Supplementary tables and figures

Table S1: *Scoring of toxicity according to CTCAE criteria. Patients were asked to select the score describing the current situation at the time of fecal sample collection*

Symptom	Score	Explanation	Symptom	Score	Explanation
Nausea	0	Not at all	Unintentional weight loss past 3-6 months	0	Not at all (less than 5%)
	1	Less appetite		1	Moderate weight loss (between 5 and 10%)
	2	Less food intake		2	Severe weight loss (between 10 and 20%)
	3	Insufficient food intake		3	Very severe weight loss (>20%)
Vomiting	0	Not at all	Constipation	0	Not at all
	1	1-2 times per 24 hours		1	Occasional complaints
	2	3-5 times per 24 hours		2	Persistent complaints
	3	6 or more times per 24 hours		3	Necessary to remove stool by hand
	4	life threatening, acute intervention needed		4	Life threatening
Fever	0	Not at all	Peripheral sensory neuropathy	0	Not at all
	1	38°C – 39°C		1	Minimal complaints
	2	39,1°C – 40°C		2	Moderate complaints
	3	More than 40°C, less than 24 hours		3	Severe complaints
	4	More than 40°C, more than 24 hours		4	Life threatening
Diarrhea (patients without stoma)	NA	Not applicable	Diarrhea (Patients with stoma)	NA	Not applicable
	0	Not at all		0	Not at all
	1	Compared to normal an increase of <4 times stool per day		1	Compared to normal a minimal increase
	2	Compared to normal an increase of 4-6 times stool per day		2	Compared to normal a moderate increase
	3	Compared to normal an increase of >7 times stool per day		3	Compared to normal a severe increase
	4	Life threatening, acute intervention needed		4	Life threatening
Fatigue	0	Not at all	Hand-foot syndrome	0	Not at all
	1	Fatigue which decreases after rest		1	Skin changes or skin inflammation without pain
	2	Fatigue which does not decrease after rest: limited possibility to normal daily functioning		2	Skin changes with pain: with limited possibility to normal daily functioning
	3	Fatigue which does not decrease after rest: limited possibility to self-care		3	Severe skin changes with pain with limited possibility to self-care
Oral mucositis	0	Not at all	Hair loss	0	Not at all
	1	No complaints or mild complaints		1	Hair loss <50%
	2	Moderate pain		2	Hair loss >50%
	3	Severe pain			
	4	Life threatening			

Table S2: *Calculation of the %tumor change before and after three cycles of capecitabine and classification of tumor response according to the RECIST categories*

Calculation of %tumor size change	
SLD before	Sum longest diameter of the target lesion before start of treatment with capecitabine
SLD after	Sum longest diameter of the target lesion after three cycles of capecitabine
Formula	$\frac{(SLD\ after - \ SLD\ before)}{SLD\ before} x\ 100\%$
RECIST categories	
Complete response	Disappearance of all target lesions
Partial response	At least 30% decrease in the sum of the target lesions
Progressive disease	At least 20% increase in the sum of target lesions
Stable disease	Small changes that did not meet above criteria

Table S3: *MUST scores and Karnofsky Performance Scores (KPS) before (T1), during (T2) and after (T3) three cycles of capecitabine. Percentages are calculated based on valid measurements (missings are excluded).*

	T1 n(%)	**T2** n(%)	**T3** n(%)
MUST – low risk	34 (77.3%)	34 (82.9%)	39 (92.8%)
MUST – medium risk	7 (15.9%)	6 (14.6%)	1 (2.4%)
MUST – high risk	3 (6.8%)	1 (2.4%)	2 (4.8%)
	T1 median (IQR)	**T2** median (IQR)	**T3** median (IQR)
KPS	90 (15)	80 (20)	80 (20)

Table S4: *Number of patients suffering from chemotherapy-induced toxicities before (T1), during (T2) and after (T3) three cycles of capecitabine. Percentages are calculated based on valid measurements (missings are excluded)*

Gastrointestinal complications			
	T1 n(%)	**T2** n(%)	**T3** n(%)
Nausea			
Grade 0	37 (84.1%)	28 (68.3%)	29 (67.4%)
Grade 1	5 (11.4%)	12 (29.3%)	11 (25.6%)
Grade 2	2 (4.5%)	1 (2.4%)	3 (7.0%)
Diarrhea			
Grade 0	34 (80.9%)	34 (85.0%)	35 (83.3%)
Grade 1	6 (14.3%)	6 (15.0%)	5 (11.9%)
Grade 2	1 (2.4%)	-	2 (4.8%)
Grade 3	1 (2.4%)	-	-
Constipation			
Grade 0	37 (84.1%)	32 (78.0%)	34 (79.1%)
Grade 1	7 (15.9%)	8 (19.5%)	8 (18.6%)
Grade 2	-	1 (2.4%)	1 (2.3%)
Oral mucositis			
Grade 0	43 (97.7%)	27 (65.9%)	31 (72.1%)
Grade 1	-	12 (29.3%)	10 (23.3%)
Grade 2	-	1 (2.4%)	1 (2.3%)
Grade 3	1 (2.3%)	1 (2.4%)	1 (2.3%)
Weight loss			
Grade 0	36 (83.7%)	34 (82.9%)	39 (90.7%)
Grade 1	7 (16.3%)	7 (17.1%)	4 (9.3%)
Peripheral sensory neuropathy			
Grade 0	38 (88.4%)	29 (70.7%)	30 (71.4%)
Grade 1	5 (11.6%)	9 (22.0%)	7 (16.7%)
Grade 2	-	2 (4.9%)	4 (9.5%)
Grade 3	-	1 (2.4%)	1 (2.4%)
Hand-foot-syndrome			
Grade 0	41 (97.6%)	23 (56.1%)	21 (50.0%)
Grade 1	1 (2.4%)	15 (36.6%)	12 (28.6%)
Grade 2	-	3 (7.3%)	7 (16.7%)
Grade 3	-	-	2 (4.8%)
Fatigue			
Grade 0	18 (41.9%)	6 (14.6%)	8 (18.6%)
Grade 1	18 (41.9%)	29 (70.7%)	29 (67.4%)
Grade 2	7 (16.3%)	5 (12.2%)	5 (11.6%)
Grade 3	-	1 (2.4%)	1 (2.3%)

Table S5: *Results from the linear mixed model testing the fixed effect of sampling timepoint, correcting for random effects produced by longitudinal sampling within patients*

SCFA	Estimate	Std. Error	t-value	2.5% - 97.5%
Acetate	0.00096	0.033	0.029	-0.065 - 0.067
Propionate	-0.06031	0.096	-0.629	-0.249 - 0.128
Butyrate	-0.1150	0.083	-1.388	-0.278 - 0.048
Valerate	-0.2423	0.082	-2.955	-0.404 – (-0.081)*
Caproate	-0.2362	0.106	-2.232	-0.444 – (-0.027)*

BCFA	Estimate	Std. Error	t-value	2.5% - 97.5%
Iso-butyrate	-0.1346	0.133	-1.012	-0.397 - 0.127
Iso-valerate	-0.05961	0.095	-0.627	-0.247 - 0.127

Table S6: *Cross-sectional differences in SCFA and BCFA concentrations*

Dose adjustments during T1 – T3 (groups: no dose adjustments, dose reduction, dose increase, dose increase and reduction)

Kruskal Wallis Test	T1			T2			T3		
	X^2	df	p-value	X^2	df	p-Value	X^2	df	p-Value
Acetate	3.6827	3	0.2978	1.7386	3	0.6284	5.3489	3	0.148
Propionate	3.296	3	0.3482	0.29991	3	0.96	4.5232	3	0.2102
Butyrate	2.9675	3	0.3967	0.76836	3	0.857	3.7203	3	0.2933
Valerate	1.378	3	0.7107	1.6757	3	0.6424	4.0907	3	0.2518
Caproate	1.7971	3	0.6156	6.227	3	0.1011	2.4859	3	0.4779
Iso-butyrate	2.7853	3	0.4259	2.6389	3	0.4507	2.2016	3	0.5316
Iso-valerate	4.1181	3	0.249	2.217	3	0.5286	2.9868	3	0.3937

Tumor response (groups: progressive disease, stable disease, partial response)

Kruskal Wallis Test	T1			T2			T3		
	X^2	df	p-value	X^2	df	p-value	X^2	df	p-value
Acetate	0.506	2	0.777	2.566	2	0.277	0.059	2	0.971
Propionate	0.448	2	0.799	2.322	2	0.313	0.148	2	0.928
Butyrate	2.087	2	0.352	3.216	2	0.200	0.306	2	0.858
Valerate	4.633	2	0.099	0.316	2	0.854	0.343	2	0.842
Caproate	3.397	2	0.183	0.418	2	0.811	1.062	2	0.588
Iso-butyrate	8.544	2	0.014*	0.779	2	0.677	0.454	2	0.797
Iso-valerate	2.184	2	0.336	1.380	2	0.501	0.602	2	0.740

*significant result of Kruskal Wallis test, subsequently a post-hoc test Dunn's test with Bonferroni correction was performed:
stable disease vs. partial response: p.adjusted=0.017
stable disease vs. progressive disease: p.adjusted=1.000
partial response vs. progressive disease: p.adjusted=0.043

Therapy continuation (groups: continuation capecitabine (4th cycle), no continuation capecitabine)

Mann Whitney U Test	T1		T2		T3	
	W	p-value	W	p-value	W	p-value
Acetate	133	0.834	82	1.0	101	0.955
Propionate	155	0.356	97	0.557	118	0.471
Butyrate	116	0.760	93	0.675	108.5	0.726
Valerate	114	0.711	86	0.897	133	0.192
Caproate	102	0.437	75	0.753	78.5	0.425
Iso-butyrate	166	0.193	76.5	0.812	112.5	0.612
Iso-valerate	155	0.348	84.5	0.948	130	0.235

Systemic treatment before T1 (groups: no previous systemic treatment, previous systemic treatment)

Mann Whitney U Test	W	p-value
Acetate	237	0.953
Propionate	230	0.825
Butyrate	242	0.972
Valerate	276	0.406
Caproate	276.5	0.393
Iso-butyrate	273	0.443
Iso-valerate	285.5	0.289

Antibiotic use last year (>3 months before inclusion, groups: no antibiotic use, antibiotic use)

Mann Whitney U Test	W	p-value
Acetate	242	1.00
Propionate	245	0.944
Butyrate	249	0.871
Valerate	274	0.456
Caproate	197	0.298
Iso-butyrate	211	0.481
Iso-valerate	268	0.541

Table S6 continued: *Cross-sectional differences in SCFA and BCFA concentrations*

Co-treatment with bevacizumab (groups: bevacizumab co-treatment, no bevacizumab co-treatment)				
Mann Whitney U Test	**T2**		**T3**	
	W	**p-value**	**W**	**p-value**
Acetate	154	0.660	197	0.098
Propionate	133	0.832	176	0.327
Butyrate	128	0.708	180.5	0.260
Valerate	131	0.778	173	0.376
Caproate	95.5	0.123	137	0.805
Iso-butyrate	147.5	0.816	135	0.759
Iso-valerate	142	0.960	113	0.311

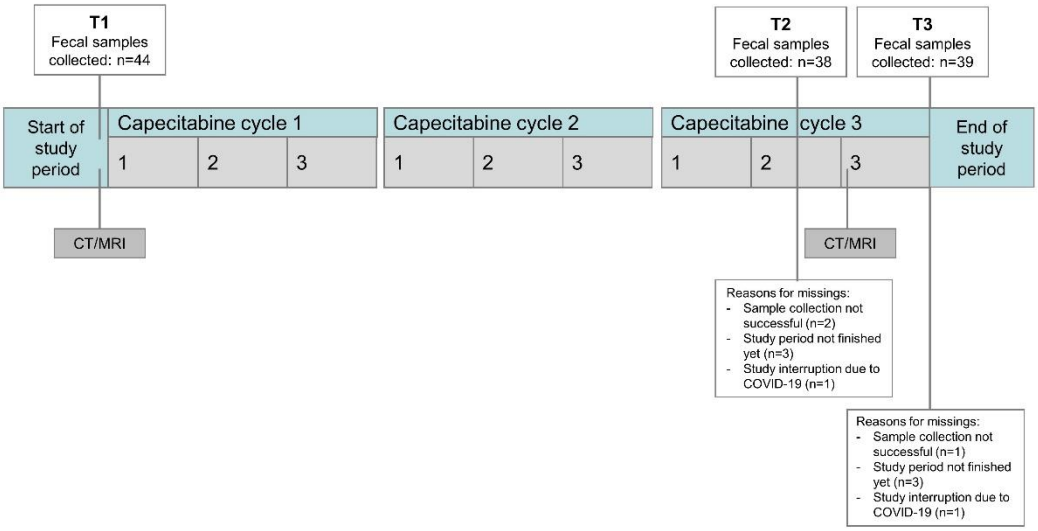


Figure S1: *Overview of study period and sampling timepoints.*

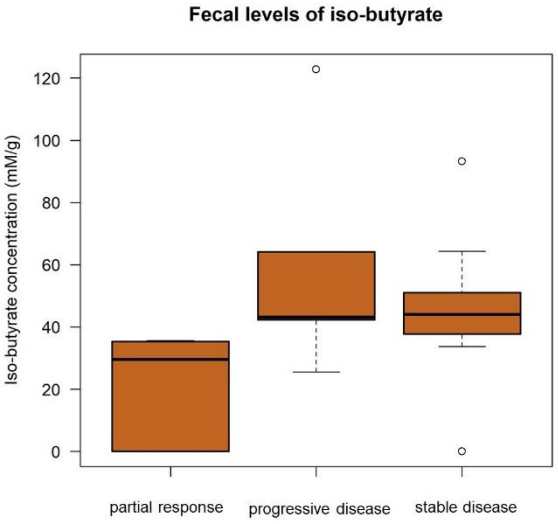
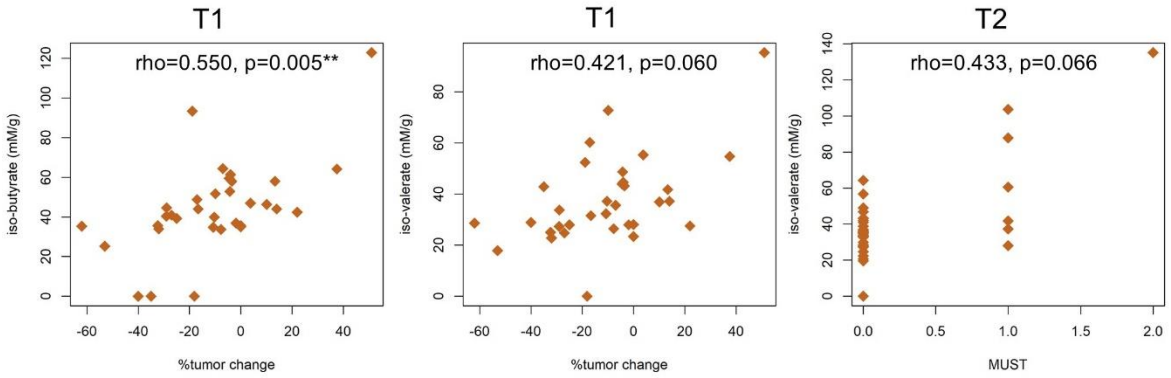


Figure S2: *Fecal levels of iso-butyrate at T1 were significantly lower in patients showing partial response compared to patients with stable disease ($p_{adjusted}$=0.017) or progressive disease ($p_{adjusted}$=0.043)*

A. Clinical parameters



B. Blood inflammatory parameters

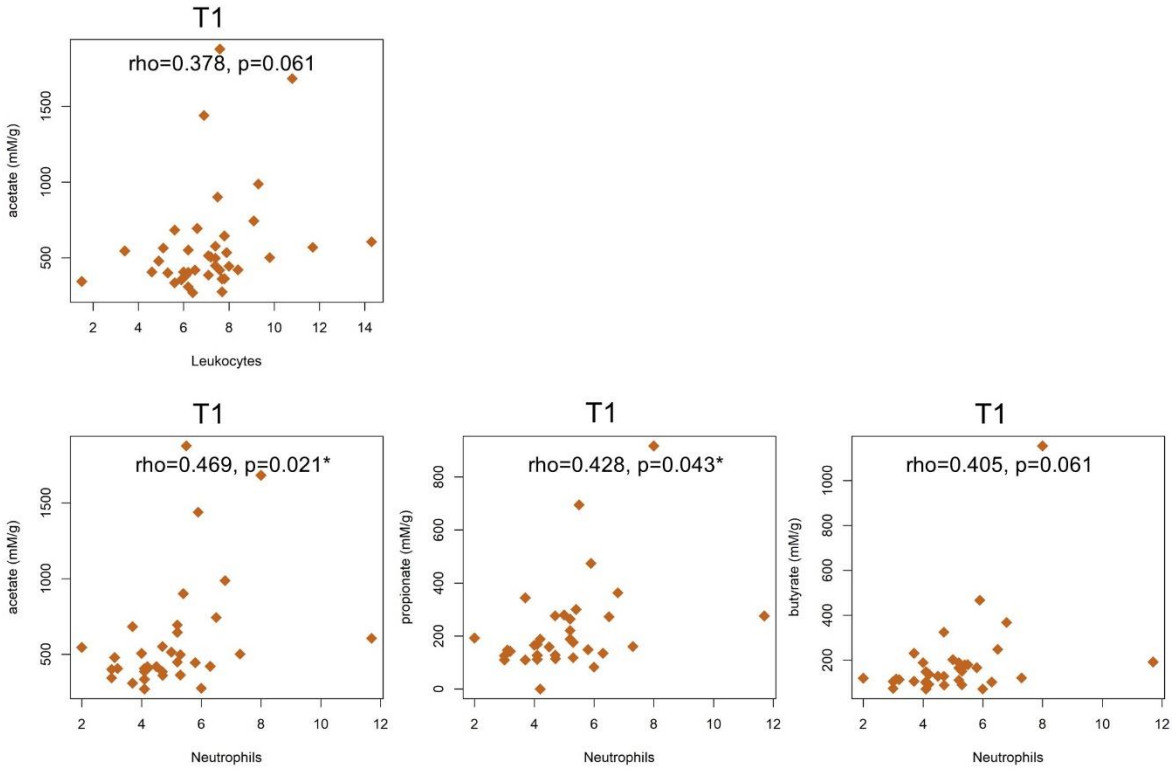


Figure S3: *Scatterplots for correlations between SCFA/BCFA and clinical as well as blood inflammatory parameters. Statistical significance is indicated with asterisks: *=p<0.05; **=p<0.01*

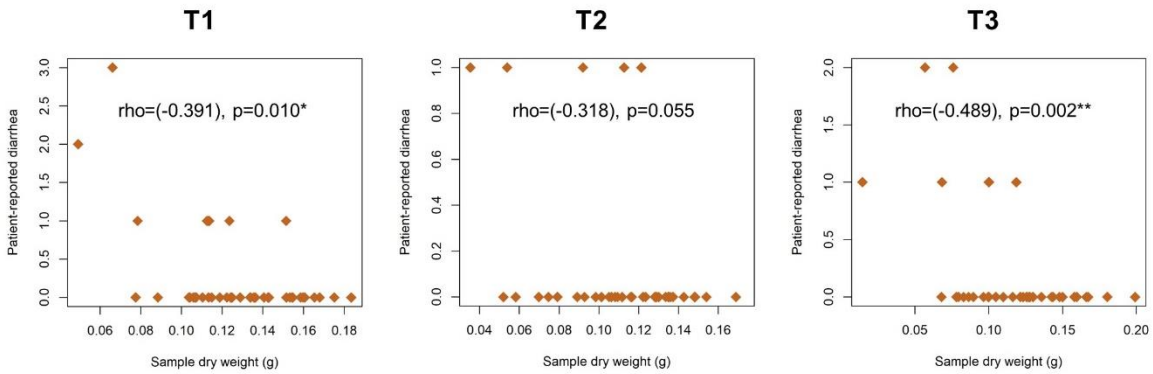


Figure S4: *Scatterplots for correlations between sample dry weight and patient-reported diarrhea. Statistical significance is indicated with asterisks: *=p<0.05; **=p<0.01*