

The gut microbiome in patients with chronic lymphocytic leukemia

Tereza Faitová,¹ Rebecka Svanberg,¹ Caspar da Cunha-Bang,¹ Emma E. Ilett,² Mette Jørgensen,² Marc Noguera-Julian,^{3,4} Roger Paredes,^{3,5} Cameron R. MacPherson² and Carsten U. Niemann^{1,6}

¹Department of Hematology, Rigshospitalet, Copenhagen, Denmark; ²PERSIMUNE Center of Excellence, Rigshospitalet, Copenhagen, Denmark; ³Institut de Recerca de la Sida-IrsiCaixa, Hospital Universitari Germans Trias i Pujol, Badalona, Catalonia, Spain; ⁴University of Vic-Central University of Catalonia, Barcelona, Spain; ⁵Infectious Diseases Department, Hospital Universitari Germans Trias i Pujol, Badalona, Catalonia, Spain and ⁶Department of Clinical Medicine, University of Copenhagen, Copenhagen, Denmark

Correspondence:

C. U. NIEMANN - carsten.utoft.niemann@regionh.dk

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Supplemental Material

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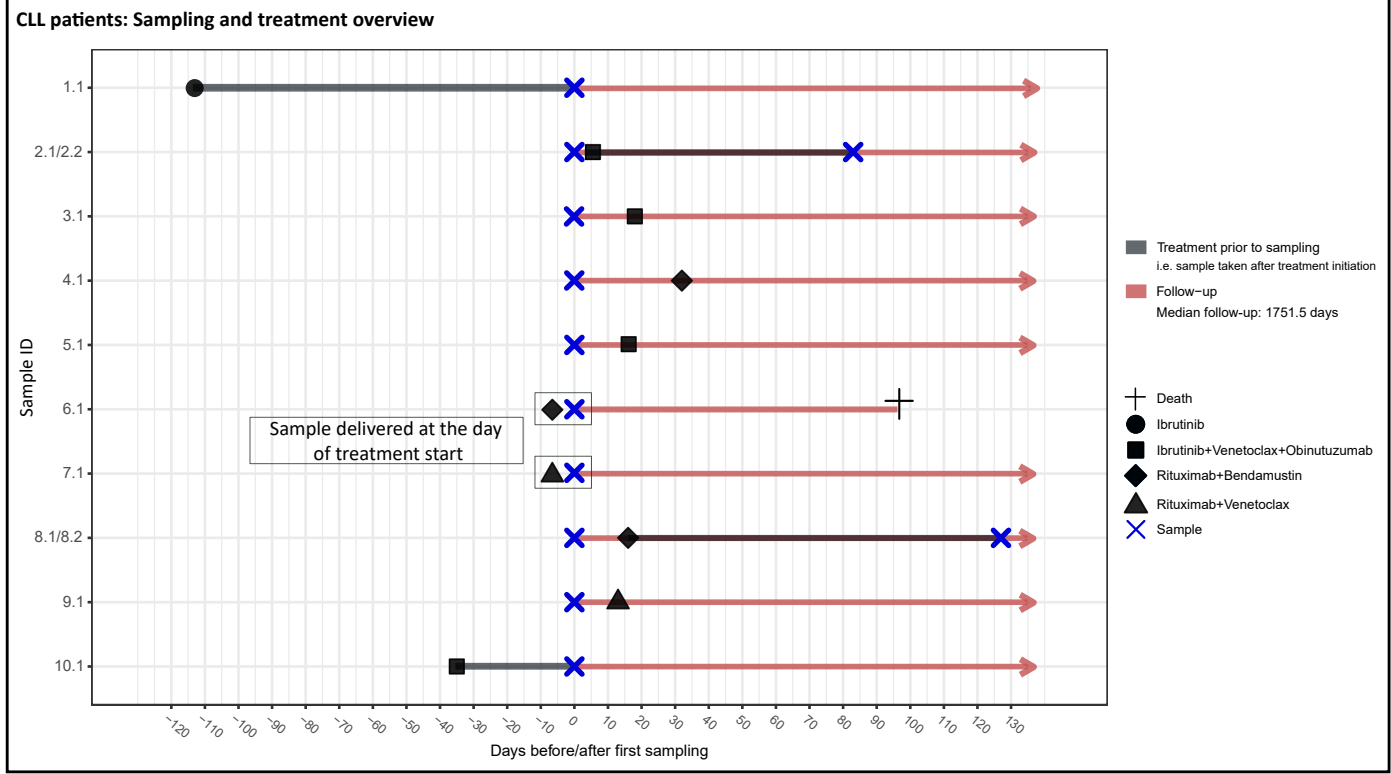
Supplemental Figure S1. Patient and controls characteristic.

Supplemental Figure S2. Heatmap visualization of the highly proportional bacterial genera and microbial signature abundance.

Supplemental Table S1. Microbial signature of CLL identified by phi proportionality-clustering method.

Supplemental Figure 1

A



B

A) CLL patients characteristics

Patient ID	Age (y)	Gender	Diagnosis - 1 st L treatment (d)	1 st L treatment	Diagnosis - Death (d)	Cause of death	Binet stage	CLL-IPI	B2M	FISH status	IGHV	TP53
1	< 65	M	1029	IBR	NA	NA	A	Interm.	< 4.0 mg/L	Normal	U-CLL	Mutated
2	< 65	M	796	IBR, VEN, G	NA	NA	A	Interm.	< 4.0 mg/L	Del13q	U-CLL	Wild-type
3	< 65	F	1000	IBR, VEN, G	NA	NA	A	Low	< 4.0 mg/L	Normal	M-CLL	Wild-type
4	> 65	M	106	RTX, BDM	NA	NA	B	High	> 4.0 mg/L	Tri12	M-CLL	Wild-type
5	< 65	F	2532	IBR, VEN, G	NA	NA	A	Low	< 4.0 mg/L	Normal	M-CLL	Wild-type
6	> 65	M	1067	RTX, BDM	1165	Liver carcinoma	A	Interm.	< 4.0 mg/L	Del13q	U-CLL	Wild-type
7	> 65	F	682	VEN, RTX	NA	NA	B	High	< 4.0 mg/L	Del13q	U-CLL	Wild-type
8	> 65	F	21	RTX, BDM	NA	NA	C	High	> 4.0 mg/L	Del11q	U-CLL	Wild-type
9	< 65	M	608	VEN, RTX	NA	NA	A	Interm.	< 4.0 mg/L	Del13q	U-CLL	Wild-type
10	< 65	M	2388	IBR, VEN, G	3093	SCLC	A	Interm.	< 4.0 mg/L	Normal	U-CLL	Wild-type

C

CLL and control groups characteristic

Characteristic	CLL no., (%)	Control group 1 no., (%)	Control group 2 no., (%)
Total patients	10 (100)	61 (100)	63 (100)
Males	6 (60)	28 (46)	37 (59)
Females	4 (40)	33 (54)	26 (41)
Median age (IQR), y	64 (62-71)	63 (56-67)	68 (65-71)

D

	Pre-treatment (% relative abundance)									Post-treatment (% relative abundance)			
	S 2.1	S 3.1	S 4.1	S 5.1	S 6.1	S 7.1	S 8.1	S 9.1	S 1.1	S 2.2	S 8.2	S 10.1	
Bacteroides	64.53	30.00	20.38	33.27	19.55	9.03	38.56	12.03	27.75	76.46 ↑	68.61 ↑	28.62	
Prevotella	0.00	0.00	0.79	0.00	0.00	25.45	9.47	73.06	0.00	0.00	0.85 ↓	4.42	
Clostridiales *	7.85	1.65	18.17	5.37	10.19	20.96	0.69	1.77	0.85	3.55 ↓	3.20 ↑	15.13	
Parabacteroides	0.00	2.11	1.25	3.41	4.08	0.17	34.48	0.97	13.03	0.00	4.79 ↓	1.15	
Enterobacteriaceae *	0.00	46.42	4.78	0.02	0.00	0.02	3.56	0.01	0.01	0.02	0.10 ↓	0.01	
Alistipes	0.04	0.65	1.94	5.01	14.23	1.35	0.05	2.25	5.37	0.00	4.81 ↑	3.48	
Roseburia	10.80	3.73	0.52	2.34	4.09	1.48	1.16	0.44	0.13	5.52 ↓	0.00 ↓	6.94	
Acinetobacter	0.00	0.00	0.00	0.00	0.00	0.12	0.00	0.00	32.72	0.00	0.00	0.04	
Clostridium	0.04	0.51	5.15	0.70	4.05	11.56	1.20	1.54	4.60	0.56	0.63	1.63	
Ruminococcaceae *	0.13	0.44	2.43	16.31	1.04	2.25	0.05	0.43	1.21	0.08	0.30	3.46	

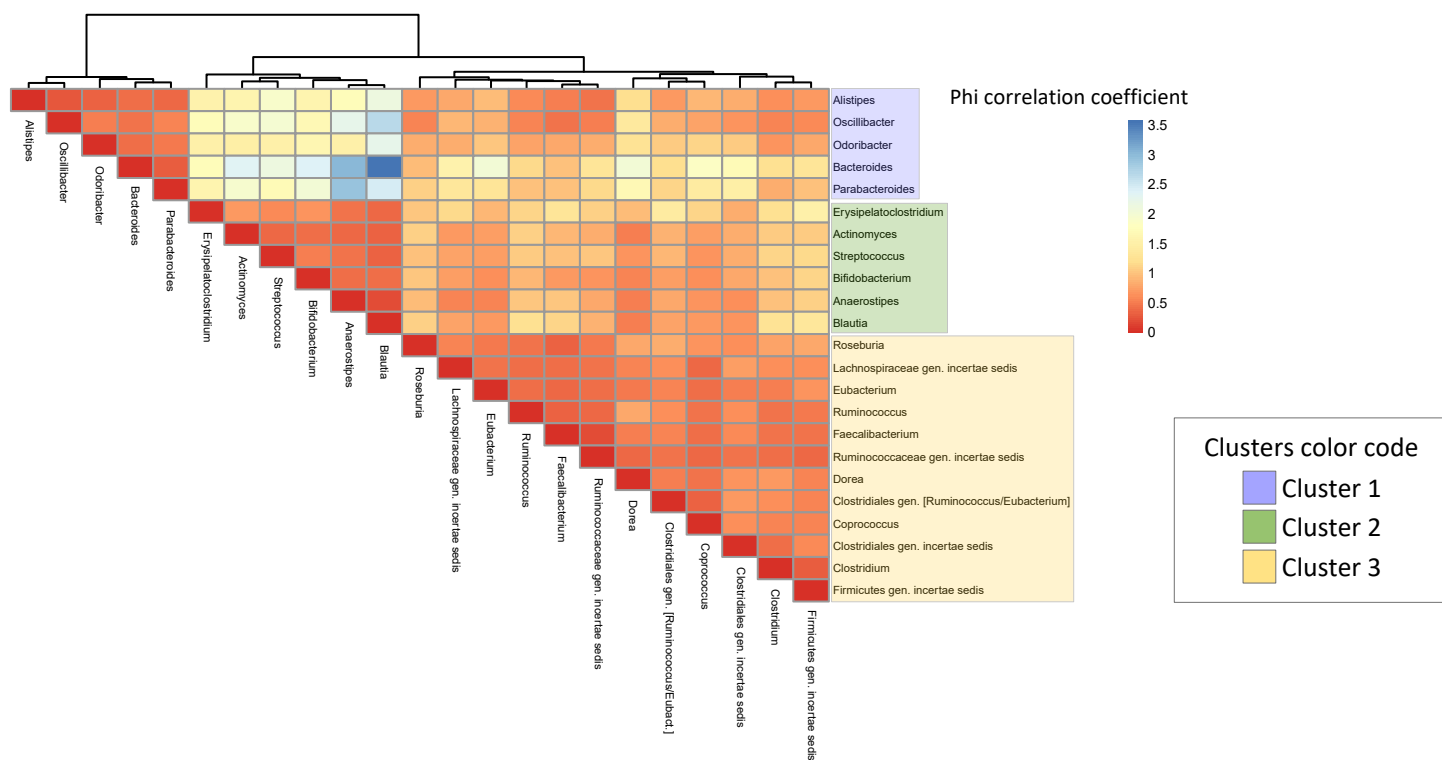
Legend:

- * Genus incertae sedis
- S Sample
- Paired-samples
- ↑/↓ Increase/decrease in abundance

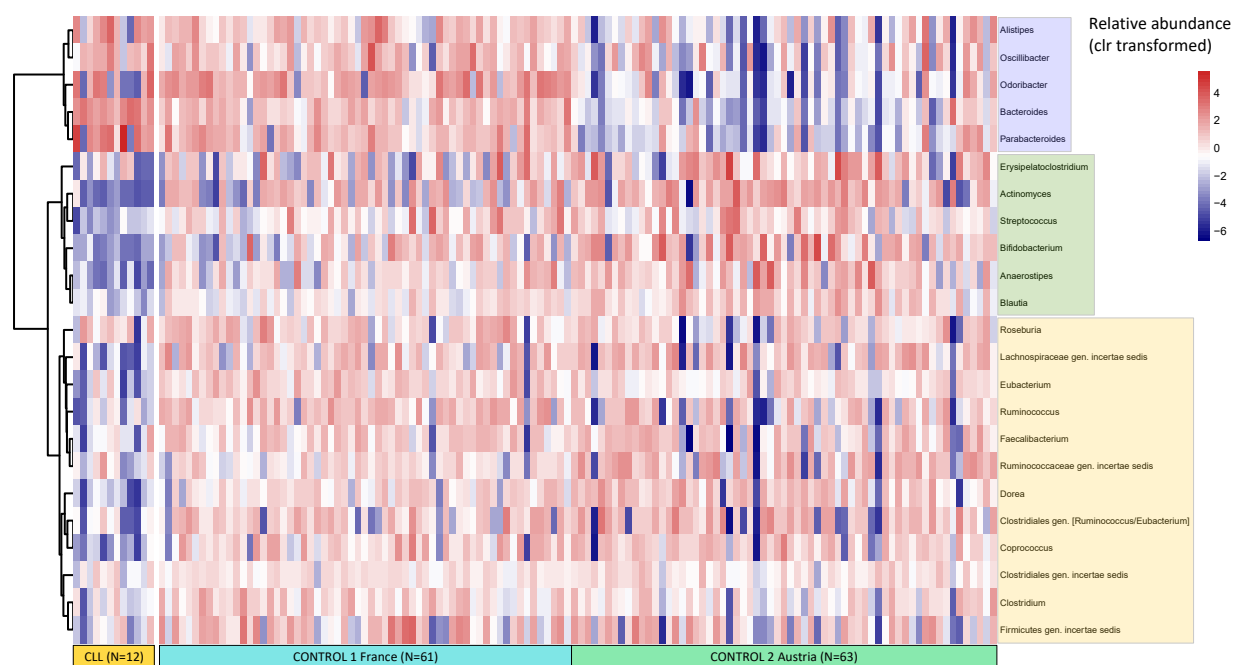
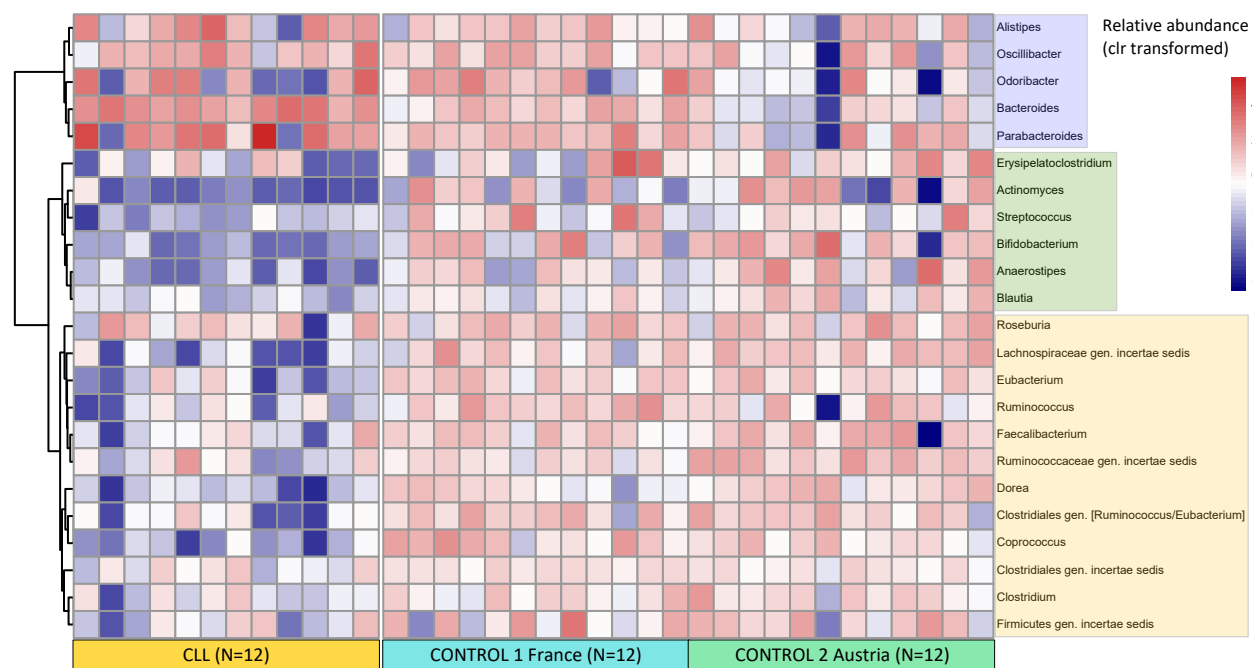
Supplemental Figure S1. Patient and controls characteristic. A) Swimmer plot illustrating the clinical course of 10 CLL patients. The treatment regimens (black shapes), sampling time points (blue crosses) and survival status are shown in this swimmer plot. Patients 2 and 8 were sampled at two time points (Sample IDs: 2.1, 2.2 and 8.1, 8.2, respectively). In total, 8 samples were collected before treatment initiation out of which 2 were delivered at the day of treatment start; and 4 samples were collected after treatment initiation (highlighted by grey bars). Follow-up time differs for each patient and continues beyond the 130 days as indicated by arrow heads. B) CLL patient characteristics. All data shown here represent the time of diagnosis, except for Diagnosis-1stL Treatment and Diagnosis-Death. Abbreviations: y, years; d, days; M, male; F, female; 1stL, first-line; Diagnosis-1stL Treatment, days from diagnosis to first-line treatment; Diagnosis-Death, days from diagnosis to death; NA, not available; SCLC, small cell lung cancer; BDM, bendamustin; IBR, ibrutinib; G, obinutuzumab; RTX, rituximab; VEN, venetoclax; Interm., Intermediate; B2M, beta-2 microglobulin; IGHV, immunoglobulin heavy chain variable region genes; U-CLL, unmutated IGHV-status; M-CLL, mutated IGHV-status; TP53, TP53 Gene - Tumor Protein P53; FISH, fluorescence in situ hybridization. C) CLL and healthy control groups characteristics. All data are no. (%) unless otherwise stated. IQR, interquartile range. D) Change in relative abundance (%) of the 10 most abundant genera in CLL samples. The samples are grouped based on collection timepoint (pre- or post-treatment). The arrows indicate increase (green) or decrease (red) in relative abundance of individual bacterial genera in the paired samples. Only change in relative abundance larger than 1% is marked. Color code of bacterial genera correspond to color code in Figure 1A. Samples collected from the same patient at 2 timepoints (paired-samples) are highlighted in light blue.

Supplemental Figure 2

A



B



Supplemental Figure S2. Heatmap visualization of the highly proportional bacterial genera and microbial signature abundance. A) Hierarchical clustering of bacterial genera showing strong proportionality in terms of co-occurrence (ϕ) proportionality coefficient < 0.4) to any other bacterial genera from CLL and healthy samples. Clustered heatmap was built using hierarchical clustering with the ϕ correlation coefficients as distance metrics. Tree cutting at second level resulted in the 3-cluster structure. The ϕ statistic¹ represented by ϕ correlation coefficient indicates strength of *proportionality* between two variables (the closer the ϕ to zero, the stronger the proportionality between two observed taxa). B) Heatmap of relative abundance of bacterial taxa from A) in CLL and healthy control groups. Clustering performed in A) was reused to cluster the bacterial genera here. Data used for visualization were clr-transformed. Upper: Twelve samples were randomly selected from each control group for better visualization. Lower: All samples from healthy controls were used for visualization.

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1. Lovell D, Pawlowsky-Glahn V, Egozcue JJ, Marguerat S, Bähler J. Proportionality: A Valid Alternative to Correlation for Relative Data. PLOS Computational Biology 2015;11(3):e1004075.

S i g n a t u r e	Group	Subgroup	Bacterial genera	Bacterial family	Identified by ANCOM (Fig. 2B)	Median relative abundance (%)			Fold change	
						CLL	CONTROL 1 France	CONTROL 2 Austria	CONTROL 1 - CLL	CONTROL 2 - CLL
C L L	Cluster 1 Suppl. Fig. 2	Alistipes	Rikenellaceae	—	2.091	0.832	0.172	-2.51	-12.16	
		Oscillibacter	Oscillospiraceae	—	0.753	0.404	0.093	-1.86	-8.10	
		Odoribacter	Porphyromonadaceae	—	0.067	0.107	0.005	-0.63	-13.40	
		Bacteroides	Bacteroidaceae	✓	29.31	7.719	0.767	-3.80	-38.21	
		Parabacteroides	Porphyromonadaceae	✓	1.675	0.532	0.03	-3.15	-55.83	
	D E P L E T E D I N C L L	Cluster 2 Suppl. Fig. 2	Erysipelatoclostridium	Erysipelotrichaceae	—	0.002	0.019	0.044	9.50	22.00
			Actinomyces	Actinomycetaceae	—	0	0.027	0.054	—	—
			Streptococcus	Streptococcaceae	✓	0.059	0.365	0.609	6.19	10.32
			Bifidobacterium	Bifidobacteriaceae	✓	0.043	0.694	2.572	16.14	59.81
			Anaerostipes	Lachnospiraceae	✓	0.056	1.422	2.454	25.39	43.82
			Blautia	Lachnospiraceae	✓	0.896	3.914	6.369	4.37	7.11
		Cluster 3 Suppl. Fig. 2	Roseburia	Lachnospiraceae	—	1.911	2.228	1.516	1.17	-1.26
			gen. incertae sedis	Lachnospiraceae	✓	0.147	2.269	3.015	15.44	20.51
			Eubacterium	Eubacteriaceae	✓	0.476	5.202	3.288	10.93	6.91
			Ruminococcus	Ruminococcaceae	✓	0.526	5.36	3.874	10.19	7.37
			Faecalibacterium	Ruminococcaceae	—	0.617	4.032	4.593	6.53	7.44
			gen. incertae sedis	Ruminococcaceae	—	0.739	2.007	4.838	2.72	6.55
			Dorea	Lachnospiraceae	✓	0.082	1.033	1.53	12.60	18.66
			Ruminococcus/Eubacterium	Ruminococcaceae/Eubacteriaceae	—	0.012	0.05	0.061	4.17	5.08
Coproccoccus	Lachnospiraceae	✓	0.028	0.555	0.789	19.82	28.18			
gen. incertae sedis	(order: Clostridiales)	—	4.458	8.604	8.811	1.93	1.98			
Clostridium	Clostridiaceae	—	1.367	5.616	4.091	4.11	2.99			
gen. incertae sedis	(phylum: Firmicutes)	—	0.138	1.006	0.983	7.29	7.12			

Supplemental Table S1. Microbial signature of CLL identified by phi proportionality-clustering method. Table showing microbial signature divided into groups 'CLL' and 'DEPLETED IN CLL' based on three-cluster structure from Supplemental Figure S2 and the fold change. Group: Microbial signature group based on fold change in relative abundance between CLL and both control groups. Subgroup: Clusters of bacterial taxa corresponding to clusters in Supplemental Figure S2. Bacterial genera/family: Taxonomical classification. Identified by ANCOM: ✓ indicate that individual bacterial taxa were identified as differentially abundant by ANCOM (Figure 2B) independently from the phi (ϕ) proportionality analysis. Median relative abundance: Median relative abundance of individual bacterial taxa in individual cohorts, values are shown in percentages (%). Fold change: Fold change of individual bacterial taxa between CLL and control cohorts; calculated from median relative abundance. Taxa identified by an overlap of the two approaches (ANCOM and phi (ϕ) proportionality analysis) are considered as a potential microbiome pattern in the CLL patients.