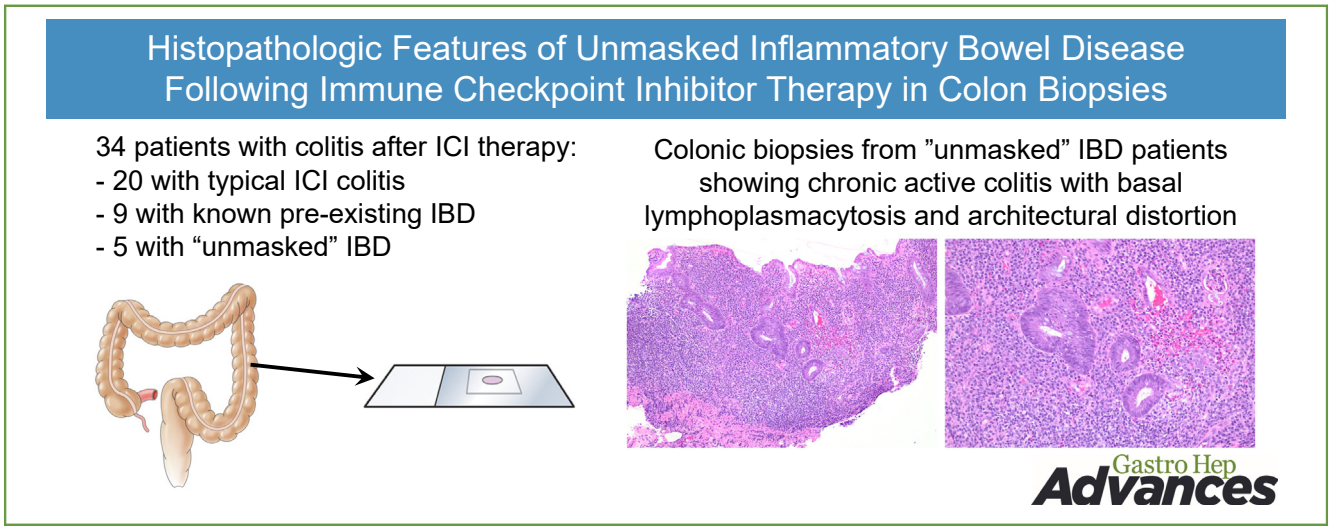
Gastro Hep Advances 2024;3:986–994

ORIGINAL RESEARCH—CLINICAL

Histopathologic Features of Unmasked Inflammatory Bowel Disease Following Immune Checkpoint Inhibitor Therapy in Colon Biopsies

M. Lisa Zhang,[1,2] Khalid Algarrahi,[1,2] Jamie DiCarlo,[3] Abigail Elvin-Ivey,[3] Michael Dougan,[2,3] and Mari Mino-Kenudson[1,2]

[1]Department of Pathology, Massachusetts General Hospital, Boston, Massachusetts; [2]Harvard Medical School, Boston, Massachusetts; and [3]Department of Gastroenterology, Massachusetts General Hospital, Boston, Massachusetts



BACKGROUND AND AIMS: Typical immune checkpoint inhibitor-induced colitis (T-ICI) has significant histomorphologic overlap with inflammatory bowel disease (IBD), a distinction further complicated in ICI-treated patients with pre-existing inflammatory bowel disease (P-IBD) and those with potentially “unmasked” inflammatory bowel disease (U-IBD) after ICI therapy. This study describes histopathologic findings seen in U-IBD colonic biopsies and assesses for distinguishing features from T-ICI and P-IBD biopsies. **METHODS:** Initial colon biopsies after symptom onset from 34 patients on ICI therapy were reviewed, and histopathologic features were tabulated. U-IBD patients were identified clinically based on rapid toxicity development post-ICI treatment with multiple recurrences after immune suppression, frequently with regional colitis (versus pancolitis). **RESULTS:** The study cohort was classified into T-ICI (n = 20), P-IBD (n = 9), and U-IBD (n = 5) groups. The predominant histological patterns were diffuse active colitis (35%) in the T-ICI, and chronic active colitis in both the P-IBD (67%) and U-IBD (60%) groups (overall $P = .003$, $P > .05$ between the two IBD groups). None of the T-ICI biopsies demonstrated chronicity features (ie, architectural distortion score 2, basal lymphoplasmacytosis, or Paneth cell metaplasia). Only U-IBD biopsies demonstrated basal lymphoplasmacytosis (60% vs 0% in T-ICI/P-IBD, $P = .002$). Among available follow-up biopsies, chronicity features were present in all (4/4) U-IBD patients, including those without chronicity seen in the initial biopsy, but none (0/7) of T-ICI patients. **CONCLUSION:** These early results show that no definite features of chronicity were seen in colon biopsies from T-ICI patients, suggesting that the presence of those features may be a clue to U-IBD in patients without a known IBD diagnosis. Frequent basal lymphoplasmacytosis seen in U-IBD may support a recent onset of mucosal injury and early architectural remodeling.

Keywords: Immunotherapy; Inflammatory Bowel Disease; Checkpoint Colitis; Unmasked IBD

Introduction

Immune checkpoint inhibitors (ICIs) targeting the programmed death receptor-1 (PD-1)/programmed death ligand-1 (PD-L1) pathway and cytotoxic T-lymphocyte-

Abbreviations used in this paper: **IBD, inflammatory bowel disease; ICI, immune checkpoint inhibitor; irAE, immune-related adverse event; P-IBD, pre-existing inflammatory bowel disease; T-ICI, typical immune checkpoint inhibitor-induced colitis; U-IBD, unmasked inflammatory bowel disease.**

Most current article

2772-5723
https://doi.org/10.1016/j.gastha.2024.05.011

associated protein-4 (CTLA-4) have been effective treatments for an expanding array of malignancies, thereby dramatically changing the cancer treatment landscape over the past decade. However, the immunomodulatory effect of ICIs can also lead to inflammatory toxicities affecting normal tissues that resemble autoimmune diseases referred to as immune-related adverse events (irAEs).[1]

While ICI-related irAEs can affect any organ system, gastrointestinal irAEs (GI-irAEs) are among the most common toxicities. As such, patients with pre-existing inflammatory bowel disease (P-IBD) were previously excluded from clinical trials and treatment with ICIs due to the risks associated with their underlying autoinflammatory disease. However, for the same reason, IBD patients are often treated with chronic immunomodulatory medications, thereby increasing their risk of malignancy and potential need for ICI therapy, particularly in situations where the anticancer benefits may outweigh potential risks. Many studies—including a recent large multicenter study[2]—have shown that patients with IBD (both ulcerative colitis and Crohn's disease), who are treated with ICI, have a significantly higher risk of experiencing more frequent (41% vs 11%) and severe GI-irAEs (ie, severe diarrhea and rare cases of colonic perforation) compared to similar patients without underlying IBD.[2–6] In addition, a recent meta-analysis found that 40% of IBD patients experienced an episode of IBD relapse following ICI treatment requiring corticosteroids and biologics, and approximately 35% of patients with IBD discontinued ICI treatment.[7] These findings indicate that IBD patients are a high-risk population that are likely to experience ICI-induced IBD flares in addition to increased GI-irAEs.

In addition, ICI-related colitis has been reported to share some similar features with IBD flares, as some cases of the former require management with additional biologic agents (eg, vedolizumab) beyond steroid/infliximab therapy.[8] Increased experience with ICI therapy in a broader patient population has led to the observation that perhaps checkpoint inhibition may trigger an initial IBD flare as a first presentation of IBD—henceforth referred to as "unmasked" inflammatory bowel disease (U-IBD)—in some patients with underlying genetic susceptibility. This phenomenon further complicates the distinction of ICI-induced colitis from IBD flares, which already have significant histopathologic overlap with one another, and a diagnosis often relies on whether the patient has a known clinical history of IBD.[9,10] Thus, in a patient without known pre-existing IBD, differentiating typical ICI-related colitis (T-ICI) from U-IBD after ICI therapy, for which morphologic features have not yet been described, may be extremely challenging.

To our knowledge, this is the first study to describe early/preliminary clinical and histopathological findings (in colon biopsies) from clinically identified U-IBD patients and systematically compare the findings from this emerging entity to those seen in T-ICI and P-IBD patients in order to assess for potential distinguishing features.

Materials and Methods

Study Population

The study was approved by the Massachusetts General Hospital (MGH) Institutional Review Board. All colon biopsies after ICI therapy from 34 patients were reviewed. Histopathologic features were tabulated, including the overall pattern of injury and semiquantitative parameters. The study cohort was classified into three groups based on clinical correlation by an expert gastroenterologist (M.D.): 1) patients with typical ICI-induced colitis (ie, pancolitis with resolution after immunosuppression and cessation of ICI therapy) and no known associated IBD (T-ICI), 2) patients with pre-existing IBD and colitis flare after ICI therapy (P-IBD), and 3) patients with potentially "unmasked" IBD after ICI therapy (U-IBD). U-IBD patients were identified clinically based on rapid toxicity development and ongoing regional colitis with at least one recurrence after appropriate immune suppression without re-exposure to ICI, as well as a need for maintenance therapy. U-IBD patients also tended to present with regional colitis rather than pancolitis, though the distribution of disease is not entirely specific for U-IBD vs T-ICI.

Histopathologic Evaluation

All colonic biopsies were evaluated and scored independently by two of the authors (K.A. and M.L.Z.); cases with discrepant findings were rereviewed and discussed under a multiheaded microscope involving another author (M.M.-K.) to arrive at a consensus. Colonic biopsies were categorized as one of the following predominant morphological patterns of injury: focal active colitis, diffuse active colitis, chronic active colitis, microscopic (lymphocytic) colitis, apoptosis only, or normal patterns.[11] Focal active colitis was defined as focal area(s) of activity (intraepithelial neutrophils ± crypt abscess) with focal/mild lamina propria lymphoplasmacytic expansion. Chronic active colitis was defined as neutrophilic activity with at least one of the following features of chronicity: 1) moderate-to-severe architectural distortion (including patterns such as deep crypt branching, sideways crypt growth, etc.), 2) unequivocal basal lymphoplasmacytosis (crypt foreshortening with a band of lymphoplasmacytic inflammation separating the base of the crypts from the muscularis mucosa), and/or 3) Paneth cell metaplasia. Diffuse active colitis was defined as multiple areas of neutrophilic activity with diffuse lamina propria lymphoplasmacytic expansion (superficially and throughout the levels of the mucosa without basal lymphoplasmacytosis) and absence of any of the above three features of chronicity. Microscopic (lymphocytic) colitis was defined by increased lamina propria chronic inflammation with significantly increased surface intraepithelial lymphocytes ± surface epithelial injury; the presence/absence of a thickened subepithelial collagen layer was also noted. The apoptosis only pattern was defined as increased crypt epithelial cell apoptoses (apoptosis score of 2 or 3, see definitions below) without other features of activity or chronicity.

Specific histopathologic features were evaluated and semiquantitatively scored (intraepithelial neutrophils, chronic inflammation, architectural distortion). Neutrophilic inflammation was scored as follows: 0-none, 1-neutrophils infiltrating into the epithelium only (mild), 2-crypt abscesses (moderate),

Table 1. Clinical Features in Initial Colonic Biopsies After ICI Therapy From T-ICI, P-IBD, and U-IBD Patients

Clinical feature	All cases	T-ICI	P-IBD	U-IBD	*P* values	*P* (U-IBD vs T-ICI)	*P* (U-IBD vs P-IBD)
Number of patients	34	20	9	5			
Demographics							
Age, mean (SD)	60.6 (12.8)	59.2 (10.6)	67.4 (11.8)	53.8 (19.0)	0.12	.39	.12
Sex, n (%)					.89	.56	1
Female	16 (47.1)	9 (45)	4 (44.4)	3 (60)			
Male	18 (52.9)	11 (55)	5 (55.6)	2 (40)			
Clinical history							
Original tumor type, n (%)							
Melanoma	21 (61.8)	16 (80)	3 (33.3)	2 (40)			
Lung adenocarcinoma	5 (14.7)	1 (5)	3 (33.3)	1 (20)			
Lung squamous cell carcinoma	1 (2.9)	0 (0)	1 (11.1)	0 (0)			
Colorectal adenocarcinoma	3 (8.8)	1 (5)	1 (11.1)	1 (20)			
Intrahepatic cholangiocarcinoma	1 (2.9)	0 (0)	0 (0)	1 (20)			
Breast invasive lobular carcinoma	1 (2.9)	1 (5)	0 (0)	0 (0)			
Renal cell carcinoma, clear cell type	1 (2.9)	0 (0)	1 (11.1)	0 (0)			
DLBCL	1 (2.9)	1 (5)	0 (0)	0 (0)			
ICI treatment, n (%)					.008	.07	.73
Ipilimumab	10 (29.4)	10 (50)	0 (0)	0 (0)			
Pembrolizumab	14 (41.2)	6 (30)	5 (55.6)	3 (60)			
Ipilimumab + pembrolizumab	5 (14.7)	2 (10)	2 (22.2)	1 (20)			
Nivolumab	2 (5.9)	0 (0)	2 (22.2)	0 (0)			
Ipilimumab + nivolumab	2 (5.9)	2 (10)	0 (0)	0 (0)			
Durvalumab	1 (2.9)	0 (0)	0 (0)	1 (20)			
Treatment duration before first biopsy (d)							
Mean (SD)	256 (286)	189 (207)	334 (417)	382 (260)	.26	.09	.82
Median	164	82	206	337	.12	.08	.30
Range (min-max)	21–1412	21–856	27–1412	67–778			

or 3-erosion/ulceration (severe). Chronic inflammation was scored as no (0), mild (1), moderate (2), or marked (3) lymphoplasmacytic expansion of the lamina propria. Architectural distortion was scored as no (0), mild (1), moderate (2), or severe (3) architectural disarray. Degree of apoptosis was scored as follows: 0-none, 1-focal (<6 apoptoses per 10 consecutive crypts), 2-increased (≥6 apoptoses in 10 consecutive crypts), or 3-extensive/marked apoptoses.[12] The presence/absence of basal lymphoplasmacytosis, Paneth cell metaplasia, intraepithelial lymphocytosis, and thickened subepithelial collagen layer were also recorded.

Statistical Analysis

Statistical analyses were performed using R version 4.2.2. Comparisons between categorical variables were made using Fisher's exact tests, and comparisons between continuous variables were made using two-sided t-tests (means) and Wilcoxon rank sum tests (medians). *P* values of < 0.05 were considered statistically significant.

Results

Clinical Features

The overall study cohort (n = 34) had a mean age of 60.6 years (standard deviation [SD]: 12.8 years), and 18 (52.9%) were male. The study cohort was classified into T-ICI (n = 20), P-IBD (n = 9), and U-IBD (n = 5) upon clinical correlation. Of the 9 P-IBD patients, 5 had ulcerative colitis (UC), 2 had Crohn's disease, and 2 had unspecified IBD. Most patients received ICI therapy for melanoma (21, 61.8%), followed by lung adenocarcinoma (5, 14.7%), colorectal adenocarcinoma (3, 8.8%), and one each of five other tumor types (Table 1).

The mean and median number of days between the last ICI treatment and the first posttreatment biopsy were 256 (SD: 286) and 164 days (range: 21–1412), respectively, with no significant differences among the three groups. Regarding the specific ICI regimen, most patients received pembrolizumab alone (14, 41.2%) or ipilimumab alone (10, 29.4%), while the remaining patients received combination ipilimumab and pembrolizumab, combination ipilimumab and nivolumab, nivolumab alone, or durvalumab alone (Table 1). There was a significant difference in treatment types among the three groups ($P = .008$), as no patients in the U-IBD or P-IBD groups received ipilimumab alone, which is standard practice for high-risk P-IBD patients.

The clinical features of the five U-IBD patients are shown in Table 2. Of note, ICI colitis typically presents as a pancolitis and resolves after immunosuppression and cessation of the offending ICI agent. However, these patients presented with an atypical regional disease distribution (usually left colon/rectosigmoid) and continued to experience symptoms despite steroid treatment, ultimately requiring maintenance therapy—essentially behaving like IBD

Table 2. Clinical Features of Five Patients With Unmasked Inflammatory Bowel Disease

Patient	Immune checkpoint inhibitor	Disease distribution at presentation	# Of Recurrences[a]	Time from initial treatment to most recent recurrence (d)	Current maintenance therapy
1	Durvalumab	Left colon	1	618	Budesonide
2	Pembrolizumab	Left colon	2	802	Budesonide, mesalamine
3	Pembrolizumab	Right and left colon	1	234	Vedolizumab
4	Ipilimumab + pembrolizumab	Left colon	1	419	Adalimumab
5	Pembrolizumab	Left colon	3	850	Adalimumab

[a]After complete resolution of initial symptoms following immunosuppression and cessation of immune checkpoint inhibitor.

patients—even years after cessation of ICI treatment. Though the distribution of disease is not entirely specific for U-IBD vs T-ICI, in our emerging experience, this is a clinical factor that has contributed to the identification of potential U-IBD patients. Comparison of the clinical and treatment course for these five U-IBD patients and the 20 T-ICI are shown in Figure 1. All patients in the T-ICI cohort demonstrated resolution of their GI-irAEs while the patients in the U-IBD cohort experienced prolonged ongoing colitis.

Histopathologic Findings in Colon Biopsies

Analysis was performed on the initial biopsies (taken during the first episode of GI-irAE) after ICI therapy for each patient (Table 3). Overall, most biopsies were obtained from a random colon site (38.2%) or the left colon (35.3% each). The distribution of biopsy locations differed between T-ICI biopsies and U-IBD biopsies (10 vs 0 random colon biopsies, $P = .041$), which follows from the pancolitis often seen with T-ICI and regional colitis typical of U-IBD. Among the initial biopsies for each patient, the predominant histological patterns were diffuse active colitis (35%) in T-ICI patients and chronic active colitis in both the P-IBD (66.7%) and U-IBD (60%) groups (overall $P = .003$) (Figure 2). In total, there were four cases of lymphocytic colitis pattern, all from T-ICI patients (Figure 2G). No biopsies demonstrated a thickened subepithelial collagen layer. There was no significant difference in histological pattern between the two IBD groups, but a significant difference between the T-ICI and U-IBD groups ($P = .016$). No differences in the degrees of neutrophilic inflammation or apoptosis were observed among the three groups. However, 100% of patients in both IBD groups had chronic inflammation scores of 1 or 2 while only 65% of patients in the T-ICI group had a chronic inflammation score of 1 (and none had a score of 2) ($P = .001$).

In contrast to the IBD groups, none of the T-ICI biopsies demonstrated features of chronicity (at least one of the following: architectural distortion score of 2–3, basal lymphoplasmacytosis, and/or Paneth cell metaplasia); only 15% showed mild architectural disarray. The U-IBD biopsies had significantly more architectural distortion compared to T-ICI biopsies ($P = .001$), but no differences were observed with regard to the presence of Paneth cell metaplasia (20% vs 0%, $P = .367$). Interestingly, only U-IBD biopsies (60%) showed unequivocal basal lymphoplasmacytosis (Figure 3), while none of the T-ICI nor P-IBD biopsies demonstrated this feature ($P < .001$). Among available follow-up biopsies, features of chronicity were present in all (4/4) U-IBD patients, including those with only diffuse active colitis seen in the initial biopsy, but none (0/7) of T-ICI patients.

Discussion

In this study, we present preliminary clinical and histopathologic descriptions from patients with clinically identified "unmasked IBD" after ICI therapy. We found that colon biopsies from U-IBD patients showed frequent basal lymphoplasmacytosis, which contrasts with the absence of this feature in P-IBD colonic biopsies as well as the lack of any definitive chronicity features seen in T-ICI biopsies. Thus, identification of basal lymphoplasmacytosis in colonic biopsies from patients with suspected GI-irAE, often in addition to at least mild architectural distortion, may suggest "unmasked" IBD in the appropriate clinical context. To our knowledge, this is the first study to describe the histologic features of the emerging "unmasked" IBD patient population and systematically compare them to T-ICI and P-IBD colon biopsies.

As ICI-related colitis is known to have significant histopathologic overlap with IBD, multiple studies have investigated the presence of chronic colitis in T-ICI biopsies. In this study, the lack of unequivocal chronicity in the T-ICI colonic biopsies is consistent with our experience in practice. However, some previous studies have reported features of chronicity in colonic biopsies from patients treated with ICIs,[11,13,14] while others have not.[15] Notably, none of these studies reported the number of cases demonstrating each specific feature of chronicity—basal lymphoplasmacytosis, crypt architectural distortion, and Paneth cell metaplasia.

Marthey et al.[13] described colonic biopsies from 27 patients with anti-CTLA-4 enterocolitis; of those, none had basal lymphoplasmacytosis and only one had "mild chronic colitis, characterized by focal crypt distortion and branching". Interestingly, of the eight patients who had follow-up colon biopsies, three reportedly showed chronic colitis.

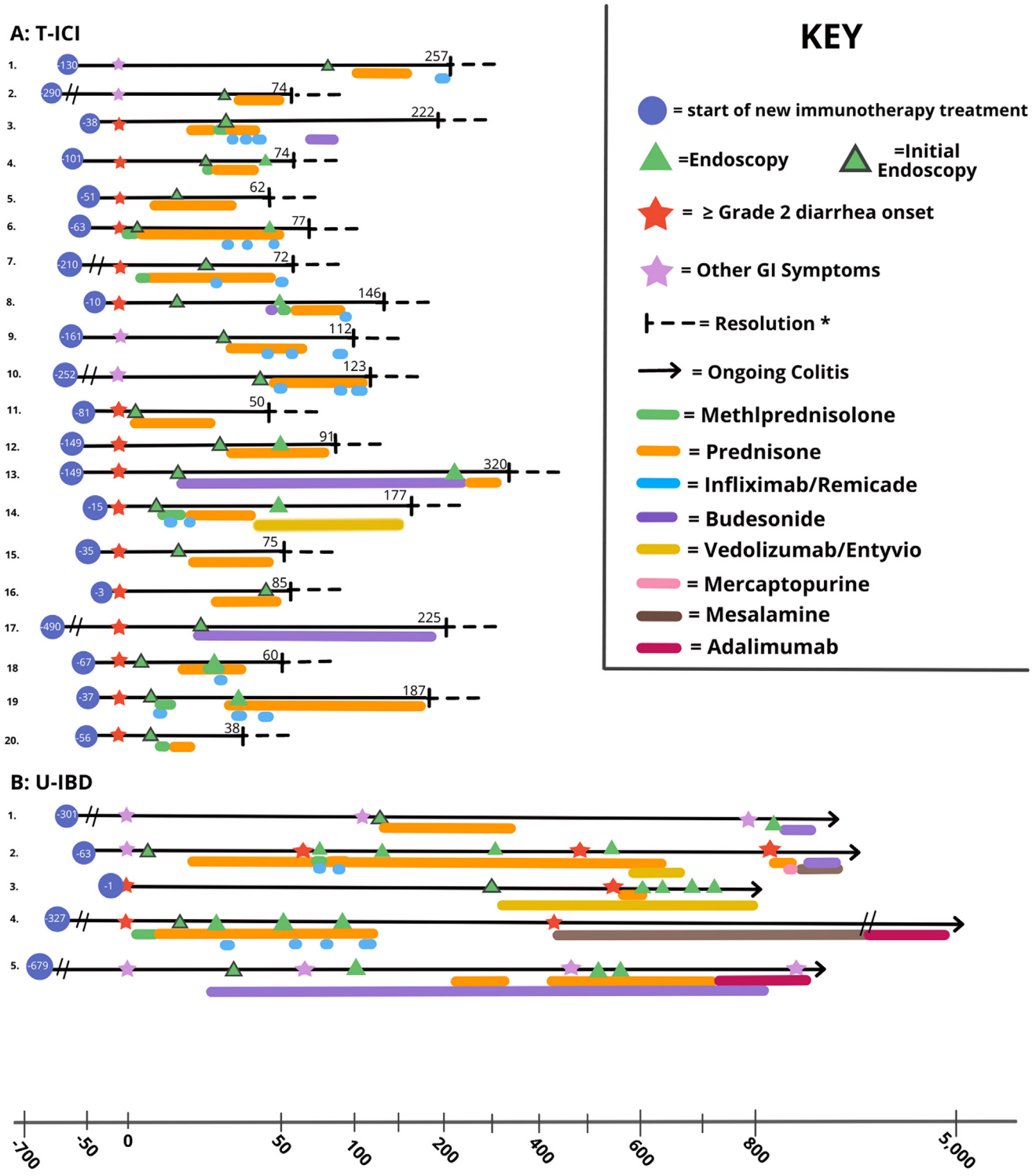


Figure 1. Treatment courses for 25 cancer patients who developed colonic mucosal inflammation after initiating immune checkpoint inhibitor (ICI) therapy. (A) Typical ICI (T-ICI) patients and (B) Unmasked IBD (U-IBD) patients. All numbers on diagram are relative to the first gastrointestinal immune-related adverse events (GI-irAE). Numbers in blue circles denote days of immunotherapy prior to ICI cessation. The first star in each treatment course indicates a patient has come off immunotherapy due to the onset of GI-irAE. Pink stars represent grade I diarrhea, blood in stool, etc. Treatments consisting of oral or IV glucocorticoids and/or monoclonal antibodies are represented by colored lines as indicated in the key. Resolution of GI-irAEs (dashed line after vertical line) for the T-ICI cohort is defined by no GI flare for three months off treatment; the number next to resolution indicates total duration of GI-irAE symptoms. For patients to be included in the U-IBD cohort, they must have had more than two periods of GI symptoms with resolution. The timeline indicates the duration in days from GI-irAE onset to marked event.

Table 3. Histopathologic Findings in Initial Colon Biopsies After ICI Therapy From T-ICI, P-IBD, and U-IBD Patients

Histological feature	All cases	T-ICI	P-IBD	U- IBD	*P* values	*P* (U-IBD vs T-ICI)	*P* (U-IBD vs P-IBD)
Number of patients	34	20	9	5			
Location of biopsies, n (%)					.16	**.04**	.66
Left colon	12 (35.3)	5 (25)	4 (44.4)	3 (60)			
Right colon	3 (8.8)	3 (15)	0 (0)	0 (0)			
Right and left colon	4 (11.8)	2 (10)	1 (11.1)	1 (20)			
Right, transverse, and left colon	2 (5.9)	0 (0)	1 (11.1)	1 (20)			
Random colon	13 (38.2)	10 (50)	3 (33.3)	0 (0)			
Histopathological features, n (%)							
Histological pattern					**.003**	**.02**	1
Chronic active colitis	9 (26.5)	0 (0)	6 (66.7)	3 (60)			
Focal active colitis	5 (14.7)	4 (20)	1 (11.1)	0 (0)			
Diffuse active colitis	11 (32.4)	7 (35)	2 (22.2)	2 (40)			
Lymphocytic colitis	4 (11.8)	4 (20)	0 (0)	0 (0)			
Apoptosis only	4 (11.8)	4 (20)	0 (0)	0 (0)			
Normal	1 (2.9)	1 (5)	0 (0)	0 (0)			
Neutrophilic inflammation					.06	.15	.60
0	9 (26.5)	9 (45)	0 (0)	0 (0)			
1	5 (14.7)	2 (10)	1 (11.1)	2 (40)			
2	14 (41.2)	6 (30)	5 (55.6)	2 (40)			
3	7 (20.6)	3 (15)	3 (33.3)	1 (20)			
Chronic inflammation					**.001**	**.009**	1
0	7 (20.6)	7 (35)	0 (0)	0 (0)			
1	20 (58.8)	13 (65)	5 (55.6)	2 (40)			
2	7 (20.6)	0 (0)	4 (44.4)	3 (60)			
3	0 (0)	0 (0)	0 (0)	0 (0)			
Architectural distortion					**<.001**	**.001**	.08
0	20 (58.8)	17 (85)	3 (33.3)	0 (0)			
1	11 (32.4)	3 (15)	3 (33.3)	5 (100)			
2	3 (8.8)	0 (0)	3 (33.3)	0 (0)			
3	0 (0)	0 (0)	0 (0)	0 (0)			
Basal lymphoplasmacytosis					**.002**	**.004**	**.03**
Yes	3 (8.8)	0 (0)	0 (0)	3 (60)			
No	31 (91.2)	20 (100)	9 (100)	2 (40)			
Paneth cell metaplasia					**.04**	.37	1
Yes	4 (11.8)	0 (0)	3 (33.3)	1 (20)			
No	30 (88.2)	20 (100)	6 (66.7)	4 (80)			
Apoptosis					.49	.25	.75
0	1 (2.9)	1 (5)	0 (0)	0 (0)			
1	9 (26.5)	7 (35)	2 (22.2)	0 (0)			
2	20 (58.8)	11 (55)	5 (55.6)	4 (80)			
3	4 (11.8)	1 (5)	2 (22.2)	1 (20)			
Intraepithelial lymphocytosis					.32	.55	N/A
Yes	4 (11.8)	4 (20)	0 (0)	0 (0)			
No	30 (88.2)	16 (80)	9 (100)	5 (100)			
Thickened subepithelial collagen layer					N/A	N/A	N/A
Yes	0	0	0	0			
No	34 (100)	20 (100)	9 (100)	5 (100)			

Bold text represents significant *P* values <.05.

Notably, the above description of "focal crypt distortion and branching" would not be considered a feature of unequivocal chronicity in our study (which was reserved for architectural distortion scores of at least 2/moderate), but rather scored as 1 (mild), which was observed in 3 (15%) of our T-ICI biopsies. Furthermore, none of follow-up colon biopsies from the T-ICI patients in our cohort (0 out of 7 patients) demonstrated features of chronicity. Chen et al.[16] described a small cohort of eight patients with anti-PD-1 colitis and found that though none of the initial colon biopsies had features of chronicity, two follow-up biopsies showed basal lymphoplasmacytosis, crypt architectural irregularity, and Paneth cell metaplasia.[16] One patient had a history of UC, and this recurrence was clinically considered a UC flare precipitated by anti-PD-1 therapy. The other patient's colitis with chronic features self-resolved and the patient remained diarrhea-free for over two years following this episode.

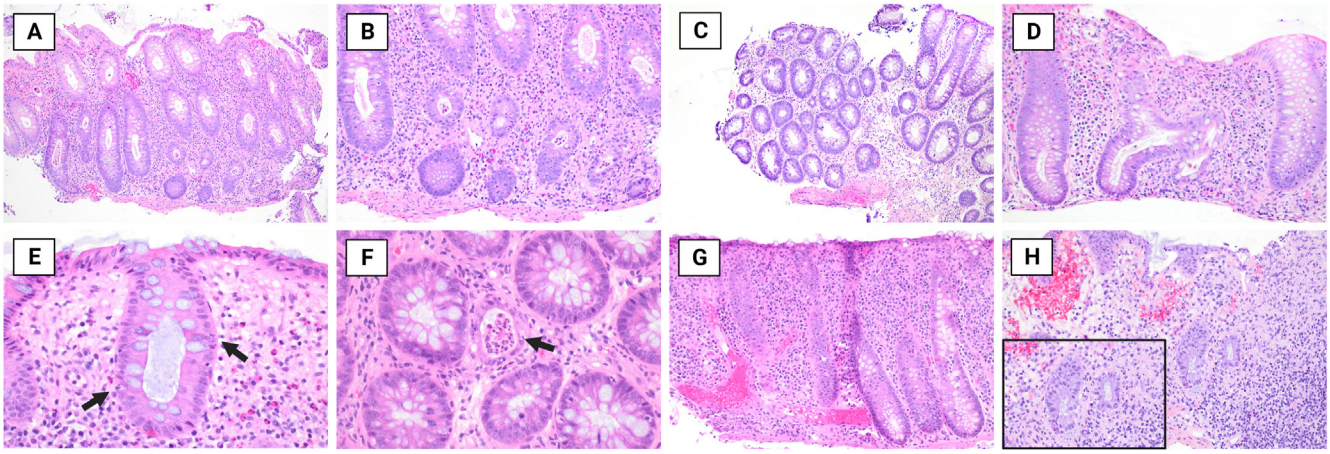


Figure 2. Histological patterns of colonic injury after immune checkpoint inhibitor therapy in T-ICI patients. A-B) Diffuse active colitis with no architectural distortion (100× and 200×). C-D) Diffuse active colitis with mild architectural disarray (score 1) but no definitive evidence of chronicity (100× and 200×). E) Focal active colitis with intraepithelial neutrophils (arrows, score 1) (400×). F) Focal active colitis with crypt abscess (arrow, score 2) (400×). G) Lymphocytic colitis (200×). H) Apoptosis pattern, score 3 (200×, inset 400×).

Wang et al.[14] performed a more detailed histologic study on a cohort of 53 patients with ICI-related colitis and graded various features at a more granular level. In their cohort, a large number of patients (32/53, 60%) had features of chronicity on initial colonic biopsies. Though basal lymphoplasmacytosis was observed, the number of cases with this feature was not reported. On histologic follow-up, 4 of 5 patients with acute inflammation only on the initial biopsy showed evidence of chronic colitis (compared to 0 of 7 follow-up colon biopsies from T-ICI patients in our cohort). Perhaps a contributing factor to these observed differences is that our comparison groups were selected based on subsequent clinical course whereas the Wang et al. study compared groups by treatment type (steroid only vs steroid + infliximab), endoscopic findings, and histologic findings.

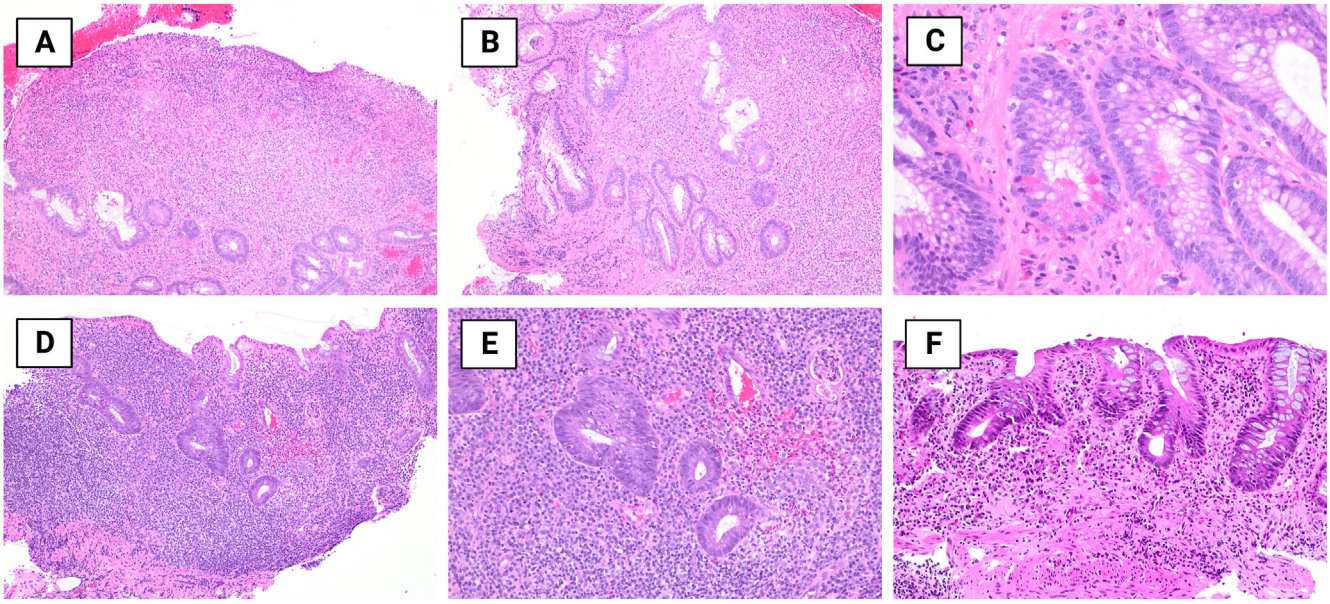


Figure 3. Features of chronicity in colonic biopsies after immune checkpoint inhibitor (ICI) therapy. A-C) Biopsy from patient with preexisting inflammatory bowel disease (P-IBD) and colitis flare after ICI treatment showing (A) severely active chronic colitis with ulceration (100×), (B) adjacent moderate architectural distortion (score 2) (100×) and (C) Paneth cell metaplasia (400×). D-F) Biopsies from patients with "unmasked" inflammatory bowel disease (U-IBD) after ICI treatment showing (D) basal lymphoplasmacytosis with moderate architectural distortion (score 2) (100×) and E) chronic active colitis with intraepithelial neutrophils (200×). Another U-IBD patient with colon biopsy showing (F) crypt branching and foreshortening (moderate architecture distortion, grade 2) (100×).

More recently, Isidro et al.[11] compiled a cohort of 86 patients with biopsy-confirmed ICI colitis and found that chronic active colitis was present in 26% of cases and significantly more frequent in patients on nivolumab vs ipilimumab (45% vs 21%, $P < .05$). Similarly, basal lymphoplasmacytosis was observed, but the number of cases was not reported. In contrast, no patients in our T-ICI group had features of chronicity, which is in line with the experience at our institution, where in an earlier study we did not identify chronic colitis in any of our ICI colitis biopsies (from patients without IBD) regardless of ICI type.[15] Overall, we feel that there is abundant overlap in the histological patterns seen with different ICI agents and that while they may play some confounding role, it is not possible to clearly identify exactly how given the variability in patterns seen across different studies and in different practices.

There are also a couple of studies comparing the histopathologic features of post-ICI colitis in patients with preexisting IBD versus those without. Adler et al.[17] examined morphologic findings in biopsies from 22 patients treated with ipilimumab and 12 patients with treatment-naïve UC. They found no difference in the frequency of cryptitis, crypt abscesses, and ulcerations, but reported significantly less basal lymphoplasmacytosis (14% vs 92%), crypt distortion (23% vs 75%), and more apoptotic bodies in the left colon (mean 17.6 vs 8.2) in T-ICI vs UC biopsies. More recently, Lo et al.[18] conducted a detailed comparison of 21 patients with T-ICI colitis who received various treatments (anti-CTLA-4, anti-PD-1, and anti-PD-L1) with an IBD group consisting of initial pretreatment colonic biopsies from five UC patients. Similar to our study design, the authors semiquantitatively scored histopathologic features on a scale from 0-3 and determined an "overall chronicity score" by adding the individual scores for crypt architectural distortion, subcryptal inflammation (ie, basal lymphoplasmacytosis), and cellularity of the lamina propria. They found that each of the individual chronicity features as well as the overall chronicity score were significantly more frequent in IBD biopsies as compared to T-ICI colitis, though the score distributions for the individual features, including basal lymphoplasmacytosis, were not reported. Histologic features of activity—epithelial cryptitis, crypt abscesses, epithelial injury—were not different between the groups. Lastly, though T-ICI colitis showed more apoptosis and surface intraepithelial lymphocytes, the differences were not significant.

It is difficult to directly compare our current study to prior ones, as our study design is unique: our P-IBD cohort was treated with ICI therapy (which reflects how we encounter these biopsies in practice) instead of focusing on treatment-naïve IBD biopsies, and we include a new U-IBD cohort. Overall, our results show similar correlations to previous studies with the exception of Wang et al,[14] which found features of chronicity in 60% of their cases. Most studies only observed features of chronic colitis in a minority of T-ICI cases, with some possibly based on only mild crypt architectural distortion (crypt architectural disarray), which would not meet the threshold for chronicity in our study. We recognize that there can be significant histopathologic overlap between IBD and ICI-related injury (among other etiologies), and that sometimes the diagnosis requires clinical history.[9] However, from our experience, we do find that identification of unequivocal features of chronicity may sometimes support IBD over ICI-induced colitis.

In addition, the prior studies did not entertain the possibility of "unmasked" IBD with detailed follow-up clinicopathologic data. In this study, we evaluated the histologic findings of the colonic mucosa in clinically identified U-IBD and identified features that can help distinguish U-IBD from T-ICI in the setting of patients without a known history of IBD. All U-IBD biopsies had some degree of neutrophilic inflammation, increased chronic inflammation/expansion of the lamina propria, and mild-to-moderate architectural distortion. In addition, 3/5 (60%) initial U-IBD biopsies had a chronic active colitis pattern (with the other two cases developing chronicity on follow-up biopsies) vs none of the T-ICI initial or follow-up biopsies. Perhaps the most interesting and helpful finding was the presence of definite basal lymphoplasmacytosis in 3/5 (60%) U-IBD biopsies but in none of the T-ICI or P-IBD biopsies; this was the only feature that appeared to distinguish U-IBD from P-IBD. The reason for this is not clear, as 67% of P-IBD biopsies still had a chronic active colitis pattern with either moderate architectural distortion or Paneth cell metaplasia. Perhaps, frequent basal lymphoplasmacytosis may support a recent onset of mucosal injury and early architectural remodeling in U-IBD patients, in contrast to P-IBD patients who have been treated for their known long-standing IBD.

We acknowledge that there are many limitations to our study, as we are contributing a small study to the only nascent experience and literature on the idea of U-IBD. Our cohort sizes are very small, particularly those of the P-IBD and U-IBD groups, as these patients are rarely encountered in practice and the phenomenon of U-IBD is only recently being recognized. We also acknowledge the possibility that another interpretation of patients with "U-IBD" who require maintenance therapy is the identification of a severe cohort of ICI colitis that is likely to become chronic and resemble IBD. The semiquantitative scoring method is inherently subject to interobserver variability, though any cases in which there was discrepancy between the two pathologists were rereviewed and adjudicated under a multiheaded scope involving an additional subspecialty GI pathologist. In addition, the pathologists were not blinded to the patient category when during histopathological evaluation. Lastly, though the identification of the U-IBD cohort was made clinically by a gastroenterologist with expertise in GI-irAE, there is a lack of literature on the precise definition of U-IBD, and there may be differences across practices as to how these patients are identified and managed given the evolving landscape in this area.

In summary, our early results suggest that definite features of chronicity on colon biopsies may help to distinguish IBD flares in U-IBD/P-IBD patients from typical ICI-related

colitis in the appropriate clinical context. Especially in patients without known pre-existing IBD, these features may be a clue to the emerging identification of these rare "unmasked" IBD patients after ICI therapy and lead to early appropriate management, such as maintenance therapy, of these patients. Larger follow-up studies, including at the molecular level, could help to further clarify the differences between "unmasked" IBD and severe chronic ICI-related colitis.

References

1. Wang SJ, Dougan SK, Dougan M. Immune mechanisms of toxicity from checkpoint inhibitors. Trends Cancer Res 2023;9(7):543–553.
2. Abu-Sbeih H, Faleck DM, Ricciuti B, et al. Immune checkpoint inhibitor therapy in patients with preexisting inflammatory bowel disease. J Clin Oncol 2020; 38(6):576–583.
3. Johnson DB, Sullivan RJ, Ott PA, et al. Ipilimumab therapy in patients with advanced melanoma and preexisting autoimmune disorders. JAMA Oncol 2016; 2(2):234–240.
4. Braga Neto MB, Ramos GP, Loftus EV Jr, et al. Use of immune checkpoint inhibitors in patients with preestablished inflammatory bowel diseases: retrospective case series. Clin Gastroenterol Hepatol 2021; 19(6):1285–1287.e1.
5. Sleiman J, Wei W, Shah R, et al. Incidence of immune checkpoint inhibitor-mediated diarrhea and colitis (imDC) in patients with cancer and preexisting inflammatory bowel disease: a propensity score-matched retrospective study. J Immunother Cancer 2021;9(6):e002567.
6. Brown LJ, Weppler A, Bhave P, et al. Combination anti-PD1 and ipilimumab therapy in patients with advanced melanoma and pre-existing autoimmune disorders. J Immunother Cancer 2021;9(5):e002121.
7. Meserve J, Facciorusso A, Holmer AK, et al. Systematic review with meta-analysis: safety and tolerability of immune checkpoint inhibitors in patients with pre-existing inflammatory bowel diseases. Aliment Pharmacol Ther 2021;53(3):374–382.
8. Siakavellas SI, Bamias G. Checkpoint inhibitor colitis: a new model of inflammatory bowel disease? Curr Opin Gastroenterol 2018;34(6):377–383.
9. Zhang ML, Deshpande V. Histopathology of gastrointestinal immune-related adverse events: a practical review for the practicing pathologist. Am J Surg Pathol 2022;46(1):e15–e26.
10. Arora K, Zhang ML, Goiburú-Chenu MB, et al. Pathology of immune checkpoint inhibitor-induced injury of the gastrointestinal tract and hepatobiliary system. Diagn Histopathol 2021;27(2):62–68.
11. Isidro RA, Ruan AB, Gannarapu S, et al. Medication-specific variations in morphological patterns of injury in immune check-point inhibitor-associated colitis. Histopathology 2021;78(4):532–541.
12. Shulman HM, Cardona DM, Greenson JK, et al. NIH Consensus development project on criteria for clinical trials in chronic graft-versus-host disease: II. The 2014 Pathology Working Group Report. Biol Blood Marrow Transplant 2015;21(4):589–603.
13. Marthey L, Mateus C, Mussini C, et al. Cancer immunotherapy with anti-CTLA-4 monoclonal antibodies induces an inflammatory bowel disease. J Crohns Colitis 2016;10(4):395–401.
14. Wang Y, Abu-Sbeih H, Mao E, et al. Endoscopic and histologic features of immune checkpoint inhibitor-related colitis. Inflamm Bowel Dis 2018;24(8):1695–1705.
15. Zhang ML, Neyaz A, Patil D, et al. Immune-related adverse events in the gastrointestinal tract: diagnostic utility of upper gastrointestinal biopsies. Histopathology 2020;76(2):233–243.
16. Chen JH, Pezhouh MK, Lauwers GY, et al. Histopathologic features of colitis due to immunotherapy with anti-PD-1 antibodies. Am J Surg Pathol 2017;41(5): 643–654.
17. Adler BL, Pezhouh MK, Kim A, et al. Histopathological and immunophenotypic features of ipilimumab-associated colitis compared to ulcerative colitis. J Intern Med 2018;283(6):568–577.
18. Lo Y-C, Price C, Blenman K, et al. Checkpoint inhibitor colitis shows drug-specific differences in immune cell reaction that overlap with inflammatory bowel disease and predict response to colitis therapy. Am J Clin Pathol 2021;156(2):214–228.

Received April 18, 2024. Accepted May 30, 2024.

Correspondence:
Address correspondence to: Mari Mino-Kenudson, MD, Department of Pathology, Massachusetts General Hospital, Warren 210, 55 Fruit Street, Boston, Massachusetts 02114. e-mail: mminokenudson@mgb.org.

Authors' Contributions:
Mari Mino-Kenudson and M. Lisa Zhang designed the study. All authors performed data acquisition. M. Lisa Zhang analyzed the data. M. Lisa Zhang, Khalid Algarrahi, and Mari Mino-Kenudson performed data interpretation. M. Lisa Zhang wrote the manuscript. All authors critically reviewed and approved the manuscript.

Conflicts of Interest:
The authors disclose no conflicts.

Funding:
The authors report no funding.

Ethical Statement:
The study was approved by the Massachusetts General Hospital (MGH) Institutional Review Board (Protocol 2019P002829).

Data Transparency Statement:
Data will not be made available publicly, but the corresponding author can be contacted to discuss data sharing.

Reporting Guidelines:
STROBE.