LETTER TO THE EDITOR

Mitigation of gastrointestinal graft-*versus*-host disease with tocilizumab prophylaxis is accompanied by preservation of microbial diversity and attenuation of enterococcal domination

Saurabh Chhabra,[1] Aniko Szabo,[2*] Annelie Clurman,[3*] Katelynn McShane,[4] Nicholas Waters,[3] Daniel Eastwood,[2] Lisa Samanas,[5] Teng Fei,[6] Gabriel Armijo,[3] Sameen Abedin,[1] Walter Longo,[1] Parameswaran Hari,[1] Mehdi Hamadani,[1] Nirav N. Shah,[1] Lyndsey Runaas,[1] James H. Jerkins,[1] Marcel van den Brink,[7] Jonathan U. Peled[7#] and William R. Drobyski[1#]

[1]Bone Marrow Transplant and Cellular Therapy Program, Department of Medicine, Medical College of Wisconsin, Milwaukee, WI; [2]Division of Biostatistics, Institute for Health and Equity, Medical College of Wisconsin, Milwaukee, WI; [3]Department of Immunology, Sloan Kettering Institute, Memorial Sloan Kettering Cancer Center, New York, NY; [4]Cancer Center Clinical Trials Office, Medical College of Wisconsin, Milwaukee, WI; [5]Department of Pharmacy, Froedtert Health, Milwaukee, WI; [6]Department of Epidemiology and Biostatistics, Memorial Sloan Kettering Cancer Center, New York, NY and [7]Adult Bone Marrow Transplantation Service, Department of Medicine, Memorial Sloan Kettering Cancer Center, Weill Cornell Medical College, New York, NY, USA

**AS and AC contributed equally as co-second authors.*
[#]JUP and WRD contributed equally as co-senior authors.

Correspondence:
S.CHHABRA - schhabra@mcw.edu
W. DROBYSKI - wdrobysk@mcw.edu

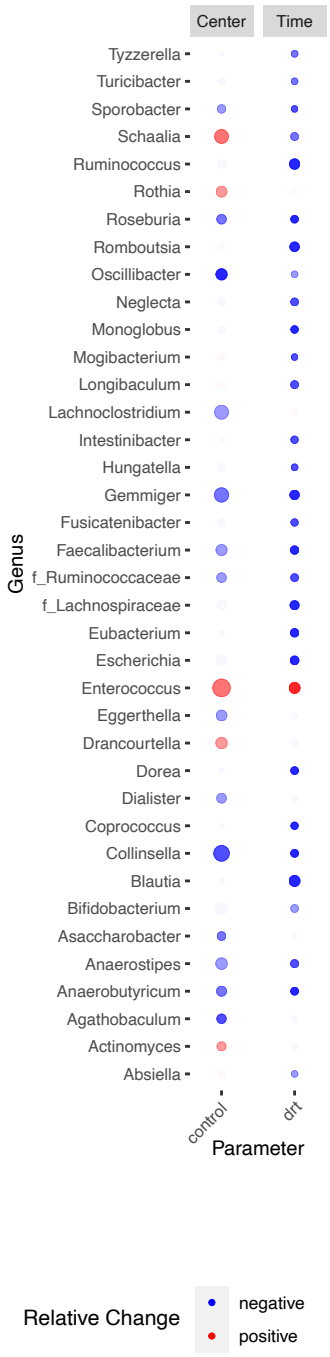
https://doi.org/10.3324/haematol.2022.281309

SUPPLEMENTAL FIGURE LEGEND

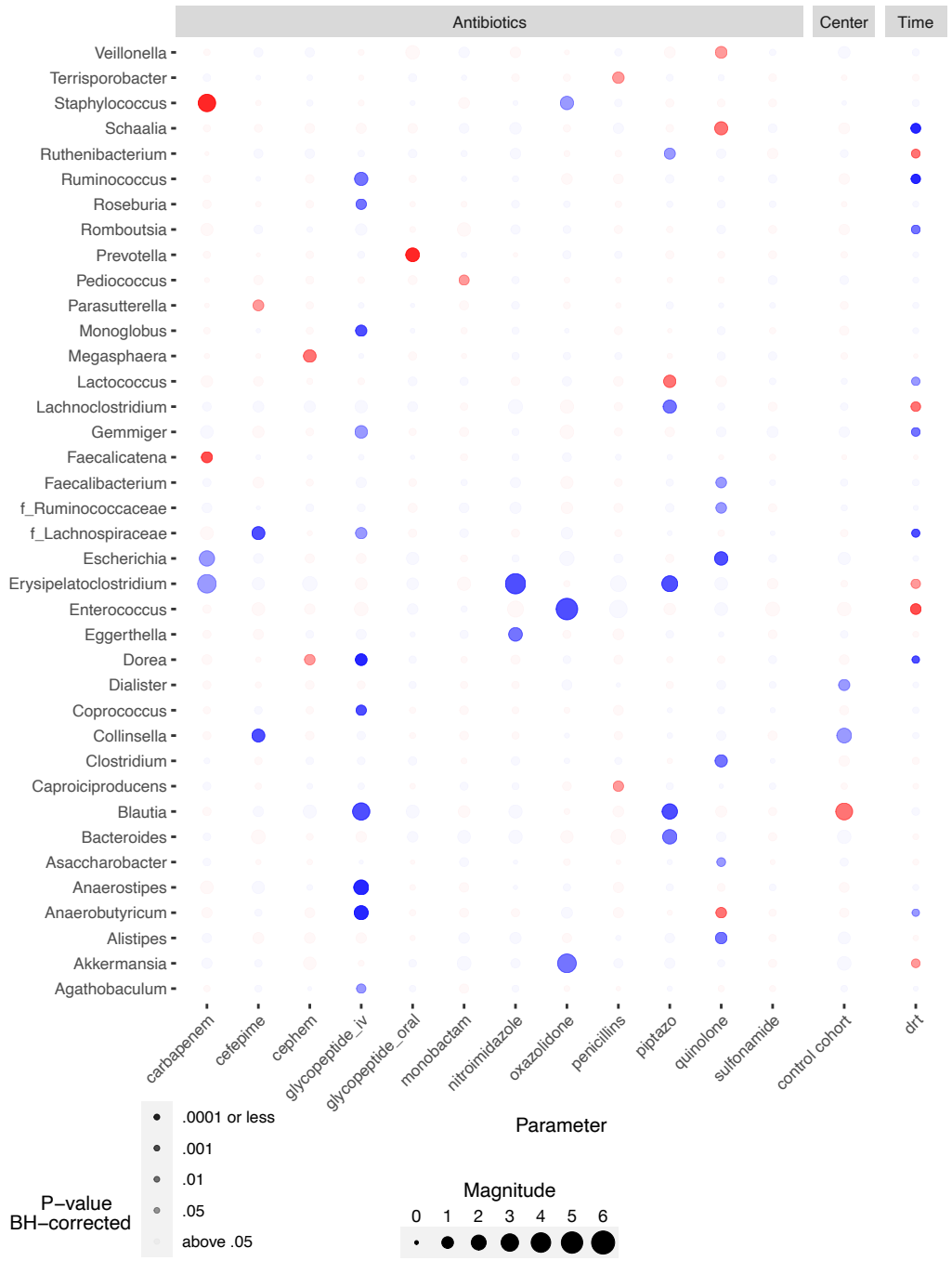
Supplemental Figure 1: **Multivariate analysis of taxonomic profiles between the tocilizumab and control cohorts.** The Maaslin2 package was used to generate mixed-effects models to determine relationships between the microbial composition and patient metadata between the two centers. We considered all post-baseline samples day 0-26, retaining genus-level abundances for taxa present in > 5% of the samples with a relative abundance >0.002. Significance after Benjamini-Hochberg correction is indicated with transparency, and the effect size and direction are shown with size and color, respectively. Patient ID is included as a random effect. (A). Bivariate model shows taxonomic changes over time and by center; data in the "Center" column indicate taxonomic changes in the control cohort relative to the reference toci center. (B). Multivariate plot of the associations between per-sample antibiotic exposures, time, and center which highlight taxonomic shifts associated with antibiotic exposures. (C). Number (and percentage) of 19 toci and 38 control patients exposed to each antibiotic; antibiotic groups included in the multivariate model are indicated by the asterisks (**).

Supplemental Figure 1

A



B



C

Antibiotic	Group	toci	control
levofloxacin	quinolone**	16 (84.2)	3 (7.9)
ciprofloxacin	quinolone**	4 (21.1)	34 (89.5)
pip-tazo	piptazo**	4 (21.1)	19 (50)
cefepime	cefepime**	4 (21.1)	5 (13.2)
metronidazole	nitroimidazole**	3 (15.8)	4 (10.5)
vancomycin(oral)	glycopeptide(oral)**	3 (15.8)	3 (7.9)
vancomycin(iv)	glycopeptide(iv)**	2 (10.5)	37 (97.4)
TMP/SMX	sulfonamide**	2 (10.5)	6 (15.8)
linezolid	oxazolidone**	2 (10.5)	2 (5.3)
aztreonam	monobactam**	1 (5.3)	3 (7.9)
cefuroxime	cephem**	1 (5.3)	2 (5.3)
meropenem	carbapenem**	1 (5.3)	2 (5.3)
amox/clav	penicillins**	1 (5.3)	-
dapsone	antibacterial(other)	1 (5.3)	-
cefazolin	cephem**	-	5 (13.2)
penicillin	penicillins**	-	3 (7.9)
ceftriaxone	cephem**	-	2 (5.3)
ampicillin	penicillins**	-	1 (2.6)
azithromycin	macrolide	-	1 (2.6)
ceftazidime	cephem**	-	1 (2.6)
cephalexin	cephem**	-	1 (2.6)
imipenem	carbapenem**	-	1 (2.6)

SUPPLEMENTAL TABLE 1. PATIENT CHARACTERISTICS OF TOCILIZUMAB TRIAL PATIENTS (N=29)

Variable	
Age, median (range)	57 (24-66)
Sex (M/F)	16/13
Disease (n, %)	
AML	15 (52)
CR1	10 (34)
CR2	4 (14)
Secondary AML, CR1	1 (3)
ALL, CR1	1 (3)
MDS	6 (21)
Myelofibrosis	5 (17)
CML	2 (7)
Donor Type (n, %)	
MRD	12 (41)
MUD	17 (59)
Preparative Regimen (n, %)	
Bu/CY	1 (3)
Flu/Bu4	25 (86)
Flu/Bu3	3 (10)
Graft Source (n, %)	
Peripheral Blood	29 (100)
Disease Risk Index (n, %)	
Intermediate	12 (41)
High	16 (55)
Very High	1 (3)
CMV Serostatus (n, %)	
Donor–/Recipient–	9 (31)
Donor+/Recipient–	1 (3)
Donor+/Recipient+	9 (31)
Donor–/Recipient+	10 (34)
HCT-CI, (n, %)	
0-1	10 (34)
2	7 (24)
3	6 (21)
4-5	6 (21)
ABO match	13 (45)
ABO mismatch	16 (55)
Major	8 (28)
Minor	7 (24)
Bidirectional	1 (3)
KPS, median (range)	80 (70-100)

Median follow up of survivors (range), months	20.9 (14.6-29.6)

AML, acute myelogenous leukemia; ALL, acute lymphoblastic leukemia; MDS, myelodysplasia; CML, chronic myelogenous leukemia; CR, Complete Remission; MRD, matched related donor; MUD, matched unrelated donor; BU, Busulfan; CY, Cyclophosphamide; Flu, Fludarabine; CMV, Cytomegalovirus; HCT-CI, Hematopoietic Cell Transplantation-Comorbidity Index

SUPPLEMENTAL TABLE 2. DEMOGRAPHIC CHARACTERISTICS OF THE TOCILIZUMAB SUBSET AND THE MSK CONTROL COHORT FOR MICROBIOME ANALYSIS

Characteristics	Total	Control	Toci*
Patients (n)	57	38	19
Age (median-range)	61.8 (24-74.7)	66.1 (27.6-74.7)	56 (24-64)
Gender			
Female	22 (38.6)	12 (31.6)	10 (52.6)
Male	35 (61.4)	26 (68.4)	9 (47.4)
Diagnosis (n, %)			
ALL	3 (5.3)	2 (5.3)	1 (5.3)
AML	33 (57.9)	23 (60.5)	10 (52.6)
MDS	14 (24.6)	11 (28.9)	3 (15.8)
MDS/MPN	2 (3.5)	0 (0.0)	2 (10.5)
MPN	5 (8.8)	2 (5.3)	3 (15.8)
Graft Source (n, %)			
PBSC	57 (100.0)	38 (100.0)	19 (100.0)
Conditioning Regimen (n, %)			
BU/CY	1 (1.8)	0 (0.0)	1 (5.3)
Flu/BU3	3 (5.3)	0 (0.0)	3 (15.8)
Flu/BU4	53 (93.0)	38 (100.0)	15 (78.9)

*Subset of the Toci patient cohort that had a pre-transplant and at least one post-transplant stool sample that were both successfully sequenced.

AML, acute myelogenous leukemia; ALL, acute lymphoblastic leukemia; MDS, myelodysplasia; MPN, myeloproliferative disorder; MRD, matched related donor; PBSC, peripheral blood stem cells