Clinical aspects of immunotherapy and targeted therapy of advanced melanoma

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Chapter 3

Immune-checkpoint inhibition-related colitis: Symptoms, endoscopic features, histology and response to management

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Background

Immune-checkpoint-inhibitors are successfully introduced as anti-cancer treatment. However, they may induce severe immune-related adverse events (irAEs). One of the most frequent irAEs is diarrhea. The main objective of this study was to analyze symptoms (i.e. grade of diarrhea), endoscopic and histological features and response to management in immune-checkpoint inhibition-related colitis (IRC).

Patients and methods

We retrospectively analyzed patients who developed diarrhea upon checkpoint inhibition and therefore underwent an endoscopy and/or were treated with corticosteroids. Patients were treated between August 2010 and March 2016 for metastatic melanoma or non-small cell lung cancer. Severity of IRC was scored using the endoscopic Mayo score and the van der Heide score.

Results

Out of a cohort of 781 patients ninety-two patients were identified who developed diarrhea and therefore underwent an endoscopy and/or were treated with corticosteroids. Patients were treated with monotherapy anti-CTLA-4, anti-PD-1, or a combination of both. All patients had symptoms of diarrhea (16% grade 1, 39% grade 2 and 44% grade 3). A complete colonoscopy was performed in 62 (67%) patients, of whom 42 (68%) had a pancolitis (≥ 3 affected segments). Ulcers were seen in 32% of endoscopies. There was no significant correlation between the grade of diarrhea at presentation and endoscopic severity scores, the presence of ulcers or histological features. In 54 episodes of diarrhea (56%) patients received one or more cycles infliximab for steroid-refractory colitis. Patients with higher endoscopic severity scores, ulcers and/or a pancolitis needed infliximab more often.

Conclusions

The correlation between grade of diarrhea and endoscopic or histological features for severity of colitis is poor. Patients with higher endoscopic severity scores, ulcers, or a pancolitis, needed the addition of infliximab more often. Therefore, endoscopy may have value in the evaluation of the severity of immune-checkpoint inhibitor-related colitis and may help in decision making for optimal management.

Significance of this study

What is already known about this subject?

- Immunotherapy can induces adverse events, which are predominantly immune-related.
- One of the most common and severe immune-related adverse events is diarrhea.
- Diarrhea is seen in 35% of patients treated with anti-CTLA-4, 20% in patients treated with anti-PD-1 and even 44% in patients treated with the combination therapy.

What does this study add?

- Patients in which ulcers were seen during endoscopy required significantly more often the addition of infliximab for steroid-refractory colitis compared to patients in which no ulcers were seen.
- Patients with a high Van der Heide score, a high Mayo score or a pancolitis required significantly more often the addition of infliximab for steroid-refractory colitis compared to patients with a low Van der Heide score, low Mayo score or no pancolitis.
- There was no significant correlation between the grade of diarrhea at presentation and endoscopic Mayo score, van der Heide score, or presence of ulcers.
- There was no correlation between the presence of abdominal pain and any endoscopic feature.
- The most common histopathological feature was an increase in lamina propria cellularity, primarily consisting of mononuclear cells. The second most common histopathological feature was neutrophilic infiltration, either intraepithelial or as crypt abscesses.

How might this impact on clinical practice?

- Algorithms to guide management of immune-related diarrhea should not be based on the grade of diarrhea.
- Endoscopic features, such as the presence of ulcers or a pancolitis, can help clinicians to intensify immune suppression more rapidly.
- Histopathology does not seem to have an added value to guide therapy beyond what is found endoscopically. Mucosal biopsies appear to mainly serve to confirm diagnosis.

INTRODUCTION

The introduction of immune-checkpoint inhibitors has changed treatment options and improved survival of patients with advanced cancer. Ipilimumab, a monoclonal antibody blocking cytotoxic T-lymphocyte antigen-4 (CTLA-4) on T cells, showed an overall survival benefit in patients with advanced melanoma [1]. Nivolumab and pembrolizumab, both antibodies blocking programmed death-receptor 1 (PD-1), improved survival compared to chemotherapy and ipilimumab [2, 3]. The combination of ipilimumab with an anti-PD-1 antibody improves overall response rate and progression free survival even further compared to single agent therapy [4]. Checkpoint inhibitors also show activity in several other types of cancer, such as metastatic non-small cell lung cancer (NSCLC) and bladder cancer [5-7]. Although efficacy and durability of response with checkpoint inhibitors has been well established, one of the major concerns is the high rate of adverse events that are predominantly immune-related.

Diarrhea

One of the most common and severe immune-related adverse events (irAEs) is diarrhea, with an incidence of 35% for anti-CTLA-4, 20% for anti-PD-1 and even 44% for the combination therapy [4, 8]. The median time to onset of diarrhea is 7 – 8 weeks after start for ipilimumab (or combinations with ipilimumab), compared to 3 – 6 months for anti-PD-1 [9-12]. The Common Terminology Criteria for Adverse Events (CTCAE version 4.03) are often used to define grades of diarrhea in patients treated in clinical trials. Grade 1 diarrhea is defined as an increase of < 4 stools over baseline, grade 2 as between 4 – 6 stools over baseline, grade 3 as ≥ 7, grade 4 as life-threatening consequences and grade 5 as death.

Treatment-algorithms

Current treatment-algorithms for immune-checkpoint inhibition-related colitis (IRC) are based on symptoms of diarrhea graded according to CTCAE [13-16]. For patients with grade 2 diarrhea delay of immunotherapy and start of symptomatic treatment with loperamide is considered. If symptoms persist for > 3 days, oral corticosteroids in a dose of 0.5 – 1.0 mg/kg are recommended. For patients with grade 3 or 4 diarrhea, discontinuation of immunotherapy (IT) and treatment with 1.0–2.0 mg/kg prednisone is advised. Steroid-refractory colitis is defined as the persistence of symptoms within 3 days of high-dose corticosteroids. These patients could be treated with the addition of 5 mg/kg infliximab. The implementation of these treatment-algorithms has resulted in a decrease of serious complications such as perforation and colectomy [17]. According to these algorithms a lower endoscopy is advised for patients with grade 3 or 4 symptoms of diarrhea, but no recommendations are provided on differential treatment based on endoscopic findings. The aim of this study was to try to correlate symptoms, endoscopic features, histology and

response to management in patients that developed diarrhea upon immune-checkpoint inhibition.

METHODS

Patients

Patients who developed diarrhea upon immunotherapy and therefore underwent an endoscopy and/or were treated with corticosteroids, were retrospectively identified. All patients were treated for melanoma or NSCLC, between August 2010 and March 2016. Patients were treated with monotherapy anti-CTLA-4, anti-PD-1, a combination of both, or the combination of anti-CTLA-4 and radiofrequency ablation (RFA). Diarrhea was scored according to CTCAE version 4.03. All patient characteristics were derived from the electronic patient records. Routinely, stools were tested for microorganisms, including SSYC, *Clostridium difficile* and viral pathogens. Severity of IRC on endoscopy was scored retrospectively using two different scoring systems (Supplementary Table 1). Endoscopic characteristics of IRC are very diverse and there are no available validated scoring systems. Often, a diffuse component of inflammation was present and therefore we used the Mayo score, which is validated for scoring diffuse inflammation seen in ulcerative colitis (UC) [18]. However, this score is not ideal in patients with ulcers among a normal or slightly friable mucosa. When ulcers were present in a further normal mucosa a Mayo score of 0 with a positive ulcer score was given in our study. We also used the van der Heide score, as it is more descriptive and therefore potentially more useful for the diverse characteristics seen in IRC. This score has been used previously for this purpose [19, 20]. However, the van der Heide score does not take into account the extensiveness of inflammation. Therefore, numbers of affected segments of the colon (recto-sigmoid, descending, transverse and ascending) were scored separately. Involvement of ≥ 3 segments was defined as pancolitis. Scores were gathered through saved images and endoscopy reports and revised by one gastroenterologist (JvD), blinded for the grade of diarrhea. As the scores may be influenced by subjectivity, the most objective endoscopic feature, namely the presence of ulcers, was analyzed as a separate variable. An ulcer was defined as a mucosal break of ≥ 0.5 centimeter. All hematoxylin-eosin (HE) stained slides of biopsies taken during endoscopies were reassessed by one gastrointestinal pathologist (PS).

Treatments

Patients treated with ipilimumab, nivolumab or pembrolizumab as monotherapy received standard or flat doses. Patients who received the combination of ipilimumab and RFA (radiofrequency ablation) underwent RFA of one liver metastasis, directly followed by four cycles of ipilimumab (depending on the cohort, either 3 mg/kg or 10 mg/kg q3 weeks). Pa-

tients received either the standard combination of ipilimumab (3 mg/kg) and nivolumab (1 mg/kg) or a sequential but overlapping scheme of 2 cycles ipilimumab 3mg/kg on day 1 and 22 followed directly by nivolumab (3mg/kg) or pembrolizumab (2mg/kg) from day 23 and onwards q2 weeks or q3 weeks respectively.

Statistical analysis

For continuous variables data are presented as median with interquartile range (IQR) and categorical variables as a number (%). Correlations between clinical symptoms and the endoscopic features were assessed using Spearman rank correlation coefficient. Associations between clinical symptoms, endoscopic features, histology and outcome of management were analyzed by Chi-square tests. A *P* value of < 0.05 was considered statistically significant. Statistical analysis was performed using SPSS version 22 (IBM corp., Armonk, NY, USA).

RESULTS

Patient characteristics

Out of a cohort of 781 patients ninety-two patients were identified who developed diarrhea and therefore underwent an endoscopy and/or were treated with corticosteroids. All patient characteristics have been summarized in Table 1. Four patients had two different episodes of diarrhea (median days between episodes 318 days; range 190–632). Mean age was 58 years (range 30–88) and 54% of patients were female. Eighty patients were treated for metastatic melanoma (87%) and 12 patients (13%) for metastatic NSCLC. Fifty-six percent (54/96) of episodes were due to anti-CTLA-4 (of which 10/54 received the combination with RFA), 22% due to anti-PD-1 and 22% due to the combination of anti-CTLA-4 and anti-PD-1. In sixteen percent of episodes patients had grade 1 diarrhea, 39% grade 2 diarrhea and 44% grade 3 diarrhea. In 48 episodes (50%) patients also experienced abdominal pain and in 29 episodes (30%) patients had bloody stools. Infectious causes for diarrhea were ruled out in 68 episodes (71%). Three patients had a positive stool culture for which they were treated with antibiotics. However, as symptoms did not resolve, an IRC component was present as well. The median time between the first cycle of immunotherapy and onset of diarrhea was 38 days (IQR 23 –62). For patients treated with ipilimumab the median time to onset of diarrhea was 33 days, for anti-PD-1 84 days and for the combination 27 days. Three patients developed a perforation of the colon, for which they underwent surgery (Supplementary Table 2). No patients died due to colitis.

Table 1. Patient characteristics

	No. (%)
Age median (range)	**58 (30 – 88)**
Gender	
Male	**42 (46)**
Female	**50 (54)**
Type of cancer	
Melanoma	**80 (87)**
NSCLC	**12 (13)**
Immunotherapy among 96 episodes	
Ipilimumab (3 mg/kg)	**44 (46)**
Ipilimumab (10 mg/kg)	**10 (10)**
Nivolumab	**11 (12)**
Pembrolizumab	**10 (10)**
Sequential ipilimumab + pembrolizumab	**7 (7)**
Sequential ipilimumab + nivolumab	**2 (2)**
Combined ipilimumab + nivolumab	**12 (13)**
Diarrhea at presentation among 96 episodes	
Grade 1	**15 (16)**
Grade 2	**37 (39)**
Grade 3	**43 (44)**
Grade 4-5	**0 (0)**
Unknown	**1 (1)**
Prednisone at start of diarrhea	
None	**4 (4)**
< 1 mg/kg	**32 (33)**
1 mg/kg	**57 (60)**
> 1 mg/kg	**3 (3)**
Budesonide	
No	**84 (87)**
Yes	**12 (13)**
Infliximab	
No	**42 (44)**
Yes	**54 (56)**
Mycophenolic acid	
No	**93 (97)**
Yes	**3 (3)**
Tacrolimus	
No	**94 (98)**
Yes	**2 (2)**

NSCLC: non-small cell lung cancer

Endoscopic results

In all but 3 episodes an endoscopy was performed. Endoscopy images were not available in one episode. These 4 patients were excluded from endoscopic and histopathologic analysis. The median time between start of diarrhea and endoscopy was 8 days (IQR 5 – 14), the median endoscopic Mayo score was 1 (range 0–3) and the median van der Heide score was 6 (range 0–12). Ulcers were seen during 29 endoscopies (32%). In the majority of endoscopies (79%) a continuous pattern of inflammation was seen. A complete colonoscopy was per-

formed in 62 (67%) patients, of whom 42 (68%) had a pancolitis (≥ 3 affected segments). No serious side-effects of colonoscopy were seen in our patients. All endoscopic features are summarized in Table 2. There was no significant correlation between grade of diarrhea

Table 2. van der Heide and endoscopic Mayo scores from 92 endoscopies

Endoscopic feature according to the van der Heide classification	No. (%)
Color	
Normal	**12 (13)**
Red	**58 (63)**
Deeply red	**22 (24)**
Vascular patter	
Normal	**18 (20)**
Partially absent	**45 (49)**
Completely absent	**29 (31)**
Friability	
Normal	**17 (19)**
Slightly friable	**48 (52)**
Severely friable	**27 (29)**
Granularity	
Absent	**23 (25)**
Fine granularity	**62 (67)**
Coarse granularity	**7 (8)**
Rectal valves	
Sharp	**46 (50)**
Swollen	**46 (50)**
Absent	**0 (0)**
Ulcers	
Absent	**63 (69)**
Few	**18 (19)**
Multiple	**11 (12)**
Spontaneous bleeding	
Absent	**87 (95)**
Discrete	**4 (4)**
Severe	**1 (1)**
Mucopurulent exudate	
Absent	**35 (38)**
Little	**35 (38)**
Much	**22 (24)**
Van der Heide score	
Low (0 – 6)	**50 (54)**
High (7 – 16)	**42 (46)**
Mayo score*	
0	**14 (16)**
1	**46 (52)**
2	**25 (29)**
3	**3 (3)**

*The Endoscopic Mayo score was available for 88 episodes (four episodes could not be classified according to the endoscopic Mayo score).

at presentation and endoscopic Mayo score (ρ 0.12; $P = 0.28$), van der Heide score (ρ 0.13; $P = 0.23$), or presence of ulcers (ρ 0.12; $P = 0.25$). Also, no correlation was found between the presence of abdominal pain and any endoscopic feature. A correlation was found between the presence of bloody stools and the endoscopic scores: Mayo ρ 0.35 ($P = 0.001$) and van der Heide ρ 0.43 ($P < 0.001$). There was no difference in the presence of ulcers in patients with grade 2 (37%) or grade 3 diarrhea (33%; $P = 0.73$). In 15 (24%) of the complete colonoscopies the ascending colon was more severely affected than the descending colon (an example can be seen in Figure 1e&f). Moreover, in 5 out of 64 colonoscopies (8%) endoscopic signs of inflammation were only seen in the ascending colon. Endoscopic features and their association with symptoms and treatment management have been summarized in Table 3.

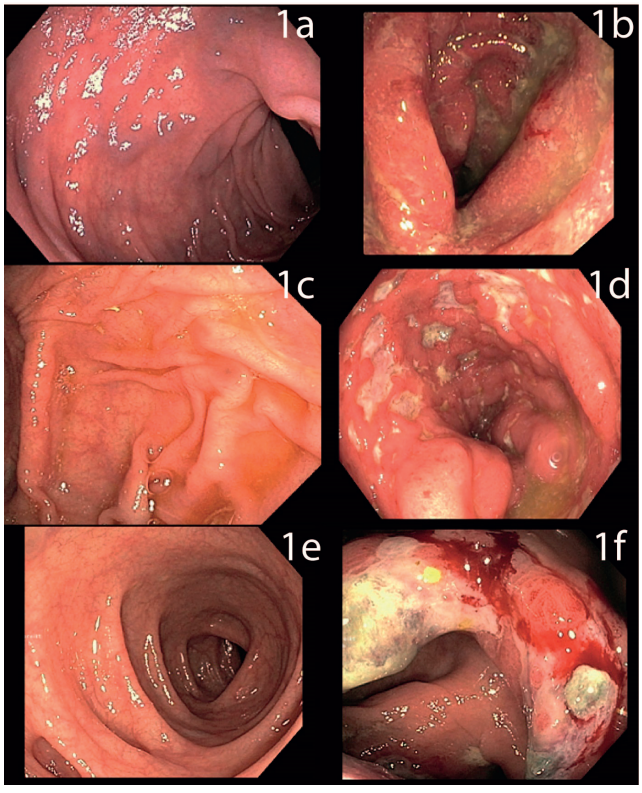

Figure 1a-1f. Examples of differences in immune-checkpoint inhibition-related colitis.
Figures 1a & 1b show two different patients with grade 2 diarrhea. Figure 1a shows no abnormalities on colonoscopy. Figure 1b shows a swollen, erosive and friable mucosa.
Figures 1c & 1d show two different patients with grade 3 diarrhea. Figure 1c shows no abnormalities on colonoscopy. Figure 1d shows a deeply red colon where the vascular pattern is partially absent, the mucosa appears severely friable and multiple ulcers can be seen.
Figures 1e & 1f show a single patient with grade 1 diarrhea. During colonoscopy the entire descending colon (1e) showed no abnormalties, while the ascending colon (1f) showed a swollen, severely friable mucosa, with deep ulcers.

Table 3. Endoscopic features and association with symptoms and treatment management in 92 episodes of diarrhea

	Total No. (%)	Grade of diarrhea† G2/G3 No. (%)	P value	Bloody stools no/yes No. (%)	P value	Need for infliximab no/yes No. (%)	P value
Endoscopic features							
Endoscopic Mayo*							
0-1 (low)	60 (68)	21 (44)/27 (56)	0.84	47 (78)/13 (22)	< 0.01	32 (53)/28 (47)	< 0.01
2-3 (high)	28 (32)	12 (46)/14 (54)		13 (46)/15 (54)		6 (21)/22 (79)	
Total van der Heide score							
0-6 (low)	50 (54)	20 (49)/21 (51)	0.53	44 (88)/6 (12)	< 0.01	29 (58)/21 (42)	< 0.01
7-12 (high)	42 (46)	15 (42)/21 (58)		19 (45)/23 (55)		12 (29)/30 (71)	
Ulcers							
No	63 (69)	22 (44)/28 (56)	0.73	47 (75)/16 (25)	0.06	35 (56)/28 (44)	< 0.01
Yes	29 (31)	13 (48)/14 (52)		16 (55)/13 (45)		6 (21)/23 (79)	
Pancolitis#							
No	20 (32)	7 (50)/7 (50)	0.36	17 (85)/3 (15)	0.13	15 (75)/5 (25)	< 0.01
Yes	42 (68)	14 (36)/25 (64)		28 (67)/14 (33)		10 (24)/32 (76)	

Cases with missing values not included in χ^2 test.

*The Endoscopic Mayo score was available for 88 episodes (four episodes could not be classified according to the endoscopic Mayo score).

#Pancolitis only available for 62 episodes in which a full colonoscopy was performed.

†Grade of diarrhea only G2 versus G3

Histological features

In 90 episodes (94%) biopsies were taken during endoscopy. Histopathological features have been summarized in Table 4. In the majority of episodes patients had received immunosuppressive drugs before the endoscopic procedure (52%, *N* = 47). Median days on high-dose steroids was 4 (IQR 2 – 6). The most common change was an increase in lamina propria cellularity (83%, *N* = 75), primarily consisting of mononuclear cells (Supplementary Figure 2). The second most common change was neutrophilic infiltration, either intraepithelial (79%, *N* = 71) or as crypt abscesses (62%, *N* = 56). In both circumstances, usually mild and patchy. Mild to prominent intraepithelial lymphocytosis was present in only 10% (*N* = 9). Small foci with minimal increase in intraepithelial lymphocytes were noted in an additional 15 cases (17%). Increased numbers of apoptotic cells were seen in the crypts in 42% (*N* = 38) but usually this was mild (*N* = 28). Outcomes of exploratory association analysis of histopathological features with various clinical and endoscopic features are displayed in Supplementary Table 3. Analogous to endoscopic features, none of the histopathological features had an association with the grade of diarrhea. However, multiple histopathological features were associated with endoscopic features (such as Mayo score and the presence of ulcers), bloody stools and the need for infliximab. Endoscopic and histopathological features had no association with different types of immune-

check point inhibitors (Supplementary Table 4). Also, histopathological features did not correlate with whether or not immunosuppressive therapy had been administered before taking the biopsies (data not shown).

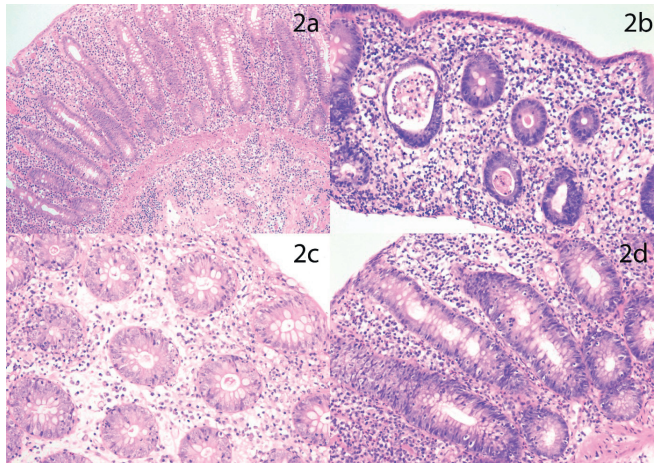

Figure 2. Representative HE sections demonstrating immune-checkpoint inhibition-related colitis
Representative HE sections demonstrating immune-checkpoint inhibition-related colitis characterized by increased lamina propria cellularity (2a, 2b and 2d). 2a, extension of the infiltrate into the submucosa. 2b, neutrophilic inflammation with a crypt abscesses, mild cryptitis, mucin depletion of epithelial cells and small foci with minimal increase in intraepithelial lymphocytes. 2c, apoptotic cells in crypt epithelium. 2d, prominent intraepithelial lymphocytosis.

Initial management of IRC

In all but 4 episodes patients received high-dose corticosteroids. Median time between onset of symptoms and start of high-dose corticosteroid therapy was 5 days (IQR 3 – 11). Of all 92 episodes of diarrhea treated with high-dose corticosteroids, 32 (35%) were initially treated with a dosage of < 1 mg/kg, 57 episodes (62%) with 1 mg/kg and 3 episodes (3%) with > 1 mg/kg. Patients used high-dose corticosteroids for a median of 44 days (IQR 29 – 73). In twelve episodes (13%) patients received budesonide as local treatment.

Steroid-refractory colitis

In 54 (56%) episodes patients required infliximab (5 mg/kg) for corticosteroid-refractory colitis, and of those patients 50% (N = 27) were given more than one cycle infliximab. Median time between start of prednisone and start of infliximab was 9 days (IQR 5 – 19.5) and median time to response on infliximab was 2 days (IQR 1 – 4). In three episodes patients required additional immunosuppressive agents such as mycophenolic acid or tacrolimus. None of 15 patients with grade 1 diarrhea required infliximab therapy. We did not see any difference in the requirement of infliximab for patients that presented with grade 2 (68%) or grade 3 (67%) diarrhea. Interestingly, in 79% of episodes in which ulcers were seen

Table 4. Histopathological features of biopsies taken in 90 endoscopies

Histopathological feature	No. (%)
Lamina propria cellularity	
Normal	**15 (17)**
Increased	
Focal	**7 (8)**
Patchy	**24 (27)**
Diffuse, superficial	**4 (4)**
Diffuse, transmucosal - mild	**24 (26)**
Diffuse, transmucosal - moderate	**16 (18)**
Diffuse, transmucosal – severe	**0 (0)**
Crypt architecture	
Normal	**58 (64)**
Irregular – mild	**23 (26)**
Irregular – moderate	**8 (9)**
Irregular – severe	**1 (1)**
Mucosal surface	
Flat/normal	**74 (82)**
Irregular	**15 (17)**
Villous	**1 (1)**
Apoptotic cells in crypt epithelium	
Absent/hardly any	**52 (58)**
Mild	**28 (31)**
Moderate	**6 (7)**
Severe	**4 (4)**
Extension of chronic inflammatory infiltrate into submucosa	
Not present	**46 (58)**
Present	**33 (42)**
Location of intraepithelial neutrophilic infiltration	
Absent	**19 (21)**
Present in crypt epithelium	**8 (9)**
Present in superficial epithelium	**16 (18)**
Present in crypt and superficial epithelium	**47 (52)**
Grade of intraepithelial neutrophilic infiltration	
None	**19 (21)**
Minimal	**15 (17)**
Mild	**46 (51)**
Moderate	**10 (11)**
Severe	**0 (0)**
Neutrophilic crypt abscesses	
Absent	**34 (38)**
Mild	**46 (51)**
Moderate	**8 (9)**
Severe	**2 (2)**
Location of intraepithelial lymphocytosis	
Absent	**66 (73)**
Present in crypt epithelium	**4 (5)**
Present in superficial epithelium	**8 (9)**
Present in crypt and superficial epithelium	**12 (13)**

Table 4. Histopathological features of biopsies taken in 90 endoscopies (*continued*)

Histopathological feature	No. (%)
Grade of intraepithelial lymphocytosis	
Absent	**66 (73)**
Minimal and patchy	**15 (17)**
Mild	**6 (7)**
Moderate	**2 (2)**
Severe	**1 (1)**
Mucin depletion of epithelial cells	
Not present	**46 (51)**
Mild	**32 (36)**
Moderate	**10 (11)**
Severe	**2 (2)**
Ulceration	
Absent	**71 (79)**
Present	**19 (21)**
Granuloma	
Absent	**85 (94)**
Present in lamina propria	**4 (5)**
Present in submucosa	**1 (1)**

during endoscopy, patients needed infliximab, while this was only the case for 44% of episodes in which no ulcers were seen (P = 0.002). Patients with a Van der Heide score between 7 and 12 (high score) received infliximab in 71% of episodes, while this was 42% in case of a Van der Heide score of 0–6 (low score; P = 0.005). Similarly, with these data, 79% of patients with a Mayo score of 2–3 (high score) received infliximab compared to 47% for patients with a Mayo score of 0–1 (low score; P = 0.005). Seventy-six percent of patients with a pancolitis (≥ 3 affected segments) required infliximab, while this was only the case in 25% of patients with < 3 affected segments (P < 0.001). In total six patients (16%) had a response (complete response (CR) or partial response (PR)) in the group that did not receive infliximab versus ten patients (22%) in the group that did receive infliximab (P = 0.53). Also when looking at disease control rate (stable disease + PR + CR) there was no significant difference in response in patients that received infliximab versus those that did not. Disease control rate was 59% (22 out of 37 patients) in the group of patients that did not receive infliximab versus 44% (20 out of 46 patients) in the group of patients that did receive infliximab (P = 0.15). No serious infliximab related side-effects were seen.

Colitis and best overall response

In total 60 patients with colitis and a cutaneous melanoma had an evaluable response. In the group of patients treated with anti-CTLA-4 monotherapy the response rate was 18% (7 out of 39 patients). In the group of patients treated with anti-PD1 monotherapy the response rate was 44% (4 out of 9 patients) and it was 33% (4 out of 12) for patients treated with the combination of anti-CLTA-4 and anti-PD1. These response rates appear

not different from what has been demonstrated in phase III clinical trials (Checkmate-069 and -067 or Keynote-006).

DISCUSSION

In this retrospective study on IRC we have shown that there is no significant difference between patients with grade 2 or 3 diarrhea with regard to endoscopic severity scores, histopathological features, the requirement for infliximab, or the presence of ulcers. This is important because IRC is usually managed based on the grade of diarrhea according to the CTCAE. Instead, we have found a correlation between endoscopic features and the need for immune suppression beyond high-dose corticosteroids. In that light our findings are relevant, as they would help the clinician to more rapidly intensify immune suppression, with the aim to reduce the time to recovery. Endoscopic characteristics in IRC are very diverse and there are no available validated scoring systems. Of note, due to the retrospective nature of our study the endoscopic findings may have influenced physicians in their choice for management. Based on our findings we suggest that these variables are taken into account in future scores for IRC. Our study suggests that the presence of ulcers and pancolitis (≥ 3 affected colon segments) are predictors of steroid-refractory colitis, perhaps warranting immediate start of infliximab upon colonoscopy.

Given the rapid improvement in symptoms after infliximab treatment (median time to response on infliximab was two days) we therefore strongly advise to consider the use of infliximab earlier, especially in patients with ulcerations or pancolitis. Furthermore, our study supports the findings by Schadendorf et al. who showed that infliximab did not seem to affect the development of a response or the durability of response [21]. The rationale of early initiation of infliximab is based on its efficacy in patients with inflammatory bowel disease (IBD). In IBD treatment with infliximab resulted in more clinical responses, mucosal healing, sparing of steroids, fewer admissions to the hospital and less surgical interventions [22, 23]. An earlier start of infliximab –top down approach– is now increasingly used in severe cases of IBD. Currently a trial is being performed that investigates early treatment with infliximab in immune-checkpoint inhibition induced colitis (NCT02763761).

In our study we have also shown that in 23% of colonoscopies the ascending colon was more severely affected than the descending colon. In these cases, the severity of IRC would have been underestimated by sigmoidoscopy only. Therefore, performing a full colonoscopy in patients that present with grade ≥ 2 diarrhea may have added value to sigmoidoscopy, as the underestimated amount and severity of colonic inflammation may

results in under treatment of patients. The choice for colonoscopy however, has to be judged in relation to other factors such as: burden for the patient and the possibility of perforation, which is about 2–4 times higher than that of sigmoidoscopy [24]. Therefore, if severe ulceration is present in the left-sided colon, assessment of the right-sided colon is not necessary for decisions on further management. CT-colonography could be used as an alternative to colonoscopy, as it has a slightly lower iatrogenic perforation rate. However, CT-colonography has low sensitivity for correct detection of acute colitis (64%), offers no possibility to take biopsies and still is a considerable burden for patients [24-26].

In this largest series to date analyzing histopathology of IRC we found that IRC is most typically described as an increase in lamina propria cellularity (83%), commonly extending slightly into the submucosa (42%), combined with patchy neutrophilic infiltrate (intraepithelial, 79% and/or crypt abscesses, 62%). In 36% of cases there were also some irregularities in the crypt architecture present but still the overall morphology appeared different from IBD. This is in line with the results of earlier analyses [20, 27]. Some cases showed an increase in intraepithelial lymphocytes and/or apoptosis but these features were inconsistent. Histopathological features neither correlate with the different types of immune-checkpoint inhibitors used nor with the time point of start of immunosuppression (before or after biopsies were taken). Many of the histopathological features were correlated with endoscopic features. Therefore, histopathology did not seem to have an added value to guide therapy beyond what was found endoscopically. Mucosal biopsies appear to mainly serve to confirm diagnosis.

Despite the information that is provided by colonoscopy and mucosal biopsies, which may help guide optimal management of diarrhea and colitis, one could argue that performing a colonoscopy will not change the management of IRC. Based on management algorithms patients will start with high-dose steroids and in case of insufficient improvement of symptoms, the addition of infliximab within several days – all without any guidance of information from colonoscopy or mucosal biopsies. In addition colonoscopies are cumbersome for patients, not without risk of perforation and the time benefit until infliximab treatment might be marginal with a waiting time of several days before a colonoscopy can be performed. Currently, it is not known whether omission of a colonoscopy affects the outcome of patients developing immunotherapy-induced gastrointestinal toxicity negatively, and therefore should be the subject of further studies. Nevertheless, patients with severe IRC should be treated promptly to prevent serious complications, such as perforation. On the other hand, patients with only mild or no active colitis, based on endoscopic findings, could be sufficiently treated with local steroids only.

Limitations of our study are the retrospective analysis, the fact that there might be classification bias due to the retrospective scoring of symptoms and endoscopic severity. Also, biopsies were taken in a non-standardized manner.

CONCLUSIONS

The correlation between grade of diarrhea and endoscopic features for severity of colitis is poor. Patients with higher endoscopic severity scores, ulcers, or a pancolitis needed the addition of infliximab more often. Therefore, endoscopy may have value in the evaluation of the severity of immune-checkpoint inhibitor-related colitis and may help in decision making for optimal management.

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REFERENCES

1. Hodi FS, O'Day SJ, McDermott DF et al. Improved survival with ipilimumab in patients with metastatic melanoma. N Engl J Med 2010; 363: 711-723.
2. Robert C, Long GV, Brady B et al. Nivolumab in previously untreated melanoma without BRAF mutation. N Engl J Med 2015; 372: 320-330.
3. Robert C, Schachter J, Long GV et al. Pembrolizumab versus Ipilimumab in Advanced Melanoma. N Engl J Med 2015; 372: 2521-2532.
4. Larkin J, Chiarion-Sileni V, Gonzalez R et al. Combined Nivolumab and Ipilimumab or Monotherapy in Untreated Melanoma. N Engl J Med 2015; 373: 23-34.
5. Brahmer J, Reckamp KL, Baas P et al. Nivolumab versus Docetaxel in Advanced Squamous-Cell Non-Small-Cell Lung Cancer. N Engl J Med 2015; 373: 123-135.
6. Motzer RJ, Escudier B, McDermott DF et al. Nivolumab versus Everolimus in Advanced Renal-Cell Carcinoma. N Engl J Med 2015; 373: 1803-1813.
7. Rosenberg JE, Hoffman-Censits J, Powles T et al. Atezolizumab in patients with locally advanced and metastatic urothelial carcinoma who have progressed following treatment with platinum-based chemotherapy: a single-arm, multicentre, phase 2 trial. Lancet 2016; 387: 1909-1920.
8. Ibrahim N, Berman D, DePril V et al. Ipilimumab safety profile: Summary of findings from completed trials in advanced melanoma. J Clin Oncol 29: 2011 (suppl; abstr 8583).
9. Weber JS, Dummer R, de Pril V et al. Patterns of onset and resolution of immune-related adverse events of special interest with ipilimumab: detailed safety analysis from a phase 3 trial in patients with advanced melanoma. Cancer 2013; 119: 1675-1682.
10. Bristol-Myers Squibb. Investigator brochure ipilimumab. Accessed in May 2017.
11. Merck. Investigator brochure pembrolizumab. Accessed in May 2017.
12. Bristol-Myers Squibb. Investigator brochure nivolumab. Accessed in May 2017.
13. Haanen JBAG, Carbonnel F, Robert C et al. Management of toxicities from immunotherapy: ESMO Clinical Practice Guidelines for diagnosis, treatment and follow-up†. Annals of Oncology 2017; 28: iv119-iv142.
14. Immune-Mediated Adverse Reactions Management Guide. Nivolumab. Accessed in May 2017.
15. Immune-Mediated Adverse Reactions Management Guide. Ipilimumab. Accessed in May 2017.
16. A Guide To Monitoring Patients During Treatment With Keytruda. Pembrolizumab. Accessed in May 2017.
17. Lin R, Yellin J, Lowy I et al. An analysis of the effectiveness of specific guidelines for the management of ipilimumab-mediated diarrhea/colitis: Prevention of gastrointestinal perforation and/or colectomy. J Clin Oncol: 26 (15) (suppl) 9063.
18. Schroeder KW, Tremaine WJ, Ilstrup DM. Coated oral 5-aminosalicylic acid therapy for mildly to moderately active ulcerative colitis. A randomized study. N Engl J Med 1987; 317: 1625-1629.
19. van der Heide H, van den Brandt-Gradel V, Tytgat GN et al. Comparison of beclomethasone dipropionate and prednisolone 21-phosphate enemas in the treatment of ulcerative proctitis. J Clin Gastroenterol 1988; 10: 169-172.
20. Verschuren EC, van den Eertwegh AJ, Wonders J et al. Clinical, Endoscopic, and Histologic Characteristics of Ipilimumab-Associated Colitis. Clin Gastroenterol Hepatol 2016; 14: 836-842.

21. Schadendorf D, Wolchok JD, Hodi FS et al. Efficacy and Safety Outcomes in Patients With Advanced Melanoma Who Discontinued Treatment With Nivolumab and Ipilimumab Because of Adverse Events: A Pooled Analysis of Randomized Phase II and III Trials. J Clin Oncol 2017; Jco2017732289.
22. Hanauer SB, Feagan BG, Lichtenstein GR et al. Maintenance infliximab for Crohn's disease: the ACCENT I randomised trial. Lancet 2002; 359: 1541-1549.
23. Jarnerot G, Hertervig E, Friis-Liby I et al. Infliximab as rescue therapy in severe to moderately severe ulcerative colitis: a randomized, placebo-controlled study. Gastroenterology 2005; 128: 1805-1811.
24. Panteris V, Haringsma J, Kuipers EJ. Colonoscopy perforation rate, mechanisms and outcome: from diagnostic to therapeutic colonoscopy. Endoscopy 2009; 41: 941-951.
25. Bellini D, Rengo M, De Cecco CN et al. Perforation rate in CT colonography: a systematic review of the literature and meta-analysis. Eur Radiol 2014; 24: 1487-1496.
26. Singh K, Narula AK, Thukral CL et al. Role of CT Colonography in Colonic Lesions and Its Correlation with Conventional Colonoscopic Findings. J Clin Diagn Res 2015; 9: TC14-18.
27. Berman D, Parker SM, Siegel J et al. Blockade of cytotoxic T-lymphocyte antigen-4 by ipilimumab results in dysregulation of gastrointestinal immunity in patients with advanced melanoma. Cancer Immun 2010; 10: 11.

SUPPLEMENTARY INFORMATION

Supplementary Table 1. Van der Heide score for the assessment of endoscopic activity of colitis

		Grade	
Endoscopic feature	**0**	**1**	**2**
Color	**Normal**	**Red**	**Deeply red**
Vascular patter	**Normal**	**Partially absent**	**Completely absent**
Friability	**Normal**	**Slightly friable**	**Severely friable**
Granularity	**Absent**	**Fine granularity**	**Coarse granularity**
Rectal valves	**Sharp**	**Swollen**	**Absent**
Ulcers	**Absent**	**Few**	**Multiple**
Spontaneous bleeding	**Absent**	**Discrete**	**Severe**
Mucopurulent exudate	**Absent**	**Little**	**Much**

A score of 0 – 6 is considered as low, while a score of 7 – 16 is considered as high

Endoscopic Mayo score for the assessment of endoscopic activity of colitis

Grade	Findings
0	**Normal mucosa**
1	**Mild: Erythema, decreased vascular pattern, mild friability**
2	**Moderate: marked erythema, absent vascular pattern, friability, erosions**
3	**Severe: spontaneous bleeding, ulcers**

Supplementary Table 2. Description of patients that underwent surgery due to bowel perforation as a complication of immune-related colitis

Patient	Tumor type	Treatment	Grade of diarrhea at presentation	Days between diarrhea and start immunosuppression	Immunosuppression	Days between diarrhea and endoscopy	Ulcers	Pancolitis	Mayo score	Addition of infliximab	Days between endoscopy and start infliximab	Days between endoscopy and surgery	Surgery
1	Uveal melanoma	Ipilimumab 10 mg/kg + RFA	2	13	Prednisone 1 mg/kg	14	yes	yes	2	yes	3	10	Left sided hemicolectomy with colostomy
2	Cutaneous melanoma	Ipilimumab 3 mg/kg	2	2	Prednisone <1 mg/kg	9	yes	yes	NA	yes	0	8	Subtotal colectomy with ileostomy
3	Cutaneous melanoma	Ipilimumab 3 mg/kg	3	14	Prednisone <1 mg/kg	14	yes	yes	2	yes	12	9	Sigmoid resection with colostomy

Abbreviations: NA; Not Available, RFA; radio-frequency ablation

Supplementary Table 3. Histopathological features and association with symptoms, endoscopic features and treatment management from 90 biopsies

Histopathological features	Total No. (%)	Grade of diarrhea† G2/ G3 No. (%)	P value	Bloody stools no/yes No. (%)	P value	Endoscopic Mayo score 0-1/2-3 No. (%)	P value	Need for infliximab no/yes No. (%)	P value
Lamina propria cellularity									
Normal	15 (16)	6 (50)/6 (50)	0.16	14 (93)/1 (7)	0.01	15 (100)/0 (0)	< 0.01	11 (73)/4 (27)	0.02
Increased									
Focal	7 (8)	3 (75)/1 (25)		6 (86)/1 (14)		7 (100)/0 (0)		5 (71)/2 (29)	
Patchy	24 (27)	8 (40)/12 (60)		19 (79)/5 (21)		14 (70)/6 (30)		8 (33)/16 (67)	
Diffuse, superficial	4 (4)	0 (0)/4 (100)		3 (75)/1 (25)		3 (75)/1 (25)		3 (75)/1 (25)	
Diffuse, transmucosal - mild	24 (27)	13 (59)/9 (41)		12 (50)/12 (50)		12 (50)/12 (50)		6 (25)/18 (75)	
Diffuse, transmucosal - moderate	16 (18)	4 (31)/9 (69)		7 (44)/9 (56)		7 (44)/9 (56)		7 (44)/9 (56)	
Diffuse, transmucosal – severe	0 (0)	0 (0)		0 (0)		0 (0)		0 (0)	
Crypt architecture									
Normal	58 (64)	21 (44)/27 (56)	0.73	48 (83)/10 (17)	< 0.001	46 (82)/10 (18)	< 0.001	30 (52)/28 (48)	0.26
Irregular – mild	23 (26)	9 (47)/10 (53)		12 (52)/11 (48)		10 (48)/11 (52)		7 (30)/16 (70)	
Irregular – moderate	8 (9)	4 (57)/3 (43)		1 (13)/7 (87)		2 (25)/6 (75)		3 (38)/5 (62)	
Irregular – severe	1 (1)	0 (0)/1 (100)		0 (0)/1 (100)		0 (0)/1 (100)		0 (0)/1 (100)	
Mucosal surface									
Flat/normal	74 (82)	29 (47)/33 (53)	0.38	56 (76)/18 (24)	< 0.001	54 (76)/17 (24)	< 0.001	34 (46)/40 (54)	0.61
Irregular	15 (17)	4 (33)/8 (67)		5 (33)/10 (67)		4 (29)/10 (71)		6 (40)/9 (60)	
Villous	1 (1)	1 (100)/0 (0)		0 (0)/1 (100)		0 (0)/1 (100)		0 (0)/1 (100)	
Apoptotic cells in crypt epithelium									
Absent/hardly any	52 (58)	17 (41)/25 (59)	0.75	39 (75)/13 (25)	0.27	37 (76)/12 (24)	0.30	30 (58)/22 (42)	0.03
Mild	28 (31)	13 (50)/13 (50)		15 (54)/13 (46)		16 (59)/11 (41)		7 (25)/21 (75)	
Moderate	6 (7)	2 (50)/2 (50)		4 (67)/2 (33)		3 (50)/3 (50)		2 (33)/4 (67)	
Severe	4 (4)	2 (67)/1 (33)		3 (75)/1 (25)		2 (50)/2 (50)		1 (25)/3 (75)	
Extension of chronic inflammatory infiltrate into submucosa									
Not present	46 (58)	16 (46)/19 (54)	0.73	36 (78)/10 (22)	0.001	39 (87)/6 (13)	< 0.001	24 (52)/22 (48)	< 0.01
Present	33 (42)	15 (48)/16 (52)		14 (42)/19 (58)		8 (26)/22 (74)		7 (21)/26 (79)	

Supplementary Table 3. Histopathological features and association with symptoms, endoscopic features and treatment management from 90 biopsies (*continued*)

Histopathological features	Total No. (%)	Grade of diarrhea† G2/ G3 No. (%)	P value	Bloody stools no/yes No. (%)	P value	Endoscopic Mayo score 0-1/2-3 No. (%)	P value	Need for infliximab no/yes No. (%)	P value
Location of intraepithelial neutrophilic infiltration									
Absent	19 (22)	6 (46)/7 (54)	0.79	17 (90)/2 (10)	0.03	17 (94)/1 (6)	0.03	16 (84)/3 (16)	< 0.001
Present in crypt epithelium	8 (9)	2 (29)/5 (71)		5 (63)/3 (37)		5 (63)/3 (37)		4 (50)/4 (50)	
Present in superficial epithelium	16 (18)	6 (43)/8 (57)		13 (81)/3 (19)		11 (73)/4 (27)		9 (56)/7 (44)	
Present in crypt and superficial epithelium	47 (52)	20 (49)/21 (51)		26 (55)/21 (45)		25 (56)/20 (44)		11 (23)/36 (77)	
Grade of intraepithelial neutrophilic infiltration									
None	19 (21)	6 (46)/7 (54)	0.59	17 (90)/2 (10)	< 0.01	17 (94)/1 (6)	< 0.001	16 (84)/3 (16)	< 0.001
Minimal	15 (17)	6 (55)/5 (45)		12 (80)/3 (20)		13 (93)/1 (7)		10 (67)/5 (33)	
Mild	46 (51)	17 (40)/26 (60)		29 (63)/17 (37)		25 (57)/19 (43)		11 (24)/35 (76)	
Moderate	10 (11)	5 (63)/3 (37)		3 (30)/7 (70)		3 (30)/7 (70)		3 (30)/7 (70)	
Severe	0 (0)	0 (0)		0 (0)		0 (0)		0 (0)	
Neutrophilic crypt abscesses									
Absent	34 (38)	12 (44)/15 (56)	0.64	26 (77)/8 (23)	0.36	25 (76)/8 (24)	0.47	22 (65)/12 (35)	0.01
Mild	46 (51)	19 (50)/19 (50)		28 (61)/18 (39)		28 (65)/15 (35)		16 (35)/30 (65)	
Moderate	8 (9)	2 (25)/6 (75)		5 (63)/3 (37)		4 (50)/4 (50)		2 (25)/6 (75)	
Severe	2 (2)	1 (50)/1 (50)		2 (100)/0 (0)		1 (50)/1 (50)		0 (0)/2 (100)	
Location of intraepithelial lymphocytosis									
Absent	66 (73)	25 (42)/34 (58)	0.27	42 (64)/24 (36)	0.55	39 (62)/24 (38)	0.32	27 (41)/39 (59)	0.70
Present in crypt epithelium	4 (5)	3 (100)/0 (0)		3 (75)/1 (25)		3 (75)/1 (25)		2 (50)/2 (50)	
Present in superficial epithelium	8 (9)	3 (43)/4 (57)		6 (75)/2 (25)		7 (88)/1 (12)		4 (50)/4 (50)	
Present in crypt and superficial epithelium	12 (13)	3 (50)/3 (50)		10 (83)/2 (17)		9 (82)/2 (18)		7 (58)/5 (42)	
Grade of intraepithelial lymphocytosis									
Absent	66 (73)	25 (42)/34 (58)	0.59	42 (64)/24 (36)	0.59	39 (62)/24 (38)	0.43	27 (41)/39 (59)	0.38
Minimal and patchy	15 (17)	7 (58)/5 (42)		11 (73)/4 (27)		11 (79)/3 (21)		7 (47)/8 (53)	
Mild	6 (7)	2 (50)/2 (50)		5 (83)/1 (17)		5 (83)/1 (17)		3 (50)/3 (50)	
Moderate	2 (2)	0 (0)/0 (0)		2 (100)/0 (0)		2 (100)/0 (0)		2 (100)/0 (0)	
Severe	1 (1)	0 (0)/0 (0)		1 (100)/0 (0)		1 (100)/0 (0)		1 (100)/0 (0)	

Supplementary Table 3. Histopathological features and association with symptoms, endoscopic features and treatment management from 90 biopsies (*continued*)

Histopathological features	Total No. (%)	Grade of diarrhea† G2/ G3 No. (%)	P value	Bloody stools no/yes No. (%)	P value	Endoscopic Mayo score 0-1/2-3 No. (%)	P value	Need for infliximab no/yes No. (%)	P value
Mucin depletion of epithelial cells									
Not present	46 (51)	15 (41)/22 (59)	0.37	37 (80)/9 (20)	< 0.001	38 (84)/7 (16)	< 0.001	24 (52)/22 (48)	0.29
Mild	32 (36)	15 (56)/12 (44)		22 (69)/10 (31)		19 (66)/10 (34)		13 (41)/19 (59)	
Moderate	10 (11)	4 (44)/5 (56)		1 (10)/9 (90)		1 (10)/9 (90)		3 (30)/7 (70)	
Severe	2 (2)	0 (0)/2 (100)		1 (50)/1 (50)		0 (0)/2 (100)		0 (0)/2 (100)	
Ulceration									
Absent	71 (79)	27 (47)/30 (53)	0.53	48 (68)/23 (32)	0.95	55 (78)/16 (22)	< 0.001	35 (49)/36 (51)	0.07
Present	19 (21)	7 (39)/11 (61)		13 (68)/6 (32)		3 (20)/12 (80)		5 (26)/14 (74)	
Granuloma									
Absent	85 (94)	31 (44)/40 (56)	0.22	58 (68)/27 (32)	0.33	57 (70)/25 (30)	0.06	38 (45)/47 (55)	0.39
Present in lamina propria	4 (5)	3 (75)/1 (25)		3 (75)/1 (25)		1 (25)/3 (75)		1 (25)/3 (75)	
Present in submucosa	1 (1)	0 (0)/0 (0)		0 (0)/1 (100)		0 (0)		1 (100)/0 (0)	

Cases with missing values not included in χ^2 test

†Grade of diarrhea only G2 versus G3

*The Endoscopic Mayo score was available for 86 episodes (four episodes could not be classified according to the endoscopic Mayo score).

Supplementary Table 4. Association of different immunotherapies with histopathological and endoscopic features from 92 endoscopies

	Total No. (%)	Anti CTLA-4 No. (%)	Anti PD-1 No. (%)	Combined anti CTLA-4 + anti PD-(L)1 No. (%)	Sequential anti CTLA-4 + anti PD-1 No. (%)	P value
Lamina propria cellularity						
Normal	15 (16)	4 (8)	5 (25)	4 (33)	2 (24)	0.12
Increased						
Focal	7 (8)	3 (6)	4 (20)	0 (0)	0 (0)	
Patchy	24 (27)	11 (22)	4 (20)	5 (42)	4 (50)	
Diffuse, superficial	4 (4)	3 (6)	1 (5)	0 (0)	0 (0)	
Diffuse, transmucosal - mild	24 (27)	17 (34)	3 (15)	3 (25)	1 (13)	
Diffuse, transmucosal - moderate	16 (18)	12 (24)	3 (15)	0 (0)	1 (13)	
Diffuse, transmucosal – severe	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	
Crypt architecture						
Normal	58 (64)	29 (58)	15 (75)	9 (75)	5 (63)	0.24
Irregular – mild	23 (26)	18 (36)	1 (5)	2 (17)	2 (25)	
Irregular – moderate	8 (9)	3 (6)	3 (15)	1 (8)	1 (12)	
Irregular – severe	1 (1)	0 (0)	1 (5)	0 (0)	0 (0)	
Mucosal surface						
Flat/normal	74 (82)	44 (88)	16 (80)	9 (75)	5 (63)	0.28
Irregular	15 (17)	6 (12)	3 (15)	3 (25)	3 (37)	
Villous	1 (1)	0 (0)	1 (5)	0 (0)	0 (0)	
Apoptotic cells in crypt epithelium						
Absent/hardly any	52 (58)	29 (58)	11 (55)	8 (67)	4 (50)	0.72
Mild	28 (31)	14 (28)	8 (40)	2 (17)	4 (50)	
Moderate	6 (7)	5 (10)	0 (0)	1 (8)	0 (0)	
Severe	4 (4)	2 (4)	1 (5)	1 (8)	0 (0)	
Extension of chronic inflammatory infiltrate into submucosa1						
Not present	46 (58)	25 (53)	13 (81)	6 (60)	2 (33)	0.15
Present	33 (42)	22 (47)	3 (19)	4 (40)	4 (67)	

Supplementary Table 4. Association of different immunotherapies with histopathological and endoscopic features from 92 endoscopies (*continued*)

	Total No. (%)	Anti CTLA-4 No. (%)	Anti PD-1 No. (%)	Combined anti CTLA-4 + anti PD-(L)1 No. (%)	Sequential anti CTLA-4 + anti PD-1 No. (%)	P value
Location of intraepithelial neutrophilic infiltration						
Absent	19 (21)	7 (14)	6 (30)	4 (34)	2 (25)	0.68
Present in crypt epithelium	8 (9)	5 (10)	2 (10)	1 (8)	0 (0)	
Present in superficial epithelium	16 (18)	9 (18)	5 (25)	1 (8)	1 (13)	
Present in crypt and superficial epithelium	47 (52)	29 (58)	7 (35)	6 (50)	5 (62)	
Grade of intraepithelial neutrophilic infiltration						
None	19 (21)	7 (14)	6 (30)	4 (33)	2 (25)	0.63
Minimal	15 (17)	9 (18)	4 (20)	2 (17)	0 (0)	
Mild	46 (51)	29 (58)	8 (40)	5 (42)	4 (50)	
Moderate	10 (11)	5 (10)	2 (10)	1 (8)	2 (25)	
Severe	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	
Neutrophilic crypt abscesses						
Absent	34 (38)	16 (32)	11 (55)	3 (25)	4 (50)	0.49
Mild	46 (51)	27 (54)	7 (35)	9 (75)	3 (38)	
Moderate	8 (9)	5 (10)	2 (10)	0 (0)	1 (12)	
Severe	2 (2)	2 (4)	0 (0)	0 (0)	0 (0)	
Location of intraepithelial lymphocytosis						
Absent	66 (74)	37 (74)	11 (55)	10 (84)	8 (100)	0.08
Present in crypt epithelium	4 (4)	3 (6)	0 (0)	1 (8)	0 (0)	
Present in superficial epithelium	8 (9)	2 (4)	5 (25)	1 (8)	0 (0)	
Present in crypt and superficial epithelium	12 (13)	8 (16)	4 (20)	0 (0)	0 (0)	
Grade of intraepithelial lymphocytosis						
Absent	66 (73)	37 (74)	11 (55)	10 (84)	8 (100)	0.16
Minimal and patchy	15 (17)	10 (20)	4 (20)	1 (8)	0 (0)	
Mild	6 (7)	1 (2)	4 (20)	1 (8)	0 (0)	
Moderate	2 (2)	2 (4)	0 (0)	0 (0)	0 (0)	
Severe	1 (1)	0 (0)	1 (5)	0 (0)	0 (0)	

Supplementary Table 4. Association of different immunotherapies with histopathological and endoscopic features from 92 endoscopies (*continued*)

	Total No. (%)	Anti CTLA-4 No. (%)	Anti PD-1 No. (%)	Combined anti CTLA-4 + anti PD-(L)1 No. (%)	Sequential anti CTLA-4 + anti PD-1 No. (%)	P value
Mucin depletion of epithelial cells						
Not present	46 (51)	22 (44)	14 (70)	6 (50)	4 (50)	0.65
Mild	32 (36)	22 (44)	3 (15)	4 (33)	3 (38)	
Moderate	10 (11)	5 (10)	2 (10)	2 (17)	1 (12)	
Severe	2 (2)	1 (2)	1 (5)	0 (0	0 (0)	
Ulceration						
Absent	71 (79)	40 (80)	17 (85)	9 (75)	5 (63)	0.60
Present	19 (21)	10 (20)	3 (15)	3 (25)	3 (37)	
Granuloma						
Absent	85 (94)	47 (94)	20 (100)	10 (84)	8 (100)	0.19
Present in lamina propria	4 (5)	3 (6)	0 (0)	1 (8)	0 (0)	
Present in submucosa	1 (1)	0 (0)	0 (0)	1 (8)	0 (0)	
Endoscopic Mayo*						
0-1 (low)	60 (68)	34 (57)	16 (84)	7 (64)	3 (43)	0.21
2-3 (high)	28 (32)	17 (33)	3 (16)	4 (36)	4 (57)	
Total van der Heide score						
0-6 (low)	50 (54)	25 (48)	16 (80)	6 (50)	3 (37)	0.07
7-12 (high)	42 (46)	27 (52)	4 (20)	6 (50)	5 (63)	

Cases with missing values not included in χ^2 test

*The Endoscopic Mayo score was available for 88 episodes (four episodes could not be classified according to the endoscopic Mayo score).