Supplementary tables

Table S1: clinical characteristics

Baseline characteristics	Total	Responders	Non-responders	*p*-value
Total no. of weeks previous systemic treatment				
Median (IQR)	0 (24)	13 (31)	0 (24)	0.436
25-75%	0-24	0-31	0-24	
Time between collection faecal sample and last systemic treatment – days				
Median (IQR)	686 (1000)	425 (1452)	705 (869)	0.446
25-75%	261-1261	140-1592	392-1261	
Therapeutic antibiotic use last year – No. (%)	8 (24)	0 (0)	8 (31)	0.296
Time between collection faecal sample and last therapeutic antibiotic treatment – days				
Mean (SD)	197 (±101)	NA	197 (±101)	NA
Range	93-394		93-394	
Prophylactic antibiotics use last year – No. (%)	10 (30)	2 (33)	7 (27)	1.000
Time between collection faecal sample and last prophylactic antibiotic treatment – days				
Mean (SD)	96 (±92)	23 (±24)	91 (±74)	0.083
Range	0-276	6-40	0-171	
Previous chemoradiation – No. (%)	6 (18)	0 (0)	6 (23)	0.564
Use of PPI at T1– No. (%)	14 (42)	1 (17)	12 (46)	0.361
Co-treatment with bevacizumab – No. (%)	27 (82)	5 (83)	21 (81)	1.000
No. of years smoking until T1				
Mean (SD)	32 (±17)	26 (±22)	32 (±17)	0.559
Range	2-68	7-50	2-68	
Type of colorectal surgery – No. (%)†				
Rectal resection	12 (41)	2 (33)	10 (46)	
Sigmoid resection	5 (17)	0 (0)	5 (23)	
Hemicolectomy left	2 (7)	1 (17)	1 (5)	
Extended hemicolectomy left	1 (3)	1 (17)	0 (0)	
Transverse resection	0 (0)	0 (0)	0 (0)	0.316
Hemicolectomy right	8 (28)	2 (33)	5 (23)	
Extended hemicolectomy right	0 (0)	0 (0)	0 (0)	
Subtotal colectomy	0 (0)	0 (0)	0 (0)	
Total colectomy	0 (0)	0 (0)	0 (0)	
Unknown	1 (3)	0 (0)	1 (5)	
Tumour mutation status – No. (%)				
KRAS/NRAS/BRAF WT	9 (27)	1 (17)	8 (31)	
KRAS mutation	13 (40)	4 (66)	9 (34)	0.865
NRAS mutation	1 (3)	0 (0)	1 (4)	
BRAF mutation	2 (6)	0 (0)	1 (4)	
Unknown	8 (24)	1 (17)	7 (27)	

Response could not be evaluated in one patient.
† Percentages do not add up to 100% due to rounding.

Table S2: clinical characteristics during chemotherapy

Baseline characteristics	Total	Responders	Non-responders	*p*-value
Antibiotic use between T1 and T2 – No. (%) ‡	5 (16)	0 (0)	5 (19)	0.555
Days antibiotics use between T1 and T2 *Median (IQR)*	7 (27)	NA	7 (27)	NA
% Capecitabine administered – *Median (IQR)* *Cycle 1* *Cycle 2* *Cycle 3*	 94 (19) 95 (16) 95 (16)	 86 (23) 84 (22) 84 (21)	 95 (17) 96 (14) 96 (15)	 0.356 0.131 0.119
Compliant at T2	24 (89)	4 (100)	20 (87)	1.000
% Tumour change *Mean (SD)* *Range*	 -13 (17) -53-22	 -34 (14) -53 - -10	 -8 (13) -29-22	 <0.001
Continuation cycle 4 – No. (%) †	28 (88)	6 (100)	22 (85)	0.566

Response could not be evaluated in one patient.
† Percentages do not add up to 100% due to rounding.
‡Between T2-T3 only one non-responder received oral amoxicillin and ciprofloxacin to treat pneumonia.

Table S3: CTCAE at T1

Toxicity grade	Total	Responders	Non-responders	*p*-value
Diarrhoea without colostomy – No. (%)				
0	19 (86)	4 (100)	14 (82)	1.000
1	3 (14)	0 (0)	3 (18)	
Diarrhoea with colostomy – No (%) †				
0	7 (70)	1 (50)	6 (75)	
1	1 (10)	0 (0)	1 (13)	0.186
2	1 (10)	0 (0)	1 (13)	
3	1 (10)	1 (50)	0 (0)	
Diarrhoea with or without colostomy – No (%)				
0	27 (82)	5 (83)	21 (81)	
1	4 (12)	0 (0)	4 (15)	0.384
2	1 (3)	0 (0)	1 (4)	
3	1 (3)	1 (17)	0 (0)	
Peripheral Sensory Neuropathy – No (%)				
0	30 (91)	5 (83)	24 (92)	0.476
1	3 (9)	1 (17)	2 (8)	
Hand Foot Syndrome – No. (%)				NA
0	32 (100)	6 (100)	25 (100)	
Fatigue – No (%) †				
0	14 (42)	3 (50)	10 (39)	0.753
1	14 (42)	2 (33)	12 (46)	
2	5 (15)	1 (17)	4 (15)	
Nausea – No (%)				
0	27 (82)	6 (100)	20 (77)	0.226
1	5 (15)	0 (0)	5 (19)	
2	1 (3)	0 (0)	1 (4)	
Oral mucositis – No (%)				NA
0	33 (100)	6 (100)	26 (100)	
Vomiting – No. (%)				NA
0	33 (100)	6 (100)	26 (100)	
Constipation – No (%)				
0	27 (82)	6 (100)	21 (81)	0.773
1	6 (18)	0 (0)	5 (19)	

Response could not be evaluated in one patient.
† Percentages do not add up to 100% due to rounding.

Table S4: CTCAE at T2

Toxicity grade	Total	Responders	Non-responders	*p*-value
Diarrhoea without colostomy – No. (%)				
0	17 (94)	3 (100)	14 (93)	1.000
1	1 (6)	0 (0)	1 (7)	
Diarrhoea with colostomy – No (%)				
0	6 (60)	0 (0)	6 (75)	0.133
1	4 (40)	2 (100)	2 (25)	
Diarrhoea with or without colostomy – No (%) †				
0	27 (84)	4 (67)	23 (89)	0.228
1	5 (16)	2 (33)	3 (12)	
Peripheral Sensory Neuropathy – No (%) †				
0	19 (59)	5 (83)	14 (54)	
1	10 (31)	1 (17)	9 (35)	0.193
2	2 (6)	0 (0)	2 (8)	
3	1 (3)	0 (0)	1 (4)	
Hand Foot Syndrome – No. (%) †				
0	17 (53)	3 (50)	14 (54)	0.800
1	12 (38)	3 (50)	9 (35)	
2	3 (9)	0 (0)	3 (12)	
Fatigue – No (%) †				
0	7 (22)	4 (67)	3 (12)	
1	20 (63)	1 (17)	19 (73)	0.067
2	4 (13)	1 (17)	3 (12)	
3	1 (3)	0 (0)	1 (4)	
Nausea – No (%)				
0	22 (69)	5 (83)	17 (65)	0.637
1	10 (31)	1 (17)	9 (35)	
Oral mucositis – No (%)				
0	19 (59)	5 (83)	14 (54)	
1	12 (38)	1 (17)	11 (42)	0.221
2	0 (0)	0 (0)	0 (0)	
3	1 (3)	0 (0)	1 (4)	
Vomiting – No. (%)				NA
0	32 (100)	6 (100)	26 (100)	
Constipation – No (%)				
0	26 (81)	5 (83)	21(81)	0.773
1	5 (16)	1 (17)	4 (15)	
2	1 (3)	0 (0)	1 (4)	

Response could not be evaluated in one patient.
† Percentages do not add up to 100% due to rounding.

Table S5: CTCAE at T3

Toxicity grade	Total	Responders	Non-responders	*p*-value
Diarrhoea without colostomy – No. (%)				
0	19 (86)	4 (100)	15 (83)	0.442
1	2 (9)	0 (0)	2 (11)	
2	1 (5)	0 (0)	1 (6)	
Diarrhoea with colostomy – No (%) †				
0	6 (60)	1 (50)	5 (63)	1.000
1	3 (30)	1 (50)	2 (25)	
2	1 (10)	0 (0)	1 (13)	
Diarrhoea with or without colostomy – No (%)				
0	25 (78)	5 (83)	20 (77)	0.592
1	5 (16)	1 (17)	4 (15)	
2	2 (6)	0 (0)	2 (8)	
Peripheral Sensory Neuropathy – No (%) †				
0	20 (63)	4 (67)	16 (62)	0.458
1	7 (22)	2 (33)	5 (19)	
2	4 (13)	0 (0)	4 (15)	
3	1 (3)	0 (0)	1 (4)	
Hand Foot Syndrome – No. (%) †				
0	13 (41)	2 (33)	11 (42)	0.784
1	11 (34)	2 (33)	9 (35)	
2	6 (19)	2 (33)	4 (15)	
3	2 (6)	0 (0)	2 (8)	
Fatigue – No (%) †				
0	5 (16)	3 (50)	2 (8)	0.026
1	22 (69)	3 (50)	19 (73)	
2	4 (13)	0 (0)	4 (15)	
3	1 (3)	0 (0)	1 (4)	
Nausea – No (%) †				
0	21 (66)	5 (83)	16 (62)	0.271
1	8 (25)	1 (17)	7 (27)	
2	3 (9)	0 (0)	3 (12)	
Oral mucositis – No (%)				
0	21 (66)	4 (67)	17 (65)	0.692
1	9 (28)	2 (33)	7 (27)	
2	1 (3)	0 (0)	1 (4)	
3	1 (3)	0 (0)	1 (4)	
Vomiting – No. (%)				NA
0	32 (100)	6 (100)	26 (100)	
Constipation – No (%)				
0	26 (81)	5 (83)	21 (81)	0.716
1	5 (16)	1 (17)	4 (15)	
2	0 (0)	0 (0)	0 (0)	
3	1 (3)	0 (0)	1 (4)	

Response could not be evaluated in one patient.
† Percentages do not add up to 100% due to rounding.

Table S6: longitudinal CTCAE

Toxicity grade	T1	T2	T3	*p*-value
Diarrhoea without colostomy – No. (%) †				
0	19 (86)	17 (94)	19 (86)	0.449
1	4 (13)	1 (6)	2 (9)	
2	0 (0)	0 (0)	1 (5)	
Diarrhoea with colostomy – No (%)				
0	7 (70)	6 (60)	6 (60)	
1	1 (10)	4 (40)	3 (30)	0.819
2	1 (10)	0 (0)	1 (10)	
3	1 (10)	0 (0)	0 (0)	
Diarrhoea with or without colostomy – No (%)				
0	27 (82)	27 (84)	25 (78)	
1	4 (12)	5 (16)	5 (16)	0.407
2	1 (3)	0 (0)	2 (6)	
3	1 (3)	0 (0)	0 (0)	
Peripheral Sensory Neuropathy – No (%) †				
0	30 (91)	19 (59)	20 (63)	
1	3 (9)	10 (31)	7 (22)	**0.002**[1]
2	0 (0)	2 (6)	4 (13)	
3	0 (0)	1 (3)	1 (3)	
Hand Foot Syndrome – No. (%)				
0	32 (100)	17 (53)	13 (41)	
1	0 (0)	12 (38)	11 (34)	**<0.001**[2]
2	0 (0)	3 (9)	6 (19)	
3	0 (0)	0 (0)	2 (6)	
Fatigue – No (%) †				
0	14 (42)	7 (22)	5 (16)	
1	14 (42)	20 (63)	22 (69)	0.154
2	5 (15)	4 (13)	4 (13)	
3	0 (0)	1 (3)	1 (3)	
Nausea – No (%)				
0	27 (82)	22 (69)	21 (66)	0.132
1	5 (15)	10 (31)	8 (25)	
2	1 (3)	0 (0)	3 (9)	
Oral mucositis – No (%)				
0	33 (100)	19 (59)	21 (66)	
1	0 (0)	12 (38)	9 (28)	**<0.001**[3]
2	0 (0)	0 (0)	1 (3)	
3	0 (0)	1 (3)	1 (3)	
Vomiting – No. (%)				NA
0	33 (100)	32 (100)	32 (100)	
Constipation – No (%)				
0	27 (82)	26 (81)	26 (81)	
1	6 (18)	5 (16)	5 (16)	0.761
2	0 (0)	1 (3)	0 (0)	
3	0 (0)	0 (0)	1 (3)	

† Percentages do not add up to 100% due to rounding.
[1] Post hoc Wilcoxon test with Bonferroni correction indicated a significant difference between T1-T2 (*p*=0.002) and T1-T3 (*p*=0.007).
[2] Post hoc Wilcoxon test with Bonferroni correction indicated a significant difference between T1-T2 (*p*=<0.001), T1-T3 (*p*=<0.001), and T2-T3 (*p*=0.002).
[3] Post hoc Wilcoxon test with Bonferroni correction indicated a significant difference between T1-T2 (*p*=<0.001) and T1-T3 (*p*=0.002).

Table S7: bone marrow toxicity

Variable	Pre	Post	*p*-value
Hemoglobin – in µ/L			
Mean (SD)	8.3 (1.1)	8.0 (1.0)	0.166
Leucocytes – in 10^9/l			
Median (IQR)	7.4 (1.8)	6.0 (2.9)	0.032
Neutrophils – 10^9/l			
Median (IQR)	5.1 (1.7)	3.7 (2.4)	0.006
Thrombocytes – in 10^9/l			
Median (IQR)	248 (111)	186 (110)	<0.001

Table S8: longitudinal data

	T1	T2	T3	
MUST score – No (%)†				
Low risk	26 (79)	25 (83)	29 (94)	0.554
Medium risk	4 (12)	4 (13)	0 (0)	
High risk	3 (9)	1 (3)	2 (7)	
Karnofsky Performance Score – No (%)†				
Median (IQR)	90 (20)	80 (20)	80 (23)	
50	1 (3)	1 (3)	1 (3)	
60	2 (7)	4 (14)	6 (20)	0.013‡
70	3 (10)	6 (21)	5 (17)	
80	6 (19)	5 (17)	8 (27)	
90	11 (36)	7 (24)	8 (27)	
100	8 (26)	6 (21)	2 (7)	
Carcino Embryonic Antigen – in µ/L				0.234
Median (IQR)	28 (100)	23 (79)	23 (64)	

† Percentages do not add up to 100% due to rounding.

‡ Post hoc Wilcoxon test with Bonferroni correction indicated a significant difference between T1-T3 (*p*=0.002).

Table S9: α-diversity at T1

α-diversity	Responders *n*=6	Non-responders *n*=26	*p*-value
Shannon effective†			
Mean (SD)	47.0 (29.8)	50.1 (24.3)	0.786
Median (IQR)	47.3 (46.7)	46.3 (36.6)	
Richness†			
Mean (SD)	200.5 (86.8)	222.4 (73.3)	0.528
Median (IQR)	198 (94.8)	209 (104.8)	

Response could not be evaluated in one patient.
† An independent t-test was performed.

Table S10: α-diversity at T2

α-diversity	Responders *n*=5	Non-responders *n*=22	*p*-value
Shannon effective†			
Mean (SD)	37.3 (23.3)	48.0 (19.6)	0.301
Median (IQR)	36.6 (32.8)	44.5 (26.6)	
Richness†			
Mean (SD)	165.2 (83.3)	220.4 (72.3)	0.145
Median (IQR)	168 (92)	215 (102.2)	

Response could not be evaluated in one patient.
† An independent t-test was performed.

Table S11: α-diversity changes over time

α-diversity	T1	T2	T3	*p*-value
Shannon effective†				
Mean (SD)	48.9 (26.2)	46.6 (19.9)	48.5 (23.8)	0.640
Median (IQR)	45.0 (39.3)	43.3 (26.2)	46.0 (31.0)	
Richness‡				
Mean (SD)	220.3 (79.5)	211.1 (75.4)	202.9 (85.6)	0.240
Median (IQR)	203 (110)	212 (104)	210 (135)	

† The Friedman test was performed.
‡ Repeated measures ANOVA was performed.

Table S12: Within-subject temporal (in)stability of β-diversity between responders and non-responders

β-diversity	T1 vs. T2	T2 vs. T3	T1 vs. T3
Generalized UniFrac†	*p*=0.8	*p*=0.9	*p*=0.3
Bray-Curtis†	*p*=0.6	*p*=0.4	*p*=0.07

†A Mann Whitney U test was performed.