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Pathologic Manifestations of Gastrointestinal and Hepatobiliary Injury in Immune Checkpoint Inhibitor Therapy

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Tumor cells survive by numerous mechanisms that include modulation of the tumor immune microenvironment. Immune checkpoint inhibitors (CPIs), including cytotoxic T-lymphocyte–associated protein 4 (CTLA-4) inhibitors and the programmed death receptor-1 (PD-1)/programmed death ligand-1 (PD-L1) inhibitors, are being increasingly used for treating many advanced malignancies. However, CPI therapy is also associated with gastrointestinal and hepatobiliary adverse effects.

Objectives.—To review the adverse effects of CPI therapy on the gastrointestinal tract and hepatobiliary system. To describe histopathologic patterns and discuss differential diagnostic considerations in the diagnosis of CPI injuries.

Data Sources.—Published peer-reviewed literature in the English language and personal experience in the diagnosis of CPI injuries.

Conclusions.—The pathologic manifestations of CPI therapy–induced gastrointestinal and hepatobiliary injury are broad. The patterns of esophageal CPI injury include lymphocytic inflammation and ulcerative esophagitis, while those of gastric injury include chronic active gastritis, lymphocytic gastritis, focal enhancing gastritis, and periglandular inflammation. The duodenal injury may present as duodenitis with villous blunting and granulomas. We also noticed active colitis, microscopic colitis, chronic active colitis, increased apoptosis, ischemic colitis, and nonspecific inflammatory reactive changes in colonic injuries. The reported histologic features of hepatobiliary injuries are panlobular hepatitis, centrilobular necrosis, portal inflammation with bile duct injury, steatosis, nodular regenerative hyperplasia, and secondary sclerosing cholangitis. In summary, we here discuss the pathologic features and differential diagnosis of CPI therapy–induced gastrointestinal and hepatobiliary injury. Recognition of CPI injury is important to determine the proper management that often includes cessation of CPI therapy; and administration of steroids or other immunosuppressive agents, based on severity of injury.

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esophageal mucosa is more resilient to CPI therapy–induced injury, compared to columnar lined mucosa in other parts of the gastrointestinal tract. Endoscopic abnormalities reported in CPI esophageal injury include erythema, ulceration, edema, and stenosis. Histopathologically, the patterns of CPI esophageal injury include lymphocytic inflammation and ulcerative esophagitis.

**Lymphocytic Inflammation.**—Esophageal injury with anti–PD-1 therapy has been reported to link to increased lymphocytic inflammation of the squamous mucosa (Figure 1, A). The biopsies showed marked increase in intraepithelial lymphocytes in the squamous epithelium with or without apoptotic keratinocytes. The infiltrating lymphocytes were predominantly CD3+ T lymphocytes with only rare CD20+ lymphocytes. Patients may present dysphagia at 6 months after nivolumab therapy. The dysphagia may significantly improve after steroids treatment. Esophageal stenosis may also develop after many doses (during a 6-month period) of nivolumab therapy. Patients may show symptomatic improvement with steroids treatment, but may experience symptomatic recurrence during steroids taper. Subsequent treatment with the interleukin 6 receptor neutralizing antibody tocilizumab could result in excellent clinical response.

**Ulcerative Esophagitis.**—Esophageal injury was also reported to include ulcerative esophagitis with anti–PD-1 therapy. In a case report, one patient was treated with 14 cycles of pembrolizumab (every 3 weeks) before presentation of ulcerative esophagitis. The patient’s dysphagia improved, and follow-up endoscopy showed partial mucosal healing after cessation of pembrolizumab and administration of steroids. Patient may present with severe mucocutaneous inflammation mimicking Stevens-Johnson syndrome with skin, mouth, esophagus, uvea, and glans involvement after 4 cycles of pembrolizumab (every 3 weeks) therapy. These adverse events could resolve after discontinuation of pembrolizumab, administration of steroids, infliximab, plasmapheresis, and intravenous immunoglobulins.

**Differential Diagnosis.**—The esophageal lymphocytic predominant inflammation (intraepithelial lymphocytic infiltrate) mimics lymphocytic esophagitis. The inflammation...

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**Figure 1.** Patterns of immune checkpoint inhibitor injury to the esophagus, stomach, and duodenum. A, Esophagitis with lymphocytic-predominant inflammation. B, Chronic active gastritis: increased lamina propria lymphoplasmacytic and neutrophilic infiltrates, neutrophilic gland abscess, and intraepithelial lymphocytosis. C, Lymphocytic gastritis: increased intraepithelial lymphocytes without significant increase of neutrophils or plasma cells. D, Duodenitis with villous blunting mimicking celiac disease: severe villous blunting, surface foveolar metaplasia, cryptitis, and reactive crypt hyperplasia (hematoxylin-eosin, original magnifications ×200 [A through C] and ×100 [D]).
in lymphocytic esophagitis is described as prominence of peripapillary intraepithelial lymphocytes accompanied by spongiosis without significant eosinophils or neutrophils. Lymphocytic esophagitis is a histologic pattern that can be seen in association with a variety of conditions including autoimmune/connective tissue diseases, inflammatory bowel disease, esophageal motility disorder, reflux esophagitis, and others. The differential diagnosis for esophageal ulceration includes severe reflux esophagitis, radiation, infections including viral and fungal, and pill/drug/toxin-related esophagitis. Reflux esophagitis on endoscopy shows more severe abnormalities in the distal esophagus than in the proximal esophagus. Changes in the squamous mucosa associated with reflux esophagitis on histology include mixed inflammation (containing lymphocytes, neutrophils, and few eosinophils), as opposed to predominantly lymphocytic inflammation in CPI injury. Detection of fungi can be assisted by periodic acid–Schiff and Grocott methenamine silver special stains or culture in case of high clinical suspicion. Viral infections cause esophagitis and ulceration as well, especially in immunosuppressed cancer patients. Identification of viral cytopathic effect on histopathology and immunohistochemical staining for herpes simplex virus (HSV) or cytomegalovirus (CMV) can help the diagnosis of HSV or CMV esophagitis. Pill esophagitis may show refractile foreign material on histologic examination.

**Stomach**

Upper gastrointestinal symptoms such as abdominal pain, nausea, and vomiting are relatively common for patients receiving CPI treatment. However, histopathologic patterns of upper gastrointestinal tract (stomach and duodenum) CPI injury have not been well characterized until recently. The most common abnormality reported on endoscopy is erythema followed by erosions. Other findings like granularity, sloughing, exudates, ulcer, atrophy, and rarely, severe hemorrhagic gastritis, have been reported. The endoscopic findings did not always correspond and rarely, severe hemorrhagic gastritis, have been reported on endoscopy. Reflux esophagitis on histology is erythema followed by erosions. Other findings include mixed inflammation (containing lymphocytes, neutrophils, and few eosinophils), as opposed to predominantly lymphocytic inflammation in CPI injury. Detection of fungi can be assisted by periodic acid–Schiff and Grocott methenamine silver special stains or culture in case of high clinical suspicion. Viral infections cause esophagitis and ulceration as well, especially in immunosuppressed cancer patients.

**Chronic Active Gastritis.**—Chronic active gastritis is the most common pattern of CPI therapy–induced gastric injury, characterized by increased lamina propria lymphoplasmacytic infiltrate and increased intraepithelial neutrophils with or without neutrophilic gland abscess (Figure 1B). There is no site predilection as it can involve both gastric body and antrum. This pathologic pattern of gastritis resembles chronic active gastritis in *Helicobacter pylori* infection. The disease activity varies from severe to mild or moderate. Although apoptosis can be seen, most of the cases do not demonstrate prominent increase of apoptosis. Concurrent increase of intraepithelial lymphocytes (intraepithelial lymphocytosis) in the foveolar as well as glandular epithelium is seen in nearly 80% of CPI chronic active gastritis cases. The number of intraepithelial lymphocytes can range from 19 to 55 per 100 epithelial cells that are predominantly CD8 T cells. Features of autoimmune gastritis such as endocrine cell hyperplasia and intestinal metaplasia are not seen in CPI-induced chronic active gastritis.

**Lymphocytic Gastritis.**—Different from concurrent intraepithelial lymphocytosis in chronic active gastritis, lymphocytic gastritis (Figure 1, C) without accompanying active gastritis has been reported. One patient with advanced melanoma and recurrent lung metastases was reported with this pattern of injury after 1 month of anti–PD-1 pembrolizumab therapy. Gastric biopsy showed lymphocytic gastritis that was not present on a gastric biopsy 2 months before the initiation of therapy. The intraepithelial lymphocytes were found to be predominantly CD8 T lymphocytes.

**Focal Enhancing Gastritis and Periglandular Inflammation.**—Focal enhancing gastritis in CPI-induced gastric injury is characterized by small collections of lymphocytes and histiocytes around a small group of actively inflamed gastric foveolae or glands mimicking gastric involvement by Crohn disease. This pattern of gastritis can involve both gastric body and antrum. Interestingly, one of the patients with focal enhancing gastritis was found to be homozygous for the autophagy-related 16-like protein (*ATG16L1*) Crohn disease–associated gene variant (rs2241880) without a clinical history of inflammatory bowel disease. It is unclear whether patients with genetic polymorphisms associated with increased risk of inflammatory bowel disease are more likely to develop severe gastrointestinal injury when receiving CPI therapy.

CPI-induced gastric injury can present as periglandular inflammation and was reported in about 40% of gastric biopsies from patients who received CPI therapy. This pattern of gastritis is characterized by inflammation in the gastric pit/isthmus/neck region composed of predominantly lymphocytes. Concurrent nonnecrotizing granulomas without relation to ruptured gastric glands can also be seen in this pattern of gastric injury. Granulomatous inflammation is uncommon in CPI therapy–induced gastric injury.

**Differential Diagnosis.**—The differential diagnosis includes *H pylori* or other infectious gastritis, lymphocytic gastritis, inflammatory bowel disease (especially Crohn disease) with gastric involvement, and granulomatous gastritis. The chronic active gastritis pattern may resemble *H pylori* gastritis. The chronic active gastritis pattern of CPI injury can be differentiated by less severe lamina propria lymphoplasmacytic infiltrate, fewer lymphoid aggregates, frequent intraepithelial lymphocytosis, with or without prominent apoptosis in comparison with *H pylori* gastritis. Of note, *H pylori* gastritis may show intraepithelial lymphocytosis (lymphocytic gastritis), but it is often focal, as opposed to the diffuse involvement of mucosa with increased intraepithelial lymphocytes in CPI chronic active gastritis. Furthermore, our unpublished data demonstrate that there are fewer lamina propria CD20 B lymphocytes and higher number of intraepithelial CD8 T lymphocytes in CPI chronic active gastritis than in *H pylori* gastritis. It is noteworthy to mention that prior empirical treatment for *H pylori* and antibiotic use for other infectious conditions may change the histologic appearance of gastritis and result in lack of detection of *H pylori*, thereby causing difficulty to favor one diagnosis over another. Lymphocytic gastritis is an entity that shows increased intraepithelial lymphocytes. Increase of intraepithelial lymphocytes can be associated with celiac disease, *H pylori* gastritis, nonsteroidal anti-inflammatory drugs, and microscopic colitis. Although typical lymphocytic gastritis has been reported, CPI–induced gastric injury with intraepithelial lymphocytosis is...
often seen in a background of chronic active gastritis, unlike the typical lymphocytic gastritis, which is rarely associated with marked neutrophilic inflammation. Viral infections such as CMV and Epstein-Barr virus (EBV) can be associated with intraepithelial lymphocytosis and/or chronic active gastritis. Additional testing including CMV immunohistochemistry or Epstein-Barr encoding region (EBER) in situ hybridization can assist in the diagnosis. Finally, CPI focal enhancing gastritis or periglandular inflammation, especially when there is concurrent granuloma, may mimic granulomatous gastritis seen in infections, sarcoidosis, or inflammatory bowel disease (Crohn disease). Recognition of the histopathologic patterns, and correlation with time course of patient symptoms post CPI therapy can help the pathologists to make a diagnosis of CPI-induced gastritis, if other causes of gastritis can be excluded.

**Duodenum and Small Bowel**

CPI injury of the duodenum and small bowel has been described in small case series and occasional case reports. The most frequent endoscopic abnormality was erythematous mucosa. Other occasional findings reported were erosions, flattening, stricture, exudate, and ulcers. Endoscopic findings did not always correspond with histologic findings. Histologic abnormalities were detected in 9 of 17 patients with mucosal erythema (53%), and in 5 of 13 patients with normal mucosa (23%) on endoscopy. The pathologic patterns of duodenal injury include duodenitis with villous blunting and granulomas.

**Duodenitis With Villous Blunting.**—Villous blunting was noted to be the most common feature of CPI injury of the duodenum and ileum. The degree of villous blunting can be mild, moderate, or severe, and is almost always accompanied by neutrophilic villitis (activity). This pattern of duodenal injury may or may not show increased epithelial apoptosis. Patchy increase of intraepithelial lymphocytes may be seen. There is expansion of the lamina propria with lymphoplasmacytic infiltrate with or without eosinophils (Figure 1, D). Lymphoid aggregates in the lamina propria, reactive crypt hyperplasia, and foveolar metaplasia of the duodenal surface epithelium may be noted.

**Granulomas.**—Nonnecrotizing granulomas with or without villous blunting can be seen in 10% to 18% of duodenal biopsies in patients who received CPI therapy. One study revealed that patients with granulomas in the duodenum had granulomas in the stomach as well, but all these patients did not have systemic granulomatous disease such as sarcoidosis, or infectious disease.

**Differential Diagnosis.**—The main differential diagnosis includes celiac disease or other drug-induced duodenal injury. Although the presence of antibodies such as tissue transglutaminase antibody on serologic testing supports the diagnosis of celiac disease, results of serologic testing may not be available when interpreting the duodenal biopsies to evaluate CPI-induced injury. The most striking morphologic difference in CPI-induced duodenal injury is the presence of activity (neutrophilic infiltrates and/or erosions) as opposed to celiac disease that rarely shows acute inflammation/activity. There was no significant difference in other morphologic features, such as degree of lamina propria inflammation, intraepithelial lymphocytosis, presence of lymphoid aggregates, crypt hyperplasia, foveolar metaplasia, or apoptosis, between celiac disease and CPI therapy duodenal injury. Clinically, the response of CPI-induced injury to treatment (cessation of CPI therapy with or without administration of steroids) is often rapid and excellent. On the contrary, celiac disease shows a slow and gradual response to gluten-free diet. Drugs other than CPI agents, such as olmesartan, can be associated with an increase in intraepithelial lymphocytes and moderate to marked villous blunting. A clinical history of hypertension treated with olmesartan may help in the differentiation from CPI injury. Autoimmune enteropathy can cause intraepithelial lymphocytosis with apoptosis and villous blunting. However, autoimmune enteropathy can be distinguished by absence of goblet cells and/or Paneth cells. Serologic testing for antibodies, such as anti-enterocyte brush border antibodies, and appropriate clinical context aid in the diagnosis.

It is noteworthy to mention that CPI therapy has been reported to induce “real” celiac disease in patients receiving anti–CTLA-4 ipilimumab therapy, or ipilimumab and anti–PD-1 (nivolumab) combination therapy. These 2 reported cases both responded well to gluten-free diet. It is unclear whether the CPI therapy exacerbated previously existing celiac disease or induced “de novo” celiac disease. The immunomodulatory effect of CPI therapy on upregulation of T-cell response and inhibition of CTLA-4/PD-1 pathway may lead to the manifestation of celiac disease.

**Colon**

The most common presenting symptom of patients with CPI therapy–induced colonic injury is diarrhea. The PD-1/PD-L1 inhibitors have fewer adverse effects and later median onset time of diarrhea/colitis than anti–CTLA-4 agents. The median onset time of diarrhea/colitis with anti–PD-1/PD-L1 agents is around 3 to 6 months, as opposed to at around 6 to 8 weeks with anti–CTLA-4 agents. Common endoscopy findings include erythema, loss of vascular pattern, granularity, ulcerations, and even normal mucosa. As endoscopic normal mucosa can show histopathologic features of CPI therapy injury, biopsies are recommended for the diagnosis when CPI therapy injury is clinically suspected. CPI injury is often a pancolitis, but involvement of descending colon only can also be seen. Severe colitis cases with perforation have been reported in both anti–CTLA-4 and anti–PD-1/PD-L1 treatments. CPI therapy–induced colonic injury can sometimes coexist with upper gastrointestinal tract injury. The CPI therapy–induced colonic injury can present in a number of histopathologic patterns including active colitis, microscopic colitis (lymphocytic and collagenous), chronic active colitis, increased apoptosis, ischemic colitis, and nonspecific inflammatory reactive changes. There can be an overlap between these patterns.

**Active Colitis.**—Active colitis is the most common histopathologic pattern of CPI-induced injury to the colon. In this pattern of colitis there is neutrophilic infiltrate of the lamina propria, neutrophilic cryptitis, and crypt abscess (Figure 2, A), with or without crypt atrophy or crypt dropout. Increased apoptosis and intraepithelial lymphocytes may or may not be present. The atrophic crypts often show attenuated crypt epithelium with intraluminal apoptotic debris admixed with inflammatory cells, similar to mycophenolate-induced colitis.

**Microscopic Colitis (Lymphocytic and Collagenous Colitis).**—In the lymphocytic colitis pattern of injury there are increased intraepithelial lymphocytes along with an increase in lymphoplasmacytic infiltrate in the lamina propria, without significant acute/active inflammation (Figure 2, B). Epithelial changes like attenuation of the
epithelium, mucin depletion, and sometimes crypt epithelial apoptosis can be present. Collagenous colitis is an uncommon pattern of CPI injury. In the collagenous colitis pattern of CPI injury there is thickened subepithelial collagen along with an increase of intraepithelial lymphocytes (Figure 2, C). A review of 15 patients with CPI therapy–induced microscopic colitis (13 lymphocytic colitis and 2 collagenous colitis) showed that patients had more aggressive disease course, requiring more treatments with oral or intravenous steroids and/or nonsteroidal immunosuppressive agents, and higher rate of hospitalization than with microscopic colitis not related to CPI therapy.

**Chronic Active Colitis.**—Chronic active colitis, a histologic pattern of inflammatory bowel disease (IBD) has been reported in CPI therapy–induced colonic injury, especially in cases with recurrent CPI treatment. In this pattern, there is increased lamina propria lymphoplasmacytic infiltrate and basal plasmacytosis, often accompanied by active inflammation in the form of cryptitis/crypt abscess. Chronic changes like crypt architectural distortion and pseudopyloric or Paneth cell metaplasia are often present (Figure 2, D). A study reported that 2 of 5 patients who initially presented with active colitis developed features of chronicity including basal plasmacytosis, mononuclear expansion of the lamina propria, crypt architectural irregularity, and Paneth cell metaplasia after recurrent CPI (anti–PD-1) therapy. Chronic active colitis can also be the first pathologic manifestation in CPI-induced injury. Three cases of nivolumab-induced colitis exhibited features similar to ulcerative colitis on both endoscopy and histopathology at initial diagnosis. In these 3 cases, treatment similar to that for ulcerative colitis was successful. Accordingly, CPI therapy–induced colitis has been proposed as a peculiar form of IBD. A recent study retrospectively reviewed 53 patients with CPI (either anti–CTLA-4 or anti–PD-1) therapy–induced colitis and found that 60% (32 of 53) of the cases had chronic inflammation (basal lymphocytic infiltrate, crypt architectural distortion, and Paneth cell metaplasia). This reported percentage is significantly higher than the previously reported.

**Increased Apoptosis.**—In this pattern of injury there is an isolated increase of crypt epithelial apoptosis without any active or chronic inflammation (Figure 2, E), mimicking graft versus host disease. Increased crypt epithelial apoptosis is often noted in association with other patterns of CPI colonic injury including active colitis, lymphocytic colitis, and chronic active colitis.

**Ischemic Colitis.**—Rarely, CPI therapy can cause changes similar to that of ischemic colitis. In this pattern of injury, the colonic mucosa shows withered crypts, reactive epithelial changes, and lamina propria fibrosis. A study reported ischemic colitis pattern in 3 of 17 patients who received anti–PD-1 therapy.

**Non-specific Inflammatory Reactive Changes.**—Besides the above–described specific patterns of CPI-induced injury, colonic mucosa may present with nonspecific inflammatory reactive changes. This pattern of injury shows increased lamina propria lymphoplasmacytic infiltrate accompanied by reactive epithelial changes, attenuation of the epithelium, and mucin depletion. Intraepithelial lymphocytes or apoptosis may be slightly increased but insufficient to support a diagnosis of lymphocytic colitis pattern or increased apoptosis pattern of CPI-induced injury (Figure 2, F). Features of active colitis or chronic active colitis are not present.

**Differential Diagnosis.**—The differential diagnosis of CPI-induced colonic injury is broad and includes infections, injury by other drugs, idiopathic microscopic colitis, graft versus host disease, and IBD. A high index of suspicion should be kept in mind when patients receiving CPI therapy develop acute onset of diarrhea. CPI-induced active colitis may coexist with infection. The possibility of infectious etiology can be ruled out by stool tests for bacterial infection, Clostridium difficile, CMV or other viruses, ova and parasites. Patients with advanced malignancies and receiving CPI therapy are often immunosuppressed. Hence, it is important to exclude viral infections by evaluation of viral cytopathic effects (eg, CMV inclusions) and immunohistochemical stains (eg, CMV) when a diagnosis of CPI-induced colitis is considered, particularly in case of active colitis. Although they are different from anti–PD-1/PD-L1 therapies, drugs such as mycophenolate mofetil and idelalisib (a phosphoinositide 3-kinase d inhibitor) disrupt/modulate immune homeostasis, resulting in diarrhea and colitis. The histopathologic changes of injury by these drugs are morphologically similar to that of CPI-induced colitis.

In the context of microscopic colitis (lymphocytic and collagenous colitis) pattern, possibilities of preexisting microscopic colitis and microscopic colitis secondary to infection or other drugs (histamine H2 receptor blockers, proton pump inhibitors, gold salts, statins) should be considered. The review of clinical history for prior gastrointestinal symptoms, medication use, and prior endoscopy and pathology findings may help to distinguish CPI-induced microscopic colitis from other causes or preexisting microscopic colitis.

As described earlier, increased colonic crypt epithelial apoptosis is a common feature in CPI colitis, although the apoptosis-only pattern exists. The presence of increased colonic apoptosis is reminiscent of other colitides including acute graft versus host disease, mycophenolate mofetil colitis, idelalisib colitis, and autoimmune enteropathy. These can be distinguished by history of transplant, drug therapy, a temporal association between the initiation of CPI drugs and the onset of diarrhea, and appropriate clinical context.

The chronic active colitis pattern of CPI colitis can mimic IBD, especially ulcerative colitis. Although most of the chronic active colitis pattern is due to recurrent CPI treatment, initial presentation as inflammatory bowel disease can also occur. The changes of basal plasmacytosis, cryptitis, crypt abscess, crypt architectural distortion, and pseudopyloric or Paneth cell metaplasia are common features in both CPI-induced chronic active colitis and IBD and can cause diagnostic dilemmas if history of CPI therapy is not available. Features like increase in intraepithelial lymphocytes, increase in apoptosis, and crypt atrophy are commonly seen in CPI colitis but generally not in IBD, which can be useful to distinguish between the 2 entities. CPI-induced chronic active colitis can occur many months after the CPI therapy is stopped; this is important to keep in mind to prevent a misdiagnosis of IBD. Owing to the immune dysregulation effects of CPI therapy, patients with autoimmune diseases and IBD were largely excluded from the CPI clinical trials. A recent study revealed that both anti–CTLA-4 and anti–PD-1/PD-L1 therapies for patients with preexisting IBD were overall safe, but patients with preexisting IBD had a higher risk of severe colonic adverse events, such as high grades of diarrhea and colitis, high rates of colonic perforation, and frequent recurrence, than did. Arch Pathol Lab Med Immune Checkpoint Inhibitor Therapy GI and Hepatobiliary Injury—Patil & Zhang 5
Figure 2. Patterns of immune checkpoint inhibitor injury to the colon. A, Active colitis: neutrophilic infiltrate in lamina propria, neutrophilic cryptitis, crypt abscess, and crypt atrophy. B, Lymphocytic colitis: increased intraepithelial lymphocytes, increased chronic inflammation in the lamina propria, epithelial attenuation, and mucin depletion. C, Collagenous colitis: thickened subepithelial collagen. D, Chronic active colitis: increased chronic inflammation in the lamina propria, basal plasmacytosis, cryptitis, crypt abscess, and crypt architectural distortion. E, Increased apoptosis: increased apoptosis without significant increase in inflammation of the lamina propria or epithelium. F, Nonspecific inflammatory reactive changes: slightly increased lymphocytes and plasma cells in the lamina propria, slightly increased intraepithelial lymphocytes, reactive epithelial changes, and mucin depletion (hematoxylin-eosin, original magnification ×200 [A through F]).
patients without preexisting IBD. The distinction between IBD flare/recurrence and CPI-induced chronic active colitis is difficult, and clinical correlation is required for optimal management.

**PATTERNS OF INJURY TO THE LIVER AND BILIARY TRACT**

CPI therapy–induced liver injury is reported in about 2% to 10% patients with CPI therapy. The injury occurs between approximately 1 to 3 months with anti–PD-1/PD-L1 agents, and between about 3 to 9 weeks with anti–CTLA-4 agents after initiation of therapy. The injury may vary with the type of CPI therapy agents used. A systematic review and meta-analysis of published data showed that CTLA-4 inhibitors (ipilimumab and tremelimumab) had higher risk of all-grade and high-grade hepatotoxicity than PD-1 inhibitors. Interestingly, liver immune-related adverse events were found to be associated with a reduced risk of cancer progression. As the CPI-induced liver injury often manifests as asymptomatic elevations in liver enzymes, it is essential to monitor liver function tests at baseline and periodically in the posttreatment period. The abnormality pattern of elevation of liver enzymes can be hepatocellular (with elevation of transaminases), cholestatic (with elevation of alkaline phosphatase and/or γ-glutamyl transpeptidase), or mixed cholestatic and hepatocellular. The severity of hepatitis is graded by degree of elevation of liver enzymes, and the grade determines further management. When the diagnosis of CPI-induced hepatobiliary injury is considered, it is important to exclude other causes like viral infections, other concurrent drug use–induced liver injury, autoimmune hepatitis, and primary or secondary biliary diseases. These can be excluded by serologic work up, history of medication use, imaging, and biopsy. The histopathologic patterns of CPI-induced hepatobiliary injury include panlobular hepatitis, portal inflammation with bile duct injury, granulomas, steatosis or steatohepatitis, nodular regenerative hyperplasia, and secondary sclerosing cholangitis.

**Liver**

**Panlobular Hepatitis.**—Panlobular hepatitis is the most commonly observed pattern of CPI therapy–induced liver injury. This pattern injury often presents with elevation of the liver enzymes aspartate aminotransferase (AST) and/or alanine aminotransferase (ALT), with proportionately lower hyperbilirubinemia. On histopathology this pattern is characterized by panlobular hepatitis with proportionately milder portal inflammation (Figure 3, A). The lobular inflammation is composed of predominantly lymphocytes and histiocytes, and there may be a few scattered plasma cells, neutrophils, and eosinophils. The histiocytic infiltrates are mostly located in the sinusoids. Two patterns of liver injury, granulomas and non-granulomatous hepatocellular, are described in occasional cases of CPI-induced liver injury. There is no significant infiltrate of plasma cells. Sometimes focal aggregates of eosinophils or occasional aggregates of macrophages without formation of granulomas may be seen. Another feature that can be present is central vein endotheliitis with centrilobular necrosis and lymphocytic infiltrates (Figure 3, B), which is reported especially in patients with anti–CTLA-4 therapy. Rarely, panlobular inflammation may be accompanied by multiple microabscesses.

**Portal Inflammation With Bile Duct Injury.**—This pattern of injury presents with a cholestatic pattern of elevation of liver enzymes with greater elevation of alkaline phosphatase and/or γ-glutamyl transpeptidase. The histopathologic findings of this pattern of injury include portal inflammation that is predominantly composed of lymphocytes with occasional eosinophils, neutrophils, and plasma cells (Figure 3, C). There is varying degree of bile duct injury in the form of cholangitis with increased intraepithelial lymphocytes, bile ductular proliferation, ductopenia, and rarely, vanishing bile duct syndrome. Recently a study of 10 CPI (anti–PD-1 pembrolizumab or anti–PD-L1 atezolizumab)–induced liver injury cases demonstrated that the portal-based inflammation and lymphocytic cholangitis resembled primary biliary cholangitis in one of these cases. Furthermore, mixed portal inflammation with portal vein endothelitis and bile duct injury mimicking acute cellular rejection has been reported in one patient treated with anti–PD-1 pembrolizumab for stage IV melanoma. Mixed canalicular and hepatocellular cholestasis may occur. Concurrent panlobular hepatitis and portal hepatitis with bile duct injury has also been reported in patients with CPI therapy.

**Granulomas.**—Granulomas can be seen in some cases of panlobular hepatitis pattern of CPI therapy injury, wherein the histiocytes in the lobular inflammation can form microgranuloma-like aggregates in addition to sinusoidal histiocytic infiltrates. Fibrin ring granulomas are more commonly seen in anti–CTLA-4 therapy or anti–PD-1 and anti–CTLA-4 combination therapy.

**Steatosis and Steatohepatitis.**—Steatosis has been reported in few case series in CPI therapy liver injury, which is indistinguishable from nonalcoholic steatosis or steatohepatitis. Cases with steatosis or steatohepatitis may represent deterioration of the underlying fatty liver disease or CPI-induced liver injury. There are insufficient data so far and more studies are required to distinguish between the 2 entities. Nonetheless, cessation of CPI (ipilimumab anti–CTLA-4 or anti–PD-1) therapy improved the abnormal liver enzyme profile in these patients. It is unclear if preexisting fatty liver disease is associated with an increased risk of liver injury from CPI therapy.

**Nodular Regenerative Hyperplasia.**—Nodular regenerative hyperplasia (NRH) belongs to the category of noncirrhotic portal hypertension, characterized by abnormal architecture in the form of nodular transformation of hepatic parenchyma. The exact mechanism of NRH is unknown but it seems to be related to endothelial injury of the hepatic microvasculature. Use of medications, such as immunosuppressant and chemotherapeutic drugs, and immunological disorders have been associated with the pathogenesis of NRH. Nodular regenerative hyperplasia is a rare pattern of CPI-induced liver injury. NRH with severe portal hypertension has been reported in 2 patients with melanoma who were treated with anti–PD-1 pembrolizumab. One of these 2 cases also had concurrent panlobular hepatitis. The other of these 2 cases was managed with transjugular intrahepatic portosystemic shunt and withdrawal of CPI therapy; the mean portosystemic pressure gradient was improved at 2.5 months follow-up. Immune dysregulation by pembrolizumab may have contributed to the development of NRH.
Differential Diagnosis.—The main histopathologic differential diagnosis of panlobular hepatitis includes autoimmune hepatitis (AIH), drug-induced liver injury (DILI) by other concurrent drugs, acute viral hepatitis, and granulomatous hepatitis. AIH and DILI are often considered clinically in patients receiving CPI therapy who develop abnormal liver enzyme profiles. AIH and DILI show an almost similar histopathologic picture as that of CPI-induced liver injury in the form of panlobular hepatitis or centrilobular necrosis with or without confluent/bridging necrosis. A recent study systematically compared histopathologic features of CPI hepatitis, AIH, and DILI. AIH often shows portal inflammation with interface activity and abundant plasma cells, as opposed to CPI-induced hepatitis that does not have a significant proportion of plasma cells. Features like hepatocyte rosettes and confluent necrosis are noted in AIH, but are rare or not seen in CPI-induced hepatitis. Furthermore, AIH can be excluded by lack of elevation of immunoglobulin G (IgG) and autoimmune hepatitis–related autoantibodies. DILI is associated with a greater proportion of eosinophilic infiltrate and confluent necrosis than CPI hepatitis. DILI can also show bile plugs that are not commonly noted in CPI-induced hepatitis. Viral hepatitis—such as hepatitis A, B, C, D, E; EBV; CMV; and HSV—can be further investigated by immunohistochemistry, in situ hybridization, viral serologies, and appropriate clinical context.

In case of CPI-induced hepatitis with granulomas, the consideration is to exclude infectious etiology. In this regard, clinical context, special stains, and testing for infectious agents using serology, culture, and polymerase chain reaction may help. The differential diagnosis of fibrin ring granulomas when observed during evaluation for CPI-induced liver injury includes certain infections like Q fever, viral infections like EBV and CMV, toxoplasmosis, and...
leishmaniasis. Diseases like lupus, and other drug reactions, may also be associated with fibrin ring granulomas. Microgranulomas are a nonspecific reaction to liver injury and are often the type of granulomas noted in CPI-induced hepatitis.

The differential diagnosis of CPI-induced portal inflammation with bile duct injury is broad. The considerations include DILI, primary biliary cholangitis, graft versus host disease, and extrahepatic biliary obstruction.

**Biliary Tract**

**Secondary Sclerosing Cholangitis.**—CPI therapy can cause injury to the biliary tract in the form of sclerosing cholangitis similar to primary sclerosing cholangitis on histopathology and radiology. Patients with this pattern of injury often present with abnormal liver function tests with either a mixed cholestasis and hepatocellular pattern of elevation of liver enzymes or a predominantly cholestatic pattern. This pattern of injury can cause diffuse damage to extrahepatic large bile duct or intrahepatic small bile duct, and can even involve the gallbladder. On histopathology, the extrahepatic or intrahepatic bile duct wall shows fibrosis and infiltrate of predominantly CD8+ T cells, and an increase of intraepithelial lymphocytes (Figure 3, D). Some portal tracts may reveal reactive bile ductular proliferation. The lobular parenchyma may reveal changes of hepatocellular and/or canalicular cholestasis. This injury may respond to immunosuppression; however, the response is slower than the response of CPI therapy–induced liver injury.

**Differential Diagnosis.**—Secondary sclerosing cholangitis should be differentiated from primary sclerosing cholangitis, extrahepatic biliary obstruction, and IgG4 cholangitis. Biliary obstruction can be further investigated by imaging. Appropriate clinical context may be helpful to make the distinction of CPI-induced from DILI-associated bile duct injury and cholangitis. The inflammatory infiltrates in DILI more commonly contain eosinophils in a greater proportion as opposed to the inflammation in CPI-induced injury, which has fewer eosinophils. IgG4-related disease is often accompanied by plasma cell infiltrate in the portal tracts, and it may or may not show additional histopathologic features of phlebitis, or storiform fibrosis. Additional workup including immunohistochemistry for the IgG4 to IgG ratio, serum IgG4 levels, and appropriate clinical context is helpful to make the diagnosis.

**Management and Follow-Up**

**CPI Therapy–Induced Gastrointestinal Injury**

The guidelines/recommendations for management of CPI-induced colitis are based on grade of abnormalities of liver enzymes and/or bilirubin. For grade 1 elevation in liver enzymes (AST or ALT <3 times and/or total bilirubin <1.5 times upper limit of normal), CPI therapy can be continued with close laboratory monitoring of liver enzymes and bilirubin. In cases with grade 2 elevation in liver enzymes (AST or ALT <5 times and/or total bilirubin <3 times upper limit of normal), CPI therapy should be withheld. Additional administration of steroids is advised if required. For grades 3 (AST or ALT <20 times and/or total bilirubin <10 times upper limit of normal) to 4 (AST or ALT >20 times and/or total bilirubin >10 times upper limit of normal) elevation in liver enzymes, CPI therapy should be discontinued permanently. Administration of steroids should start immediately. If steroid refractory or no improvement after 3 days, administration of mycophenolate mofetil should be considered. Infliximab should not be used to treat CPI-induced hepato-biliary injury given the potential risk of liver failure. In cases of CPI therapy–induced secondary sclerosing cholangitis, treatment with steroids, ursodeoxycholic acid, and endoscopic intervention has been suggested.

**CPI Therapy–Induced Hepato-biliary Injury**

The guidelines/recommendations for management of CPI-induced hepatitis are based on grade of abnormalities of liver enzymes and/or bilirubin. For mild (grade 1) colitis, CPI therapy can be continued with close laboratory monitoring of liver enzymes and bilirubin. In cases with grade 2 elevation in liver enzymes (AST or ALT <5 times and/or total bilirubin <3 times upper limit of normal), CPI therapy should be discontinued permanently. Administration of steroids should start immediately. If steroid refractory or no improvement after 3 days, administration of mycophenolate mofetil should be considered. Infliximab should not be used to treat CPI-induced hepato-biliary injury given the potential risk of liver failure. In cases of CPI therapy–induced secondary sclerosing cholangitis, treatment with steroids, ursodeoxycholic acid, and endoscopic intervention has been suggested.

**Summary**

The exact mechanisms of CPI therapy–induced injury to the digestive system are still unclear. It is postulated that immune checkpoint inhibition reduces the regulation of autoreactive T cells and eventually results in immunemediated adverse effects and injury. There are some overlapping histopathologic patterns of CPI-induced injury with challenging differential diagnoses in the gastrointestinal tract and hepato-biliary system. The pathologic patterns of CPI-induced injury are summarized in the Table. Of note, these pathologic patterns are nonspecific and exclusion of other causes is essential. Recognition of histopathologic patterns of CPI-induced injury helps to form a timely and accurate pathologic diagnosis for effective patient management and appropriate therapy. The management of CPI-induced injury includes cessation of CPI therapy, or cessation of CPI therapy with administration of steroids or other immunosuppressive agents, based on severity of injury.
| Site                        | Pattern                        | Key Histopathologic Features                                                                 |
|-----------------------------|--------------------------------|---------------------------------------------------------------------------------------------|
| **Esophagus**               | Lymphocytic inflammation       | Increased intraepithelial lymphocytes                                                        |
|                             | Ulcerative esophagitis         | Ulceration and granulation tissue                                                            |
| Stomach                     | Chronic active gastritis       | Lamina propria lymphoplasmacytic infiltrate with increased intraepithelial neutrophils       |
|                             | Gland abscess ±               |                                              |
|                             | Increased apoptosis ±          |                                              |
|                             | Increased intraepithelial lymphocytes ± |                                              |
| Lymphocytic gastritis       | Increased intraepithelial lymphocytes |                                              |
| Focal enhancing gastritis   | Small collections of lymphocytes and histiocytes around glands |                                              |
| Focal periglandular inflammation | Inflammation with predominantly lymphocytes in the pit/isthmus/neck region of glands | Granulomas ± |
| **Duodenum**               | Duodenitis with villous blunting | Villous blunting                              |
|                             | Acute inflammation and villitis |                                              |
|                             | Increased apoptosis ±          |                                              |
|                             | Increased intraepithelial lymphocytes ± |                                              |
|                             | Granulomas |                                              |
|                             | Villous blunting ± |                                              |
| **Colon**                  | Active colitis                 | Lamina propria neutrophilic infiltrate                                                     |
|                             | Cryptitis and/or crypt abscess |                                              |
|                             | Crypt atrophy and crypt dropout ± |                                              |
|                             | Increased apoptosis ±          |                                              |
|                             | Increased intraepithelial lymphocytes ± |                                              |
| Microscopic colitis (lymphocytic colitis) | Increased intraepithelial lymphocytes |                                              |
|                             | Increased lamina propria lymphoplasmacytic infiltrate |                                              |
|                             | Increased apoptosis ±          |                                              |
|                             | Epithelial changes like attenuation of the epithelium and mucin depletion ± |                                              |
| Microscopic colitis (collagenous colitis) | Thickened subepithelial collagen |                                              |
| Chronic active colitis      | Lamina propria lymphoplasmacytic infiltrate |                                              |
|                             | Basal plasmacytosis            |                                              |
|                             | Neutrophilic cryptitis and/or crypt abscess |                                              |
|                             | Crypt architectural distortion |                                              |
|                             | Pseudopyloric or Paneth cell metaplasia ± |                                              |
| Increased apoptosis         | Increased crypt epithelial apoptosis without inflammation |                                              |
| Ischemic colitis            | Withered crypts, reactive epithelial changes, and lamina propria fibrosis |                                              |
| Nonspecific inflammatory reactive changes | Increased lamina propria lymphoplasmacytic infiltrate | Reactive epithelial changes, attenuation of the epithelium and mucin depletion |
|                             | No active colitis              |                                              |
|                             | No crypt architectural distortion or metaplasia |                                              |
|                             | Increased apoptosis ±          |                                              |
|                             | Increased intraepithelial lymphocytes ± |                                              |
| **Liver and biliary tract** | Panlobular hepatitis           | Panlobular inflammation                      |
|                             | Lobular disarray               |                                              |
|                             | Acidophil bodies and necrosis ± |                                              |
|                             | Centrilobular necrosis ±       |                                              |
|                             | Central vein endotheliitis ±   |                                              |
| Portal inflammation with bile duct injury | Portal inflammation | Bile duct injury  |
|                             | Bile duct injury               |                                              |
|                             | Ductular proliferation ±       |                                              |
|                             | Ductopenia ±                   |                                              |
|                             | Canalicular and/or hepatocellular cholestasis ± |                                              |
Abbreviation: ±, may or may not be present.

References
1. Havel JJ, Chouvell D, Chan TA. The evolving landscape of biomarkers for checkpoint inhibitor immunotherapy. Nat Rev Cancers. 2019;19(3):133–150.
2. Hargadon KM, Johnson CE, Williams CJ. Immune checkpoint blockade therapy: from mechanisms to management. J Clin Oncol. 2018;36(6):576–583.

Continued

| Site                      | Pattern                  | Key Histopathologic Features |
|---------------------------|--------------------------|------------------------------|
| Granulomas                |                          | Fibrin ring granulomas ±     |
| Secondary sclerosing cholangitis |                    | Granulomas or microgranulomas |
| Steatosis or steatohepatitis |                        | Bile duct fibrosis           |
| Nodular regenerative hyperplasia |                | Bile duct inflammation       |
|                           |                          | Increase in bile duct intraepithelial lymphocytes |
|                           |                          | Steatosis                    |
|                           |                          | Steatohepatitis ±            |
|                           |                          | Nodular parenchyma without cirrhosis |
|                           |                          | Portal hypertension ±        |

Abbreviation: ±, may or may not be present.

References
1. Havel JJ, Chouvell D, Chan TA. The evolving landscape of biomarkers for checkpoint inhibitor immunotherapy. Nat Rev Cancers. 2019;19(3):133–150.
2. Hargadon KM, Johnson CE, Williams CJ. Immune checkpoint blockade therapy: from mechanisms to management. J Clin Oncol. 2018;36(6):576–583.
47. Miller ED, Abu-Sbeih H, Sytskel B, et al. Clinical characteristics and adverse impact of hepatotoxicity due to immune checkpoint inhibitors. *Am J Gastroenterol*. 2020;115(2):251–261.

48. Kumar V, Chaudhary N, Garg M, Floudas CS, Soni P, Chandra AB. Current diagnosis and management of immune related adverse events (irAEs) induced by immune checkpoint inhibitor therapy. *Front Pharmacol*. 2017;8:49.

49. Tian Y, Abu-Sbeih H, Wang Y. Immune checkpoint inhibitors-induced hepatitis. *Adv Exp Med Biol*. 2018;995:159–164.

50. Wang W, Lie P, Guo M, He J. Risk of hepatotoxicity in cancer patients treated with immune checkpoint inhibitors: a systematic review and meta-analysis of published data. *Int J Cancer*. 2018;141(5):1018–1028.

51. Parlati L, Vallet-Pichard A, Batista R, et al. Incidence of grade 3-4 liver injury under immune checkpoint inhibitors: a retrospective study. *J Hepatol*. 2018;69(6):1396–1397.

52. De Martin E, Michot JM, Papouin B, et al. Characterization of liver injury induced by cancer immunotherapy using immune checkpoint inhibitors. *J Hepatol*. 2018;69(6):1181–1190.

53. Zen Y, Yeh MM. Hepatotoxicity of immune checkpoint inhibitors: a histology study of seven cases in comparison with autoimmune hepatitis and idiosyncratic drug-induced liver injury. *Mod Pathol*. 2018;31(6):965–973.

54. Zhang D, Hart J, Ding X, et al. Histologic patterns of liver injury induced by anti-PD-1 therapy. *Gastroenterol Rep*. 2020;8(1):50–55.

55. Koya Y, Shibata M, Shinohara N, et al. Secondary sclerosing cholangitis with hemobilia induced by pembrolizumab: case report and review of published work. *Hepatol Res*. 2019;49(8):950–956.

56. Kono M, Sakurai T, Okamoto K, et al. Efficacy and safety of chemotherapy following anti-PD-1 antibody therapy for gastric cancer: a case of sclerosing cholangitis. *Intern Med*. 2019;58(9):1263–1266.

57. Kleiner DE, Berman D. Pathologic changes in ipilimumab-related hepatitis in patients with metastatic melanoma. *Dig Dis Sci*. 2012;57(8):2233–2240.

58. Jhencilla M, Misraji J, Pratt DS, et al. Ipilimumab-associated hepatitis: clinicopathologic characterization in a series of 11 cases. *Am J Surg Pathol*. 2015;39(8):1075–1084.

59. Doherty GJ, Duckworth AM, Davies SE, et al. Severe steroid-resistant anti-PD1 T-cell checkpoint inhibitor-induced hepatotoxicity driven by biliary injury. *ESMO Open*. 2017;2(4):e000268.

60. Zen Y, Chen YY, Jeng YM, Tsai HW, Yeh MM. Immune-related adverse reactions in the hepatobiliary system: second-generation check-point inhibitors highlight diverse histological changes. *Histopathology*. 2020;76(3):470–480.

61. Gelsomino F, Vitale G, Ardizzoni A. A case of nivolumab-related cholangitis and literature review: how to look for the right tools for a correct diagnosis of this rare immune-related adverse event. *Invest New Drug*. 2018;36(1):144–146.

62. Avozan K, Long GV, Sinclair EC, Kench JG, McKenzie CA. Histopathology of pembrolizumab-induced hepatitis: a case report. *Pathology*. 2017;49(7):789–792.

63. Everett J, Srivastava A, Misraji J. Fibrin ring granulomas in checkpoint inhibitor-induced hepatitis. *Am J Surg Pathol*. 2017;41(1):134–137.

64. LoPiccolo J, Brener ML, Oshima K, Lipson EJ, Hamilton JP. Nodular regenerative hyperplasia associated with immune checkpoint blockade. *Hepatology*. 2018;68(6):2431–2433.

65. Hadzic N. Letter to the editor: is nodular regenerative hyperplasia an immune-mediated phenomenon? *Hepatology*. 2019;69(6):2717–2718.

66. Zen Y, Yeh MM. Checkpoint inhibitor-induced liver injury: a novel form of liver disease emerging in the era of cancer immunotherapy. *Semin Diagn Pathol*. 2019;36(6):434–440.

67. Kawakami H, Tanizaki J, Tanaka K, et al. Imaging and clinicopathological features of nivolumab-related cholangitis in patients with non-small cell lung cancer. *Invest New Drug*. 2017;35(4):529–536.

68. Majem M, Garcia-Martinez E, Martinez M, et al. SEOM clinical guideline for the management of immune-related adverse events in patients treated with immune checkpoint inhibitors (2019). *Clin Transl Oncol*. 2020;22(2):213–222.

69. Thompson JA, Schneider BJ, Brahmer J, et al. Management of immuno-therapy-related toxicities, version 1.2019. *J Natl Compr Canc Netw*. 2019;17(3):255–289.