Supplementary data

Methods

Extraction protocol H, recommended by the International Human Microbiome Consortium (IHMS_SOP_07; http: //www.microbiome-standards.org).

- Total DNA was extracted from 0.2 g of fecal samples.
- Frozen feces were treated with 250 µL of guanidine thiocyanate and 40 µL of N-lauroyl sarcosine 10%. After thawing, 500 µL of N-lauroyl sarcosine 5% was added and the samples incubated at 70 °C for 1 hour for cell lysis.
- To improve cellular lysis, 750 µL glass beads (0.1-mm diameter) were added, and agitation carried out at maximum speed for 10 min in a Bead Beater™ (Biospec, Bartlesville, OK).
- Polyvinylpolypyrrolidone (15 mg) was added to ensure removal of polyphenol contamination that could inhibit subsequent qPCR reactions. Samples were vortexed and centrifuged at 18 000 g for 5 min, and the supernatant was recovered.
- The remaining pellet was washed with 500 µL of TENP [50 mM Tris (pH 8), 20 mM EDTA (pH 8), 100 mM NaCl, 1% polyvinylpolypyrrolidone] and centrifuged at 18 000 g for 5 min. The washing step was repeated twice more and the resulting supernatants pooled.
- Nucleic acids were precipitated by addition of one volume of isopropanol, storage 15 min at room temperature, and centrifugation at 18 000 g for 10 min.
- The pellet was resuspended in 450 µL of phosphate buffer and 50 µL of potassium acetate and incubated at 4°C for 1h30. Samples were centrifuged at 18 000 g for 30 min and the supernatant containing the DNA was transferred to a clean 2 mL tube.
- For RNA digestion, 2 µL of RNase (10 mg/mL) was added and samples incubated for 30 min at 37 °C.
- For DNA purification, 50 µL of sodium acetate and 1 mL of 100% fresh ethanol (stored at 20 °C) were added and leave overnight at -20 °C. Ethanol was then removed after centrifugation for 30 min at 18 000. Two wash were carried out by resuspending the pellets in 1 mL of 70% ethanol, and removing the ethanol after centrifugation at 18 000 g for 5 min.
- DNA was resuspended in 200 µL of TE buffer.

Table S1. Primers used in this study for microbiota analyze by quantitative real-time PCR.

Target bacteria	Primers and probes	Sequence 5'- 3'	Reference
All bacteria[1]	F_Bact 1369	CGGTGAATACGTTCCCGG	Suzuki *et al*. 2000
	R_Prok1492	TACGGCTACCTTGTTACGACTT	
	P_TM1389F	6FAM-CTTGTACACACCGCCCGTC	
Bacteroides / Prevotella[1]	F_Bacter 11	CCTWCGATGGATAGGGGTT	Furet *et al*. 2009
	R_Bacter 08	CACGCTACTTGGCTGCTTCAG	
	P_Bac303	VICAAGGTCCCCCACATTGMGB	
Cluster IV (*Clostridium leptum* group)[1]	F_Clept 09	CCTTCCGTGCCGSAGTTA	Furet *et al*. 2009
	R_Clept 08	GAATTAAACCACATACTCCACTGCTT	
	P-Clep 01	6FAMCACAATAAGTAATCCACC	
Cluster XIVa (*Blautia coccoides*)[2]	MatsuF	CACATTGGGACTGAGACACG	Matsuki *et al.* 2004
	MatsuR	TAAATCCGGATAACGCTTGC	
Cluster I/II[2]	Cluster I F1	TACCHRBAGGAGGAAGCCAC	Song *et al*., 2004
	Cluster I F2	GTTCTTCCTAATCTCTACGCAT	
Enterococcus sp[2]	F_Entero	CCCTTATTGTTAGTTGCCATCATT	Rinttilâ *et al*, 2004
	R_Entero	ACTCGTTGTACTTCCCATTGT	
Lactobacillus / Leuconostoc / Pedioccocus[2]	F_Lacto 05	AGCAGTAGGGAATCTTCCA	Furet *et al*. 2009
	R_Lacto 04	CGCCACTGGTCYTCCATATA	
Escherichia coli[2]	E.coli F	CATGCCGCGTGTATGAAGAA	Furet *et al*. 2009
	E.coli R	CGGGTAACGTCAATGAGCAAA	

Detection type: [1]Taqman; [2]SYBR®

Supplementary references:

Suzuki MT, Taylor LT and DeLong EF. Quantitative analysis of small-subunit rRNA genes in mixed microbial populations via 5'-nuclease assays. *Appl Environ Microbiol*. 2000;11: 4605–4614.

Furet JP, Firmesse O, Gourmelon M, Bridonneau C, Tap J, Mondot S, Dore J and Corthier G. Comparative assessment of human and farm animal faecal microbiota using real-time quantitative PCR. *FEMS Microbiol Ecol*. 2009;68:351-362.

Matsuki T, Watanabe K, Fujimoto J, Takada T and Tanaka R. Use of 16S rRNA gene-targeted group-specific primers for real-time PCR analysis of predominant bacteria in human feces. *Appl Environ Microbiol*. 2004;70:7220-7228.

Song Y, Liu C and Finegold SM. Real-time PCR quantitation of clostridia in feces of autistic children. *Appl Environ Microbiol*. 2004;70:6459-6465.

Rinttilä T, Kassinen A, Malinen E, Krogius L, Palva A. Development of an extensive set of 16S rDNA-targeted primers for quantification of pathogenic and indigenous bacteria in fecal samples by real-time PCR. *J Appl Microbiol*. 2004;97(6):1166-77.

Table S2. Characteristics of study participants.

	aGVHD (n = 35)	**Control** (n = 35)	**Total** (n = 70)
Pre-transplant characteristics			
Age, mean in y [range]	49.2 [16.4-69.0]	46.7 [18.2-68.9]	47.9 [16.4-69.0]
Gender (male/female)	22/13	19/16	41/29
Underlying disease, n (%)			
Leukemia	11 (31.4)	22 (62.8)	33 (47.1)
Myelodysplastic syndrome	17 (48.6)	5 (14.3)	22 (31.4)
Lymphoma	5 (14.3)	3 (8.6)	8 (11.4)
Bone marrow failure	2 (5.7)	4 (11.4)	6 (8.6)
Other	0 (0.0)	1 (2.9)	1 (1.4)
Transplant characteristics, n (%)			
Myeloablative conditioning	8 (22.8)	16 (45.7)	24 (34.3)
Total body irradiation	7 (20.0)	9 (25.7)	16 (22.9)
Steam cell source			
Peripheric blood	27 (77.1)	26 (74.3)	53 (75.7)
Bone marrow	5 (14.3)	7 (20.0)	12 (17.2)
Cord blood	3 (8.6)	2 (5.7)	5 (7.1)
HLA match			
Identical sibling	11 (31.4)	17 (48.6)	28 (40.0)
Matched unrelated	16 (45.7)	6 (17.1)	22 (31.4)
Mismatched unrelated	7 (20.0)	12 (34.3)	19 (27.2)
Mismatched relative	1 (2.9)	0 (0.0)	1 (1.4)
Dead at 1 year, n (%)	16 (45.7)	9 (25.7)	25 (35.7)
Main cause of death, n (%)			
Disease	4 (25.0)	8 (88.9)	12 (48.0)
aGVHD	11 (68.8)	0 (0.0)	11 (44.0)
Infection	1 (6.2)	1 (11.1)	1 (8.0)

Abbreviation: GVHD, graft-versus-host disease.

Table S3. Characteristics of aGVHD episodes according to gut severity.

Number of aGVHD	**Stage 1** n = 18	**Stage 2 or 3** n = 17	**Total** n = 35
Post-transplant onset time (median, [range]) (day)	17 [10-45]	12 [7-27]	14 [7-45]
Localization, n (%)			
Gut	1 (5.6)	4 (23.5)	5 (14.3)
Gut and skin	10 (55.5)	9 (53.0)	19 (54.2)
Gut and liver	1 (5.6)	0 (0.0)	1 (2.8)
Gut, skin and liver	6 (33.3)	4 (23.5)	10 (28.6)
Stage 1 digestive signs, n (%)			
Vomiting	9 (50.0)		
Diarrhea	11 (61.1)		
Unknown	2 (11.1)		
Cortico-resistance, n (%)	6 (33.3)	7 (41.2)	13 (37.1)
Dead at 1 year, n (%)	9 (50.0)	7 (41.2)	16 (45.7)
Main cause of death, n (%)			
Disease	4 (44.4)	0 (0.0)	4 (25.0)
GVHD	4 (44.4)	7 (100)	11 (68.8)
Infection	1 (11.2)	0 (0.0)	1 (6.2)

Table S4. Results of 16S negative control.

Number of OTUs and reads identified in the negative control, and their detection in patient samples according to the data processing. A filter was applied to sequencing data obtained for patient samples to limit the impact of potential contaminants: least abundant OTUs, representing only 1% of the cumulative sequences, and present in less than five subjects were excluded for the analysis.

Negative control	No of OTUs	No of reads
Total OTUs	128	2525
Absent from samples	55	1664
Present after filtrering	21	93
OTUs with >5 reads	65	2375
Absent from samples	30	1602
Present after filtrering	6*	58
OTUs with >10 reads	39	2166
Absent from samples	22	1533
Present after filtrering	2†	29

*Firmicutes: *Streptococcus* (15 reads)†, *Faecalibacterium* (14 reads)†, *Dialister* (9 reads); Bacteroidetes: *Barnsiella* (7 reads), *Parasutterella* (7 reads), *Ruminococcus 2* (6 reads).

Figure S1. Antibiotic treatment at the time of stool collection in GVHD patients and controls.

GVHD patients were sampled at the time of acute GVHD diagnosis, varying from 1 to 6 weeks post-HSCT. Control patients sampled at the same post-transplant week were studied.
Glycopeptide: vancomycin or teicoplanine; Tazo: piperacillin-tazobactam; SMZ: sulfamethoxazole-trimethoprim; Metro: metronidazole; Amox: amoxicillin alone or in combination with ofloxacin; Cefta*: ceftazidime; Fep*: cefepime; Penem*: imipenem or meropenem. *alone or in combination with a glycopeptide.

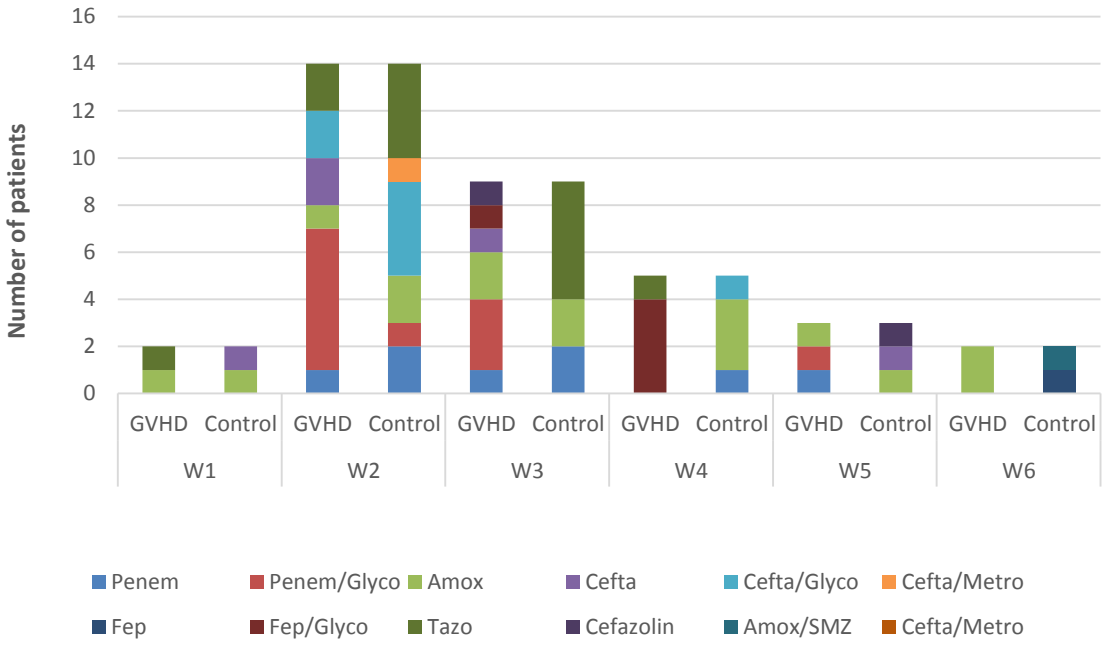

Figure S2. Enteric microbiome in HSCT patients according to severity of gut aGVHD.

(A) Relative abundance of sequence reads at the phylum level, and (B) at the genus level assigned to different bacterial taxa in each patient according to clinical status (control, GVHD 1 or GVHD 2-3).

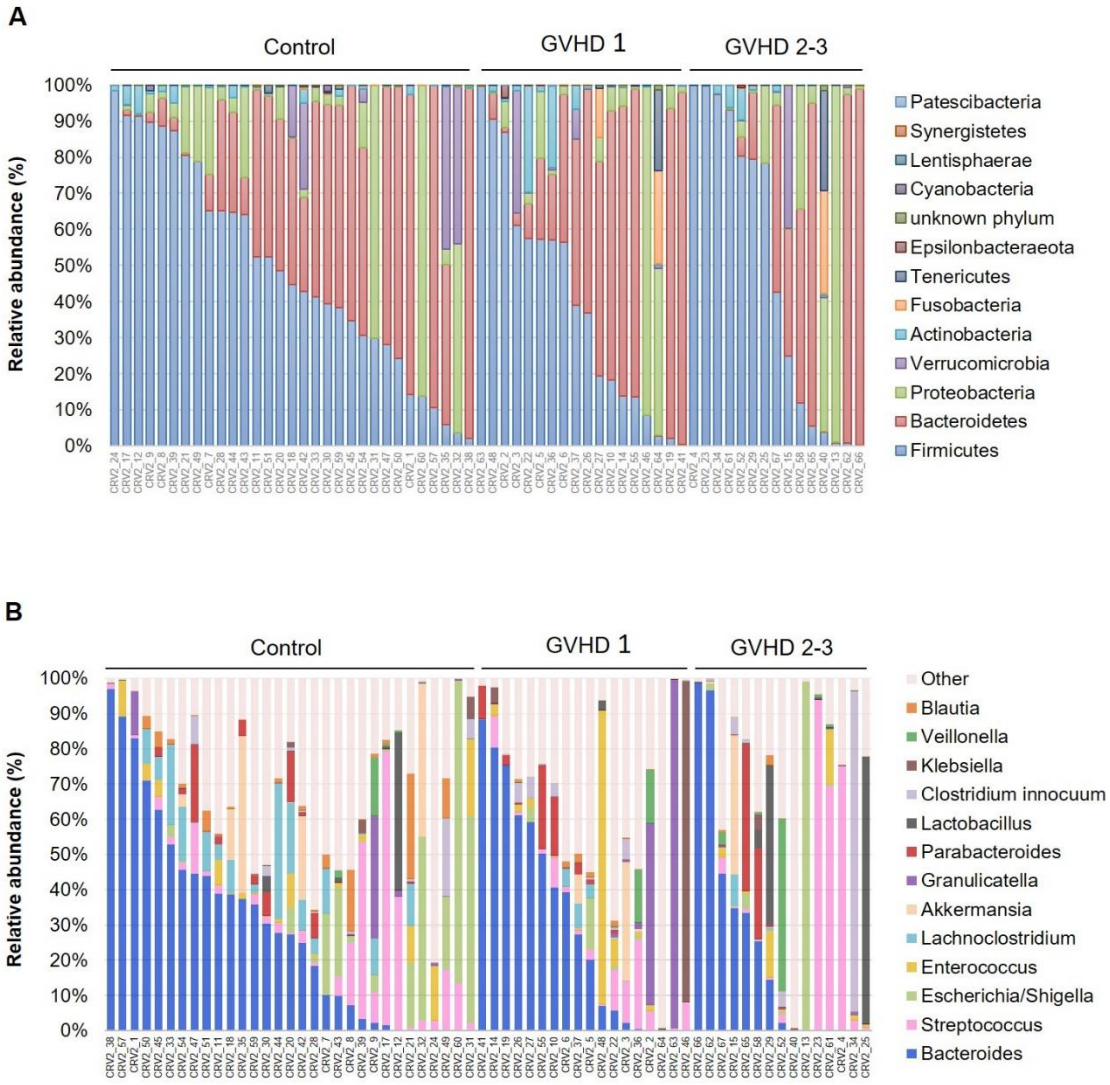

Figure S3. Quantitative microbiota shifts according to gut aGVHD severity.

Absolute count of sequences of the 16S rRNA gene was measured using quantitative-PCR targeting 7 major bacterial groups or species of the human gut microbiota. The median level of Cluster XIVa (*Blautia coccoides* group) showed a significant decrease between control subjects and severe aGVHD patients (p = 0.007), and between mild and severe gut aGVHD patients (p = 0.036). The decreasing trend observed in the levels of *Enterococcus* and *Bacteroides* was not significant after adjustment. Statistical analyses were performed using non-parametric Wilcoxon-Mann-Whitney test with false discovery rate (FDR) correction for multiple testing: FDR p *** <0.001, **<0.01, *<0.05.