

**Supplementary Figure 1.** 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.

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

Endoscopic feature	Grade		
	0	1	2
Color	Normal	Red	Deeply red
Vascular patten	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

<b>Histopathological features</b>	<b>Total No. (%)</b>	<b>Grade of diarrhea<sup>†</sup> G2/G3 No. (%)</b>	<b>P value</b>	<b>Bloody stools no/yes No. (%)</b>	<b>P value</b>	<b>Endoscopic Mayo score 0-1/2-3 No. (%)</b>	<b>P value</b>	<b>Need for infliximab no/yes No. (%)</b>	<b>P value</b>
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)	
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)	

<b>Histopathological features</b>	<b>Total No. (%)</b>	<b>Grade of diarrhea<sup>†</sup> G2/G3 No. (%)</b>	<b>P value</b>	<b>Bloody stools no/yes No. (%)</b>	<b>P value</b>	<b>Endoscopic Mayo score 0-1/2-3 No. (%)</b>	<b>P value</b>	<b>Need for infliximab no/yes No. (%)</b>	<b>P value</b>
Grade of intraepithelial neutrophilic infiltration	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
None	15 (17)	6 (55)/5 (45)		12 (80)/3 (20)		13 (93)/1 (7)		10 (67)/5 (33)	
Minimal	46 (51)	17 (40)/26 (60)		29 (63)/17 (37)		25 (57)/19 (43)		11 (24)/35 (76)	
Mild	10 (11)	5 (63)/3 (37)		3 (30)/7 (70)		3 (30)/7 (70)		3 (30)/7 (70)	
Moderate	0 (0)	0 (0)		0 (0)		0 (0)		0 (0)	
Severe									
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)	
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  $\chi^2$  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 submucosa <sup>1</sup>	46 (58)	25 (53)	13 (81)	6 (60)	2 (33)	0.15
Not present	33 (42)	22 (47)	3 (19)	4 (40)	4 (67)	
Present						
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)	

	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
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)	
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)	



	<b>Total No. (%)</b>	<b>Anti CTLA-4 No. (%)</b>	<b>Anti PD-1 No. (%)</b>	<b>Combined anti CTLA-4 + anti PD- 1 No. (%)</b>	<b>Sequential anti CTLA-4 + anti PD-1 No. (%)</b>	<b>P value</b>
<b>Endoscopic Mayo*</b>						
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)	
<b>Total van der Heide score</b>						
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  $\chi^2$  test

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