metastatic disease, an additional level of suspicion must be present for any subtle changes in the patient's history, physical exam, or laboratory values since recurrence rates after initial hepatectomy are still 50–80 %. After initial hepatectomy, patients should be followed with serial liver function tests and CEA levels. Although CEA level has variable sensitivity and specificity regarding detection of hepatic metastases as discussed previously, it can be used as an adjunct in the postoperative period to measure response to surgical treatment or to prompt further evaluation when a rise in levels is observed. When a recurrence is suspected, a biphasic abdominal and pelvic CT scan and chest X-ray should be ordered. In addition, CT/PET should be performed for its higher specificity and sensitivity in detecting extrahepatic disease. In those who an isolated second hepatic recurrence is detected, we recommend an algorithm based on the EORTC 40983 trial. Since a substantial portion of patients recur not only in the liver but at extrahepatic sites as well, patients should undergo neoadjuvant FOLFOX ± bevacizumab therapy in order to ascertain responsiveness to chemotherapy and thus test tumor biology but also to detect further hepatic or extrahepatic disease in an interim period prior to resection. Should the patient demonstrate hepatic disease regression or stability after undergoing FOLFOX treatment, then that patient should undergo hepatic resection ± RFA, followed by additional adjuvant therapy. For those individuals who have progression of disease or remain unresectable, an alternate chemotherapy regimen should be instituted based on k-ras status. It is in those individuals with negative prognostic factors such as elevated

preoperative CEA level, presence of extrahepatic disease, and possibly the size of recurrent metastases, that nonresponsiveness to chemotherapy indicates a more aggressive pathology and one in which little survival benefit will be gained by offering any type of surgical resection.

Appendix

See Tables 1, 2, and 3.

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Minimally Invasive Surgery of the Liver

Michael White, Yuman Fong and Laleh Melstrom

Abstract

Operations on the liver have been undertaken for centuries for numerous indications including trauma, infections, and even for malignancy, but it was not until the past few decades that rates dramatically increased. This expanse in liver operations is due to a multitude of factors, including broader indications as well as improved safety. Our understanding of metastatic disease to the liver, especially colorectal cancer metastases, has vastly amplified the number of patients who would be candidates for hepatic resections and liver-directed therapies. We will focus our discussion here on planned minimally invasive operations for benign and malignant tumors as the majority of the literature relates to this setting.

Keywords

Minimally invasive liver · Robotic liver resection

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1 Introduction

The first documented planned anatomic liver resection has been credited to Lortat-Jacob in 1952. This was a right hepatic lobectomy for metastatic colon cancer [1]. However, major anatomic resections such as this did not become common for a prolonged period of time due to high operative morbidity and mortality rates initially [2]. Operations on the liver have been undertaken for centuries for numerous indications including trauma, infections, and even for malignancy, but it was not until the past few decades that rates dramatically increased. This expanse in liver operations is due to a multitude of factors, including broader indications as well as improved safety. Our understanding of metastatic disease to the liver, especially colorectal cancer metastases, has vastly amplified the number of patients who would be candidates for hepatic resections and liver-directed therapies. Improvements in liver imaging, knowledge of liver anatomy, anesthetic perioperative techniques to maintain a low central venous pressure, and technology have reduced the morbidity and mortality associated with liver operations. Operative mortality is now less than 2 % in many centers for operations that were once thought to have prohibitively high morbidity and mortality [2, 3]. The ability to perform liver operations safely with open techniques now leads to the next push for improved morbidity outcomes and thus the use of minimally invasive approaches.

We will focus our discussion here on planned minimally invasive operations for benign and malignant tumors as the majority of the literature relates to this setting.

2 Laparoscopic Assisted Hepatectomy

Laparoscopic surgery has now been fully embraced among surgeons for many abdominal operations after initial success in laparoscopic cholecystectomy which allowed for smaller incisions, lower wound infection rates, faster recovery with less pain, and shorter hospitalizations [4]. Numerous surgical studies assessing minimally invasive techniques have demonstrated reductions in postoperative pain, shorter hospital lengths of stay, and greater cost effectiveness without sacrificing oncologic outcomes [5–9]. Laparoscopic liver surgery has been slower to take hold. The liver has intricate vascular and biliary anatomy that if injured can result in significant bleeding and complex repairs. Due to concerns for major bleeding that would require rapid conversion to an open operation, the complex anatomy, and the fragile liver parenchyma, many surgeons are hesitant to utilize laparoscopic approaches for both major and minor liver resections [10]. Additionally, there was initial concern for oncologic compromise of margins as well as port site seeding [11]. In Table 1 we have listed the published studies of laparoscopic liver resections since 2010 that have included more than 50 patients.

Table 1 Published studies of laparoscopic liver resections for malignancy with more than 50 patients since 2010

Reference	Year	*n*
Jiang et al. [12]	2015	50
Meguro et al. [13]	2015	60
Cipriani et al. [14]	2015	209
Takahara et al. [15]	2015	436
Shehta et al. [16]	2015	232
Beppu et al. [17]	2015	171
Heuer et al. [18]	2015	90
Nomi et al. [19]	2015	173
Han et al. [20]	2015	88
Cauchy et al. [21]	2015	223
Ferretti et al. [22]	2015	142
Hasegawa et al. [23]	2015	100
Martin et al. [24]	2015	100
Nomi et al. [25]	2015	183
de'Angelis et al. [26]	2015	52
Hirokawa et al. [27]	2015	135
Shelat et al. [28]	2015	52
Tranchart et al. [29]	2014	140
Yamashita et al. [30]	2014	63
Goh et al. [31]	2014	147
Ahn et al. [32]	2014	51
DiFabio et al. [33]	2014	156
Kim et al. [34]	2014	70
Chan et al. [35]	2014	100
Montalti et al. [36]	2014	57
Ettorre et al. [37]	2014	105
Cai et al. [38]	2014	365
Long et al. [39]	2014	173
Tsung et al. [40]	2014	114
Cannon et al. [41]	2014	52
Troisi et al. [42]	2014	265
Choi et al. [43]	2013	57
Abu Hilal et al. [44]	2012	133
Topal et al. [45]	2012	81
Yoon et al. [46]	2012	89
Shafaee et al. [47]	2011	66
Lai et al. [48]	2011	56
Belli et al. [49]	2011	65
Nguyen et al. [50]	2011	108
Kazaryan et al. [51]	2011	107
Dagher et al. [52]	2010	163
Martin et al. [53]	2010	65
Yoon et al. [54]	2010	69

There has been a well-established learning curve for complex minimally invasive operations, but once appropriate proficiency in this skill set is obtained, conversion rates, operative times, blood loss, and incidence of complications can be minimized [55, 56]. A study from the Annals of Surgery in 2009 by Vigano and colleagues looked specifically at determining what this learning curve entailed for laparoscopic hepatic resection. They evaluated conversion rates, operative time, blood loss, and morbidity rates to determine that a learning curve for laparoscopic hepatectomies was approximately 60 cases. As skill sets improved, more complex procedures were performed [11]. For the 174 cases included in their analysis, 90.2 % were completed through a minimally invasive approach. The rates of major hepatectomy done via laparoscopy compared to open increased during the time period of their study from 1.1 % initially to 8.5 % by the end of their study in 2008, including 13.1 % of their right hepatectomies [11].

A world review of the literature on laparoscopic liver resections including more than 2800 patients published in 2009 was able to demonstrate that laparoscopic liver resections were able to be performed safely with conversion rates of less than 5 %, more than 50 % of which were for malignancy, with comparable 3- and 5-year oncologic outcomes to reported rates for equivalent open hepatic resections [8]. This demonstrates that in experienced hands with appropriately selected patients, a minimally invasive approach is reasonable in performing hepatic resections.

Numerous authors have looked at comparisons in outcomes between laparoscopic and open operations on the liver in recent years, however mostly in smaller, single-institution series [30, 57–59]. The Louisville Statement of 2008 from the World Consensus Conference on Laparoscopic Surgery developed recommendation guidelines for laparoscopic liver surgery. Indications included patients with solitary lesions that were smaller than 5 cm and within the peripheral segments. Major hepatectomies were to be performed only in specialized centers by those highly skilled in both hepatobiliary surgery as well as with advanced laparoscopic skills [60]. The left lateral section has been noted to be the most common of anatomic resections performed in many of these smaller series due to accessibility as well as easier sonographic evaluation of the left lobe and smaller parenchymal transection required. As experience has grown and more studies have been published, a second International Consensus Conference on Laparoscopic Liver Surgery was held in Morioka. This conference used the Zurich-Danish consensus conference model that utilized an expert panel as well as nine-member jury to grade questions using the Balliol Classification [61]. This provided a systematic review of the literature, expert opinion including videos and updated the consensus guidelines in this rapidly evolving field. Recommendations were made on the basis of meta-analysis, multiple case series, and retrospective reviews as no randomized controlled clinical trials are yet to be performed comparing minimally invasive approaches to open hepatic resections. The authors noted that the use of laparoscopic approaches for liver resections is rapidly increasing worldwide. For the purposes of these recommendations, resections were broken down into minor, two or fewer Couinaud segments, or major, three or more segments or resections involving the posterior superior segments. The recommendations were typically based upon low quality of

Table 2 Guideline recommendations from the second international consensus conference on laparoscopic liver surgery

1	Laparoscopic liver resection for minor resections is not inferior to open surgery for operative mortality
2	Laparoscopic outcomes for minor resections has superior outcomes for some areas of postoperative complications and is equivalent in other areas
3	Laparoscopic minor resections are not inferior in terms of margin status for oncologic resections
4	Laparoscopic minor resections have a superior length of stay for minor resections
5	Overall survival for laparoscopic minor resections are not inferior to open liver resections
6	Operative mortality for laparoscopic major resections are not inferior to open resections for 30 and 90 day mortality rates
7	Postoperative complications in major laparoscopic resections are not inferior to rates in open liver resections
8	Laparoscopic outcomes for margin negativity in major resections are not inferior to open resections
9	Length of stay data for laparoscopic major hepatic resections are superior to open liver resections
10	Overall survival data for major laparoscopic liver resections are not inferior

evidence due to the small volume of studies and the lack of randomized trials. Despite these limitations, the recommended guidelines are highlighted in Table 2.

Many other conclusions were also made during this consensus conference. The panel felt as though literature results for estimated blood loss and short-term recovery were unreliable. Additionally, measurements of cost and pain were variable and felt to be unreliable. Quality of life for both major and minor laparoscopic resections was felt to be not different from open approaches.

In regards to technical considerations, several additional recommendations were made. To begin laparoscopic liver operations, technical expertise in both hepatobiliary surgery as well as advanced laparoscopic skills are necessary. Surgeons should start with minor resections prior to attempting major resections. A hand assist or hybrid technique can help to minimize conversion rates. Use of a caudal approach facilitates dissection of the hilum and parenchymal division in major and anterior resections. The lateral approach provides better access to the posterior segments. Use of low central venous pressure in combination with an insufflation pressure of 10–14 mm Hg with CO_2 helps minimize bleeding during laparoscopic liver resections. Numerous energy devices are available for dividing hepatic parenchyma, but these do not substitute for adequate knowledge of liver anatomy, precise dissection, and sealing of vascular structures under direct visualization. Either individual hilar dissection or Glissonian approaches for hilar control are options in laparoscopic surgery [61].

3 Minimally Invasive Robotic Assisted Hepatectomy

One topic from this conference with insufficient data for recommendations was the use of robotics in both minor and major hepatectomies. Robotic surgery is a progressively developing approach to minimally invasive liver resections. Robotics has developed to address many of the limitations of laparoscopic instruments. Robotics allows for superior ergonomics with increased dexterity and fine movements mimicking the human hand. This allows for more precise suturing in times of hemorrhage and handling of delicate structures in small, tight spaces [3]. Natural human tremor is removed with robotics to allow for fine surgical movements and precise suture placement [10]. Optics and visualization are improved with three-dimensional viewing at the console compared to two-dimensional screens routinely utilized for laparoscopy. The primary surgeon also has control of three working arms as well as the camera at the console and thus has the ability to overcome limitations in retraction and exposure from inexperienced assistants or fatigued camera holders. The segments generally considered off limits for laparoscopy, segments 7, 8 and 1, are more ideally suited for resection robotically due to the improved ergonomics and reach with robotic instrumentation that mimic accessibility of an open operation [3].

Robotic surgery is not without its disadvantages. Surgeons must have significant experience to develop "visual haptic feedback" to understand how much force they are using in retraction and grasping of human tissues to prevent iatrogenic injuries. The mechanics of current robotics do not provide tactile sensory feedback of tension applied to tissues or sutures. The robot also must be docked once ports are placed. In order to adjust patient positioning such as reverse Trendelenburg or lateral rotation, the robot has to be undocked and then redocked which adds significant amounts of time to the operation. Most studies have also shown that robotic surgical operations generally take longer than open or laparoscopic equivalent operations due to time for docking, instrument exchanges, and patient setup [40]. These differences are likely to decrease with increased user experience. Costs including purchase and maintenance fees, limitations in operative field, and the requirement of a skilled bedside assistant are also reported as disadvantages [10].

The only current robotic platform available in the United States with approval for use by the United State Food and Drug Administration is the da Vinci Surgical System (Intuitive Surgical, Inc., Sunnyvale CA). There are three different devices available including the *S*, *Si*, and the recently released *Xi*.

Ocuin et al. published an elegant review of the existing robotic hepatectomy literature encompassing studies that included more than nine patients and was published in 2015 in the Journal of Surgical Oncology [10]. The authors reviewed 14 major series that reported on perioperative outcomes. Of the 439 patients included in these studies, 72 % were performed for malignancies with the two most predominant indications being hepatocellular carcinoma and colorectal liver metastases. The overall conversion rate was 7 % and included indications of bleeding, adhesions, concern over margin status, and technical complications. The

complication rate overall was 21 % with a range of 0–43 % with a 0 % perioperative mortality rate. Postoperative hospital length of stay ranged from 4 to 12 days and was similar to laparoscopic hepatectomies but 3 days shorter than for open operations in one study [62]. Limited data was reported for margin status and survival, but the limited long term follow-up data suggested equivalency to laparoscopic outcomes for recurrence rates [8, 10]. No port site recurrences were reported in these series. Cost analysis is dependent on numerous factors and difficult to truly define, thus definitive statements regarding cost are not truly feasible, but the studies reviewed by the authors that included cost information suggest that robotic approaches did have increased cost compared to open and laparoscopic approaches [10].

Eight series were reviewed by Ocuin et al. in their paper that statistically compared robotic and laparoscopic liver resections. Collectively, these papers suggested that robotic approaches had equivalent safety and feasibility for both major and minor hepatic resections with similar oncologic outcomes when compared to laparoscopic data.

There are numerous technical challenges that exist with minimally invasive laparoscopic approaches. Rigid instrumentation restricts access to certain areas, especially the posterior sector and caudate lobe (Couinaud segments 7, 8, and 1). This is one reason that these resections have been considered major resections even if two or fewer Couinaud segments are removed. A few authors have looked to address the feasibility and safety of these resections via either laparoscopic or robotic approaches and have found these approaches to be feasible, safe, and noninferior in terms of postoperative and oncologic outcomes [63–65]. Montalti and colleagues compared robotic and laparoscopic approaches directly for resections of the posterosuperior liver segments via a propensity score-matched comparison in 2015. Resections of Couinaud segments 7, 8, 4a, and 1 were included. A total of 36 robotic cases were matched for comparison with 72 laparoscopic cases. No significant difference was noted in their study in estimated blood loss, length of stay, R0 margin status, and mortality. Robotic cases did involve a longer Pringle time compared to laparoscopic cases but morbidity outcomes were not statistically different. A trend toward increased complication rates was seen in robotic cases [66]. To summarize, the authors felt that both minimally invasive approaches yielded similar safety and feasibility for these complicated hepatic resections.

The principles associated with liver resections are similar when performed open or through a MIS technique. Specific differences for each type of resection are beyond the scope of this chapter, but technical approaches have been described in detail in numerous publications [63, 65–68]. In general, for patients to be a candidate for a minimally invasive hepatectomy, they must have similar characteristics to those undergoing any major laparoscopic operation. The patient needs to have adequate cardiovascular and pulmonary function to tolerate prolonged periods of intra-abdominal insufflation. The tumors must be in an area that is accessible to either a laparoscopic or robotic dissection to obtain control of inflow and outflow vessels as well as division of the parenchyma in the case of anatomic segment or

lobe resections. Significant previous abdominal operations may be prohibitive if extensive adhesiolysis would be necessary. A benefit to MIS liver operations however is that, if a potential reoperative condition presents, adhesions can be expected to be less than with open hepatic resections. We conclude that in appropriately selected patients, both laparoscopy and robotic approaches can provide equivalent if not superior outcomes for both minor and major hepatic resections. The literature and patient sample size for these fields is rapidly expanding and will continue to produce evolving recommendations and guidelines as well as technical considerations.

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Locoregional Therapies for Primary and Secondary Hepatic Malignancies

Ahsun Riaz, Robert J. Lewandowski and Riad Salem

Abstract

Management of hepatic malignancies is a multidisciplinary task with the involvement of hepatologists, medical/surgical oncologists, transplant surgeons, and interventional radiologists. The patients should be selected for a specific targeted therapy after multidisciplinary consensus. Interventional oncology has established its role in the management of hepatic malignancies. Image-guided locoregional therapies decrease the rate of systemic toxicity without compromising tumoricidal effect.

Keywords

Locoregional therapies · Primary hepatic malignancies · Secondary hepatic malignancies

1 Introduction

1.1 Hepatic Malignancies

1.1.1 Primary Hepatic Malignancies

Primary hepatic malignancies include hepatocellular carcinoma (HCC) and intrahepatic cholangiocarcinoma. The incidence of HCC has increased in the past few decades [1].

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Hepatocellular Carcinoma (HCC)

The details of diagnosis and staging of HCC are beyond the scope of this chapter. Laboratory tests to assess liver function status and radiologic studies (CT or MRI with contrast) to diagnose/stage HCC are of optimal importance. Serum tumor markers for HCC, i.e., alpha-fetoprotein (AFP) and protein induced by vitamin K absence (PIVKA-II) have a role in diagnosing HCC.

Surgical resection is curative but possible only if liver function is well compensated. Patients with unresectable HCC within the Milan criteria (UNOS stage T2), i.e., single lesion ≤ 5 cm or up to 3 lesions ≤ 3 cm, are eligible for orthotopic liver transplantation (OLT) [2]. Due to a variety of reasons, the foremost of which is limited donor organs, the role of OLT is limited. Bridging (maintaining patient's UNOS stage T2 and hence OLT candidacy) by locoregional therapies is being established.

Patients with unresectable HCC who are beyond the Milan criteria are candidates for targeted therapies. Transarterial therapies can downstage HCC to within transplant criteria [3], which allows patients who were initially outside Milan criteria to be eligible for OLT.

Systemic therapy with sorafenib has been shown to have statistically significant improvement in survival of patients with advanced HCC [4]. Patients who have advanced disease may also benefit from some targeted therapies. The presence of PVT excludes these patients from the transplant criteria. Radioembolization can be administered in patients with malignant PVT [5]. The presence of distant metastases, i.e., lung and adrenals, is a contraindication to these treatments, as there has not been a survival benefit seen in this group of patients.

Intrahepatic Cholangiocarcinoma (ICC)

Interventional oncology has been shown to be effective in the treatment of HCC but its role in the management of ICC has not been extensively studied.

1.1.2 Secondary Hepatic Malignancies

Secondary hepatic malignancies occur frequently and surgical resection is the only curative option available. Systemic therapeutic agents are considered first and second line therapies. External beam radiation therapy also has a role in a select group of patients.

Metastatic Colorectal Carcinoma (mCRC)

Less than ten percent patients have hepatic mCRC that are resectable [6]. Systemic chemotherapy for this disease is considered first and second line therapy. Locoregional therapies have established their role in the treatment of hepatic mCRC [7].

Metastatic Neuroendocrine Tumors (mNET)

Neuroendocrine tumors commonly metastasize to the liver. Hormone production potentially makes these malignancies symptomatic. Carcinoid, VIPomas, gastrinomas, and somatostatinomas are some examples. Systemic chemotherapy and

ablative procedures have been shown to have a modest benefit in these patients. Patients with unresectable disease are candidates for transarterial therapies.

Other Secondary Malignancies

The presence of hepatic metastases from primary malignancies other than the ones listed above will be discussed under the heading of other secondary malignancies. Many other secondary malignancies have been treated using locoregional therapies but only metastatic breast cancer has been studied in detail.

1.2 Interventional Oncology

Interventional oncology has established its role in the management of hepatic malignancies. Image-guided locoregional therapies decrease the rate of systemic toxicity without compromising tumoricidal effect. Table 1 summarizes the various image-guided locoregional therapies available and their uses.

1.3 Hepatic Anatomy

Hepatic malignancies are the predominant target of interventional oncologists, partly due to favorable anatomy making image-guided approaches to tumors possible. The liver is a relatively superficial organ and thus percutaneous techniques are possible in which image-guided targeting permits direct access to the lesion.

The liver is supplied by the hepatic artery and the portal vein. Hepatic malignancies are predominantly supplied by the hepatic artery. The normal hepatic parenchyma is supplied, in contrast, by the portal vein.

Table 1 Summary of locoregional therapies

Treatment type	Tumoricidal effect	Treatment modality
Percutaneous ablative therapies	Thermal	Radiofrequency ablation (RFA)
		Microwave ablation
		Laser ablation
		Ultrasound ablation
		Cryoablation
	Chemical	Ethanol ablation
		Acetic acid ablation
	Other	Irreversible electroporation (IRE)
Transarterial therapies	Ischemic	Transarterial bland embolization
	Ischemic/chemoembolic	Transarterial chemoembolization
	Minimally ischemic/chemoembolic	Transarterial bland embolization with drug eluting beads
	Minimally ischemic/radioembolic	Radioembolization

An overview of the arterial supply of the liver is important to understand the use of transcatheter therapies for the treatment of liver malignancies. Conventionally, the right and left hepatic arteries are branches of the proper hepatic artery, which is a branch of the common hepatic artery arising from the celiac trunk. There are many variations to this conventional hepatic arterial anatomy which are beyond the scope of this chapter. Hepatic malignancies are hypervascular structures which get their blood supply predominantly by parasitizing arterial flow from the surrounding tissue. They are not limited to the surrounding hepatic parenchyma and may get arterial supply from adjacent organs.

1.4 Multidisciplinary Effort

Management of hepatic malignancies is a multidisciplinary task with the involvement of hepatologists, medical/surgical oncologists, transplant surgeons, and interventional radiologists. The patients should be selected for a specific targeted therapy after multidisciplinary consensus.

1.4.1 Monitoring Response to Treatment

Image-guided locoregional therapies use imaging modalities for pre-procedure planning, the procedure itself, and post-procedure evaluation. The response to treatment is monitored clinically and radiologically. Regular laboratory follow-up includes liver function tests and tumor markers such as AFP for HCC [8].

The first radiologic study is done one month after treatment to assess response or lack thereof. The patients are then followed with scans every three months to assess response to treatment or progression of disease. The use of the WHO size criteria and the EASL necrosis criteria are used to assess response in the target lesions [9]. Conventional anatomic imaging studies may not be able to assess tumor response until six weeks have elapsed after treatment. Functional MRI and/or PET CT may have a role in earlier detection of tumor response [10].

2 Catheter-Based Transarterial Therapies

2.1 Bland Embolization

2.1.1 Introduction

This is a therapy which occludes the blood vessels and induces tumoral ischemia by transcatheter injection of bland embolic material into the tumor feeding vessel. This was first used in the 1950s and is the basic concept behind transcatheter therapies [11].

2.1.2 Procedure

After reviewing pre-procedure imaging, the vascular anatomy of the region and the tumor is meticulously studied using conventional angiography. After localizing vascular supply of the tumor, the bland embolic material is injected into the tumor

using the feeding vessel(s). Some embolic agents used are polyvinyl alcohol particles, Embospheres; BioSphere Medical Inc, Rockland, MA: or Gelfoam; Pharmacia and Upjohn Company, Kalamazoo, MI). Bland embolization has been shown to induce tumor ischemia and cell death but may also lead to neoangiogenesis due to an increase in angiogenic factors [12].

There is a trend toward an increased survival using transarterial chemoembolization when compared to bland embolization but no study has been able to demonstrate a difference in survival between the two treatments [13, 14].

2.1.3 Uses of Bland Embolization

A randomized controlled trial comparing bland embolization with symptomatic treatment failed to show a survival benefit of using bland embolization in patients with HCC belonging to Child Turcotte Pugh Class A [15]. As mentioned above, the use of TACE has not shown to have a difference in survival when compared to bland embolization. Maluccio et al. concluded that bland embolization was effective in treating patients with unresectable HCC, and that bland particles may be the critical component of intra-arterial embolotherapy [16]. Bland embolization has also been used for pain relief and control of symptoms in hepatic metastases from neuroendocrine tumors and sarcomas [17].

2.1.4 Complications

Post-embolization Syndrome

Patients may experience a mild post-embolization syndrome which consists of the following clinical signs and symptoms; fatigue, nausea, vomiting, anorexia, fever, abdominal discomfort, and cachexia. There might be right upper quadrant pain. This is usually mild and resolves without clinical intervention. In selected cases it may require inpatient care.

Groin Injury

Injury to the groin when gaining access or when closing the access to the vessel is a potential complication of all transcatheter techniques. This may lead to the formation of a hematoma or pseudoaneurysm. The femoral nerve may also be injured during these therapies [18].

Abscess

Given the arterial occlusive nature of this technique, abscesses may form. This complication risk is higher in patients who have had ampulla violating procedures.

2.2 Transarterial Chemoembolization (TACE)

2.2.1 Introduction

TACE allows the delivery of a high dose of chemotherapeutic agents to the tumor with minimal systemic toxicity. It was first used in 1980. This is usually performed as an inpatient procedure.

2.2.2 Procedure

After meticulous evaluation of the baseline characteristics and stage of the patient using clinical and radiologic data, conventional angiography is performed to study the vascular anatomy of the region and the tumor. The chemotherapeutic drug is mixed with lipiodol (radio-opaque poppy seed oil) and injected into the tumor feeding vessel during angiography. Lipiodol (with the chemotherapeutic agent) is concentrated in the tumor and is retained for weeks while the normal hepatocytes are able to remove it in seven days. There is stasis associated with lipiodol and hence there is an embolic effect associated with this conjugate. Lipiodol is visualized on post treatment CT scans in the target lesion. Lipiodol/chemotherapeutic mixture injection is followed by the injection of bland embolic particles to prevent washout of the drug and to induce ischemic necrosis [19].

2.2.3 Uses of TACE

Relative contraindications include serum bilirubin (>2 mg/dL), lactate dehydrogenase (>425 U/L), aspartate aminotransferase (>100 U/L), tumor burden exceeding >50 % of the liver, ascites, bleeding varices, and thrombocytopenia or cardiac or renal insufficiency. Doxorubicin alone as the chemotherapeutic agent is commonly used worldwide. The best patients for TACE are those with good performance status, preserved liver function, and no evidence of vascular invasion or extrahepatic metastasis. Llovet et al. studied the survival outcomes in patients treated with fixed interval (intention to treat) chemoembolization, embolization, and conservative measures [20]. The authors of this study concluded that TACE and embolization significantly improved survival in select patients with unresectable HCC. Takayasu et al. published data from a large cohort study of 8510 HCC patients treated with TACE and showed that prognosticators of survival included degree of liver damage, AFP value, maximum tumor size, number of lesions, and portal vein invasion [21].

There are limited data on patients treated with TACE for metastatic disease. Geschwind et al. demonstrated that TACE can prolong survival of patients with colorectal metastases even in patients who had not responded to systemic chemotherapy [22]. Liapi et al. analyzed imaging responses and determined outcomes of 26 patients with neuroendocrine metastases treated with TACE and showed a mean patient survival was 78 months [23]. The imaging response to treatment using WHO (bidimensional) and RECIST (unidimensional) criteria was seen in only 1/3 of the cohort. Patients with breast metastasis to the liver unresponsive to standard of care chemotherapy have been treated with TACE and the survival benefit seen is not impressive as most patients develop extrahepatic metastases [24, 25]. Burger et al. have reported on their experience of treating 17 patients with unresectable cholangiocarcinoma treated with TACE and concluded that TACE was effective in prolonging survival in these patients [26].

2.2.4 Combination Therapies

TACE/RFA

TACE has been used prior to RFA to decrease the tumor size and vascularity making it more susceptible to the effects of RFA [27]. TACE may be given before RFA (TACE-RFA) in tumors 3–5 cm, to decrease the size and perfusion of the tumor leading to potentially increased sensitivity to RFA. However, the largest analysis on TACE-RFA presented controversial data [28].

TACE/Surgery

TACE has the potential of bridging patients to OLT allowing longer waiting times. It also has shown ability to downstage patients to the Milan criteria thus allowing them to undergo OLT [29].

TACE/Ethanol Ablation

A randomized control trial comparing TACE alone with the combination of TACE and ethanol ablation showed significantly higher response rates and less recurrence following the combination [30].

2.2.5 Complications

Post-embolization Syndrome

Post-embolization syndrome is seen after TACE. Patients are usually admitted following TACE to manage post-embolization syndrome. It is usually not severe enough to require further hospitalization.

Hepatobiliary Complications

Hepatic failure is a potential complication following TACE and proper patient selection (preserved liver function) is important for this procedure. Bilomas may form after TACE. Bile duct injury (ischemic) may occur following this treatment and may lead to severe complications [18]. Up to 5.3 % patients may develop biliary complications following TACE [31]. The occurrence of liver abscesses is rare but is possible after TACE especially in patients who have undergone ampulla violating biliary procedures [18]. Abscesses can be prophyllactically managed by antibiotics and may require drainage if they occur. Drugs such as moxifloxacin may be considered in these patients given biliary excretion.

Gastrointestinal Complications

There is a risk of inadvertent spread of the chemotherapeutic drug and the bland embolic material to the gastrointestinal tract. This may cause duodenal or gastric ulcers and may even lead to perforation in severe cases [18].

Vascular Injury

TACE may cause injury to the hepatic vasculature leading to spasm. The intra-arterial approach increases the risk of vascular injury in all transcatheter

techniques [18]. Chemotherapeutic agents may also have a role in causing this complication [32].

Other Complications

There have been incidences of tumor rupture (0.15 %) following TACE [18]. The time period between treatment and rupture was variable (0–45 days). Pulmonary artery embolism may occur and the patient may present with cough and dyspnea [18].

2.3 TACE with Drug-Eluting Beads (DEB TACE)

2.3.1 Introduction

The concept of DEB TACE is to load microspheres with chemotherapeutic agents and deliver them intra-arterially. This allows the delivery of the agent in a controlled and sustained manner.

2.3.2 Procedure

The procedure is similar to TACE and is delivered after conventional angiographic evaluation of the vascular anatomy. Studies have shown significant reductions in the peak plasma concentrations after TACE with DEB when compared to conventional TACE [33].

2.3.3 Uses of DEB TACE

The doxorubicin loaded DEBs have a promising role in the treatment of inoperable HCC [34–36]. Patient selection is similar to that for conventional TACE. Poon et al. reported a 63 % response using the modified RECIST criteria which takes tumor necrosis into account [37]. A recent randomized controlled trial on 200 patients comparing conventional TACE with DEBs failed to show a significant survival benefit (PRECISION V). There were fewer adverse events seen after TACE with DEBs when compared to conventional TACE. DEBs loaded with irinotecan are being investigated for the treatment of patients with colorectal hepatic metastases [38].

2.3.4 Complications

The toxicity profile is similar to that seen after TACE and preliminary data according to the PRECISION V trial may indicate that this is a safer therapy when compared to conventional TACE.

2.4 Radioembolization

2.4.1 Introduction

Radioembolization uses various vehicles carrying radionuclides for delivery of a concentrated radiation dose to the target lesion. There is a very high incidence of

radiation-induced liver disease after external radiation and radioembolization minimizes the incidence of this complication. Radioembolization is an outpatient procedure.

2.4.2 Procedure

Pre-procedure Angiography/Tc-99m MAA Scan

The inadvertent spread of radioactive microspheres is prevented by meticulous study of the vascular anatomy of the liver and collateral non-target flow [39]. Coil embolization of non-target vessels may be necessitated to decrease the unintended deposition of microspheres. Technetium-99 m labeled macroaggregated albumin (^{99m}Tc-MAA) is used to assess splanchnic shunting and pulmonary shunting. This pretreatment nuclear scan is important to prevent the complications associated with the treatment and to calculate the lung shunt fraction (LSF). All the hepatic vessels are assessed during the angiogram and the arteries feeding the tumor are studied in detail.

Radioembolization

Radioembolization is performed approximately 1 week following the pre-procedure angiography. This allows time for dose calculation and ordering a tailored dose for the patient. There are experienced centers which are performing the pre-procedure angiography/^{99m}Tc-MAA scan and radioembolization on the same day in selected cases [40].

2.4.3 Available Devices

Yttrium-90 Microspheres (Glass/Resin)

^{90}Y is a pure beta emitter with a half life of 64.2 h. It decays into the stable element Zirconium-90. The range of tissue penetration of the emissions is 2.5–11 mm. It is the most commonly used radionuclide. These spheres have minimal embolic effect and thus have deeper tissue penetration. Its use in presence of malignant PVT is possible because these devices do not compromise the blood supply of the normal hepatic parenchyma by occlusion of the portal venous flow. The technical details of the procedure and dosimetry are beyond the scope of this chapter [33]. The procedure is performed on an outpatient basis and the patient is discharged on the same day [41]. There are two forms available.

TheraSphere®

TheraSphere® (BTG, London, UK) consists of non-biodegradable glass microspheres that have a diameter between 20 and 30 microns. It was approved by the FDA in 1999 as a bridge to transplantation and recently has been approved for use in HCC patients with PVT. Each microsphere has an activity of 2500 Becquerel at the time of calibration. The activity of the vial varies inversely with the time elapsed after calibration.

SIR-Spheres

SIR-Spheres consist of resin microspheres that are biodegradable. The spheres have slightly increased diameter and lower specific gravity per microsphere than TheraSphere®. The use of SIR-Spheres was approved by the FDA for metastatic colorectal cancer to the liver in 2002.

Iodine-131 Labeled Iodized Oil (I-131 Lipiodol)

I-131 is a beta and gamma emitter. The iodine moiety of lipiodol can be substituted for the radionuclide, ^{131}I. It has a half life of 8 days. Extreme caution has to be taken during administration as the dissolution of the plastic catheters has been noted. The thyroid gland, due to its use of Iodine to make the thyroid hormones, is susceptible to the toxicity of ^{131}I. The thyroid gland is blocked before and after the treatment to minimize toxicity to the gland. The radionuclide is excreted in urine and thus the patient has to be hospitalized for six days after treatment as a radioactivity safety measure. Patients are told to refrain from possible conception until six months after treatment. The use of ^{131}I-Lipiodol has been studied in a prospective trial and compared in efficacy to chemoembolization and has been shown to have similar outcomes with significantly decreased adverse events [42].

Rhenium-188 HDD Labeled Iodized Oil

Transarterial radionuclide therapy (TART) with Rhenium-188 (^{188}Re) 4-hexadecyl-1, 2, 9, 9-tetramethyl-4, 7-diaza-1, 10-decanethiol (HDD)-labeled iodized oil has been recently studied for use in inoperable HCC. ^{188}Re has a shorter half life (16.9 h), high beta energy (maximum: 2.1 meV) and low gamma energy (155 keV) emissions, and is available through a Tungsten-188 (^{188}W-^{188}Re) generator [43]. The iodized oil, i.e., lipiodol is discussed above. The quantity of ^{188}Re HDD iodized oil (lipiodol) administered is based on the radiation absorbed dose (RAD) to critical organs, which is calculated after administration of test dose of the radioconjugate, transarterially [43]. The incidence of serious adverse events is low. There is a survival benefit for HCC reported in published literature [43].

Phosphorus-32 Glass Microspheres

Phosphorus-32 (^{32}P) is a radioisotope which emits high-energy beta particles during decay. It has a half life of 14.28 days and a maximum tissue penetration of 8 mm with an average of 3.2 mm [44]. It is administered as an integral constituent of non-biodegradable glass microspheres (GMS). The half life of this radionuclide gives it an advantage of a lower dose requirement and a potential of having a decreased radiation to handlers as compared to the other radionuclides available. It has been shown to be tolerated well in animal and human trials. Its role in the management of liver tumors is yet to be established.

Milican/Holmium-166 Microspheres (HoMS)

The use of Holmium/chitosan complex (Milican, Dong Wha Pharmaceutical Co., Seoul, Korea) has been shown to be effective in treating small HCC in a novel study from Korea [45]. Chitosan is a unique substance derived from chitin. It has the

ability to liquefy in an acidic environment and form a gel in basic environments. The gel has embolic effects. The use of Holmium-165 Poly (L-lactic acid) microspheres is being studied in animal models and has been shown to be safe. Its use in humans has not been studied as of yet.

2.4.4 Uses of Radioembolization

HCC

Radiation Lobectomy/Segmentectomy: Radioembolization can be administered via a lobar or segmental artery resulting in radiation lobectomy or segmentectomy, respectively [46, 47].

Bridging: Radioembolization has been shown to limit progression of the HCC. This allows the patient more time to wait for donor organs [48] and thus increases their chance of undergoing OLT. Thus, it has a role of bridging the patient to OLT.

Downstaging: Patients beyond transplant criteria have been shown to be downstaged to be eligible for transplant after radioembolization. There is an increase in overall survival in these patients as well [48].

Malignant PVT: Patients with PVT have been shown to have a good response to treatment after radioembolization. A survival benefit has been shown with the use of radioembolization in patients with malignant vascular involvement [5].

ICC

A pilot study analyzing the use of ^{90}Y in 24 patients with biopsy proven ICC has shown a favorable response to treatment and favorable survival outcomes [49]. The patients with a better performance status according to the Eastern Cooperation Oncology Group (ECOG) had a significantly better survival in this study.

mCRC

The patients who have unresectable disease and are on systemic chemotherapy or have failed to respond to first line or second line chemotherapeutic agents are considered as candidates for radioembolization. The combination of radioembolization with systemic chemotherapy has been shown to have a significantly better tumor response, a longer time to progression, survival benefit, and an acceptable safety profile. Radioembolization of fluorouracil-refractory patients with hepatic mCRC with concomitant systemic irinotecan chemotherapy has also demonstrated safety and efficacy [50, 51]. Radioembolization alone has also been published in many series and has shown promising results [52]. Dose escalation studies have shown a better response with increasing doses [53].

mNET

Radioembolization of metastatic disease to the liver from a neuroendocrine neoplasia has been shown to be effective and safe. A prolonged response to treatment, i.e., greater than 2 years has also been seen [54, 55]. Patients also have decreased symptoms and decreased requirement of sandostatin following radioembolization.

Other Secondary Malignancies

Breast cancer is the most common cancer in women. It has a tendency to metastasize to the liver. Radioembolization is an efficacious treatment for unresectable breast cancer metastasis to the liver [56]. There is a significant radiologic response after radioembolization but the survival benefit of this treatment in these patients has not been established. Radioembolization has been used to treat secondary liver tumors from various primary sources. This mode of treatment gives an effective alternative to patients who have failed chemotherapy or have become chemorefractory [57]. The data suggests a similar benefit in survival and tumor response in metastatic liver tumors from the mixed neoplasia.

2.4.5 Complications

Post-embolization Syndrome

The post-embolization syndrome occurs less commonly after radioembolization due to the small size of the particles and these seldom require hospitalization [58–60]. Some serious adverse events related to radioembolization are explained below.

Hepatobiliary Toxicity

Radiation-induced liver disease usually occurs between four and eight weeks after radioembolization. The biochemical toxicity rates following radioembolization have been between 15 and 20 % [61, 62]. The clinical appearance of ascites and jaundice may be seen. The histologic hallmark of veno-occlusive disease may be seen in severe cases. The hepatic toxicity may be severe and lead to significant morbidity and mortality [61]. The presence of various factors such as a deranged hepatic function at baseline, age and activity delivered may predispose patients to the hepatotoxic effects of radioembolization.

Biliary Complications

The biliary tract is also susceptible to toxicity by radioembolization. According to Atassi et al., less than 2 % of patients required intervention for the biliary toxicity induced by radioembolization [10]. These included drainage of three bilomas, one abscess and two cholecystectomies. Radiation-induced cholangitis has been reported following ^{90}Y as well [10].

Fibrosis

Radioembolization has been shown to cause fibrosis that may lead to portal hypertension by changing volume of the treated lobe. The time for development of portal hypertension is variable [63]. It is more often associated with bilobar treatment and its incidence is increased in patients who have chemotherapy associated steatohepatitis (CASH). The presence of preexisting cirrhosis leading to portal hypertension in most HCC patients makes them more susceptible to the aggravation of this complication as well.

Radiation Pneumonitis

Caution has to be taken when the LSF is greater than 13 % [64]. A restrictive pulmonary dysfunction is seen after radioembolization in a few cases with a predisposing high LSF if treated with resin microspheres. The LSF is used to calculate the dose that would be administered to the lung. An absolute contraindication to radioembolization is the predicted administration of a dose greater than or equal to 30 Gray to the lungs in a single treatment or greater than 50 Gray as a cumulative dose after multiple treatments [65]. Recently, sorafenib has been shown to decrease the LSF.

Gastrointestinal Complications

Gastrointestinal complications after radioembolization have been reported. The inadvertent spread of microspheres to the gastrointestinal tract is responsible for complications such as ulceration [66, 67]. This complication is severe and may require surgery for treatment. It can be prevented by meticulous mapping of the blood vessels to look for aberrant vasculature arising from branches of the hepatic artery which supply the gastrointestinal tract. The prophylactic use of proton pump inhibitors is recommended.

Vascular Injury

Transcatheter ^{90}Y radioembolization is an invasive procedure. The incidence of vascular injury is very low and mostly has been seen in patients with prior systemic chemotherapy [68].

3 Thermal Ablative Therapies

3.1 Radiofrequency Ablation

3.1.1 Introduction

Radiofrequency (RF) refers to alternating current (AC) signals with frequencies between 3 Hertz (Hz) and 300 Giga-Hertz (GHz). RF ablation (RFA) uses energy of 450–500 kilo-Hertz (kHz) for hyperthermic ablation of liver tumors. This is the most commonly used and the most studied hyperthermic percutaneous ablative therapy.

3.1.2 Procedure

The applicator used in RFA is called an electrode. The electrode creates an AC electric field in the tumor. The resistance of the tissue to ionic flow leads to frictional heat around the electrode. The ground pads (dispersive electrodes) are usually placed on the thighs. They have a large surface area when compared to the electrode(s) placed in the tumor. This allows the generated heat to be focused around the needle electrode(s) [69]. RFA is performed under ultrasound/CT guidance.

The perfusion mediated tissue cooling ("heat sink" effect) is a concept observed in all hyperthermic ablation techniques. It may be minimized by embolizing the tumor feeding vessel beforehand. Additionally, tumors in close proximity to large vascular structures are considered poor candidates. The soft tumor has a dense rim and this pseudocapsule serves to trap the heat within the tumor. This has been termed as the "oven effect" [70].

The approach to the tumor may be difficult percutaneously in some cases and may need laparoscopy. Small vessels surrounding the tumor are cauterized by the heat generated in all hyperthermic ablation techniques, which in turn has the advantage of minimizing the dissemination of toxic substances released after tissue death following ablation.

The needle electrodes are of various types. The multi-tined expandable electrode is the first type and this term refers to the electrodes that have multiple electrode tines that expand from a centrally positioned larger needle [71]. The second type, i.e., internally cooled electrodes, have a perfusate flowing in internal channels where the fluid does not come in contact with the tissue. Cluster electrodes are a type of internally cooled electrode devices in which three or more closely spaced electrodes are used simultaneously. The perfusion electrodes are the third type and they actually allow fluid to be injected into the tissues before treatment. Electrodes which do not require grounding pads are also under investigation.

3.1.3 Uses of RFA

RFA is used for patients with HCC that is ≤ 3 cm. Tumor size and presence of large abutting vessels (>3 mm) significantly affect the treatment efficacy [69]. The presence of multifocal disease also limits the use of this treatment. The location of the tumor close to major blood vessels or ducts may lead to complications by damaging these structures. The selection of tumors for RFA with metastatic disease from the colon is very similar to that of HCC [69]. This treatment modality is also safe and efficacious in the treatment of neuroendocrine metastases to the liver [72].

3.1.4 Complications

The following complications are seen after all hyperthermic ablation techniques:

Hemorrhage

The occurrence of postprocedural hemorrhage is possible after all ablation techniques but is rarely clinically significant. There has been a study showing a statistically significant increased incidence of bleeding after RFA using cluster RF electrodes when compared to that using single cryoprobes or single RF electrodes [73].

Tumor Lysis Syndrome

Tumor lysis syndrome is a complication that has been associated with RFA and cryoablation. It is a systemic process resulting from the systemic absorption of necrotic debris. It can lead to severe coagulopathy and multi-organ failure.

Liver Abscess

Abscesses may form after tumor ablation. A history of biliary interventions where there is violation of the ampulla of Vater (e.g., sphincterotomy) increases the risk of abscess formation [74]. Bacteremia due to any cause is another risk factor [74]. Preprocedural antibiotics can decrease the incidence of abscess formation in these patients.

Tumor Seeding

The rate of tumor ablation has been around 0.5 % in a large multicenter trial [75]. This is more than that associated with biopsy and may be associated with the larger bore needles used in these thermal ablation techniques and multiple punctures that may sometimes be performed in these procedures.

Post-ablation Syndrome

Post-ablation syndrome is a systemic constellation of symptoms including low-grade fever and general malaise. The symptoms correlate with the volume of tissue ablated [76].

Biliary Complications

Lesions located near the porta hepatis should be approached with caution as damage to central biliary structures could lead to clinically significant toxicities.

Grounding Pad Burns

Grounding pad burns are potentially seen with RFA but the risk is significantly reduced with proper application.

3.2 Microwave Ablation

3.2.1 Introduction

The term microwave refers to alternating current signals with frequencies between 3 and 300 GHz.

3.2.2 Procedure

The applicator in the case of microwave ablation is termed as antenna. Ablation using multiple antennae with simultaneous activation has been shown to be effective in the treatment of tumors greater than 3 cm [77]. This procedure is performed under ultrasound/CT guidance.

If the feeding vessel is identified before the treatment using color Doppler sonography, it is destroyed using the same technique to target the vessel [78]. This minimizes the heat sink effect. Advantages of microwave over RFA include faster ablation times, high intratumoral temperatures and improved convection profiles [79].

3.2.3 Uses of Microwave Ablation

Similar rates of complete ablation and complications have been observed with the use of RFA and microwave in a randomized control trial. The zone of coagulation following microwave is less than that following RFA and hence may necessitate multiple sessions to target the tumor [80]. Microwave ablation has been shown to be effective in the treatment of HCC less than 5 cm with a favorable location, i.e., distant from major duct or vessel. The presence of multifocal disease is also seen to limit the efficacy of this treatment [78]. Microwave ablation has recently been shown to have a role in metastatic CRC treatment [81]. The role of microwave ablation in the treatment of other metastatic lesions to the liver is yet to be established.

3.2.4 Complications

The spectrum and rate of complications following treatment are similar to those seen after RFA. Please note that no grounding pads are used in microwave ablation.

3.3 Laser Ablation

3.3.1 Introduction

This technique is also a thermal ablation technique in which high energy, i.e., light (electromagnetic radiation), is delivered to the target site using optical fibers which are inserted into the tumor.

3.3.2 Procedure

Laser induced thermotherapy (LITT) is another term used for laser ablation. The fibers are inserted into the needles and the number (1–4) and arrangement of the needles is chosen in accordance with the size, shape, and location of the lesion [82]. Multiple treatments (illuminations) by using the pull-back technique can be administered during one session. The delivery of the laser is terminated using a diffuser. The tumor is targeted using US or MR guidance. Coagulation necrosis is achieved using the neodymium–yttrium aluminum garnet (Nd–YAG) laser.

3.3.3 Uses of Laser

Nolsoe et al. published a pilot clinical study in which they studied the effect of laser ablation in 11 patients with metastatic colorectal carcinoma to the liver [83]. The efficacy and safety of this treatment modality has been shown in many studies in secondary liver tumors [84]. It has shown to have very promising results in the treatment of metastatic breast cancer [85]. Its use in primary liver tumors has not been studied extensively [82]. The patient population with the best outcomes is similar to that for RFA, i.e., small tumors away from large vasculature [82].

3.3.4 Complications

The rate and spectrum of complications following laser ablation is very similar to the other hyperthermic ablation techniques, i.e., RFA and microwave ablation.

Pleural effusions have been frequently observed (7 % of cases) after laser ablation but seldom require interventions [84].

3.4 Ultrasound Ablation

Ultrasound (US) ablation uses high energy, cyclic sound pressure pulses to heat the tumor leading to coagulation. This also acts by creating a hyperthermic zone leading to coagulation necrosis. Extracorporeal application of high-intensity focused ultrasound (HIFU) has limited role in treatment of liver tumors due to the organ's movement with respiration [86]. The use of general anesthesia and mechanical ventilation has allowed the use of this modality. The preliminary data shows that it is a safe and efficacious procedure with possible added benefits of treating larger lesions proximal to major vasculature [87, 88]. The use of interstitial applicators by direct penetration of the tumor may also have a promising role, but this carries the risk of needle tract seeding.

3.5 Cryoablation

3.5.1 Introduction

Cryoablation is the hypothermic ablation of a tumor. The ice ball formed during this procedure leads to necrosis of the tumor.

3.5.2 Procedure

Cryoablation can be administered intraoperatively, laparoscopically, and percutaneously [89]. The applicators in this technique are termed cryoprobes. The cryoprobes are inserted into the tumor under ultrasound guidance. Cryoablation has the disadvantage of not confining the thermal ablation to the tumor and exposing the surrounding tissue to the ablative effects of the treatment. This may be considered an advantage as cryoablation may be able to target satellite nodules around the target lesion. This technique is also not associated with the cauterization of the surrounding vessels leading to some of the complications discussed below. The presence of major vessels around the tumor leads to perfusion mediated tissue heating and decreases the efficacy of this procedure.

3.5.3 Uses of Cryoablation

Patients with HCC within Milan criteria can be considered for cryoablation. There is no data to support the use of hypothermic ablation techniques over the use of hyperthermic ablation techniques. The patients selected for cryoablation with liver metastasis are the patients who would be candidates for resection but cannot undergo surgery due to comorbidities [74].

3.5.4 Complications

The risk of needle tract seeding is also present after cryoablation. There is an increased risk of "cryo-shock" (multi-organ failure and severe coagulopathy) which is actually tumor lysis syndrome, after cryoablation. The interesting phenomenon of cracking after cryoablation has also been seen [74]. The additional complications are biochemical. The biochemical toxicities increase with the increase in the volume of the tumor ablated. Aspartate aminotransferase (AST) and alanine aminotransferase (ALT) may rise after treatment but is seen to resolve without intervention. The decrease in platelet count seen is related to the increase in AST and ALT after treatment. There is a significant increase in myoglobin levels after cryoablation but is not severe enough to lead to nephrotoxicity [90].

4 Chemical Ablative Therapies

4.1 Ethanol Ablation

4.1.1 Introduction

This is a chemical ablative therapy in which dehydrated alcohol (95 % alcohol) is injected into the tumor. It induces tumor necrosis by cellular dehydration, protein denaturation, and small vessel thrombosis.

4.1.2 Procedure

Conventional ethanol ablation entails the ethanol being injected under local anesthesia at a rate of 10 ml/session. "One shot" ethanol ablation is done under general anesthesia and the ethanol is injected at a rate of 30–50 ml/session. This can allow the use of this technique for larger tumors [75]. It can also be administered transarterially but carries a very high risk of complications [91]. It is performed percutaneously using narrow gauge single needle with multiple placements and could also be performed using a multiport needle [89], under ultrasound or CT guidance. Multiple sessions (≥ 2) are needed to target the tumor.

4.1.3 Uses of Ethanol Ablation

Ethanol ablation is used to treat tumors ≤ 3 cm in diameter which are closely adjacent to critical structures such as central bile ducts and bowel [89]. Livraghi et al. have reported high tumor recurrence rates (up to 17 %) in tumors up to 5 cm treated with ethanol ablation [75]. RFA has been shown to be superior to ethanol ablation in terms of recurrence free survivals in a randomized controlled trial [92].

4.1.4 Complications

As with all percutaneous techniques, the risk of tract seeding after ethanol ablation is present. The risk is lower than that of RFA [93]. Abdominal pain occurs in up to 48 % of the cases after ethanol ablation [91]. Opioids administered to alleviate the

pain may cause constriction of the sphincter of Oddi leading to pancreatitis. This is a severe but rare complication seen after opioid administration following ethanol ablation [91].

4.2 Acetic Acid Ablation

Ethanol ablation has limited applications because of the presence of septa in the tumor nodule which does not allow uniform distribution of the ethanol and hence necessitates multiple treatments. Ethanol is unable to dissolve the fibrous capsule [37]. Acetic acid has a low pH and hence can dissolve the dissociation of collagen cross-links. Percutaneous acetic acid is a promising alternative [94]. Randomized controlled trials comparing acetic acid ablation to ethanol ablation have shown significantly better survivals and decreased recurrence rates following acetic acid ablation [95].

5 Other Ablative Therapies

5.1 Irreversible Electroporation (IRE)

IRE utilizes high voltage electrical pulses applied through adjustable needles to increase cell membrane permeability, which results in cell death and necrosis [96]. IRE offers promise as the extracellular matrix is not damaged and hence, blood vessels and bile duct damage is minimized. This is in contradistinction to thermal ablative techniques [97].

6 Conclusion

As detailed above, image-guided therapeutic percutaneous and transarterial interventions are establishing their roles in selected patients. Proper patient selection and procedure allocation in collaboration with a multidisciplinary team is of optimal importance. Further analyses are needed to institute some of these therapies into the management algorithms of hepatic malignancies.

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Pathologic Features of Primary and Metastatic Hepatic Malignancies

Kristina A. Matkowskyj, M. Sambasiva Rao and Guang-Yu Yang

Abstract

In the mammalian liver, 60 % of the cellular components are hepatocytes while the remainder (35 %) includes biliary epithelium, Kupffer cells, endothelial cells, fat storing cells and connective tissue cells. Although neoplasms of hepatocytes are the most common, a significant number of both benign and malignant primary liver neoplasms arising from other cell types can develop, such as tumors of bile duct epithelium (Table 1). In addition, the liver is one of the most susceptible sites for metastatic tumors arising from other organs of the body. Not too long ago, liver tumors were left untreated because the liver was considered a complex and mysterious organ inaccessible to surgery. Advances in imaging procedures and surgical techniques over the past 40 years have revolutionized the approaches to the treatment of benign and malignant liver tumors. Subsegmentectomy, segmentectomy, lobectomy, and transplantation are routinely performed for the treatment of primary and metastatic liver tumors with minimal morbidity and mortality. Since accurate diagnosis remains the key to clinical and surgical management, the emphasis of this chapter is on classification, morphological features and differential diagnosis of malignant neoplasms of the liver.

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Keyword

Hepatocellular carcinoma · Cholangiocarcinoma · Hepatoblastoma · Undifferentiated (embryonal) sarcoma · Epithelioid hemangioendothelioma · Metastatic tumors

1 Primary Liver Tumors

See Table 1.

Table 1 Classification of primary liver tumors and tumor-like conditions

	Benign	Malignant
Epithelial tumors and tumor-like lesions	Macroregenerative nodule	Hepatocellular carcinoma (HCC)
	Dysplastic nodule	Cholangiocarcinoma
	Hepatocellular adenoma	Combined HCC/Cholangiocarcinoma
	Focal nodular hyperplasia	Billiary cystadenocarcinoma
	Bile duct adenoma	Malignant IPMN-B
	Bile duct hamartoma	Squamous cell carcinoma
	Bilary cystadenoma/mucinous cystic neoplasm (MCN)	Lymphoepithelioma-like carcinoma
	Biliary cyst	Hepatoblastoma
	Intraductal papillary mucinous neoplasm of bile ducts (IPMN-B)	
	Fatty change	
	Heterotopia	
	Pseudocyst	
Non-epithelial tumors	Hemangioma	Epithelioid hemangioendothelioma
	Angiomyolipoma	Angiosarcoma
	Infantile hemangioendothelioma	Fibrosarcoma
	Mesenchyma hamartoma	Germ cell tumors
	Solitary fibrous tumor	Embryonal sarcoma
	Inflammatory pseudotumor	Rhabdomyosarcoma
	Benign cystic mesothelioma	Leiomyosarcoma
	PEComa	Liposarcoma
	Paraganglioma	Kaposi sarcoma
	Glomangioma	
	Leiomyoma	Neuroendocrine carcinoma
	Lipoma	
	Myelolipoma	
	Lymphangioma	

1.1 Hepatocellular Carcinoma

Hepatocellular carcinoma (HCC) is one of the most common malignant liver tumors in the world with an estimated 1 million new cases each year and an estimated 650,000 deaths. Although the incidence is much less in western countries such as Europe and the United States, a progressive threefold increase has been seen over the last 30 years [4]. The increased incidence of HCC noted in the United States has been attributed to the high incidence of hepatitis C virus (HCV) infection, particularly in patients with alcoholic liver disease and cirrhosis [110]. Other etiologies and risk factors have also been identified as being responsible for the high incidence of HCC and possibly explain uneven geographic distribution throughout the world (Table 2). Although HCC may develop in non-cirrhotic livers, the majority of patients (about 85 %) with HCC have cirrhosis [21, 22]. The pathogenesis of HCC development in cirrhosis remains unknown. The possible mechanism is likely multifactorial and includes factors such as the increased susceptibility of dividing hepatocytes to oxidative stress resulting from infections [hepatitis B virus (HBV) and HCV)], chronic inflammation, alcohol, cigarette smoking, metabolic diseases (hemochromatosis or α_1-antitrypsin), and exposure to environmental carcinogens (Thorotrast, aflatoxins, oral contraceptives) leading to the initiation phase of the neoplastic process [42, 144]. In addition, there are epigenetic (hypermethylation of tumor suppressor genes) [51] and genomic alterations (c-myc amplification, telomere shortening, aneuploidy, gain/loss of chromosomes, and point mutations) [42, 130, 162] as well as metabolic and circulatory perturbations in the cirrhotic liver that may contribute to the promotion and progression of tumor development.

Table 2 Risk factors associated with HCC

Factor	Possible mechanism of tumor induction
Dietary contamination by mycotoxins	Direct DNA damage
Alcoholic liver damage	Hepatitis, free radical generation, oxidative DNA damage, cirrhosis
Hepatitis C virus infection	Chronic necroinflammatory changes, free radical generation, oxidative DNA damage, cirrhosis
Synergistic effects of HCV infection + alcoholic hepatitis	Inflammation, free radical generation, oxidative DNA damage, cirrhosis
Hepatitis B virus infection	Chronic necroinflammatory changes, cell proliferation, free radical generation oxidative DNA damage, cirrhosis, activation of oncogenes by adjacent viral insertion or inactivation of tumor suppressor genes
Iron overload (Hemochromatosis, hemosiderosis)	Hydroxyl radical generation, oxidative DNA damage, cirrhosis
Copper overload (Wilson's disease, Indian childhood cirrhosis)	Chronic hepatitis, free radical generation, cirrhosis
Other inherited disorders (α1-antitrypsin deficiency, porphyria cutanea tarda, tyrosinemia)	Chronic inflammation, hepatocyte regeneration, cirrhosis
Cirrhosis (secondary to any cause)	Increased hepatocyte proliferation, circulatory disturbances, and other factors as already mentioned above

Microarray technology, including global gene expression arrays, proteinomic and metabolic arrays, etc., have revolutionized our understanding of the molecular basis of hepatocellular carcinoma. Many studies have identified a number or profile of candidate genes useful as biomarkers in cancer staging, prediction of recurrence and prognosis, and treatment selection, as well for identifying the unknown primary from a metastatic malignancy. Some of these target molecules have been used to develop new serum diagnostic markers and therapeutic targets against HCC in order to benefit patients. Recently, analysis of transcriptional gene expression profiling has identified aberrant expression of *MARKL1* and *MARK3* [64], *VANGL1* [156], *PEG10* [108], *BMAL2* [157], *DDEFL1* [109], *RhoC* [150], *GEP* [15], *HLA-DR* [90], *Claudin10* [14], and *Ephrin A1* [53] in HCC, which had not been previously reported. Through comprehensive expression analysis, *GPC3* [95], *ROBO1* (Ito et al. [59], and *SP-5* [13] are up-regulated and are potential diagnostic markers for HCC. By extensively reviewing genomic profiling data, four signature classes have been identified in HCC, including classes of prediction, phenotype, function, and molecular targets [153]. These signature distinctions may help to address the application of genomic data from the research bench to the bedside.

Pathology of Hepatocellular Carcinoma

Although all HCCs arise from hepatocytes, their biological behavior is quite variable and unpredictable. Despite tremendous advances in molecular biology that have revolutionized our understanding of hepatocellular carcinogenesis, reliable molecular clues to predict the behavior of HCC are still lacking, but may be available in the near future given the tremendous progress in deciphering the human genome. Currently, traditional features such as size, encapsulation, large vein invasion, differentiation, and cytological features are still used for prognostication of these tumors. Here we present a practical scheme for gross and microscopic classification of HCC (Table 3).

1.1.1 Classical-Type HCC

Gross Pathology

Growth pattern of HCC, irrespective of the size, can provide useful information on the prognosis [111]. Approximately 50 % of HCC grow by expansion and are

Table 3 Classification of HCC

Gross	Microscopic
Encapsulated	Classical HCC
Infiltrating	Variants of HCC • Fibrolamellar • Sclerosing/Scirrhous • Clear cell • Sarcomatoid/Spindle cell • Pleomorphic/Giant cell
Multifocal	
Pedunculated	
	Combined HCC/Cholangiocarcinoma

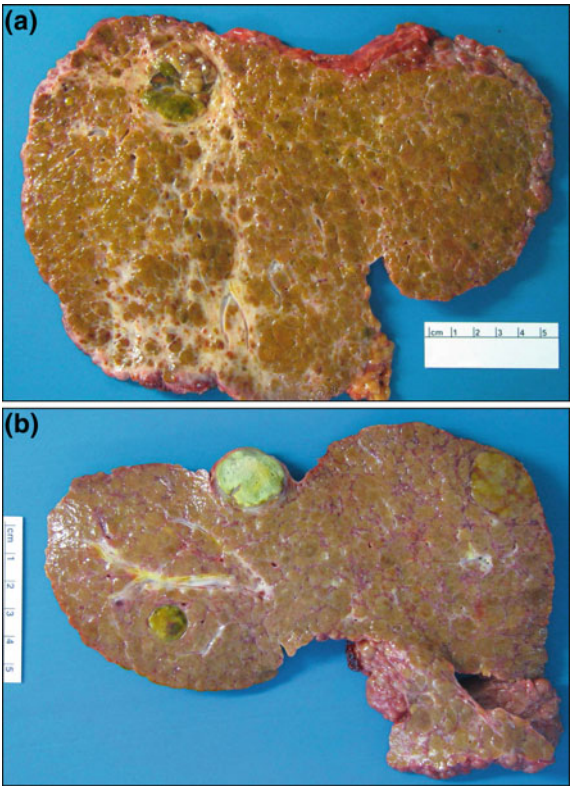

Fig. 1 Hepatocellular carcinoma. **a** Small, encapsulated HCC in a cirrhotic liver. **b** Multifocal HCC with 3 discrete nodules arising in a cirrhotic liver

separated from the adjacent parenchyma by a well-formed pseudocapsule. These encapsulated tumors can be large or small and can be seen in both cirrhotic (Fig. 1a) and non-cirrhotic livers. Encapsulated tumors often lack invasion beyond their capsule, or vascular permeation and are associated with better survival in patients after surgery [104]. Formation of a capsule around the tumors is considered to be a host defense mechanism rather than compression and collagenization of adjacent stroma. In contrast, infiltrating (spreading) HCCs are poorly delineated with invasion into the adjacent parenchyma. These tumors also show frequent vascular invasion. Multifocal (diffuse) type of HCC invariably develops in cirrhotic liver and tumors of various sizes are scattered throughout the liver (Fig. 1b). Small HCC may be difficult to distinguish from macroregenerative nodules seen in cirrhosis [73]. Multifocality probably represents a combination of multicentric tumor development and intrahepatic metastasis. Pedunculated HCC is a rare polypoid tumor connected to the hepatic surface by a stalk. Some pedunculated HCC may represent tumors arising in accessory lobes, or ectopic hepatic tissue or

peri-adrenal metastasis that fuse with the liver. Irrespective of growth pattern, HCC's are soft, tan in color with areas of yellow and green depending on the extent of bile production. The cut surface of small tumors is usually homogeneous and fleshy, whereas large tumors may exhibit hemorrhage and necrosis.

Microscopic Pathology of Classical-Type HCC

This is the most frequent type of HCC displaying cytoarchitectural features of hepatocytes. Tumor cells are usually arrayed in a trabecular pattern of variable cell thickness. Plates ranging from 2 to 8 cells and more than 8-cells thick are referred to as microtrabecular and macrotrabecular patterns, respectively. Trabeculae are separated by sinusoids lined by endothelial cells and Kupffer cells (Fig. 2a). In some areas, there may be acinar or pseudogland-like spaces containing bile as a result of canalicular dilatation. The tumor cells are polygonal with eosinophilic (pink), basophilic (blue), or amphophilic (purple) cytoplasm. In addition, the cytoplasm may contain a variety of inclusions such as fat droplets, Mallory bodies and protein globules normally synthesized by hepatocytes (αl-antitrypsin, α1-chymotrypsin, α-fetoprotein, fibrinogen, albumin, and ferritin). In some tumors, there may be excessive accumulation of glycogen in the cytoplasm imparting clear cell features.

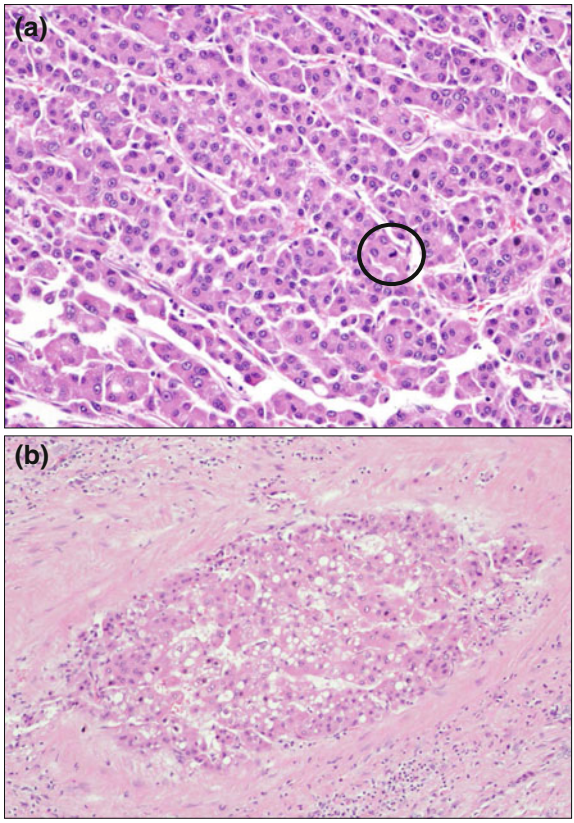

Fig. 2 Microscopic pathology of classical-type HCC. **a** HCC showing a trabecular architectural pattern and a rare mitosis (*black circle*). **b** HCC demonstrating large vessel invasion

Between the tumor cells, well-formed bile canaliculi are present that may not be readily identifiable by light microscope, but can be clearly demonstrated by immunoperoxidase technique using polyclonal carcinoembryonic antigen (CEA), CD10 or electron microscope. Depending on the functional differentiation of tumor cells, bile may be present in the cytoplasm and/or canaliculi. The nuclear features vary with the degree of tumor differentiation and they contain prominent eosinophilic nucleoli. Mitotic activity is often variable. Some HCCs may be associated with a marked lymphocyte infiltration that reflects the host response to the tumor and better prognosis or in rare cases, may be associated with Epstein-Barr virus (EBV) infection [94]. Vascular invasion of HCC is not uncommon and should always be critically evaluated (Fig. 2).

In classical HCC, the stromal component is minimal and the reticulin framework is delicate and sparse. In high grade HCC, the diagnostic trabecular architectural feature is frequently lost and these tumors may be difficult to differentiate from other primary liver tumors or metastatic lesions. In such cases, immunohistochemical stains using antibodies to liver specific proteins or other proteins such as α-fetoprotein, α1-antitrypsin, α1-microglobulin, albumin, erythropoeitin associated antigen, des-γ-carboxy prothrombin, ferritin, fibrinogen, HepPar-1, cytokeratins, (CK) and polyclonal carcinoembryonic antigen (CEA), glypican-3, and CD34 are helpful for correct identification of tumors [27, 33, 37]. Although a positive reaction for liver specific proteins in tumors is highly specific for the diagnosis of HCC, unfortunately the sensitivity is low.

Grading of Hepatocellular Carcinoma

A four tier grading system was originally proposed by Edmondson and Steiner [28] based on cytological and architectural features. More recently, a four tier grading system based on the Working Group Consensus Conference at the International Agency for Research on Cancer (Lyon, 2009) and published by the World Health Organization (WHO) is utilized [146]. Well-differentiated HCC, or Grade 1, is the most common and is typical of tumors less than 2 cm in size. With the advent of sophisticated imaging techniques, these small hepatocellular lesions are readily identified and biopsied. In tumors >3 cm, well-differentiated HCC is identified occasionally at the periphery of the tumor. Well-differentiated tumors are characterized by a normotrabecular pattern, microacinar/pseudogland formation, mild elevation in nucleus-to-cytoplasm ratio, mild atypia, and frequently fatty change [73–75]. Moderately differentiated HCC is most common in lesions that are larger than 3 cm. These tumors have well-formed trabeculae that are greater than 3 cells thick, cells have more abundant eosinophilic cytoplasm with prominent nucleoli, and frequent pseudogland formation with inspissated bile or proteinaceous fluid. Poorly-differentiated HCC grows in a solid pattern and is exceedingly rare in small tumors. There is loss of the trabecular pattern and only slit-like spaces are identified within the large tumor nests. The neoplastic hepatocytes exhibit a high nuclear-to-cytoplasm ratio, show marked cellular pleomorphism and contain frequent mitoses. Undifferentiated HCC tumor cells contain minimal cytoplasm, they

lose architectural and cytological resemblance to hepatocytes, appear spindled in shape and may resemble other non-hepatic tumors. Okuda et al. proposed that the degree of tumor differentiation is inversely proportional to the size of the tumor, however, this is not related to prognosis [56].

It is generally believed that development of HCC in humans, like in experimental animals, develops in a stepwise fashion and is preceded by the formation of precursor lesions [32, 73, 110]. This phenomenon is well documented in livers with cirrhosis [52, 145]. Hepatocytes undergoing regeneration can give rise to dysplastic foci ($\sim$1 mm) with small and large cell change, the smallest morphologically recognizable precursors of HCC. These dysplastic foci progress to dysplastic nodules (DNs) which can be grossly identified as lesions <2 cm, but which must be differentiated from small HCC. The high-grade DNs are felt to be direct precursors of HCC that have accumulated genetic and epigenetic alterations in an injured liver secondary to growth factor and cytokine stimulation. These alterations lead to the activation of proto-oncogenes and the inactivation of tumor suppressor genes, ultimately resulting in increased cell proliferation. Unfortunately, studies have demonstrated genetic heterogeneity within a single focus of HCC, suggesting multiple molecular pathways which are likely to be involved in hepatocarcinogenesis (see Table 4).

Predictors of Prognosis

The survival and prognosis of patients with HCC depends on several factors. Traditionally used pathological criteria for evaluating the prognosis in HCC are presence/absence of cirrhosis, tumor size, encapsulation, infiltration into the adjacent parenchyma, portal vein infiltration, margin status, lymph node, and distant metastases. Of these, small size and tumor encapsulation are associated with better prognosis after surgical treatment. In addition, other factors such as expression of CK19, the presence of a TP53 mutation, AFP levels greater than 100 ng/ml and portal vein thrombosis impact patient prognosis. For lesions less than $\sim$5 cm and

Table 4 Grading systems of HCC according to the WHO [146]

WHO	Microscopic features
Well-differentiated	Hepatocytes are slightly smaller than normal, 1–3 cell thick hepatic trabeculae, negligible to minimal atypia, abnormal reticulin framework, frequent fatty change
Moderately-differentiated	Hepatocytes are larger than normal with more eosinophilic cytoplasm and distinct nucleoli, hepatic trabeculae are >3 cells thick, frequent pseudogland formation
Poorly-differentiated	Hepatocytes exhibit a high nuclear-to-cytoplasmic ratio, marked cellular pleomorphism, hyperchromatic nuclei, loss of hepatic trabeculae, solid growth pattern, frequent mitoses
Undifferentiated	Hepatocytes contain minimal cytoplasm, loss of architectural and cytological resemblance to hepatocytes, marked anaplasia, frequent spindling of cells which may resemble other non-hepatic high-grade tumors

without evidence of large vessel invasion, liver transplantation is an effective therapy. For patients with contraindications for surgery including inoperable tumors, cryoablation and radioactive- or chemoembolization have been used. Imaging procedures and serum tumor markers like α-fetoprotein are routinely used for the identification of small or recurrent HCC.

1.2 Variants of HCC

Although the incidence of variants of HCC is infrequent, recognition of these variants is important not only because they pose diagnostic problems but also their natural behavior and clinical manifestations can be quite different from classical HCC.

1.2.1 Fibrolamellar Carcinoma (FLC)

This variant of HCC is a slow growing tumor, often develops in children and young adults (<25 years of age) in non-cirrhotic livers and is associated with excellent prognosis [7, 20, 118]. The etiology and risk factors of these tumors are unknown and no association with viral infections or alcohol consumption has been identified. Underlying chronic liver disease was observed in only 20 % of the patients with fibrolamellar carcinomas. For unknown reasons, the left lobe is involved in two-thirds of cases.

Pathologic Features

Grossly, most of the tumors are large, solitary, and well circumscribed with focal encapsulation. However, in some tumors satellite nodules may be present. The cut surface is yellow to pale tan, firm with a central scar in 75 % of cases and connecting fibrous septa (Fig. 3a). Necrosis and hemorrhage may be present. These tumors show unique features that include distinct stroma and epithelial cells. The stromal component is abundant and is composed of thin multilayered strands of collagenous fibers arranged in parallel bands (lamellae) separating the epithelial cells into trabeculae and nodules (Fig. 3b). The epithelial cells are polygonal with abundant granular eosinophilic cytoplasm and vesicular nuclei containing prominent nucleoli (Fig. 3c). Mitoses are infrequent. The granularity of tumor cells is due to abundant number of mitochondria (oncocytic features) [31]. Other findings include calcification, gland-like formation, or cytoplasmic eosinophilic globules and/or pale bodies which represent the accumulation of α-antitrypsin and fibrinogen, respectively [20, 82]. The tumor cells may also contain bile, copper, copper-associated protein, and express both hepatic (CK 8 and 18) and biliary (CK 7 and 19) cytokeratins [8, 82]. Tumor cells may contain mucicarmine or alcian blue-positive secretions and should not be misclassified as combined HCC/cholangiocarcinoma; thus careful attention to clinical history and microscopic findings are imperative. The prognosis for FLC is better than classical HCC arising in a cirrhotic liver and similar to HCC arising in a non-cirrhotic liver. FLCs are

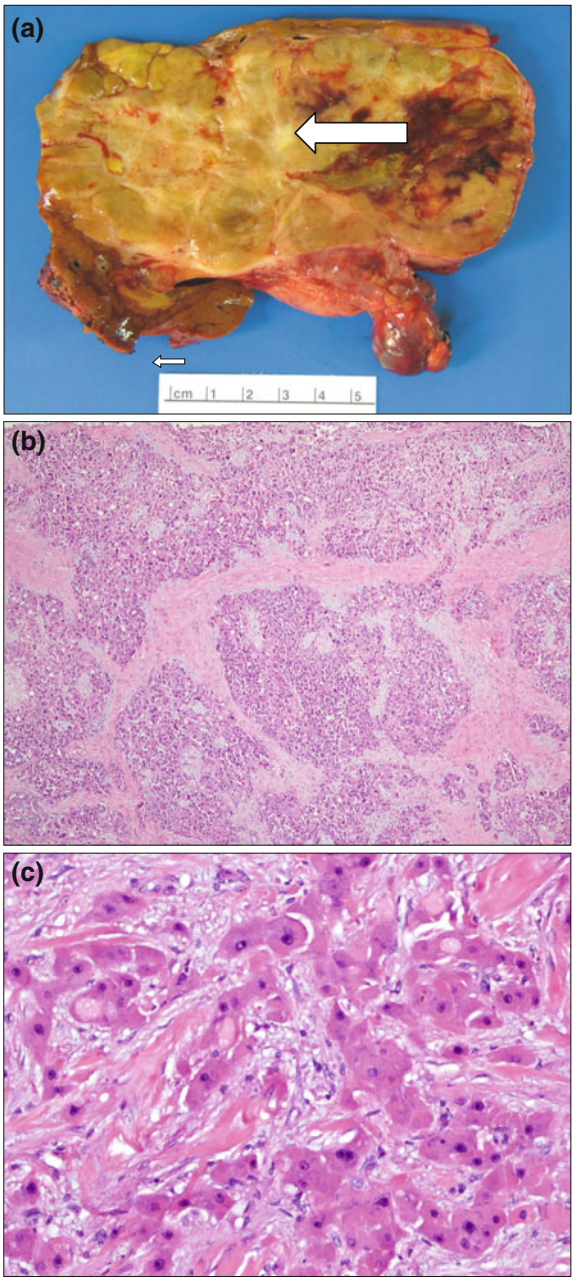

Fig. 3 Fibrolamellar carcinoma. **a** Large, yellow to pale tan, well-circumscribed lesion with central scar (*white arrow*). **b** Lamellar fibrosis runs in parallel bands between nests of oncocytic tumor cells. **c** Tumor cells contain abundant eosinophilic cytoplasm, vesicular nuclei and prominent nucleoli, characteristic features of FLC

frequently associated with increased serum levels of neurotensin and vitamin B_{12}-binding globulin and normal levels of α-fetoprotein, which are useful in monitoring disease and detecting recurrence.

1.2.2 Sclerosing (Scirrhous) HCC

The scirrhous type of HCC accounts for <5 % of all hepatocellular carcinomas, develops in older patients without coexisting cirrhosis, is located directly beneath the liver capsule and is often associated with hypercalcemia [112, 114]. As the name indicates, the tumor is associated with abundant non-lamellar, fibrotic stroma containing tumor cells arranged in atrophic trabeculae forming a pseudoglandular pattern. The neoplastic cells express higher levels of CK7 and lower levels of HepPar1 compared to classical HCC [91, 139]. Clinical behavior and prognosis of scirrhous HCC is similar to that of classical HCC [69].

1.2.3 Clear Cell HCC

This is a rare histological subtype of HCC which has a frequency between 0.4 and 37 %. The diagnostic criteria for this subtype are ill-defined, possibly accounting for the variability in frequency. Foci of clear cell can be detectable in greater than 50 % of HCCs, however, it is extremely rare to see a tumor with >90 % clear cells. Conservatively speaking, in order to classify a clear cell HCC, between 30 and 50 % of the cells must have clear cytoplasm. These clear cells are medium in size and polygonal in shape and form either a solid or trabecular growth pattern. The cytoplasm is "clear" due to cytoplasmic accumulation of glycogen and/or fat droplets [113, 154, 155]. The nuclei appear generally bland with a central or slightly eccentric location and dense chromatin. In small biopsy specimen, it may be difficult to differentiate clear cell HCC from other clear tumors metastatic to the liver such as kidney, adrenal, or ovary. In this instance, foci of classical HCC should try to be identified along with immunostains such as pCEA, CD10 and HepPar1 (liver), EMA and LeuM1 (kidney), inhibin and calretinin (adrenal), and CK7 and CD15 (ovary) to differentiate the tissue of origin [97]. In addition, the majority of patients with clear cell HCC will have an elevated serum α-fetoprotein, hypoglycemia, and/or hypercholesterolemia [113, 143]. Surgical resection is an effective manner way to achieve favorable outcomes and long-term survival. The prognosis of patients with clear cell HCC is still controversial as some studies have reported a similar prognosis to classical HCC [30, 80], while others report a better prognosis [87].

1.2.4 Sarcomatoid (Spindle Cell) HCC

These tumors are also referred to as spindle cell HCC or HCC with sarcomatous change [56, 88]. The clinical findings and gross morphology are similar to classical type of HCC. Histologically, these tumors contain a spindle cell, sarcomatous component as well as areas of conventional HCC. It is more common to see sarcomatoid change in lesions treated with chemotherapy or chemoembolization

[72]. Sarcomatoid areas contain spindle cells arranged in fascicles and/or a storiform pattern with variable amounts fibrous stroma and can resemble fibrosarcoma, leiomyosarcoma, rhabdomyosarcoma, or osteosarcoma and contain osteoclast-like or anaplastic giant cells. These spindle cells often stain positively for epithelial markers [44, 88] including CK8 and CK18 as well as AFP [36, 44]. Sarcomatoid HCC is very rare and the clinical behavior and survival are based on a small series of cases. These tumors tend to have an aggressive behavior characterized by frequent intrahepatic, lymph node, and lung metastases and a dismal prognosis after resection, similar to high-grade sarcomas.

1.2.5 Pleomorphic (Giant Cell) HCC

This variant of HCC is very rare (< 1 %) and only a few cases have been reported. Histologically, these tumors contain scattered areas of a malignant epithelial component arranged in a solid sheet with the majority of tumor cells exhibiting bizarre nuclear features and two strikingly different types of multi-nucleated giant cells. The first type often occupies an extensive area of the lesion and is benign-appearing osteoclast-like giant cells with small, uniform nuclei. The second type are less numerous and contain large, vesicular, hyperchromatic nuclei with malignant features similar to those observed in the epithelial component. These tumors typically do contain foci of classical HCC, but extensive sampling may be required [76]. In one study of these tumors, the authors found no evidence of hepatocyte differentiation in the benign-appearing osteoclast-like giant cells [96]. Therefore, it is crucial to differentiate HCC with osteoclast-like giant cells from the more rare HCC with pleomorphic tumor giant cells [21, 22, 48, 76].

1.3 Cholangiocarcinoma

Cholangiocarcinoma is an adenocarcinoma arising from biliary epithelium anywhere in the biliary tract and classified according to its location along the biliary tree. The majority of tumors are located in the upper third of the biliary tract, while the remainder involves the bifurcation of the common hepatic duct [23]. Tumors arising in the small peripheral intrahepatic ducts (peripheral cholangiocarcinomas) are the least common and represent less than 10 % of cases. Bile duct tumors that involve the common hepatic duct bifurcation are referred to as Klatskin tumors or hilar cholangiocarcinoma regardless of whether they arise from the intrahepatic or extrahepatic portion of the biliary tree. These hilar/peri-hilar tumors account for ~65 % of all cholangiocarcinomas. The remaining 25 % of tumors arise in the distal extrahepatic bile ducts and are referred to as extrahepatic cholangiocarcinomas [98, 158]. Presenting systems vary with the anatomic location of the tumor; peripheral lesions are frequently asymptomatic until the tumor is at an advanced stage, while patients with hilar, peri-hilar, and extrahepatic lesions present clinically with biliary obstruction. The incidence of cholangiocarcinoma is much less common than HCC, and usually affects both the sexes equally over the age of 50. The incidence of biliary tract cancers increases with age with most patients presenting

between 50 and 70 years of age. However, patients with a history of primary sclerosing cholangitis (PSC) and/or choledochal cysts typically present in their 30s–50s, nearly two decades earlier [10, 149]. New diagnostic methods used for obstructive jaundice can now identify biliary malignancies which previously might have gone undiagnosed may account for the rise in cholangiocarcinomas [61]. Unfortunately, the rising rates have not been associated with an increase in the detection of early stage or smaller size lesions [133]. Additional studies suggest that the increasing incidence may be related to a concomitant increase in certain risk factors such as cirrhosis, alcoholic liver disease, and HCV infection [134]. Exposure to thorotrast and infestation with liver flukes has also been causally linked to cholangiocarcinoma [58, 132]. Several additional predisposing factors such as Caroli's disease, polycystic liver disease, inflammatory bowel disease, and hepatolithiasis have been identified.

A variety of molecular defects involving both oncogenes (β-catenin, Kras, BRAF, HER2/neu, and EGFR) and tumor suppressor genes (TP53, p16, and SMAD4) have been described in specimens of invasive biliary tract tumors. Approximately 30 % of tumors overexpress p53, and another 10–54 % contain activated Kras mutations [47]. These genetic alterations appear to be associated with a more aggressive tumor phenotype [54, 102]. The contribution of these genetic changes to the development of cholangiocarcinoma and the relationship to chronic inflammation, ethnic background, and carcinogen exposure continues to remain uncertain. However, the identification of altered gene expression in cholangiocarcinoma is becoming more readily available with the availability of array technologies. Copy number gains were recently identified in several genes including ERBB2, MEK2, PDGFB, MTOR, VEGFR 3, PDGFA, RAF1, VEGFA, and EGFR allowing for molecular stratification and a more tailored therapeutic treatment of the patient [93]. Similarly, global gene expression analysis has identified 52 genes that were commonly up-regulated and 421 that were down-regulated [107]. These genes may provide for a better understanding of the tumorigenesis of cholangiocarcinoma and to the development of diagnostic and therapeutic strategies.

Pathologic Features

Grossly, peripheral cholangiocarcinomas are tan-gray to white, firm, unencapsulated tumors that present as a large solitary mass or multiple nodules (Fig. 4a), whereas hilar and extrahepatic cholangiocarcinomas present as strictures or diffusely infiltrative lesions. Microscopically, irrespective of location, cholangiocarcinomas show features of mucin-producing adenocarcinomas with abundant desmoplastic stroma. Intrahepatic cholangiocarcinomas that arise in a background of chronic biliary disease are often characterized by precancerous lesions including dysplasia and carcinoma in situ. Based on the architectural and cytological differentiation these invasive tumors are classified as well-differentiated (Grade 1), moderately-differentiated (Grade 2), or poorly-differentiated (Grade 3). Well-differentiated cholangiocarcinomas reveal tubular, papillary, or tubulo-papillary architecture. The lining epithelium is cuboidal to low columnar with basally located uniform nuclei

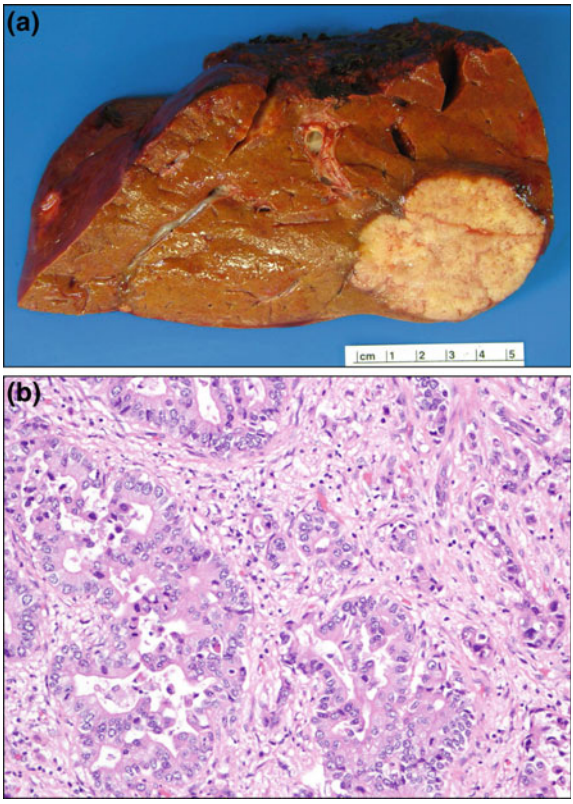

Fig. 4 Cholangiocarcinoma. **a** Tan-gray to white, firm, unencapsulated peripheral tumor. **b** Microscopically, the neoplastic cells have a tubular architecture composed of cuboidal to low columnar cells with basally located uniform nuclei and infrequent mitosis

(Fig. 4b). Mitotic activity is infrequent. In poorly differentiated carcinomas, gland formation is infrequent, and tumor cells are arranged in clusters or cords. Tumor cells show marked nuclear pleomorphism and frequent mitoses. Moderately differentiated cholangiocarcinomas have features ranging from well differentiated to poorly differentiated tumors. These tumors have a slow growth rate, a high rate of local invasion, and a tendency to invade perineural sheaths and spread along nerves. The incidence lymph node metastasis in peripheral cholangiocarcinomas is quite high compared to the hilar type, whereas distant metastases are overall uncommon in cholangiocarcinoma. Occasionally, cholangiocarcinomas can show uncommon histological types that include signet ring cells, clear cells, undifferentiated small cells, mucinous carcinoma, and adenosquamous features. Poorly differentiated cholangiocarcinomas are difficult to differentiate from metastatic carcinomas and HCC. Proper identification of these tumors is possible with immunohistochemical stains using antibodies to different types of cytokeratins (pan-keratins, CK7, CK19, CK20), carcinoembryonic antigen, epithelial membrane antigen, CA 19-9 and α-fetoprotein [18, 27, 46, 127]. In addition, more recent studies have indicated that stem cell markers including C-kit and p63 can be used to identify

cholangiocarcinoma [89, 106]. Finally, serum levels of carcinoembryonic antigen and carbohdrate antigen CA 19-9 are almost always increased in cholangiocarcinomas [60].

1.4 Intraductal Growth of Intrahepatic Cholangiocarcinoma Arising from Intraductal Papillary Mucinous Neoplasm of the Bile Ducts

Mucin-producing intraductal papillary neoplasm of the bile duct is becoming recognized as a specific type of neoplasm. This neoplasm shares similar pathologic features to intraductal papillary mucinous neoplasm of the pancreas [68, 160, 161] and therefore the term intraductal papillary mucinous neoplasm of the bile duct (IPMN-B) is frequently utilized. Cases previously reported as biliary mucinous neoplasm without ovarian-like stroma, intraductal papillary neoplasia of the liver (IPN-L), mucin-hypersecreting biliary papillomatosis, mucin-hypersecreting papillary cholangiocarcinoma, mucin-producing cholangiocarcinoma, and mucin-hypersecreting bile duct tumor are now believed to be IPMN-B. IPMN-B is characterized by numerous papillary projections composed of columnar ductal epithelial cells lining a fibrovascular core [86]. The tumor grows slowly, tends to be multifocal and grows along the intra- and extrahepatic bile ducts [70, 79, 84]. IPMN-B may invade the ductal wall and penetrate to its exterior [70, 83] and is then categorized as malignant IPMN-B or intraductal growth of intrahepatic cholangiocarcinoma. Although histologically benign in most cases, IPMN-B are generally regarded clinically as borderline or low-grade malignant tumors because of their tendency to recur, their multicentricity, and their ability to undergo malignant transformation and, rarely, to metastasize [21, 22]. Malignant IPMN-B is a rare disease, which accounts for <10 % of cholangiocarcinoma [142]. In contrast to other cholangiocarcinomas, malignant IPMN-B can be successfully resected and demonstrates a more favorable prognosis [12, 55, 100, 115, 140]. However, it is associated with significant morbidity and mortality due to recurrent cholangitis, obstructive jaundice, and sepsis [115]. These intermittent symptoms are characteristic of IPMN-B and are unusual for other bile duct tumors. Interestingly, malignant IPMN-B differs from mass-forming cholangiocarcinomas with respect to genetic alterations and phenotypic changes such that malignant IPMN-B has a lower frequency of K-ras and p53 mutations [1, 62].

Pathologic Features

Grossly, the bile duct contains multiple foci of tumor that has a soft, polypoid appearance and results in duct dilation. A large amount of mucin is produced, especially when associated with foci of mucinous carcinoma [84, 99]. The tumor is friable and tends to slough, causing the intermittent clinical symptoms previously described [16, 68, 86]. Microscopically, the tumor cells are columnar in shape and surround a fibrovascular core. The nuclei are round to oval, basally located, and

without stratification. The cytoplasm is generally abundant and mucinous, but reports of clear or oncocytic cytoplasm as well as intestinal metaplasia have been identified. Mitotic figures are typically infrequent.

1.5 Biliary Cystadenocarcinoma Arising from Biliary Mucinous Cystic Neoplasm

Biliary mucinous cystic neoplasms (MCNs) also known as mucinous cystadenomas are a well-known mucin-producing neoplasm in the bile duct [135, 152]. The cystic tumor usually does not communicate with the bile ducts, and the mucin which is secreted by the tumor is confined. As in the pancreas, the presence of ovarian-like stroma is required to establish the diagnosis of biliary MCN [160, 161]. Biliary cystadenocarcinoma, a rare cystic malignant neoplasm with less than 100 cases reported can develop from a benign biliary cystadenoma and accounts for 20–40 % of the reported cases of hepatobiliary cystic tumors [18, 124].

Pathologic Features

These tumors are well-circumscribed, large multiloculated cysts ranging in size from 5 to 20 cm and separated from the adjacent liver by a fibrous tissue capsule. The cysts contain clear mucinous or hemorrhagic fluid. Microscopically, the tumors show benign, borderline, and malignant areas. In the benign areas, the lining epithelium is cuboidal to columnar with nuclei that are basally oriented. No cellular atypia is present. In borderline areas, there is cellular and nuclear pleomorphism, stratification of nuclei and increased mitosis. In the malignant areas, atypical cellular features are identified along with a multilayered epithelium that may form papillary fronds and show invasion into underlying stroma and adjacent liver. Biliary cystadenocarcinoma has a much better prognosis after surgical excision when compared to cholangiocarcinoma.

1.6 Combined Hepatocellular Carcinoma-Cholangiocarcinoma

Only the tumors that exhibit features of classical HCC and cholangiocarcinoma with transitional areas should be included in this category [105]. This mixed tumor accounts for up to 5 % of all primary carcinomas of the liver [41]. HCC with pseudoglandular features secondary to dilatation of bile canaliculi should not be easily confused with this variant. In this combined tumor, the hepatocellular areas show a cytokeratin profile consistent with hepatocytes (CK8 and CK18 positive), while in the glandular areas the neoplastic cells express a bile duct epithelium pattern (CK7, CK19, and CA19-9) and are positive staining for mucin [74, 75]. In the transitional areas, the cells are often cuboidal to columnar with amphophilic cytoplasm, inconspicuous nucleoli, gland formation and with mucin production. The WHO defines the hepatic component by the presence of bile production, bile

canaliculi, and a trabecular pattern of growth. The glandular component is defined by the presence of true gland formation with mucin production. Collision tumors, in which these elements are clearly separated in the tumor mass, or are situated side by side, are not considered true HCC/CC tumors according to the WHO Bile production by tumor cells, or immunoperoxidase positivity for markers specific for hepatocytes, such as AFP, polyclonal CEA, and hepatocyte antibody, are useful to reveal the hepatocellular component, but a lack of staining for CK7, CK19, or MOC-31 may be evidence of hepatocytic differentiation as well, because many HCC markers may not be positive in poorly differentiated components of HCC. In a case report by Fischer et al. [34] cells in transitional areas showed keratin profile similar to that of bile duct epithelium. Bidirectional differentiation of combined tumors supports the hypothesis of existence of bipotential stem cells in the human liver and possible origin of these tumors from those cells.

1.7 Malignant Pediatric Tumors

1.7.1 Hepatoblastoma

Hepatoblastomas (HB) are most common in children under the age of 3 years, but are occasionally seen in older children and adults [5, 71, 123, 137]. In young children, hepatoblastomas are more common in males than in females (2:1 ratio) while in older children there is no sex difference. The etiology of hepatoblastoma is not known and these tumors arise in livers without any preceding chronic liver disease. One third of the patients have various congenital anomalies, including an increased incidence in children with familial adenomatous polyposis syndrome [38, 50] and Beckwith–Weidemann [148] have been reported. The histogenesis of hepatoblastoma is controversial. It is generally believed that both the epithelial and mesenchymal elements are neoplastic and are possibly derived from a common precursor (stem) cell [126]. Using cultured chemically transformed rat epithelial cells Tsao and Grisham [147] have produced tumors with features of hepatoblastoma in rats. These findings clearly demonstrated multidirectional differentiation potential of hepatic epithelial cells. Serum AFP is a useful marker of tumor recurrence or metastasis as it is almost always elevated.

Pathological Features

Hepatoblastoma most commonly appears in non-cirrhotic livers as a single bulky mass surrounded by a pseudocapsule. Because the capsule is indistinct, microscopic invasion may be found beyond the main lesion. Occasionally, hepatoblastoma may present as a multifocal lesion. The cut surface of the tumor has variegated appearance depending on the microscopic type. Tumors with pure epithelial component are homogeneous with a brown to green cut surface, whereas tumors with epithelial and mesenchymal components show areas of hemorrhage and necrosis. Since distinct tumor nodules can contain different histologic components, extensive tissue sampling is highly recommended. Histologically, hepatoblastomas are subdivided into pure epithelial (fetal and/or embryonal), epithelial-mesenchymal, small

cell undifferentiated, and macrotrabecular types [40, 77, 137]. In the fetal subtype, as in the fetal liver, small hepatocytes with prominent nucleoli and eosinophilic cytoplasm are arranged in 2–3 cell thick trabeculae. Extramedullary hematopoiesis is frequently seen in the sinusoids. In the embryonal subtype, the tumor cells are less differentiated than fetal type and may not resemble hepatocytes. The cells are small with a high nuclear-to-cytoplasm ratio, marked nuclear pleomorphism, and increased mitoses. Tumor cells are arranged in cords, sheets, and acini and may show areas of squamous differentiation (Fig. 5a). Foci of hematopoiesis can also be observed. Just as the name suggests, mixed epithelial and mesenchymal hepatoblastomas consist of epithelial cells admixed with mesenchymal elements. The mesenchymal components include immature spindle cells, rhabdomyoblasts of varying maturity, osteoid and cartilagenous tissue (Fig. 5b). Areas of metaplastic squamous epithelium and mucinous epithelium may also be identified. Small cell undifferentiated type contains small, anaplastic cells arranged in sheets and may mimic neuroblastomas. These tumors are associated with a poor prognosis. The macrotrabecular type consists of epithelial cells arranged in thick trabeculae and

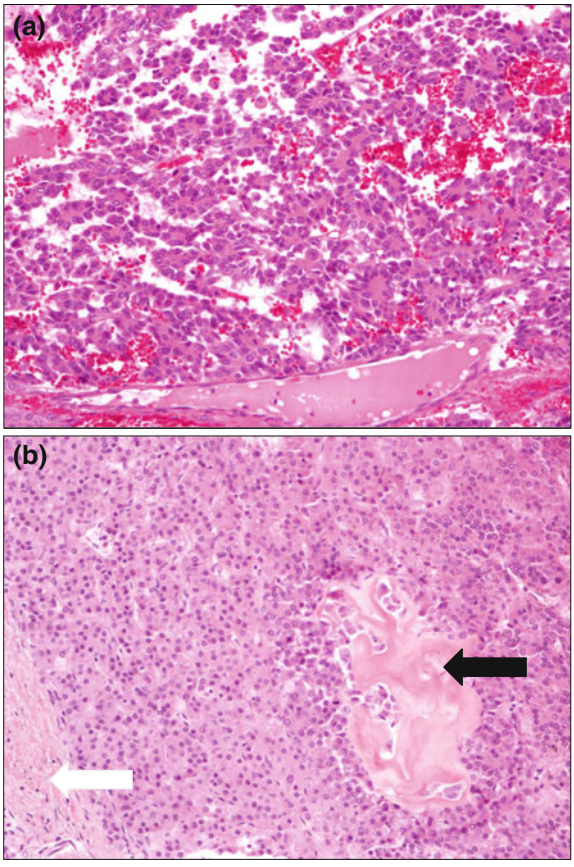

Fig. 5 Hepatoblastoma, epithelial-mesenchymal type. **a** In one area of the tumor, the epithelial cells were of embryonal subtype; less differentiated, with a high nuclear-to-cytoplasmic ratio, and arranged in acini. **b** In a separate area, the fetal subtype was identified with the tumor cells appearing as small hepatocytes with prominent nucleoli and eosinophilic cytoplasm arranged in 2–3 cell thick trabeculae. Mesenchymal elements such as spindle cells (*white arrow*) and osteoid (*black arrow*) were also identified within the same lesion

often may resemble classical HCC. Irrespective of histological subtype, hepatoblastomas are associated with high levels of serum α-fetoprotein and this protein is readily demonstrable in fetal and embryonal cells by immunoperoxidase technique. The impact of histopathology on the prognosis of hepatoblastoma is not entirely clear; however, it has been reported that patients with the purely fetal type histology seem to have the most favorable prognosis after resection [45], while those with small cell undifferentiated or macrotrabecular subtypes have a worse prognosis [19]. Prognosis is directly related to completeness of surgical excision, tumor-free margins, age at presentation, tumor size, and involvement of adjacent organs [19].

1.7.2 Undifferentiated (Embryonal) Sarcoma

Embryonal sarcoma, also referred to as undifferentiated sarcoma, is a rare tumor that commonly afflicts children between the ages of 6 and 10 years and occasionally, older children and adults [138]. Although no precursor lesions or predisposing factors have been identified, more recent molecular studies have identified chromosomal abnormalities in 19q, suggesting a possible relationship between this lesion and benign mesenchymal hamartoma [9, 81].

Pathologic Features

Grossly, these tumors are large, soft, well circumscribed, and usually single. On cut section, the tumor has a variegated appearance with gelatinous, myxoid, cystic, hemorrhagic, and necrotic areas (Fig. 6a). Microscopically, the tumor is composed of primitive, anaplastic, mesenchymal cells arranged in fascicles, sheets or without any pattern (Fig. 6b). Typical and atypical mitoses are very common. The matrix is myxoid, rich in acid mucopolysaccharides. PAS-positive, diastase resistant eosinophilic globules, and considered to be α1-antitrypsin, can be seen in the cytoplasm and in the stroma. Entrapped dilated bile ducts and islands of extramedullary hematopoiesis may be present within the tumor. Embryonal sarcomas stain positive for vimentin, α_1-antitrypsin, and α_1-antichymotrypsin [67, 78]. Tumors are often amenable to surgery and the prognosis is considered good [6, 71, 138].

1.8 Malignant Vascular Tumors

Although benign vascular tumors (hemangioma) are very common in the liver, malignant vascular tumors are very rare. Epithelioid hemangioendothelioma and angiosarcoma, two distinct histological types of malignant vascular tumors of endothelial origin, which strongly stain for factor VIII-related antigen, CD31 and CD34, with different biological behavior develop in the liver.

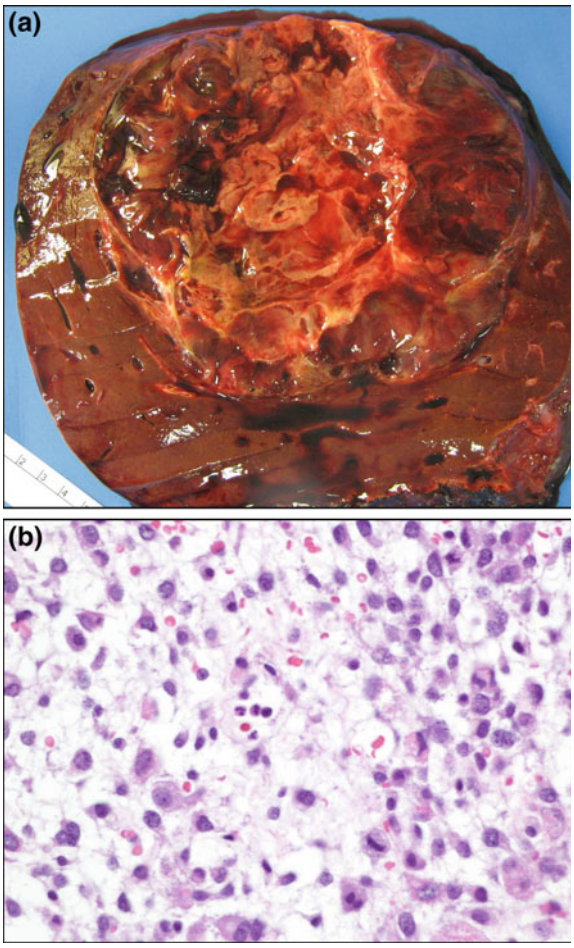

Fig. 6 Embryonal sarcoma. **a** A single, large tumor with a variegated cut surface containing cystic and myxoid to gelatinous areas. **b** Anaplastic, spindle to oval-shaped cells with ill-defined borders in a myxoid stroma containing numerous thin-walled vessels and frequent mitotic activity

1.8.1 Epithelioid Hemangioendothelioma (EH)

These rare, low-grade tumors are more common in females (ratio of females to males of 2:1) and occur over a wide age range (2nd–8th decade) [57]. There is no associated chronic liver disease and these lesions are frequently found incidentally, but may also present as upper abdominal discomfort or mass lesion. Oral contraceptives have been suggested as a possible etiology of epithelioid hemangioendothelioma [24, 57].

Pathologic Features

The tumors often present as multiple, ill-defined, white to yellow, firm nodules of varying sizes involving both the lobes. The cut surface of the lesion is homogeneous with infiltrative margins and a gritty consistency (Fig. 7a). Microscopically, the tumor consists of epithelioid to spindle shaped cells occurring singly or arranged in nests or short cords (Fig. 7b). The epithelioid cells contain abundant

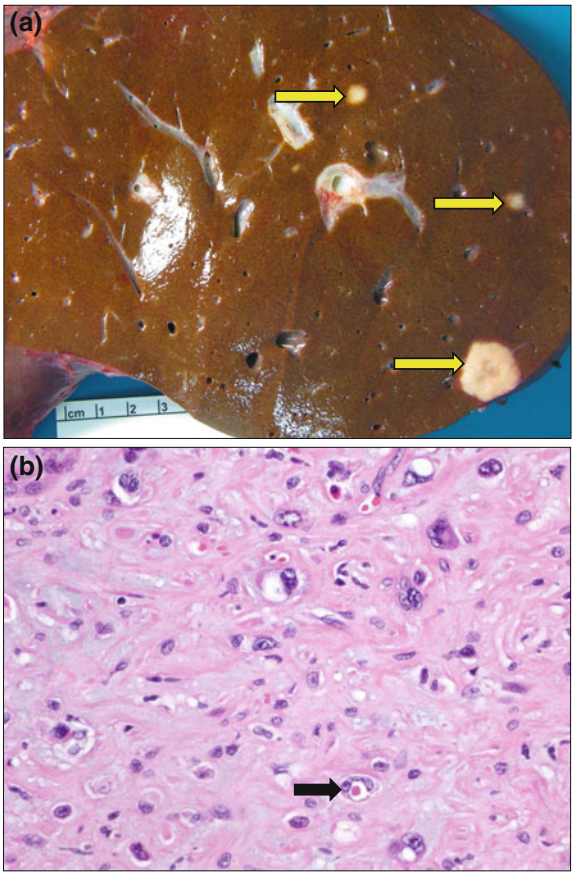

Fig. 7 Epithelioid hemangioendothelioma. **a** Multiple, ill-defined, white to yellow, firm, and gritty nodules (*yellow arrows*) are present in the liver parenchyma. **b** The lesion is composed of spindle to epithelioid-shaped cells containing abundant eosinophilic cytoplasm and prominent nuclei in a myxoid stroma. Intracytoplasmic vacuoles containing red blood cells can be identified in some of the cells (*black arrow*)

eosinophilic cytoplasm and prominent nuclei. In some cells intracytoplasmic vacuoles containing red blood cells can be seen. Mitotic activity is variable. The tumor contains abundant stroma with areas of dense fibrosis, myxoid change, and calcification. Areas of necrosis and a scattered inflammatory infiltrate can be seen. Extension of tumor cells into large veins and intraluminal growth is frequent. Differentiation of epithelioid hemangioendothelioma from angiosarcoma or epithelial tumors with sclerosis is important because epithelioid hemangioendothelioma has a better prognosis after lobectomy or transplantation [66] compared to other tumors.

1.8.2 Angiosarcoma

This tumor affects men more often than women (M:F ratio of 4:1), mostly in the sixth and seventh decades. There have been rare cases reported in children often in the setting of infantile hemangioendothelioma [11, 101, 120, 128]. There is good

correlation between exposure and thorotrast, vinyl chloride, and arsenic and the development of angiosarcoma [58, 119]. In addition, there is also some evidence that radium, inorganic copper, and anabolic steroids may play some role in the pathogenesis of angiosarcoma. However, in many patients no risk factors can be identified. There are no effective therapies available, thus prognosis is poor with a survival of ~6 months [71].

Pathologic Features

Angiosarcoma is often a multifocal disease involving both the lobes and appears as nodules of variable sizes. The cut section shows poorly delineated lesions with gray-white to hemorrhagic areas. Histologically, these tumors consist of predominantly spindle cells arranged in a combination of four basic patterns: sinusoidal, papillary, cavernous, and solid. In the sinusoidal pattern, the tumor cells proliferate along sinusoids and there is varying degree of sinusoidal dilatation and atrophy of the liver cell cords. The neoplastic cells are larger, more numerous and more hyperchromatic that the normal endothelial cells. In the papillary pattern, tumor cells line nodules of which protrude into a lumen. In the cavernous pattern, the sinusoids become markedly dilated with loss of hepatocytes, forming large blood-filled spaces. The tumor cells are pleomorphic and multilayered. In the solid pattern, cells grow in sheets without forming vascular spaces and display marked cellular atypia and increased mitoses, frequently resembling fibrosarcoma. Extramedullary hematopoiesis may be present. Infiltration of tumor cells into portal and hepatic veins is common. Endothelial cells in the liver adjacent to the tumor usually shows hyperplastic and dysplastic changes.

1.8.3 Kaposi Sarcoma

Kaposis sarcoma (KS) was originally described by Hungarian dermatologist Moritz Kaposi in 1872 [63]. KS is a systemic disease which frequently presents with cutaneous lesions with or without internal organ involvement. Four subtypes have been described and include classic KS (middle aged Mediterranean and Jewish men), African endemic KS, KS in immunosuppressed patients (iatrogenic), and AIDS-related KS. In all subtypes, human herpesvirus 8 (HHV-8) is responsible. Liver lesions frequently occur in AIDS-related KS, in immunosuppressed patients and less frequently in the other subtypes.

Pathologic Features

The tumors are multifocal, spongy, hemorrhagic, nodules ranging in size from ~5 to 7 cm. Microscopically, the lesions are frequently centered around portal tracts and are composed of bland appearing spindle cells containing hyaline globules and forming slit-like spaces with extravassated red blood cells. Within the liver, the neoplastic cells growth into the sinusoidal spaces at the periphery of the tumor. The tumor cells are positive for endothelial markers (CD31, CD34) and human herpes virus 8 latency associated nuclear antigen-1 (LANA-1).

2 Other Malignant Primary Tumors

Various other tumors including rhabdomyosarcoma, leiomyosarcoma, germ cell tumors, plasmacytoma, fibrosarcoma, liposarcoma, squamous cell carcinoma, rhabdoid tumor, and carcinoid occur very rarely as primary tumors in the liver [21, 22, 25, 43, 103, 116, 117, 151] and shows morphological features similar to those occurring elsewhere in the body. When these tumors are seen in the liver, the possibility of metastasis should be considered and ruled out.

3 Metastatic Tumors

In the United States and Europe, metastatic tumors of the liver are far more common than primary tumors in the non-cirrhotic liver. The liver is considered a fertile soil for metastasis from any tumor, although tumors from some organs such as the colon/rectum, stomach, pancreas, lung and breast, as well as lymphomas, melanomas and sarcomas are the lesions that most frequently will metastasize to the liver [17]. Liver specific metastasis from these tumors is dependent on several factors such as portal-venous drainage, microenvironment, extracellular matrix proteins, cell adhesion molecules, and hepatocyte-derived growth factors [17, 65]. Because of liver specific factors, circulating tumor cells are likely to seed the liver and grow, thus resulting in liver only metastasis. Under these circumstances, surgical therapy, local ablation, regional chemoradiation therapy, or chemo/radioembolization may result in palliation, local tumor growth control or even potential cure [141]. With recent advances in imaging techniques, liver lesions of smaller size that represent both primary tumors and metastatic tumors from known and unknown primaries are increasingly identified. Because of significant difference in the treatment of these various tumors, proper pathological diagnosis and classification are very important. The differentiation of primary tumors from metastatic tumors and proper identification of metastatic tumors is possible based on characteristic gross and microscopic morphological features, and special procedures such as histochemical, immunohistochemical, and ultrastructural studies. In the current practice of surgical pathology, a myriad of immunohistochemical markers are available to help in proper identification of tumors (Table 5). It is important to always include HCC in the differential diagnosis, even without a background of cirrhosis. Thus, immunohistochemical markers such as CK7, CK19, CA19-9, CK20, and CDX2 (colon and other GI primaries), PSA and PSAP (prostatic markers), ER/PR (breast), TTF-1 (lung and thyroid) are useful to determine epithelial tissue of origin. HMB-45, S100, CD34, pankeratin, and CD117 can be helpful when non-epithelial and spindle cell lesions are encountered.

Grossly, metastatic tumors may be single and circumscribed or multiple with poorly defined margins. The cut surface of the tumor is variable depending on the origin of the tumor. Melanomas are usually black, choriocarcinomas are hemorrhagic, and undifferentiated tumors and lymphomas display a “fish-flesh” appearance.

Table 5 Immunohistochemical stains for identifying common primary tumors and specific metastatic tumors to the liver

Tumor	Marker
Hepatocellular carcinoma	CK8, CK18, polyclonal CEA, Hep-Par1, glypican-3, CD34, fibrinogen, α1-antitrypsin, AFP
Cholangiocarcinoma	Pan-keratin, CK7, CK19, CK20, CEA, CA19-9, MOC-31, c-kit, p63
Vascular tumors	Factor VIII-related antigen, CD31, CD34
Choriocarcinoma/germ cell tumors	Pan-keratin, inhibin, human chorionic gonadotropin (HCG), human placental lactogen (HPL)
Lymphoma	Leucocyte common antigen (CD45); CD3, CD4, CD8 (T cell); CD20 and Pax-5 (B cell)
Neuroendocrine tumors	Neuron specific enolase (NSE), chromogranin-A, synaptophysin, CD56, MOC-31
Metastatic colonic adenocarcinoma	CK7, CK20, CDX2
Metastatic breast adenocarcinoma	Pan-keratin, CK7, Gross cystic disease fluid protein (GCDFP-15/BRST-2), estrogen receptor (ER), progesterone receptor (PR), mammoglobin
Metastatic melanoma	S100, Melan-A, HMB-45, MiTF, Mart-1, vimentin
Metastatic prostate carcinoma	Prostate specific antigen (PSA), prostate specific acid phosphatase (PSAP), p501S
Metastatic gastrointestinal stromal tumor (GIST)	CD117, CD34, DOG-1

Microscopically, well and moderately differentiated tumors retain the morphologic features of the primary tumors. Metastatic tumors from the breast and pancreas may incite a desmoplastic reaction and may be difficult to differentiate from cholangiocarcinoma. A sinusoidal growth pattern is commonly seen with small cell carcinoma of lung and occasionally with other tumors. The liver parenchyma adjacent to the metastatic tumors may often show cholestasis, sinusoidal dilatation, and atrophy of liver cell cords as secondary changes.

Some of the more frequently encountered liver metastases include colonic adenocarcinomas, GI and pancreatic neuroendocrine tumors, breast adenocarcinomas and gastrointestinal stromal tumors (GIST) and these entities will be discussed briefly.

3.1 Metastatic Colonic Adenocarcinoma

Approximately half of the patients diagnosed with colon cancer will have liver metastases either at the time of initial diagnosis or as progression of their disease. Resectable liver metastases with a negative resection margin results in 5-year survival rates of 25–40 % in non-randomized studies [3, 35, 39, 49, 125, 131]. Those with initially unresectable metastases may occasionally become candidates

for resection if they have a good response to chemotherapy. For these patients, the 5-year survival rates are similar to those with that had initially resectable disease [85]. These lesions present as large, solitary, or multifocal nodules with central umbilication, and variable amounts of fibrosis, necrosis, and calcification (Fig. 8a). If well or moderately differentiated, the tumor cells may resemble the primary lesion with columnar cells arranged in tubular, papillary, or cribriform patterns. The tumor cells have basophilic cytoplasm, hyperchromatic and elongated nuclei, extensive necrosis and mitosis (Fig. 8b).

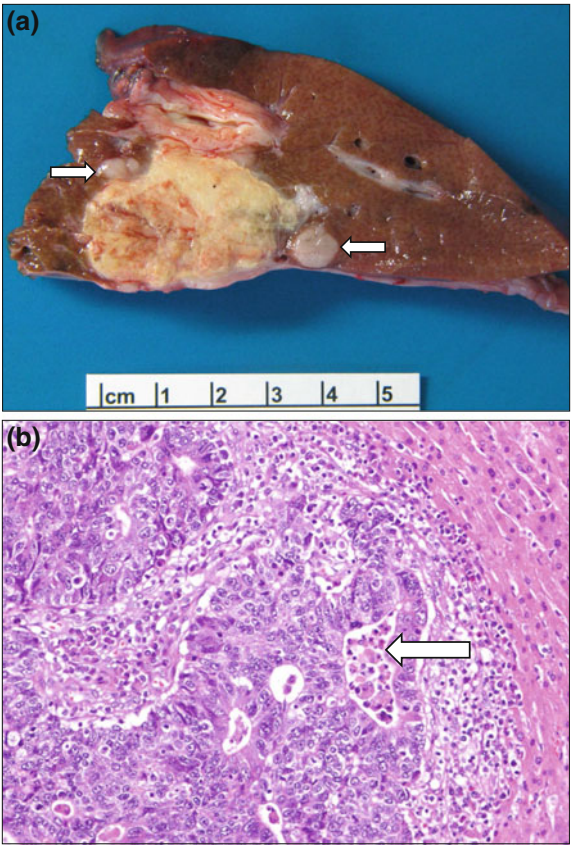

Fig. 8 Metastatic colonic adenocarcinoma. **a** Multifocal, unencapsulated, well-circumscribed, yellow-tan lesions with central umbilication, necrosis and minimal hemorrhage. The smaller satellite lesions (*white arrow*) have a more mucoid appearance. **b** Moderately-differentiated tumor cells forming complex and irregular tubules. The tumor cells have hyperchromatic nuclei that show a loss of polarity and the glands are filled with necrotic debris (*white arrow*). The lesion is associated with inflammatory cells, particularly at edge of tumor

3.2 Metastatic Neuroendocrine Tumor/Carcinoma

Gastrointestinal and pancreatic neuroendocrine tumors (NETs) are rare neoplasms presenting complex challenges to diagnosis and treatment. NETs typically have a protracted course and can produce hormones and/or amines leading to specific clinical signs and symptoms. Once the tumors are metastatic, they are referred to as neuroendocrine carcinomas (NECs). The vast majority of metastatic NECs fall into two nearly distinct categories, those from the gastrointestinal tract, previously referred to as carcinoids, and those from the pancreas which were previously referred to as islet cell tumors. The clinical course of patients with metastatic NEC is quite variable. Some untreated patients remain symptom-free for years, while others have symptomatic disease. With metastatic NEC from the GI tract, the secretion of serotonin and other vasoactive substances can result in carcinoid syndrome which includes flushing, wheezing, diarrhea, and heart failure. Carcinoid syndrome, associated with small bowel and appendiceal NET, almost exclusively occurs in the setting of metastatic liver disease. Treatment for metastatic NETs is based on numerous factors and includes liver resection, ischemic therapy, embolization, chemoembolization, a variety of medical therapies and rarely, liver transplantation. Grossly, these tumors present as numerous are gray to yellow lesions that can range in size from 2 mm to >5 cm (Fig. 9a). Microscopically, the lesion is composed of polygonal cells with granular eosinophilic cytoplasm in an insular, trabecular, glandular, or mixed growth pattern. The nuclei have finely granular chromatin with inconspicuous nucleoli (Fig. 9b).

3.3 Metastatic Breast Adenocarcinoma

Liver metastases in breast cancer patients are an indication of disseminated disease, and as such carries a relatively poor prognosis. However, in a small subgroup of these patients, liver metastases are the only site of distant disease. Long-term survival after chemotherapy and/or radiation is rarely observed, whereas favorable survival rates have been reported after hepatectomy for metastatic breast cancer and recent studies suggest a role for surgical resection in a subset of patients with restricted metastases [2, 29, 121, 129, 136, 159]. Similar to metastatic colonic adenocarcinomas, metastatic breast cancers can be single or multiple. These lesions, however, tend to have less necrosis and hemorrhage when compared to those metastatic from the colon (Fig. 10a). Microscopically, the tumor cells often resemble the primary breast tumor (Fig. 10b) with corresponding immunohistochemical profile (Fig. 10c and d).

3.4 Metastatic Gastrointestinal Stromal Tumor (GIST)

GISTs arise from interstitial cell of Cajal, which are involved in the pacemaker activity of the gut and represent the most common mesenchymal tumors in the gastrointestinal tract. These lesions were originally described to be of neural origin

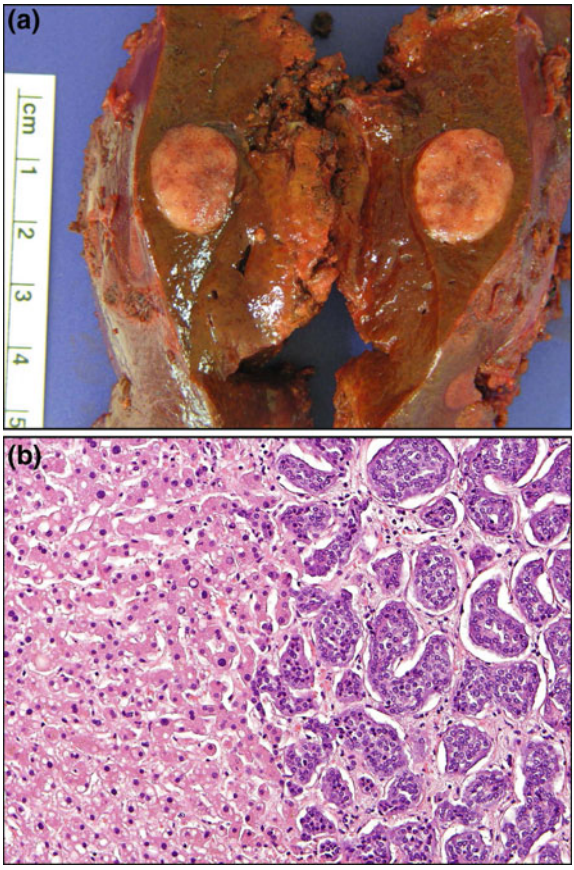

Fig. 9 Metastatic neuroendocrine carcinoma. **a** Single, well delineated, pink-tan, homogenous tumor without a well-defined capsule. No hemorrhage or necrosis is appreciated. **b** Nests of polygonal cells with moderate eosinophilic. The tumor cells are arranged in a trabecular pattern and demonstrate "salt and pepper" chromatin

in 1983 [92] and reflect a specific tumor type which harbors an activating mutation in one of the receptor protein tyrosine kinases, either KIT (CD117) or platelet-derived growth factor receptor alpha (PDGFRA). These tumors can occur throughout the entire gastrointestinal tract, however, the most common location is the stomach, while those in the esophagus are exceedingly rare. The biological behavior and metastatic potential of these tumors are quite variable and may be difficult to predict in some cases based on clinicopathologic features. However, several factors including anatomic site, tumor size, mitotic rate, tumor cell morphology, and exon-specific KIT mutations are currently used to determine prognosis. Recent molecular studies have suggested that the status of *c-KIT* or *PDGFRA* mutations may be helpful to predict response to selective tyrosine kinase inhibitor therapy (imatinib and/or sunitinib) in primary and metastatic GISTs.

For recurrent or metastatic GIST, the standard treatment is imatinib. Since recurrence and/or metastasis frequently occur within 2 years of starting imatinib, surgery has been added to management of patients with metastatic GIST to delay/prevent recurrence. One study examining surgery after imatinib therapy on

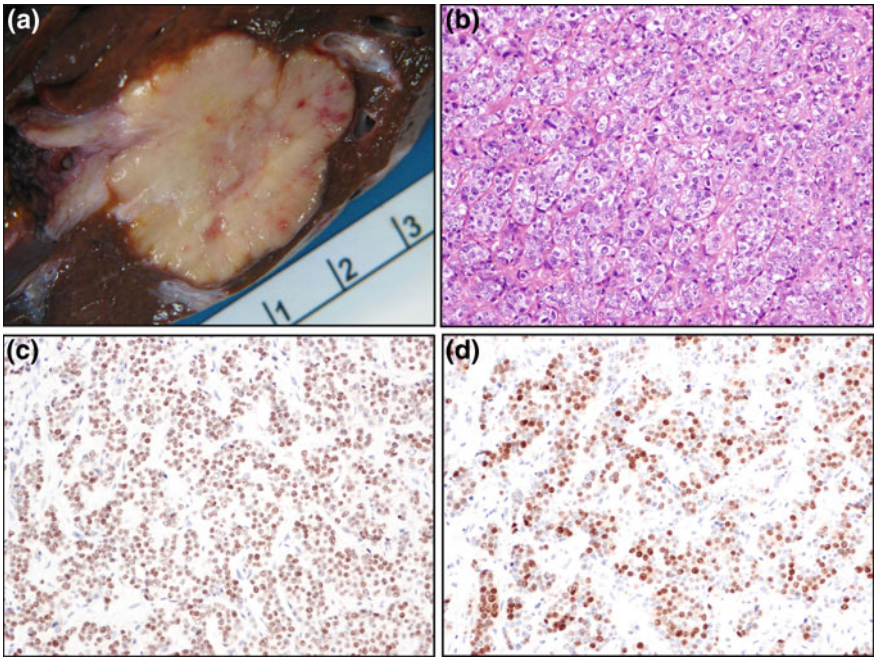

Fig. 10 Metastatic breast adenocarcinoma. **a** Unencapsulated, well-circumscribed, white-tan lesion with central umbilication. **b** The tumor cells are arranged in a nested growth pattern and exhibit marked pleomorphism. The tumor cells have a slightly vacuolated cytoplasm and the nuclei have a finely granular chromatin with multiple nucleoli. The tumor cells stain positively for estrogen receptor **c** and progesterone receptor **d**

the impact of survival for advanced GIST found that survival correlated with initial response to imatinib [122]. Unfortunately, the benefit of this treatment modality is anecdotal and has not been proven in randomized clinical trials. Current guidelines suggest that cytoreductive surgery in metastatic GIST should be considered when gross metastatic tumor resection is possible and the disease is stable/responsive to imatinib therapy, if there is an isolated progression while on imatinib therapy after an initial response, or if hemorrhage, perforation, obstruction, or abscess have or may be expected to occur [26]. Radiofrequency ablation (RFA), hepatic artery embolization, and liver transplantation are other alternative options for treating liver metastases.

Pathologic Features

Grossly, the lesion is multifocal, unencapsulated, but well circumscribed with either a whorled cut surface or a blue-white mucoid appearance (Fig. 11a). Large lesions can exhibit areas of necrosis and cystic degeneration. Histologically, the cells can be spindled, epithelioid, or mixed. Spindled cells have a pale to eosinophilic fibrillar cytoplasm with minimal nuclear pleomorphism in a storiform or fascicular

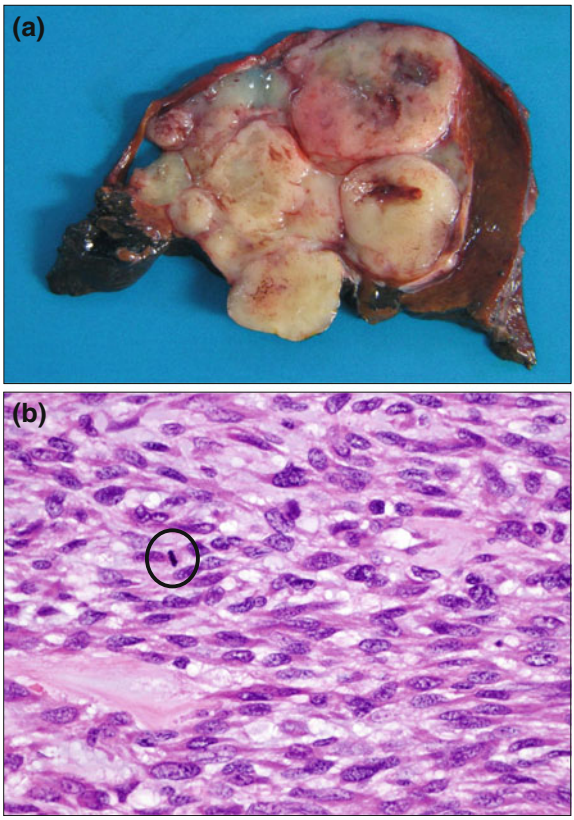

Fig. 11 Metastatic gastrointestinal stromal tumor (GIST). **a** A multinodular, unencapsulated lesion with a tan, mucoid appearance and focal cystic degeneration and hemorrhage. **b** Plump, spindle cells with fibrillary eosinophilic cytoplasm in a myxoid and focally hyalinized stroma. The cells exhibit perinuclear vacuoles and mitosis is identified (*black circle*)

pattern (Fig. 11b). The lesion may be extremely cellular or paucicellular with a myxoid background. The nuclei are oval with fine chromatin and inconspicuous nucleoli, prominent pallisading, perinuclear vacuoles that indent the nucleus, and extensive stromal hyalinization. The epithelioid cells have a round to polygonal shape with a condensed rim of eosinophilic cytoplasm adjacent to the nucleus and peripheral cytoplasmic clearing, well-defined cell membranes with round nuclei and small nucleoli. Epithelioid cells may commonly exhibit bi- or multinucleation with more significant atypia when compared to the spindle cell GIST. In the mixed pattern, epithelioid cells are interspersed with plump spindled cells of similar size and nuclear characteristic. Greater than 90 % of GISTS are immunohistochemically CD117/c-kit positive. Other commonly expressed markers include DOG1, CD34, SMA, desmin, and S100 protein and the immunophenotype is dependent on the anatomic location.

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Robotic Rectal Cancer Surgery

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Abstract

There are an estimated 39,000 new cases of rectal cancer in the United States per year which makes it the third most prevalent cancer when paired with colon cancer. Given its complexity, there are now multiple modalities available for its successful treatment. This includes innovative chemotherapy, radiation, transanal resection techniques, and minimally invasive surgery. Robotic surgery for the treatment of rectal cancer represents the current pinnacle of minimally invasive technology for this disease process.

Keywords

Robotic surgery · Minimally invasive surgery · Rectal cancer

1 Background

There are an estimated 39,000 new cases of rectal cancer in the United States per year which makes it the third most prevalent cancer when paired with colon cancer [1]. Given its complexity, there are now multiple modalities available for its successful treatment. This includes innovative chemotherapy, radiation, transanal resection techniques, and minimally invasive surgery. Robotic surgery for the treatment of rectal cancer represents the current pinnacle of minimally invasive technology for this disease process. However, this has been an evolution in technique spanning more than two decades. The first step was to investigate laparoscopic surgery for colon cancer and ensure its safety and reproducibility. This was

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accomplished through multiple randomized controlled trials in the mid-2000s including the COST, COLOR, and CLASICC trials [2–4]. The end result was that laparoscopic surgery produces equivalent outcomes when compared to open surgery for colon cancer. The next step was to prove that the outcomes are the same when using laparoscopic surgery for rectal cancer. The first randomized controlled trial to report outcomes was the MRC CLASICC trial from the United Kingdom [5]. Although not significant, the circumferential margin was positive in 12 % versus 6 % of all the open cases. These results gave surgeons pause about the laparoscopic method, and opened the opportunity for newer methods including robotic surgery.

The robot was designed to offer superior views and maneuvering in tight spaces. Therefore it was a natural match for the pelvis and urologists and gynecologists were the first to incorporate its use into widespread practice. Colorectal surgeons lagged behind in the use of the robot. The first robotic rectal surgeries were first reported around 2004 [6]. The next decade witnessed the evolution of robotic rectal surgery for cancer. This began with the perfection of technique, optimization of docking methods, and advancement of the robot technology. Numerous single institution comparative studies followed; the majority of which showed favorable outcomes with the robot. This brings us to the present day where the field is rapidly expanding. The most recent numbers suggest around 50 % of colorectal procedures are done through a minimally invasive approach [7]. The newest robotic platform, the Da Vinci Xi (Intuitive Surgical, Sunnyvale, CA), has made the docking process much easier. Finally, two randomized trials comparing laparoscopic to robotic rectal cancer surgeries are just closing [8, 9]. The results of these along with surgeons' acceptance and long-term cost analyses will determine the future of robotic rectal surgery. This review will discuss the surgical technique behind robotic surgery followed by data on short-term outcomes, long-term outcomes, the surgeon learning curve, and finally costs involved in robotic rectal surgery.

2 Surgical Technique

Robotic surgery was incorporated into rectal surgery to overcome several limitations that were present in laparoscopic and open surgery. The biggest problem with open surgery is the limited visualization especially in a long narrow pelvis. Laparoscopic surgery is able to magnify the field and look into small spaces, but its largest fallibility is the limited reach of straight instruments deep in the pelvis. The robot offers several advantages. Increased magnification and 3D views make the field easier to see. The robot also delivers seven ranges of motion which allows the user to more reliably reproduce the wrist motions necessary to work deep in the pelvis. Finally, the motion scaling of the robot allows for more fine tuned movements in small spaces.

Numerous techniques have been described for using the robot for rectal surgery. These all vary based on three criteria. (1) The model of robot used, (2) The amount of laparoscopic surgery used in conjunction with the robot, and (3) The method of

specimen extraction. However, at its core, a robotic rectal cancer surgery follows the same principles as an open or laparoscopic procedure. The procedure begins with the patient in low lithotomy position. The patient will need some form of immobilization (bean bag, shoulder supports, tape) as the patient will need to be rotated into steep Trendelenberg and tilted toward the right. This is essential in keeping the small bowel out of the field. Ports are then placed based on the model being used and the surgeon's preference. The newest robot models have a camera and three arms. One to two additional ports are placed in the right upper quadrant for the assistant to provide retraction and suction. For a total robotic procedure, the robot is then docked and the colon and splenic flexure are mobilized. The Xi model allows for a single docking along the side of the patient, while the S models require multiple docks [10, 11]. The mobilization can also be performed laparoscopically as a hybrid approach [12].

The next step is the total mesorectal excision (TME) which represents the gold standard in optimal rectal surgery technique [13]. If not already docked, the robot will be brought in along the side of the patient or between the legs. The assistant provides retraction of the colon out of the pelvis while the excision is performed using a monopolar and bipolar instrument in each hand. The third arm provides extra retraction on the rectum or the bladder/uterus. Once down to the pelvic floor, the rectum is then divided. The newer models have robotic staplers however endoscopic ones placed through the right lower quadrant port work as well. The third option is a small Pfannenstiel incision and open stapling. The specimen is then removed via a Pfannenstiel incision, left lower quadrant incision, the proposed ileostomy site, or through a natural orifice (anus/vagina) [14]. The anastomosis is completed transanally using the standard techniques.

3 Short-Term Outcomes

3.1 Operative Characteristics

Key intraoperative findings for a successful surgery include operative time and blood loss. Earlier studies of robotic proctectomies found operative times from 180 to 360 min in length [15]. The values can be broken down into specific robot parameters, namely console time and docking time. Hara described a total time of 270 min which encompassed 135 min of console time (the time operating the robot at the surgeon console) and 5 min of docking time (the time to attach and detach the instruments in and on the patient) [16]. Total robotic procedures will meet with a much higher console time. Docking times can be minimized with team experience. Robotic operative times are significantly higher when compared to open surgery. This has been validated in three comparative studies where the average robotic time was 239 min versus 180 open [17–19]. However, when comparing robotic operative time to laparoscopic operative time, there is more variability. There are several studies where the robotic time is on average 58 min longer, but the majority of studies do not find a significant difference in time [17, 20, 21]. One recent

meta-analysis of eight studies found the robot to add 21 min when compared to laparoscopic surgery, but the result was not significant [22].

Intraoperative blood loss has been reported much more infrequently in the literature compared to operative time. Given this, there is much more variability in the data. Blood loss has been reported between 59 cc up to 283 cc [15, 23]. Only one open versus robot comparative study looked at blood loss and found it to be significantly lower in the robotic group, 187 versus 273 cc [24]. This was validated in a meta-analysis of 7 studies where robot use resulted in a reduction of blood loss by 47 cc [25]. However, multiple other studies have failed to find any significant differences [17, 26, 27]. The most important finding in terms of blood loss is that overall, regardless of approach, this is a relatively bloodless surgery and the numbers are low compared to other more complex surgeries.

The final intraoperative outcome is conversion. One main theoretical advantage of the robot is that it can get to places where laparoscopic surgery cannot and therefore conversions should be reduced. This is supported by the literature for the most part. A systematic review from 2012 found only one conversion in 241 robotic rectal surgeries across 19 studies [28]. A multicenter study showed a conversion rate of 4.9 % among three centers [29]. Indeed, when laparoscopic proctectomy is compared to robotic, there is a significantly lower rate of conversion in the majority of studies. For example, a 2015 comparison of 133 robotic to 84 laparoscopic procedures found 0 robotic versus 6 laparoscopic conversions [27]. This is supported by a meta-analysis where an odds ratio of 0.25 in favor of robotic conversions compared to laparoscopic was reported [22]. This significant result is seen in three other meta-analyses as well [25, 30, 31]. The data above do suggest that conversion rate is lower in robotic versus laparoscopic rectal surgery.

3.2 Postoperative Characteristics

The main advantages seen when laparoscopic colon surgery was compared to open was a reduced hospital stay and a quicker return to bowel function [32]. The robotic studies have attempted to answer these questions as well. The hospital stay for a robotic rectal procedure lasts anywhere from 4 to 11 days [15]. One study does show a reduced length of stay when using the robot compared to open, 10 versus 12 days [33]. This should be expected; however, the more interesting question is if the same holds true when comparing laparoscopic to robotic surgery? There are four studies which all show a hospital stay reduction of about one to two days [17, 26, 27, 34]. However, when the data was pooled into four separate meta-analyses, there was no significant difference in overall length of stay [22, 25, 30, 31]. There is only one randomized controlled trial to date to compare robotic and laparoscopic rectal cancer surgery. Eighteen patients were randomized to each group. The only significant finding in this paper was a reduced length of stay for the robotic group, 6.9 versus 8.7 days [35].

Return of bowel function can be measured in several different ways. This can be first passage of flatus, first bowel movement, or first time tolerating regular diet.

This data is harder to pool as each study will vary with their measurements. For this reason, only two meta-analyses report this data and both showed no difference in passage of flatus when comparing robot to laparoscopic rectal surgery [25, 30]. Another complicating factor when measuring this field is the presence of a diverting ileostomy which is often placed as part of a rectal cancer surgery. For example, Bianchi found bowel movements occurring one day sooner with robotic procedures; however, they also had two times as many diverting ileostomies, 10 versus 5 [36].

The final postoperative outcome is complications. Rectal cancer surgery is a morbid procedure with postoperative complications averaging 39 % in large trials [37]. The most dreaded complication is the anastomotic leak, however, other common ones include, intrabdominal abscess, wound infection, ileus, and cardiac/pulmonary complications. Fortunately, mortality is much less with rectal surgery averaging 1 % [37]. There has not been any evidence to show any difference when comparing robotic surgery to open or laparoscopic surgery [19, 25]. This should be expected as all three operations expose the patient to the same risks for developing complications. As the robot works in a narrow field and requires the passage of multiple instruments, it has been suggested the accidental intestinal perforation rate to be higher with the use of a robot. This does not appear to be significantly higher when compared to laparoscopic surgery and is a very rare complication overall [36].

3.3 Pathologic Characteristics

Although the robot is used for benign rectal disease, including rectal prolapse and inflammatory bowel disease, its main draw is the ability to perform an optimal rectal cancer surgery. The increased visibility and freedom of movement will hopefully lead to a more complete oncologic resection. This has been studied via three variables: number of lymph nodes harvested, circumferential resection margin (CRM), and distal resection margin (DRM).

The robot is able to remove a sufficient number of lymph nodes in the rectal cancer specimen. Lymph node harvest numbers vary from 13 up to 20 nodes in various studies [15]. However, when comparing the harvest to open or laparoscopic surgery, there is no significant difference in the number of nodes retrieved in the majority of studies published. Park most recently reported obtaining 16 lymph nodes in robotic as well as laparoscopic resections [27]. This should make sense though as the optimal treatment for rectal cancer is the TME. For this reason, all lymph nodes should remain within the mesorectal envelope and it should not matter how the mesorectum is removed. The most important variable should be the circumferential margin which is a surrogate for a complete TME.

The CRM is a marker of a complete mesorectal excision. It is often noted to be positive if it less than 1 mm from the margin. For smaller cancers, this is a meaningful measurement for completeness of resection. However, when the tumor becomes large, it grows into and beyond the normal anatomic planes, and the CRM will always be positive regardless of the quality of surgery. The CRM is widely

reported in the literature when describing robotic rectal surgery. There are no differences in CRM when comparing robotic to open rectal cancer resections [19, 24]. This also holds true for the robotic versus the laparoscopic approach based off of several meta-analyses [22, 25]. There is only one study in the literature which refuted this and did find robot use leading to fewer positive CRMs. D'Annibale reported 50 case-matched operations and found no positive CRMs in the robot group versus six positive ones in the laparoscopic group. However, the definition for a positive CRM was 2 mm rather than the more standard 1 mm [17]. Another way to describe the completeness of resection is to grade the quality of the TME. This is often described as completely intact, partially intact, or incomplete [38]. These numbers were reported in two studies. Shiomi described 113 consecutive robotic proctectomies and was able to retrieve a completely intact specimen in all 113 [39]. Baik was able to compare TME specimens from robotic and laparoscopic specimens and found significantly more complete resections in the robotic group, 93 % versus 75 % [34].

The DRM is the final pathologic outcome. This number is also somewhat unclear when describing a good operation. This is because the length of the margin not only depends on the amount of dissection but also the distance of the tumor from the sphincter complex. However, the margin should never be positive as this would mandate an abdominal perineal resection. Fortunately, current studies do not report any positive distal margins and instead report the distal margin length. In several robotic versus open rectal resection studies, the DRM was significantly longer in the robotic specimen, 2.7 cm versus 1.9 cm and 2.8 cm versus 2.3 cm in another study [19, 33]. While the results are significant, it is difficult to determine if the extra 0.5–0.9 cm is meaningful long term. The DRM is reported in many laparoscopic versus robotic rectal resection studies and there is no difference seen among the groups [22, 25, 31].

4 Long-Term Outcomes

4.1 Survival

As the robotic platform enters its second decade of use for rectal surgery, more studies are now reporting on long-term oncologic outcomes. The most recent randomized trial on laparoscopic rectal cancer surgery, COLOR II, revealed 3 year disease free survival rates (DFS) of 74 % and overall survival (OS) of 86 % in the laparoscopic rectal cancer resections. This is comparable to the similar rates seen with open surgery in that trial, 70 % DFS and 83 % OS [40]. The next phase of robotic rectal cancer research sought to see if long-term oncologic outcomes would compare to these rates.

There are multiple single institution studies which have reported on survival rates. Local recurrence rates at 3 years varied between 3.6 and 4 % [39, 41, 42]. Two of these studies looked at survival at three years and found DFS at 79 % in both studies, while the OS was 90 and 93 % in the same studies. A multi-institutional report from

2010 found three year DFS to be 77 % and OS at 97 % [29]. Finally, Hara examined five year data from robot rectal surgery and found DFS to be 81 % and OS to be 92 % [16]. Survival rates are just now being reported in comparative studies. Park in 2015 compared oncologic outcomes in robotic versus laparoscopic rectal cancer surgery and found no significant differences in all three variables at five years. Local recurrence was 2.3 % in the robot group and 1.2 % in the laparoscopic group. DFS was 81 and 78 % while OS was 92 and 93 % in the respective groups. The surgery technique was found not to decrease survival on univariate analysis [27]. Another study validated these numbers with no significant difference in survival rates at three years. This study only looked at ultra low anterior resections [26]. Based on all data available to date, robotic rectal surgery produces comparable long-term oncologic outcomes compared to open or laparoscopic surgery.

4.2 Sexual and Urinary Function

Sexual and urinary dysfunction is a large problem after a rectal cancer resection. The majority of patients will experience at least temporary dysfunction of the reproductive and urinary systems. Meticulous technique is required to identify and avoid injury to the main nerves serving these functions. This potential problem is injury to the hypogastric nerves which lie near the aortic bifurcation and can be injured during the ligation of the inferior mesenteric artery. The second location of injury is low in the pelvis during the lateral dissection of the mesorectum where the nervi erigentes and pelvic plexus are located. The final area of potential injury are the cavernous nerves which run anterior close to the prostate deep in the pelvis [43]. Injury to any of these nerves can lead to problems with erectile dysfunction, ejaculatory problems, and bladder voiding problems. Large trials have yet to show any advantage of laparoscopic surgery in being able to reduce this dysfunction [44].

There have been several studies which have specifically looked at sexual and urinary dysfunction with robotic rectal surgery. Questionnaires sent to 74 patients undergoing robotic resections found sexual satisfaction decreased significantly at one month in both men and women, however, it returned to baseline by one year. In addition, urinary complaints were unchanged at one year [45]. A significant potential advantage of robotic surgery is its ability to better visualize and avoid the nerve bundles as compared to laparoscopic surgery. Sexual and urinary function has been studied in several reports comparing robotic and laparoscopic surgery. Sexual function has been measured by the international index of erectile function (IIEF) score, while urinary function was measured with the international prostate symptom score (IPSS). Park found no difference in IPSS scores, but there was a significant difference in IIEF scores at six months where 85 % of men still complained of moderate to severe erectile dysfunction in the laparoscopic groups as compared to 49 % in the robotic group [46]. Kim also investigated this in 2012 and found the urinary function took three months to return to normal in the robotic group as opposed to six months in the laparoscopic group. Reduced scores were also seen in sexual function where the IIEF did not return to normal in the robotic group until

six months versus 12 months in the laparoscopic group [47]. While both these studies suggest the robot provides an advantage in this regard, the study samples were small with 20–40 patients in each cohort. To help bolster these numbers, one meta-analysis has been performed to look at sexual and urinary function [48]. Four studies were included which resulted in 152 and 161 patients in the robot and laparoscopic groups, respectively. The final analysis did favor robotic surgery over laparoscopic surgery in improved IIEF and IPSS scores over the postoperative period. Although the data is still small, robotic surgery does appear to offer superior results in terms of sexual and urinary function in rectal cancer surgery.

5 Learning Curve

The ability to master a new technique has many implications for the surgeon, the patient, and the credentialing institution. Therefore, there has been much research into the appropriate learning curve for robotic rectal surgery. The learning curve is complex as it requires the synthesis of two different advanced procedures. Before attempting any robotic rectal surgery, the surgeon must be comfortable and have mastered the TME technique. In order for any rectal cancer surgery to be successful, this first must be accomplished. This is often accomplished through open surgery during an advanced fellowship. The next step is to become familiar with the robot and how it works. Some people recommend starting with more straightforward surgeries (i.e., right colectomies) until one is comfortable with the robot controls. The final step is to merge the two into robotic rectal surgery. The one caveat to this is that there must be appropriate volume. This surgery requires continual practice which is greatly aided in a steady supply of rectal cancer patients. In fact, it has been shown that complications, length of stay, and cost are significantly higher for low volume robotic colorectal surgeons as compared to average or high volume surgeons [49]. In this 2013 study by Keller, low volume was classified as less than 5 procedures during an 18 month period which included robotic colon and rectal surgery, while high volume was greater than 15 procedures. Complication rates were 15 % with high volume surgeons as opposed to 26 % for low volume surgeons. Unfortunately this is a hard problem to correct as the number of high volume surgeons is low. Only 16 % of the cases were performed by high volume surgeons.

The actual learning curve, or number of cases required to master the procedure, has been defined by several different studies. The majority of the studies use the cumulative sum (CUSUM) method to determine milestones in the learning curve. The CUSUM is a sequential analysis technique which detects deviations in a progressive curve. It has been validated as a viable determinant for acquisition of surgical skills [50]. The CUSUM uses the operative time as the determinant of proficiency. In the case of robotic skills, it is the surgeon console time used rather than the total operative time. Bokhari found the initial proficiency to take place after 10 cases [51]. While, Sng found the initial learning curve to be longer at 35 cases

[52]. A systematic review from 2014 found six studies which investigated the learning curve and found the number to vary from 15 to 30 cases [53]. The next phase is a mastery of the robotic surgery which represents the next phase of the CUSUM analysis. The Bokhari study noted this to be at 25 cases, while Sng again was longer at 128 cases. Of note, while surgeon proficiency takes some time, the operative team performing a robotic rectal surgery attains proficiency much quicker. This was measured by docking time by Sng. They found that after 35 cases the docking had hit optimal performance [52].

Finally, learning curves have been directly compared between laparoscopic and robotic rectal surgery. Eighty-nine patients in each group were analyzed through the CUSUM method and the results showed similar learning curves for laparoscopic, 41 cases, and robotic surgery, 44 cases [54]. The one downside to all of these studies is that it represents the pioneers in robotic rectal surgery. The typical surgeon in these studies was already proficient in laparoscopic rectal surgery and was actively advancing the field of robotic rectal surgery. As more studies come out, it will be more revealing to see what the learning curve is for the novice robotic colorectal surgeon.

6 Cost

One of the largest determinants in the long-term viability of robotic rectal surgery is the cost associated with performing the procedure. As with any new technology, the robot is expensive. The cost of a single unit is about $2 Million. It also costs about $200,000 per year to maintain. Instruments are reusable but expire after about 10 uses [55]. It is these factors which increase the cost of using the robot. The largest expense with laparoscopic surgery is the consumables, while in robotic surgery it is the cost of the operation and equipment which make up 60 % of the total cost [56]. In addition to this, there is no extra income received for a robotic procedure. There is no CPT code for robotic colectomy, instead it is coded using the laparoscopy code 44207 (Laparoscopy, surgical; colectomy, partial, with anastomosis, with coloproctostomy) [57]. In Korea, the national insurance does not pay extra for the robotic procedure. In one study, patients were counseled preoperatively that in order to undergo a robotic procedure versus laparoscopic one, they would pay an extra $6000 US [27].

Every study which has examined cost has shown the robotic approach to be more expensive for rectal cancer surgery versus laparoscopic surgery. Baek found the robot procedure to cost more, $14,467 versus $9978. The payments to the hospital were also significantly more; $11,540 versus $3956, however, the overall profit was significantly less for the robot versus laparoscopic rectal surgery, $689 versus $1671 [56]. This was confirmed later by Park where the mean cost was $12,742 for robot versus $10,101 for laparoscopic rectal surgery in Korea [27]. A small meta-analysis of three studies also found robotic rectal surgery to cost

significantly more than laparoscopic surgery [25]. Finally, Keller looked at a national inpatient database and compared costs for robotic rectal surgery versus laparoscopic surgery in the United States. As in the Korean studies, the robotic surgery cost significantly more, $23,810 versus 17,530 [55].

7 Summary and Future Directions

Robotic surgery remains at the forefront of surgical technology for rectal cancer. The tight confines of the deep pelvis make the robot a good match for rectal surgery. The robot may be used to do the entire procedure or it may be paired with laparoscopic surgery in a hybrid approach. It has also been shown to allow for more natural orifice specimen extractions (transanal or transvaginal). The robot has a learning curve as with most other surgeries which ends up falling in the 30 case ranges. The robot has been shown to decrease conversions as compared to laparoscopic surgery in the majority of studies. It has also been shown to decrease the amount of postoperative sexual and urinary dysfunction. However, the robot takes significantly longer to perform in most studies, and it also costs significantly more money to perform. In all other respects, it is equivalent to laparoscopic surgery. Hospital stay and return of bowel function are nearly the same. Pathologic outcomes including lymph node harvest and CRM are similar. Finally, all long-term survival data show no difference between the robot and the laparoscopic method. Some of the major studies cited in this chapter are summarized in Table 1.

The future of robotic rectal cancer surgery is still uncertain. There are two major randomized controlled trials which are just about completed. The ROLARR trial is a worldwide, 12 center, study comparing robotic rectal cancer surgery to laparoscopic surgery. It is the first and only large study to do this [9]. The ACOSOG Z6051 study is also almost completed [8]. This is another randomized controlled trial in the United States looking at minimally invasive approaches versus open surgery for rectal cancer. The minimally invasive arm does allow for robotic surgery. Results from these studies will help better determine the role of the robot. As the medical payment structures are vastly changing and the nation is becoming more cost conscious about medical care, the increased cost and time required to perform robotic surgery will be under increasing scrutiny. The final aspect driving the future of robotic rectal surgery is acceptance from the surgical community. While there are many surgeons who have accepted and incorporated the robot into their practice, there are also many opponents. The most recent edition of the American Society of Colon and Rectal Surgeons textbook cautions the use of the robot and views it as a crutch for more inexperienced surgeons who cannot perform a laparoscopic resection [58]. The counterargument to this would be that the robot broadens the access for patients to undergo a quality minimally invasive rectal cancer surgery rather than restrict care to a select few advanced laparoscopic surgeons. In conclusion, robot rectal cancer surgery is a rapidly advancing field with an exciting but uncertain future.

Table 1 Selected comparative rectal surgery studies

References	Surgery type	# of patients	OR time (min)	EBL (cc)	Conversions (%)	LOS (days)	Time to flatus (days)	Complications (%)	Lymph nodes	CRM+	DRM (cm)
deSouza [24]	Robot	36	337	187	0	7.0	NR	30	15	0	NR
	Open	46	273	273	NA	7.3	NR	32	16	3	NR
Kim et al. [19]	Robot	100	188	NR	0	7.1	2.1	11	20	1	2.7
	Open	100	103	NR	NA	6.9	3.1	14	19	1	1.9
Kim [18]	Robot	21	197	NR	0	7.6	2.1	19	20	0	NA
	Open	27	161	NR	NA	7.7	2.8	33	16	4	NA
Park et al. [33]	Robot	52	232	NR	0	10.4	3.2	19	19	1	2.8
	Open	88	233	NR	NA	12.8	4.4	20	18	2	2.3
	Lap	123	158	NR	0	9.8	3.0	12	15	3	3.2
Baek et al. [26]	Robot	47	352	190	2	9.0	NR	19	10	1	1.1
	Lap	37	360	302	16	11.0	NR	27	14	3	1.6
Baik et al. [34]	Robot	56	190	NR	0	5.7	1.9	10	18	4	4.0
	Lap	57	191	NR	10	7.6	2.1	19	18	5	3.6
Bianchi et al. [36]	Robot	25	240	NR	0	6.5	NR	16	18	0	NR
	Lap	25	237	NR	4	6.0	NR	24	17	1	NR
D'Annibale et al. [17]	Robot	50	270	NR	0	8.0	NR	5	16	0	3.0
	Lap	50	280	NR	12	10.0	NR	11	13	6	3.0
Park et al. [20]	Robot	41	231	NR	0	9.9	2.9	29	17	2	2.1
	Lap	82	168	NR	0	9.4	2.7	23	14	3	2.3
Park et al. [23]	Robot	40	235	45	0	10.6	2.4	15	12	3	1.4
	Lap	40	185	59	0	11.3	2.5	12	13	2	1.3
Park et al. [27]	Robot	133	205	77	0	5.8	2.4	7	16	9	2.7
	Lap	84	208	82	7	6.5	2.4	9	16	6	2.8
Tam et al. [21]	Robot	165	228	NR	7	4.5	NR	12	NR	NR	NR
	Lap	369	167	NR	21	4.7	NR	8	NR	NR	NR

EBL Estimated blood loss, *LOS* Length of stay, *CRM+* Involved circumferential resection margin, *DRM* Distal resection margin, *NA* Not applicable, *NR* Not reported

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Pathologic Features of Primary Colon, Rectal, and Anal Malignancies

Kaitlin E. Sundling, Ranran Zhang and Kristina A. Matkowskyj

Abstract

In the United States, colorectal cancer is the third most commonly diagnosed cancer in both men and women, as well as the third leading cause of cancer deaths (Colorectal cancer facts & figures 2014–2016, 2014 [2]). Worldwide, colorectal cancer is the fourth leading cause of death and causes almost 700,000 deaths each year (Cancer: fact sheet No. 297, 2015 [55]). This chapter discusses the clinical and pathologic features of the spectrum of epithelial, hematolymphoid, and mesenchymal malignant tumors of the colon, rectum, appendix, and anus.

Keywords

Tubular adenoma · Sessile serrated adenoma · Traditional serrated adenoma · Conventional colorectal adenocarcinoma · Medullary carcinoma · Hereditary colon cancer syndromes · Neuroendocrine tumors · Lymphomas · Gastrointestinal stromal tumors · Appendiceal neoplasms · Anal carcinoma

In the United States, colorectal cancer is the third most commonly diagnosed cancer in both men and women, as well as the third leading cause of cancer deaths [2]. Worldwide, colorectal cancer is the fourth leading cause of death and causes almost 700,000 deaths each year [55]. Fortunately, due to the colon's surgical and endoscopic

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accessibility and functional redundancy, colorectal cancer is very treatable. Colonoscopic surveillance has the potential to not only providing tissue for the diagnosis of precancerous polyps and invasive carcinoma, but also to prevent development of invasive carcinoma by the removal of precancerous lesions. This chapter discusses the clinical and pathologic features of the spectrum of epithelial, hematolymphoid, and mesenchymal malignant tumors of the colon, rectum, appendix, and anus.

1 Conventional Colorectal Adenocarinoma

Ninety to 95 % of colorectal cancers are adenocarcinomas, originating from the epithelial cells of the colonic crypts [21]. There is a 5 % lifetime risk of developing colorectal cancer, and most colon cancers occur in people over the age of 50 [2, 8].

1.1 Risk Factors for Colorectal Cancer

A number of risk factors have been identified that affect a patient's risk for developing colorectal cancer. Family history of colorectal cancer is a significant risk factor, and ten to thirty percent of patients with colorectal cancer have a positive family history, without an identifiable hereditary cancer syndrome [33]. Inflammatory bowel disease is an important risk factor for colorectal cancer, with cancer risk increasing with both duration of colitis and the severity of the inflammation. This suggests a possible link between colorectal cancer and inflammation [44]. Increased surveillance is recommended for patients with inflammatory bowel disease, and the distinction between dysplasia related to polypoid and flat lesions is important for consideration of treatment options (continued surveillance vs. proctocolectomy). Diets high in red meat and low in fruits, vegetables, and fiber are associated with increased risk of colorectal cancer, suggesting a possible role for gut bacterial interactions in colorectal cancer pathogenesis [49]. Additional risk factors include lack of physical activity, obesity, tobacco use, and alcohol use [8].

1.2 Precursor Lesions of the Colon and Rectum

Given that more than 95 % of colorectal adenocarcinomas arise from precursor polyps, the diagnosis of these polyps after colonoscopic removal plays an important role in colorectal cancer risk stratification and determination of screening intervals [6].

1.2.1 Tubular Adenoma (Conventional Dysplasia)

Tubular adenomas range in size from subcentimeter to several centimeters in size and can be sessile, raised, or pedunculated. Microscopically, these dysplastic polyps reveal cigar-shaped, pseudostratified nuclei with hyperchromatic nuclei and clumped chromatin. There is often depletion of the surface mucin, resulting in an overall blue appearance at low power (Fig. 1a). Adenomatous polyps can develop

areas of elongated parallel crypts, described as tubulovillous adenomas or villous adenomas depending on the fraction of villous component. This is particularly common in larger polyps. The risk of finding invasive carcinoma within a tubular adenoma increases with the size of the polyp. Areas of back-to-back crypts without intervening lamina propria and with complete loss of cellular polarity indicate high-grade dysplasia and should prompt detailed evaluation of the specimen for invasive carcinoma. On the molecular level, the hallmark of these precancerous polyps is *APC* mutation, which occurs somatically in contrast to the germline mutations seen in patients with familial adenomatous polyposis (FAP) [18].

1.2.2 Sessile Serrated Polyp/Adenoma

Sessile serrated adenomas (also known as sessile serrated polyps and thus referred to as sessile serrated polyp/adenoma) are predominantly right-sided lesions and as the name suggests, are often flat, sessile lesions. Microscopically, sessile serrated adenomas show serrations involving the base of the crypts, with widened, boot-shaped, and bifurcating crypt bases (Fig. 1b). There is typically no cytologic dysplasia, although the presence of tubular adenoma-like dysplasia may represent progression of the lesion. These lesions can develop into microsatellite instability-high (MSI-H) colorectal carcinomas, some of which may maintain their serrated morphology (serrated carcinomas). Polyps of this type may progress much more rapidly than tubular adenomas, necessitating shorter follow-up intervals [38].

1.2.3 Traditional Serrated Adenomas

Traditional serrated adenomas are flat or villous polyps with slit-like serrations and pink cytoplasm. The nuclei are crowded but there is no tubular adenoma-like dysplasia (Fig. 1c). These polyps are rarer than sessile serrated adenomas or tubular adenomas, with a reported incidence of approximately 2 %. These precancerous polyps may give rise to colorectal adenocarcinoma with a molecular characteristic of CpG island methylation, which results in epigenetic silencing of genes [48]. Current recommendations for screening after the identification of a traditional serrated adenoma is similar to that for tubular adenomas [38].

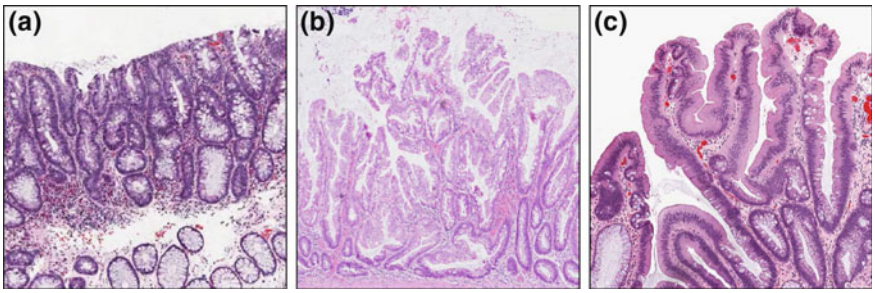

Fig. 1 Colorectal polyps. **a** Tubular adenoma with conventional dysplasia, in contrast to the normal crypts at the bottom right edge; **b** Sessile serrated polyp/adenoma with widening of the crypt bases and boot-like crypts; **c** Traditional serrated adenoma with villous projections, pink cytoplasm, crowded nuclei, ectopic crypt formation and serrated glands

1.3 Invasive Adenocarcinoma

1.3.1 Clinical Presentation

The clinical presentation of colorectal adenocarcinoma varies with the location of the mass. Right-sided carcinomas may be asymptomatic with unrecognized bleeding from the tumor resulting in anemia. Left-sided carcinomas, however, are more likely to present with obstructive symptoms, hematochezia and decreased stool caliber, particularly for rectal carcinomas. On imaging, carcinoma may present as an eccentric or circumferential narrowing of the bowel lumen, resulting in the characteristic "apple core" or "napkin ring" signs [42].

1.3.2 Gross Pathology

Typically, colorectal adenocarcinoma appears grossly as a firm, light tan, mucosal-based mass. Smaller carcinomas may present as an umbilicated depression within a polyp, while large carcinomas may be fungating or ulcerated with rolled borders (Fig. 2a). The cut surfaces of the tumor are usually firm and may be friable in areas of necrosis. Depth of invasion and lymph node involvement are important prognostic factors. Involvement of peritoneal surfaces is associated with poorer survival [47]. The predictive value of lymph node metastasis increased with the number of lymph nodes recovered surgically and on pathologic exam. Current data suggests that the probability of finding a metastasis increases with the number of lymph nodes examined. As a practical matter, 12 lymph nodes is an accepted minimum goal for pathologic examination [3], however it is strongly recommended that all lymph nodes are recovered and examined.

1.3.3 Microscopic Pathology

Colorectal adenocarcinoma is typically composed of irregular, angulated glands that infiltrate into the lamina propria, with stromal desmoplasia. "Dirty necrosis" containing neutrophils and nuclear debris within the lumen of malignant glandular structures is characteristic of, but not specific for, colorectal adenocarcinoma (Fig. 2b). Immunohistochemically, colorectal adenocarcinoma is positive for CK20 and CDX2 and usually negative for CK7. Histologic grade is determined by assessment of the degree of gland formation generated by the tumor, with low-grade (well to moderately-differentiated) tumors having well-formed glands while high-grade (poor to undifferentiated) tumors having little or no gland formation. The differential diagnosis of poorly-differentiated or undifferentiated low-lying rectal tumors includes poorly-differentiated anal squamous cell carcinoma, which may be delineated by immunohistochemical positivity for p63 and p16 in these HPV-related anal cancers.

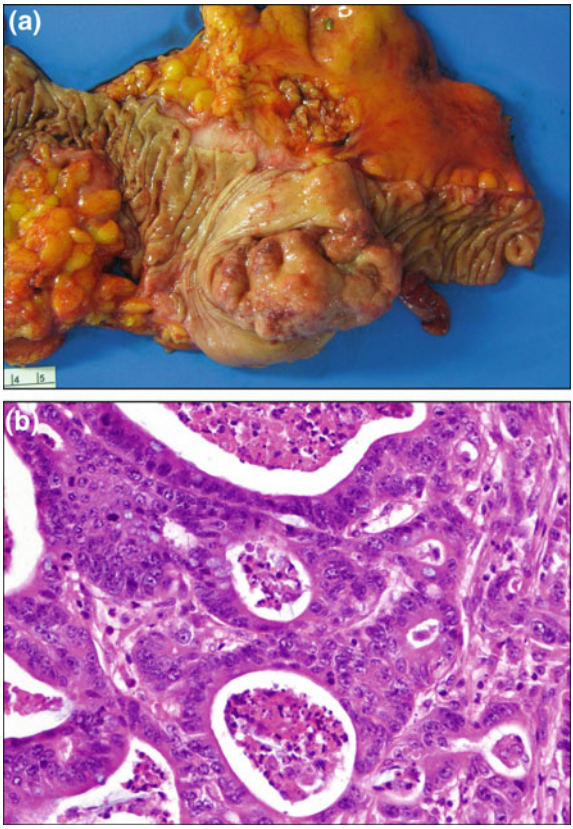

Fig. 2 Colorectal adenocarcinoma. **a** Gross image of fungating mucosal tumor; and **b** microscopic image of invasive carcinoma with irregular moderately-differentiated glands containing "dirty necrosis"

2 Colorectal Carcinomas with Microsatellite Instability

2.1 Histologic Features

Some colorectal adenocarcinomas carry high-frequency microsatellite instability (MSI) and are histologically distinct (Table 1). MSI has a high level of variability in the length of these sequences due to defects in mismatch repair proteins, whether they are germline (Lynch syndrome) or acquired. Tumors can be stratified as MSI-High (MSI-H), MSI-Low (MSI-L), or Microsatellite Stable (MSS) colon cancers, and those with MSI-H had a more positive prognosis by 15 % compared to MSI-L or MSS tumors. Silencing of MLH1 expression via promoter silencing is an acquired cause of MSI-H tumors [51]. This phenotype should trigger additional molecular testing for mutations of mismatch repair proteins, which not only identifies Lynch syndrome patients and families, but also facilitates risk stratification

Table 1 Histologic findings associated with MSI-H carcinomas

Histologic finding associated with MSI-H adenocarcinoma	Description
Tumor-infiltrating lymphocytes	Brisk lymphocytic infiltrate within the tumor
Medullary growth pattern	Circumscribed tumor with fleshy gross appearance, rimmed by abundant lymphocytes, solid growth pattern with a pushing border
Crohn-like lymphocytic reaction	Exuberant peritumoral lymphoid aggregates
Mucinous/signet ring differentiation	Foci of tumor with lakes of mucin, or presence of signet ring cells (single infiltrating cells with an eccentric nucleus indented by a single large mucin droplet)

and treatment planning for patients with sporadic colorectal adenocarcinomas. MSI-H tumors tend to carry a better prognosis, but do not respond well to fluorouracil-based chemotherapy [39]. Histologic types associated with MSI-H tumors include those listed in Table 1, and are illustrated in Fig. 3 [51]. It is worth noting that, when these patterns predominate within a carcinoma, the carcinoma

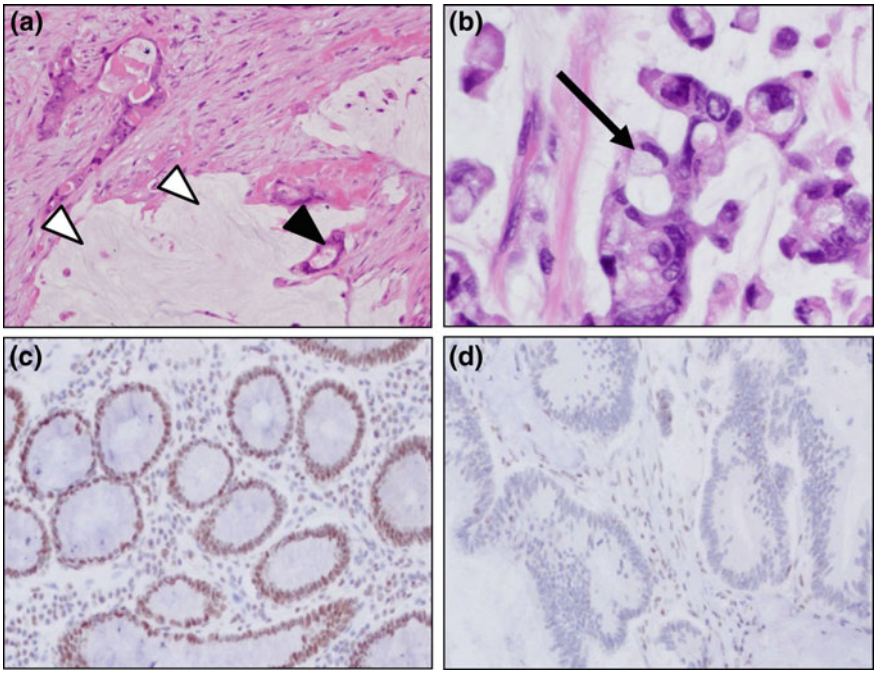

Fig. 3 Colorectal adenocarcinoma with features of microsatellite instability (MSI). **a** Mucinous features with lakes of mucin (*white arrowheads*) containing malignant cells (*black arrowhead*); **b** signet ring cells (*arrow*); **c** PMS2 positive control immunohistochemistry showing brown nuclear staining and **d** tumor cells with loss of PMS2 nuclear immunoexpression indicating MSI-H phenotype

may be further subclassified as medullary carcinoma, mucinous carcinoma, or signet ring cell carcinoma (see sections below). In the presence of any features of MSI-H carcinoma, options for further testing include immunohistochemistry for mismatch repair proteins (MLH1, MSH2, MSH6, and PMS2) or nucleic acid testing for the length of microsatellites, which are short repetitive nucleotide sequences.

2.2 Histologic Variants of MSI-H Colorectal Adenocarcinoma

2.2.1 Medullary Carcinoma

Medullary carcinomas (MCs) were also referred to as "large cell minimally differentiated carcinoma" due to the morphological similarity between MCs and poorly to undifferentiated carcinomas. However, comparing to undifferentiated carcinoma, MCs typically have less lymph node metastasis and a relatively better prognosis, which justifies their separation as a distinct entity. These are rare tumors with a frequency about 5–8 cases per every 10,000 colon cancers diagnosed. They typically affect the elderly population, with mean age at diagnosis being around 70. They are twice as common in females, who also present at a later age. Interestingly, compared to the male population, females who suffer from MCs tend to present at a lower stage with a trend of more favorable prognosis. In terms of tumor locations, MCs typically occur in the cecum and ascending colon, followed by transverse and sigmoid colon [50].

Gross Pathology

Grossly, MCs tend to present with large and fleshy tumors with expansive borders. The median tumor size is 7 cm. The surface mucosa is usually ulcerated, and tumor necrosis is common. There is frequent tumor invasion into adjacent structures [53].

Microscopic Pathology

Microscopically, MCs are characterized by sheets of polygonal cells with vesicular nuclei, prominent nucleoli, and abundant cytoplasm. Perineural and angiolymphatic invasion is common, but only 60 % of cases demonstrate lymph node metastasis at the time of diagnosis [50]. One prominent feature of MCs is that they tend to exhibit a significant peritumoral lymphoid infiltrate, similar to that of MSI-H tumors [37] (Fig. 4a–c). Not surprising, when compared to colorectal cancers that typically show KRAS and TP53 mutations, MCs are indeed more likely to harbor defects in DNA mismatch repair [20]. With this said, most MCs are sporadic and the link between MCs and MSI-high syndromes (e.g., Lynch syndrome) is only speculative. Due to its relatively favorable prognosis, it is important to distinguish MCs from undifferentiated carcinomas. In addition to morphological features, MCs are usually cytokeratin 20 (CK20) negative, occasionally CK7 positive, and often show reduced CDX2 expression. Calretinin is more likely to be positive in MCs compared to undifferentiated carcinomas [20, 54].

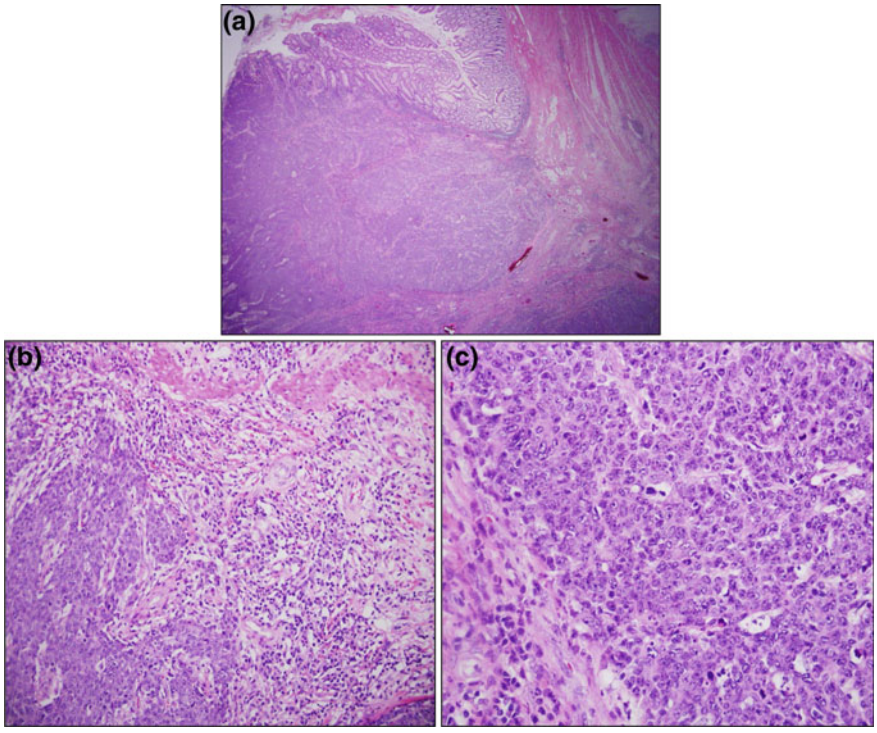

Fig. 4 Colorectal medullary carcinoma. **a** Low magnification of medullary carcinoma with pushing boarder. **b** Tumor with significant peritumoral lymphocytes. This particular case has loss of nuclear expression of MLH1 and PMS2 (immunohistochemistry not shown). **c** Tumor cells with large vesicular nuclei, prominent nucleoli, frequent mitotic features and amphophilic cytoplasm

2.2.2 Mucinous Carcinoma

Mucinous carcinoma is a variant of colorectal adenocarcinoma in which greater than 50 % of the tumor is composed of lakes of mucin (Fig. 3a). The mucin may be grossly apparent upon examination of the colon on endoscopy or the time of resection. MSI-H mucinous carcinomas behave as low-grade adenocarcinomas, while microsatellite-stable mucinous carcinomas have similar behavior to conventional poorly differentiated colorectal adenocarcinoma [23].

2.2.3 Signet Ring Cell Carcinoma

Signet ring cell carcinoma is a variant of colorectal adenocarcinoma will the tumor composed of greater than 50 % signet ring cells, which are round to ovoid tumor cells containing a large mucin vacuole which indents the nucleus to one side (Fig. 3b). These cells tend to infiltrate singly into the lamina propria or muscularis propria. This histology is identical to signet ring cell carcinoma of the stomach. These tumors may be MSI-H, however the prognosis for signet ring cell carcinoma

is worse than for mucinous carcinoma [23]. As with mucinous carcinoma, MSI-H signet ring cell carcinomas have a better prognosis than their microsatellite-stable signet ring cell carcinomas counterparts.

3 Hereditary Colon Cancer Syndromes

3.1 Autosomal Dominant

Lynch Syndrome

Lynch syndrome (also known as hereditary non-polyposis colon cancer, HNPCC) is caused by defects in mismatch repair proteins (MLH1, MSH2, MSH6, and PMS2) and results in predominantly right-sided colon cancers that have a genetic alteration termed microsatellite instability (see Invasive Colorectal Adenocarcinoma, section MSI-H Histologic Features). Patients with Lynch syndrome develop colorectal cancer in the absence of florid polyposis, although polyps can occur in these patients [33]. In addition to colorectal cancer, patients with Lynch syndrome may develop cancers of the pancreas, biliary tract, small intestine, upper urinary tract, endometrium, ovary, brain (glioblastoma in Turcot syndrome), and skin (sebaceous glands and keratoacanthomas in Muir-Torre syndrome) [51].

Familial Adenomatous Polyposis

Familial adenomatous polyposis (FAP) is an autosomal dominant disorder caused by mutation in the adenomatous polyposis coli (APC) tumor suppressor gene. The physiologic role of APC is to antagonize the WNT signaling pathway, and mutations cause inappropriate activation of this pathway via interaction with beta-catenin [4]. Patients develop thousands of adenomatous polyps in their teenage years, and nearly all patients will develop colon cancer by age 50 if left untreated. Attenuated forms of FAP have been identified, in which fewer polyps may manifest at an older age. Colectomy is the standard treatment for patients with FAP, once polyposis is evident. Extracolonic manifestations include desmoid tumors, osteoid osteomas, fundic gland polyps, and adenomas of the stomach, duodenal adenomas, papillary thyroid cancer, and hepatoblastoma of the liver [33].

Peutz-Jeghers, Cowden and Juvenile Polyposis Syndromes

Peutz-Jeghers, Cowden, and juvenile polyposis syndromes are hamartomatous polyposis syndromes with differing risks for intestinal carcinoma. Hamartomatous polyps are composed of tissue elements normally found at that site, but growing in a disorganized manner. Peutz-Jeghers syndrome is characterized by multisystem involvement by hamartomatous growths and a very high lifetime risk of developing cancer. The causative mutation occurs in the Serine-Threonine Kinase 11 gene (STK11) and is inherited in an autosomal dominant fashion. Characteristic findings include mucocutaneous hyperpigmentation and hamartomatous polyps of the

gastrointestinal tract, nares, pelvis, bladder, and lungs. Almost any organ in the body can develop cancer, and the relative risk of developing colorectal cancer is approximately 84-fold higher than that of the general population [46]. Notably, cancers do not necessarily develop from the hamartomatous growths. Cowden syndrome is a rare syndrome resulting in multiple hamartomatous polyps throughout the gastrointestinal tract without a clear increased risk of intestinal cancer. Patients with Cowden syndrome are, however, at an increased risk of breast and thyroid carcinomas [46]. Finally, juvenile polyposis syndrome results in multiple hamartomatous polyps occurring at a young age, with an increased risk of colorectal cancer as well as other gastrointestinal cancers. Colonoscopic screening beginning in the teenage years is recommended for these patients [46].

3.2 Autosomal Recessive

MUTYH Polyposis

MUTYH polyposis is an autosomal recessive disease caused by mutation in mutY DNA glycosylase, an enzyme involved in base excision repair. Defective repair results in a specific KRAS mutation, with microsatellite stability generally preserved. The disease phenotype usually results in fewer adenomatous polyps than FAP and variable extracolonic manifestations [5]. Thus in young patients with multiple adenomatous polyps, this entity should be included in the differential diagnosis.

4 Neuroendocrine Tumors

Neuroendocrine cells in the gastrointestinal (GI) tract constitute the largest endocrine system in the body [1]. Neuroendocrine tumors (NETs) in the GI tract are relatively rare (4.7 per 100,000), although the incidence is rising recently, partially due to increased detection on radiographic imaging and endoscopy [56]. Except for tumorlets (lesions measuring less than 0.5 cm), all NETs should be considered as having malignant potential. In the colon, NETs are more commonly found in the rectum, followed by cecum and sigmoid colon [31, 32]. They tend to affect patients in their sixth to seventh decade of life and most of these tumors are nonfunctional. Compared to rectal NETs, colonic NETs are more likely to be seen in women, are often large, symptomatic at diagnosis, and have a relatively aggressive course [7, 26, 31]. The majority of NETs are sporadic, although they can be associated with familial syndromes, including multiple endocrine neoplasia I, Von Hippel-Lindau syndrome, tuberous sclerosis, and neurofibromatosis type 1. The classification of NETs has undergone several revisions during the last two decades. According to the 2010 WHO classification [40], NETs are graded into three categories based on tumor proliferation rate (Table 2).

Table 2 WHO 2010 classification for neuroendocrine tumors

Classification	Definition	Mitosis/HPF	Ki-67 index (%)
Well-differentiated neuroendocrine tumor, Grade 1	Well-differentiated, little change from the cytology of the native neuroendocrine cells	<2 mitosis/10 HPF	<2
Well-differentiated neuroendocrine tumor, Grade 2		2–20 mitosis/10 HPF	3–20
Poorly differentiated neuroendocrine carcinoma, Grade 3	Poorly-differentiated, with either pleomorphic large cells with prominent nucleoli or abnormal small cells with hyperchromatic nuclei and occasional crush artifact	>20 mitosis/10 HPF	>20

HPF High-Power Field (40X objective)

In an attempt to risk stratify, there is a trend to further reduce the categories into well-differentiated (low and intermediate grade) NETs and poorly differentiated (high-grade) neuroendocrine carcinomas. It is worth noting that there is significant heterogeneity within both categories and neither grading nor staging can accurately predict the clinical course [24]. The clinical setting, primary location, cell type, stage, grade, and other tumor features (e.g., hormone production) are important for the selection of treatment modalities.

4.1 Gross Pathology

Grossly, most of NETs are mucosa-covered, well demarcated and grow with pushing borders, forming broad-based, polypoid lesions, with the bulk of lesions in the submucosa or extending to the muscular layer. Cut sections reveal a fleshy, homogenous, red-to-tan color mass lesion. Necrosis, mucosal ulceration, and invasion into the muscularis propria can be seen in more advanced cases and are typically signs of more aggressive behavior (Fig. 5a).

4.2 Microscopic Pathology

Microscopically, well-differentiated NETs have a characteristic "organoid" arrangements of the neoplastic cells forming nests, acini, rosettes, ribbons, festoons, or trabeculae intermixed with a delicate vasculature. Gland formation by the tumor cells can be seen. The cells are relatively uniform, with round to oval nuclei, coarsely stippled chromatin ("salt-and-pepper" chromatin pattern), and moderate, finely granular cytoplasm (Fig. 5b, c). Clear cell, oncocytic, and spindle change can occasionally be seen. Poorly-differentiated neuroendocrine carcinomas (PDNECs)

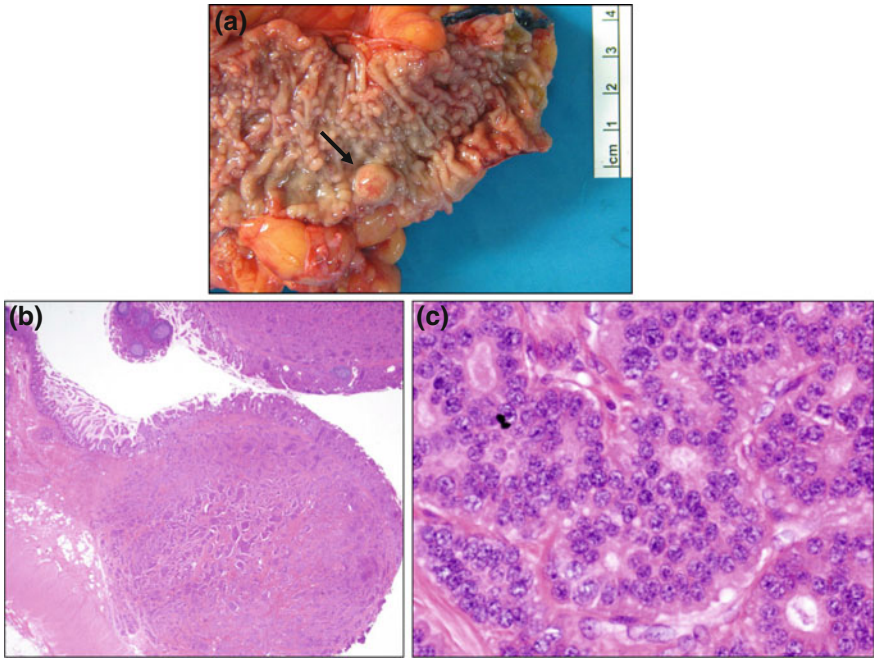

Fig. 5 Well-differentiated neuroendocrine tumor. **a** A well-delineated, mucosal covered polypoid lesion (*black arrow*). **b** Neoplastic cells have an "organoid" or nested arrangement. **c** The tumor cells are uniform with round to oval nuclei, "salt-and-pepper" chromatin, and finely granular cytoplasm

usually demonstrate marked cellular atypia, frequent necrosis and high proliferative rate. They have a more sheet-like or diffuse growth pattern, irregular nuclei, and less cytoplasmic granularity. Some PDNECs are nearly identical to pulmonary small cell carcinomas, exhibiting small cells with scant cytoplasm, round to ovoid nuclei, coarse chromatin, inconspicuous nucleoli, and nuclear moulding. Other PDNECs are more like large-cell neuroendocrine carcinomas with large cells, highly atypical and vesicular nuclei as well as prominent nucleoli. In view of the heterogeneity among WDNETs and PDNECs, several biomarkers have been proposed for the prognostication of GI NETs and include CK19, CD117, $p27^{Kip1}$, CD99, and PAX8 [16, 34, 45, 57]. However, their reliability is yet to be verified with larger case series and may not be applicable to metastatic tumors.

5 Primary Colorectal Lymphoma

Primary GI lymphomas are defined by the following five components: (1) absence of palpable, superficial lymph nodes at presentation; (2) absence of enlarged mediastinal lymph nodes on chest X-ray; (3) normal range for white blood cell

count including total and differential; (4) only regional lymph node involvement at time of surgery; and (5) liver/spleen are without disease [9]. Although extranodal lymphomas account for one-third of all cases of non-Hodgkin lymphoma (NHL), with the GI tract being the most common extranodal site; primary colorectal lymphomas are exceedingly rare and account for less than 1 % of all colorectal malignancies [36]. The incidence of primary colorectal lymphomas increases with age and peaks between 50 and 70 years of age with a slight male predominance [58]. Risk factors include inflammatory bowel disease, chronic immunosuppression including HIV, post-transplantation, and chronic infection with Epstein Barr virus [11]. Patient present with nonspecific symptoms including abdominal pain, weight loss, and less commonly change in bowel habits or lower GI bleeding. The vast majority of colorectal lymphomas are proximal to the hepatic flexure, with the cecum being the most frequent location, thought to be due to the presence of abundant lymphoid tissue. Intussusception may be seen with bulky lymphomas in the ileocecal region [19].

Currently, the WHO classification recognizes five major NHL categories in the GI tract: diffuse large B-cell lymphoma (DLBCL), extranodal marginal zone lymphoma (MZL) (also known as mucosa-associated lymphoid tissue (MALT)-associated lymphoma), mantle cell lymphoma (MCL), Burkitt lymphoma, and follicular lymphoma (FL). The most common lymphomas in the colorectum are the more aggressive DLBCL and Burkitt lymphomas, although recently the incidence of MCLs and MZLs appear to be increasing [41]. Despite the presence of multiple staging systems, the Ann Arbor staging system is the most widely accepted system currently used for lymphomas. Although the primary treatment modalities for lymphomas have been cytotoxic chemotherapy regimens and/or targeted therapies with or without radiation, resection is the mainstay of treatment for colorectal lymphoma in the absence of metastatic disease [19].

In general, the morphological and molecular features as well as the clinical behaviors of primary colorectal lymphomas are similar to those occurring elsewhere in the body. Considering the vast diversity of lymphomas, discussing the features of each subtype is beyond the scope of this chapter. Here, we focus the discussion on DLBCL, the prototype of the more aggressive and the most common primary colorectal lymphoma, as well as MCL, an entity that has been recognized with increasing frequency in the colon and rectum.

5.1 Diffuse Large B-Cell Lymphoma

5.1.1 Gross Pathology

Primary colorectal diffuse large B-cell lymphomas (DLBCLs) grossly appear similar or larger than low-grade lymphomas. They are usually elevated or infiltrative, with or without ulceration of the surface mucosa, and are typically transmurally invasive. Perforation at presentation can be seen.

5.1.2 Microscopic Pathology

Microscopically, most DLBCLs are composed of centroblasts (activated B cells), often with an admixture of immunoblasts (larger lymphocytes with a paler and wider rim of cytoplasm), although some cases are composed almost exclusively of immunoblasts. It is worth noting that occasionally, a component of low-grade lymphomas, especially MZL, can be found with primary colorectal DLBCL, which is consistent with large-cell transformation of a low-grade lymphoma elsewhere in the body [14]. It is important to note that most intestinal DLBCLs have a germinal center B-cell (GBC) immunophenotype (CD10+ or CD10−, Bcl6+, MUM1−), which typically conveys a better prognosis compared to the non-GCB immunophenotype (either CD10−, Bcl6+, MUM1+ or CD10−, Bcl6−), which is also called activated central blast (ABC) immunophenotype [10]. The genetic heterogeneity of DLBCLs is maintained in primary colorectal DLBCLs, partially reflecting the process of transformation from other lymphomas with distinct translocations [14].

5.2 Mantle Cell Lymphoma

Primary colorectal mantle cell lymphomas (MCLs) were thought to be relatively rare compared to other forms of primary colorectal lymphomas. However, the incidence is increasing recently, possibly due to improved detection [41]. They usually affect multiple sites and manifest as widespread disease with mesenteric lymph node involvement at presentation. Although there is usually a positive response to chemotherapy in patient suffering from MCLs, relapses are common, rendering MCLs one of the lymphomas with poor prognosis.

5.2.1 Gross Pathology

Endoscopically, MCLs often take the form of innumerable fleshy-white nodules or polyps, 0.5–2 cm in greatest dimension, mainly involving the mucosa but sometimes with superficial submucosal involvement, which is termed multiple lymphomatous polyposis [25]. Less commonly, mantle cell lymphoma takes the form of a discrete mass or an ulcerated lesion. The largest polyps are usually seen at ileocecal region. It is worth noting that not all MCLs present with multiple lymphomatous polyposis, such as in the rare cases of MCLs with concurrent adenocarcinoma [22]. Moreover, other types of primary colorectal lymphomas such as FL and MZL, can occasionally present with multiple lymphomatous polyposis [25].

5.2.2 Microscopic Pathology

Microscopically, monotonous lymphoid cells form band-like infiltrates or multiple ill-defined nodules. The lymphomas tend to displace and obliterate intestinal glands, but formation of true lymphoepithelial lesions is not a feature.

Cytologically, MCL cells have scant cytoplasm and are without conspicuous nucleoli. They are slightly larger and more irregular than normal lymphocytes. Immunophenotyping typically shows CD20+, CD5+, CD43+, CD10−, CD23−, with antibodies to follicular dendritic cells such as CD21 highlighting a loose, expanded dendritic network. MCLs are associated with t(11;14)(q13;q32), with fusion of cyclin D1 (also called bcl-1, PRAD1, CCND1) and IgH in 90 % of cases. This translocation of cyclin D1 and immunoglobulin heavy chain leads to the overexpression of cyclin D1 that can be detected by positive nuclear staining by immunohistochemistry [14]. Of note, in-situ hybridization for cyclin D1/bcl-1 is more sensitive and specific than immunostains. Mucosal homing receptor, α4β7, is thought to suggest GI involvement in MCL patients [17].

6 Gastrointestinal Stromal Tumors

Gastrointestinal stromal tumors (GISTs) are thought to originate from either interstitial cells of Cajal or precursors of these cells. Approximately 4500–6000 GISTs are diagnosed annually in the United States. However, most cases arise in the stomach or small intestines, and only 5–10 % of the cases are colorectal primary [29]. Compared to GISTs arising from the stomach, colorectal GISTs are more likely to have distant disease at presentation, higher rate of local recurrence, higher morbidity and worse overall survival [13, 27]. Interestingly, colorectal GISTs tend to be smaller in size (mostly less than 10 cm). The standard treatment includes surgical resection and/or targeted therapy (e.g., imatinib).

6.1 Pathologic Features

Similar to GISTs elsewhere, colorectal GISTs grossly can appear solid or cystic and can be seen at subserosal, intramural, intraluminal, or external masses [28] (Fig. 6a). The cut section may appear hemorrhagic or necrotic. However, these features are not indicative of malignancy. The histomorphology varies greatly and includes pure spindle cell (Fig. 6b, c), pure epithelioid cell, and mixed spindle and epithelioid cell types. It is worth noting that although smooth muscle tumors of the GI tract are much less common than GISTs in general, leiomyomas are more common than GISTs in the colorectum as well as esophagus [12]. Greater than 95 % of GISTs will stain positively for c-KIT (CD117), DOG1, and/or CD34 by immunohistochemistry. Approximately 85 % of GISTs are associated with an abnormal c-KIT pathway, the tyrosine kinase function of which is important in the medical therapy (imatinib, sunitinib, regorafenib) for GISTs. GISTs with non-mutated *c-KIT* instead have a mutation in another gene, platelet derived growth

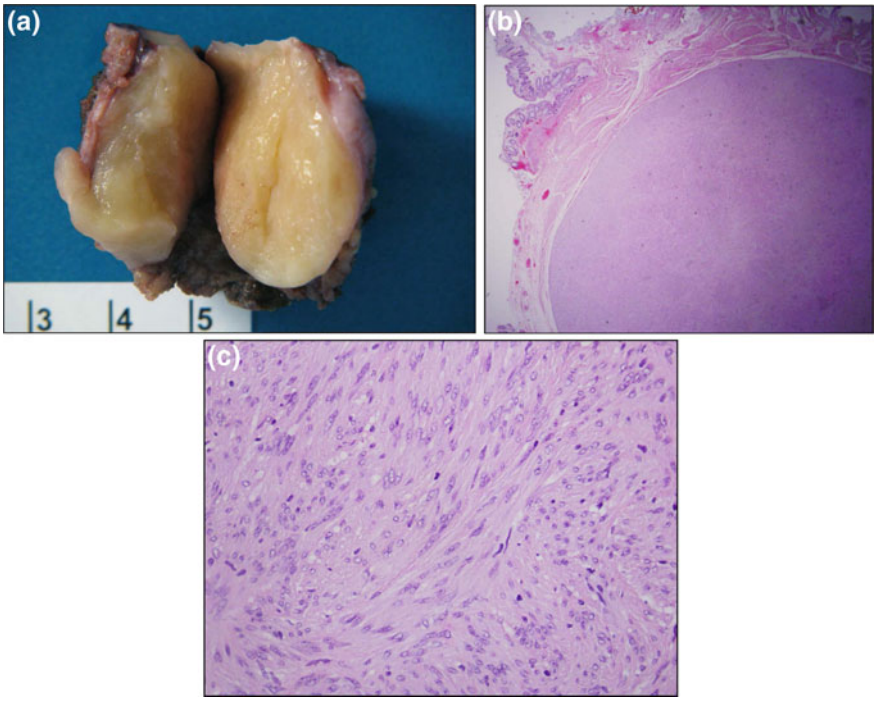

Fig. 6 Gastrointestinal stromal tumor, spindle cell type. **a** The tumor have a solid, tan, homogenous cut surface. **b** The tumor is well circumscribed with the neoplastic cells located deep to the submucosa and arising from the muscularis propria. **c** Spindle tumor cells with modest amounts of pale, eosinophilic, fibrillary cytoplasm

factor receptor alpha (*PDGFR-α*), also a tyrosine kinase. Mutations in *c-KIT* and *PDGFRA* are mutually exclusive.

7 Appendiceal Neoplasms

Cancer of the appendix is very rare. It is found in less than 1 % of the appendectomy specimens and accounts for less than 1 % of gastrointestinal malignancies [30, 43]. Mucinous neoplasms and NETs are the most common neoplasias encountered.

7.1 Mucinous Neoplasms of the Appendix

Mucinous neoplasms with pushing or "dissecting" acellular mucin are classified as low-grade appendiceal mucinous neoplasm (LAMN). The acellular mucin can dissect between the smooth muscle fibers and be contained within the appendix or

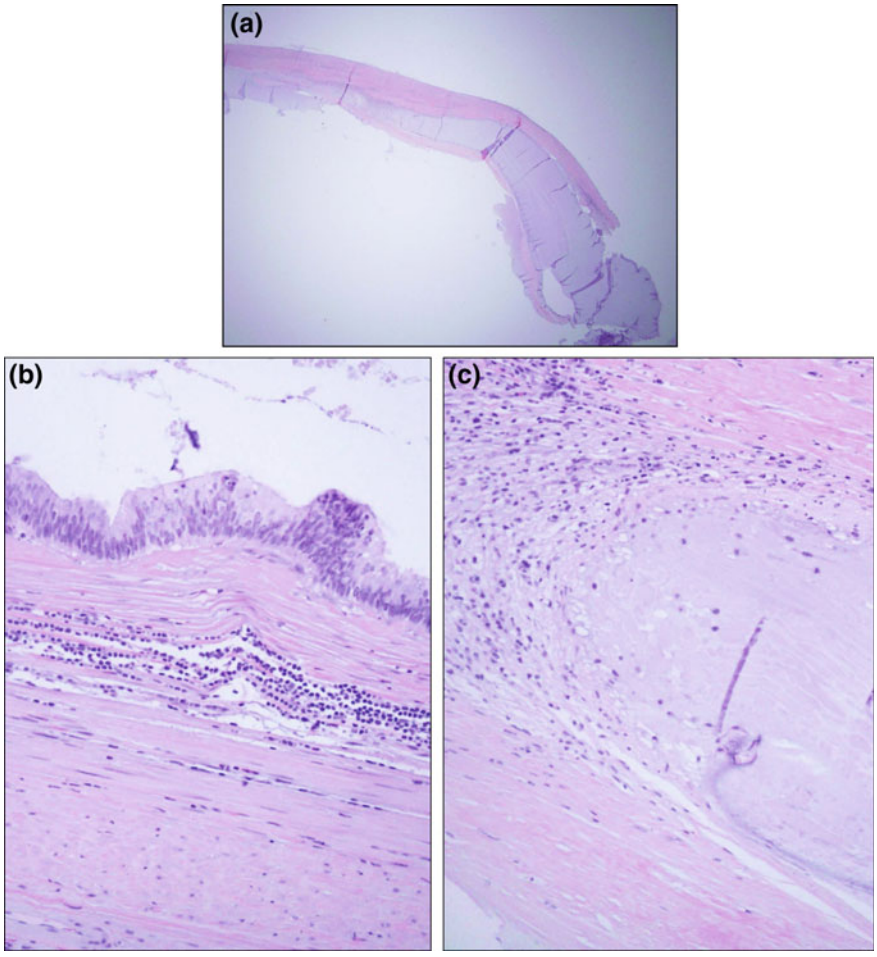

Fig. 7 Low-grade appendiceal mucinous neoplasm (LAMN). **a** Low magnification image reveals acellular mucin dissecting the appendiceal wall. **b** High magnification of the mucinous appendiceal lining reveals columnar epithelial cells with low-grade atypia resting atop of fibrotic stroma. There is no residual lamina propria present. **c** The areas with dissecting mucin also demonstrate a mild inflammatory infiltrate composed of lymphocytes and plasma cells without evidence of neoplastic cells

can extend beyond the appendix and into the peritoneum (Fig. 7a–c). When there is extension and accumulation of acellular mucin beyond the appendix, it is clinically termed pseudomyxoma peritonei. The presence of neoplastic cells within the dissecting mucin or tumor cells within the peritoneum indicates a worse prognosis and

the entire appendix should be examined for evidence of invasive carcinoma. Appendiceal neoplasms that have neoplastic cells within the mucin, an infiltrative pattern through the appendiceal wall and beyond are best classified as mucinous adenocarcinomas. These tumors have a more aggressive course, similar to invasive carcinomas of the colon and rectum.

7.2 Neuroendocrine Tumors of the Appendix

Most of the neuroendocrine tumors (NETs) in the appendix are well-differentiated and were previously referred to as "carcinoid tumor" (See the section of Neuroendocrine Tumors for a more detailed discussion). It is important to distinguish pure NETs from mixed glandular and endocrine neoplasms, including goblet cell carcinoid and mixed adenoneuroendocrine carcinoma (MANEC), which exhibit glandular differentiation, mucin production and behave in a more aggressive fashion with a worse prognosis.

8 Squamous Cell Carcinoma (SCC) of the Anus

Carcinomas of the anal canal are rare but are increasing in frequency and currently account for about 5 % of cases of large intestine and anal malignancies [35]. Squamous cell neoplasms of the anal canal in general are associated with human papillomavirus (HPV), although HPV itself is thought to be insufficient to induce cancerous transformation without other risk factors, including anal-receptive intercourse, heavy smoking, history of sexually transmitted diseases, HIV-positive status, and immunosuppression [15, 35]. Interestingly, the epidemiology and clinical courses differ between SCCs arising above and below the dentate line. SCCs below the dentate line are more commonly seen in males, and usually coexist with precursor lesions such as dysplasia. On the other hand, SCCs above the dentate line are slightly more common in females and often arise in the absence of any known pre-existing condition [35].

8.1 Pathologic Features

The gross and microscopic features of SCCs of the anal canal are heterogeneous, but resemble SCCs seen elsewhere in the body and share similar precursors of squamous dysplasia termed either low-grade or high-grade squamous intraepithelial lesions (Fig. 8a–d). Grossly, SCCs of the anal canal can be large, exophytic, or ulcerating, with or without bleeding. Microscopically, there are several morphological variants, including conventional, non-keratinizing, basaloid, and verrucous (Figs. 7 and 8). However, due to the fact that most of the SCCs are diagnosed on

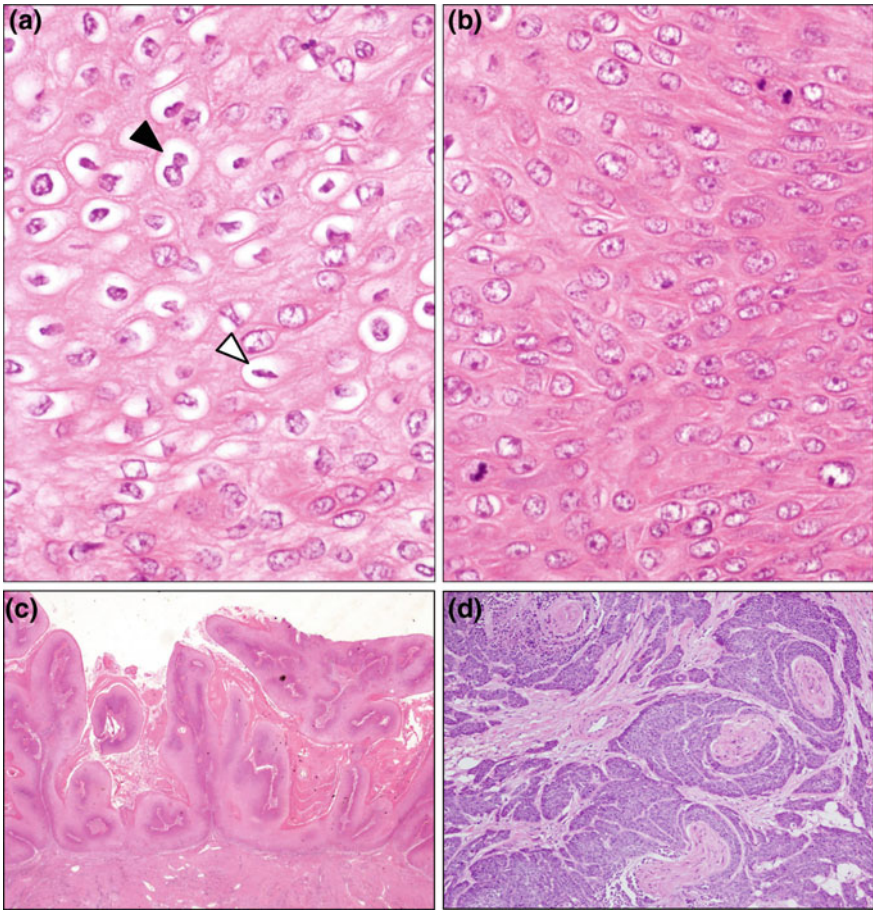

Fig. 8 Anal squamous neoplasms. **a** Low-grade squamous intraepithelial lesion (previously referred to as condyloma acuminatum) demonstrate HPV cytopathic effect including raisinoid nuclei and cytoplasmic clearing/koilocytosis (*white arrowhead*) as well as binucleation (*black arrowhead*). **b** High-grade squamous intraepithelial lesion (previously referred to as anal intraepithelial neoplasia [AIN2 and AIN3]) with loss of orderly maturation of the epithelium, cellular atypia and abundant mitoses. **c** Verrucous carcinoma with pushing growth pattern without evidence of tumor cell invasion. **d** Invasive squamous cell carcinoma reveals an infiltrative growth pattern and perineural invasion

small biopsies which may not reflect the features of the entire lesion, the 2010 WHO classification encourages the use of a more general diagnosis of invasive SCC with additional descriptors, such as degree of differentiation, predominant cell size, basaloid features, degree of keratinization, or adjacent dysplasia [52]. Terms such as basaloid, transitional cell, cloacogenic, or mucoepidermoid carcinoma are strongly discouraged.

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Importance of Adequate Lymphadenectomy in Gastrointestinal Cancer

Andrew Benjamin and Ryan P. Merkow

Abstract

One of the most important factors influencing cancer-specific survival in the field GI oncology is the presence of positive lymph nodes. Although it remains controversial, adequate lymph node examination is required for accurate staging such that patients can receive correct adjuvant treatments and for stratification in clinical trials. Nevertheless, wide variation in the quality of lymph node examination exists in the US and many centers are not meeting guideline treatment recommendations.

Keywords

Lymphadenectomy · Lymph node examination · Gastrointestinal cancer · Esophogeal cancer · Gastric cancer · Colon cancer · Rectal cancer · Pancreatic cancer

1 Background

In 2010 there were an estimated 1.5 million incident cancer cases in the United States and nearly 600,000 deaths. [1] Approximately 17 % of all new cases originated in the gastrointestinal (GI) tract, however, these cases represented

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one-quarter of all cancer-related deaths. Certain GI malignancies have an especially poor prognosis, particularly esophageal (5-year survival: 20 %) and pancreatic (5-year survival: 7 %) tumors [1]. The most common GI malignancy, colorectal cancer, represents nearly 10 % of all diagnosed malignancies and cancer related deaths in the United States [1].

Given these statistics, it is imperative to identify strategies that will improve the quality of cancer care in the field of GI oncology. It is clear that one of the most important predictors of survival is the presence of lymph node metastasis, however, the precise number of examined lymph nodes needed for accurate staging for each cancer site is variable and not always clearly defined. Nevertheless, the number of lymph nodes examined after surgical resection will considerably affect staging accuracy, which is relevant for treatment decisions and when entering patients in clinical trials. In addition, evidence suggests the extent of lymphadenectomy could be a surrogate of other unmeasured factors such as the quality of surgical technique, more thorough pathologic examination, or both [2]. Therefore, to address these issues, the objective of this chapter is to explore the current evidence and controversies with respect to lymph node staging standards in GI oncology.

2 History

By the mid-1800s, the concept that lymph nodes may act as functional barriers and channels of cancer spread began to be accepted. William Halsted, an American born surgeon, popularized the idea that cancer spreads in an orderly manner by way of lymphatic channels. Based on his own theory, Halsted advocated radical mastectomy as the corner stone of treatment for women with breast cancer, an operation which removes the breast, underlying muscle, and all lymphatic tissue in the axilla [3]. In this manner, Halsted believed he was removing all locoregional disease, particularly cancer cells harbored in axillary lymph nodes, which he postulated would decrease recurrence and ultimately improve survival.

The role of lymph node sampling in the natural history of cancer continued to evolve. In contrast to the "Halstedian" theory, a new concept emerged, championed by Bernard Fisher and Blake Cady [4]. Fisher believed that lymph node involvement was an indicator of cancer biology rather then a step in the sequence toward distant metastases. He did not believe variations in locoregional therapy were as important as addressing the systemic disease, and advocated for less radical surgical treatments [4]. Fisher would go on to cofound the National Surgical Adjuvant Breast and Bowel project, and conduct a series of landmark randomized controlled trials that demonstrated, among other important findings, that en bloc resection of axillary lymph nodes and radical surgery in all women with breast cancer was not associated with better outcomes [5].

Out of these and other discussions, a critical concept emerged—that standardizing the classification of specific cancers into separate stages was important for both prognostic and treatment purposes, and that nodal status was an integral

component of overall stage. In 1959, six founding organizations (American Cancer Society, American College of Surgeons, American Society of Clinical Oncology, Centers of Disease Control, National Cancer Institute and College of American Pathologists) convened to launch the American Joint Commission on Cancer (AJCC) [6]. The objective of the AJCC was to define cancer-staging groups that optimized prognostic estimates based on tumor size, nodal status and presence of distant metastasis. Standardizing cancer staging has allowed researchers to reliability evaluate many important questions in the field of cancer surgery, including those specific to the optimal extent of lymphadenectomy.

3 Esophageal Cancer

The extent of lymphadenectomy in esophageal cancer has important prognostic and potentially therapeutic implications, however, a clear lymph node threshold has not been defined. Studies performed have been heterogeneous with variable study inclusion and exclusion criteria. In addition, as with other GI malignancies, lymph node metastases may be simply a marker of systemic disease. Nevertheless, it is clear that the presence of positive lymph nodes is a powerful prognostic indicator and numerous reports now show the benefits of adequate lymph node examination, with thresholds ranging from 10 to 40 nodes [7–11]. The NCCN [12] currently recommends the examination of at least 15 nodes, and the AJCC staging manual identifies four distinct groups (N0–N3) of nodal metastasis, highlighting the need for adequate nodal examination for staging accuracy [13].

Similar to rectal cancer, the majority of stage II and III esophageal cancer patients are treated with neoadjuvant therapy [14], yet, almost all studies examining lymph node thresholds exclude these patients from analysis [9]. In one recent study, 161 patients from the CROSS trial were examined to determine the survival benefit of node resection in esophagectomy patients with and without neoadjuvant chemoradiation therapy. The median number of resected nodes was 18 and 14 respectively. The number of nodes retrieved was associated with survival in the group without neoadjuvant therapy, however, there was no prognostic impact in the patients who had received neoadjuvant chemotherapy [15]. Current guidelines continue to recommend retrieval of at least 15 nodes for adequate staging [12]. While certainly necessary in patients undergoing surgery alone, further studies need to address the optimal lymph node yield in patients treated with chemoradiation.

Few studies have been performed regarding adherence to the 15 lymph node guideline. However, a recent retrospective review of 13,995 patients from the National Cancer Data Base (NCDB) undergoing esophagectomy from 639 hospitals showed that only 28 % of patients had at least 15 lymph nodes examined, and only 7 % of hospitals examineda median of 15 nodes. Hospital type, volume, and geographic location were all predictors of meeting the guideline of at least 15 nodes examined (Fig. 1) [16].

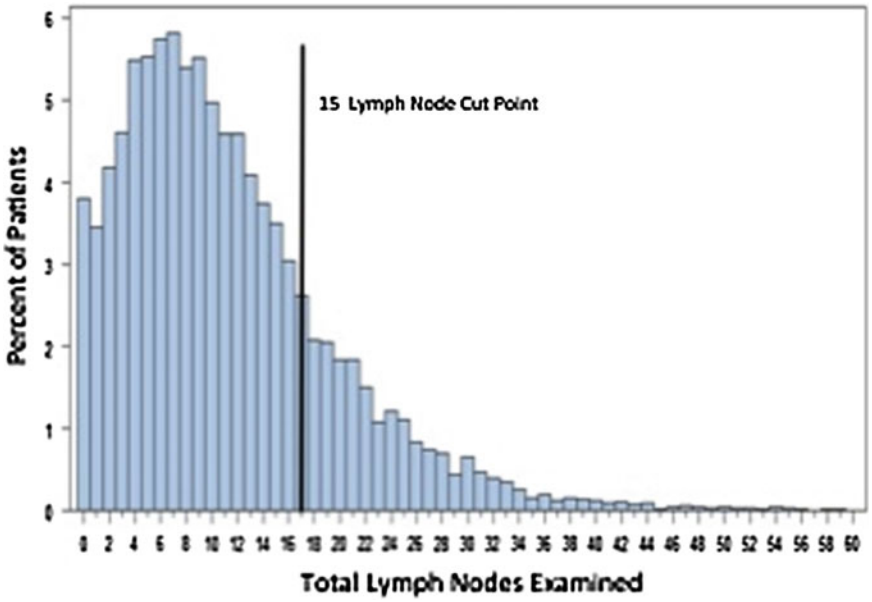

Fig. 1 Proportion of patients receiving a lymph node evaluation of at least 15 nodes from 1998 to 2007

4 Gastric Cancer

The presence of positive lymph nodes in gastric cancer has long been recognized as one of the most important prognostic factors. In 1889, Mikulicz, a Polish–Austrian surgeon was one of the first to promote the importance of extended lymphadenectomy in gastric cancer [17]. Like Halsted, Mikulicz believed that cancer required aggressive locoregional control if there was any hope for cure.

Yet, more than a century later, one of the most controversial debates in the field of GI oncology remains the extent of lymphadenectomy in gastric cancer. Principally, there are three main lymph node resection categories: D0 (incomplete removal of perigastric nodes), D1 (complete removal of perigastric nodes) and D2 (extended lymphadenectomy including common hepatic, left gastric, celiac and splenic nodes with or without splenectomy and distal pancreatectomy). The debate echoes the "Halstedian" vs. "Fisherian" arguments. Proponents of the D2 resection believe that cancer cells spread in an orderly fashion, passing through lymphatics, and thus removing all of this tissue should portend a survival advantage. Opponents believe this extensive surgical procedure only adds potential morbidity, no survival advantage, and is necessary only for staging purposes. Many trials have attempted to address this question, unfortunately, with variable results [18–22]. Recently, the long-term results of the Dutch randomized trial were reported that supported the D2 resection [22], which is consistent with current consensus guidelines

[12]. Although the traditional D2 resection appears to have a survival benefit, the complication rate is significantly increased as well. A prospective, randomized trial conducted by the Italian Gastric Cancer Study Group showed that a modified, spleen and pancreas preserving D2 resection was associated with a similar survival benefit, but decreased complication rate, with morbidity and mortality similar to D1 rates seen in Dutch and UK trials [22–24].

A related issue is determining the optimal number of lymph nodes to evaluate during gastrectomy. Many analyses have addressed this question, and it appears that evaluating at least 15 lymph nodes provides a reliable estimate of pathologic N stage, and is consistent with current guidelines [12, 22, 25–27]. Interestingly, in a study by Karpeh et al. [25], it was not the location of positive lymph nodes that was important, but simply the overall number of positive nodes. Current guidelines recommend a D1 or modified D2 resection with the goal of examining at least 15 or more lymph nodes. Given the technical difficulty of a D2 resection, these procedures should be preformed at high-volume centers [12].

Few studies have evaluated hospital performance with respect to the 15-lymph node benchmark. Bilimoria et al. [28], in a study from the NCDB that included over 3000 patients, found that the median number of nodes examined was only 7, but higher at NCCN-NCI hospitals compared community hospitals (12 vs. 6). In addition, this study reported only 23.2 % of patients undergoing gastrectomy had at least 15 nodes examined (Fig. 2). In a separate population-based study using SEER data, the median number of nodes was 10 and only 32 % of patients underwent an adequate lymph node evaluation [29]. However, other reports from high-volume specialty centers have documented lymph node evaluation rates exceeding 15 nodes in nearly 80 % of patients [25]. These data suggest the majority of patients are not being adequately staged, which may partially explain the survival differences between specialized cancer centers and community centers.

5 Pancreatic Cancer

Pancreatic cancer continues to pose substantial diagnostic and treatment challenges. Among GI cancers, it has the worst overall survival, and is most commonly diagnosed at later stages of disease when curative resection is not possible. Nonetheless, in patients who have resectable disease, lymph node staging has been shown to be one of the most important prognostic indicators [30]. Similar to other GI malignancies, it is unclear if this survival advantage reflects stage migration, or if there is in fact a therapeutic benefit of lymphadenectomy. In addition, the value of extended lymphadenectomy has also been questioned. In a randomized trial comparing patients who underwent standard versus extended retroperitoneal lymphadenectomy for periampullary cancers, there was no difference in survival [31]. Nevertheless, establishing the presence of lymph node involvement is especially important in pancreatic cancer for clinical trial stratification especially as chemotherapy improves. Moreover, Tomlinson et al. [32] demonstrated well-staged node-negative

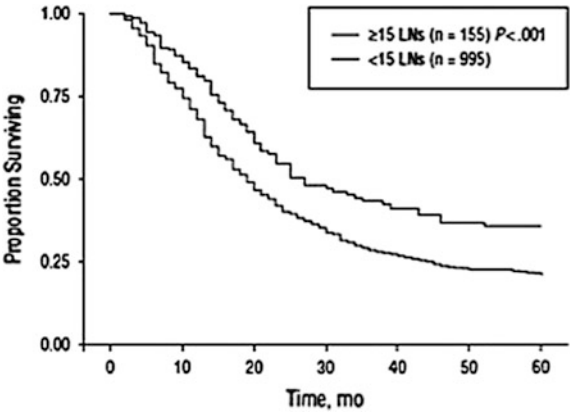

Fig. 2 Kaplan–Meier survival curves of node-negative pancreatic cancer patients with ≥ 15 lymph nodes and <15 lymph nodes examined after pancreatectomy. From: Tomlinson et al. [32]

patients had a median survival benefit of 8 months over less well-staged node-negative patients (Fig. 2). Of note, this improvement in survival is greater than any adjuvant therapy.

A number of studies have also investigated the optimal number of lymph nodes that should be examined in pancreas cancer. One study, performed by Schwarz and colleagues using SEER data from 1973 to 2000, demonstrated that harvesting 10–15 nodes in node-negative patients improved survival. Two separate SEER studies [32, 33] using different methodologies and patient populations, essentially confirmed this threshold of 10–15 nodes. Currently, both the NCCN and AJCC support this recommendation.

Despite the importance of lymph node staging, evidence suggests that the majority of hospitals in the US do not meet the 15 lymph node benchmark. In a 2008 study from the NCDB, Bilimoria et al. [28] showed that in 2004, only 16.4 % of patients with pancreatic cancer had at least 15 nodes examined after surgery (Fig. 3). In this study, the median number of nodes examined was only 7, far less than what is recommended. Patients were found to be significantly more likely to have an adequate resection at NCCN-NCI centers, yet, even at these experienced centers, patient-level lymph node examination rates remained low at only 27 %. Consistent with this study, a separate report using SEER data from 1988 to 2003, found that approximately 70 % of patients had fewer than 15 lymph nodes examined after surgical resection [33]. These statistics are striking in a disease where the only hope for cure is with surgery. Although high-volume centers met the 15-node benchmark more frequently, there is still substantial room for improvement.

For patients with positive lymph nodes, lymph node ratio has been a measure shown to correlate with survival. One study showed that with <15 % positive nodes 5 year survival was 21.7 % versus 5.2 % with >15 % positive nodes [34]. Another study by Malleo et al. showed that lymph node number and location was also correlated with survival, with lymph node metastases along the proximal superior mesenteric artery having the worst prognostic value as well as increased number of

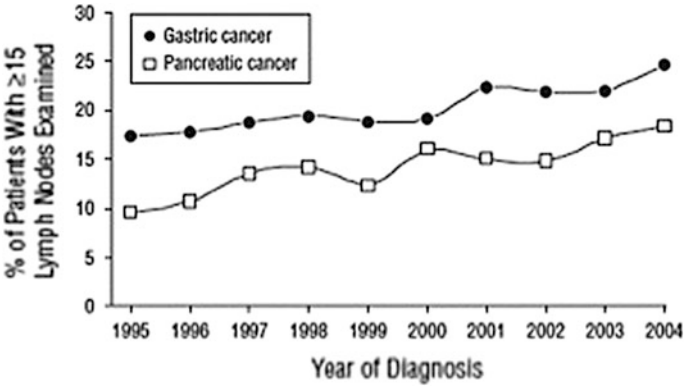

Fig. 3 Trends in lymph node evaluation for gastric and pancreatic cancer from the NCDB (1995–2004). From: Bilimoria et al. [28]

metastatic locations [35]. This study suggests that lymph node metastasis number and pattern may offer valuable prognostic value in addition to positive lymph node ratio, and may help in considering patients for adjuvant or neoadjuvant therapies.

6 Colon Cancer

Colon cancer is the 4th most common malignancy diagnosed in the United States, and approximately 80 % of patients present with potentially resectable disease. Nodal metastases have long been recognized as the most important factor predicting long-term survival [1] and is an important determinant in the decision to administer adjuvant chemotherapy. With the demonstration over the last decade of highly effective systemic therapies for colon cancer, it is essential to ensure that all patients who would benefit from such treatment are identified by appropriate nodal staging [36].

Numerous studies have shown an improvement in disease specific and overall survival when increasing numbers of lymph nodes are examined for colon cancer [37–39]. This improvement in outcomes is likely due in part to stage migration or more accurate staging that allows for increased utilization of adjuvant chemotherapy. Although estimates have varied widely, numerous studies and consensus guidelines (e.g., College of American Pathologists Consensus Statement 1999 [40]) have suggested that examination of 12 regional lymph nodes is a reasonable minimum for adequate nodal evaluation for colon cancer [12, 41, 42]. Despite these findings, population-based assessments have shown that the majority of patients in the United States do not have 12 or more nodes examined [43, 44]. This motivated the American College of Surgeons (ACS), American Society of Clinical Oncology (ASCO), and the National Comprehensive Cancer Network (NCCN) to harmonize a quality measure requiring resection and pathological examination

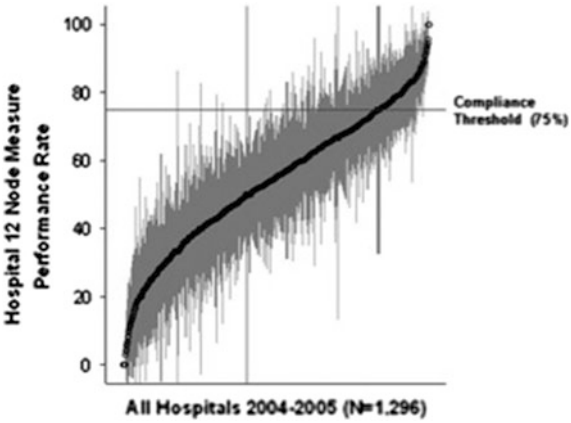

Fig. 4 Proportion of Commission on cancer hospitals meeting 12 node measure performance rate in 2004–2005. From: Bilimoria et al. [44]

of 12 or more lymph nodes for colon cancer [45]. Subsequently, the National Quality Forum (NQF) endorsed the 12-node measure for quality surveillance [41].

In examining treatment of colon cancer patients who underwent colectomy at 1296 hospitals using data from the NCDB, Bilimoria and colleagues [44] found that although the proportion of compliant hospitals increased considerably during the study period, 60 % of hospitals failed to comply with the 12-node measure (Fig. 4). Prior studies conducted at the level of individual patients have demonstrated that only approximately 37–50 % of colon cancer patients in the United States have 12 or more nodes examined [43].

Review of 90,203 patients from the SEER database from 1998 to 2009 show that over that time period, with implementation of a variety of consensus guidelines, the number of patients with at least 12 lymph nodes examined increased from 34 to approximately 75 %. Although significant improvements have been made, roughly 25 % of patients are still not receiving guideline compliant care (Fig. 5) [46]. Nodal evaluation is likely to continue to improve further following the development of the 12-node quality measure and as physicians and hospitals recognize that a requirement to examine 12 or more nodes may affect referral, reimbursement, and will likely soon be reported publicly.

7 Rectal Cancer

Rectal cancer exemplifies the "Halstedian" theory of cancer progression—that is, in an orderly fashion through surrounding lymphatic channels. However, lymph node resection in rectal carcinoma presents some distinct challenges. In 1979, Heald first described the total mesorectal excision (TME) [47], a procedure that removes all regional lymph nodes in the mesorectum. TME has since been demonstrated to substantially decrease tumor recurrence and may also improve survival [48]. However, even among experienced surgeons, the adequacy of TME may vary

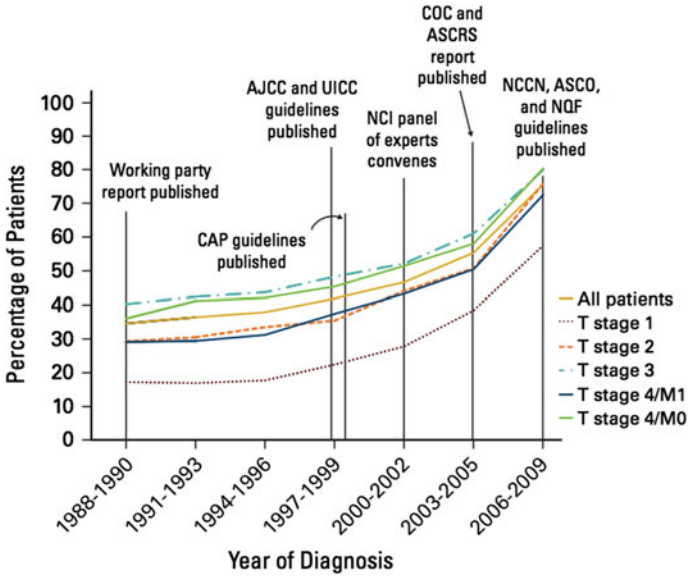

Fig. 5 Percentage of patients receiving guideline-recommended lymph node evaluation by year and T stage with timing of guideline implementation. (*AJCC* American Joint Committee on Cancer; *ASCO* American Society of Clinical Oncology; *ASCRS* American Society of Colon and Rectal Surgeons; *CAP* College of American Pathologists; *COC* Commission on Cancer; *NCI* National Cancer Institute; *NCCN* National Comprehensive Cancer Network; *NQF* National Quality Forum; *UICC* Union for International Cancer Control). From: Parsons et al. [46]

considerably. For example, in the Dutch TME trial [49], even after surgical standardization was performed among all participating surgeons, the quality of TME specimens were inadequate in as many as 1/3 of patients who were treated with abdominoperineal resection [50].

A second challenge in rectal cancer is determining the optimal number of lymph nodes that should be examined after neoadjuvant therapy. Although treatment guidelines are similar to colon cancer in indicating at least 12 nodes should be examined [6, 12], in contrast to colon cancer, it is standard of care for patients with T3/T4 or node positive rectal cancers to receive neoadjuvant therapy. Research has shown that preoperative chemoradiation reduces the number of nodes available for examination [51]. Because preoperative chemoradiotherapy appears to reduce the number of nodes available for examination, it is unclear the precise number of nodes that should be optimally removed [52]. Estimates in the literature are variable, and range between 0 and 30 [53–55]. A study looking at 63,381 patients from the SEER database undergoing surgery for rectal cancer from 1995 to 2009 showed that eighteen nodes were required for patients treated without neoadjuvant radiation versus 16 for those treated with neoadjuvant radiation to prevent stage migration, which the authors defined as detection of Stage III disease [56]. Although these numbers are higher than current guidelines, it does demonstrate the continued

importance of lymph node evaluation despite decreased yield in patients undergoing neoadjuvant therapy. Further studies have shown that number of lymph nodes retrieved is independently associated with disease free survival in patients with rectal cancer who underwent neoadjuvant chemoradiation [57].

It is clear that lymph node staging is important whether or not neoadjuvant therapy is administered. At present, we continue to advocate that surgeons and pathologist continue to follow guidelines recommending examining at least 12 lymph nodes, however further investigation is necessary to determine optimal lymph node yield following neoadjuvant treatment.

Similar to colon cancer, hospital-level performance with respect to lymph node examination rates in rectal cancer is variable. Baxter et al., in one of the few population-based studies evaluating the number of colorectal cancer patient with at least 12 lymph nodes examined, found that rectal cancer was an independent factor associated with decreased lymph node harvests. In addition, this study reported that overall, only 23 % of stage I patients received adequate lymph node evaluation [43]. Nevertheless, rectal carcinoma poses unique challenges, and further research is required to elucidate these controversies.

8 Conclusions

Twenty-five percent of all cancer-related deaths are a result of a gastrointestinal malignancy, and the presence of lymph node metastases is one of the most powerful indicators of poor survival. Only by adequate lymph node evaluation will patients be appropriately staged. Although there is still debate between the "Halstedian" and "Fisherian" philosophies regarding the extent of lymphadenectomy, it remains an important part of cancer staging. Given the current evidence, there appears to be a concerning lack of adequate lymph node examination in the US. Organizations like the American College of Surgeons Cancer Programs have developed numerous quality measures based on adequate lymphadenectomy, and are further developing rapid feedback mechanisms to cancer centers such that hospitals can identify and address potential problems in real time [58]. Future studies should investigate both structural and process related factors that could potentially improve the quality of lymphadenectomy at poor performing centers.

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Circulating Tumor Cells in Gastrointestinal Cancer: Current Practices and Future Directions

Colin M. Court, Jacob S. Ankeny, Shonan Sho and James S. Tomlinson

Abstract

GI cancers are the leading cause of cancer-related death worldwide primarily due to a combination of late presentation and aggressive biology. The lack of adequate biomarkers for screening, diagnosis, staging, and prognosis confounds clinical decision-making and delays potentially effective therapies. Circulating tumor cells (CTCs) are a new biomarker with particular promise in GI cancers, potentially offering clinicians and researchers real-time access to tumor tissue in a reliable, safe, and cost-effective manner. Preliminary studies have investigated the potential clinical utility of CTCs for all GI cancer types with promising results. Furthermore, advances in single cell analytics have been successfully applied to CTCs, allowing for exciting new clinical and research applications. In this chapter, we will review the current state of CTC research in GI cancers as well as the potential future applications that are currently being developed.

Keywords

Cirulating tumor cells · Pancreatic cancer · Diagnosis · Staging · Single Cell Sequencing · Liquid biopsy

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1 Introduction

GI cancers are among the most prevalent cancers, and are the leading cause of cancer-related death, worldwide [38]. Due to their late presentation and aggressive nature, progress in GI malignancies has proven to be slower than for other major cancers. To date, the majority of the progress made in GI cancers has been due to the detection of lesions at an earlier stage through better screening programs or by treatment of cancer-related infectious disease [62]. Unfortunately, screening programs for GI cancers either do not exist, in the case of pancreatic and gastric cancer, or suffer from poor compliance, in the case of colon and liver cancer. Historically, the chemo-resistant nature of many GI cancers has hampered advances in life expectancy and treatment. However, the successes of new multidrug chemotherapeutic regimens and targeted therapies have the potential to make an impact in the treatment of advanced GI malignancies. Unfortunately, treatment response rates vary considerably between patients secondary to differences in tumor biology that most likely are reflected in the mutational profile of each individual tumor [21]. Furthermore, as patients live longer the relevance of the genetic makeup of their primary tumor has decreasing value, and additional biopsies are needed to adjust to the changing mutational landscape of the metastatic tumor deposits. Therefore, with the age of personalized and precision medicine seemingly approaching, there is a pressing need for the longitudinal evaluation of tumors and their response to therapy. CTCs theoretically have the biomarker attributes that are required to satisfy this need.

2 Circulating Tumor Cells

Circulating tumor cells (CTCs), which are thought to originate from primary tumors or metastatic sites, invade into a blood vessel or "intravasate," and then circulate in the blood (Fig. 1) [5]. Although this concept of circulating tumor cells was first introduced by Ashworth [8] while investigating the blood of a widely metastatic breast cancer patient in 1869, it has only been over the past two decades that technological advances have allowed for reliable capture and identification of these rare cells in the blood. These advances have led to an explosion of research: A PubMed search for "circulating tumor cell" returned 16,070 publications, and a search of ClinicalTrials.gov resulted in 615 clinical trials, as of April, 2015. The first CTC platform to gain FDA approval was the CellSearch platform, which looks at CTC enumeration as a prognostic biomarker in metastatic lung, breast, and colon cancer [5]. Beyond simple capture and enumeration, second generation CTC platforms offer the ability to isolate CTCs for further testing in a manner similar to fine needle aspiration (FNA). Therefore, CTCs hold particular promise for GI cancers due to the difficulties involved in obtaining tumor tissue from visceral organs for the diagnosis, staging, prognosis, and management of these tumors. As a

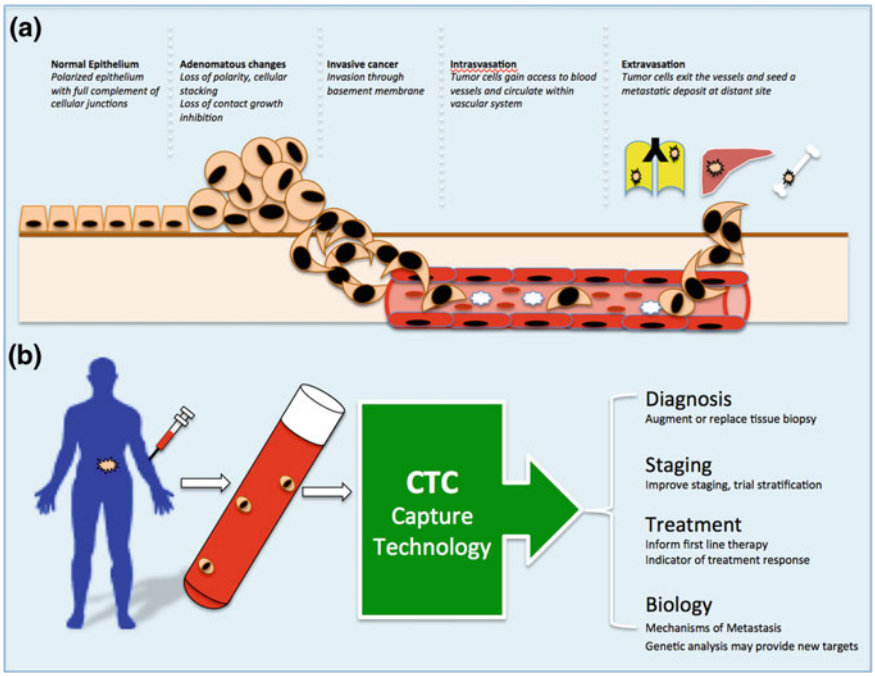

Fig. 1 **a** Adenocarcinoma development and steps of metastases: normal polarized epithelial cells undergo loss of polarity, cellular stacking and loss of contact inhibition of cell division as premalignant "adenomatous" changes. Invasive cancer occurs with invasion through the basement membrane. The classic steps of the "metastatic cascade" are outlined and start with the step of "intravasation" or tumor cells gaining access to the vascular system. CTCs are tumor cells that have achieved at least the "intravasation" step of metastasis. Their potential to achieve extravasation and seeding a distant metastatic deposit remains unknown. **b** Potential of CTCs as a biomarker in GI cancers: CTCs obtained from a single peripheral blood draw have the potential to function as a biomarker to improve cancer diagnosis, staging, treatment guidance and assessment of treatment response, and hopefully will shed more light on the biology of metastasis

potential "liquid biopsy," CTCs may offer clinicians access to tumor tissue in a safe, convenient, and repeatable fashion from a simple peripheral blood draw.

2.1 Enrichment and Detection of CTCs

The difficulties involved in isolating rare CTCs have led to a vast array of different platforms for the enrichment and detection of CTCs. One recent review found over 50 unique techniques, more than 10 of which were first described in the last 5 years [76]. The rarity of CTCs, representing just a few cells in the millions of white blood cells and billions of red blood cells per milliliter of blood, is the primary difficulty that CTC detection methods must address. Most platforms involve a combination of

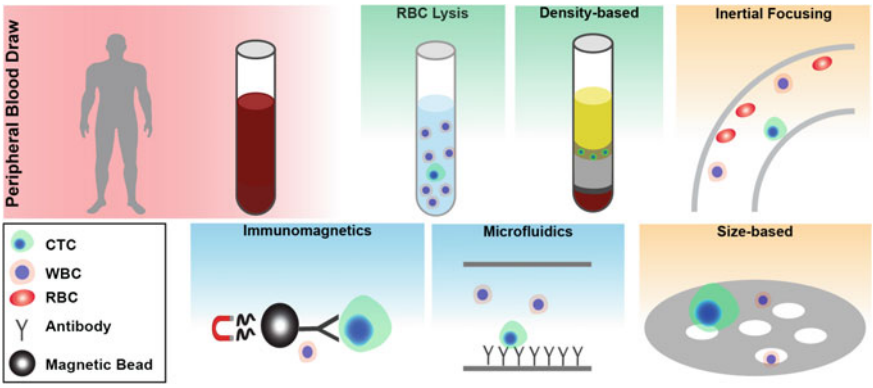

Fig. 2 Methods for the enrichment and isolation of CTCs. *Peripheral Blood Draw* peripheral blood is obtained from patients, usually 2–20 mL. *RBC lysis* involves incubation of whole blood with a mixture of ammonium chloride, potassium carbonate, and EDTA resulting in lysis of RBCs but not WBCs or CTCs. *Density-based* density gradient centrifugation uses a density medium to separate mononuclear cells from RBCs and granulocytes based on cell density. *Inertial focusing* using a spiral device, cells are separated by size based on different flow patterns due to inertial microfluidics. *Immunomagnetics* antibodies are bound to magnetic beads allowing for the capture of CTCs as well as their subsequent manipulation. *Microfluidics* antibody-coated nanofabricated microfluidic channels use various methods to ensure cell-antibody interactions, allowing for the capture and manipulation of CTCs. *Size-based* CTCs are generally larger than RBCs and WBCs and can be trapped by filtration on a micropore membrane. *CTC* circulating tumor cell, *WBC* white blood cell, *RBC* red blood cell, *EDTA* Ethylenediaminetetraacetic acid

different techniques to first enrich the CTC population against that of the RBC and WBC background followed by identification and isolation of CTCs.

Methods used to both enrich and identify CTCs can broadly be broken down into those that utilize physical properties and those that utilize biological ones (Fig. 2). Physical properties such as size, density, and stability are commonly used. Additional properties including electrical charge, optical characteristics, photoacoustics, and deformability have also been studied. In general, enrichment of CTCs by physical properties alone is fast and relatively inexpensive, but does not allow for the level of enrichment seen with biological properties. Thus, many technologies use an initial physical enrichment before more specific biologic enrichment and detection is performed. Biologic properties, including protein expression, as well as DNA and RNA signatures, offer highly specific enrichment and isolation of CTCs. In particular, methods utilizing cell surface markers for enrichment and capture of CTCs are currently the most common. Of the cell surface label-based techniques, the majority use antibodies against the epithelial marker EpCAM to positively select the epithelial cells. While highly specific, some researchers have shown that the use of these anti-EpCAM antibodies results in the loss of CTCs that do not express EpCAM, especially CTCs that have undergone epithelial to mesenchymal transition (EMT) [84]. Therefore, newer technologies use tumor-specific or

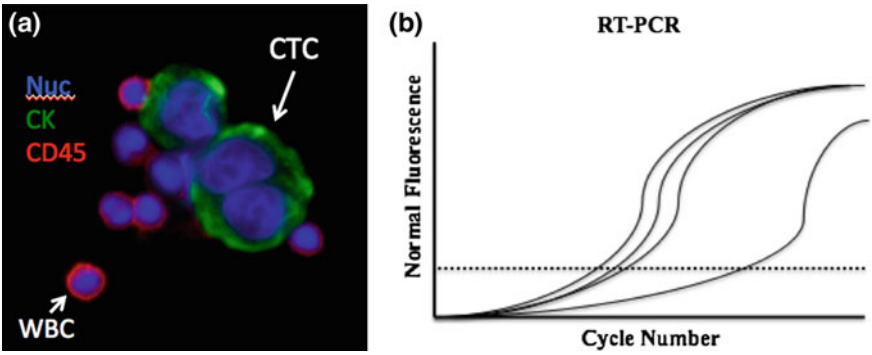

Fig. 3 CTC identification and enumeration methods: **a** ICC: representative image of immunocytochemical staining obtained form the Nanovelcro platform from a patient with pancreatic cancer. CTCs are distinguished form other mononuclear cells based on differential immunostaining and cytologic characteristics. The *blue* nuclear stain (DAPI) identifies all nucleated cells. The epithelial marker cytokeratin is identified by *green* fluorescence (AlexaFluor 488) on CTCs while the hematopoietic marker CD45 is identified by *red* fluorescence (AlexaFluor 555) on WBCs. **b** RT-PCR: the figure shows a representation of a readout from a real-time quantitative RT-PCR study. The PCR reaction is specific for a CTC-associated mRNA transcript (e.g., CEA or hTERT). The graph plots the PCR cycle number versus the normalized relative fluorescent signal emitted at a given cycle count. In these types of studies, thresholds are calculated based on the baseline variance, and CTC positivity is defined as fluorescence greater than that threshold at a predetermined cycle count. If the fluorescent signal is greater than the calculated threshold at a cycle count lower than the cutoff, then the sample is positive for a CTC-associated transcript. *ICC* Immunocytochemistry, *CTC* circulating tumor cell, *WBC* white blood cell, *Nuc* nuclear stain, *CK* cytokeratin, *DAPI* 4′,6-diamidino-2-phenylindole, *RT-PCR* reverse transcription polymerase chain reaction

mesenchymal cell surface markers to allow for the enrichment and capture of these additional CTC subtypes.

Following enrichment, the identification of CTCs must be confirmed. Techniques for doing so can broadly be broken down into visual and molecular categories. The visual identification of CTCs by immunocytochemistry (ICC), which combines cytometry and immunofluorescence, is the most common technique used today. The most common visual definition of a CTC in use today is based on 3-channel immunofluorescence. It utilizes a nuclear stain (usually DAPI), cytokeratins (CK) as an epithelial marker, and CD45 as a hematopoietic marker (Fig. 3). Thus, a Nuclear+/CK+/CD45− cell is a CTC whereas a Nuclear+/CK−/CD45+ cell is a WBC. While these categories are widely accepted, the discovery of Nuclear+/CK+/CD45+ cells, with both epithelial and hematopoietic markers, by many different CTC platforms has led to some uncertainty in the ICC definition of a CTC. Studies to date seem to indicate that these cells may represent nonspecifically stained macrophages or polymorphoneucleocytes, or occur due to technical antibody processing errors [57].

Advances in molecular biology have made molecular detection techniques very sensitive, allowing for the detection of a single CTC in a background of thousands

to hundreds of thousands of WBCs. The most common methods in use today are reverse transcriptase polymerase chain reaction (RT-PCR)-based technologies that identify CTCs based on the expression of tumor-associated mRNA transcripts. Unfortunately, RT-PCR-based techniques are not as specific as ICC due to illegitimate transcription by WBCs that can result in tumor-associated transcripts being found in the blood of normal patients [27]. Therefore, the only truly tumor-specific molecular biomarkers are those looking at gene fusion products or ubiquitous driver mutations. While most studies to date have been limited to RT-PCR of a single transcript, advances in multiplexing and single cell sequencing are increasingly being explored.

2.2 Clinical Implications of CTCs

Despite the large body of research on CTCs, basic questions about the detection, isolation, enumeration, meaning, and clinical utility of CTCs remain to be answered. Additionally, important fundamental questions about the source of CTCs and their importance in tumor biology, especially the metastatic cascade, are yet to be definitively answered. CTCs are very heterogeneous, both between patients and among CTCs of a single patient, expressing markers of both epithelial and mesenchymal origin [54]. While studies have proven the viability of some CTCs through culture and functional assays, many CTCs appear to be apoptotic [1, 4]. Additionally, their half-life appears to vary considerably between studies, from hours to weeks, in studies of patients with resected tumors. Perhaps the most important unanswered question is why CTCs do not correlate directly with the presence of metastasis. For example, patients with peritoneo-venous shunts do not immediately develop widespread metastasis despite large numbers of circulating tumor cells [95]. One possibility is that CTCs demonstrate considerable metastatic inefficiency, likely due to the issues outlines above. In fact, studies have demonstrated that only 2.5 % of CTCs can form micrometastases and only 0.01 % of them can form macrometastases [20, 58, 74]. Thus, CTCs as a whole represent tumor cells that have accomplished the metastatic step of intravasation, however, whether these cells have the ability to extravasate from the vascular system and seed a metastastic distant metastatic colony is not clear. The isolation of the subset of CTCs with metastatic potential remains an active area of research [27]. One relevant area of research has been the study of intraoperative CTC counts to determine if surgical technique may affect the risk of metastasis following surgery. Studies have found that pressure, biopsies, and other invasive manipulations during surgery can transiently increase CTC counts up to 66 fold [39]. However, despite the development of “touchless” techniques that decrease the shedding of CTCs into the blood stream, studies have not consistently demonstrated an associated decrease in survival.

3 CTCs as a Biomarker in Gastrointestinal Cancers

Studies to date have found several promising clinical applications for CTCs in GI cancers. As a biomarker in GI cancers, CTCs have a very high specificity coupled with a lower sensitivity. Their high specificity, equivalent to that of an FNA, may eventually allow CTCs to function as a liquid biopsy. However, several important limitations have been found. While false positive CTCs are rarely found in healthy controls, circulating epithelial cells have been detected in patients with benign diagnoses such as cystic pancreatic lesions and inflammatory bowel disease [75, 81]. While newer molecular techniques may allow for the confirmation of tumor origin of circulating epithelial cells, studies on patients with inflammatory and cystic diseases are yet to be performed. Additionally, GI cancers generally have lower CTC counts than other malignancies. The filtering or "first pass" effect of the liver is thought to be the culprit and this theory has been demonstrated in a mouse model [85]. Additional support for this theory comes from several studies that found higher CTC counts in portal blood versus systemic blood in patients during surgery [22]. Currently, the only FDA-approved CTC application is the enumeration of CTCs as a prognostic biomarker in patients with colorectal cancer (CRC). However, the success of recent clinical studies makes the approval of CTCs as a biomarker in other GI cancers likely. Furthermore, molecular data obtained through analysis of CTCs may also shed light on new molecular therapeutic targets as well as provide guidance on clinical therapeutic decision-making.

3.1 Overview of Studies

While CTCs have been studied in the context of almost every clinical parameter, only the association of CTC positivity with poor prognosis has been universally found in all GI cancers. The inconsistency in clinical findings is primarily due to the large variability in CTC platforms and detection techniques coupled with small sample sizes. As discussed above, methods for enriching and identifying CTCs vary considerably, resulting in numerous CTC definitions. As an example, when comparing an RT-PCR-based CTC platform with an ICC-based CTC platform, the detection of a tumor-associated transcript by RT-PCR is viewed as equivalent to the detection of a circulating epithelial cell by ICC. Obviously, both have different sensitivities of detection of CTCs coupled with different sources of errors making comparisons very difficult between studies of the same tumor type utilizing different CTC isolation technologies. Even within studies using the same platform, the definition of CTC positivity varies. For example, for the FDA-approved CellSearch system, CTC positivity is defined as ≥ 5 CTCs in breast and prostate cancer versus ≥ 3 CTCs in colon cancer. Furthermore, the amount of blood analyzed in each study can vary considerably. While most CTC platforms require between 5 and 10 mL, they range from as low as 1 mL to as high as 1500 mL. All of these variables make comparing studies difficult, limiting the ability of clinicians and

Table 1 Esophageal CTC studies

Study	Patient type (*N*)	CTC platform	Results Summary
Nakamura (2000)	Pretreatment, SCC (47)	Dynabeads; DGC and ICC	38 % of patients were CTC+. CTC+ patients had worse survival after both surgery and chemotx
Nakashima (2003) 69	Preoperative, SCC (54)	DGC and RT-PCR for CEA mRNA	31/54 (57.4 %) patients were CTC+. CTC+ patients had more positive nodes, more recurrences, and worse prognosis
Ito (2004)	Pretreatment, SCC (28)	RT-PCR for CEA and CK-20 mRNA	CEA mRNA found in 7/28 patients; CK-20 mRNA found in 3/28 patients
Liu (2007) 55	Preoperative, Adeno/SCC (53)	DGC and RT-PCR for CEA mRNA	Of CTC+ patients, 50 % recurred within 1 year versus 14.3 % of CTC− patients
Hiraiwa (2008)	Preoperative, SCC (38)	CellSearch	0/10 nonmetastatic and 5/23 metastatic patients were CTC + ($P < 0.121$). CTC+ associated with worse prognosis in metastatic pts ($P < 0.001$)
Yin (2012)	Preradiotherapy, SCC (72)	DGC and RT-PCR for CEA, CK-19, and survivin mRNA	54.2 % (39/72) and 38.9 % (28/72) of patients were CTC+ pre- and post radiotherapy, respectively. CTC+ associated with worse survival
Bobek (2014)	Preoperative, Adeno/SCC (43)	MetaCell; size-based and ICC	27/43 (62.8 %) patients CTC+. 69.2 % resectable patients CTC +; 42.9 % unresectable patients CTC+
Reeh (2015)	Preoperative, Adeno/SCC, (100)	CellSearch	18/100 (18 %) of patients CTC+. CTC+ patients had shorter relapse-free and overall survival ($P < 0.001$)

researchers to draw conclusions about the applicability of CTCs in various clinical contexts. Therefore, we have reviewed the important studies on CTCs in GI cancers individually in Tables 1, 2, 3, 4, 5 and 6 while discussing the implications and clinical applicability in the text of this chapter.

3.2 CTCs in Esophageal Cancer

Esophageal cancer incidence is increasing the fastest among all cancers in the US today [12]. It is usually diagnosed late, and is noted for its aggressive course. Micrometastases are hypothesized to be responsible for the high rates of recurrence in postoperative patients in long-term follow up studies [56]. While a moderate

Table 2 Gastric CTC studies

Study	Patient type (*N*)	CTC platform	Results Summary
Hiraiwa (2008)	Preoperative, SCC (38)	CellSearch	2/14 nonmetastatic and 15/27 metastatic patients were CTC+ ($P < 0.006$). CTC+ associated with worse prognosis in metastatic patients ($P < 0.039$)
Matsusaka (2010)	Pre- and postchemotx (52)	CellSearch	Patients who were CTC+ at 2- and 4-week point of chemotx had shorter PFS and OS
Uenosono (2013)	Consecutive clinic patients (265)	CellSearch	10.8 % of resectable patients and 60.2 % of unresectable patients were CTC+. CTC+ patients had worse survival and earlier recurrence ($P < 0.001$)
Tang (2013)	Meta-analysis (1030)	RT-PCR-based studies	As a diagnostic, the sensitivity and specificity of CTCs was 42 and 99 %, respectively. Summary AUC = 0.97

number of RT-PCR-CTC studies exist, relatively few ICC-based CTC studies have been conducted. Overall, studies using both techniques have consistently demonstrated low CTC counts, with only 18–66.7 % of patients demonstrating CTC positivity.

Among ICC studies, the largest study to date found CTCs in 18 % of 100 patients undergoing surgical resection [79]. CTC presence was strongly associated with worse relapse-free ($P < 0.001$) and overall survival ($P = 0.007$). Furthermore, multivariate analysis demonstrated that CTCs are an independent prognostic indicator of tumor recurrence (HR = 5.063; 95 % CI = 2.233–11.480; $P < 0.001$). This study demonstrates the utility of CTCs as an adjunct preoperative staging marker in determining the potential benefit of surgery and perhaps informing adjuvant therapy utilization. Of note, an earlier study found that CTC count did not correlate with resectability. Bobek et al. [12] used MetaCell, a sized-based isolation platform that allows for the cultivation of live CTCs, and found CTCs in 62.8 % of 43 patients with esophageal cancer. They found more CTCs in patients with adenocarcinoma than with squamous cell carcinoma, and, paradoxically, found more CTCs in resectable patients than in nonoperative ones. Other studies using ICC support the poor prognosis associated with CTC positivity. Using the CellSearch system, Hiraiwa et al. demonstrated that patients with higher CTC counts had a worse prognosis and were significantly more likely to develop pleural dissemination [30]. A study by Nakamura et al. [68] used the Dynabeads platform on 47 patients with esophageal SCC and found that 38 % of them were CTC positive, and that CTC positivity correlated with worse survival after both surgery and chemotherapy. Similarly, Matsushita et al. [61] found that the presence of CTCs following chemotherapy predicted progressive disease in patients with SCC.

Molecular studies in esophageal cancer have utilized RT-PCR of numerous cancer-associated mRNAs including CEA, survivin, SCC, and cytokeratin [32, 56, 93, 108]. Similar to the findings with ICC techniques, RT-PCR studies have found

Table 3 Pancreas CTC studies

Study	Patient type (*N*)	CTC platform	Results Summary
Soeth (2005)	Preoperative (154)	DGC and RT-PCR for CK-20 mRNA	52/154 (33.8 %) patients were CTC+. CTC+ correlated with higher stage and worse survival
Zhou (2011)	All types (25)	Immunomagnetic and RT-PCR for C-MET, hTERT, CK20, and CEA mRNA	The positive expression rates of C-MET, hTERT, CK20, and CEA in the group were 80 % (20/25), 100 % (25/25), 84 % (21/25), and 80 % (20/25), respectively. One benign control positive for CK20. CTC+ correlated with stage and lymph node status
Ren (2011)	Pre chemotx (41)	CD45 depletion and ICC	Found CTCs in 80.5 % of patients before chemotx but only 29.3 % after. Postchemotx CTCs showed more apoptosis
Khoja (2012)	Stage III or IV (54)	ISET; Size-based and RT-PCR for EpCAM, CK, vimentin and E-cadherin	Compared ISET to CellSearch. CellSearch detected CTCs in 40 % of patients versus 93 % for ISET. ISET found CTCs in higher numbers, possibly mesenchymal CTCs
Iwanicki-Caron (2013)	Prediagnosis (40)	ScreenCell; Size-based and cytopathology	Compared CTC to EUS-FNA. For EUS-FNA, sensitivity, specificity, and accuracy were 77.8, 100, and 85 %, respectively. For CTCs, sensitivity, specificity, and accuracy were 55.5, 100, and 70 %, respectively
Ankeny et al. [7]	Prediagnosis (71)	Nanovelcro; DGC and ICC	39/52 (75 %) patients CTC+ at time of diagnosis. CTCs correlate with stage, outperforming CA19-9 at determining local versus metastatic disease
Rhim (2014)	Prediagnosis (31)	GEM chip; microfluidics and ICC	7/21 (33 %) of patients with cystic lesions but no diagnosis of cancer were CTC+. 8/11 (73 %) of cancer patients and no healthy controls CTC+. CTCs did not correlate with stage or tumor/cyst size
Yu (2014)	Stage III and IV (50)	CTIC platform; ICC and invasiveness	50/50 (100 %) patients CTC+. Used gene expression profiles to predict response to chemotherapy and tracked the development of resistance over time

Table 4 Biliary and neuroendocrine CTC studies

Study	Patient type (*N*)	CTC platform	Results Summary
Khan (2011)	GI-NETS (79)	CellSearch	43 % of midgut NETs and 21 % of pancreatic NET patients were CTC+. CTCs associated with stage and disease progression
Khan (2013)	Metastatic GI-NETS (176)	CellSearch	49 % patients had $\geq$ one CTC, 42 % had $\geq$ two CTCs, and 30 % had $\geq$ five CTCs. Presence of CTCs was associated with increased burden, increased tumor grade, and elevated CgA
Al Ustwani (2012)	Biliary cancers (16)	CellSearch	3/13 cholangiocarinomas and 1/3 gallbladder cancers CTC+
Mataki (2004)	Preoperative biliary cancers (54)	DGC and RT-PCR for CEA mRNA	CTC+ patients recurred 75 % of the time versus 5.4 % of CTC− patients. CTCs outperformed other tumor markers and rose before recurrence could be found by imaging
Leelawat (2012)	Cholangiocarcinoma (40)	DGC and RT-PCR for CK-19 and hTERT mRNA	45 % of patients CTC+. CTC+ associated with worse survival

correlations with multiple prognostic indicators including advanced disease, relapse, and recurrence, as well as reduced disease-free and overall survival. Unfortunately there has been a large degree of variability between studies making conclusions difficult to draw. For example, rate of CTC detection using RT-PCR examining CEA mRNA in the blood of preoperative esophageal SCC patients varied from 25 to 62 % [104].

3.3 CTCs in Gastric Cancer

Gastric cancer is the second most common cause of cancer deaths worldwide [19]. While 5-year survival for early stage disease is almost 90 %, the 5-year survival for advanced gastric cancer is less than 4 % [94]. Unfortunately, no effective screening regimen exists, and the majority of patients are diagnosed with advanced disease. Similar to esophageal cancer, there have been few ICC studies but numerous molecular ones. Overall, both ICC and molecular studies have demonstrated a strong correlation between CTC presence and survival. Unfortunately, despite a large number of studies looking at CTCs as a screening biomarker in gastric cancer, the sensitivity is consistently too low for clinical use.

Table 5 Liver CTC studies

Study	Patient type (*N*)	CTC platform	Results Summary
Waguri (2003)	All types (55)	Immunomagnetic beads and RT-PCR for hTERT mRNA	29/55 (53 %) HCC patients were CTC+, and CTCs correlated with disease extent
Vona (2004)	Nonmetastatic HCC (44)	ISET; Size-based, cytology and ICC for AFP	23/44 (52.3 %) patients were CTC+. CTCs associated with tumor diffusion, PVT, shorter OS
Fan (2011) 18	Preoperative (82)	DGC, Flow cytometry	CTCs associated with risk of both intrahepatic and extrahepatic recurrence after hepatectomy
Xu (2011)	All types (85)	DGC and ASGPR-based binding enrichment, ICC	69/85 (81 %) patients CTC+, no CTCs found in healthy or cirrhotic controls, or in patients with other types of cancer. CTCs correlated with tumor size, PVT, differentiation, TNM stage. HER-2 amplification and TP53 mutations detected by single cell analysis
Kim (2012)	Preoperative, resection (17) or liver transplant (6)	DGC and RT-PCR for hTERT mRNA	No difference in hTERT mRNA levels found between HCC and control groups
Sun (2013)	Pre- and postoperative (123)	CellSearch	66.67 % of patients CTC+, and preoperative CTCs were associated with increased risk of recurrence. Postoperative decline in CTCs associated with decreased risk of recurrence
Schulze (2013)	All types (59)	CellSearch	18/59 (30.5 %) of HCC patients CTC+ versus 1/19 (5.3 %) of patients with cirrhosis or benign hepatic tumors. CTCs associated with shorter OS and correlated with AFP levels
Nel (2013)	All types (11)	DGC and ICC for mesenchymal and epithelial markers	An increase in the ratio of mesenchymal to epithelial type CTCs was associated with faster progression

Table 6 Colon CTC studies

Study	Patient type (*N*)	CTC platform	Results Summary
Allard (2004)	All types (196)	CellSearch	30 % of patients were CTC+
Cohen (2008)	Baseline and during chemotherapy (430)	CellSearch	26 % of patients were CTC+. Pretreatment CTC+ associated with worse PFS and OS. Conversion from CTC+ to CTC− during chemotherapy associated with better PFS and OS
Sastre (2008)	All types (94)	CellSearch	34/94 (36.2 %) patients were CTC+. CTCs correlated with stage but not location, differentiation, and LDH or CEA level
Katsuno (2008)	Preoperative, meta-analysis (646)	RT-PCR of CEA mRNA	CTCs associated with positive lymph nodes, hepatic metastases, and worse DFS 1 year post-resection
Tol (2010)	Baseline and during chemotherapy (467)	CellSearch	129/467 (29 %) patients were CTC+. Presence of CTCs at both baseline and during chemotherapy were associated with worse PFS and OS
Rahbari (2010)	All types, meta-analysis (3094)	RT-PCR and ICC	CTCs associated with worse DFS and OS
Gazzaniga (2011)	Stage II/III (37)	CellSearch	22 % of patients were CTC+ after surgery and before adjuvant therapy. CTCs associated with lymph node involvement and stage
Thorsteinsson (2011)	Pre- and postoperative (20)	CellSearch	1/20 (5 %) patients CTC+ preoperatively, and 0/20 (0 %) patients CTC+ postoperatively
Deneve (2013)	All types (75)	RosetteSep CD45 depletion; CellSearch; Epispot culture-based CK19 releasing assay	41/74 (55.4 %) patients were found to have CTCs by the RosetteSep/Epispot assay system versus 20/69 (29.0 %) patients by the CellSearch system. CTCs detected by Epispot were found to be viable based on culture and CK-19 releasing assay

(continued)

Table 6 (continued)

Study	Patient type (*N*)	CTC platform	Results Summary
Groot Koerkamp (2013)	Meta-analysis (1491)	RT-PCR and ICC	CTCs associated with worse PFS and OS
Shigeyasu (2014) 89	All types (8)	RBC lysis and adenovirus GFP-based FACS	2/8 (25 %) of patients had CTCs and KRAS/BRAF mutations were confirmed by sequencing. KRAS/BRAF mutations from CTCs and primary tumors matched
Huang (2015)	Meta-analysis (1847)	CellSearch	CTCs are significantly more common among metastatic than nonmetastatic patients. CTCs associated with worse PFS and OS, as well as worse response rate during chemotherapy

Several studies have examined the use of the CellSearch system in gastric cancer. In the largest study to date, Uenosono et al. [100] looked at CTC enumeration in 265 consecutive patients with gastric cancer. They found CTCs in 10.8 and 60.2 % of resectable and unresectable patients, respectively. The presence of CTCs was associated with worst overall survival ($P \leq 0.0001$) and was an independent factor in determining overall survival in multivariate analysis. The presence of CTCs was also associated with shorter time to recurrence in patients who underwent resection. A study by Hiraiwa et al. [30] resulted in a similar conclusion; CTCs were associated with worse prognosis in patients with metastatic disease. The utility of CTCs in predicting response to chemotherapy has had mixed results. Kolodziejczyk et al. [47] showed that the number of CTCs decreased following preoperative chemotherapy in potentially resectable gastric cancer patients. However, while chemotherapy eliminated CTCs from the blood in 14 of 32 patients (44 %), there was no correlation with 3-year survival. However, similar work done by Matsusaka [60] did find significance; patients found to have CTCs at 2 and 4 weeks after induction of chemotherapy had worse progression-free survival.

The majority of CTC molecular studies to date have focused on the utility of CTCs as a screening test. A meta-analysis pooling data from 20 different studies by Tang et al. examined the utility of RT-PCR-based techniques in a total of 1030 patients and 668 controls [94]. They found a pooled sensitivity and specificity of 42 and 99 %, respectively, and a summary ROC curve of 0.97 (95 % CI, 0.95–0.98). Unfortunately, this low sensitivity makes it unlikely that CTCs will be the screening biomarker that is desperately needed in gastric cancer. Other studies have found associations between molecular CTC markers and postoperative recurrence [77, 88], metastatic disease [64, 66], major vascular invasion [49], and tumor invasion depth [66, 99].

3.4 CTCs in Pancreatic Cancer

Pancreatic ductal adenocarcinoma (PDAC) is the 10th most common cancer but the 4th most common cause of cancer-related death in the US [90]. The overall prognosis is dismal due to late presentation and aggressive biology, with a 5-year survival of 5 % for all patients and only 2 % for those with metastatic disease [31]. However, the recent successes of multidrug regimens like FOLFIRINOX and Gemzar-Abraxane have energized the research and clinical community to search for improved biomarkers in patients with pancreatic cancer. While historically surgery has been offered to almost all patients with even borderline resectable cancers, 5-year survival after surgery remains only 15–25 % for most studies due to high recurrence rates, indicative of the failure of current staging systems [82]. Due to these statistics, there is a pressing need for better biomarkers for screening, diagnosis, staging, prognosis, and management of pancreatic cancer. Studies on CTCs

in PDAC to date have been small, but several promising studies provide insight into the potential future applications of CTCs in pancreatic cancer.

In the original CellSearch study, pancreatic cancer was noted to have one of the lowest levels of CTCs amongst all solid tumors [5, 97]. Additional studies using the CellSearch system in pancreatic cancer have consistently resulted in low CTC detection rates, with CTCs found in only 5–19 % of preoperative patients and between 39–42 % of metastatic patients [9, 43, 48]. Due to these low yields, newer platforms have looked at a variety of novel methods for increasing CTC counts. The most promising studies have used size-based filtration, microfluidic devices, or molecular techniques with CTCs showing significance in several clinical parameters.

Several small studies have demonstrated the potential utility of CTCs as an initial diagnostic biomarker in pancreatic cancer. The current gold standard, cross-sectional imaging and EUS-FNA, has a combined sensitivity of 68–92 % in PDAC due to the relative paucity of tumor tissue within the dense stromal background [37]. Therefore, multiple biopsies and even multiple endoscopies are commonly needed to ensure a tissue diagnosis. Iwanicki-Caron et al. [37] used the ScreenCell size-based filtration platform in 40 patients undergoing EUS-FNA and determined that CTCs have a sensitivity and specificity of 55.5 and 100 %, respectively. Given the high costs and potential risk associated with endoscopic biopsy, the authors conclude that CTCs may offer clinicians a safer initial biopsy option prior to EUS-FNA. Overall, as a diagnostic biomarker, CTCs are less sensitive than EUS-FNA; however, CTCs are cheaper, less invasive, and virtually risk free. CTCs are therefore a potential first line diagnostic assay in patients with suspicion of PDAC prior to EUS-FNA.

The study of CTCs in the staging of pancreatic cancer has generated mixed results [7, 11, 37, 81, 91, 112]. Due to differences in the classification of stage between the studies, specifically the lack of pathologic confirmation of stage in 2 of the 6 studies, conclusions are difficult to make. However, in studies that looked at postoperative pathologic stage, preoperative CTC positivity consistently correlated with a higher stage. Furthermore, CTC positivity has been shown to correlate with risk of recurrence. Together, these findings make a case that CTCs may be a useful staging biomarker, providing clinicians with additional support in deciding optimal first line therapy in patients with PDAC.

Similar to all GI tumors, CTCs have consistently demonstrated prognostic utility using newer CTC platforms. Both ICC and molecular studies have demonstrated the association between CTC positivity and worse disease-free and overall survival. In a meta-analysis of nine studies looking at 623 patients with pancreatic cancer, CTC positivity was associated with worse progression-free survival (HR = 1.89, 95 % CI = 1.25–4.00, $P < 0.001$) as well as overall survival (HR = 1.23, 95 % CI = 0.88–2.08, $P < 0.001$) [28]. Additionally, in subgroup analysis by treatment type CTC positivity was associated with worse progression-free survival and overall survival in all three treatment modalities: surgery alone, chemotherapy alone, and surgery combined with chemotherapy. However, larger studies using

established biomarker validation protocols are required before these conclusions can be considered clinically translatable.

Two studies have also looked at the potential for CTCs to assist in chemotherapeutic decision-making. Yu et al. [109] used a novel cell invasion-based CTC capture platform to isolate cells that were thought to have an invasive phenotype. In a trial of 50 patients with locally advanced or metastatic disease, they were able to isolate CTCs in all 50 patients. Following CTC isolation, they used mRNA microarray-based expression profiling to generate a pharmacogenomics drug sensitivity profile (PGx) to predict response to a number of different chemotherapy regimens. The study is significantly limited by the fact that they were not able to confirm whether or not isolated cells were in fact cancer cells or just leukocytes by the authors own admission. No staining or genetic analysis of driver mutations was performed. A different study used immunomagnetic bead enrichment followed by ICC staining with CK and CA19-9 to look for apoptotic CTCs following 5-FU therapy in 41 metastatic patients [80]. They found CTCs in 80.5 % of patients before treatment but only 29.3 % of patients 1 week after initiating therapy. Furthermore, 20 % of the CTCs after initiation of therapy displayed apoptotic changes by TUNEL staining. While the potential utility of these studies is difficult to assess, they represent an interesting potential application of CTCs informing and evaluating therapy.

3.4.1 CTCs in Neuroendocrine Tumors

Neuroendocrine tumors (NETs) were once thought to be rare entities but now comprise 2 % of all malignancies worldwide, the majority of them being gastrointestinal in origin [86]. While functional NETs have well-established biomarkers, nonfunctional tumors, which comprise 40–60 % of all NETs, have only seen partial success with currently available biomarkers [101]. Furthermore, Chromogranin A (CgA), the only widely used marker, is not specific for NETs as it is elevated in a number of nonmalignant conditions, such as rheumatoid arthritis, pancreatitis and inflammatory bowel disease among others [41]. CTCs have been examined by a series of studies from a single group in GI-NETs. After demonstrating the high degree of EpCAM expression in NETs, Khan et al. used the CellSearch system to look at CTCs as a prognostic biomarker in patients with metastatic NETs and compared their findings to the current gold standard, tumor grade [42, 42]. Of 176 patients with liver metastases, they found CTCs in 49 % of patients, 51 % with midgut NETs and 36 % with pancreatic NETs. They found that the CTC positivity was associated advanced stage, CgA level, as well as worse progression-free and overall survival. Furthermore, in multivariate analysis they found that while CTCs were associated with increased tumor burden and grade, as a biomarker, CTCs outperformed both of these measures as predictors of progression-free and overall survival.

3.5 CTCs in Liver Cancer

Hepatocellular carcinoma currently ranks third worldwide in cancer-related mortality and its incidence is expected to double over the next 10–20 years in the United States [19, 44]. Unfortunately, despite the implementation of screening programs, over 50 % of HCC is diagnosed late. Of the patients who are eligible for resection of their tumor, 70 % will experience a recurrence. Even with the application of orthotopic liver transplantation as the ultimate local/regional therapy for HCC in the setting of cirrhosis, up to 20 % of patients will experience a recurrence following transplantation [2]. These data highlight the need for development of improved biomarkers that could predict recurrence and thus inform treatment decisions given the paucity of available allografts for transplantation.

CTC counts in HCC have generally been higher than in other gastrointestinal cancers, presumably due to the filtering effect of the liver on other GI cancers. The CellSearch system has been used by several studies in HCC and has consistently demonstrated the association of CTC positivity with worse prognosis and overall survival. Sun et al. [92] looked at 123 patients undergoing liver resection and found that 41.5 % of patients were CTC positive. On multivariate analysis, CTC positivity was an independent risk factor for recurrence and was further associated with early recurrence. Schulze et al. found that 30.5 % of 59 patients with HCC were CTC positive, as well as 1 of 18 healthy controls [87]. They also found that CTC positivity was associated with worse overall survival. Additionally, they found that CTC positivity correlates with staging and AFP level, as well as macroscopic and microscopic vascular invasion.

One concern with using the CellSearch system in HCC is that only a third of HCC tumors are positive for EpCAM and only 10–20 % will stain for the most common cytokeratin markers [17]. This likely represents the more mesenchymal phenotype of hepatocytes versus the ductal and epithelial cells responsible for most GI malignancies [52]. In order to capture these CTCs, different methods must be utilized such as size-based enrichment methods or capture based on mesenchymal markers. Nel et al. [72] used immunomagnetic bead enrichment to isolate CTCs with both epithelial and mesenchymal characteristics. They looked at the clinical implication of phenotypically mesenchymal or epithelial CTCs separately, and found that an increase in the ratio of mesenchymal to epithelial CTCs was associated with shorter progression-free survival. A different approach looked at ICAM-1 positive cells, a cancer stem cell marker postulated to indicate a higher metastatic potential [54]. They found these circulating stem cells in 30 of 60 patients (50 %) and found an association with both disease-free and overall survival. Similarly, a study by Vona et al. [102] used the size-based ISET platform combined with cytomorphologic analysis and found CTCs in 23 of 44 (52.3 %) nonmetastatic patients. CTC positivity was associated with shorter survival as well as tumor diffusion and portal vein thrombosis. They then used molecular techniques to find that only 3/60 CTCs isolated had β-catenin mutations and concluded that the Wnt/β-catenin pathway was unlikely to be important for tumorigenesis; however, studies since then have tended to disagree [53].

One novel approach used the hepatocyte-specific ASGPR receptor system for CTC capture as a means of bypassing the issues of EMT. The first study from the group used the target of ASGPR, asialofetuin, as a capture substrate to bind CTCs [105]. They found CTCs in 69 of 85 patients (81 %), and found that both CTC presence and enumeration correlated with tumor size, portal vein thrombosis, differentiation status, and stage. While not statistically significant, among seven patients who underwent transplant, 3 of the 4 CTC positive patients had a recurrence while the remaining negative CTC negative patients did not experience a recurrence in follow up. A second study from the group used anti-ASGPR antibodies to capture the CTCs [51]. They found CTCs in 89 % of 27 patients with HCC.

Molecular studies of HCC CTCs have primarily used RT-PCR of AFP mRNA; however, small studies have also examined hTERT, alpha albumin, and CK19. Studies using AFP mRNA to detect CTCs have generally had poor results, with sensitivities ranging from 10 to 73 % [10, 14, 26]. Additionally, AFP detection is complicated by the 12.5 % of cirrhotic patients who express AFP [104]. Studies looking at other transcripts have had mixed results. A study looking at hTERT mRNA was able to detect CTCs in 29 of 55 (53 %) of cases, and found an association with clinical stage [103]. However, a similar study by another group in 23 patients undergoing resection did not find prognostic significance for hTERT [45].

3.6 CTCs in Biliary Cancers

There has been one study looking at CTCs in biliary cancers using the CellSearch system [3]. CTCs were detected in 3 of 13 (23.1 %) cholangiocarcinomas and 1 of 3 (33.3 %) gallbladder carcinomas. Unfortunately, the number of patients in the study was too low to draw any conclusions about the utility of CTCs in biliary cancers. Molecular studies have demonstrated an association between CTCs and prognosis using a variety of different tumor-associated transcripts. The utility of CEA mRNA was assessed by a study using density centrifugation followed by nested RT-PCR and compared its utility with that of the serum tumor markers CEA and CA19-9 in the detection of recurrence following surgery [59]. They found that CTCs outperformed both serum CEA and CA19-9 in the detection of recurrence, and that levels of CEA mRNA rose months before recurrence was detected by imaging. Most importantly, CTCs were found in 75 % of patients who relapsed but only 5.4 % of those who did not ($P < 0.001$). A similar study using CK19 and hTERT mRNA found CTCs in 45 % of 40 patients with cholangiocarcinoma and was associated with worse overall survival [50].

3.7 CTCs in Colon Cancer

Colorectal cancer (CRC) is the second leading cause of cancer-related deaths in the United States [15]. While advances in screening protocols have dramatically increased early detection, patient-related factors and cost continue to make compliance with recommended screening an issue. Furthermore, available biomarkers are inadequate for staging, management, and prognosis. There are more studies of CTCs in CRC than in all other gastrointestinal malignancies combined. This is primarily due to the prevalence of CRC, but also due to FDA approval of the CellSearch platform in CRC. Studies using the CellSearch, and other ICC-based, platforms have demonstrated the utility of CTCs for a number of clinical parameters. The majority of studies have looked at the association between CTC enumeration and prognosis; however, CTCs have also been associated with regional lymph node involvement, stage, and postoperative recurrence. The original CellSearch study by Allard et al. looked at 196 metastatic CRC patients and found CTCs in 30 % of them [5]. Other CTC enumeration studies have found similar results: from 5 to 24.1 % of nonmetastatic and 37–60.7 % of metastatic patients will have CTCs [16, 23, 63, 83, 96]. Sastre et al. [83] looked at CTC enumeration in 97 patients with CRC, and found that CTC positivity correlated with stage. They found CTCs in 34 (36.2 %) patients including 20.7 % of stage II patients, 24.1 % of stage III patients, and 60.7 % of stage IV patients. There was no correlation between CTC count and tumor location, CEA or LDH level, or tumor grade. Similar studies have also demonstrated the correlation between CTC presence and increased stage in both pre- and postoperative patients [46]. The association between stage and CTC count is not always found due to the overall low yield of CTCs in early stage patients. Thorsteinsson et al. [96] looked at 20 patients with stage I–III CRC and found a CTC in only 1 of 20 patients (5 %). Overall, ICC-based studies have consistently found an association between CTC positivity and worse prognosis. A prospective multicenter study using the CellSearch platform on 430 patients with metastatic CRC looked at CTC enumeration at baseline as well as after first, second, and third-line therapies [15]. They found that 26 % of patients had CTCs at baseline, and that these patients had significantly shorter progression-free and overall survival. Conversely, during therapy, patients who converted from being positive for CTCs to negative had significantly longer overall survival than patients who continued to have CTCs following treatment. They also found that higher CTC enumeration 3–5 weeks after chemotherapy was associated with a greater risk of progression or death. Two meta-analyses have found similar results. First, a meta-analysis of 11 studies employing the CellSearch platform in CRC found a strong correlation with prognosis: patients with CTCs had a twofold increased risk of progression, recurrence, and death, as well as a significantly lower response rate to chemotherapy [35]. A different meta-analysis by Groot Koercamp et al. [25] looked at all twelve ICC-based enumeration studies in patients with CRC which totaled 1329 patients. They concluded that patients with CTCs had a 2.5-fold increased risk of death and a twofold increased risk of progression or recurrence.

Despite the strong association between CTC enumeration and prognosis, CTC counts have not uniformly demonstrated a strong association with treatment response. In the largest study of the CellSearch platform to date, CTC positivity at baseline and 1–2 weeks after starting systemic therapy with capecitabine and either bevacizumab or cetuximab had a sensitivity/specificity in predicting response to therapy of 16.7 %/70.1 % (bevacizumab arm) and 20.0 %/95.1 % (cetuximab arm), respectively [98]. A possible explanation for these findings was discovered by Gazzaniga et al. [24], who found that the anti-VEGF agent bevacizumab significantly decreased CTC count despite progression of the patient's disease. In a group of 27 patients with a median of 2.7 CTCs/7.5 mL of blood prior to treatment, 89 % of patients had no CTCs and 100 % had ≤ 1 after 6–12 weeks of treatment with bevacizumab. Despite these findings, 56 % of patients had progressive disease. Nearly the opposite response was found among 20 patients treated with cetuximab: 6/6 patients with ≥ 3 CTCs after 12 weeks of treatment had progressive disease whereas 13/14 (93 %) of patients with decreased CTC enumeration had at least a partial response to therapy. The authors hypothesized that the tumor hypoxia induced by the anti-VEGF agents may alter the CTC phenotype to a mesenchymal one, as has been shown in head and neck cancers [106].

Results of RT-PCR-based molecular studies of CTCs in CRCs have had mixed results due to the large variability in study design, technique, and markers used [96]. While the overwhelming majority of studies looked at CEA mRNA, other markers have been studied with some success. Both CK and CD133 mRNA expression studies found associations between CTC positivity and worse progression-free and overall survival in Dukes stage B or C cancer [36]. For CEA mRNA studies, detection has ranged from 4 to 75.9 % depending on the method and stage of the patients sampled [40, 96]. A meta-analysis of CEA mRNA RT-PCR studies in CRC found that CTC positivity correlated with lymph node positivity, hepatic metastases, and disease-free survival [40]. Similarly, another meta-analysis looked at a total of 36 studies with 3094 patients and found that only CEA mRNA studies were significantly associated with disease-free survival [78]. They further found a strong correlation between CTC positivity in the blood and prognosis: a 2.7-fold increased risk of death and a 3-fold risk of recurrence. These results are almost identical to the results of the meta-analysis of ICC techniques performed by Groot Koerkamp et al.

4 Molecular Characterization and Sequencing of CTCs

Perhaps the most exciting potential applications of CTCs is as a "liquid biopsy," allowing researchers and clinicians real-time access to tumor tissue by a safe, repeatable, and cost-effective method. Such a liquid biopsy holds particular promise for GI malignancies given the difficulty, cost, and potentially risk associated with endoscopic and image-guided biopsies. Moving beyond the potential diagnostic value of such a liquid biopsy, advances in molecular biology have dramatically increased the amount of information that can be obtained from the small number of

CTCs present in the blood. Over the past 5 years the development of whole genome amplification (WGA) and whole transcriptome amplification (WTA), in tandem with the advent of next generation sequencing, has created the field of single cell sequencing [71]. Since 2011, researchers have successfully performed genome-wide studies including array CGH [29], copy number variation [73], whole exome [33, 111], whole transcriptome [13], and whole genome sequencing [70] on single CTCs in a variety of cancers. Furthermore, single cell techniques have allowed for the reliable culture and characterization of CTCs [107]. Initial studies on single cell sequencing of CTCs have demonstrated the possibility of answering important questions about metastasis and tumor heterogeneity in addition to providing insight into the role of CTCs in the metastatic cascade. Clinically, access to and characterization of a patient's tumor tissue is vitally important to take advantage of the advances in personalized medicine and targeted therapies. Thus, while studies to date have been small, the potential utility of a liquid biopsy for both clinical and research applications is enormous.

For GI cancers, the application of single cell sequencing to CTCs has already demonstrated potential clinical applications in targeted therapy decision-making as well as providing researchers with an important tool for studying tumor heterogeneity and the biology of metastasis. Anti-EGFR therapies have shown dramatic increases in life expectance for the subset of CRC patients with wild-type KRAS status, with between 17 and 40 % of wild-type patients responding versus 0 % of those with mutant KRAS responding [6, 67]. Thus, most insurance companies will require that a patient's KRAS mutation status be known prior to authorizing anti-EGFR therapies. Currently, KRAS status is determined by bulk analysis of the primary tumor; however, studies on tumor heterogeneity in CRC have shown that bulk analysis is not always accurate at determining the true KRAS status of the different clones within the tumor. Mostert et al. [67] showed that in a study of 49 patients with metastatic CRC, there was discordance between the primary and metastatic tumor's *KRAS* and *BRAF* status 23 and 7 % of the time, respectively. Huang et al. [34] looked at discordance between a patient's primary tumor and CTC KRAS status. In 18 patients with wild-type primary tumors, 4 (22.2 %) had discordance between the primary tumor and CTCs. In an important study, Misale et al. [65] looked at the development of *KRAS* mutations in patients following the development of secondary resistance to anti-EGFR therapy. All patients' primary tumors were wild type for *KRAS*, and, following the development of resistance, 6 of ten patients demonstrated a new *KRAS* mutation and one additional patient had an amplification of the *KRAS* gene. These studies together show that there is significant tumor heterogeneity in CRC, and that historic KRAS testing of the primary tumor may not accurately reflect the current genomic landscape of many patients tumors. Additional studies are needed to determine if molecular characterization of CTCs better determines response to anti-EGFR therapy, and, as important, whether CTCs can be used to detect resistance to targeted therapies.

While there are numerous studies on the molecular characterization of CTCs in gastrointestinal cancers, three of the initial genome-wide single cell studies are of particular note. Heitzer et al. used a combination of the CellSearch platform and

micromanipulation to isolate 37 single CTCs from 21 patients. They used next generation sequencing and found multiple regions of copy number variation [29]. Additionally, they used massively parallel sequencing on CTCs, as well as patient matched primary tumors and metastasis, to determine a 68 CRC-associated gene panel to compare the mutational landscape from these tissue sources. They found that while driver mutations such as *APC*, *KRAS*, and *PIK3CA* mutations were commonly shared between CTCs, metastases, and primary tissue, the CTCs often had additional mutations in other known cancer genes. In order to determine if these new mutations were in fact unique to the CTCs or represented a subclone within the primary tumor, they performed additional deep sequencing of the primary tissue. For almost all cases, the mutations that were unique to the CTCs by parallel sequencing were actually also present in the primary tissue at a subclonal level. This finding lends important support to the hypothesis that CTCs do in fact originate from the primary tumor tissue and can therefore potentially function as a biopsy equivalent. Furthermore, CTCs may actually provide a better representation of the aggressive subclones of the primary tumor than a traditional biopsy. In a therapeutic target discovery paper, Yu et al. [110] isolated single CTCs in a mouse model of pancreatic cancer. They performed single cell mRNA sequencing (RNASeq) to obtain a digital gene expression profile that revealed upregulation of WNT2 gene expression in the murine CTC population. They then used the same methodology to look at *WNT2* expression status in human PDAC CTC population and found increased WNT signaling in 5 of 11 patients. This study is representative of the new types of therapeutic target discovery possible with single cell CTC sequencing. In another recent study, researchers used a whole exome single cell sequencing method on 63 single cells from a single CRC tumor and found two distinct groups of tumor cells [111]. While controversial, the authors used hierarchical clustering to conclude that these two groups of cells evolved from a "biclonal origin," meaning two independent normal cells evolved into separate cancers [71]. These studies provide insight into the potential of CTCs as a biomarker, and demonstrate how powerful the application of single cell sequencing techniques to CTCs can be especially when considering the clonal heterogeneity of the primary tumor.

5 Conclusions

The search for better biomarkers to assist with the screening, diagnosis, staging, management, and prognosis of gastrointestinal cancers is an active area of research. While initial studies of CTCs in GI cancers have demonstrated their potential value, important issues remain to be addressed. With the exception of colon cancer, studies to date have been too small to determine the applicability of these studies to clinical practice. Furthermore, the enormous variability in techniques used by different CTC platforms makes comparisons between studies difficult. That being said, several CTC applications have already demonstrated their potential, and several conclusions can be made. First, the presence of CTCs has consistently been associated with recurrence and worse prognosis across all GI cancers, irrespective of the

CTC platform or the type of cancer. This is consistent with defining CTCs as tumor cells that have at least achieved the step of "intravasation" of the classic metastatic cascade (Fig. 1). Second, CTCs may represent a better first line diagnostic modality given the minimal cost and risk associated with a blood draw. As an example, consider the relative difficulty of obtaining a tissue diagnosis with EUS-FNA in patients with pancreas cancer. The high specificity, associated with CTCs and the ability to confirm the tumor origin with molecular characterization make CTCs a reliable biomarker for diagnosis of pancreatic cancer. Therefore, for the 30–70 % of patients who have CTCs, no further studies would be needed, saving the patient time, money and a complex endoscopic procedure. While larger studies are needed, it seems likely that CTCs could replace endoscopic biopsies in the determination of KRAS mutation status in various GI cancer patients. The results of multiple studies on CTCs for the detection of KRAS mutational status indicate that they may provide a better window into the mutational profile of the tumor clones actually responsible for metastases. Finally, the application of single cell sequencing to CTCs provides researchers and clinicians with an important tool in the study of tumor heterogeneity and metastasis. Thus, for GI cancers, CTCs represent a potentially useful clinical biomarker as well as a liquid biopsy, providing clinicians and researchers with access to tumor tissue in a safe, repeatable manner with minimal cost or risk to the patient.

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Cost-Effectiveness Analysis in Cancer Care

Alex Chang and Daniel E. Abbott

Abstract

With the increasing complexity of modern medical therapies, it is becoming imperative to recognize the marginal cost and gains of increasingly sophisticated (and expensive) interventions. By understanding the incremental cost of a given intervention, investigators must help answer questions about healthcare resource utilization that are not answered by randomized clinical trials. The continued funding of biomedical research and pharmaceuticals will require more objective study of the return on investment for any given treatment modality, and cost-effectiveness analyses will be instrumental in providing solutions to the inequalities in healthcare delivery.

Keywords

Cost-effectiveness · Cancer · Medical decision-making · Policy

1 Introduction

Comparative effectiveness research has long been used to weigh various interventions against each other using metrics primarily related to clinical outcomes. Cost-effectiveness analysis (CEA), a subset of comparative effectiveness research, is a method of evaluating healthcare outcomes and resource utilization by providing

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an understanding of the incremental cost of healthcare decisions [1]. These analyses bridge the gap between knowledge gained from clinical trials and the fiscal realities of delivering modern healthcare delivery. For example, simple comparative effectiveness may study the use of various antiplatelet agents and their role in preventing stroke, or whether specific chemotherapy regimens impact overall survival. In contrast, CEA compares the relative total healthcare cost between interventions and their relative healthcare gains; a more comprehensive and pragmatic approach. The importance of these studies cannot be underestimated; a recent Harvard University study found that 587 life-saving interventions could potentially save 636,000 more life-years in the United States if we as a country shifted resources from the least cost-effective interventions to the most cost effective [2]. As fiscal realities of the growing healthcare industry lead to requisite restraint, more expansive utilization of cost-effectiveness analyses has been (and must continue to be) integrated into healthcare policy.

With the increasing complexity of modern medical therapies, it is becoming imperative to recognize the marginal cost and gains of increasingly sophisticated (and expensive) interventions. By understanding the incremental cost of a given intervention, investigators must help answer questions about healthcare resource utilization that are not answered by randomized clinical trials. The continued funding of biomedical research and pharmaceuticals will require more objective study of the return on investment for any given treatment modality, and cost-effectiveness analyses will be instrumental in providing solutions to the inequalities in healthcare delivery.

As cost-effectiveness research matures, there are challenges in the conduction and implementation of its findings, especially given the culture of U.S. consumerism. First, there are many inconsistencies in cost-effectiveness research methodologies, making it difficult to interpret the results from individual studies. Methodologic guidelines created by experts in decision analysis and comparative effectiveness analyses have been established, though they are often not adhered to. This leads to challenges in interstudy comparison of cost-effectiveness analyses due to a lack of standardization in parameter definitions, data collection, and reporting of results. Second, reliable cost data are often difficult to gather, and the distinction between hospital charges, cost, and payment/reimbursement—while critical—still frequently confuses many investigators and their targe audience. Third, some have criticized the use of cost effectiveness as a gross oversimplification of costs and benefits into a highly subjective value index [3]. Many critics cite a 1990 attempt from the Oregon Health Services Commission to prioritize healthcare services to be included in universal coverage for Oregonians using CEA. When the initial draft was released, there was massive public criticism of the proposal, as it generally gave higher priority to less-expensive interventions while placing relatively low priority on more expensive but likely life-saving interventions. For instance, the treatment of headaches was given a high priority, whereas appendectomy was given a low priority. This highlights the fact that cost-effectiveness analyses cannot comprehensively account for competing values outside of healthcare economics, particularly with regard to intangible measures of well-being [4].

Despite its shortcomings, CEA will play a role in the future of health services research and policy. As this genre of health services research continues to grow, and the therapies for contemporary maladies become increasingly complex, it is imperative to have an understanding of how to interpret and utilize its findings.

2 Methodology

The core of cost-effectiveness research is determining the value of a practice or intervention. In a general sense, value is defined as the cost of an intervention juxtaposed to the clinical benefit (Fig. 1). This cost-effectiveness (C/E) ratio relies heavily on the careful definition of the numerator and denominator, understanding whether the proposed study warrants absolute or incremental costs and/or outcomes. The numerator of the C/E ratio is most frequently simplified to include the net expenditure of healthcare resources, i.e., the dollar cost of an intervention. Indirect and non-monetary measures such as opportunity costs in lost economic productivity, education, and effect on quality of life are generally (but not always) not included. Frequently these are reserved for the denominator. Costs can also be divided into direct and indirect fractions if the investigator so chooses. Direct costs are those required for the immediate delivery of an intervention (drug costs, operating costs, etc.), while indirect costs are those associated with the infrastructure of healthcare delivery, including maintenance of healthcare facilities, and staffing of auxiliary services not directly related to a given intervention. These are typically difficult to determine for individual patients and interventions and therefor excluded in CEA. Of note, other monetary values attached to healthcare

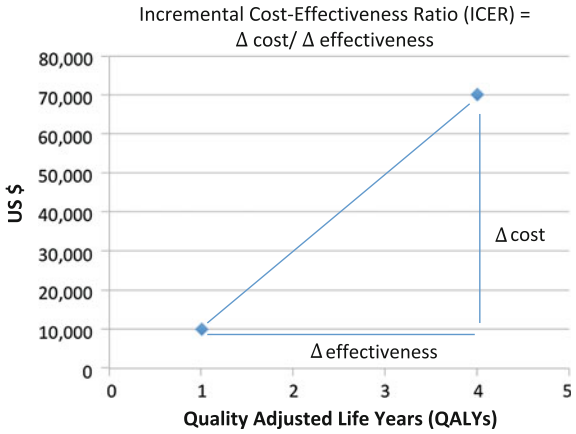

Fig. 1 Generic cost-effectiveness plot relating cost to outcome (quality-adjusted life-years, in this example). The incremental cost-effectiveness ratio is determined by dividing the marginal cost by the marginal effectiveness; the ICER is represented by the slope of the line between two outcomes. In this example, the ICER is $20,000/QALY ($70,000–$10,000/4–1 QALYs)

intervention, such as hospital charges, can be distracting and rarely useful; these are dependent on proprietary institutional accounting practices and are most frequently ignored. Finally, and when required, it is occasionally useful to evaluate an institution's ratio of cost to charge (RCC) to arrive at more meaningful cost data if charges are the only reliable data available.

Costs must be reported in constant dollars, necessitating an adjustment for inflation and deflation of cost data gathered across multiple years. Both the consumer price index as well as its medical care sector adjustment are used in this circumstance. There are many other cost categories including future healthcare costs, opportunity costs, and non-healthcare costs resulting from added life-years that are both theoretically and empirically difficult to assess and are frequently excluded from CEA research [5].

Similarly, the denominator of the C/E ratio—effectiveness—can be measured in a variety of ways. It can be as simple as a single metric (e.g., readmission) or as complicated as a quality-adjusted life-year (QALY), adjusting for utilities and health states over a long-term horizon. For more sophisticated and impactful analyses, failing to take into account opportunity costs and morbidity related to healthcare intervention will inadequately address the question of cost effectiveness. This notion is particularly important, as many studies may not have the primary goal of prolonging survival with their intervention. Health-related quality of life (HRQL) becomes critical in these circumstances, designed to encompass all effects of healthcare-related morbidity, including lost income and leisure. These utilities reflect individuals' preferences and directly affect QALYs. Similarly, disability adjusting is a common practice that corrects for the health state of a life-year by providing a weighted correction for the residual burden of the disease or therapy.

Disability-adjusted life-years (DALY) and QALYs increase the value of many interventions that would not be captured using survival and life-years alone. For instance, amputation compared to limb preserving for extremity sarcoma would add no increased value in life-years gained, but would provide significant decrease in DALY. Similar vision preserving therapy for retinoblastoma is highly valued when adjusted for disability. When using quality-adjusted or DALY, the analysis can be termed cost-utility analysis.

Another important factor is the recognition that a given intervention, performed on a heterogeneous population, occasionally requires adjustment for future uncertainty. For example, a life-saving intervention in an infant is not equivalent to a life-saving intervention on an elderly patient. The health gains over time are not constant, as the infant can be expected to incur both additional quality-adjusted life as well as unforeseen health-care costs over time. To project and account for these situations, the Disease Control Priorities Project uses a discount of 3 % per year when considering interventions to decrease infant mortality (a common discounting rate both for survival and cost). As a result, even with discounting, addressing infant lives has relatively high utility when compared to elderly lives. Ultimately, the measure of incremental cost-effectiveness ratio (ICER) is most important—expressed as marginal costs per marginal quality-adjusted outcome.

3 Modeling

With its increasing application in healthcare research, there is a large amount of variability in the methods employed as well as the conclusions that can be drawn from CEA. Models of cost-effectiveness studies also vary in the degree of complexity. The simplest model is a "flat tree," in which patients progress in a linear fashion through time until a terminal state is reached. Each branch point in a flat tree model is associated with a respective cost and probability of clinical outcome which result to the eventual outcomes. This method incorporates both cost and clinical outcomes, however fails to take into consideration changes in health states that occur during the study duration.

Markov modeling is a more complex methodology that incorporates the changes in health states that occur through the time horizon of the study (Fig. 2) [1]. These models give a more complete assessment of the costs and utilities over the duration

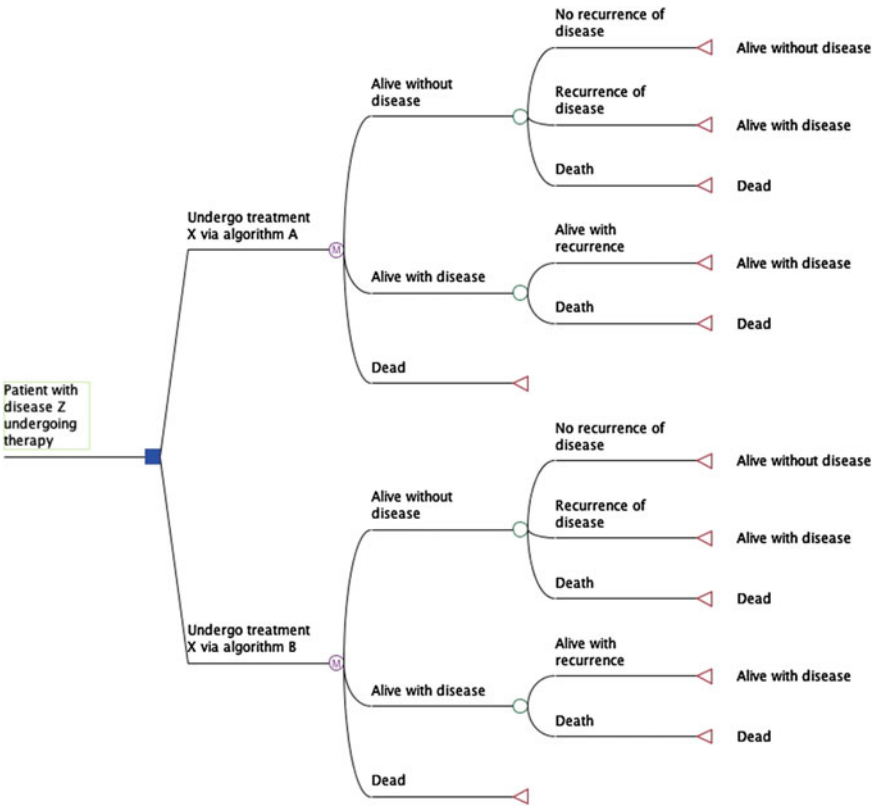

Fig. 2 Example of a Markov model, comparing two different treatment algorithms for a given disease process. Core concepts of the model are (1) specific health states for each arm ("alive without disease," "alive with disease," and "dead" in this circumstance), and (2) a set cycle length (can be weeks, months, years, etc.) through which the model runs repeatedly. For each cycle and/or health state, there can be initial and incremental costs and/or benefits inherent to a given treatment strategy

of analysis, and allow for transitions in a patients life following a given intervention. Yet another technique used in CEA is Monte Carlo simulation, in which hypothetical patients are generated to "walk" through the model. By testing potential scenarios with thousands of iterations, Monte Carlo simulation is able to highlight the distribution of outcomes that one would expect to encounter. However, these models are complicated by the lack of certainty when predicting the probability of outcomes and costs, and sophisticated probabilistic sensitivity analysis is needed to mitigate these limitations. Because the predicted cost and gains associated with an intervention are subject to these uncertainties, sensitivity analyses are a critical component of these models. Whether varying a single component of the model (one-way sensitivity analyses) or multiple variables (reflected in a tornado diagram) these sensitivity analyses allow for testing the robustness of the investigator's conclusions.

When these models are completed, a variety of outcomes are possible. One is that an intervention may be both less costly and more effective. In this situation, that intervention "dominates" other strategies in the study, and this is clearly the most cost-effective strategy. Conversely, a strategy can be both more costly and less effective—it is "dominated". The more common situation is that an intervention associated with a clinical gain is also associated with the greatest costs. Thus, this scenario results in an ICER—an incremental gain at an incremental cost. Interpretations of ICERs can be challenging, especially in the United States, as healthcare systems—and society—have lacked transparency in costs, with poor agreement of what a QALY should reasonably cost. Societal preferences and norms have not been established, a frustration for patients and providers alike.

4 Willingness to Pay

These discussions about ICERs directly lead to a critical component of CEA—willingness to pay (WTP). Most commonly, WTP is viewed from a societal (payer) perspective, though can certainly be taken from a patient perspective. In the 1980s, a C/E ratio of $50,000 per QALY began to emerge as a benchmark threshold for what society was willing to pay for in the United States. This ceiling was originally determined by how much Medicare reimbursed providers for one year of dialysis therapy for end stage renal disease. With inflation, as well as increasingly expensive medical care, recent reports suggest that more realistic upper and lower bounds of $109,000–$297,000 per QALY may more accurately reflect societal preferences in the United States today [6].

Globally, cost-effectiveness research has also become an area of interest to the World Health Organization (WHO). In their 2003 report, the WHO addressed several concerns regarding applying general cost-effectiveness methods outside of the developed world [7]. The WHO currently publishes thresholds for specific regions of the world based on GDP in that area. Interventions can be generalized into highly cost effective (less than the GDP per capital in the area), cost effective (between one and three times the GDP per capital), and not cost effective (more

than three times GDP per capita). Applying this to the United States, the WTP for a given intervention would be $159,000–164,000/QALY.

In 2010, the United States enacted the Affordable Health Care Act in efforts to increase the quality and affordability of health insurance, partially in response to the increasing cost of healthcare delivery without commensurate improvement in health outcome metrics. With the growing national and international pressure to increase the economic efficiency of healthcare delivery, high-cost interventions are being increasingly scrutinized.

5 In Practice: Cost-Effectiveness Analysis in Pancreatic Adenocarcinoma

There are a multitude of gastrointestinal disease processes that can—and are—subject to cost-effectiveness analyses. One particular pathology—pancreas adenocarcinoma—is a disease that is ripe for CEA, due to many therapeutic options, high cost, and generally poor outcomes. In 2014, the American Cancer Society reported an incidence of 46,420 new cases and 39,590 deaths from pancreatic adenocarcinoma; [8] these patients have an overall survival of less than 6 % [9], with 5 year survival of 18–24 % even after complete surgical resection [10]. These poor outcomes are complicated by significant cost; a 1996 study of pancreaticoduodenectomies (PD) performed at an urban teaching hospital in the United States showed the total cost of PD was $17,252 ± 8142 per surgical case, [11] with significantly higher costs incurred in patients who experienced complications (including pancreatic leak, chylous ascites, and marginal ulceration). Here, we will explore examples of what is known about cost effectiveness in pancreas cancer, which should serve both to explore this specific scenario but also highlight the importance and opportunities of these types of analyses

6 Multimodality Therapy

Because surgical intervention, systemic chemotherapy, and radiation therapy all play a role in pancreatic cancer, and each has both a different effectiveness and cost profile, the application of these modalities—and their sequence—can be thoroughly studied. At one extreme, some believe that any intervention is too aggressive, given the poor outcomes associated with almost all patients. To evaluate this, we performed a CEA comparing a variety of treatment strategies [12]. For patients with resectable tumors, surgery alone cost $33,539 for 6.8 QALMs versus $58,166 for 10.2 QALMs with adjuvant chemotherapy. Compared to no intervention, the ICER for chemotherapy alone was $3022/QALM, while the ICER for surgery alone was $8011/QALM. Finally, adjuvant therapy following resection was associated with an ICER of $7663/QALM, compared to no therapy. Based on these analyses, surgical resection with adjuvant therapy fits within WTP thresholds in the United States, and support the use of surgical resection in this patient population.

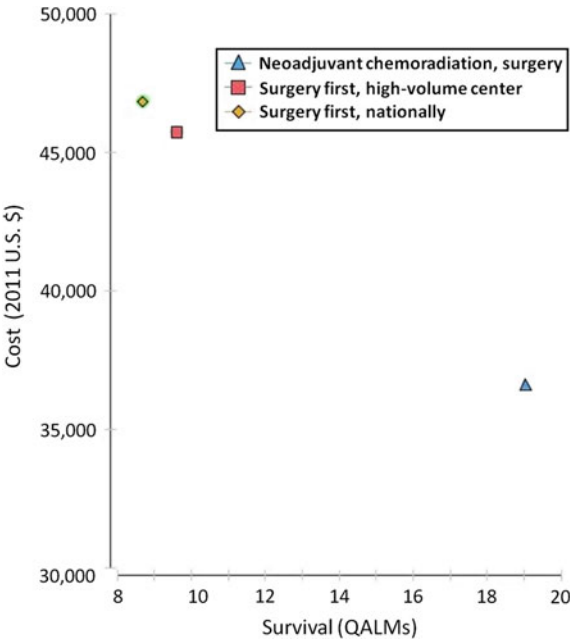

Fig. 3 Cost-effectiveness plot comparing (1) neoadjuvant chemoradiation followed by surgery, (2) a surgery first approach from a high-volume center, and (3) a surgery first approach from representative nation data for a patient with resectable pancreas cancer

But there are always opportunities to improve the cost-effectiveness profile of any multimodality care. In 2013, we published a CEA of neoadjuvant chemoradiation compared to a surgery first approach. A cost of $46,830 yielded a survival increase of 8.7 quality-adjusted life-months in the surgery first group compared to $36,583 and 18.8 QALMs in the neoadjuvant first group (Fig. 3). In the neoadjuvant group, patients who ultimately underwent resection (71 %) cost $45,673 for 23.4 QALMs, a most optimal outcome at essentially the same cost of a surgery first approach for all patients. This analysis supports the use of neoadjuvant chemotherapy for resectable pancreatic cancer from a utilitarian perspective, reducing the number of costly resections in the setting of aggressive tumor biology [13]. Many earlier comparative effectiveness studies have shown that neoadjuvant chemoradiation strategies are effective in reducing positive margin resections and early recurrence without delaying or increasing the morbidity of resection; these economic benefits seem to be just another reason to pursue this neoadjuvant strategy [14].

7 Surgical Technique and Hospital Volume

Not only does sequence of therapy directly affect cost effectiveness, but provider and hospital characteristics certainly play a role. For example, current studies of distal pancreatectomy have looked at whether laparoscopic techniques require significantly more intraoperative costs, but achieve equivalent outcomes. This does

appear to be the case, however, costs appear to largely be saved by a shorter postoperative stay [15, 16]. In larger studies using national databases, a median cost of $44,741 versus $49,792 for laparoscopic versus open distal pancreatectomy, respectively, was associated with fewer complications and shorter length of stay in the laparoscopic group [17]. Studies on robotic distal pancreatectomies and minimally invasive PD are less common and more difficult to interpret due to limited expertise and availability of these techniques.

In 2002, Birkmeyer et al. published a national study linking higher volume hospitals to lower operative mortality rates from cardiovascular and major cancer procedures, including pancreatic resection. Highest quintile centers demonstrated an odds ratio of 0.18 compared to lowest quintile centers, with observed mortality of 3.8 % compared to 17.6 % [18]. Additional studies have corroborated this finding in pancreaticoduodenectomy. In a national cost-effectiveness study, the ICER for pancreatic resection was significantly lower for high-performing centers ($5991/QALM) than for low-performing centers ($9144/QALM). Compared to centers in the highest quintile for pancreaticoduodenectomy volume, low volume centers have higher perioperative mortality as well as 10.9 % higher cost per patient [19].

8 Complications and Pancreatic Fistula

Even in experienced hands, perioperative mortality following pancreaticoduodenectomy remains 1–4 %. Morbidity remains as high as 30–50 % due to delayed gastric emptying, pancreatic leak or fistula, deep and superficial wound infections, and hemorrhage [20]. Postoperative complications after pancreatectomy in the National Inpatient Sample occurred in 23 % of patients, and were associated with increased length of stay, increased likelihood of death, with no significant decline over time.

Undoubtedly, these complications following PD significantly increase the cost of care [11]. In a large study of infectious complications following pancreatic resections, 31 % of cases met that endpoint, of which 11 % were considered major complications [21]. Total hospital costs incurred by those with major infectious complications were on average $15,000 greater than those without, with a much greater cost incurred with high grade infections. In yet another study, the cost of any complication increased the median cost of PD from $29,038 to $56,224, with a large portion of added costs arising from pharmacy costs [22]. As complications greatly increase recourse utilization following pancreaticoduodenectomy, complication rates have become highly scrutinized metrics in complex surgical care, and will increasingly be used for value-based purchasing.

Specific to pancreas surgery—and germaine to complications and cost—pancreatic fistula deserves special mention. Grade A fistulas do not appear to increase the cost ($18,075 vs. $18,209) or outcome compared to those with no complication. However, as grade of fistula increases, costs begin to increase profoundly. A grade C fistula adds $119,083 of cost to each patient, 43 % of which can be attributed to increases in ICU utilization, while the costs incurred by grade B fistulas was $34,555

and $27,778 after distal pancreatectomy and PD, respectively [23]. Due to the cost and morbidity related to pancreatic fistula, many studies have evaluated the use of somatostatin analogue prophylaxis for prevention of this complication. While there is controversy regarding the effectiveness of octreotide therapy in preventing post-operative pancreatic fistula, two studies have investigated the potential cost savings associated with its use. In 1999, a report from Canada projected the cost savings of octreotide therapy, assuming a reduction of pancreatic fistula from 23.4 to 10.7 %. Using a per-diem cost analysis, an average cost savings of $853 per patient could be achieved with octreotide; when the model utilized the total average cost of care, the average savings was $1642 [24]. The authors concluded that octreotide could be a cost-effective strategy when given to high-risk patients. This was followed by a single center study that tested this model in 227 consecutive PDs from 2001 to 2007 and found that when stratified for high-risk patients, there was the potential for a total hospital stay cost savings of $12,000 per patient [25]. A newer somatostatin analogue, Pasireotide (Novartis Signifor), which has been approved for use in Cushing's disease, was more effective than placebo in decreasing clinically significant pancreatic fistula, leak, or abscess [26]. This effect was significant in all risk stratified groups. High-quality CEA in pancreatic resections is not yet available for this drug, however critics have cited affordability issues, with Pasireotide therapy cost estimate of nearly $45,000 per prophylactic dose.

Since 2003, the measures now known as the Surgical Care Improvement Project (SCIP) were implemented nationally to standardize specific components of the care of the surgical patient. Despite high adherence to these measures, high-cost complications remain, especially with pancreatic resections. The group from MD Anderson has reported a cost analysis on clinical pathways designed to improve patient care in several surgical procedures, [27] and determined that the total cost after the implementation of a clinical pathway was $36,627, compared to a pre-pathway cost of $47,515 with a statistically significant decrease in length of stay, but not in mortality or readmission rate. More efforts, like these, will be required as we consistently strive for more cost-effective delivery of care.

9 Readmissions

Readmissions following major abdominal surgery deserve special mention, as they are both costly and an intense focus of both investigators and regulatory agencies. Initially viewed as a surrogate of low quality care, most authors believe readmission to be a more complex metric [28]. A study of pancreatectomies from 1992 to 2003 showed overall readmission rates of 16 % at 30 days and 53 % at 1 year. Authors observed that early readmissions were typically due to operative complications or patient complaints, while late complications were more commonly related to recurrence of disease [29]. A more recent multi-institutional investigation of patients undergoing pancreaticoduodenectomy from 2005 to 2010 reports 30- and 90-day readmissions at 15 and 19 %, respectively, with the largest portion of readmissions were due to infectious causes [30]. Addressing readmission with more

sophisticated cost-effectiveness analyses will be important as healthcare reform rapidly evolves, as few investigators have accurately delineated the added cost of readmission following pancreatectomies. In 2011, a study of 578 pancreatic resections from 2001 to 2009, with a 30-day readmission rate of 19 %, showed an average cost of readmission to be $10,000 [31]. Additionally, the cost of the index hospitalization of those patients was $6000 greater, and 21 % of readmitted patients in the study were readmitted multiple times. Clearly, identifying and eliminating modifiable predictors of readmission will benefit patients, and providers, both clinically and fiscally.

10 Palliative Therapy

Unfortunately, 53 % of cases of pancreatic adenocarcinoma are diagnosed at a late stage, with an associated 5-year survival of 2 % in 2014 [8]. Addressing the question of whether cross-sectional imaging is sufficient (and cost effective), a report from Duke University Medical Center showed preoperative CT scanning accurately staged 86.8 % of patients, suggesting staging laparoscopy may not be cost effective, though this debate continues [32]. For patients with tumors that do not allow for surgical resection with curative intent, palliative chemotherapy is standardly used, and is itself a useful clinical scenario to perform cost-effectiveness analyses. This is particularly true given recent advances in systemic therapy for pancreas cancer, and the cost of these new therapies will need to be consistently evaluated, being weighed against their clinical benefit.

Based on older chemotherapy regimens for patients with unresectable disease, chemotherapy alone was associated with a cost of $10,361 for 5.9 QALMs (2011 US$) [12]. A more recent Canadian study evaluated the cost effectiveness of more modern medical therapies for metastatic pancreatic cancer, comparing Gem-Cap, Gem-E, and FOLFIRINOX as first line therapy. These regimens were associated with ICERs of $84,299, $153,631, and $133,184 per QALY, respectively. These authors emphasize that the most effective strategy therefor is dependent on the societal WTP, concluding that the most effective regimen is FOLFIRINOX only if societal WTP is above $130,000/QALY. This highlights, again, the importance of having payers, patients and providers developing an agreed-upon threshold at which we—as society—will or will not pay for medical care.

11 Surveillance

As pancreatic cancer recurrence portends a poor survival despite therapy, the utility of surveillance following curative resection remains controversial and understudied. Tzeng and colleagues have shown that increasing the frequency of surveillance and adding additional radiologic and laboratory studies increased the cost of surveillance but did not confer significant survival benefit. Their findings showed that clinical evaluation and serial CA19-9 monitoring were associated with an ICER of

$5364/life-year while adding abdominal CT and CXR at the same intervals added $3465 to the cost of care, without increasing overall survival in a clinically meaningful way. Increasing the frequency of follow-up to every 3 months increased the ICER of surveillance to $127,680/life-year, and $294,696/life-year if imaging was included [33]. These data, however, do not incorporate patient anxiety and stress—certain quality of life distractors—and further investigation into patient preferences with regards to serial imaging, and its importance in timing of treatment, will be critical to surveillance protocols of the future.

12 Societal Implications

Due to a certain level of nihilism and disappointing oncologic outcomes in pancreas cancer, and despite improvements in surgical and medical therapy for this disease, there is growing concern about sustainability of healthcare resources and the societal utility in treating pancreatic adenocarcinoma. In a critical review of pancreatic cancer therapy, with respect to costs, Gudjonsson's 1995 meta-analysis concluded that an estimated $150,000 was incurred per resection [34]. These figures have been disputed by other authors, including a Swedish group that estimated a total cost of curative therapy for pancreatic cancer at 35,000 Euro per QALY, which compares favorably to other accepted therapies [35]. In yet another report, performed from a societal perspective, the broader loss of productivity of 287,420 Euro per patient suggests another layer of complexity that is usually not incorporated into traditional cost-effectiveness analyses; how loss of life impacts societal contribution [36]. Again, it is abundantly clear that standardization of the methods and reporting of cost is necessary before any definitive statements can be made regarding the utility of therapy for pancreatic adenocarcinoma.

13 Cost Effectiveness in Other Gastrointestinal Malignancies

Pancreas cancer, of course, is not the only gastrointestinal malignancy in which cost-effectiveness analyses are required. Colorectal cancer (CRC), a disease affecting approximately 150,000 Americans per year, is an important disease process based on sheer patient burden alone, with its attendant invasive interventions and cost. Investigations into screening for CRC abound, evidence by one group of reviewers examining 424 citations regarding cost effectiveness of CRC screening, including fecal occult blood testing (FOBT), sigmoidoscopy, and colonoscopy at various time intervals. While there was significant methodologic variability, as well as wide variability in life-years gained due to screening, these authors found that CRC screening was cost effective, with costs ranging from a net cost savings, to $57,000/life-year gained [37]. This speaks to the difficulty of comparing results from different studies, even when clinical treatment strategies are well accepted.

There are many other examples in the literature about screening for other gastrointestinal malignancies, though the cost effectiveness of their methods is less uniform. Several studies have examined the cost effectiveness of biochemical and ultrasound screening of high-risk patients for hepatocellular carcinoma, showing that risk stratification is paramount in achieving adequate cost-effective ratios [38, 39]. Bolondi and his colleagues reported that their surveillance algorithm had an incremental cost of $17,934 per treatable HCC detected, however this increased to a cost of $112,993/life-year gained. This dichotomy is not only important due to the high cost associated with treatment, but also highlights the potential for variability as treatment of HCC evolves.

Even with similar intervention, the specific disease pathology can significantly alter the effectiveness of even palliative intervention. In cholangiocarcinoma, many of the cost-effectiveness analyses are limited to palliative therapy, due to the frequent unresectability of this challenging malignancy; endoscopic stenting for unresectable hilar cholangiocarcinoma (HCA) is one critical component of such palliation. In a recent report, bare metal stenting of HCA achieved an ICER of $6318/QALY [40]. This value is in contrast to findings in an analysis of metal stenting for biliary obstruction from unresectable pancreatic cancer; stenting in this scenario was associated with an ICER of $613/QALY [41].

14 Conclusion

In summary, CEA will play an increasingly important role in the future of oncologic therapy. While the discipline is well established, its application in the medical arena has been limited by complex biology, a general lack of transparency in the price of health care, and disagreement—at a policy level—about how much *should* be spent to achieve improvement in duration and quality of life. As policy makers will be required to reign in ever-expanding healthcare costs, it is important that those involved in healthcare delivery and health services research develop and deploy tools to aid us in making fiscally and ethically responsible decisions.

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Gastrointestinal Malignancy: Genetic Implications to Clinical Applications

Nicole E. Lopez and Jen Jen Yeh

Abstract

Alterations in the DNA sequences of genes, or mutations, have traditionally been viewed as the primary factors driving tumor progression, however, epigenetic evidence would suggest that some heritable traits are mediated by changes in DNA expression that are not dependent upon alterations in the primary DNA sequence. Advances in the genetic understanding of cancer have, in some instances, allowed for more precise administration of anti-neoplastic therapy. Targeted therapies, the aim of which are to target *specific* cellular proteins or processes used by the cancer cells, have been advocated to avoid the adverse side effects attributable to a lack of cell specificity associated with traditional chemotherapy. Here we aim to describe the current state of understanding regarding the genetic related causes of cancers, the targeted therapies aimed at killing them and the inter-relationship between these two.

Keywords

Targeted therapy · Molecular medicine · Personalized medicine

1 Introduction

The first known mention of cancer was in an Egyptian document dating back to 3000 BCE. This document, describes the removal tumors or ulcerations of the breast, noting that 'there is no treatment'. Centuries went by with minimal gains in

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the advancement of understanding and treating cancer. By the early twentieth century it remained that only small tumors amenable to complete surgical resection were curable.

1.1 Development of a Genetic Basis for Understanding Cancer

Ideas regarding the relationship of genetics to neoplastic development began to surface around the turn of the century, and in 1903 Borrel proposed his theory for a viral etiology of cancer [1]. By 1911, Rous had demonstrated the transmission of sarcoma via cell free tumor extracts, indicating that cancer was transmissible by subcellular particles, likely viral in nature. Viruses became the first known causative agents of neoplastic processes. Aside from being an important cause of neoplastic processes, many current concepts in the genetics of cancer, particularly with regard to cell signaling and growth control pathways were derived from studies in viral carcinogenesis [2].

In the 1970s, advances in molecular techniques allowed for improved chromosomal analysis and tumor genesis was linked to specific genes [3, 4]. For example, in a landmark study, published in 1976, Stehelin et al. [5] describe genetic similarity between avian sarcoma viruses (ASV) and normal chicken DNA. After demonstrating evolutionary conservation of the *src* gene among avian species and showing that *src* genes from normal chicken cells are not capable of transforming normal cells into sarcoma, they inferred that these genes, termed 'proto-oncogenes', must incur mutations in order to produce neoplasms. Advances in technology also allowed for the identification of chromosomal breakpoints commonly effecting proto-oncogenes, as well as translocations and inversions resulting in fusion proteins often involving transcription factors that would go on to activate downstream genes [6]. Thus, viruses were instrumental in the identification of oncogenes, or gain-of-function mutations that result in carcinogenesis.

While gain-of-function mutations were the first to be elucidated, loss-of-function mutations are, perhaps, more common among inherited cancers since a normal second allele generally maintains the usual phenotype until it becomes mutated. Conversely, a normal allele would not commonly have the capacity to dampen the effects of a gene with a gain-of-function mutation, making gain-of-function mutations less likely to be inherited due to a higher likelihood that they would result in lethality during embryogenesis. Definitive evidence for the role of loss-of-function mutations in carcinogenesis came in 1983, when a series of experiments revealed that two mutational events inactivating each of two chromosomal copies of Rb gene, located on chromosome 13, were responsible for the rare childhood eye tumor, retinoblastoma [7–9]. This proved the 'two-hit hypothesis' generated by Knudson over a decade prior, and Rb gene became the prototype of the so-called 'tumor suppressor' genes [10, 11]. Traditionally, these mutations have been detected by comparison of germline DNA, which would be heterozygous (with a deleterious mutant allele and a normal allele), to tumor DNA of the same individual.

A deletion or mutation within the normal allele would result in a cell that is either hemizygous (with one deleterious allele and one deleted allele) or, more commonly, homozygous for the mutated allele. Either of these findings would confirm loss of heterozygosity (LOH). However, commonly, there is no LOH to explain the loss of expression of tumor suppressor genes. While haploinsufficiency might offer one plausible explanation of this phenomenon, there is growing support for a significant role of epigenetic mechanisms in the silencing of tumor suppressor genes [4, 12].

Alterations in the DNA sequences of genes, or mutations, have traditionally been viewed as the primary factors driving tumor progression, however, epigenetic evidence would suggest that some heritable traits are mediated by changes in DNA expression that are not dependent upon alterations in the primary DNA sequence. These epigenetic changes can result from DNA hypomethylation and hypermethylation, as well as from modifications of histone patterns. Promoter hypermethylation, also known as CpG hypermethylation, is perhaps the best understood of these mechanisms and is most relevant to our understanding of how these changes effect tumor progression with regard to their effects on tumor suppressor genes. CpG islands commonly exist in the promoter regions of DNA, and their hypermethylation is associated with inappropriate transcriptional silencing of genes. This type of promoter hypermethylation has been found in many neoplasms [13]. Indeed, almost half of well-known tumor suppressor genes, which cause inherited forms of cancer when germline mutations are identified, have also been found to undergo silencing by hypermethylation in sporadic forms of cancer [13–15].

Finally, while environmental factors have been found to account for 80–90 % of human cancers, studies such as that of El-Omar et al. [16, 17], describing increased risk of gastric cancer due to *Helicobacter pylori* among those with a genetic polymorphism in the interlukin-1 gene, suggest that genetic alterations may affect host responses to environmental pathogens thereby contributing to carcinogenesis.

1.2 Development of a Genetic Basis for Cancer Treatment

Surgical resection was the only chance for cure in cancers at the beginning of the century, but radiation was soon added to help control residual disease after incomplete resections. The use of radiation in the treatment of cancers was based on experimental regression of radiated tumors. The mechanism by which these effects were achieved was largely unknown.

Similarly, the use of nitrogen mustards to treat hematologic malignancies was primarily based on the leukopenia it produced in soldiers who had been exposed to it during World War I [18]. In 1947, Auerbach et al. reasoned that nitrogen mustard might combine with the materials composing genes. However, the double helical structure of DNA was not described until 1953, and it was not until 1960s that investigators could explain its efficacy, showing that nitrogen mustard induced DNA cross-linking leading to the prevention of denaturation and cell arrest [18–20]. Improved understanding of the mechanisms by which chemotherapy works, and

increased availability led to its widespread use in the treatment of unresectable cancers and systemic metastases.

Cytotoxic chemotherapies have traditionally functioned by disrupting DNA synthesis or cellular division. This causes detrimental effects on rapidly dividing cancer cells, but also harms normal cells that divide rapidly at baseline, such as intestinal mucosa, bone marrow and hair follicles. Therefore, systemic side effects are usually most pronounced in these tissues.

Advances in the genetic understanding of cancer have, in some instances, allowed for more precise administration of anti-neoplastic therapy. Targeted therapies, the aim of which are to target *specific* cellular proteins or processes used by the cancer cells, have been advocated to avoid the adverse side effects attributable to a lack of cell specificity associated with traditional chemotherapy. In general, targeted therapies come in two forms: small molecule and antibodies. These drugs have had varied degrees of success in treating cancers and this has, in several circumstances, been associated with tumor genotype. With continued advances in the understanding of the genetic basis of cancer we hope to gain insights into its pathogenesis and improve therapies by identifying new biomarkers and novel treatment targets.

Here we aim to describe the current state of understanding regarding the genetic related causes of cancers, the targeted therapies aimed at killing them and the inter-relationship between these two.

2 Gastrointestinal Stromal Tumors (GIST)

2.1 Epidemiology and Clinical Manifestations

GISTs are the most common mesenchymal tumor of the gastrointestinal tract. According to a study of the SEER database annual age-adjusted incidence was 0.78 per 100,000 with 5-year overall and GIST specific survival rates of 65 and 79 %, respectively [21]. Previously identified as leiomyomas, leimyosarcomas or schwannomas, differentiation from these tumors by immunohistochemistry became convention after 1998 when Hirota et al. [22] found a strong association with KIT protein expression and *KIT* proto-oncogene mutation. Expression of KIT protein indicates that the tumor originates from the interstitial cells of Cajal (ICC), which are found in the myenteric plexus and are known for their function as pacemaker cells in gastrointestinal motility [23]. GISTs can be found throughout the gastrointestinal tract but are most commonly located in the stomach and small bowel. A review of SEER data from 1992–2000 revealed that among 1458 cases of GIST diagnosed in this time period, 51 % were in the stomach, 36 % small intestine, 7 % colon, 5 % rectum, and 1 % in the esophagus [24]. Finally, GISTs tend to occur in older adults with a median age at diagnosis of 60–65 years old [25].

Surgical resection with negative margins is the mainstay of therapy for GISTs. They are highly resistant to traditional chemotherapy and radiation. As such, historically, the prognosis for advanced GISTs has been poor [26]. However, with the

introduction of tyrosine kinase inhibitors this has changed drastically. Two-year overall survival has improved from approximately 25 % before the imatinib era to roughly 75 % since its introduction [27, 28]. Likewise, median survival increased from 18 to 57 months [28]. Indeed, with the success of tyrosine kinase inhibitors in managing GIST, this disease now represents the paradigm for targeted therapy. Here we will review the mutations key to the development of GIST and the relationship between these mutations and tumor responses to targeted therapies.

2.2 Identification of High-Risk Patients and Diagnosis

GISTs are generally considered to be sporadic, however, rare familial forms of the disease have been reported in which germline *KIT* mutations predispose patients to developing GISTs. Familial GISTs present at earlier ages in conjunction with hyperpigmented skin lesions, and dysphagia [29–31].

Patients with neurofibromatosis-1 (NF-1) are also at increased risk of developing GISTs. Tumors in patients with NF-1 tend to be multicenteric and are most commonly found in the small bowel [32–34]. Additionally, in comparison to sporadic tumors, these tumors show a female predilection, increased propensity to metastasize, younger age of onset and unpredictable behavior [34].

Pediatric GISTs are rare, however, they can be seen in association with other syndromes. Syndromic diseases, such as Carney-Stratakis and Carney triad, in which succinate dehydrogenase is altered, can present with GIST, paragangliomas and in Carney triad, pulmonary chondromas [34–36].

Due to similar epithelioid and spindle cell features among other mesenchymal tumors of GI origin, the pathologic diagnosis of GIST is based on a combination of morphologic and cytologic characteristics. Approximately 95 % of GISTs overexpress KIT (CD117) [25]. Staining with CD117 and/or DOG1 is characteristic, and therefore, diagnostic of GIST [37, 38]. In cases when these markers are negative, but GIST is suspected, immunohistochemistry for succinate dehydrogenase complex B (SDHB), and mutational analysis of KIT and PDGFRA can be helpful in establishing a diagnosis [38]. *SDH* and *BRAF* mutational analysis can also be helpful in assessing genetic status of wild-type tumors.

2.3 Molecular Genetics

While 95 % of GISTs overexpress KIT, only approximately 80 % are found with *KIT* mutations. The majority of remaining tumors are wild-type (~15 %), but nearly 5 % can have *PDGRFA* mutations [25, 39–47] (Table 1). KIT and PDGFRA are homologous receptor tyrosine kinases with genes located adjacently at chromosome 4q12 [48]. Mutations alter the proteins causing ligand independent dimerization and constitutive activation. This induces activation of downstream PI3, MAPK, and STAT signaling, resulting in increased proliferation and decreased apoptosis [46, 47, 49].

Table 1 Distribution of mutations in GISTs

Year, Study	*n*	KIT mutation *n* (%)	Exon 9 *n* (%)	Exon 11 *n* (%)	Exon 13 *n* (%)	Exon 17 *n* (%)	PDGFR mutation *n* (%)	Exon 12 *n* (%)	Exon 18 *n* (%)	WT *n* (%)
Taniguchi [39]	124	71(57)		71(57)	NR	0(0)	NR	NR	NR	NR
Rubin [40]	48	44(92)	6(13)	34(71)	2(4)	2(4)	NR	NR	NR	NR
Heinrich [41]	127	112(88)	23(18)	85(67)	2(1.6)	2(1.6)	6(4.7)	1(0.8)	5(3.9)	15 (12)
Antonescu [42]	120	94(78)	13(11)	81(67)	0(0)	0(0)	NR	NR	NR	NR
Emile [43]	241	205(85)	14(5.1)	93(39)	NR	NR	5(2.1)	3(1.2)	2(0.8)	31 (12)
Andersson [44]	177	108(61)	6(3.4)	101(57)	0(0)	1(0.6)	6(3.4)	3(1.7)	3(1.7)	63 (36)
Debiec-Rychter [45]	377	315(83)	58(15)	248(66)	6(1.6)	3(0.8)	10(2.7)	NR	NR	52 (14)

2.4 KIT

Mutations are known to occur at four locations in the *KIT* gene—exons 9, 11, 13, and 17. Most *KIT* mutations are in exon 11. This region codes for an intracellular juxtamembrane domain that normally inhibits KIT function [50, 51]. Some recognize *KIT* exon 11 deletions in association with high risk, malignant tumors and poor outcomes [44, 52]. However, tumors with exon 11 mutations are also known for their exquisite sensitivity to imatinib [41, 53].

Though they are responsible for a much smaller portion of tumors, exon 9 mutations are the second most frequently occurring mutation in *KIT*. This mutation occurs at the end of the extracellular domain and almost exclusively causes small intestinal GIST [44, 54]. Exon 9 mutations have also been associated with larger tumor size, unfavorable outcomes and increased recurrence; however this may be partially related to the nature of small intestinal tumors [42, 45, 55]. Relative to GISTs with exon 11 mutations, GISTs with exon 9 mutations respond weakly to imatinib, however, in comparison to tumors without an identified mutation, *KIT* exon 9 mutations are nonetheless a significant predictor of response to imatinib [41].

Exon 13 and 17 mutations are rare. They appear to be associated with increased risk of progression and response to imatinib therapy, though small sample size makes these assertions difficult to impart with certainty [25, 56, 57].

2.5 PDGFRA

PDGFRA mutations are uncommon, but can occur in three regions of the gene: exon 12, 14 and 18. GISTs with *PDGFRA* mutations tend to be of epithelioid morphology and often stain weakly, if at all, for c-KIT on immunohistochemistry. The stomach is the primary site of GISTs with *PDGFRA* mutations and they typically follow a benign course [58, 59].

Exon 18 mutations are responsible for more than 80 % of *PDGFRA* mutations and result in alterations in the activation loop of the protein. The majority of exon 18 mutations occur as a result of the D842V substitution. Resistance is specific to this substitution and other mutations in exon 18 are sensitive to imatinib [41, 46, 60].

Mutations in exon 12 (juxtamembrane domain) and exon 14 (first tyrosine kinase domain) represent 13.7 and 3.7 % of *PDGRFA* mutations, respectively [46]. One study found that mutations in exon 14 tended to follow an indolent course even despite tumor characteristic suggesting moderate to high malignant potential [61]. Other data regarding associated findings with these mutations is difficult to extract given their relative rarity.

Wild-type (WT) tumors account for approximately 12 % of GISTs and encompass all tumors without identified *KIT* or *PDGFRA* mutations [62]. Recently, tumors with *BRAF* mutations have also been excluded from classification as WT

tumors. Overall though, WT tumors are a heterogenous group of tumors that generally show poor response to imatinib.

2.6 Succinate Dehydrogenase Complex (SDH)

Loss-of-function mutations in the SDH complex have been noted in a subset of GISTs that are WT for *KIT* and *PDGFRA*. This fraction accounts for approximately 40 % of GISTs designated as WT [63].

Germline mutations in SDH were found to occur in 12 % of patients with WT GIST and no family history of GIST [64, 65]. This finding can alert providers to a patient's increased risk for development of other tumors associated with *SDH* mutations, such as paragangliomas and subsequent GISTs [64].

It often originates in the stomach and has a tendency to present with multiple synchronous tumors and has a predilection for earlier onset than traditional GIST. Histologic findings include multinodular architecture with epithelioid or mixed morphology [63, 66, 67]. Additionally, the course of disease in patients is generally indolent, even in the setting of metastases [63, 66, 68, 69].

The SDH complex has four subunits (A, B, C, D) and is part of the mitochondrial respiratory chain that participates in metabolic processes such as oxidative phosphorylation and the citric acid cycle. Loss-of-function mutations in the SDH complex have been linked to tumor susceptibility [70]. SDHB is a ubiquitously expressed mitochondrial protein. Loss of any SDH subunit protein destabilizes the complex and results in loss of SDHB. As such, absence of SDHB on immunohistochemistry has been confirmed as a reliable marker for deficiency of any SDH subunit [71].

Though exact mechanism of tumorigenesis is unclear, alterations in cellular metabolism result in activation of hypoxia pathways and overexpression of both hypoxia inducible factor 1α(HIF-1α), and it's target genes, including VEGF [72, 73]. Lack of activated receptor tyrosine kinases driving pathogenesis of SDH deficient GIST may explain the absence of response to imatinib [41, 45]. Conversely, the overexpression of VEGF in these tumors may partially explain tumor response to sunitinib, an orally dosed multi-targeted receptor tyrosine kinase inhibitor that blocks signaling from all three isoforms of VEGF in addition to KIT and PDGFRA [74, 75]. A phase III trial, currently underway, investigating the efficacy of bevacizumab in combination with imatinib for unrespectable and metastatic GIST (NCT00324987) may further clarify the role of VEGF antagonists in treating GISTs.

Additionally, overproduction of HIF-1α in SDH deficient tumors has been implicated as a source of IGFR protein overexpression in these tumors. Consequently, groups have investigated potential for therapeutic targeting of this protein [76–78]. Unfortunately, an abundance of dangerous side effects including dehydration, hyperglycemia, and treatment related deaths as indicated by a phase III clinical trial investigating the utility of targeted IGFR therapy in non-small-cell lung cancer may preclude further development of IGFR targeted treatments [79].

2.7 BRAF

The *BRAF* gene encodes the B-raf protein, a proto-oncogene [80]. It is a member of the RAF family of serine/threonine protein kinases that are important effectors in mitogen-activated protein kinase (MAPK) pathways involving RAS/RAF/MEK/ERK signaling. This pathway is fundamental in transcriptional regulation, and plays an important role in carcinogenesis. *BRAF* has been primarily recognized for its relevance in melanoma where it is mutated in up to 66 % of tumors [81, 82].

While encountered with far less frequency in GIST, primary *BRAF* mutations have been found in 7–13 % of WT GISTs lacking *KIT/PDGFRA* mutations [80, 83, 84]. Additionally, its occasional presence in imatinib resistant tumors suggests that *BRAF* mutations may represent an alternate mechanism for imatinib resistance in GIST [83, 85]. Similar to most melanomas, these GISTs have exon 15 V600E mutations [80, 83, 84]. Given the success of BRAF inhibitors in treating melanoma, it is reasonable to propose a similar treatment strategy for BRAF mutant GISTs [86].

Indeed, a single report of successful management of a *BRAF* mutant GIST with a BRAF inhibitor was reported in 2013 [87]. Further studies regarding the targeted use of BRAF inhibitors in GIST are warranted, however, the relative rarity of this type of tumor may make such efforts challenging.

Existing data suggest that mutational status has prognostic value and can have considerable effects on the tumor response to therapy, however, the role of mutational testing in guiding therapy is not currently well defined (Table 2).

2.7.1 Targeted Therapies in GIST

Several targeted therapies are FDA approved for use in treating GISTs. Mutational analysis is not standard in the treatment of GIST tumors. As such, imatinib is generally used as the first-line treatment, followed by sunitinib when resistance develops, and regorafenib may be used after failure of both imatinib and sunitinb. Several therapies are listed in NCCN guidelines for use after failure of these medications, which are not mentioned in depth here, but include sorafenib, nilotinib, dasatinib, and pazopanib [88].

Imatinib (Gleevec)

Imatinib, also known by its investigational name, STI-571, is an orally bioavailable small molecule inhibitor of BCR-ABL, KIT and PDGFR tyrosine kinases. Its efficacy as a tyrosine kinase inhibitor (TKI) was initially established in the targeting of Philadelphia chromosome positive chronic myelogenous leukemia (CML) in 2001. In the following year, it was approved for use in advanced GISTs and in 2012 it was approved for prevention of recurrence in resected GISTs.

FDA Approval and Dosing

Imatinib was approved for use in GIST based on a phase II randomized, multicenter trial evaluating the safety and efficacy of imatinib used at 400 and 600 mg daily doses. 147 patients were randomized to 400 or 600 mg of imatinib daily, 53.7 %

Table 2 Mutation-dependent behavior of GISTs

Gene	Mutation site	Mutation frequency	Common disease sites	Behavior	Imatinib response	Sunitinib response	Regorafenib response	Other response
KIT	Exon 9	10–15 % [41]	Intestine	Better RFS and OS compared to exon 11 mutations [97]	Intermediate response [97]	Increased response compared to other mutations [98]	Responsive [99]	Some response to sorafenib [100]
	Exon 11	70 % [41]	Stomach, intestine	Worse RFS and OS in comparison to exon 9 mutations [97]	Responsive [97]	Decreased sensitivity to second line sunitinib [90]		Some response to sorafenib [100, 101]
	Exon 13	1–3 % [41]	Small intestine		Responsive [97]	Perhaps more beneficial than imatinib [97]		
	Exon 14	Usually a secondary mutation			Usually resistant	Perhaps more beneficial than imatinib [97]		
	Exon 17	<1 % [45]	Small intestine		Unknown	Unknown		
PDGFRA	Exon 12	1 % [46]	Stomach	Indolent	No exon specific data. Exon 18 D842 V mutations demonstrate resistance. All other mutations, including other exon 18 mutations appear responsive [46]	No exon specific data. Exon 18 D842V mutations demonstrate resistance. All other mutations, including other exon 18 mutations appear responsive [46]	Responsive [99]	
	Exon 14	<1 % [46]	Stomach	Indolent				
	Exon 18		Stomach	Indolent				

(continued)

Table 2 (continued)

Gene	Mutation site	Mutation frequency	Common disease sites	Behavior	Imatinib response	Sunitinib response	Regorafenib response	Other response
KIT/PDGFRA WT		12–15 % [41]	Stomach		Resistant [102]	Some activity	Responsive [99]	Some response to sorafenib [101] Some evidence for response to dasatinib [103]
SDH	Usually SDHB or SDHA [64]	7.5 % [67]	Stomach	Indolent. Good survival and slow progression of disease despite frequent lymph node metastases [68]	Resistant [68]	Resistant [68]	Reported responses in a small number [99].	Nilotinib [104]
BRAF	V600E [83]	<1 % [83]	Small intestine	High risk of malignancy [80]	Resistant [85]	Resistant [85]	Unknown	Vemurafenib may be effective (based on melanoma data) [86]

had a partial response, 27.9 % had stable disease, and the remaining 4.8 % could not be evaluated. There were no complete responses. Common side effects included edema, diarrhea, and fatigue. There were no significant differences in toxicity or response between the two doses, though this study was likely underpowered to assess these differences [89]. This lack of distinction among dosing regimens led the FDA to approve both doses (400 and 600 mg daily) for patients with GISTs in 2002.

A phase III multicenter randomized controlled trial then evaluated the effect of twice daily dosing (800 mg) of imatinib versus once daily dosing (400 mg) on PFS. Patients who progressed on the once daily regimen were permitted to crossover to the twice daily regimen at the time of progression. At a median follow-up of 2 years, twice daily dosing of imatinib significantly improved PFS with 50 % of patients having progression in twice daily regimen versus 56 % in the once daily regimen (HR 0.82, $P = 0.026$). However, twice daily dosing required more dose reductions (16 vs. 60 %) and treatment interruptions (40 vs. 64 %). Overall, 5 % patients achieved a complete response, 47 % a partial response, and 32 % had stable disease. There was no detectable difference in responses between the groups. Median time to best response was 107 days. Survival for once daily versus twice daily dosing was 85 % versus 86 % at 1 year and 69 % versus 74 % at 2 years. This data suggested that while there was no difference in initiating a response between the dosing regimens, the extended PFS provided by the twice daily regimen might make twice daily dosing worthwhile for selected patients [27].

A second phase III randomized controlled trial with longer follow-up (median follow-up 4.5 years) compared the effect of twice daily dosing (800 mg) of imatinib versus once daily dosing (400 mg) and found no statistically significant differences in PFS, OS, or ORR. Median PFS for once daily versus twice daily dosing was 18 month versus 20 months ($P = 0.13$). Median OS was 55 months versus 51 months, respectively ($P = 0.83$). Dose reductions were more common ($P = 0.0001$) in patients treated with the twice daily regimen. Among patients with disease progression on once daily dosing, approximately 30 % had a partial response or stable disease with dose escalation to twice daily dosing affording an additional 5 months PFS [28]. Results of this study imply that initiating therapy at the lower, once daily dosing regimen and increasing the dose to twice daily in cases of tumor progression is an appropriate dosing strategy.

Mutation Dependent Response to Imatinib

Closer evaluation of pretreatment tumor samples from the formerly mentioned phase III trial published in 2004 by Verweij et al. [27], revealed *KIT* or *PDGFRA* mutation status correlated with clinical outcome and showed differential responses to imatinib dependent on mutation type. Specifically, *KIT* exon 9 mutations are less sensitive to imatinib and benefit from the 800 mg daily dose of the drug, demonstrating significantly improved progression-free survival ($P = 0.0013$). The study also found an increased relative risk of progression and death among patients with exon 9 mutations in comparison to exon 11 mutations (171 %, $P < 0.0001$ and 190 %, $P < 0.0001$, respectively). The study found analogous increases in relative

risk for tumor progression and death among patients with wild-type tumors 108 % ($P < 0.0001$) and the relative risk of death by 76 % ($P = 0.028$) [45].

A similar assessment of the effect of GIST mutational status and response to dosing in the North American phase III trial revealed improved outcome for patients with *KIT* exon 11 mutations in comparison to patients with *KIT* exon 9 mutations or WT tumors. *KIT* exon 11 mutants had objective response rates of 71.7 % while KIT exon 9 and wild-type (WT) genotypes were 44.4 % ($P = 0.007$); and 44.6 % ($P = 0.0002$), respectively. The median time to tumor progression for *KIT* exon 11 mutants was 24.7 months versus 16.7 in exon 9 mutants and 12.8 months in WT tumors and median OS was 60.0 months versus 38.4 and 49.0 months, respectively [90]. A meta-analysis of the two trials also demonstrated a slight benefit of the higher dosing regimen that was more apparent in exon 9 mutations [91]. Given these data, the NCCN currently recommends dosing to start at 400 mg daily with dose escalation as tolerated in *KIT* exon 9 mutations [88].

Duration of Treatment and Progression After Interruption of Imatinib Treatment

In 2010 Le Cesne et al. published a Phase III randomized controlled trial investigating the role of treatment interruption in rapid progression amongst patients with stable GISTs who had undergone 3 years of treatment. The study randomized 50 patients with non-progressive disease after 3 years of treatment with imatinib to either continue, or interrupt dosing. At 35 months median follow-up, 2-year PFS was 80 % in the continuation group and 16 % in the interruption group ($P < 0.0001$). The group concluded that discontinuation of treatment results in a high risk of progression and recommended continuing treatment in patients with unresected GISTs to avoid this [92].

Imatinib Resistance

Primary resistance occurs within the first 6 months of treatment and describes progression of disease while on therapy. GIST with *KIT* exon 9 mutations, and WT GIST (particularly *PDGFRA* exon 18 D842V and SDH deficient GISTs) are most susceptible to primary resistance to imatinib [41, 45, 93, 94]. Notably, dasatinib is recommended for use in patients with the D842V substitution in exon 18 of *PDGFRA*, which are known to exhibit primary imatinib resistance [46, 88, 95, 96] (see Table 2).

Secondary resistance develops after an initial response or stabilization of disease. It occurs in patients who have been treated for more than 6 months, and is signaled by progression of disease. Resistance to imatinib commonly occurs after 2 years of treatment [27, 45, 105]. The most accepted mechanism for imatinib resistance is the development of secondary *KIT* mutations that render protein conformations incompatible with drug binding [106, 107]. When resistance develops, treatment options include imatinib dose escalation and switching to sunitinib [108, 109].

Sunitinib Malate (Sutent)

Sunitinib is an orally dosed multi-targeted receptor tyrosine kinase inhibitor blocking signaling by KIT, PDGFRs, VEGF 1-3, Fms-like tyrosine kinase-3 receptor (FLT3), and the receptor encoded by the ret proto-oncogene RET [74, 75, 110, 111].

Sunitinib was FDA approved for use in GIST in January 2006 after Demetri et al. [109] published results of a randomized controlled trial to assess efficacy and tolerability of sunitinib in patients with imatinib resistant GIST. 312 patients with GISTs that were resistant or who were unable to tolerate imatinib were randomized to receive sunitinib or placebo. This trial was unblinded early due to interim analysis indicating a significantly longer time to progression in the sunitinib group, median PFS was 27.3 weeks in patients treated with sunitinib compared to 6.4 weeks in those receiving placebo (HR 0.33; $P < 0.0001$). These results confirm the efficacy of sunitinib to control disease in patients who have developed resistance to imatinib and support its use in this population.

Sunitinib Resistance

Through mechanisms similar to that involved in imatinib resistance, GISTs can develop resistance to sunitinib as well [112]. In this circumstance, regorafenib may be used as the third line treatment for GIST.

Regorafenib (Stivarga)

Regorafenib is a small molecule multikinase inhibitor (VEGFR 1-3, KIT, PDGFR-α/β, RET, FGFR1/2, TIE2, DDR2, TrkA, Eph2A, RAF-1. BRAF, SAPK2, PTK5, and Abl).

In February of 2013 regorafenib was FDA approved for use in GIST that cannot be surgically resected and does not respond to imatinib or sunitinib. This approval was based on a trial in which 199 patients with metastatic or unresectable GIST with progression after failure of imatinib and sunitinib were randomized to receive regorafenib or placebo, with crossover to regorafenib permitted at the time of progression. Median PFS was 4.8 months and 0.9 months, respectively (HR 0.27; $P < 0.0001$). OS was similar (HR 0.77; $P = 0.199$). Drug-related adverse events were reported in 130 patients (98.5 %) with grade 3 and 4 reactions in 79 (59.8 %). Reactions most commonly consisted of hypertension, hand–foot skin reaction, and diarrhea [99]. Despite a fairly high rate of adverse reactions, the authors note that these were well managed by dose reductions and anti-hypertensives. Additionally, the high rate (85 %) of crossover after progression in placebo-treated patients likely confounds the results, which suggest a similar OS between groups, since there was an almost 4 month improvement in PFS in the regorafenib group. Therefore, with careful attention to managing adverse reactions, regorafenib offers a valuable option for patients with unrespectable or metastatic GIST with resistance to imatinib and sunitinib.

2.8 Management

Patients with GIST should be treated at centers where multidisciplinary planning is possible. While surgical resection with negative margins is the primary treatment of choice for GIST tumors, surgical resection alone is associated with 40 % recurrence at 2 years and 50 % disease specific survival at 5 years [113]. Neoadjuvant and adjuvant targeted therapies can significantly improve recurrence rates and survival.

2.8.1 Asymptomatic, Incidentally Discovered GIST <2 cm

Small, asymptomatic GISTs are common and can be found at autopsy in approximately 20 % of adults over the age of 50 [114]. Data regarding the natural history of incidentally discovered small GISTs are insufficient to make strong recommendations with regard to management. However, close endoscopic surveillance at 6–12 month intervals may be an option for small tumors in the absence of high-risk features (irregular border, cystic spaces, ulceration, echogenic foci, and heterogeneity) on endoscopic ultrasound [115].

2.8.2 Symptomatic GIST and All GIST >2 cm

All symptomatic GISTs and GISTs greater than 2 cm should be surgically resected. Due to a very low rate of lymph node metastasis there is no need for lymph node dissection [116]. The goal for resection is R0 margins, however, R1 margins do not require repeat resection as there is no difference in recurrence free survival in patients undergoing R1 versus R0 resections [117]. However, avoiding tumor rupture and associated peritoneal seeding is perhaps the most important technical aspect of surgery for GISTs as rupture has been found to be a significant factor in tumor metastasis and recurrence [118, 119]. As such GISTs are suitable for laparoscopic resection only if this approach does not increase the risk of compromising the surgery by tumor rupture. Additionally, tumor extraction should be performed with the assistance of a nonpermeable bag to reduce risk of seeding [120].

2.9 Post-operative Imatinib

A phase III randomized controlled trial investigated the utility of imatinib in the adjuvant setting for resected GIST tumors over 3 cm in size. The primary endpoint was recurrence free survival (RFS), but the trial was stopped early due to interim analysis showing the clear benefit of imatinib in comparison with placebo; 1 year RFS was 98 % for the imatinib group versus 83 % with placebo (HR 0.35, $P < 0.0001$) [121]. Based on this initial trial, demonstrating the efficacy of imatinib in the post-operative setting, it might be reasonable to conclude that all patients with resected GIST greater than 3 cm should receive adjuvant therapy [121].

However, this trial left several issues to be addressed. First, follow-up was insufficient to draw conclusions regarding survival benefit. Further, the inclusion of known risk stratification measures such as size, mitotic index, and primary tumor

Table 3 Modified NIH consensus criteria

Risk	Tumor size (cm)	Mitotic index (per 50 hpf)	Primary tumor site
Very low risk	<2.0	≤5	Any
Low risk	2.1–5.0	≤5	Any
Intermediate risk	2.1–5.0 <5.0 5.1–10	>5 6–10 ≤5	Gastric Any Gastric
High risk	Any >10.0 cm Any >5.0 cm 2.1–5.0 5.1–10.0	Any Any >10 >5 >5 ≤5	Tumor rupture Any Any Any Nongastric Nongastric

Adapted from [122]

site might allow for the improved ability to identify subgroups of patients who would derive most benefit from therapy [122]. Finally, the trial used a 1 year treatment course and noted the recurrence of tumors approximately 6 months following the discontinuation of adjuvant imatinib, suggesting longer duration of treatment may be advantageous [121].

To address these issues, Joensuu et al. conducted a phase III randomized controlled trial investigating the effect of duration of adjuvant therapy in patients with resected high risk GIST. High risk GIST was defined according to modified NIH consensus criteria (see Table 3). The study found that three years of adjuvant treatment with imatinib significantly improved RFS and OS: 5-year RFS was 65.6 % with 3 years versus 47.9 % for 1 year (HR, 0.46; 95 % CI 0.32–0.65, $P < 0.001$), and 5 year survival was 92.0 % for 3 years versus 81.7 % for 1 year (HR, 0.45; 95 % CI 0.22–0.89, $P = 0.02$) [123]. This study demonstrated that extending the duration of imatinib therapy to 3 years was beneficial, but the optimal duration of treatment is still to be determined. Thus, the NCCN currently recommends three years of treatment with imatinib in the adjuvant setting for patients with high risk GIST [88].

2.9.1 Locally Advanced GIST or GIST in Critical Locations

Minimizing functional morbidity is the key in considering the resection of GIST tumors. When the morbidity of resection is increased due to large tumor size or when a tumor is located in an area in which resection might compromise critical structures or function, patients should be treated with neoadjuvant therapy.

A review of patients with locally advanced, nonmetastatic GIST included in the phase III BFR14 trial, revealed that PFS and OS of patients that were resected after treatment with neoadjuvant imatinib were similar to local tumors. Further, PFS and OS were improved in comparison to patients with locally advanced tumors who

were treated with imatinib, but not resection [124]. A similar study concluded that in bulky tumors, neoadjuvant imatinib enhances resectability and lessens surgical morbidity [125].

Optimal duration neoadjuvant imatinib is not well established. An early study on the efficacy of imatinib in advanced GIST described a median time to objective response of 13 weeks [89]. PET is superior to CT in detecting early response to therapy and can identify significant changes in uptake as early as 24 h after a single dose of imatinib, though more commonly, PET/CT has been found to reliably detect imatinib response at 1–2 months after initiation of treatment [126–128]. While PET is superior in terms of detecting a metabolic response, CT is still preferred to determine changes in tumor mass for purposes of operative planning. Some authors suggest considering surgery at the time of maximal tumor response, which often occurs between 6 and 12 months of therapy, and is defined by a lack of further improvement between successive CT imaging [129]. The NCCN recommends assessing for resectability within 3 months of treatment initiation [88]. Still, due to the lack of data with regard to optimal surgical timing, multidisciplinary review of all interval imaging with critical consideration of treatment planning is imperative. Finally, evidence of progression on CT imaging is an indication for surgical intervention [130].

Currently, the use of imatinib as neoadjuvant therapy should be a decision made by clinicians in a multidisciplinary setting, taking into account patient specific factors. When imatinib is used as neoadjuvant therapy it can be continued until immediately before surgery and should be restarted as soon as patents tolerate oral intake. On the other hand, due to their multikinase inhibitor functions and VEGF antagonism, sunitinib, and regorafenib should be stopped at least one week prior to surgery, and restarted based on clinical judgment [88] (Table 4).

2.9.2 Recurrent and Metastatic GIST

Imatinib is the primary therapy for recurrent and metastatic GIST [27, 28, 88]. Interruption of dosing has been shown to result in a rapid recurrence of disease [92, 131]. This ‘flare phenomenon’ has been noted even in disease that appears to be refractory to treatment with imatinib, suggesting that there is still be a population of cells that continue to be responsive [132]. Consequently, in the setting of metastatic disease, even with disease progression on imatinib, dosing should be continuous, with options for proceeding including imatinib dose escalation and switching to sunitinib [108, 109].

While there are no definitive data regarding the benefit of surgical debulking in the setting of resectable, metastatic GIST, some studies have suggested that selected patients with metastatic disease, stable on tyrosine kinase inhibitors, may benefit from resection [133, 134] (Table 4).

Table 4 Targeted therapyies in the management of GISTs

	Mechanism	When to use	Optimal duration of neoadjuvant, assessing response and perioperative management	Optimal duration of therapy
Imatinib	TKI of *ABL*, *CKIT* and *PDGFR*	Preoperative GIST in anatomically sensitive areas Preoperative GIST, when the tumor is large Positive margins after resection High risk GIST after resection Unresectable or metastatic GIST	PET/CT has been found to reliably detect imatinib response at 1–2 months after initiation of treatment [126–128] NCCN assess within 3 months of treatment initiation [88] CT is preferred to determine changes in tumor mass for purposes of operative planning. Some authors suggest considering surgery at the time of maximal tumor response, which often occurs between 6 and 12 months of therapy, and is defined by a lack of further improvement between successive CT imaging [129] Imatinib may be stopped the day of surgery and resumed as soon as oral intake is possible [88]	Three years for completely resected high-risk GIST [88, 123] Continued treatment in patients with unresected GISTs to avoid the 'flare phenomenon' [92, 132]
Sunitinib	TKI of all *PDGFR* and *VEGFR*	GIST after intolerance of, or progression on imatinib	Stop sunitinib at least one week prior to surgery with resumption based on clinical recovery [88, 135]	
Regorafenib	Multikinase inhibitor targets kinases involved in angiogenesis (*VEGFR1/VEGFR2/VEGFR3, PDGFR-β, FGFR-1*) and oncogenesis (*KIT, RET, BRAF*) [136]	Surgically unresectable GIST after intolerance of, or progression on both imatinib and sunitinib	Stop at least 2 weeks prior to surgery and resume based on clinical judgment and adequate wound healing [137]	

3 II Hereditary Syndromes

3.1 Lynch Syndrome (LS)—Previously Known as Hereditary Nonpolyposis Colon Cancer (HNPCC)

3.1.1 Epidemiology and Clinical Manifestations

LS is the most common cause of inherited colorectal cancer (CRC), occurring in approximately 1 in 370 people in the U.S [138]. It is inherited in an autosomal dominant manner and accounts for approximately 3 % of colorectal cancers [139, 140]. The lifetime risk of developing colorectal cancer in LS is dependent upon multiple factors, including sex and which mismatch repair (MMR) gene is mutated, accordingly, lifetime risk can range from 30 to 74 % [141].

3.1.2 Identification of High-Risk Patients and Diagnosis

Estimates suggest that LS is vastly under-diagnosed with only 1.2 % of individuals with this disease in the U.S. currently carrying a diagnosis [138]. Traditionally, patients who are at risk for LS have been identified according to family history with the assistance of clinical criteria set forth in the Amsterdam criteria or Bethesda guidelines (Tables 5 and 6). Amsterdam criteria are commonly recalled using the "3-2-1" rule—3 relatives, 2 generations, 1 under 50 years old. Unfortunately, these criteria are imperfect at best, with sensitivity and specificity of Amsterdam II criteria 22 and 98 %, respectively. Revised Bethesda guidelines are somewhat better in terms of sensitivity (82 %), but this comes at the expense of decreased specificity (77 %) [141]. Though these clinically based criteria are well established and easily

Table 5 Amsterdam II criteria

≥3 relatives with LS associated cancers • One should be a first-degree relative of the other two • ≥2 successive generations are effected • ≥1 diagnosis prior to age 50
FAP should be excluded
Pathologic tumor verification

Adapted from [148]

Table 6 Revised Bethesda Guidelines

1. CRC in a patient less than 50 years old
2. Presence of synchronous or metachronous CRC or other LS associated tumor at any age
3. CRC with MSI-H histology in a patient <60 years old
4. CRC in a patient with ≥1 first-degree relative with a LS related cancer diagnosed under age 50
5. CRC in ≥2 first- or second-degree relatives with a LS related cancer diagnosed at any age

Adapted from [149]

recalled, multiple computational models are available that may offer improved risk assessment in the general population: MMRpredict, MMRpro, and $PREMM_{1,2,6}$. In a study of patients at high risk for having mismatch repair defects all models performed superior to Bethesda criteria. MMRpredict was most accurate with sensitivity and specificity of 94 and 91 %, respectively [142].

In patients who have developed tumors, immunohistochemistry (IHC) for MLH1/MSH2/MSH6/PMS2 proteins and/or testing for microsatellite instability (MSI) in tumors can also be performed in order to assist in establishing a diagnosis of LS. In a study comparing the efficacy of IHC versus MSI in which 500 colorectal carcinomas were examined, 98 (19.6 %) were found to be MSI positive, with 64 (12.8 %) MSI-H. IHC failed to identify 6 (9.3 %) of MSI-H tumors, and 15 tumors that were MSS had abnormal IHC. Of the 64 patients with MSI-H tumors, 18 (28.1 %) were found to have LS on further genetic testing and only 1 (5.6 %) patient with LS was not identified by IHC [143]. Other studies have conferred a similarly high degree of concordance between IHC and MSI testing, supporting its use in screening tumors for LS.

BRAF mutations and promoter hypermethylation are almost never seen in LS, therefore, when loss of MLH1 is identified by IHC, further testing for BRAF mutation or MLH1 hypermethylation can assist with determining whether MMR deficiency is due to LS [141, 144, 145]. IHC is a cost effective initial means by which to assess tissue for BRAF mutation, however many patients will need additional genetic testing to confirm or rule out the diagnosis [146].

To increase diagnosis of LS, both National Comprehensive Cancer Network (NCCN) and Healthy People 2020 have advocated for universal testing of all patients with newly diagnosed colorectal cancer. With growing national support for universal testing, feasibility is imperative and while IHC is less sensitive and specific in comparison to MSI, its relative widespread availability may make universal testing a more attainable goal [143]. In a cost analysis of tumor evaluation for LS, Ladabaum et al. [147] prefer IHC followed by BRAF mutation testing as indicated, describing acceptable cost when performed on patients ≤70 years old and a newly diagnosed CRC. A consensus statement released by the U.S. Multi-Society Task Force on Colorectal Cancer recommends either (1) universal MMR deficiency testing on all newly diagnosed CRC or (2) testing on CRC diagnosed in patients 70 years old or younger, and in patients over 70 years old, but who have a family history suggestive of LS [141] (Table 7).

Ultimately, the diagnosis of LS is established by the finding of germline mutations in MLH1, MSH2, MSH6, PMS2, or EPCAM genes. U.S. Multi-Society Task Force on Colorectal Cancer recommends genetic testing should be performed on all patients fulfilling Amsterdam I/II criteria, Revised Bethesda criteria or patients with more than 5 % chance of mutation by prediction models. Additionally patients with a known LS mutation in the family or patients diagnosed with uterine cancer prior to the age of 50 should undergo genetic testing [141].

Table 7 U.S. Multi-Society Task Force on Colorectal Cancer recommendations for genetic screening for Lynch syndrome

Amsterdam I/II criteria
Revised Bethesda criteria
>5 % chance of mutation by prediction models
Known LS mutation in the family
Personal history of a tumor with evidence of MMR deficiency that tested negative for BRAF mutation and hypermethylation of MLH1
Uterine cancer <50 years old

Adapted from [141]
LS associated tumors: Colorectal, endometrial, gastric, ovarian, pancreatic, ureter/ renal pelvis, biliary tract and brain (usually glioblastoma), tumors, sebaceous gland adenomas and keratoacanthomas and carcinoma of the small bowel
MSI-H histology: Tumor infiltrating lymphocytes, Crohn's-like lymphocytic reaction, and mucinous/signet-ring differentiation or medullary growth pattern

3.1.3 Molecular Genetics

Carcinogenesis in LS is a result of germline mutations in mismatch repair (MMR) genes. Mutations in MMR genes are inherited in an autosomal dominant fashion. Mutation, loss of heterozygosity, or epigenetic silencing through hypermethylation can result in acquired inactivation of the remaining allele causing MMR defects.

Mismatch repair genes are responsible for maintaining the integrity of DNA by identification and repair of errors due to slips in DNA replication or DNA damage. The four MMR genes commonly associated with LS and their respective chromosomal locations are as follows—*MLH1* (chromosome 3p21-23), *MSH2* (chromosome 2p21), *MSH6* (chromosome 2p16), and *PMS2* (chromosome 7p22.2)—with the majority of mutations occurring in MLH1and MSH2. A review of multiple studies estimated overall proportions of gene mutations in LS to be 32 % *MLH1*, 39 % *MSH2*, 14 % *MSH6*, and 15 % *PMS2* [150].

More recently, the epithelial cell adhesion molecule (*EPCAM*) gene, located upstream of the *MSH2* gene, has been implicated in indirect epigenetic inactivation of *MSH2* gene. Deletion of 3′ end of *EPCAM* causes hypermethylation, and thereby, inactivation of *MSH2* [151, 152].

DNA MMR deficiency promotes microsatellite instability (MSI), a characteristic of LS [153]. Defective MMR proteins are unable to detect and repair insertion–deletion loops (IDLs), which occur when DNA polymerase slips during replication of short repetitive sequences of DNA, referred to as microsatellites. In absence of a reliable repair mechanism, this type of error often causes frameshift mutations that can lead to downstream nonsense mutations and result in the production of truncated, nonfunctional proteins [154].

MSI is, however, not specific to LS, and approximately 15 % of sporadic CRCs display MSI as well [140, 155]. In contrast to the mechanism described above, MSI in sporadic CRCs develops as a result of the CpG methylator phenotype pathway

(CIMP) [156]. In this pathway, promoter regions of key tumor suppressors, such as *MLH1*, are epigenetically inactivated by hypermethylation. Tumors that result from this pathway are referred to as CIMP+ tumors, and have been closely associated with *BRAF* mutations, explaining the utility of *BRAF* mutation to rule out the presence of LS [155].

Though patients with LS are at increased risk of developing multiple malignancies, they are at highest risk of developing CRC. In general the risk is slightly lower in female patients and varies depending on which MMR protein is mutated [141]. Mutations in *MLH1* and *MSH2* are most common, and the risk of developing any LS associated cancer is most common with these mutations [150, 157–161]. In patients with *MLH1* and *MSH 2* mutations the median age of onset for colorectal cancer is between the ages of 45– 47 [161–164]. Mutations in *MSH6* and *PMS2* are less frequent, and though data are limited, they seem to present later in life and be associated with lower risk of progressing to cancer [165, 166].

LS associated CRC have high-risk morphologic features such as poorly differentiated histology with extracellular mucin and tumor infiltrating lymphocytes [167, 168]. Additionally, the polyp dwell time is substantially shorter than that seen in sporadic CRC (36 months versus 10 years) [164, 169, 170]. Still, these tumors tend to have improved prognosis in comparison to sporadic CRC, which can likely be attributed to genetic characteristics including MSI-H status of tumors associated with MMR deficiency in this syndrome [171, 172].

With improved identification and screening of at risk patients the risk of mortality associated with CRC is being minimized, and the risk of other carcinomas becomes increasingly pertinent. Therefore, monitoring of symptoms at other sites at risk for cancer and identifying surveillance strategies that might similarly prevent mortality associated due to extracolonic malignancy and is imperative [173, 174].

Chemoprevention

A randomized controlled trial investigating the effects of daily aspirin (600 mg) and resistant starch (30 mg) in the prevention of adenoma and carcinoma among patients with Lynch syndrome found no effect with aspirin, resistant starch, or both [175]. However, when dosing was extended to 25 months and follow-up to 55 months, the protective effect of aspirin became apparent [176]. These results support a delayed protective effect of aspirin on the development of colorectal cancer, which becomes evident after 3–4 years. Further studies to confirm these protective effects and establish the optimal dosing of aspirin are necessary before aspirin can be formally recommended as a chemoprevention strategy for patients with LS [144].

3.1.4 Management

Colorectal malignancy is the most common malignancy associated with LS and with an average age of diagnosis of 45 [161–164]. Studies have shown that screening colonoscopy is associated with decreased rate of detected CRC, decreased stage of detected CRC and decreased mortality in patients with LS.

Additionally, some studies suggest that increasing frequency of screening to annually might further enhance this effect [177–179].

Guidelines set forth by the US Multi-Society Task Force on Colorectal Cancer and the American College of Gastroenterology (ACG) state that patients at risk or diagnosed with LS should begin screening colonoscopy at the age of 25–25 years or 2–5 years prior to the youngest age of diagnosis in a family member, whichever comes first. Colonoscopy should be repeated every 1–2 years, and annually among patients with a known MMR germline mutation [141, 144]. Due to the lower risk of cancer and later age at diagnosis among patients with *MSH6* and *PMS2* mutations, screening in patients with these mutations may be postponed until the age of 30 years in *MSH6* and 35 years in *PMS2*, unless there is a family history of earlier onset [141, 161, 165, 166, 180].

Prophylactic colectomy is unnecessary and uncommon among patients with LS and an unaffected colon. However, it may have increased merit among unaffected carriers of LS who have a very high incidence of colorectal cancer in their family, or in whom colonoscopy is difficult, therefore practitioners should discuss this option. On the other hand, colectomy is indicated for patients who develop colon cancer or those with endoscopically unrespectable polyps.

Total abdominal colectomy with ileorectal anastomosis (IRA) is the recommended procedure due to a high rate of metachronous CRC among patients undergoing segmental colectomy, though segmental colectomy is an acceptable alternative [144]. Metachronous lesions occur in approximately 15 % at 10 years from the index procedure, but this increases to almost 70 % at 30 years [181–183]. Additionally, some models predict a gain of 2.7 years of life for young patients with LS who undergo subtotal colectomy versus partial colectomy in comparison to a gain of only 0.3 years for 67 year olds who undergo the more extensive procedure [184]. These data support the recommendation or IRA in young patients and suggest that relaxing this recommendation to segmental colectomy in older patients is a rational approach.

Polyposis Syndromes

Adenomatous Polyposis Syndromes

Familial adenomatous polyposis (FAP), attenuated familial adenomatous polyposis (AFAP) and MUTYH-associated polyposis (MAP)

3.1.5 Epidemiology and Clinical Manifestations

Familial adenomatous polyposis (FAP) is a rare autosomal dominant disease that is characterized by greater than 100 colorectal adenomas on endoscopy [144, 185]. It is caused by a mutation in the *APC* gene at chromosome 5q21 [186]. Penetrance is nearly 100 %, with most affected patients developing colorectal cancer by the age of 40 [187]. Extracolonic manifestations of the disease include gastric, duodenal and periampullary polyps as well as increased incidence of thyroid cancer, hepatoblastoma, desmoid tumors, adrenal tumors, osteomas, and dental abnormalities [144].

A milder variant of the disease, called attenuated familial adenomatous polyposis (AFAP) is defined as having fewer than 100 adenomas [144]. Among patients with AFAP, onset of colorectal carcinoma is generally later, presenting at approximately 55 years. The number of adenomas varies greatly, but there is a clear right-sided

predominance with adenomas that tend to be flat, features that are distinct from FAP [188–190]. A third, variant was found to be inherited in an autosomal recessive manner and is known as autosomal recessive familial adenomatous polyposis or, more commonly, *MUTYH*-associated polyposis (MAP). There is limited information regarding the spectrum and frequency of disease, however, many of these patients present with features of AFAP or have been diagnosed with atypical FAP [191].

3.1.6 Identification of High-Risk Patients and Diagnosis

Patients with more than 10 polyps, or with extracolonic manifestations associated with FAP (most commonly upper gastrointestinal polyps, but other findings may include malignancies of the thyroid, brain, adrenal glands, liver, and pancreas. Benign tumors such as desmoids, lipomas, fibromas, sebaceous, and epidermoid cysts, osteomas, dental abnormalities, and congenital hypertrophy of the retinal pigment epithelium (CHRPE), have also been described) should be considered for testing [192]. When there is no known family history of a specific genetic mutation, patients should be tested for mutations in both *APC* and *MUTYH*. Family members of patients with known mutations should undergo specific genetic testing for the known familial mutation.

A failure to identify a mutation in an index case, does not exclude a diagnosis, rather, more extensive genetic testing may be appropriate.

3.1.7 Molecular Genetics

Familial diffuse adenomatous polyposis can be the result of genetic mutations in two genes: *APC* and *MUTYH*.

APC

Adenomatous polyposis coli (*APC*) is a tumor suppressor gene that has functions in both cell adhesion and regulation of cell cycle. Through interactions with E cadherin in adherens junctions, APC promotes cell adhesion. Additionally, APC acts in a complex with other proteins to degrade intracellular β-catenin, which would otherwise go on to the nucleus to act as a transcription factor. Thus, with loss of function in APC, cells both lose adhesive properties and gain transcriptional activation.

Mutations in *APC*, located at chromosome 5q21, are commonly implicated in the development of sporadic cancers, but germline mutations in *APC* manifest as a syndrome of diffuse polyposis and extracolonic manifesteations identified as FAP [186]. Malignant degeneration of polyps occurs with somatic damage to the second allele. This inactivation of *APC* results in a progression of adenoma to carcinoma in a pathway similar to that of sporadic cancers, whereby mutations accrue in other important genes such as *KRAS*, *p53*, and regions of chromosome 18 [193, 194]. This is an autosomal dominant process. Penetrance of the mutation with respect to development of CRC is nearly 100 % by age 40. Though many cases of FAP are familial, approximately 10 % of *APC* mutations occur de novo [195].

Mutations are known to occur throughout the *APC* gene and phenotypic severity is affected according to mutation location. For example, in traditional FAP, *APC* mutations occur in the mutation cluster region (MCR), a region that affects

β-catenin binding [196, 197]. Clustering of mutations that subject patients to more severe forms of colorectal disease or duodenal disease occur in distinct regions as well [198]. Similarly, the less severe form of FAP, AFAP, is associated with mutations at the very proximal or distal ends of the gene [196, 198].

MUTYH

The *MUTYH* gene is located on chromosome 1p35 and produces a protein that is responsible for repair of DNA by base excision of damage caused by oxidative damage [191]. Failure of repair results in alterations in multiple genes, particularly *APC* and *KRAS*. Mutations in the *MUTYH* gene can result in a familial polyposis syndrome referred to as *MUTYH*-associated polyposis (MAP). MAP is inherited in an autosomal recessive manner making it much less likely to appear in a familial pattern. Homozygous germline mutations in the *MUTYH* gene result in polyposis that is similar to AFAP in that it is less severe, has lower penetrance, and later onset in comparison to FAP [191].

3.1.8 Chemoprevention

There has been some interest in the value of chemoprevention with sulindac, a nonsteroidal anti-inflammatory (NSAID) for patients with FAP. In 1993 Giardiello et al. [199] conducted a randomized controlled trial showing a reduction in size and number of polyps discovered among patients with FAP that were treated with sulindac for 9 months and followed with colonoscopy for a year. However, the same group published a follow-up study in 2002, which failed to show benefit in prevention of polyp formation among patents with FAP who had undergone treatment with sulindac for four years, suggesting that the benefit of sulindac in FAP is temporary [200]. Additionally, the mechanism of polyp reduction is poorly understood, though studies suggest its effects are due to both COX inhibition and β-catenin inhibition with induction of apoptosis [201, 202].

It follows that COX-2 inhibition has generated similar attention. In 1996, investigators used a mouse model of human FAP, to show that COX-2 inhibition resulted in a decrease in polyp size and number in $Apc^{\Delta 716}$ mice. This finding spurred enthusiasm for a targeted approach to treatment in FAP [203]. In 2000, Steinbach et al. published a randomized controlled study investigating the effect of celecoxib, a COX-2 inhibitor, in 77 patients with FAP. After 6 months of treatment, celecoxib significantly reduced the polyp burden in patients with FAP [204]. Shortly thereafter the FDA approved celecoxib as an adjunct in the treatment of FAP. Unfortunately, multiple studies since then have demonstrated that celecoxib induces a significant, dose dependent cardiovascular risk [205–207]. There is some evidence that the cardiovascular risk among patients with low baseline cardiovascular risk is not affected by COX-2 inhibition [206]. Further studies would therefore be necessary prior recommending COX-2 inhibition as long-term chemoprophylaxis for patients with FAP.

The negative cardiovascular side effects of COX-2 inhibitors led Burn et al. to concentrate on the preventative effects of aspirin, an NSAID with a favorable cardiovascular profile. In an international multicenter randomized controlled trial,

the group showed a trend toward reduced polyp burden in 206 patients with FAP randomized to 600 mg of aspirin per day versus resistant starch/placebo [208].

While these studies suggest a promising role for NSAIDs in the progression of polyposis, their roles in cancer prevention are less certain, and therefore cannot replace current recommendations for surveillance and prophylactic colectomy [144]. However, they may have some role as an adjunct for prevention of other cancers after colectomy.

3.1.9 Management

Patients suspected of having genetic polyposis syndromes should be referred to a geneticist undergo testing for *FAP* and *MUTYH* mutations.

Screening endoscopy with prophylactic colectomy significantly decreases mortality due to CRC in FAP and this is supported by increased survival in relatives of probands who undergo screening [209–211].

The British Society of Gastroenterology recommends beginning annual surveillance with alternating colonoscopy and sigmoidoscopy at the age of 12–15 for patients with FAP or who are at increased risk of having FAP, but have no identified genetic marker to confirm it. For patients who do not have a genetically confirmed diagnosis, this surveillance should continue until the age of 20, at which point surveillance frequency can be extended to every 3–5 years until the age of 60. Patients with genetically or clinically confirmed FAP should continue annual surveillance until polyp burden requires a colectomy. Prophylactic colectomy should be performed between the ages of 16 and 25 years to avoid the higher risk of developing cancer thereafter. Surgical options include proctocolectomy with ileal pouch and anal anastomosis or total abdominal colectomy with ileorectal anastomosis [212].

The American College of Gastroenterology (ACG) recommends annual surveillance colonoscopy for patients at risk for FAP or with a diagnosis of FAP. This should begin at puberty or at the time of symptom onset, if this is sooner. In patients with FAP sigmoidoscopy is an acceptable alternative to colonoscopy as polyps tend to be evenly distributed. However, in AFAP and MAP polyps tend to be more proximally located making a full colonoscopy necessary [189, 213–215]. Colonoscopy should be performed with attention to polyp number, size, and location. Additionally, several polyps should be chosen for biopsy.

CRC in FAP generally presents prior to the age of 40 with polyps appearing at a mean age of 16. In contrast, AFAP is characterized by a delayed onset of polyposis, often not occurring until 40 years of age, with CRC equally postponed until approximately 55 years [185, 187, 216, 217]. Therefore, screening in AFAP can be delayed until 20–25 years of age with subsequent screening exams to be performed every 1–2 years [144]. Polyposis among patients with MAP is similarly slow to progress, and patients are at less risk of developing colorectal cancer, therefore they can follow a screening protocol to similar to patients with AFAP [191, 218].

3.1.10 Prophylactic Colectomy

The ACG recommends that patients with FAP to undergo prophylactic colectomy in the late teens, early twenties, or sooner, if there is suspicion of cancer. Other indications to consider colectomy sooner include polyps greater than 1 cm in size, polyps with dysplasia, polyps that significantly increase in number between exams, multiple adenomas greater than 6 mm in size, and inability to appropriately survey the colon due to multiple diminutive polyps [144]. Similar indications for prophylactic colectomy apply to patients with AFAP and MAP, however, prophylactic colectomy in these cases can often be delayed many years as polyps can be managed endoscopically for some time, with rare patients never requiring a colectomy [190].

Surgical options for colectomy include total abdominal colectomy with ileorectal anastomosis (IRA) and proctocolectomy with ileal pouch, anal anastomosis (IPAA). With either surgery lifelong endoscopic surveillance is required, however the risk of rectal cancer with IRA can range from 12–32 % [219–221].

Polyp number can be helpful in guiding decisions with regard to which surgery is most appropriate [221]. Patients with more than 20 rectal polyps or more than 1000 colonic polyps would be at high risk for developing cancer in the rectal remnant should undergo IPAA, but patients with fewer polyps have a choice of procedure [144]. In addition to severity of polyposis, surgical decision-making can be guided by distribution of polyps, and if there are few polyps in the rectum, performing an IRA might be a reasonable approach. This strategy is most applicable in patients with AFAP and MAP, as they tend to have a decreased severity of polyposis, with a predisposition for proximal location [189, 213–215]. Similarly, some have suggested that genetically stratifying patients according attenuated, intermediate, and severe genotype groups may aid surgical decision-making [222, 223]. Other genetic considerations include whether a predisposition for developing desmoids in surgical scars among patients with genetic mutations in *APC* occurring between codon 1445 and 1580 warrants delaying surgical resection since this mutation is associated with mild polyposis, or whether it warrants expedited IPAA since desmoids can cause shortening of the mesentery and make it difficult to convert an IRA to an IPAA [185, 224]. Still, more practical considerations regarding the effect of IPAA on female fertility and incontinence issues must also be considered and discussed with patients [225–228].

In conclusion, surveillance endoscopy scheduled at short intervals from an early age with a well-timed prophylactic colectomy significantly reduces colorectal mortality among patients with FAP. Decisions with regard to surgical approach for colectomy are ultimately left to the patient. Because this is a complex decision involving many factors, practitioners must ensure that patients are fully informed.

Juvenile Polyposis Syndrome (JPS)

3.1.11 Epidemiology and Clinical Manifestations

Juvenile polyposis syndrome (JPS) is a rare autosomal dominant inherited disorder with variable penetrance [229]. It was first described by McColl et al. [230]. JPS is characterized by multiple hamartomatous polyps involving the upper and lower

gastrointestinal tracts. Polyps generally arise in patients prior to the age of 20 [231]. Congenital defects have been noted to occur in 15 % of affected individuals, though the actual number may be higher [231–233].

Despite presentation in childhood, the term '*juvenile*' refers to polyp characteristics—edematous lamina propria and mucous filled cysts lined by cuboidal to columnar epithelium with reactive changes—rather than juvenile onset [234, 235]. A study of 272 patients with JPS revealed the following frequency of distribution by site: colon and rectum (98 %), stomach (14 %), jejunum and ileum (7 %), and duodenum (2 %) [236].

Initial presentation is commonly with chronic anemia or rectal bleeding, though patients can occasionally present with rectal prolapse of polyps, protein losing enteropathy, intussusception, or obstructive symptoms as well [231, 237]. Juvenile polyps are not generally considered malignant, yet they are associated with an estimated 50 % risk of gastrointestinal malignancy in JPS [237, 238]. Indeed, the mechanism by which malignancy occurs in this syndrome is incompletely understood [229, 235].

3.1.12 Identification of High-Risk Patients and Diagnosis

The diagnosis of JPS is primarily based on clinical criteria, which were initially established by Jass et al. [239], and remain unchanged to date. Patients with more than five juvenile polyps of the colon or rectum, juvenile polyps throughout the gastrointestinal tract, or any number of juvenile polyps with a family history of juvenile polyposis should be suspected of having JPS [239]. Reasons to undergo genetic testing include distinguishing the syndrome from other conditions in which juvenile polyps form, to confirming the diagnosis, and allowing for testing of family members (Table 8). Patients with findings consistent with JPS may be tested for mutations in SMAD4 and BRMP1.

3.1.13 Molecular Genetics

SMAD4 or *BMPR1A* mutations can be detected in approximately 40 % of patients with JPS [241, 242]. The third gene, *ENG*, is less well recognized and the true extent of its role in JPS is yet to be determined [243, 244]. These varied germline mutations are common in their alteration of TGF-β signal transduction, which results in disruption of normal processes of cell growth inhibition and apoptosis [245, 246]. The lifetime risk of developing gastrointestinal cancers is dependent upon mutation type and can range from 9 to 50 % [231, 237]. Nearly 25 % of newly diagnosed patients with JPS have no family history of disease and are found with de novo mutations, leaving only 75 % of patients with JPS who exhibit a

Table 8 Clinical criteria for diagnosis of JPS

Any one of the following:
1. More than five juvenile polyps of the colon or rectum
2. Juvenile polyps in other parts of the GI tract
3. Any number of juvenile polyps and a positive family history

Adapted from [239, 240]

family history of disease. Finally, testing for genetic mutations in JPS is not currently universally recommended since more that 50 % of patients with a clinical diagnosis have no identifiable mutation [247].

SMAD4

In 1998 a gene associated with JP was localized to chromosome 18q21.1 and identified as *SMAD4* [248, 249]. SMAD family genes encode cytoplasmic mediators of the transforming growth factor-β (TGF-β) signaling pathway. The TGF-β signaling pathway is known to play a significant role in regulating colonic epithelial growth [250]. TGF-β activates a serine–threonine kinase transmembrane receptor, which then phosphorylates SMAD proteins [251, 252]. The phosphorylated SMAD proteins form a heteromeric complex with SMAD4 which then migrates to the nucleus and regulates transcription, performing important growth inhibitory functions [253, 254].

Though some genetic mutations display distinct genotype-phenotype relationships, there is no such correlation in patients with JPS. However, there is some suggestion that mutations in *SMAD4* may be associated with an increased risk of upper gastrointestinal malignancy [255]. A study in 80 patients with JPS revealed that patients with SMAD mutations (73 %) were significantly more likely to have gastric polyps in comparison to those with *BMPR1A* mutations (8 %) [256]. Furthermore, two studies have directly associated *SMAD4* mutations with increased risk of upper gastrointestinal malignancy [240, 257].

SMAD4 mutations have also been linked to hereditary hemorrhagic telangiectasia (HHT). As such, patients with this mutation should be screened for HHT [255]. Clinical diagnostic criteria for HHT include history of epistaxis, telangiectasias, visceral lesions and an appropriate family history. The presence of three of these criteria is diagnostic, while two criteria are suggestive of the diagnosis [258].

BMPR1A

A second actor in TGF-β superfamily was identified in association with JP in 2001 [259]. Bone morphogenetic protein receptor type IA (BMPR1A) protein, also designated CD292, is a serine–threonine kinase type I receptor involved in bone morphogenetic protein (BMP) signaling. It is encoded by the *BMPR1A* gene on chromosome 10q22-23 and responds to ligands of the TGF-β superfamily [259].

There are no specific risks identified with this mutation, though a very severe form of JPS, has been related to contiguous deletions in both *PTEN* and *BMPR1A*. This form is described in infants with diffuse polyposis, and is often accompanied by other congenital abnormalities with death frequently occurring in early childhood [260, 261]. Independent mutations in *PTEN* have also been suggested. But the role of *PTEN* is controversial, and may be more consistent with undiagnosed Cowden syndrome, with extra-intestinal manifestations [262].

ENG

Mutations in a third gene, *ENG* (endoglin), may also be responsible for the development of JPS [243, 244]. *ENG* is located on chromosome 9q34.11 and

encodes a surface membrane protein, endoglin, which is part of the TGF-β receptor complex. This protein is primarily recognized for its role in angiogenesis and for the hereditary hemorrhagic telangiectasia (HHT) that develops when it is mutated. However, some studies have noted *ENG* mutations in children with early onset JP [243, 244].

3.1.14 Management

Once a clinical diagnosis is accomplished patients should be referred for genetic counseling to discuss whether to proceed with genetic testing (*SMAD4* or *BMPR1A* mutations), risk of GI malignancy, and surveillance strategies [229].

While polyps in JPS are not generally considered malignant, the lifetime risk of developing colorectal cancer in JPS is 16–39 % with average age of onset at 42–44 years [231, 235, 237]. As such, screening of patients at risk for this condition is rational, however, there are few data to drive decision-making with regard to efficacy and outcomes of surveillance. As a result, there are no widely accepted guidelines for screening colonoscopy in JPS. The goals of colonoscopy for surveillance in JPS are to minimize polyp related morbidity and to prevent or detect cancers [240].

The British Society of Gastroenterology recommends screening colonoscopy of at-risk patients beginning at 15–18 years of age, or earlier if patients become symptomatic. Screening should continue at 1–2 years intervals until the age of 70 in affected individuals. Screening interval can be relaxed in at risk individuals who are without signs of disease at the age of 35. Additionally, they recommend consideration of prophylactic colectomy in patients with multiple polyps that cannot be endoscopically controlled, polyps with adenomatous change, when colon cancer is a feature of JPS in family members and in the presence of symptoms such as bleeding or anemia [212, 263].

The American College of Gastroenterology has similar, though slightly more aggressive recommendations for at risk populations. They recommend initiating surveillance at age 12–15 years (earlier if symptoms are present) with repeat colonoscopy every 1–3 years thereafter depending on degree of polyposis. They state that all polyps 5 mm and larger should be removed at the time of endoscopy and suggest surgical resection when polyps are too numerous to be controlled endoscopically, in the presence of uncontrolled symptoms such as bleeding or diarrhea, and when there is a suspicion of cancer [144].

In cases that require it, surgical options include total abdominal colectomy with ileorectal anastomosis and total proctocolectomy with ileal pouch and anal anastomosis. In contrast to FAP, the presence of rectal polyps does not assist with decision-making as to which procedure is most appropriate, and 50 % of patients that initially undergo total abdominal colectomy with ileorectal anastomosis require a second procedure for total proctectomy within 10 years [144, 264].

Patients with identified *SMAD* mutations or clinical characteristics consistent with HHT (telangiectiasis of the lips, nose, oral cavity, or fingers, arteriovenous malformations of internal organs or recurrent epistaxis) should have an electrocardiogram, a chest X-ray, and echocardiogram prior to undergoing anesthesia.

These tests can help to identify heart failure associated with significant right–left shunt due to pulmonary arteriovenous malformations, which occur in many patients with HHT [240, 258].

Peutz-Jeghers Syndrome (PJS)

3.1.15 Epidemiology and Clinical Manifestations

PJS is a rare autosomal dominant inherited disorder characterized by hamartomatous gastrointestinal polyps and mucocutaneous pigmentations [265]. It is associated with germline mutations in the serine–threonine kinase 11 gene (*STK11/LKB1*), a tumor suppressor gene [266]. Histologic features particular to PJS polyps include a central core of smooth muscle proliferation extending into the polyp in an arborizing pattern and cystic gland dilatation extending into the submucosa or muscularis propria [267]. Patients present with polyp related symptoms consisting of bleeding, anemia, abdominal pain, intussusception, and obstruction. These symptoms usually develop by the age of 20 years with malignancies developing at an average age of 42 years [267, 268]. The lifetime risk of developing colorectal cancer in patients with PJS is 39 % [268–270] Other common sites of cancer in this syndrome include stomach, small bowel, pancreas, lung, breast, uterus, ovary, cervix, and testes, resulting in a cumulative cancer risk of 76 % at 70 years old [268, 270]. Despite having a clearly heightened risk of cancer among patients with PJS, the mechanism of progression to cancer in PJS polyps is poorly understood [271, 272].

3.1.16 Identification of High-Risk Patients and Diagnosis

The American College of Gastroenterologists (ACG) recommends genetic evaluation for PJS in patients with perioral or buccal pigmentation, two or more histologically characteristic GI hamartomatous polyps, or a family history of PJS [144] (Table 9). The Mallorca European Consensus and World Health Organization (WHO) have similar recommendations for establishing a clinical diagnosis [273]

Table 9 ACG recommendations for genetic evaluation of PJS

Perioral or buccal pigmentation
Two or more histologically characteristic GI hamartomatous polyps
Family history of PJS

Table 10 WHO recommendations for clinical diagnosis of PJS

Positive family history of PJS, and • Any number of histologically confirmed PJS polyps or • Characteristic, prominent, mucocutaneous pigmentation
Negative family history of PJS, and • Three or more histologically confirmed PJS polyps, or • Any number of histologically confirmed PJS polyps and characteristic, prominent, mucocutaneous pigmentation
Histology PJS polyps: A central core of smooth muscle that shows tree-like branching, covered by the mucosa native to the region which is heaped into folds producing a villous pattern

Adapted from [273]

Table 11 Mallorca European Consensus recommendations for clinical diagnosis of PJS

Two or more histologically confirmed PJ polyps
PJ polyp(s) detected in an individual who has a family history of PJS
Mucocutaneous pigmentation in an individual who has a family history of PJS
PJ polyp(s) in an individual who also has mucocutaneous pigmentation

Adapted from [267]

(Tables 10 and 11). Mucocutaneous pigmentation is one of the most consistent findings in PJS, with >95 % of patients displaying 1–5 mm buccal or perioral pigmented macules [144]. Additionally, polyps in PJS have characteristic histologic features such as a central core of smooth muscle with tree-like branching, covered by the mucosa folding in a villous pattern. While family history is probably one of the most helpful factors in determining who is at risk for PJS 20–60 % of cases can be sporadic with de novo mutations [268, 274] (Table 12).

Genetic testing of patients at risk for PJS should include assessment for mutation of STK11.

3.1.17 Molecular Genetics

The *STK11*(*LKB1*) mutation is localized to chromosome 19p13.3 [266]. *STK11* mutations are present in 78–94 % of patients with PJS [275, 276]. 20 % of mutations are thought to be de novo [268]. Cancers likely develop in patients with PJS when a germline mutation in STK11 is present and there is loss of heterozygosity at the second allele. Genotype–phenotype correlations among patients with mutations in the *STK11* gene have not yet been identified [277].

Under normal conditions STK11 inhibits the mammalian target of rapamycin (mTOR) pathway via activation of adenine monophosphate–activated protein kinase. The mTOR pathway is an important regulator of cell growth and proliferation. Absence of inhibition of the mTOR pathway by STK11 results in uncontrolled proliferation and reduced apoptosis, explaining the role of STK11 mutations in carcinogenesis.

mTOR pathway inhibition can be achieved with several existing drugs, termed rapalogs, which include drugs such as rapamycin and its analogues everolimus and temsirolimus. These drugs are commonly used for immunosuppression in organ transplantation, but more recently, their anti-tumor activity has been investigated [278]. Specifically, mTOR inhibitors used in mouse models of PJS have been shown to decrease the number of polyps, suggesting therapeutic potential for these drugs in PJS [279]. This, and other promising evidence for mTOR inhibitors, leads to a clinical trial investigating its activity in patients with PJS. The trial, NCT00811590, opened in 2008, but was closed in 2013 due to lack of accrual.

The identification of COX-2 up-regulation in PJS polyps has lead to similar investigations regarding its potential as a target for chemoprevention [280]. A mouse model of PJS, using LKB1 knockout mice, confirmed its efficacy showing significantly decreased polyp burden in mice treated with celecoxib, a COX-2

Table 12 Summary of hereditary syndromes

	Associated genetic mutation	Who to test	What to test	Screening Colon	To note
Adenomatous polyposis syndromes					
LS	MLH1, MSH2, MSH6, PMS2, or EPCAM genes Autosomal dominant	• All CRC Or • All CRC <70 yo and CRC ≥70 who meet revised Bethesda criteria [141, 283, 284]	Start with MMR (IHC) and/or MSI	• Begin screening colonoscopy at the age of 25–25 years or 2–5 years prior to the youngest age of diagnosis in a family member, whichever comes first • Colonoscopy should be repeated every 1–2 years, and annually patients with a known MMR germline mutation [141, 144] • In patients with MSH6 and PMS2 mutations, screening in patients with these mutations may be postponed until the age of 30 years in MSH6 and 35 years in PMS2, unless there is a family history of earlier onset [141, 161, 165, 166, 180]	• Risk of endometrial, ovarian, small bowel, pancreas, urinary tract cancers
FAP	APC Autosomal dominant	• Patients with more than 10 polyps, or with extracolonic manifestations associated with FAP [192]	APC and MUTYH	• Begin screening colonoscopy at puberty (earlier if symptoms) • Repeat exam annually • Flexible sigmoidoscopy is an alternative with classic FAP, until a polyp is found [144, 283]	• >100 polyps • Risk of small bowel, stomach, pancreas, thyroid, liver, and brain cancers • CHRPE, osteomas, desmoids, gastric fundic gland polyps
AFAP	APC Autosomal dominant			• Begin screening colonoscopy at puberty, (earlier if symptoms) • Repeat examinations every 1–2 years if negative [144, 283]	• 10– <100 polyps • Risk of small bowel, stomach, thyroid cancers • Right-sided polyps are predominant
MAP	MUTYH Autosomal recessive			• Begin screening colonoscopy at 25–30 years • Repeated every 1–2 years if negative [144, 283]	• <100 polyps • Often no family history • Risk of small bowel, stomach, thyroid cancers

(continued)

Table 12 (continued)

	Associated genetic mutation	Who to test	What to test	Screening Colon	To note
Hamartomatous polyposis					
JPS	SMAD4 BRMP1A ENG (possibly) Autosomal dominant	Any one of the following: • More than 5 juvenile polyps of the colon or rectum • Juvenile polyps in other parts of the GI tract • Any number of juvenile polyps and a positive family history [239, 240]	SMAD4 BRMP1A	• Surveillance at age 12–15 years (earlier if symptoms) • Repeat colonoscopy every 1–3 years thereafter depending on degree of polyposis • All polyps 5 mm and larger should be removed at the time of endoscopy • Surgical resection when polyps are too numerous to be controlled endoscopically, in the presence of uncontrolled symptoms such as bleeding or diarrhea, and when there is a suspicion of cancer [144]	• Risk of cancers of the small bowel, pancreas and HHT • SMAD4 mutation: Higher risk of HHT and gastric cancer
PJS	STK11 Autosomal dominant	• 2 or more histologically confirmed PJ polyps • PJ polyps in a person with a family history of PJS • Mucocutaneous pigmentation in a person with a family history of PJS • PJ polyps in a person who also has mucocutaneous pigmentation [267]	STK11	• Initial surveillance colonoscopy at age 8 years • If polyps present, this should be repeated every 3 years • If no polyps are seen, surveillance colonoscopy can be resumed at age 18, then continued every 3 years, with colonoscopy performed sooner in the presence of symptoms • This should continue until age 50, at which point, screening frequency should increase to every 1–2 years [144, 267]	• Risk of caner of the small bowel, pancreas, breast, lung, ovary, uterus, cervix, and testes cancers

inhibitor. Similarly, they achieved decreased gastric polyposis in 2 of 6 human patients treated with celecoxib [281]. These results are promising, but the use of COX-2 inhibitors in the prevention of polyposis in PJS requires further clinical investigation.

3.1.18 Management

Patients meeting clinical criteria for PJS should be referred to a genetic counselor and tested for the *STK11* mutation. A lack of mutation does not exclude disease, however, detection of a mutation is an indication for family members to undergo mutation specific testing.

The lifetime risk of developing CRC in patients with PJS is 39 % [268–270] Symptoms of disease generally become apparent by the age of 20 years with malignancies often developing by the age of 42 years [267, 268]. While it is widely recommended, there is little evidence to support improved outcomes with surveillance colonoscopy [267].

The British Society of Gastroenterology recommends screening colonoscopy to begin at the age of 25 years with repeat colonoscopy every 2 years thereafter [212].

The American College of Gastroenterology recommends an initial screening colonoscopy at age 8 years. If polyps present, this should be repeated every 3 years. If no polyps are seen, surveillance colonoscopy can be resumed at the age of 18, and continued every 3 years, with colonoscopy performed sooner in the presence of symptoms. This should continue until age 50, at which point, screening frequency should increase to every 1–2 years [144, 267]. Given the rarity of dysplasia in PJS polyps, the value of polypectomy is likely to be mostly owing to decreased polyp related morbidity, therefore there is no specific recommendation with regard to polypectomy in PJS [282]. As with other hamartomatous polyposis syndromes, colectomy may be indicated if polyps cannot be managed endoscopically, for control of polyp related symptoms or for suspicion of cancer [144].

4 III Colorectal Cancer (CRC)

4.1 Epidemiology and Clinical Manifestations

Worldwide, CRC is the third most common cause of cancer in men and the second most common cancer in women, with an approximately 2 % cumulative risk of developing colorectal cancer by the age of 75 [285]. In the US, however, lifetime risk of developing CRC is approximately 4.5 % [286]. Though incidence rates have been declining at a rate of 3 % per year, the incidence rate among people younger than 50 has been increasing [285, 287, 288]. In the U.S., screening colonoscopy has significantly contributed to decreasing mortality rates [289]. However, many patients continue to present with advanced, symptomatic disease consisting of pain, anemia, bleeding, obstruction, or perforation, and up to 20 % of patients have metastatic disease at the time of presentation [290, 291].

4.2 Identification of High-Risk Patients and Diagnosis

Risks associated with developing CRC can be inherited and environmental. Aside from distinctly heritable colon cancer syndromes, a family history of CRC is one of the most significant risk factors for developing CRC [292]. Inflammatory bowel disease (IBD) also predisposes patients to developing CRC [293]. Similarly, age greater than 50 puts patients at significantly increased risk for getting CRC [294, 295]. Risks due to family history of disease, IBD status and increasing age are unavoidable. However, there are many modifiable risk factors for patients who wish to minimize CRC risk. Obesity, cigarette smoking, consumption of red and processed meats, low physical activity, and low fruit/vegetable intake have all been associated with increased risk of developing CRC and therefore represent opportunities for improvement in risk [296–299].

Molecular Genetics

Colorectal cancer is a heterogeneous disease. Familial risk and inherited syndromes account for approximately 25 % of CRC. Despite 25 % of CRCs demonstrating a familial predisposition, only around 5 % result from known germline mutations [216, 300]. Heritable diseases associated with these known mutations, including hereditary polyposis and nonpolyposis syndromes, are discussed elsewhere. The remaining 75 % of CRCs are sporadic. Sporadic CRCs result from acquired somatic mutations. Genetic instability associated with these mutations is commonly attributed to one of three pathways: chromosomal instability (CIN), CpG Island methylator (CIMP), or microsatellite instability (MSI) pathways.

4.2.1 Chromosomal Instability Pathway (CIN)

CIN is the most common pathway and is thought to be responsible for 65–70 % of sporadic CRC [301, 302]. The meaning of chromosomal instability, and how best to measure it, is poorly defined. However, CIN is generally associated with an increased rate of gains and losses of whole chromosomes or fractions of chromosomes. The detection of tumor polyploidy or aneuploidy is suggestive of CIN, and loss of heterozygosity (LOH) is often used as a surrogate for determining CIN since a true measurement of the rate of chromosomal change in vivo is impractical [303–305].

The CIN pathway generally follows the adenoma–carcinoma sequence, a multistep genetic model for carcinogenesis in CRCs. This sequence was initially described by Fearon and Vogelstein in 1990 [193]. Inactivation of the tumor suppressor gene, *APC*, activates the Wnt-β-catenin signaling pathway, initiating the tumorigenic sequence, and leading to the formation of aberrant crypt foci (ACF). Activating mutations of *KRAS*, mutations in *p53*, and LOH at chromosome 18q (*DCC* and *SMAD2/4*) then follow, and result in progression to larger adenomas and carcinomas. Mutations in other genes, such as *PI3 KCA*, can occur late in a small number of tumors [193].

The cause of CIN is also not well delineated, though it may involve defects in mitosis (such as microtubule instability or kinetochore dysfunction), alterations in cell cycle checkpoints, defective double strand DNA repair, or telomere dysfunction

[306, 307]. Whatever its cause, CIN results in aneuploidy, LOH and chromosomal amplifications [301]. Chromosomes 1, 5, 8, 17, and 18 are the most frequently effected by LOH [308].

Exact measurements of the rate of chromosomal change would require sequential in vivo measurements and are therefore impractical. Instead, currently used means for assessing CIN utilizes the degree of aneuploidy to infer the degree of CIN, though this is neither a standardized process nor a perfect gauge of CIN. Measurement of aneuploidy with flow cytometry is a crude technique, though often employed in studies, since use of more precise methods such as array comparative genomic hybridization (CGH) are not feasible for large series [309]. Other approaches include polymerase chain reaction (PCR), fluorescence in situ hybridization (FISH), and traditional karyotyping [310, 311]. Given the variety of diagnostic procedures and lack of uniform criteria to designate CIN positive tumors, the identification and resulting interpretation of CIN is complex [301].

4.3 Prognostic Value of CIN Status

Investigators have been hopeful that CIN status might prove to be of some prognostic value and multiple studies have shown that CIN+ tumors are associated with worse outcomes [306, 312]. A large meta-analysis evaluating the prognostic significance of CIN in 10,126 patients with CRC revealed that patients with CIN+ disease have worse prognosis (HR 1.45; $P < 0.001$) [309]. Some authors have proposed further categorization of CIN+ and CIN− tumors, arguing that a subgroup exists within those tumors currently categorized as CIN−, which has characteristics that are more consistent with CIN+ tumors [313]. Indeed, CIN− tumors were able to be subclassified according to a bimodal distribution into CIN-low (≤9 changes) and CIN-stable (≤6 changes) [314]. The clinical implications of such a distinction are highlighted in a similar study by Watanabe et al., in which they report a significant difference in disease free and overall survival in patients with CIN high-mild versus CIN high-severe tumors. The authors conclude that CIN phenotype may prove beneficial in determining the utility of chemotherapy in patients with stage II CRC [315]. The discrepancy in nomenclature between these two studies reflects the lack of convention for defining and studying CIN, which is central to the difficulty in understanding these results, and undermines the clinical utility of CIN status.

4.4 Efficacy of Chemotherapy in CIN+ Tumors

In phase II trials taxanes have been shown to have little efficacy in patients with CRC [316, 317]. This observation may, in part, be attributable to the high rate of CIN in CRCs and the fact that taxanes target microtubules, the dysfunction of which are thought to be the source of CIN [318–320]. For example, Aurora A kinase, a protein serine–threonine kinase with activity in mitosis and meiosis, has

been shown to be independently associated with CIN in CRC, and its amplification has been shown to be capable of overriding mitotic spindle checkpoint inhibitors conferring resistance to paclitaxel [311, 321]. A phase II European trial, the CINATRA (Chromosomal Instability and Anti-Tubulin Response Assessment) trial, sought to delineate a differential response to the taxol based chemotherapy dependent on CIN status. Unfortunately the drug was associated with high levels of toxicity and showed no evidence of efficacy [322].

This resistance to chemotherapy may not be specific to taxols, as CIN+ status in CRC cell lines has been associated with multidrug resistance. Of interest, this includes all thymidylate synthase inhibitors, suggesting that the worse prognosis of CIN+ tumors may, in part, be explained by a lack of sensitivity to 5-FU, a main component of chemotherapy for CRC. Unfortunately, there is no data regarding the efficacy of 5-FU based chemotherapy in CIN+ patients in comparison to no treatment, making it difficult to assess the benefit of fluoropyrimidine-based therapy in this group of patients [309, 323].

These studies indicate that there may be prognostic and predictive value in CIN status of tumors, which could eventually guide decision-making regarding the treatment of patients with these tumors. However, at this time there are too many uncertainties regarding CIN status and recommendations from The American Society of Clinical Oncology do not support testing for aneuploidy [324].

4.4.1 CpG Island Methylation Phenotype (CIMP)

CIMP+ tumors account for approximately 15–20 % of sporadic CRC [302, 325–327]. This classification of tumors was recognized in 1999, when Toyota et al. [156] found that a subset of colorectal tumors result from transcriptional inactivation of tumor suppressor genes via aberrant methylation of promoter regions of CpG islands, and referred to these tumors as CpG Island Methylation Phenotype (CIMP). Hypermethylation in these regions results in epigenetic silencing of a large array of tumor suppressors, however smaller panels are usually tested. Traditional markers for DNA methylation consist of *MINT1, MINT2, MINT31, CDKN2A* (p16 gene), and *MLH1* [155, 156, 328, 329]. Among these markers, *MLH1* is imperative, as it is often mutated, and a common cause of the associated MSI-H phenotype [155].

Originally, using the five-marker panel, CIMP+ tumors were defined as having ≥4/5 methylated genes [326]. However, more recent studies have indicated that an eight-gene marker panel including *RUNX3, CACNA1G, IGF2, MLH1, NEUROG1, CRABP1, SOCS1, and CDKN2A* provides improved sensitivity and specificity [326, 330]. These extended marker panels have been useful in proposing further classification of tumors into CIMP-high, CIMP-low groups based on a bimodal distribution of methylation patterns. CIMP-high tumors have 65–70 % of the genes methylated (≥5/8 or 6/8 genes) and characteristics consistent with those of tumors previously regarded as CIMP+ (≥4/5 genes on the five gene panel) [326]. CIMP-low is a term that designates tumors with fewer methylated genes, which were previously described as CIMP- tumors. Currently regarded as an intermediate

subgroup, CIMP-low tumors have 1/8–5/8 methylated genes, whereas, CIMP- tumors are without any methylated genes [330, 331].

The cause of CIMP and mechanism by which genes are selected for methylation in aging and cancer are unknown [328]. However, methylation has been associated with environmental and lifestyle factors such as smoking [332, 333]. In comparison to CIMP- tumors, CIMP+ tumors tend to occur more often in advanced age and female populations. They are frequently poorly differentiated tumors occurring in the proximal colon, and present at higher stages. They are also associated with microsatellite instability (MSI), *BRAF* mutations and tend to be *p53* wild-type [326, 327, 334, 335]. CIMP is closely linked to MSI and accounts for almost all cases of *BRAF* mutation [155]. Additionally, multiple studies have shown that CIMP+ tumors with *BRAF* mutations have a low frequency of *KRAS* mutations [155, 326, 334, 336].

Despite this, occasional studies have found increased numbers of *KRAS* mutations among tumors with promoter hypermethylation [327, 337]. Further investigation into this finding has revealed a few possible explanations. First, in cases where CIMP+ tumors also harbor *KRAS* mutations, hypermethylation of the MMR gene is associated with a G to A mutation in the *KRAS* gene, which may partially explain the infrequent finding [338]. However, the more likely explanation is that *KRAS* mutations are instead associated with the subclassification of CIMP+ tumors known as CIMP-low tumors [326, 339]. CIMP-high and CIMP-low tumors are therefore more accurately presented as a continuum whereby, at the one end, CIMP-high tumors are associated with women, often have *BRAF* mutations, MSI predominates, and both *p53* and *KRAS* mutations are rare [328]. At the other end of the spectrum, CIMP-low tumors are more common in men, often have *KRAS* and *p53* mutations, are more likely MSS, *BRAF* mutations are unusual [326, 340].

CIMP+ tumors are thought to progress through a pathway distinct from the classical adenoma–carcinoma pathway, which is commonly known, and characteristic of CIN+ tumors. Similarities among proximal hyperplastic polyps, serrated adenomas, and CIMP+ tumors, such as degree of methylation, frequency of *BRAF* mutations and serrated histology, suggest that CIMP+ tumors follow the serrated polyp pathway [329, 341, 342].

Indeed, activation of the RAS-MAPK oncogenic pathway via *BRAF*, or less frequently, *KRAS* mutations, is characteristic of most CIMP+ tumors [328, 340, 343]. As expected, activation of these oncogenes initially induces a burst of MEK dependent proliferation causing hyperplastic aberrant crypt foci [344, 345]. Activation of the MAPK pathway then prompts increased activity of tumor suppressors, such as p16 and p53, which counter this proliferative drive and induce cell senescence, thereby limiting the polyp to a small stable lesion [344, 346–348]. Subsequently, methylation induced silencing of these tumor suppressors allows for cellular escape from senescence and results in malignant transformation [347, 349–351].

4.4.2 Microsatellite Instability (MSI)

MSI occurs in approximately 15 % of sporadic CRCs [352, 353]. MSI describes an increased rate of single nucleotide mutations and alterations in small repetitive lengths of DNA, referred to as microsatellites. These types of errors occur regularly

during DNA replication, however, in the case of MSI they persist due to a deficiency of mismatch repair proteins (MMR-D) [354]. Accordingly, MSI is commonly defined by an absence of DNA mismatch repair proteins, occurring not by germline mutations as with LS, but via hypermethylation of promoter region DNA of MMR genes—usually hMLH1 [155, 325, 352, 355, 356].

When the DNA MMR system is undermined, innumerable small deletions accumulate at rates many times faster than normal and can result in frame shift mutations. These frame shift mutations preferentially occur in short repetitive tracts, and produce truncated, nonfunctional proteins. Among these, *TGF-βRII,* the receptor for the growth inhibitory signaling protein, TGF-β, is the most well-known. Inactivating mutations in *TGF-βRII* can be found in up to 90 % of MSI+ tumors [357–359]. Likewise, IGFR2, a protein prerequisite for the activation of TGF-β, is truncated in approximately 10 % of MSI tumors allowing for increased tumor cell growth due to an absence of suppression by TGF-β [359, 360]. As results of these studies might suggest, MSI-H tumors generally express either one of these mutations, but not both. Therefore, these mutations likely represent serial points along a central pathway in carcinogenesis in MSI-H tumors [359].

BAX, is another gene commonly mutated due to MSI. The BAX protein, known for its pro-apoptotic activity, can be mutated in 50 % of CRCs, providing yet another mechanism by which MSI can promote tumorigenesis [361]. Similarly, activin type II receptor (*ACVR2*) is mutated in almost 60 % of MSI-H CRCs and its loss has been found to correlate with increased tumor size [360, 362]. Along with mutations in *TGF-βRII*, *IGFR2*, and *ACVR2*, six other commonly occurring mutations were identified in a genome wide study of CRC with MSI: *SEC63* (48.8 %), *AIM 2* (47.6 %), a gene encoding a subunit of the NADH-ubiquinone oxidoreductase complex (27.9 %), *hMSH3* (26.2 %), a homologue of mouse cordon-bleu (23.8 %), *EBP1/PA2G4* (20.9 %). With the exception of *hMSH3*, the function of these proteins is largely unknown [360].

MSI-H tumors are generally diploid and lack a loss of heterozygosity at loci containing tumor suppressor genes implicated in the classic adenoma to carcinoma pathway, such as 5q (*APC*; 0 %), 17p (*p53*; 0 %), and 18q (*DCC*, *SMAD2/4*; 12.5 %) [363, 364]. Additionally, MSI-H tumors have lower rates of mutation in *APC* and *p53* as compared to MSI-L or MSS tumors [365, 366]. They are more prevalent in women and have improved 5-year OS compared with MSS/MSI-L [367]. Morphologically they often show poorly differentiated histology, mucinous phenotype, and marked lymphocytic infiltration [368].

4.5 Measuring Microsatellite Instability

MSI can be measured either indirectly by immunohistochemistry (IHC) or directly by polymerase chain reaction (PCR) to evaluate for MSI. Though IHC is slightly less sensitive and specific in comparison to PCR, the tests are generally concordant [369]. Additionally, the near universal availability of IHC, and its cost effectiveness make it an attractive first option [143, 147]. IHC testing employs immunologic

staining to identify deficiencies in MMR proteins including MLH1, MSH2, MSH6, and PMS2. If MLH1 or PMS2 is lost, IHC for BRAF, which is rarely mutated in LS, can be used to further assess the likelihood for LS versus sporadic MSI-H/MMR-D CRC [370]. Mutated BRAF therefore indicates that CpG promoter hypermethylation (CIMP) is likely responsible for MMR-D [155, 156].

Alternatively, identification of MSI by PCR is traditionally utilizes the Bethesda panel, which includes two mononucleotide markers (BAT 25 and BAT 26) and three dinucleotide microsatellites (D5S346, D2S123, and D17S250) [354]. Original Bethesda guidelines suggested the use of a five-marker panel, with tumors designated as MSI-high (MSI-H) if 2 or more microsatellite sequences in tumor DNA are mutated or MSI-low (MSI-L) if one marker is mutated. Tumors with no microsatellite mutations are considered microsatellite stable (MSS) [354]. This recommendation was amended in 2002, when a consensus meeting revised the guidelines, stating that if a tumor is designated as MSI-L, or only dinucleotide repeats are mutated, a second panel of mononucleotide markers (i.e., BAT40 and/or MYCL) should be run to clarify the MSI status. The change was recommended based on the fact that mononucleotide markers are more sensitive for identifying MSI than di- or tri-nucleotide markers, which could result in misclassification of MSI-H and MSI-L tumors [149]. Testing requires that the genotype of tumor tissue to be compared against that of normal tissue in order to identify mutations consistent with MSI.

However, testing of a pentaplex of mononucleotide markers suggested by Suraweera et al. [371, 372] (BAT26, BAT25, NR21, NR22, and NR24) revealed superior sensitivity and specificity in comparison to the Bethesda panel without the need to compare results to normal tissue. Thus, many centers use a commercially available panel markers (MSI Analysis System, Promega) consisting of AT-25, BAT- 26, NR-21, NR-24, and MONO-27 with pentanucleotide repeats Penta C and Penta D for sample identification [373].

4.6 Prognostic Value

MSI-H tumors in sporadic CRC are epidemiologically distinct from MSI-H tumors in HNPCC. They are more commonly found in women, patients over the age of 50, and smokers. However, they are similar in that they are often located proximal to the splenic flexure, and that morphologically, they tend to be mucinous, poorly differentiated, and show lymphocytic tumor infiltration [373–377]. There is some evidence to suggest that this lymphocytic infiltration associated with MSI-H tumors is representative of an immune response to malignancy, which may be responsible for the improved outcomes in these tumors [378].

Despite having a tendency to be more poorly differentiated, MSI has been associated with improved prognosis since it was initially described in 1993 [364, 379, 380]. This association was firmly founded in 2003 with the seminal article by Ribic et al., who analyzed 570 tumors collected from five phase III trials of adjuvant therapy for stage II and III colorectal cancer. They found 95 (17 %) MSI-H tumors.

Among patients not receiving adjuvant therapy, MSI-H status conferred significant improvement in survival in comparison to MSI-L or microsatellite stable (MSS) tumors (HR 0.31, 95 % CI 0.14–0.72; $P = 0.004$) [352]. Shortly thereafter, a large meta-analysis including 1277 patients with MSI found a comparable survival benefit among patients with MSI (HR 0.65, 95 % CI 0.59–0.71) [355]. In a pooled analysis of five trials that randomized patients with stage II and III CRC to surgery and chemotherapy with a fluorouracil-based regimen versus surgery alone, Sargent et al. found 15 % of tumors with deficient MMR (MMR-D)tumors. In comparison to patients with proficient MMR (MMR-P) tumors, they were unable to show a prognostic effect of MMR-D status on outcomes, however, they did show a strong protective effect of MMR-D in CRC in patients who underwent treatment with surgery alone (DFS MMR-D vs. MMR-P: HR, 0.58; 95 % CI, 0.26–1.28; $P = 0.17$; OS MMR-D vs. MMR-P: HR, 0.62; 95 % CI, 0.28–1.37; $P = 0.24$) [353]. This improved prognosis was again confirmed in patients with stage II CRC in a subset analysis of the Quick and Simple and Reliable (QUASAR) trial, which showed decreased risk of recurrence in tumors with MMR-D (RR 0.53, 05 % CI 0.4–0.7; $P < 0.001$) [376]. Additionally, evidence from the QUASAR trial and CALGB 9581 and 89,803 studies suggests a more benign biology of disease among MMR-D tumors given their increased likelihood of being stage II rather than stage III disease, in spite of being more poorly differentiated [376, 381].

4.7 Value of MSI in Predicting Response to Chemotherapy

4.7.1 5-FU

While documentation for improvement in survival among patients with MSI-H/MMR-D tumors has been fairly uniform, the benefit of fluorouracil-based chemotherapy in these patients is highly disputed. Fluorouracil-based regimens are the mainstay of adjuvant therapy for colorectal cancer. 5-fluorouracil (5-FU) is a pyrimidine that is incorporated into RNA, inhibits thymidylate synthase, and can be incorporated into DNA [382].

CRC cell lines with MMR-D demonstrate resistance to 5-FU, and 5-FU even confers relative growth and survival advantage in MMR-D cells in comparison to MMR-P cells [383]. Sensitivity to 5-FU in vitro can be restored to MMR deficient cell lines by demethylation with 5-Aza-2′-deoxycytidine (5 aza-dC), which is accompanied by reexpression of the hMLH1 protein [384]. Additionally, MMR proteins have the capacity to recognize and bind 5-FU modified DNA, and cytotoxicity of 5-FU in vitro has been shown to be dependent upon functional DNA MMR [385, 386]. Though an exact mechanism is not well defined, these observations support a critical role for MMR proteins in establishing chemosensitivity to 5-FU and are paralleled by findings in clinical studies.

Early studies based on retrospective reviews reported significant survival benefit of adjuvant therapy in patients with MSI-H tumors [387, 388]. However, in 2003 Ribic et al. reported results from analysis of phase III trials showing a lack of

benefit of fluorouracil-based chemotherapy in patients with MSI-H tumors. Among patients with MSI-H tumors, 5-year OS was 70.7 % for patients with MSI-H tumors treated with adjuvant versus 80 % for patients not receiving adjuvant ($P = 0.07$), implying that there may even be a detrimental effect of chemotherapy in patients with MSI-H tumors [352]. In 2010, Sargent et al. confirmed and expanded on these findings, reporting on tumors collected from five other phase III trials. Patients with MMR-D tumors receiving chemotherapy had no improvement in DFS in comparison to those who did not get chemotherapy (HR 1.10, 95 % CI 0.42–2.91; $P = 0.85$). Additionally, after analyzing data from 457 patients in their study, they then included the 570 patients from Ribic's study to perform a pooled subset analysis on stage II and III patients with MMR-D tumors. They found a lack of benefit of adjuvant therapy in either stage, with a trend toward a detrimental effect of chemotherapy in stage II disease (Stage II: HR, 2.30; 95 % CI, 0.85–6.24; $P = 0.09$; Stage III HR, 1.01; 95 % CI, 0.41–2.51; $P = 0.98$) [353]. It should be noted that these findings come in the setting of fluoro uracil-based therapy only, whereas current practice dictates the use of fluorouracil-based therapy in combination with oxaliplatin or irinotecan. Whether the addition of these agents may modify efficacy among patients with MMR-D/MSI-H tumors is yet to be determined.

In contrast, several studies have been unable to show a similar predictive value for response to chemotherapy. A study of patients enrolled in four randomized NSABP colon cancer treatment trials did not show that MSI status was a predictive marker for benefit of chemotherapy. However, a direct comparison of outcomes among patients with MSI-H tumors who received versus who did not receive chemotherapy was not possible [389]. The QUASAR study, which examined only stage II CRC, also found no association between MMR status and response to fluorouracil-based adjuvant therapy [376]. However, the benefit of chemotherapy in stage II CRC is a subject of controversy, and not generally as great as in stage III patients, perhaps complicating further discrimination on the basis of MSI status in this trial [390, 391]. Similarly, evidence from the CALGB 9581 (stage II CRC) and 8903 trials (stage III CRC) suggests that MMR status does not predict a response to therapy (Stage II: edrecolomab and Stage III: either FU/LV or IFL), though there were no specific comparisons among patients with MMR-D tumors who did, and did not receive adjuvant therapy [381].

Currently, European Society for Medical Oncology (ESMO) and National Comprehensive Cancer Network (NCCN) consensus guidelines support MSI status for use as an adjunct for clinical decision-making in patients with stage II CRC [392, 393].

4.7.2 *BRAF* in MSI-H Tumors

BRAF mutations, which are present in approximately 20 % of sporadic CRCs, are highly correlated with MSI-H status through a strong association with CIMP [155, 335, 341, 394, 395]. In one study, the odds ratio for association between CIMP and BRAF was 203 [155]. *BRAF* mutations occur in approximately 40 % of MSI-H tumors and almost 100 % of MSI-H CIMP + tumors [326, 396, 397]. While *BRAF*

mutations are commonly seen in sporadic MSI-H tumor they are almost never seen in LS, making it a useful test in the diagnosis of LS [394, 398]. Additionally, *BRAF* mutation can be associated with worse prognosis, particularly in MSI-L tumors [396, 399–402].

Targeted Therapies in Sporadic CRC

Clearly, the genetic interactions involved in the initiation and progression of carcinogenesis in CRC are complex. We have just recently begun to appreciate the mechanisms by which specific genetic characteristics might contribute to differential prognoses and responses to standard chemotherapy regimens. The development of therapeutics targeting specific aspects of these pathways has progressed in parallel, and we are thus starting to understand how targeted therapies might be selected according to specific genetic attributes of tumors. It is foreseeable that we may eventually prescribe targeted drugs as the initial therapies for CRC, according to the precise genetic signature of each tumor. However, our knowledge of these details remains rudimentary, and the use of targeted therapies is currently limited to the metastatic setting where an occasional genetic marker may steer decision-making.

Epidermal Growth Factor Receptor (EGFR)

EGFR is a member of the Erb family of receptors and serves as a cell surface receptor for growth factors such as EGF and TGF-α. Upon activation, the monomer dimerizes and proceeds to phosphorylate intracellular proteins activating pathways involved in proliferation, apoptosis, angiogenesis, migration, adhesion, and invasion. The PI3K-AKT, STAT, and RAS-MAPK pathways are all activated by EGFR [403].

EGFR is upregulated or overexpressed in approximately 80 % of CRC, and overexpression of EGFR is associated with poor prognosis [404–407]. These observations provided the rationale for investigating the role of targeted EGFR therapy in CRC. EGFR antagonism has been accomplished through anti-EGFR monoclonal antibodies or small-molecule EGFR tyrosine kinase inhibitors (TKIs) [408].

Cetuximab

Cetuximab is a chimeric human-murine monoclonal antibody. It binds to EGFR with higher affinity than endogenous EGFR ligands (TGF-α and EGF). Upon binding, cetuximab promotes EGFR internalization and degradation without inducing receptor phosphorylation, thereby, prohibiting activation. This results in receptor down-regulation and decreased cell surface expression of EGFR [409, 410].

4.7.3 Cetuximab as Third Line Therapy in Refractory EGFR Expressing (EGFR+) Metastatic CRC (mCRC)

Cetuximab was originally FDA approved for use in EGFR+ mCRC in patients who were refractory to, or intolerant of irinotecan-based therapy. Approval came in 2004 in response to results of the BOND-1 trial. In this landmark study, Cunningham et al. showed that among patients with mCRC who had progressed on irinotecan-based regimens, cetuximab alone or in combination with irinotecan

improves response rate and time to progression without significant additional adverse events. In comparison to cetuximab alone, combination therapy offered significant improvement in both measures, however median survival was similar. Response rates of cetuximab in combination with irinotecan versus cetuximab alone were 22.9 and 10.8 %, respectively ($P = 0.007$). The combination was more effective at prolonging median time to progression as well (4.1 vs. 1.5 months; $P < 0.001$). Despite these improvements, median survival was similar between groups (8.6 vs. 6.9 months; $P = 0.48$) [411]. Due to the superior performance of the combination, cetuximab was initially approved for use in combination with irinotecan in patients who had developed resistance to irinotecan alone, though it could be used as monotherapy in patients who were intolerant of irinotecan.

In 2007, Jonker et al. confirmed the efficacy of cetuximab as single agent therapy in patients with EGFR+ mCRC refractory to fluoropyrimidine, irinotecan, and oxaliplatin. In comparison with best supportive care (BSC), cetuximab improved progression free survival (HR 0.68; $P < 0.001$), median OS (6.1 vs. 4.6 months, HR 0.77; $P = 0.005$) and preserved quality of life [412]. Accordingly, the FDA approved cetuximab for use as a single agent in EGFR+ mCRC in patients who were refractory to both irinotecan- and oxaliplatin-based chemotherapy.

4.7.4 1st Line Treatment of KRAS WT, EGFR+ mCRC

In 2012, the FDA approved cetuximab for use in patients with *KRAS* WT, EGFR expressing mCRC. The approval was a result of retrospective analysis of tumor samples from patients enrolled in several trials (CRYSTAL, OPUS, CA225025), which revealed that among patients with *KRAS* WT tumors, cetuximab in addition to either chemotherapy, or BSC, improved objective response rate (ORR), overall survival (OS), and progression free survival (PFS) [413–415].

The Cetuximab Combined with Irinotecan in First-Line Therapy for Metastatic Colorectal Cancer (CRYSTAL) trial investigated the efficacy of FOLIRI alone or in combination with cetuximab as first-line therapy in patients with EGFR+ mCRC. Retrospective evaluation of the data revealed that 37 % of tumors were positive for KRAS mutations. Among patients with *KRAS* WT tumors, the addition of cetuximab to FOLFIRI resulted in improved median OS (23.5 vs. 20.0 months; HR, 0.796; $P = 0.0093$), median PFS (9.9 vs. 8.4 months; HR, 0.696; $P = 0.0012$), and ORR (57.3 vs. 39.7 %; OR, 2.069; $P = 0.001$), in comparison to FOLFIRI alone. This benefit was not seen among *KRAS* mutant tumors [413].

These findings were confirmed by tumor analysis from the CA225025 trial, which investigated the efficacy of cetuximab versus BSC in patients with previously treated EGFR+ mCRC. *KRAS* mutations were found in 42 % of patients. Similar to findings in the CRYSTAL study, patients with *KRAS* WT tumors displayed improved median OS (9.5 vs. 4.8 months; HR, 0.55; $P < 0.001$) and median PFS (3.7 vs. 1.9 months; HR, 0.40; $P < 0.001$) with cetuximab as compared with BSC alone. None of these improvements were seen in patients with mutant *KRAS* tumors [414]. Though cetuximab was not used in a first-line setting in this trial, the importance of *KRAS* status in predicting response to cetuximab was used to support the FDA requirement of WT *KRAS* status for use in this setting.

Analogous findings resulted from a review of collected tissues from OPUS, a phase II trial investigating the value of adding cetuximab to FOlFOX-4 as first-line treatment for mCRC. In 2011, Bokemeyer et al. showed improved PFS (HR 0.567; $P = 0.0064$) and ORR (57 vs. 34 %, OR 2.551; $P = 0.0027$), with a promising effect on median OS (22.8 vs. 18.5 months, HR 0.855; $P = 0.39$) in patients with *KRAS* WT tumors treated with FOLFOX-4 and cetuximab versus FOLFOX-4 alone. This effect was specific to patients with *KRAS* WT tumors and patients with *KRAS* mutant tumors had no similar benefit with the addition of cetuximab [415].

Panitumimab

Panitumumab is a humanized IgG2 kappa monoclonal antibody specific to EGFR with a mechanism of action similar to cetuximab [416].

4.7.5 3rd Line Treatment in Refractory EGFR+ mCRC

Panitumumab was FDA approved in 2006 for the treatment of patients with EGFR + mCRC who were refractory to treatment with fluoropyrimidine-, irinotecan-, and oxaliplatin-based regimens. This approval came after trial results published by Van Cutsem et al. [417] displayed improved PFS (8.0 vs. 7.3 weeks, HR 0.54; $P < 0.001$) and ORR (10 vs. 0 %; $P < 0.0001$) with panitumumab when compared with BSC in patients with EGFR+ chemorefractory mCRC. A follow-up study analyzing the role of tumor *KRAS* status on efficacy of panitumumab revealed *KRAS* mutations were present in 43 % of patients. Additionally, *KRAS* WT tumors showed significant improvements in PFS (12.3 vs. 7.3 weeks, HR 0.45; $P < 0.0001$) with panitumumab in comparison with BSC, an effect that was not seen in *KRAS* mutant tumors. Improved survival was seen in patients with *KRAS* WT tumors in comparison to those with *KRAS* mutant tumors, but this effect was independent of treatment (HR 0.67, 95 % CI 0.55–0.82). The OS for patients with WT *KRAS* was not affected by treatment with panitumumab (HR 0.99, 95 % CI, 0.75–1.29), however this number may have confounded by crossover (90 of 118, 75 % of patients with WT *KRAS* status) to panitumumab treatment by patients who progressed on BSC [418].

The Panitumumab, Irinotecan, and Ciclosporin in COLOrectal cancer (PICCOLO) trial was designed to assess the response with the addition of panitumumab to irinotecan in patients with previously treated advanced CRC. Midway through the trial Amado et al. [418] published their data highlighting the lack of efficacy of panitumumab in patients with *KRAS* mutant tumors. Therefore, the aims of the trial were amended to focus on quantification of benefit and the identification of other biomarkers among patients with *KRAS* WT tumors. Results of the trial indicated that for patients with *KRAS* WT tumors, the addition of panitumumab to irinotecan improved PFS (HR 0.78, 95 % CI 0.64–0.95, $P = 0.015$), however, panitumumab did not increase OS (HR 1.01, 95 % CI 0.83–1.23; $P = 0.91$) in comparison to irinotecan alone. The authors concluded that with the aid of more directed molecular markers, further improvements in patinet selection might increase significance in results with anti-EGFR therapy [419].

4.7.6 1st Line Therapy for KRAS WT mCRC

The Panitumumab Randomized Trial in Combination With Chemotherapy for Metastatic Colorectal Cancer to Determine Efficacy (PRIME) was designed to evaluate the efficacy and safety of panitumumab plus FOLFOX-4 versus FOLFOX-4 alone as initial treatment for mCRC. Published in 2010, this phase III trial demonstrated that panitumumab-FOLFOX4 was well tolerated and significantly improved PFS (9.6 vs. 8.0 months, HR 0.8; $P = 0.02$) in patients with *KRAS* WT tumors. For this group, there was also a trend toward improved median OS (23.9 vs. 19.7 months, HR 0.83; $P = 0.072$), and ORR (55 vs. 48 %, OR 1.35; $P = 0.068$). Importantly, combination therapy with panitumumab-FOLFOX4 appeared to have a detrimental effect on patients with *KRAS* mutant tumors, with shortened PFS (7.3 vs. 8.8 months, HR 1.29; $P = 0.02$), and a trend toward decreased OS (15.5 vs. 19.3 months, HR 1.24; $P = 0.068$) in comparison to FOLFOX-4 alone [420]. These results highlight the importance of determining KRAS status in patients with mCRC prior to making decisions regarding initiation of targeted therapy such as panitumumab.

EGFR

4.8 EGFR Expression Does not Predict Response to Anti-eGFR Therapy

EGFR expression was an inclusion criteria for many of the trials with anti-EGFR therapy. Accordingly, EGFR positivity is mentioned as a requirement in the of FDA approvals for both cetuximab and panitumumab. However, subset analysis in the BOND-1 trial showed that the extent of EGFR staining was unrelated to cetuximab response [411]. Similarly, phase II trials and case series indicate that patients with EGFR- tumors can have a significant response to anti-EGFR therapy [421, 422]. Still, some patients with EGFR- tumors may face difficulties with obtaining reimbursement for anti-EGFR therapy from insurance companies [423]. As such, NCCN has been careful to recommend against EGFR testing, and excluding patients from therapy on the basis of EGFR status [393].

4.9 EGFR Associated Rash May Be Indicative of Response to Therapy

Skin reactions are the most commonly recognized adverse effect in patients treated with anti-EGFR therapy. It is thought that skin breakdown is a result of decreased function of EGFR in performing its typical role in maintenance of the skin barrier, and therefore may be reflective of the efficacy of anti-EGFR therapy. Indeed, several trials have found skin reactions to be associated with increased response rate and this was confirmed in a systematic review and meta-analysis [411, 424].

4.10 KRAS Exon 2 Mutations Predict a Lack of Response to Anti-EGFR Therapy…KRAS Mutations Outside of Exon 2 as Well as NRAS Mutations Likely Predict a Similar Lack of Response

Only 10–20 % of unselected CRC tumors respond to anti-EGFR therapy [425, 426]. Given that activating *KRAS* mutations occur downstream to EGFR in the RAS-MAPK pathway, it is logical to conclude that anti-EGFR therapy might not be capable of effecting tumors with this genetic characteristic [399]. *KRAS* gene, encodes a small GTPase transductor protein. KRAS is an important signaling protein in the MAPK pathway, which regulates cell division and differentiation.

Activating mutations occur at *KRAS* exon 2 (codons 12 and 13) in approximately 40 % of CRC [425–427]. Indeed, multiple studies have confirmed a lack of efficacy with anti-EGFR therapy among patients with tumors possessing mutations in these locations [418, 425–432]. Accordingly, in July 2009, the FDA changed labeling requirements for cetuximab and panitumumab to exclude use in tumors with *KRAS* exon 2 (codon 12 and 13) mutations.

More recently, investigators are increasingly interested in the implications for anti-EGFR response among mutations in the *KRAS* gene, at locations other than exon 2, as well as other RAS family mutations. These 'extended *RAS*' or 'expanded *RAS*' mutations include *KRAS* and *NRAS* codons 12 and 13 (exon 2), 59 and 61 (exon 3), and 117 and 146 (exon 4) [433]. Sorich et al. [434] found that approximately 20 % of *KRAS* exon 2 WT tumors had an 'expanded *RAS* mutation', representing an additional 11 % of all CRC, which may not benefit form anti-EGFR therapy. Retrospective analyses of the PRIME trial data indicate that mutations in *KRAS*, other than those involving exon 2, and mutations in *NRAS* may predict a lack of response to EGFR targeted therapy [435]. Similarly, a meta-analysis of randomized controlled trials evaluated the relationship of other *RAS* mutations (*KRAS* exons 3 and 4, and NRAS exons 2, 3 and 4) with likelihood to respond to anti-EGFR therapy and found that tumor responses in patients with these 'expanded *RAS* mutations' were improbable [434]. Interestingly, patients with p.G13D mutations appear to be responsive to cetuximab, a fact that highlights the importance of continued efforts to improve patient selection for anti-EGFR therapies [436].

In 2013 the Evaluation of Genomic Applications in, Practice Prevention (EGAPP) recommended *KRAS* testing (without remark about testing outside of codons 12 and 13), but found insufficient evidence to support *NRAS* testing. Currently, the FDA approved test for using cetuximab or panitumumab assesses *KRAS* mutations on codons 12 and 13 only [437]. However, the NCCN guidelines suggest testing for any *KRAS* or *NRAS* mutation in all patients with stage IV CRC, stating that patients with these mutations should not be treated with anti-EGFR therapies [393].

4.11 BRAF Mutations are a Marker of Poor Prognosis But Data Regarding Their Capacity to Predict Response to Anti-EGFR Therapy is Limited

BRAF is a signaling molecule that functions immediately downstream to KRAS. *BRAF* mutations are known to occur in cancers that commonly exhibit *RAS* mutations, such as CRC. Similar to *KRAS*, mutations causing constitutive activation of *BRAF* results in stimulation of the RAS-MAPK pathway [81, 438]. Of note, mutations in *BRAF* and *KRAS* have been found to be mutually exclusive, suggesting both that the RAS-MAPK pathway is central in carcinogenesis of these tumors and that stimulation of the pathway can be accomplished by activating mutations at various levels of the pathway, but need not be more than one. Additionally, it implies that excluding patients with *BRAF* mutations might further refine patient selection for anti-EGFR therapy [81, 439, 440]. However, in contrast to clinical data with regard to *KRAS*, evidence concerning the efficacy of anti-EGFR therapy in *BRAF* mutated CRC is conflicting.

BRAF mutations occur in 7–14 % of CRCs [155, 430, 441–443]. Several studies have indicated a lack of response to anti-EGFR therapy in mCRC among patients with tumors that harbor mutant *BRAF* [441, 443]. However, the smaller number of *BRAF* mutated tumors has often prevented definitive conclusions regarding the ability of *BRAF* status to predict response to anti-EGFR therapy. Alternatively, data regarding *BRAF* as a prognostic marker are more certain, as patients with this mutation have been consistently shown to have poor outcomes in relation to those with WT *BRAF* [401, 413, 435, 442, 444, 445].

Guidelines put forth by the EGAPP Working Group in 2013 state that the lack of conclusive evidence pertaining to the utility of *BRAF* outside of prognostication left them unable to support or refute testing for *BRAF*. Instead they encouraged further studies to clarify its role in predicting response to anti-EGFR therapy [446]. In contrast, current NCCN recommendations include testing of tumors for *BRAF* mutations in stage IV disease, though this is of prognostic value only since lack of sufficient evidence precludes specific recommendations with regard to how this information should be used in guiding treatment choices [393]. Still, testing in these circumstances may yield additional benefit to patients in terms of identifying those who might be eligible to participate in trials with BRAF inhibitors, which is likely to be of more clinical utility than determining their response to EGFR inhibitors [433].

Genotyping can be performed on either primary tumor tissue or metastases, though the degree of concordance between *BRAF* WT tumors and their metastases is significantly higher than that of *BRAF* mutant primaries and their metastases since *BRAF* mutant primaries frequently have *BRAF* WT metastases [393, 447, 448]. These studies are limited due to the infrequency of *BRAF* mutations, however, since *BRAF* WT may indicate a benefit with anti-EGFR therapy, and the possible discordance of BRAF status at the primary site, it may be of benefit to sample tumor at metastatic site.

4.12 PI3KCA and PTEN Have an Undefined Influence on Response to EGFR Therapy

In addition to activating the MAPK pathway, EGFR activates the PI3-AKT pathway, which plays a central in cell survival and invasion. *PI3KCA* mutations can be found in approximately 15 % of CRC [440]. Though in vitro studies have determined that cell lines with mutations in *PI3KCA* and *PTEN* show significant resistance to cetuximab in comparison to WT, this has been more difficult to assess in clinical conditions [449]. In a multicenter retrospective analysis of 1022 tumor samples, De Roock et al. [440] found a differential response of *PI3KCA* exon 9 and 20 mutations, with exon 9 mutants showing no impact on response, but exon 20 mutations showing a lack of response to anti-EGFR therapy. In a much smaller study, *PI3KCA* mutation was significantly associated with a lack of response to EGFR targeted therapy [450]. On other hand, some studies show no predictive effect of *PI3KCA* [445, 451].

Most studies investigating *PTEN* evaluate expression based on IHC rather that genetic mutation. Perhaps the best study, by Laurent-Puig et al. [452, 453] suggested *PTEN* may be related to worse survival, but response to anti-EGFR therapy was not determined.

Similar to their stance on *BRAF*, the EGAPP concluded there is insufficient evidence to recommend for or against testing for mutations in *PI3KCA* or *PTEN* [446]. The NCCN does not mention either *PI3KCA* or *PTEN* in their recommendations for genetic testing.

4.12.1 Vascular Endothelial Growth Factor (VEGF)

VEGF is a signaling protein that promotes angiogenesis via binding to VEGF R-1 and VEGF R-2. This angiogenic stimulus plays a central role in tumorigenesis [454]. One of the major mechanisms by which it accomplishes this is by causing increased vascular permeability. This not only results in decreased drug delivery but also causes increased interstitial hypoxia then signals further increases in VEGF levels [455, 456]. In comparison to adjacent normal tissues, gastrointestinal adenocarcinomas were shown to have increased levels of VEGF expression, providing the rationale for testing the efficacy of VEGF inhibition in the treatment of CRC [457].

Bevacizumab

Bevacizumab is recombinant humanized monoclonal antibody to VEGF-A. Its anti-neoplastic effects result from inhibition of angiogenesis, regression of tumor-associated neovascualture, normalization of blood flow allowing more effective delivery of chemotherapy, and direct effects on tumor cells [458].

1st Line Treatment for mCRC

Bevacizumab was first FDA approved for use in CRC in 2004 after Hurwitz et al. showed a survival advantage with the addition of bevacizumab to irinotecan, fluorouracil, and leucovorin (IFL) in patients with previously untreated mCRC. In this trial (AVF2107) Patients treated with IFL plus bevacizumab had improved

median OS (20.3 vs. 15.6 months, HR 0.66; $P < 0.001$) in comparison to IFL alone. Median PFS (10.6 vs. 6.2 months, HR 0.54; $P < 0.001$) and ORR (44.8 vs. 34.8 %; $P = 0.004$) were also improved in the combination group in comparison to IFL [459]. These results were met with enthusiasm and bevacizumab was approved for use in as first line in combination with IFL for the treatment of mCRC.

Second-Line Treatment of mCRC in Combination with FOLFOX

The FDA extended indications for use of bevacizumab to include second line therapy for mCRC in 2006, after Giantonio et al. showed a survival advantage with the addition of bevacizumab to FOLFOX-4 in patients with previously treated mCRC. The ECOG E3200 trial consisted of three treatment groups: FOLFOX-4 and bevacizumab combined FOLFOX-4 only, bevacizumab only. Median OS in the three groups were 12.9 months for FOLFOX-4 and bevacizumab, 10.8 months for FOLFOX-4 (HR 0.75; $P = 0.0011$) and 10.2 months for bevacizumab. Median PFS was 7.3 versus 4.7 versus 2.7 months, respectively (HR 0.61; $P < 0.0001$ for FOLFOX-4 with bevacizumab vs. FOLFOX-4). ORR were 22.7, 8.6 and 3.3 % ($P < 0.0001$ for FOLFOX-4 with bevacizumab vs. FOLFOX-4). Grade 3 or 4 vomiting, hypertension and bleeding were more common with the addition of bevacizumab [460]. Thus, despite more frequent adverse events, bevacizumab in combination with FOLFOX-4 showed a clear survival advantage in comparison to FOLFOX-4 alone, while bevacizumab used as a single agent displayed little efficacy.

Second-Line mCRC in Combination with Fluoropyrimidine-Based Therapy (After Failure of 1st Line Therapy with a Regimen Containing Bevacizumab)

In 2013 the FDA approved bevacizumab for use in combination with fluoropyrimidine-irinotecan or fluoropyrimidine-oxaliplatin for patients with mCRC and disease progression on first-line treatment with a bevacizumab-containing regimen. The trial randomized patients who had progressed on first-line therapy with a bevacizumab-containing regimen to chemotherapy with or without bevacizumab. The chemotherapy was switched to either irinotecan- or oxaliplatin-based fluoropyrimidine based on the failed first-line regimen. Patients who received bevacizumab in addition to chemotherapy had significantly improved overall survival in comparison to chemotherapy alone (11.2 vs. 9.8 months HR 0.81, 95 % CI 0.69–0.94; $P = 0.0062$). The authors concluded that maintaining angiogenesis inhibition beyond the time of disease progression, while switching chemotherapy, is a beneficial strategy in patients with mCRC [461]. This study emphasizes the difference in resistance patterns between traditional cytotoxic chemotherapy and VEGF inhibition.

No Benefit in Addition to Adjuvant for Stage II or III CRC

In 2011 Allegra et al. published the results of NSABP protocol C-08, assessing the efficacy of 1 year of bevacizumab in addition to 6 months of mFOLFOX6 as adjuvant for stages II and III carcinoma of the colon. The trial showed a lack of benefit with the addition of bevacizumab to mFOLFOX6. DFS with mFOLFOX6 plus bevacizumab was similar to mFOLFOX6 alone (HR, 0.89; 95 % CI, 0.76–

1.04; $P = 0.15$). They observed a transient significant effect of bevacizumab during the first 15 months of the study, but this effect was not seen after that time point. The investigators hypothesized that the temporary benefit seen with bevacizumab during the first 15 months was perhaps due to a cytostatic effect that occurs only during drug exposure [462]. A follow-up study at five years confirmed these findings and revealed no difference in median OS (HR, 0.95; 95 % CI, 0.79–1.13; $P = 0.56$). The group concluded that they were unable to recommend bevacizumab [463].

A second trial, the AVANT trial (BO17920), reported similar findings despite limiting their study population to high-risk stage II and stage III CRC. Also, in addition to testing FOLFOX-4 and FOLFOX-4 plus bevacizumab, they included a third treatment regimen, XELOX plus bevacizumab. There was no improvement for DFS for either the FOLFOX-4 plus bevacizumab, or the XELOX plus bevacizumab treatment group in comparison to FOLFOX-4 (HR 1.17, 95 % CI 0.98–1.39; $P = 0.07$, and HR 1.07, 95 % CI 0.90–1.28; $P = 0.44$, respectively). Similarly, OS was not improved (HR 1.27, 95 % CI 1.03–1.57; $P = 0.02$, and HR 1.15, 95 % CI 0.93–1.42; $P = 0.21$, respectively). The investigators concluded that given these results, bevacizumab should not be recommended for inclusion in treatment regimens for stage II and III CRC [464].

With two trials demonstrating a lack of efficacy and one of them suggesting a possible detrimental effect of bevacizumab in stage II and III CRC, the NCCN currently recommends against the use of bevacizumab as adjuvant therapy [393].

4.13 VEGF

There are currently no biomarkers that predict response to bevacizumab inCRC. Circulating factors such as ANG2, VEGF, PlGF, sVEGFR1, IL-6, and circulating endothelial cells have all been associated with response to bevacizumab and as have DNA polymorphisms in VEGF and the VEGF pathway [465–470].

Due to its anti-angiogenic properties there are several areas for increased alert with use of bevacizumab. Meta-analyses have shown that bevacizumab significantly increases the risk morbidity and treatment related deaths, which is largely associated with hypertension, bleeding, neutropenia and gastrointestinal perforation [471, 472]. In particular, there may be reason to exercise special caution with the use of rectal stents in patients receiving bevacizumab, as the risk of perforation in these circumstances appears to be particularly high. A retrospective review showed a 19.6-fold increase in the risk of perforation with stenting among patients treated with bevacizumab [473]. This number is likely an overestimate since a meta-analysis revealed an overall 7.4 % risk of perforation with stenting, which was increased to 12.5 % among patient treated with bevacizumab and chemotherapy, but unaffected by chemotherapy alone [474]. Still, both studies indicate that increased caution and perhaps avoidance of use of stents in patients on bevacizumab may be prudent. Additionally, in a pooled analysis of five RCTs, bevacizumab was associated with arterial thromboembolism [475]. Accordingly, in 2013 the FDA updated its safety label warning to include arterial thromboembolic

events and in 2014 additional warnings consisting of hemorrhage, gastrointestinal perforation, fistulae, venous thrombotic events, and posterior reversible encephalopathy (PRES) were incorporated [476].

Bevacizumab should be held for 4–6 weeks prior to elective surgery, and may be resumed 28 days after surgery [393, 476].

Ziv-Aflibercept

Ziv-Aflibercept is a recombinant fusion protein with the binding portions of VEGFR1 and VEGFR2 fused to Fc portion of human IgG1. It therefore inhibits VEGF by binding its ligands and preventing them from binding and activating their endogenous receptors.

4.14 Second-Line in Treatment of mCRC in Combination with FOLFIRI

In 2012 ziv-aflibercept was FDA approved for use in combination with FOLFIRI for the treatment of mCRC that is resistant to or has progressed despite treatment with an oxaliplatin-containing regimen.

FDA approval of ziv-aflibercept came in response to a phase III study, in which Van Cutsem et al. investigated the effect of adding ziv-aflibercept to FOLFIRI in patients who hade mCRC that was previously treated with an oxaliplatin. Prior therapy with bevacizumab was permitted and was found to have no association with treatment response to ziv-aflibercept. The addition of ziv-aflibercept to FOLFIRI improved median OS in comparison to FOLFIRI alone (13.5 vs. 12.06 months, HR 0.817, 95 % CI, 0.713–0.937; $P = 0.0032$). Similar improvement was seen in PFS (HR, 0.758; 95 % CI, 0.661–0.869; $P = 0.0001$) and ORR (19.8 vs. 11.1 %; $P = 0.0001$) [477]. A follow-up of this study reported continued safety and durability of response at a median follow-up of 22.3 months [478]. The side effect profile of ziv-aflibercept is similar to that of bevacizumab.

A toxic combination: cetuximab/panitumumab and bevacizumab

The CAIRO2 and PACCE trials were phase III studies published in 2009 in which cetuximab was added to chemotherapy (oxaiplatin-based and oxaliplatin- or irinitecan-based, respectively) and bevacizumab to explore the benefit of cetuximab in addition to standard first-line therapy for mCRC. Both trials showed the addition of cetuximab resulted in significantly decreased PFS and increased frequency of adverse effects [429, 432]. Therefore, use of either cetuximab or panitumumab in combination with bevacizumab is strongly discouraged [393].

In KRAS WT tumors, cetuximab and bevacizumab may both be used as 1st line or treatment of mCRC

Two trials have investigated, whether bevacizumab or cetuximab produces superior results in combination with standard chemotherapy as first-line therapy for mCRC; FIRE-3 and CALGB/SWOG 80,405 [479, 480]

FIRE-3 is a phase III study that investigated the benefit of addition of either cetuximab or bevacizumab to FOLFIRI as first line-treatment for *KRAS* exon 2 WT mCRC. PFS (10.0 vs. 10.3 months, respectively; HR 1.06, 95 % CI 0.88–1.26, $P = 0.55$) and OS (28.7 vs. 25.0 months, respectively; HR 0.77, 95 % CI 0.62–0.96; $P = 0.017$) were similar between groups. The authors proposed that despite statistically comparable results there is a trend toward increased survival with cetuximab [479].

CALGB/SWOG 80405, a similar study, was a phase III trail examining the effect of the addition of either cetuximab or bevacizumab to chemotherapy as first-line therapy for *KRAS* exon 2 WT mCRC. Chemotherapy was per provider preference, and consisted of either mFOLFOX6 or FOLFIRI, though significantly more patients received mFOLFOX6 (73.4 %). OS was similar for the bevacizumab/chemotherapy versus cetuximab/chemotherapy groups (29.0 vs. 29.9 months, HR = 0.92, 95 % CI 0.78 1.09; $P = 0.34$). PFS was comparable as well, 10.8 months for the bevacizumab group versus 10.5 months for the cetuximab group [480].

Results from these trials indicate that either cetuximab or bevacizumab can be used to obtain similar outcomes in patients with *KRAS* exon 2 WT mCRC. However, two caveats are worth mentioning. First, the trend toward significance for improved OS with cetuximab in the FIRE-3 trial could become clinically significant with better patient selection by excluding patients with tumor characteristics (i.e., patients with 'extended RAS mutations', or BRAF mutations) that have demonstrated a lack of response to anti-EGFR therapy. Second, in light of MRC COIN trial results suggesting a lack of efficacy of oxaliplatin-based therapy in combination with cetuximab, the preponderance of patients treated with oxaliplatin-based therapies in the CALGB/SWOG 80405 study makes it difficult to accept these results with certainty [427]. Still, until further data regarding this topic are available, cetuximab and bevacizumab in combination with chemotherapy can be considered equivalent for first-line treatment of *KRAS* exon 2 WT mCRC [393].

Regorafenib

Regorafenib is an oral multikinase inhibitor that targets kinases involved in angiogenesis (VEGFR1/VEGFR2/VEGFR3, PDGFR-β, FGFR-1) and oncogenesis (KIT, RET, BRAF) [136].

4.15 Last Line of Defense in mCRC

Regorafenib was FDA approved in 2012 after Grothey et al. published results of the regorafenib monotherapy for previously treated mCRC (CORRECT) trial. The trial was designed to assess the efficacy of regorafenib in patients with mCRC in whom all other standard therapies had been exhausted (fluoropyrimidine, oxaliplatin, irinotecan, and bevacizumab, as well as cetuximab or panitumumab in patients with *KRAS* WT tumors). Patients were randomized to receive regorafenib or placebo. Regorafenib improved median OS (6.4 vs. 5.0 months, HR 0.77; 95 % CI 0.64–

0.94; $P = 0.0052$). PFS was also improved (1.9 vs. 1.7 months, HR 0.049, 95 % CI 0.42–0.58; $P < 0.0001$). Grade 3 and 4 adverse reactions occurred more commonly in the regorafenib group and consisted of skin reaction, fatigue, diarrhea, and hypertension. Dose reduction or interruption resulted in successful management of these symptoms [481].

4.16 Management

The role of genetics and targeted therapies in the treatment of CRC are expanding (Tables 13 and 14). Currently, MSI, CIN, and CIMP are recognized as distinct, though occasionally overlapping, genetic pathways implicated in the pathogenesis of CRC. These pathways appear to effect both prognosis and response to therapies. Tumor genotype may hold similar value in terms of prognostication and ability to predict a response to therapy. Still, further studies are needed to confirm and define these roles, and at this time only MSI and KRAS/NRAS are recommended for use in clinical decision-making. MSI status is used to make decisions regarding the

Table 13 Genetics in colorectal cancer

Tumor marker	When to test	How to test	Indications and implications	Theoretical implications
MSI/DNA MMR	All CRC Or All CRC <70 yo and CRC ≥70 if Bethesda criteria	Start with either IHC or MSI testing	Screening for LS Indicates improved prognosis [355, 376, 381] May aid decision to proceed with chemotherapy in stage II CRC	May indicate lack of benefit from 5-FU
CIN	Retrospective studies or clinical trials	Test for loss of heterozygosity	Indicates worse prognosis [309]	May indicate multidrug resistance [323]
CIMP	Retrospective studies or clinical trials	Test for markers of DNA methylation	Indicates worse prognosis [482]	Closely associated with MSI Unclear implications for response to 5-FU
EGFR	Not recommended	Usually tested by IHC	Not useful in predicting response to anti-EGFR therapy	
KRAS exon 2	Stage IV CRC		Predicts lack of response to anti-EGFR therapy	

(continued)

Table 13 (continued)

Tumor marker	When to test	How to test	Indications and implications	Theoretical implications
		Test for genetic mutation	Independent predictor of poor prognosis [483]	
KRAS other	Stage IV CRC	Test for genetic mutation	Predicts lack of response to anti-EGFR therapy	
NRAS	Stage IV CRC	Test for genetic mutation	Predicts lack of response to anti-EGFR therapy	
BRAF	Stage IV CRC	Test for genetic mutation	Indicative of poor prognosis, may indicate lack of response to anti-EGFR therapy	

need for chemotherapy in early stage CRC and wild-type KRAS status is required to benefit from anti-EGFR therapy in mCRC.

4.17 Multigene Assays

Multigene assays, including Oncotype DX, ColoPrint and ColDx may be used for risk stratification and predicting recurrence. However, therapeutic utility of these assays in terms of guiding indications or choices of chemotherapy remains to be determined. As such, there are no recommendations for the use of these tests [393].

5 Conclusion

Technologic advances and increasing access to technology have led to a rapid development in our understanding of cellular processes. With the introduction of imatinib, we have seen a tremendous potential for targeted therapy. Yet, the challenges we face in overcoming imatinib resistance, regardless of its efficacy, highlight the need for improved understanding of biologic systems.

In the last decades, we have developed powerful investigational tools and acquired the capacity to produce and collect tremendous amounts of data. Still, we are limited in our abilities to fully assess complex biologic interactions and also to translate these findings into clinically applicable endeavors. Improved collaboration and consensus for standardizing techniques and nomenclature, as well as attention to novel strategies for sharing, interpreting, and navigating complex data systems will be critical for continued progress in developing personalized strategies for cancer care.

Table 14 Targeted therapies for CRC

Therapy		What is it?	When do you use it and what is the evidence?				Biomarkers	To note…
			Stage II/III	Stage IV				
				1st line	2nd line	3rd line		
EGFR	Cetuximab	Chimeric human-murine monoclonal antibody to EGFR		2012 FDA approved KRAS WT (CRYSTAL trial) [413]		2004 FDA approved for patients progressing on, or intolerant of irinotecan-based therapy. (BOND-1 trial) [411] 2007 FDA approved for resistance or progression on all standard therapies. (NCIC CO17) [412]	EGFR expression is not associated with response to therapy [411, 421, 422] and should not be tested [393] KRAS, NRAS and BRAF should be tested in all stage IV patients. Patients with KRAS or NRAS mutations should not be treated with anti-EGFR therapy [393] Patients with BRAF mutations have poor prognosis and an undetermined response to EGFR therapy [393]	Cetuximab may not add benefit when combined with oxaliplatin-based chemotherapy in 1st line for mCRC [427, 430] Skin reactions are common and may be associated with response to therapy [411, 424] FDA warnings for cardiopulmonary arrest, dermatologic toxicity, hypomagnesemia and other electrolyte abnormalities [484]
	Panitumumab	Humanized monoclonal antibody to EGFR		Not FDA approved, but NCCN supports use. KRAS WT. (PRIME trial) [420]		2006 FDA approved monotherapy for resistance or progression on all standard therapies [417]		

(continued)

Table 14 (continued)

Therapy		What is it?	When do you use it and what is the evidence?				Biomarkers	To note…
			Stage II/III	Stage IV				
				1st line	2nd line	3rd line		
VEGF	Bevacizumab	Humanized recombinant monoclonal antibody to VEGF-A	Recommend against this. (NSABP C-08) [462, 463] and (AVANT trial) [464]	2004 FDA approved (AVF2107 trial) [459]	2006 FDA approved in combination with FOLFOX (ECOG E3200 trial) [459] 2013 FDA approved in combination with fluoropyrimidine–irinotecan- or fluoropyrimidine–oxaliplatin-based chemotherapy for patients with progression on first-line treatment with a bevacizumab-containing regimen. (ML18147 trial) [461]		No biomarkers though many are under investigation [465–470]	FDA warnings for arterial thromboembolic events, hemorrhage, gastrointestinal perforation, wound healing/surgical complications, fistulae, venous thrombotic events and posterior reversible encephalopathy (PRES) [476] Recommend stopping therapy 4–6 weeks prior to surgery and waiting 28 days to resume therapy after surgery [393, 476]
	Ziv-Aflibercept	Recombinant fusion protein with the binding portions of VEGF 1 and VEGF 2 fused to Fc portion of human IgG1			2012 FDA approved in combination with FOLFIRI for resistance or progression on oxaliplatin containing regimen [478]		No biomarkers	FDA warnings for hemorrhage, gastrointestinal perforation and compromised wound healing [485]

(continued)

Table 14 (continued)

Therapy		What is it?	When do you use it and what is the evidence?				Biomarkers	To note…
			Stage II/III	Stage IV				
				1st line	2nd line	3rd line		
Multikinase	Regorafenib	Multikinase inhibitor that targets kinases involved in angiogenesis (VEGFR1/VEGFR2/VEGFR3, PDGFR-β, FGFR-1) and oncogenesis (KIT, RET, BRAF) [136]				FDA approved monotherapy for resistance or progression on all standard therapies (CORRECT trial) [481]	No biomarkers	FDA warnings for severe, occasionally fatal hepatotoxicity [137]
Combination	Cetuximab/ panitumumab + Bevacizumab		Recommend against this combination in any circumstance. (CAIRO2 and PACCE trials) [429, 432]					

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