Microbiome and Immunotherapy of Cancer

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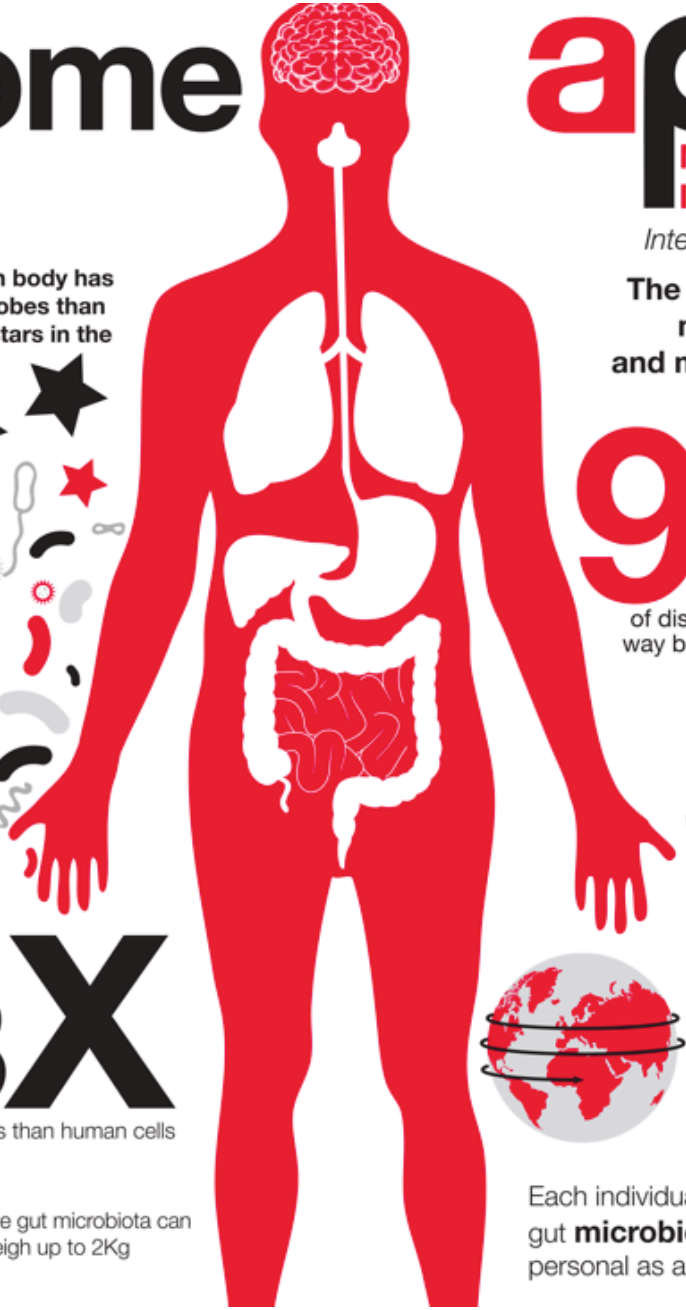
*Disclosures for **Jonathan Peled***

Seres Therapeutics (IP licensing and research support)
DaVolterra (consulting)
MaaT Pharma (consulting)
CSL Behring (consulting)
Parker Institute for Cancer Immunotherapy
Merck/Society for Immunotherapy of Cancer (prior research support)
Postbiotics+ Research LLC (advisory, equity)
Prodigy Biosciences (advisory, equity, research support)

MSK has a financial interest in Seres Therapeutics

Microbiome

IN NUMBERS



Microbiome Ireland

Interfacing Food & Medicine

100 Trillion

symbiotic microbes live in and on every person and make up the human microbiota

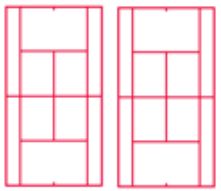
The human body has more microbes than there are stars in the milky way

95%

of our microbiota is located in the GI tract

150:1

The genes in your microbiome outnumber the genes in our genome by about 150 to one



The surface area of the **GI tract** is the same size as 2 tennis courts

You have 1.3X

more microbes than human cells

>10,000

Number of different microbial species that researchers have identified living in and on the human body

2kg

The gut microbiota can weigh up to 2Kg

The microbiome is more medically accessible and manipulable than the human genome

90%

It is thought that of disease can be linked in some way back to the gut and health of the microbiome

5:1

Viruses:Bacteria in the gut microbiota

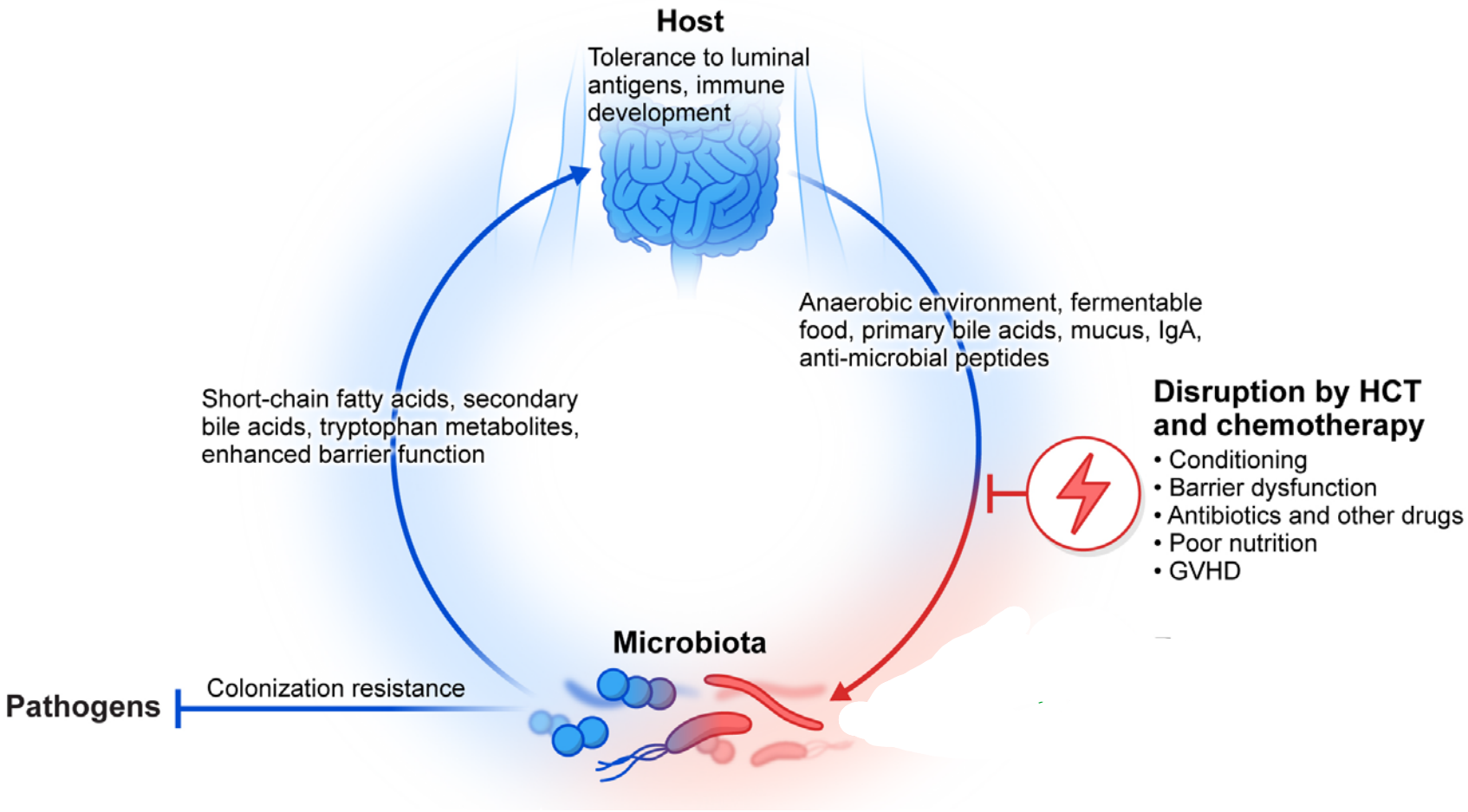
2.5

The number of times your body's microbes would circle the earth if positioned end to end

Each individual has a unique gut **microbiota**, as personal as a fingerprint

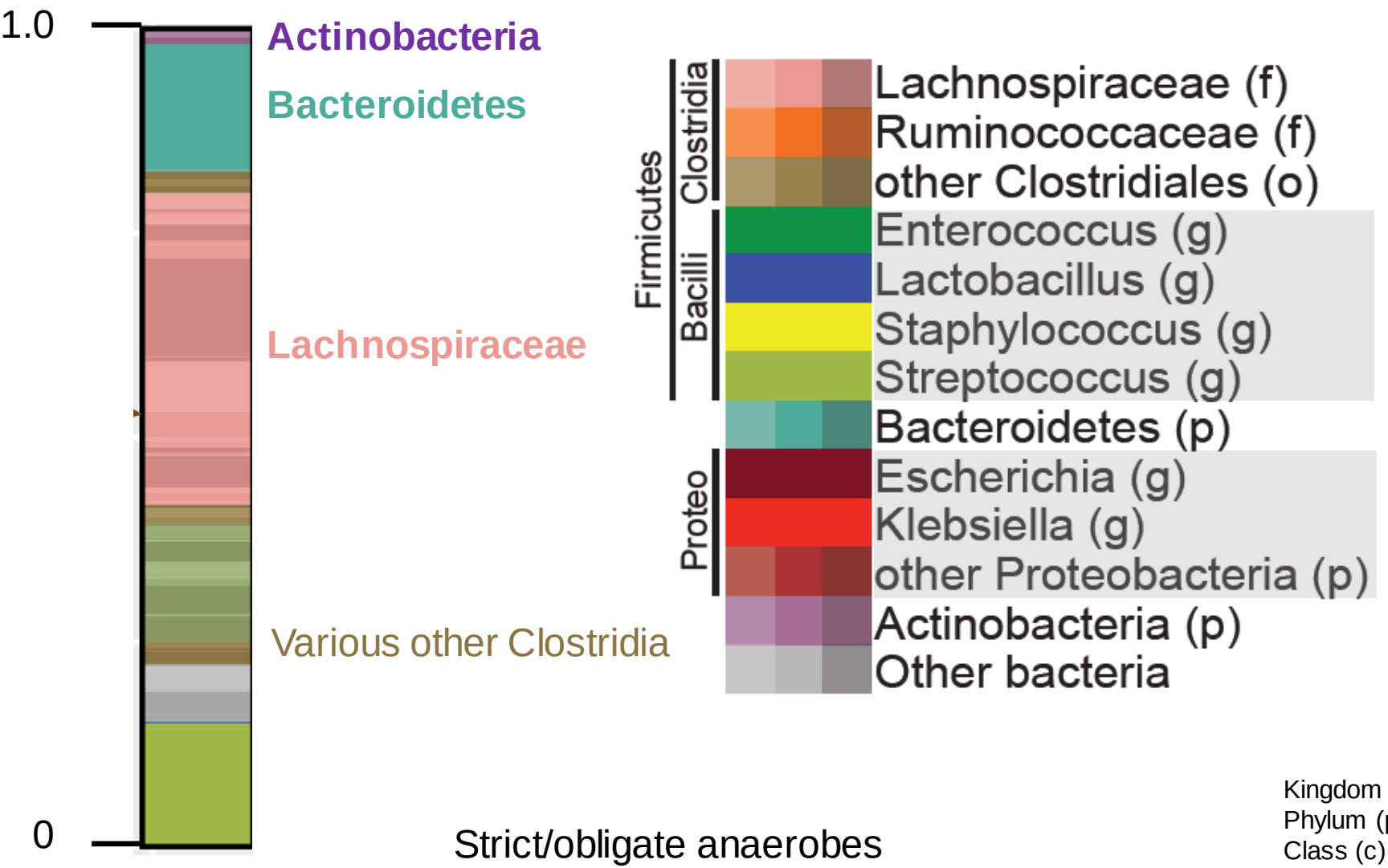
worldmicrobiomeday.com/human-microbiome

Homeostatic feedback between mammalian host and its intestinal microbiome



Peled *et al. J Clin Oncol* 2023

Example of a healthy stool sample profile

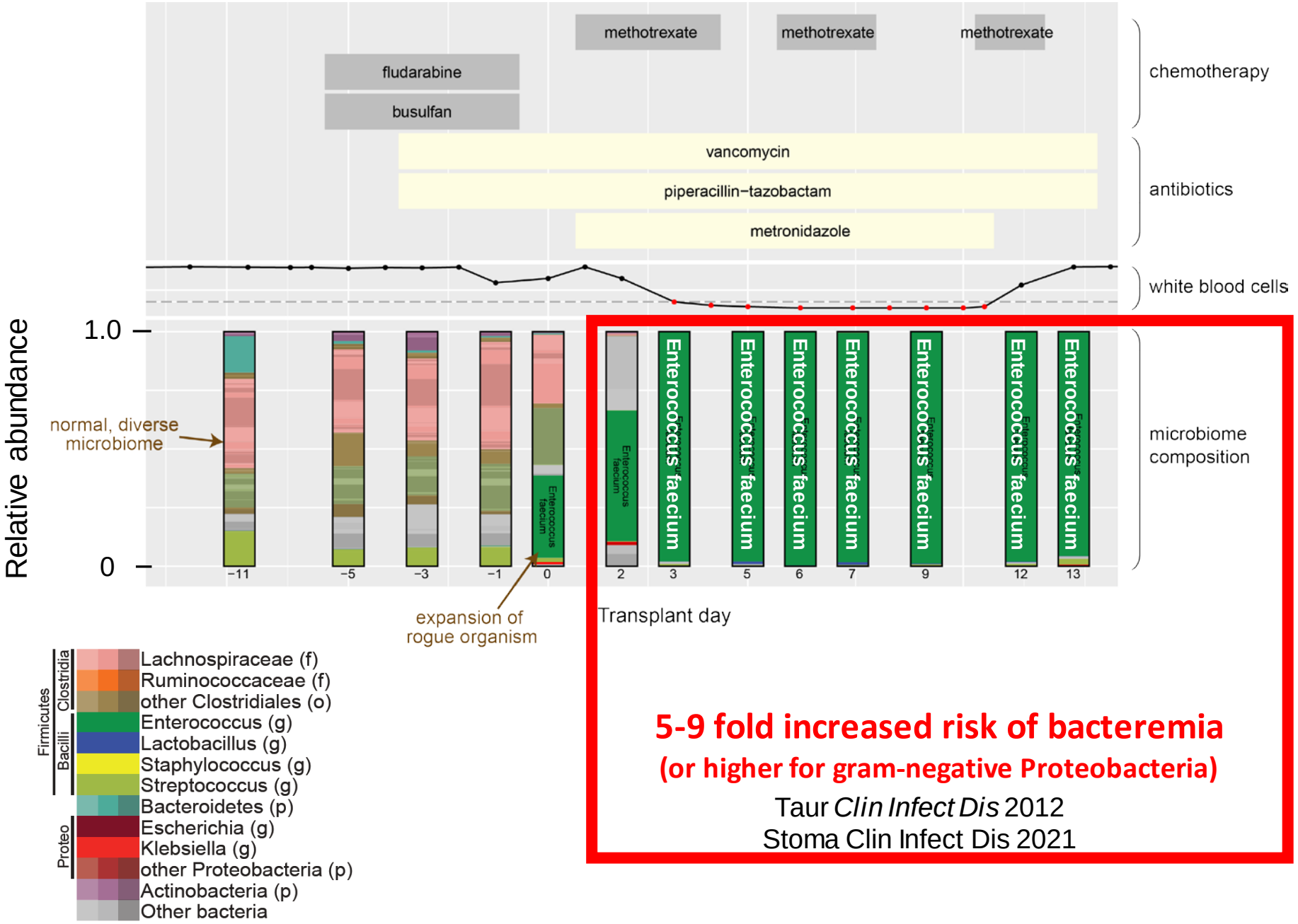


Strict/obligate anaerobes

Facultative/pathobionts

Kingdom	King
Phylum (p)	Phillip
Class (c)	Came
Order (o)	Over
Family (f)	From
Genus (g)	Great
Species (s)	Spain

Major shifts are observed in the microbiota during allo-HCT admissions



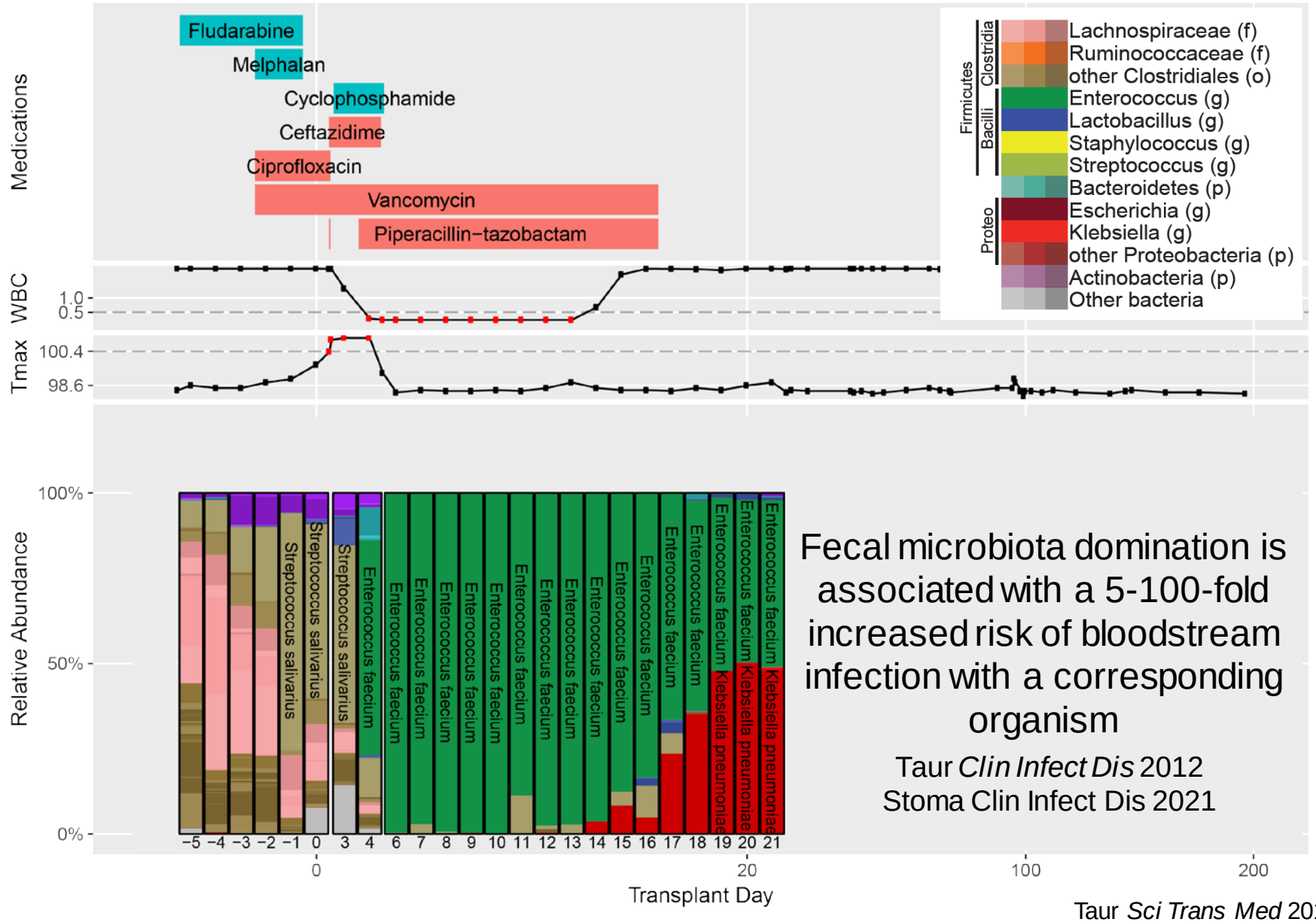
5-9 fold increased risk of bacteremia

(or higher for gram-negative Proteobacteria)

Taur *Clin Infect Dis* 2012

Stoma Clin Infect Dis 2021

Microbiota Disruption in Common in allo-HCT



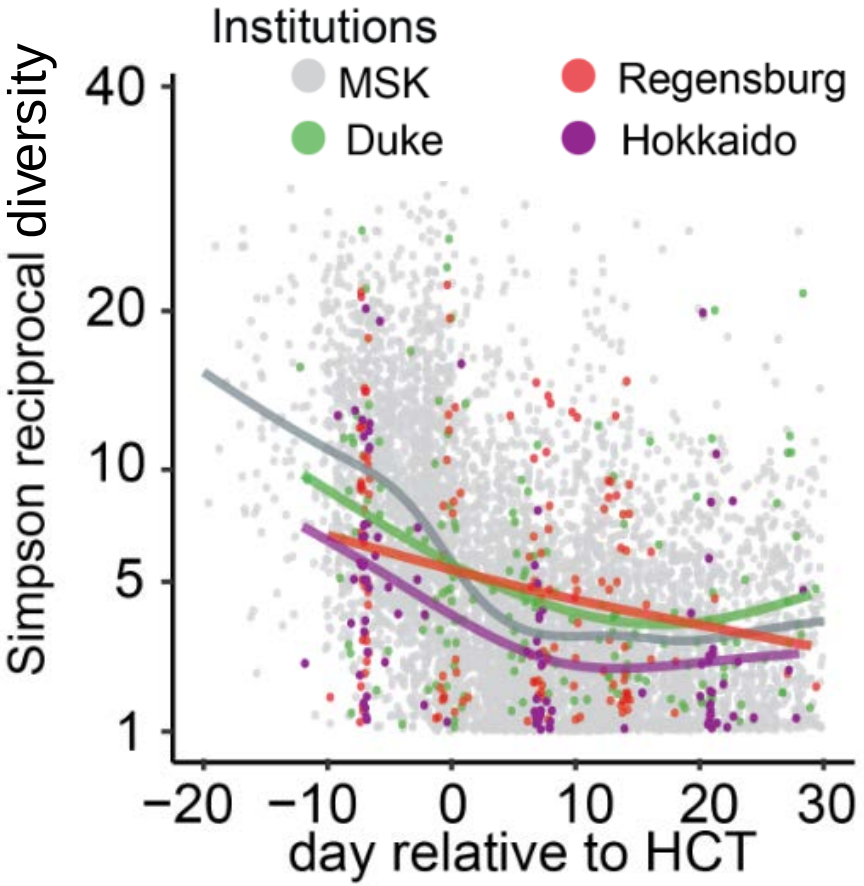
Fecal microbiota domination is associated with a 5-100-fold increased risk of bloodstream infection with a corresponding organism

Taur *Clin Infect Dis* 2012
Stoma Clin Infect Dis 2021

Taur *Sci Trans Med* 2018

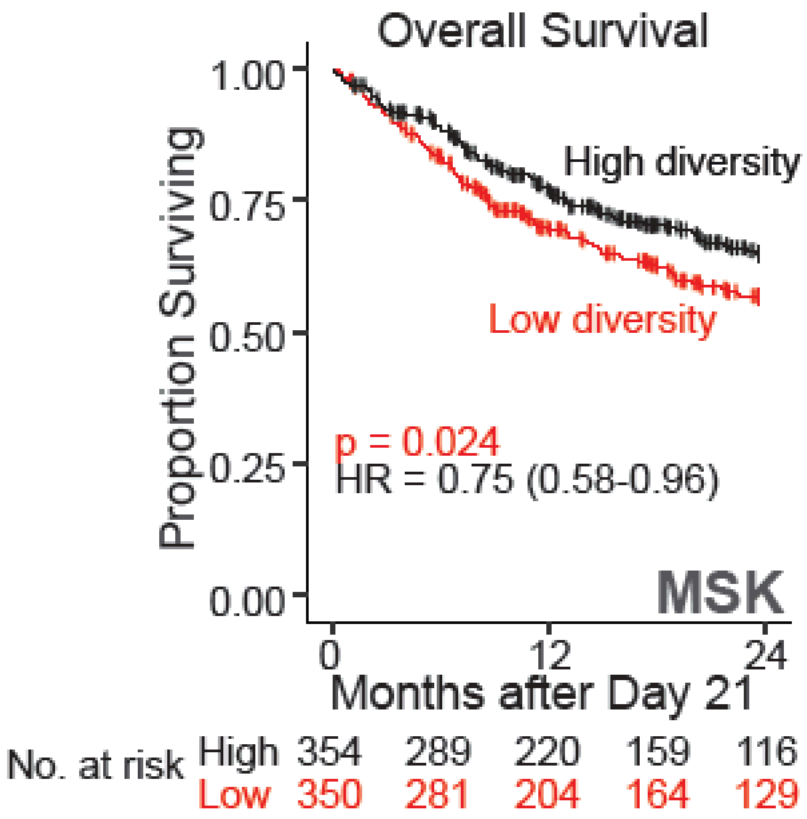
intestinal microbiota injury and clinical outcomes after allo-HCT are reproducible across geography

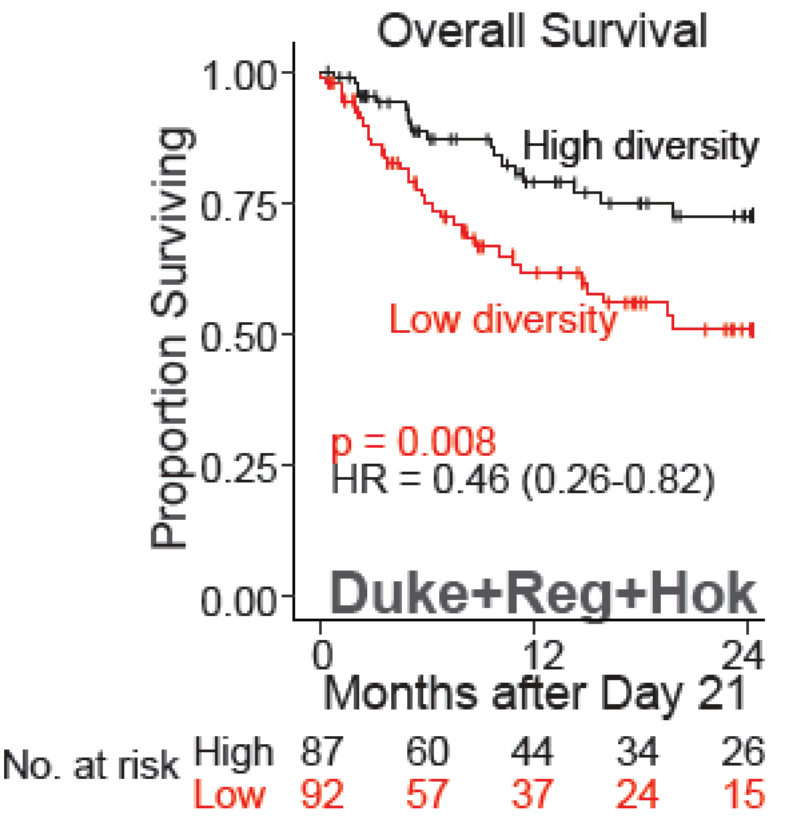
- 8,768 samples from 1,362 HCT recipients from 4 international institutions were centrally analyzed
- Overall survival was reproducibly associated with diversity pre-HCT & peri-neutrophil engraftment
- This relationship required the presence of T cells in the graft
- A composition-based risk score was predictive of clinical outcome



Peled *et al*. *NEJM* 2020

The association of OS with intestinal microbial diversity peri-neutrophil-engraftment is reproducible



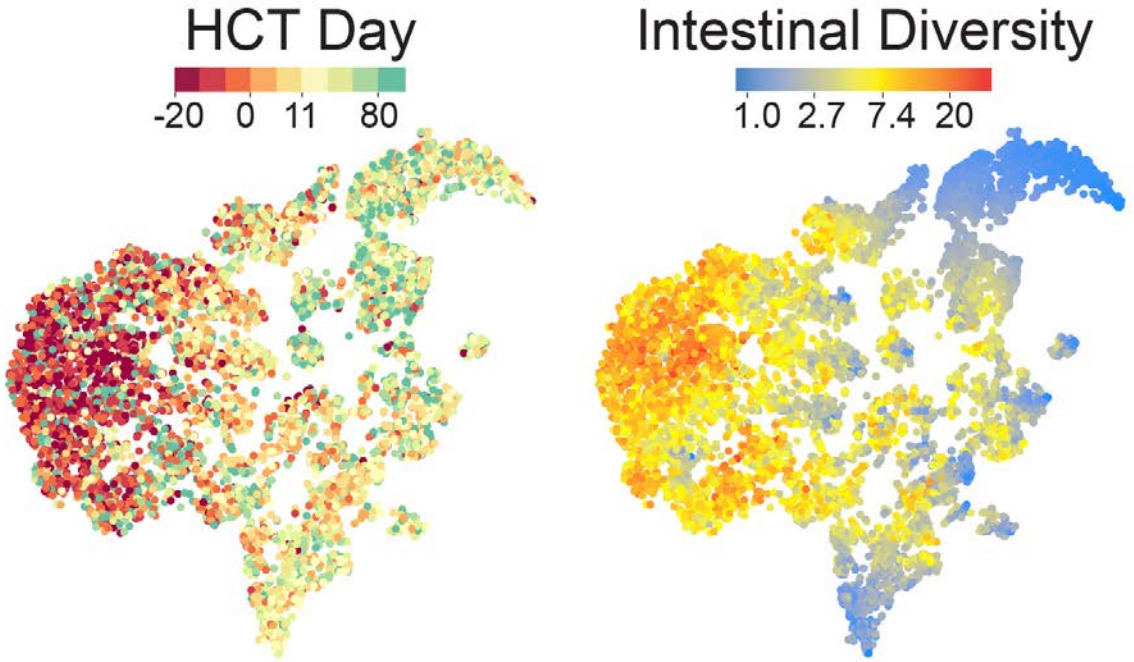


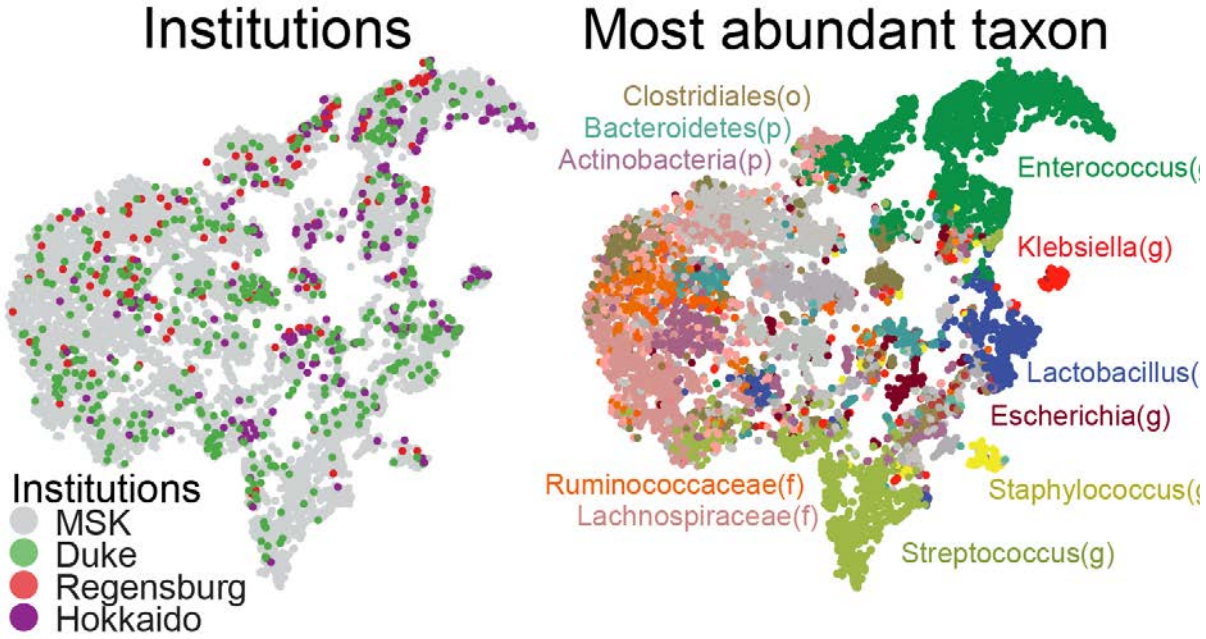
Fecal Microbiome Diversity
Low
High

stratified by above- and below-median Simpson Reciprocal Index in each cohort
single sample per patient, collected day 14 +/- 7

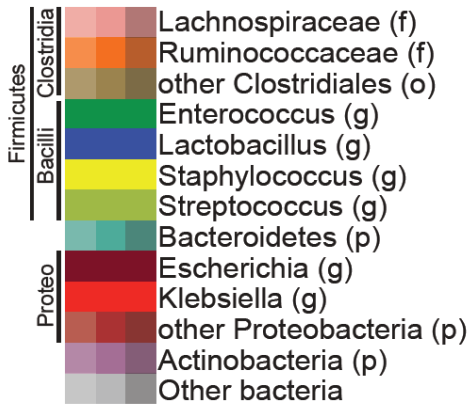
Peled *et al*. *NEJM* 2020

Microbiota injury patterns are comparable across geography



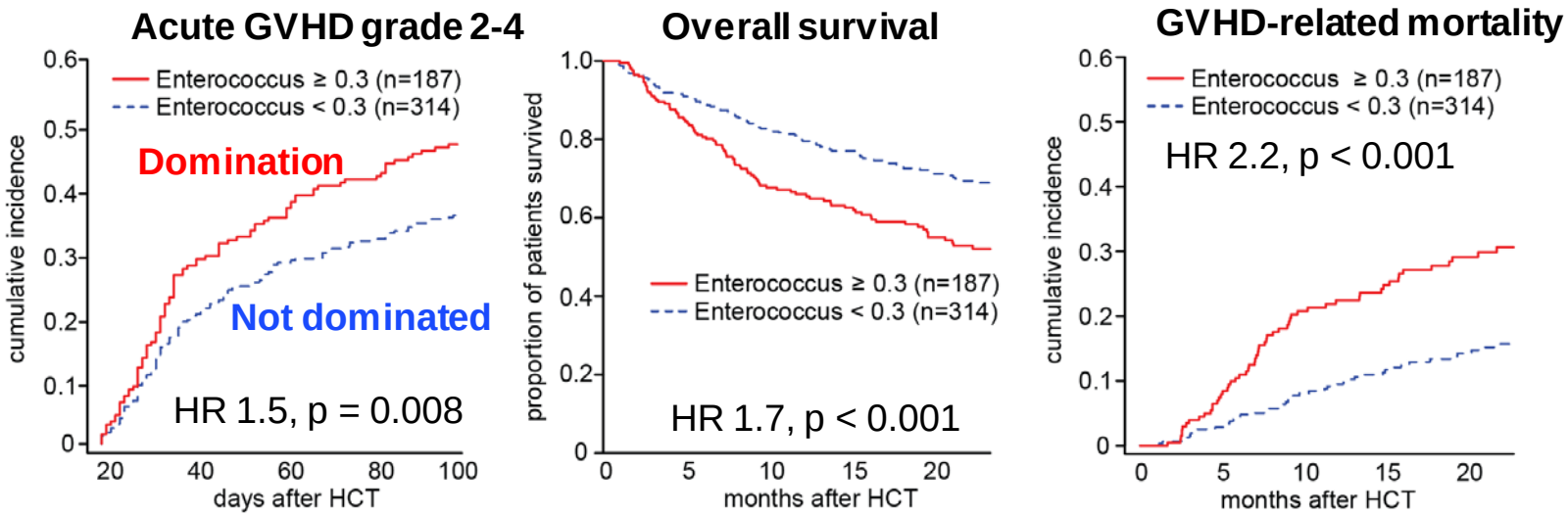


8,691 samples
1,361 HCT recipients
4 institutions



tSNE2
tSNE1

Enterococcus domination of gut flora increases risk for acute GVHD and reduces survival

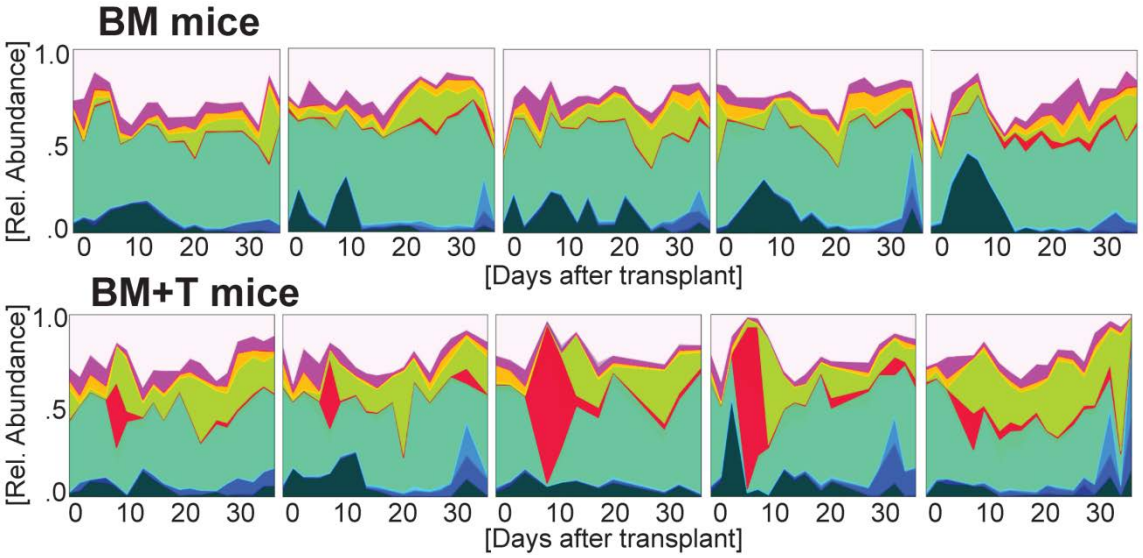


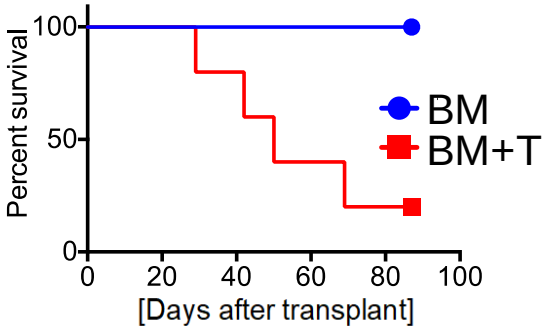
Day 0-21 fecal samples.
N = 501 BM/PBSC (72%) and cord-blood (28%)
TCDs excluded.

Stein-Thoeringer… Peled*, van den Brink* *Science* 2019

Enterococcus dominates the gut microbiota early after allo-HCT across several different mouse models

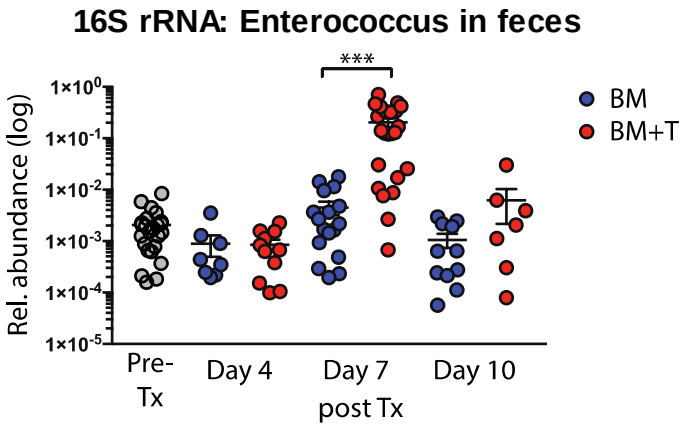
B6→ 129 (MHC-matched)



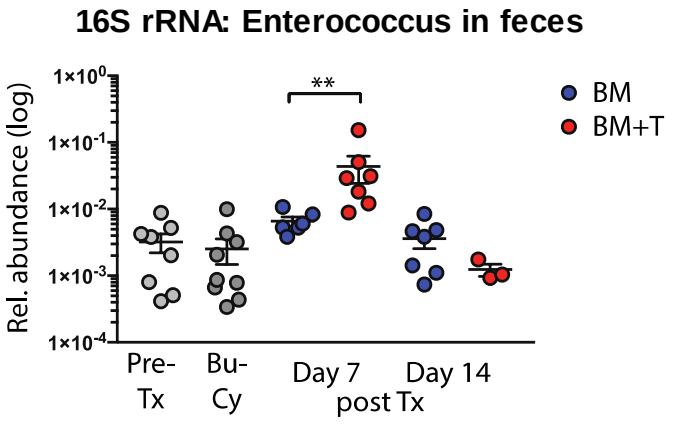


Enterococcus
Clostridia
Allobacullum
Lactobacillus
Oscillospira
Bacillus
Roseburia

B6→ BALB/c (MHC-disparate)

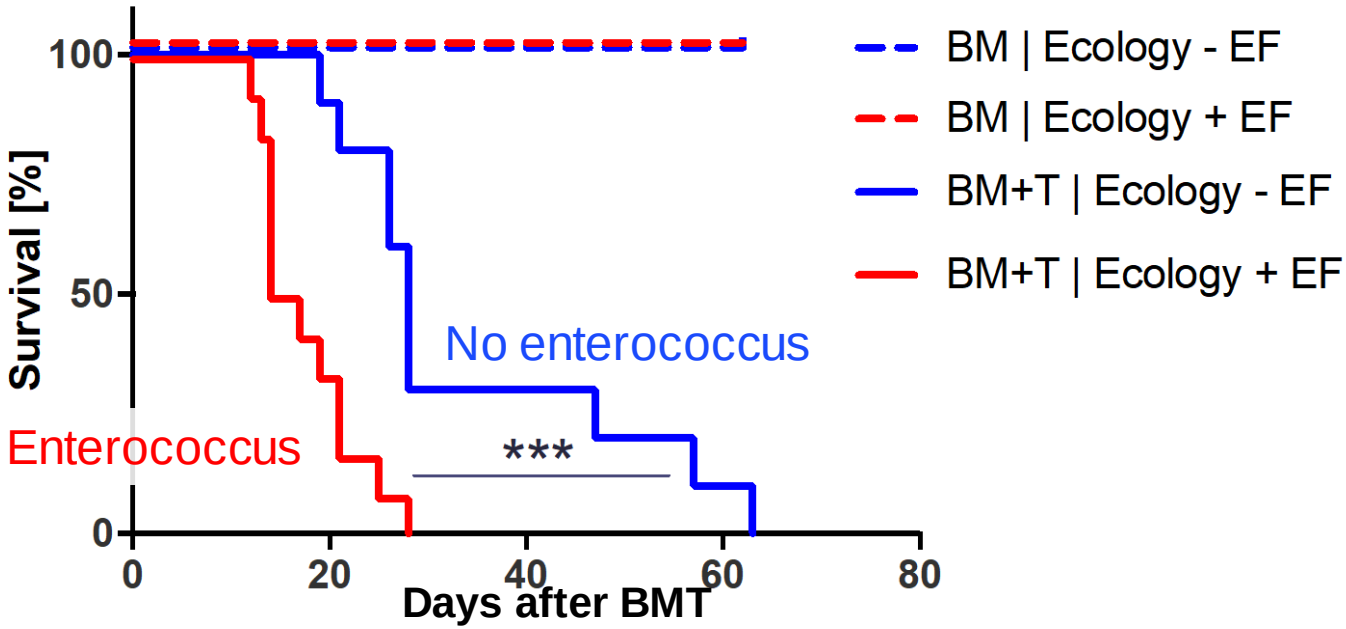


LP→ B6 (MHC-matched; Bu-Cy conditioning)



Enterococcus faecalis aggravates lethal GVHD in a gnotobiotic mouse model

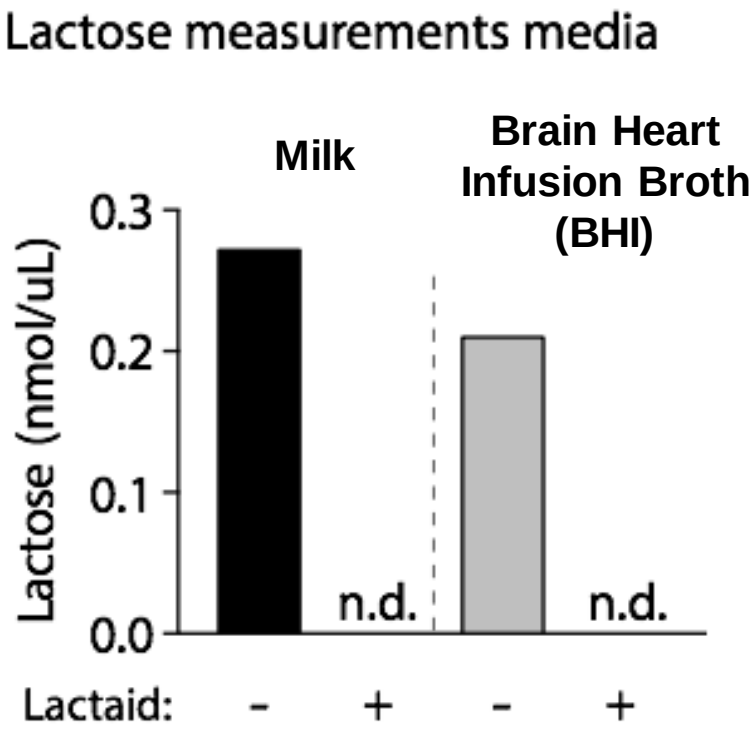
germ-free C57BL/6 mice
Colonized with with a 6-strain minimal bacterial ecology prior to allo-HCT
+/- Enterococcus faecalis

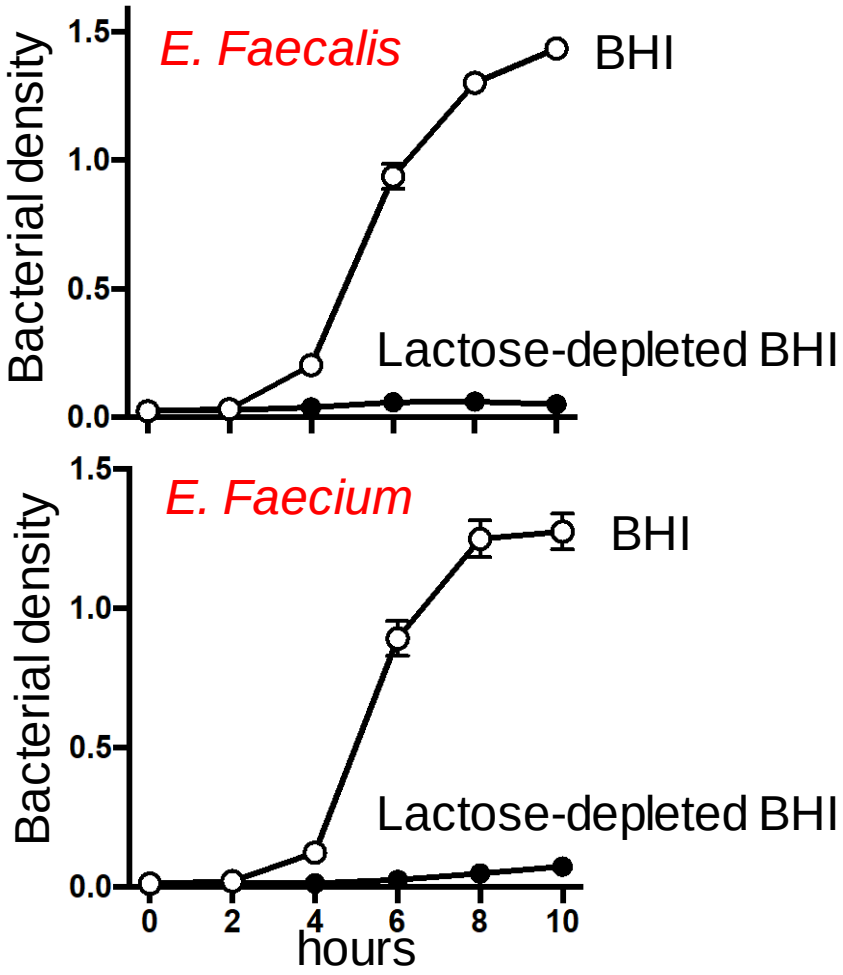


MHC-matched BMT
LP → C57BL/6 Bu-Cy conditioning
N = 10-12/group

Stein-Thoeringer… Peled*, van den Brink* *Science* 2019

E. faecalis and VRE encode lactose- and galactose-degradation enzymes and require lactose for growth *in vitro*

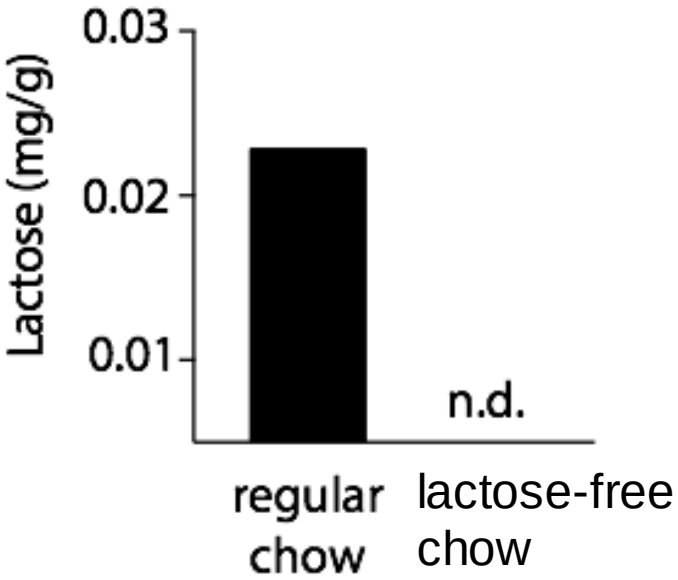




Standard laboratory mouse diet contains lactose

INGREDIENTS

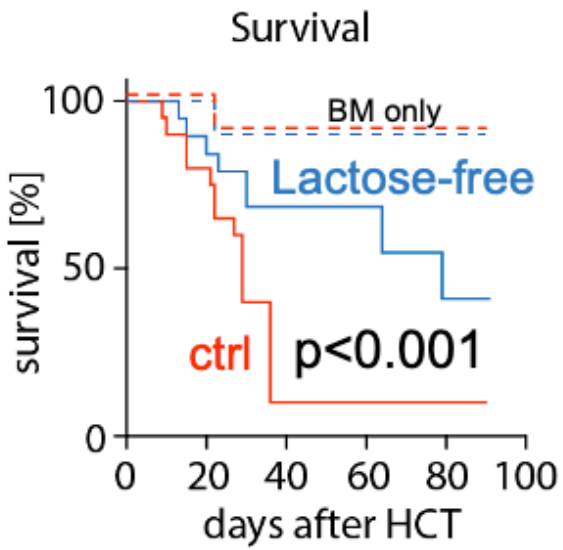
Ground Corn, Dehulled Soybean Meal, Wheat Middlings, Whole Wheat, Fish Meal, Dried Beet Pulp, Wheat Germ, Cane Molasses, Brewers Dried Yeast, Ground Oats, Dehydrated Alfalfa Meal, Soybean Oil, Whey, Calcium Carbonate, Salt, DL-Methionine, Menadione Dimethylpyrimidinol Bisulfite (source of Vitamin K), Choline Chloride, Pyridoxine Hydrochloride, Cholecalciferol, Vitamin A Acetate, DL-Alpha Tocopheryl Acetate (Form of Vitamin E), Biotin, Folic Acid, Thiamine Mononitrate, Vitamin B-12 Supplement, Nicotinic Acid

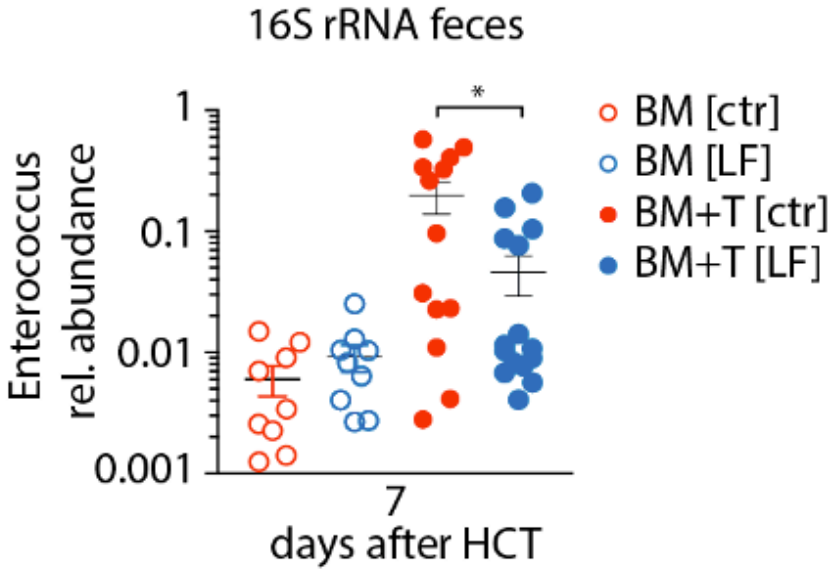


Nitrogen-Free Extract (by difference), %	**53.4**
Starch, %	28.2
Glucose, %	0.19
Fructose, %	0.24
Sucrose, %	3.25
Lactose, %	1.34

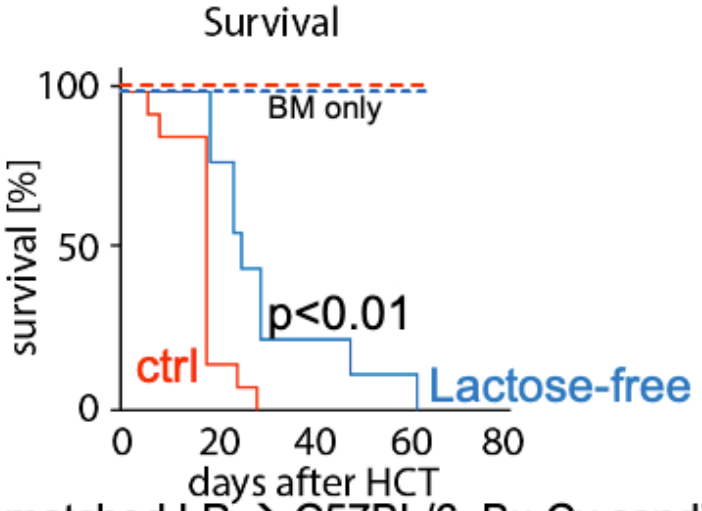
PicoLab Rodent Diet 20 #5053

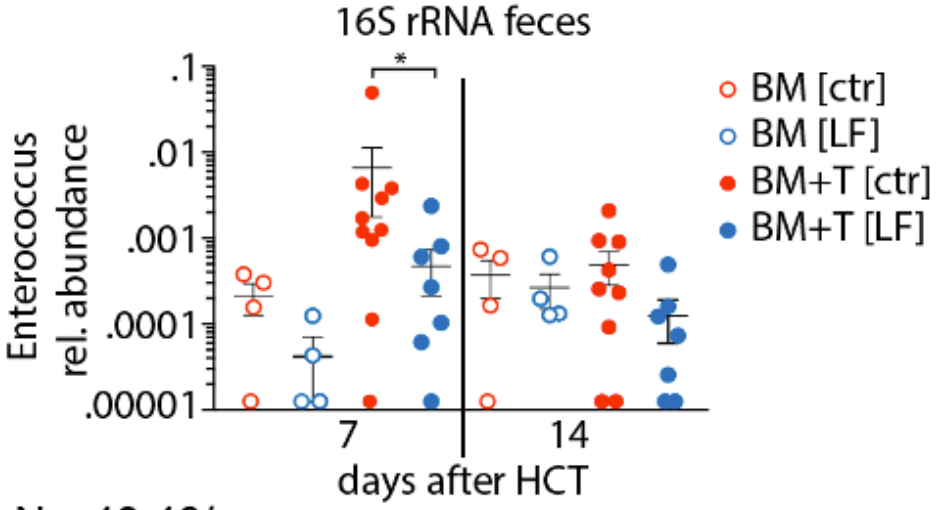
Enterococcus *bloom* after allo-HCT is significantly attenuated by feeding mice lactose-free chow





MHC-disparate, B6 → BALB/c XRT, N = 20/group





MHC-matched LP → C57BL/6, Bu Cy conditioning, N = 13-19/group

Factors hypothesized to influence microbiome composition in cancer patients

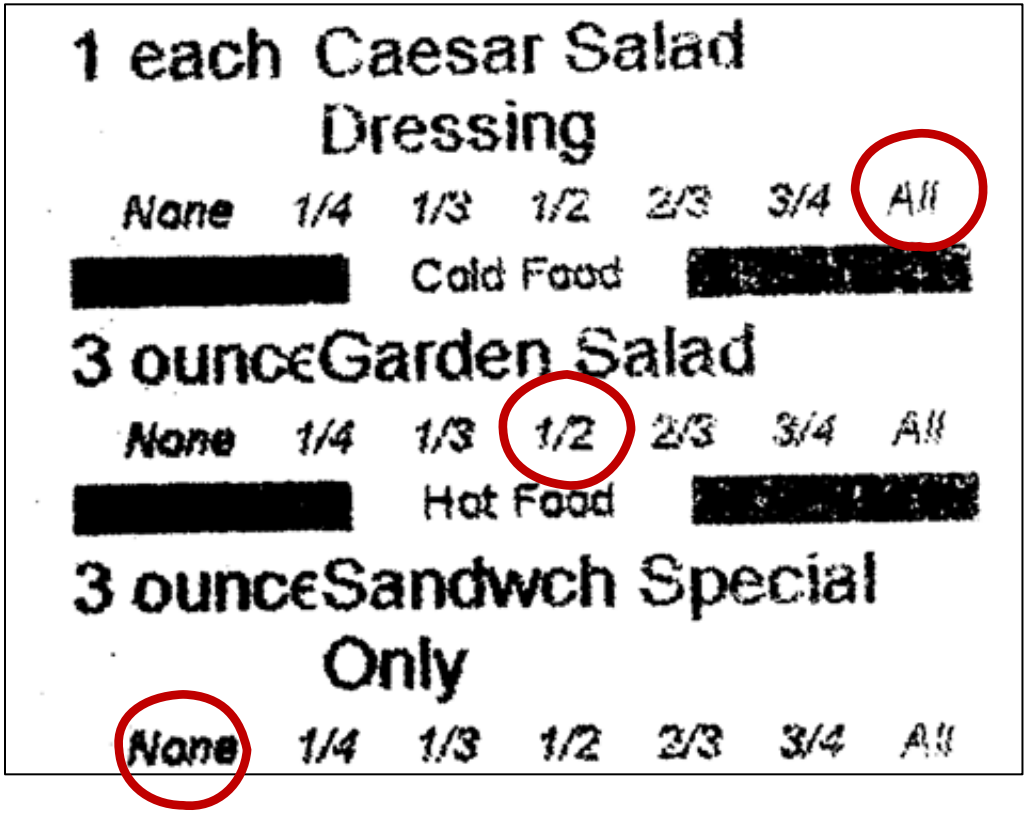
- antibiotics
- chemotherapy/irradiation
- intestinal inflammation
- other drugs
- diet

Data were collected via the hospital kitchen computer system, which contains recipes and nutritional information for each meal


07/22/16 11:14 AM
Reprint

Fri 07/22/16 LU1
Test,
Patient
DOB:
Rm: 1031B
Diet: Regular

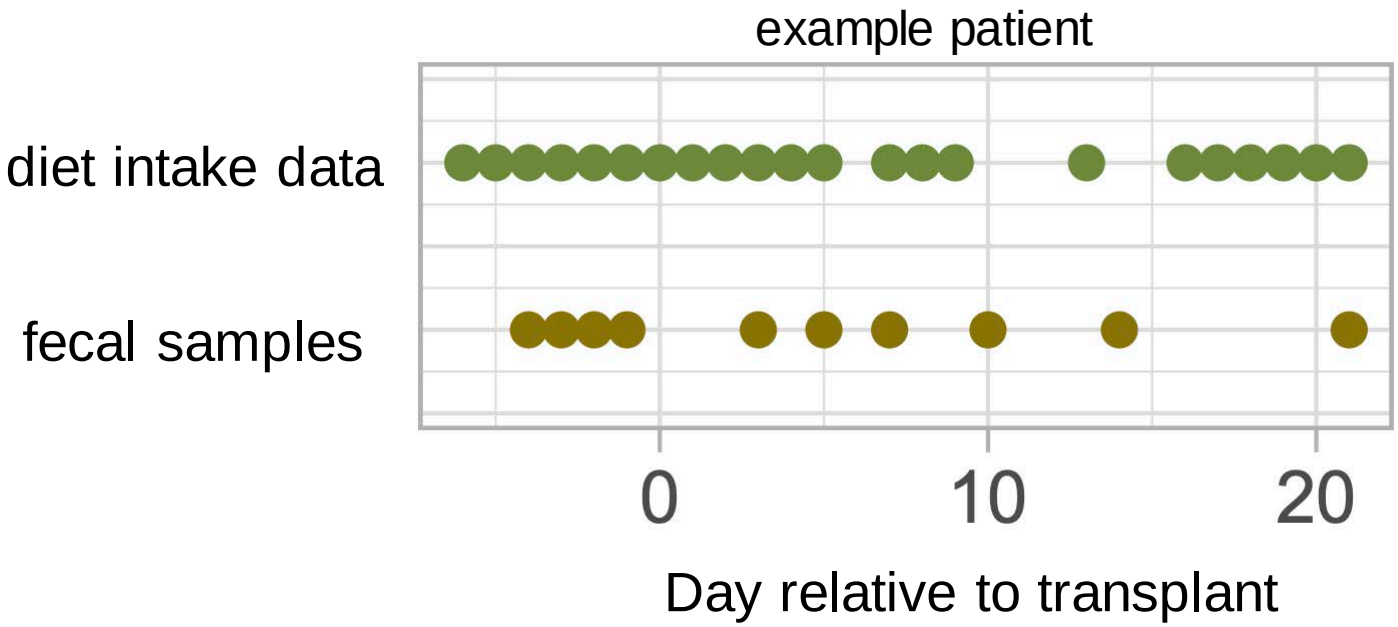
[redacted] Middle [redacted]
6 ounce Soup Special Only
(78 gm)
None 1/4 1/3 1/2 2/3 3/4 All
3 pkt Creamy Ital Drsg Pkt
None 1/4 1/3 1/2 2/3 3/4 All
1 each Caesar Salad Dressing
None 1/4 1/3 1/2 2/3 3/4 All
[redacted] Cold Food [redacted]
3 ounce Garden Salad
None 1/4 1/3 1/2 2/3 3/4 All
[redacted] Hot Food [redacted]
3 ounce Sandwch Special Only
None 1/4 1/3 1/2 2/3 3/4 All
1 serv FILLET OF SOLE
None 1/4 1/3 1/2 2/3 3/4 All
1 serv BUTTERNUT FARRO
None 1/4 1/3 1/2 2/3 3/4 All


1 each Caesar Salad Dressing
None 1/4 1/3 1/2 2/3 3/4 All
[redacted] Cold Food [redacted]
3 ounce Garden Salad
None 1/4 1/3 1/2 2/3 3/4 All
[redacted] Hot Food [redacted]
3 ounce Sandwch Special Only
None 1/4 1/3 1/2 2/3 3/4 All

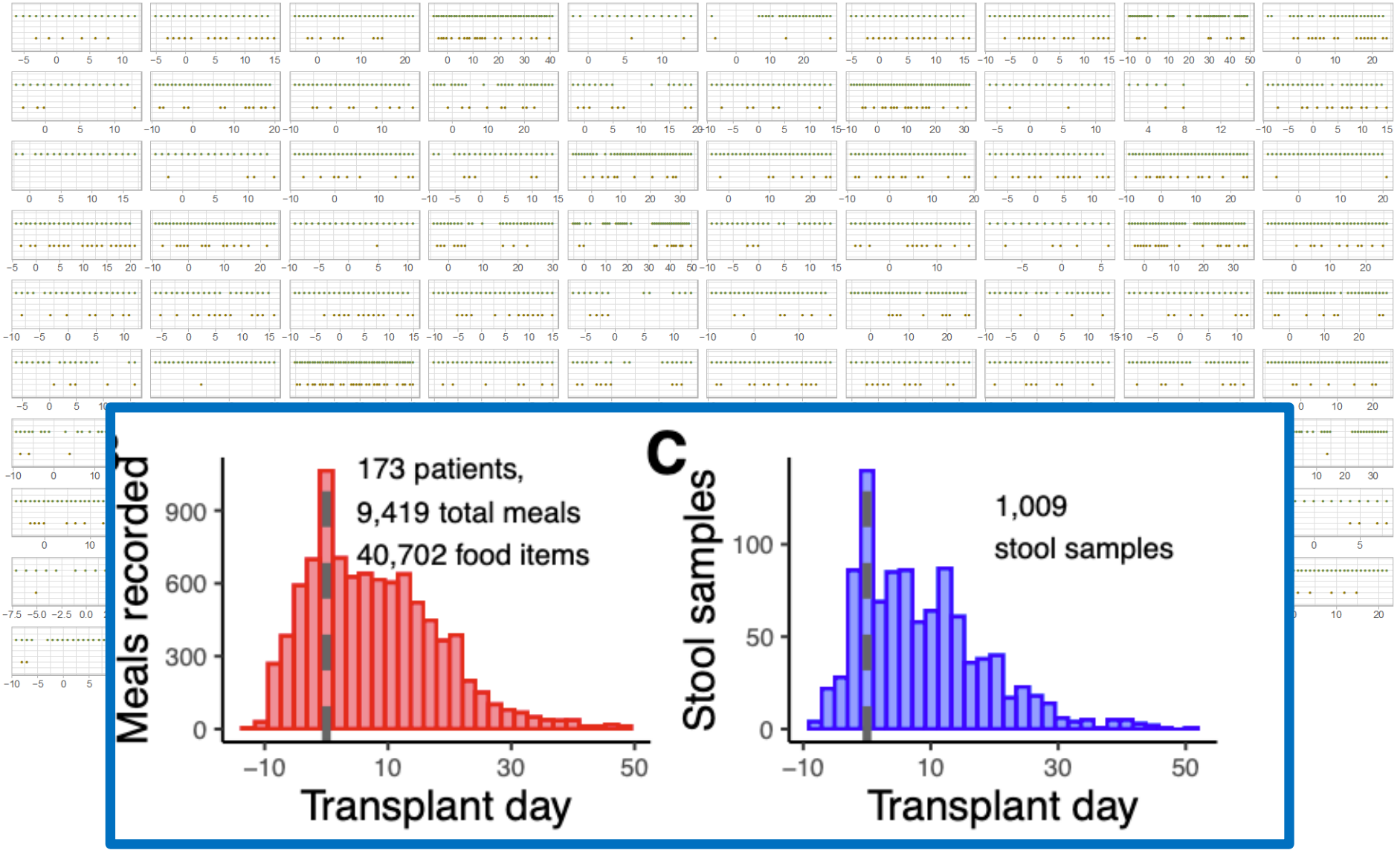
This is in contrast to traditional approaches:

- Food-frequency questionnaire
- Dietary recall survey
- Assignment of volunteers to prescribed diets

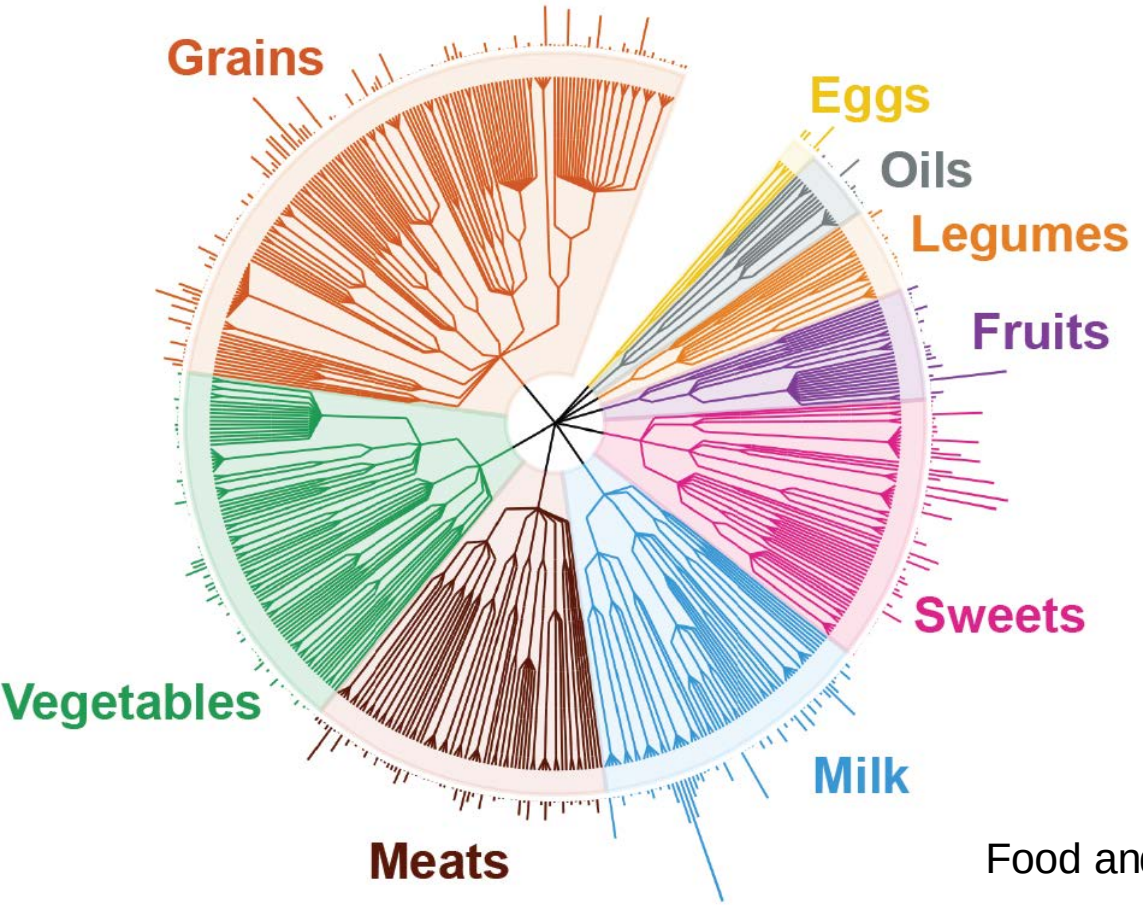
Serial stool samples and near-daily dietary intake data were collected



We analyzed 40,702 food entries and 1,009 fecal samples from 173 patients



A hierarchical food taxonomy facilitates application of ecological metrics to complex food data



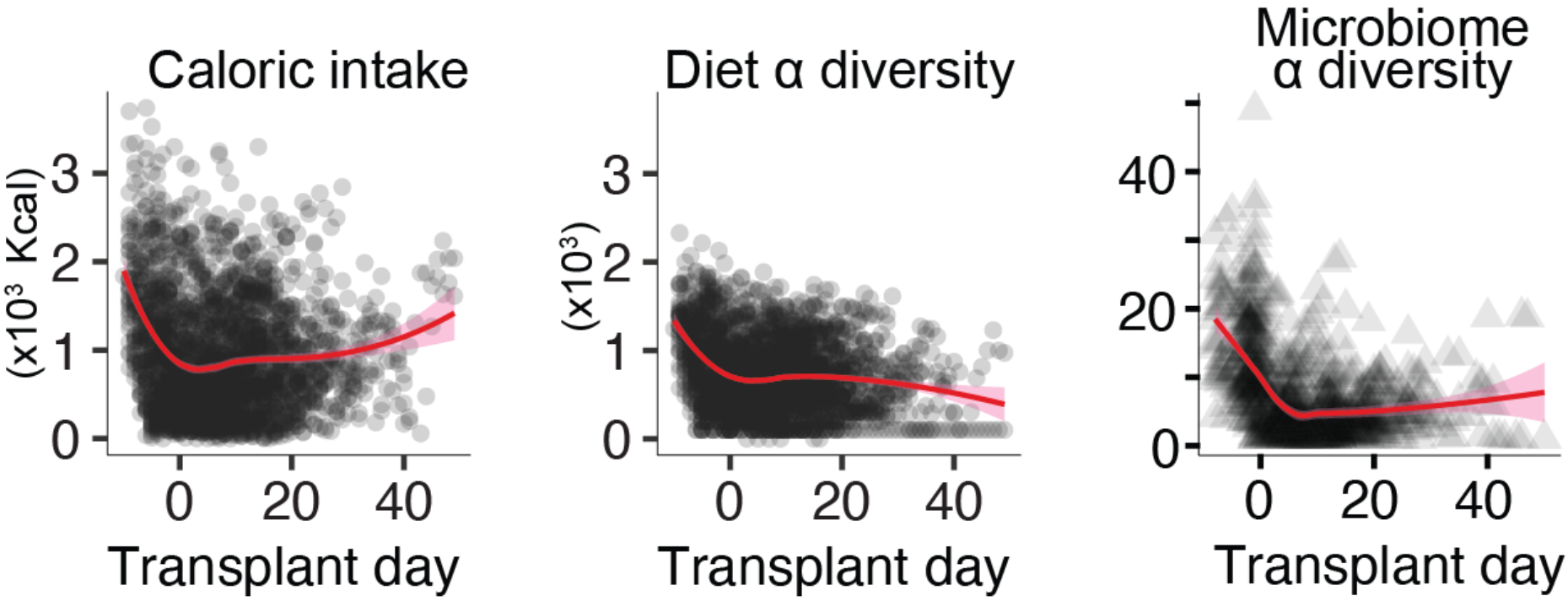
Level.code	Main.food.description
3	Eggs
31	Eggs
311	Chicken_eggs
3110	Egg_whole_or_white
3111	Egg_yolk_only
312	Other_poultry_eggs
32	Egg_mixtures
321	Egg_dishes_made_with_whole_eggs
3213	Egg_omelet_or_scrambled
322	Egg_sandwiches
3220	Egg_sandwiches
32201	Fried_egg_sandwhich
32202	Egg_with_cheese_or_meat
32203	Egg_salad_sandwhich
32204	Scrambled_egg_sandwhich
323	Egg_soups

Food and Nutrient Database for Dietary Studies (FNDDS)

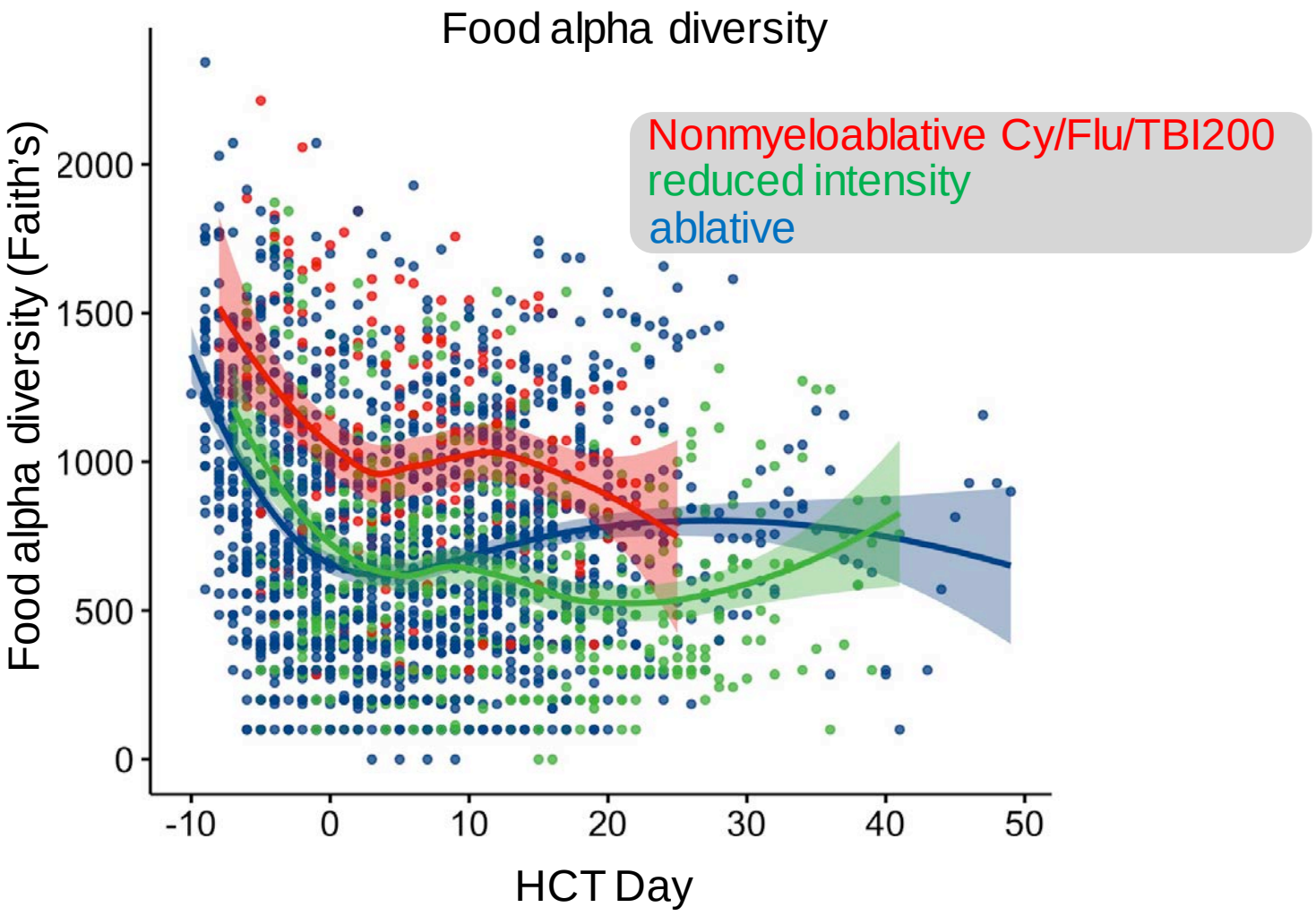
U.S. Department of Agriculture

Johnson *Cell Host Microbe* 2019

Nutrition intake declines early in transplant



Dietary alpha diversity declines during transplant in a conditioning-regimen-specific fashion



A Bayesian mixed-effects model was constructed to identify diet components associated with the outcome of fecal microbiome diversity

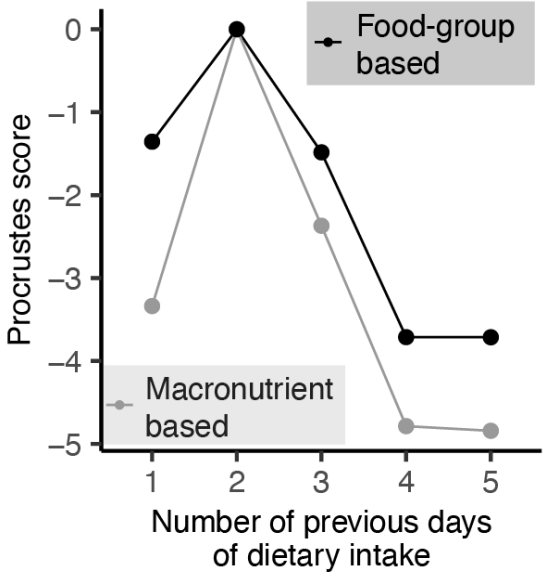
- *Outcome variable:*

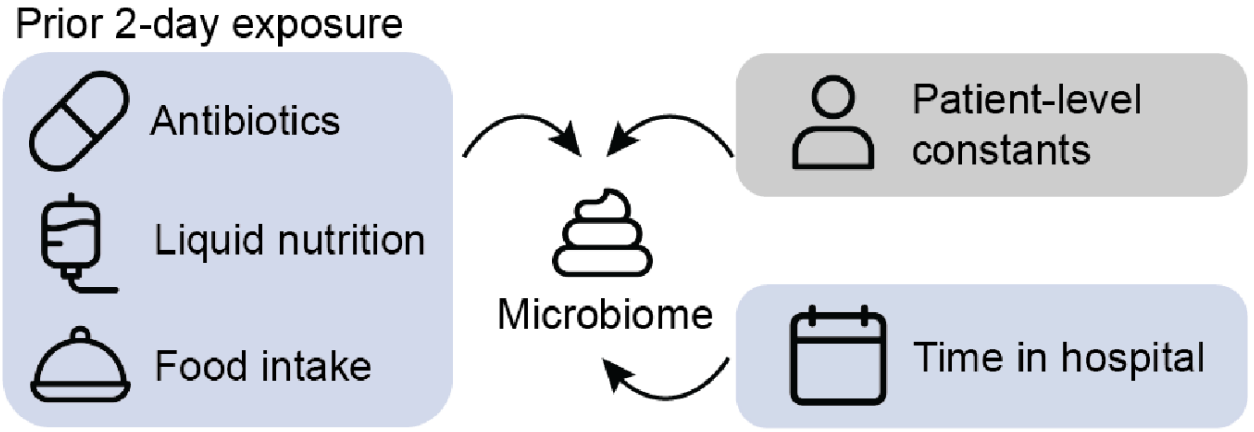
  Log transformed fecal alpha diversity (Simpson's reciprocal)

- *Predictors:*

Fixed effects
- Food consumption
- Exposure to enteral nutrition (tube feeds) or total parenteral nutrition (TPN)
- Conditioning regimen intensity
- Exposure to empirical antibiotics*

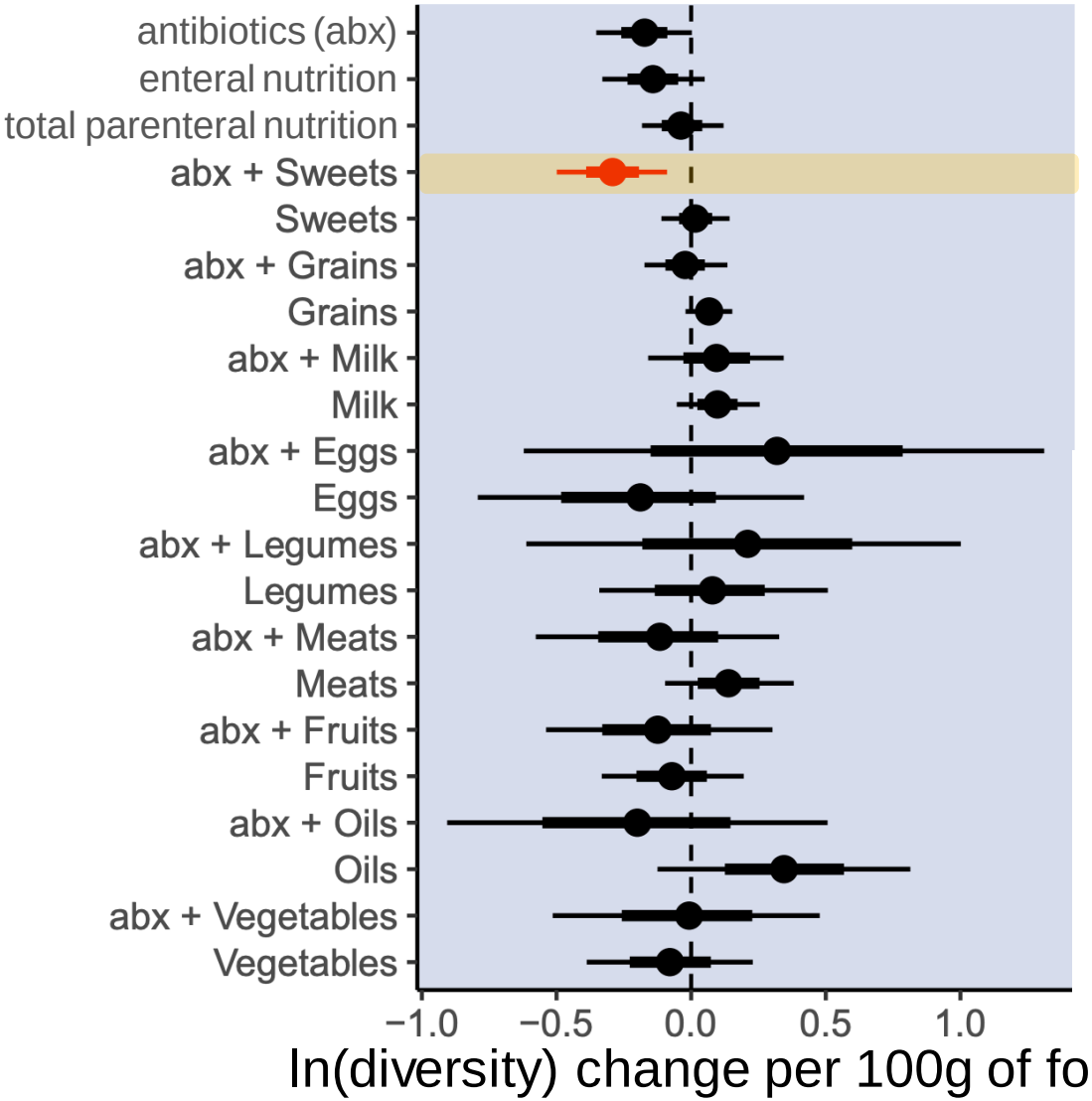
Random effects
- Patient-level term
- Time (week of fecal sample collection relative to HCT)

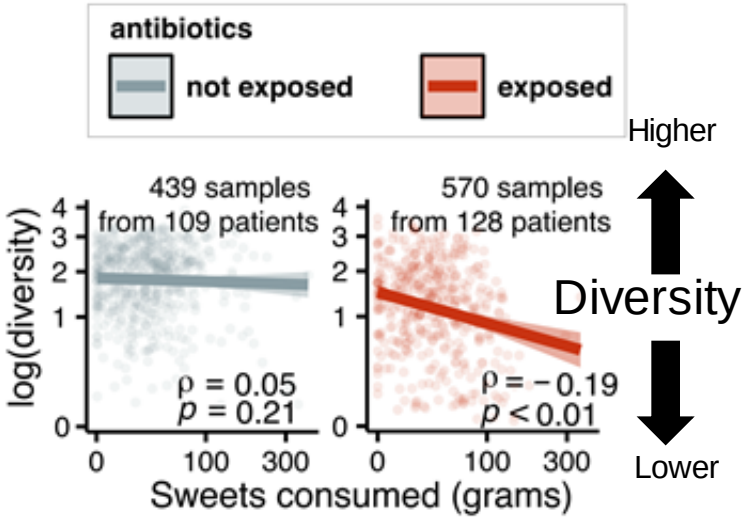




*Empirical antibiotics for neutropenic fever (pip/tazo, carbapenems, cefepime, linezolid) & *C. diff* (oral vancomycin, metronidazole). Prophylactic abx were ignored.

Low fecal diversity is most strongly associated with foods rich in simple sugars (interacting with abx) in the prior two days

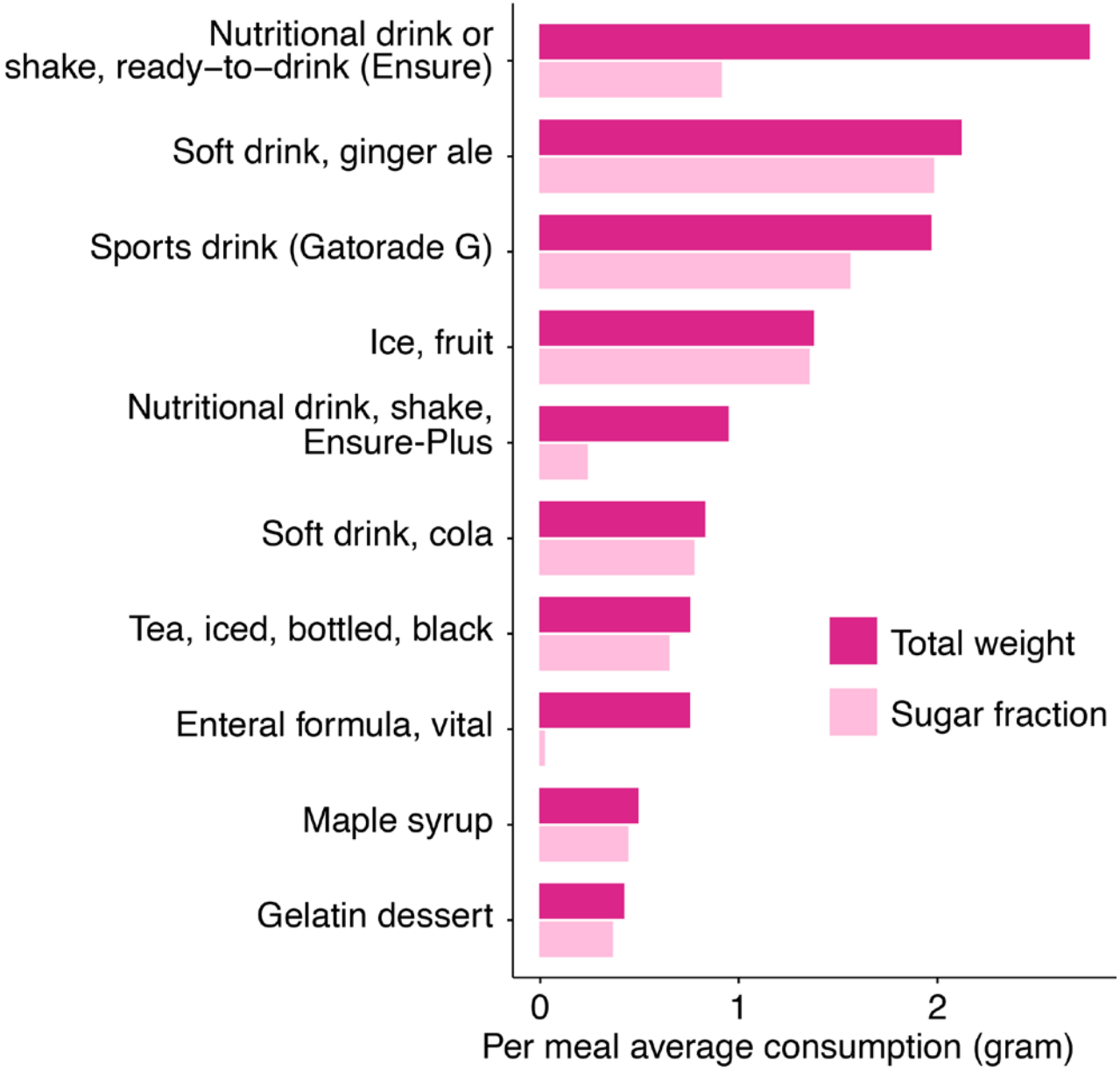




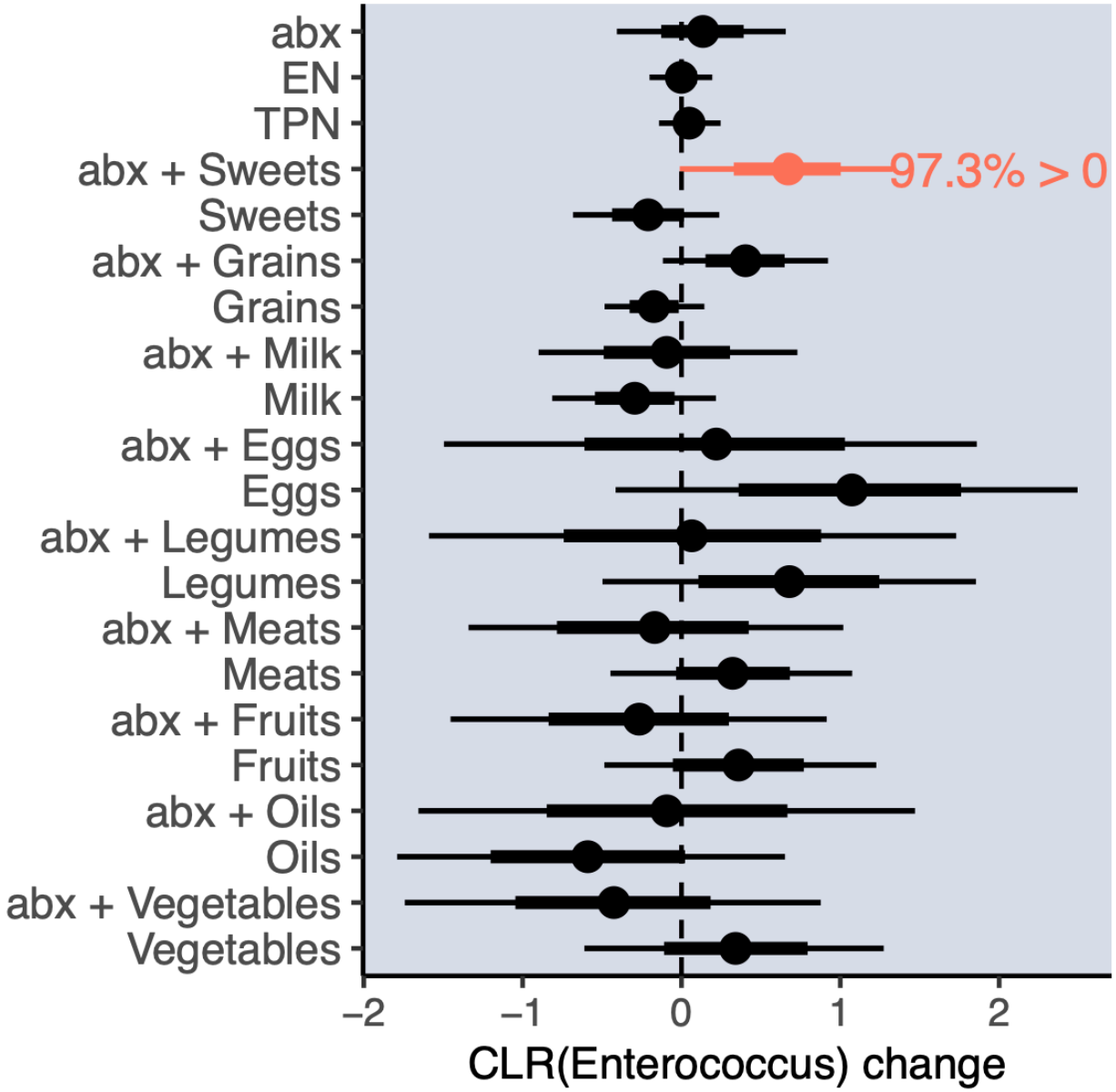
- Regression coefficients of posterior distributions from a Bayesian multilevel model; whiskers 95% confidence intervals.
- Expected diversity change (in log inverse Simpson units) per gram of indicated food in the preceding two days.
- Similar results obtained by linear mixed model fit by maximum likelihood

unpublished

The “sweets” category includes nutritional supplements



“Sweets” consumption (interacting with antibiotics) is associated with Enterococcus expansion

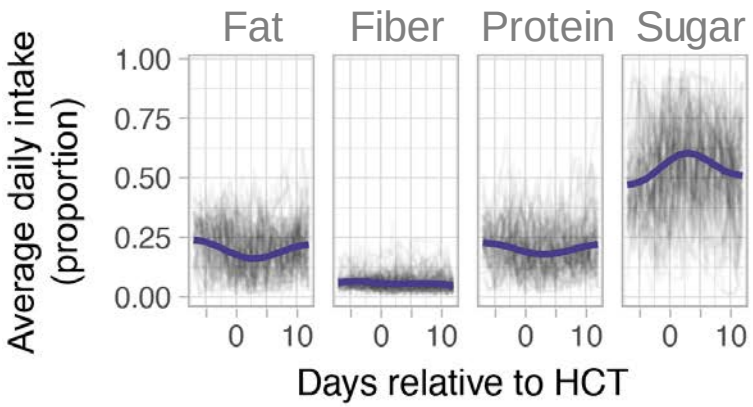


Enterococcus

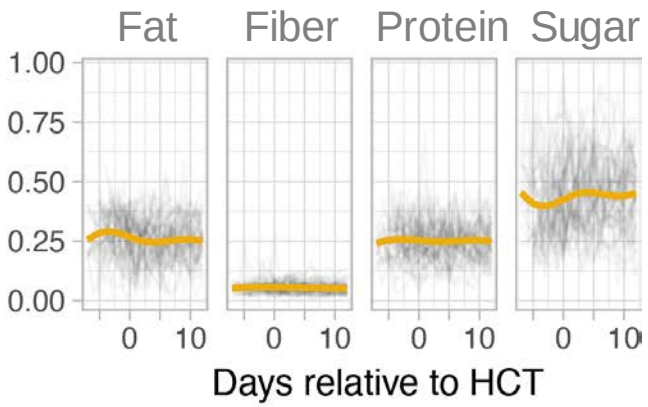
lower ← → higher

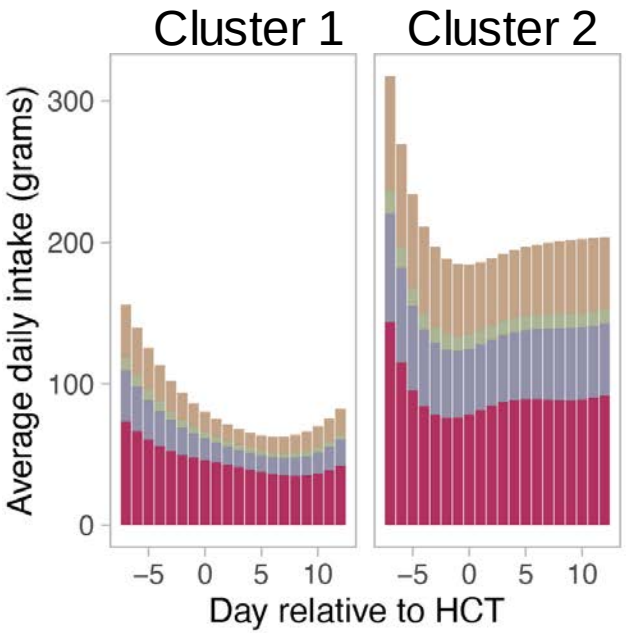
Patients could be clustered according to nutritional-composition trajectories

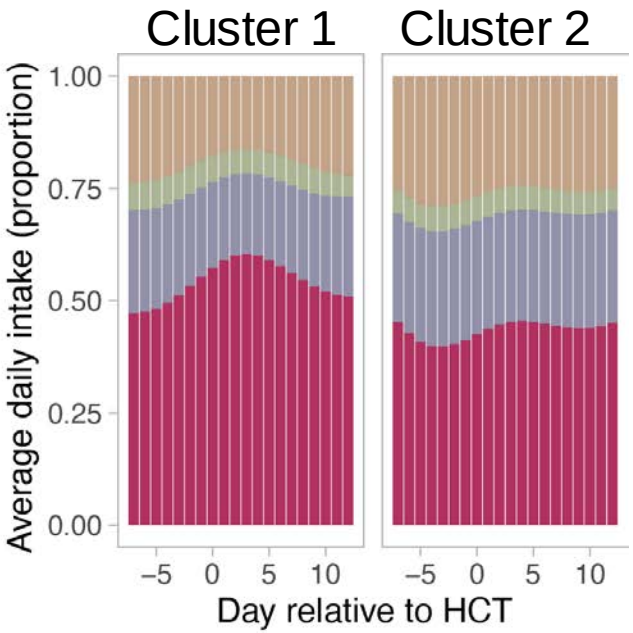
Cluster 1: low intake / sugar enriched



Cluster 2: higher intake







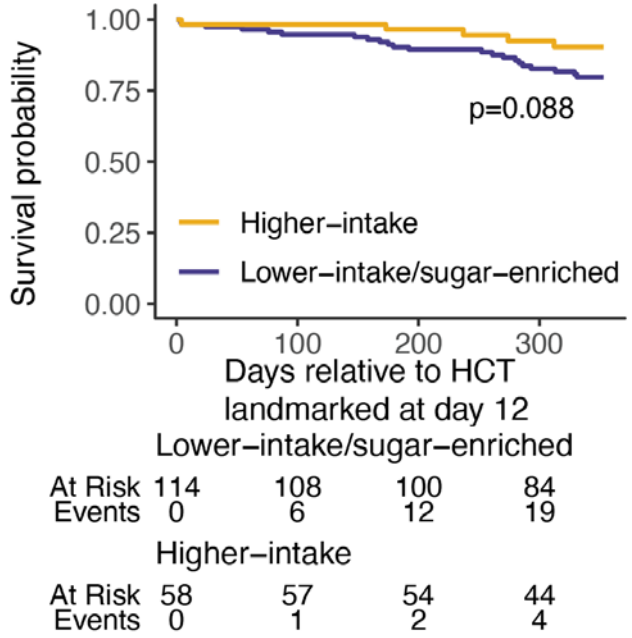
latent trajectory class analysis; Hart, **Fei T**, *et al. Biometrics* 2020

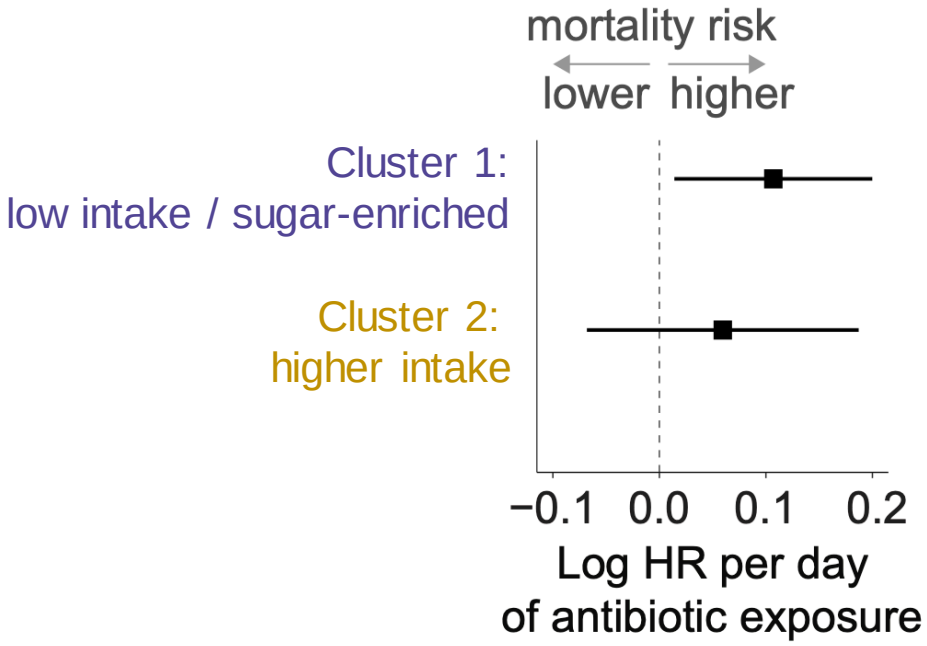
Patients could be clustered according to nutritional-composition trajectories

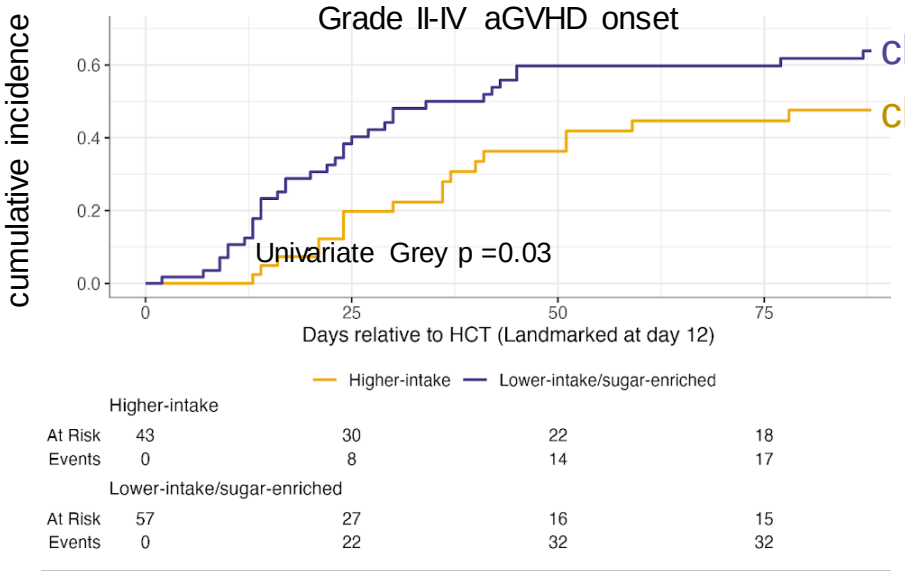
Characteristic	cluster 1, N = 114[1] (Cluster 1: low intake / sugar enriched)	cluster 2, N = 59[1] (Cluster 2: higher intake)
Age	60 (51, 65)	62 (49, 68)
Sex		
Male	50 (44%)	45 (76%)
Female	64 (56%)	14 (24%)
Disease		
Acute myeloid leukemia	47 (41%)	21 (36%)
MDS/MPN	38 (33%)	19 (32%)
Non-Hodgkin's lymphoma	6 (5.3%)	11 (19%)
Acute lymphoid leukemia	11 (9.6%)	1 (1.7%)
Other	7 (6.1%)	4 (6.8%)
Myeloma	4 (3.5%)	1 (1.7%)
Chronic lymphocytic leukemia	1 (0.9%)	2 (3.4%)
Graft type		
Unmodified bone marrow or PBSC	47 (41%)	41 (69%)
T-cell depleted PBSC	57 (50%)	15 (25%)
Cord blood	10 (8.8%)	3 (5.1%)
Intensity of conditioning regimen		
Ablative	71 (62%)	29 (49%)
Reduced intensity	39 (34%)	13 (22%)
Nonmyeloablative	4 (3.5%)	17 (29%)
GvHD prophylaxis		
CNI-based	36 (32%)	23 (39%)
PTCy-based	21 (18%)	21 (36%)
T-cell depleted PBSC	57 (50%)	15 (25%)
Days exposed to broad-spectrum antibiotics	9.0 (2.0, 13.0)	3.0 (0.0, 12.0)

[1] Median (IQR); n (%)

In the low-intake/sugar-enriched cluster, duration of antibiotic exposure predicted mortality



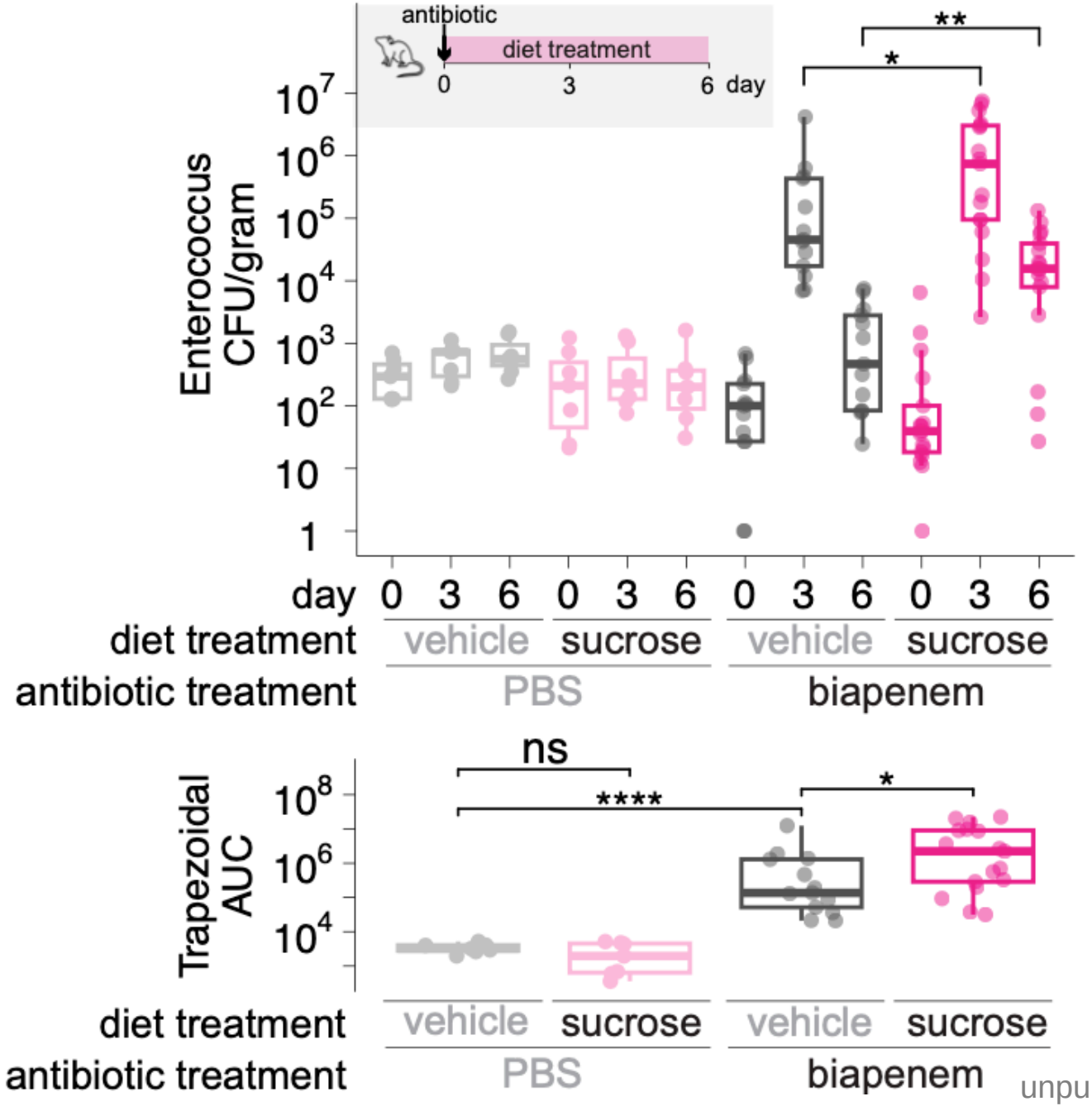




cluster 1: low intake / sugar-enriched

cluster 2: higher intake

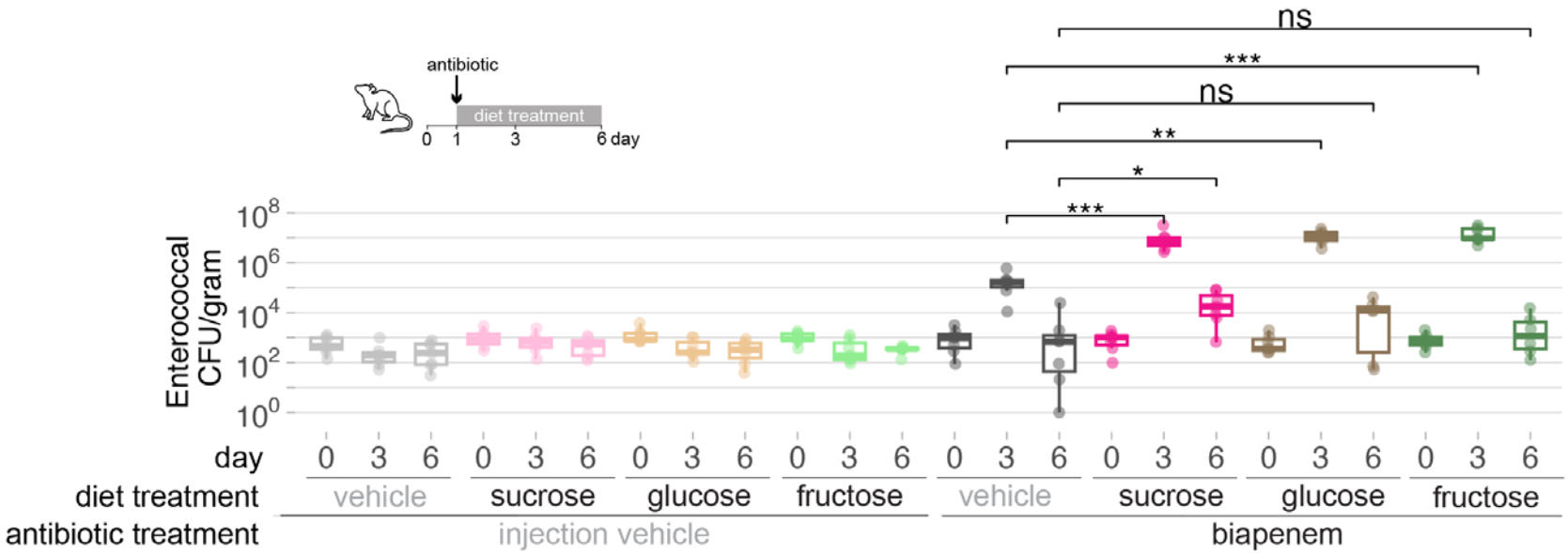
Dietary sucrose exacerbates antibiotic-induced Enterococcus expansion



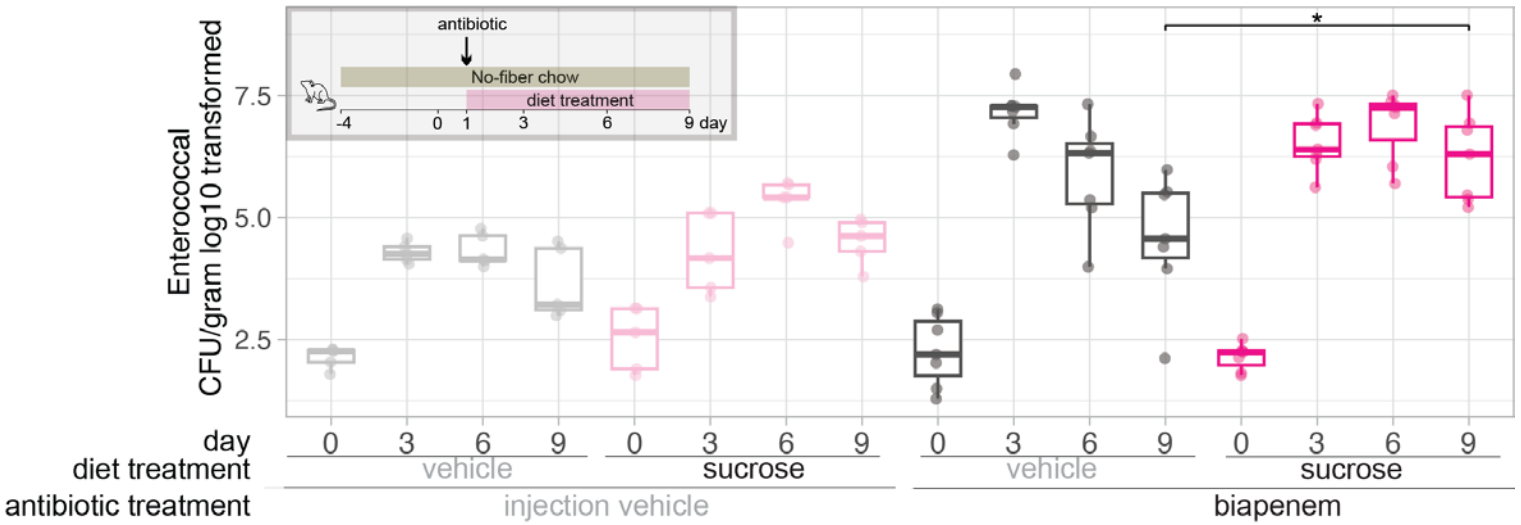
Hypotheses for how sucrose synergizes with antibiotics to disrupt Enterococcus colonization resistance

Direct utilization
Nutrient Competition
Toxin production
Host-mediated effects

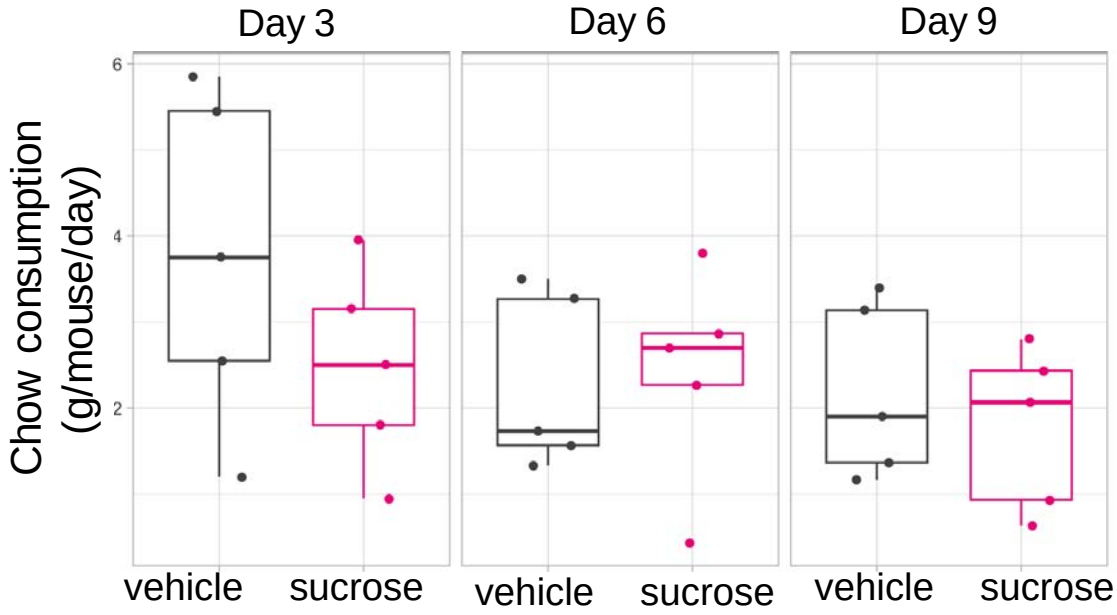
Several monosaccharides can exacerbate antibiotic-induced Enterococcus expansion



The sucrose effect is not explained simply by a reduction in fiber intake

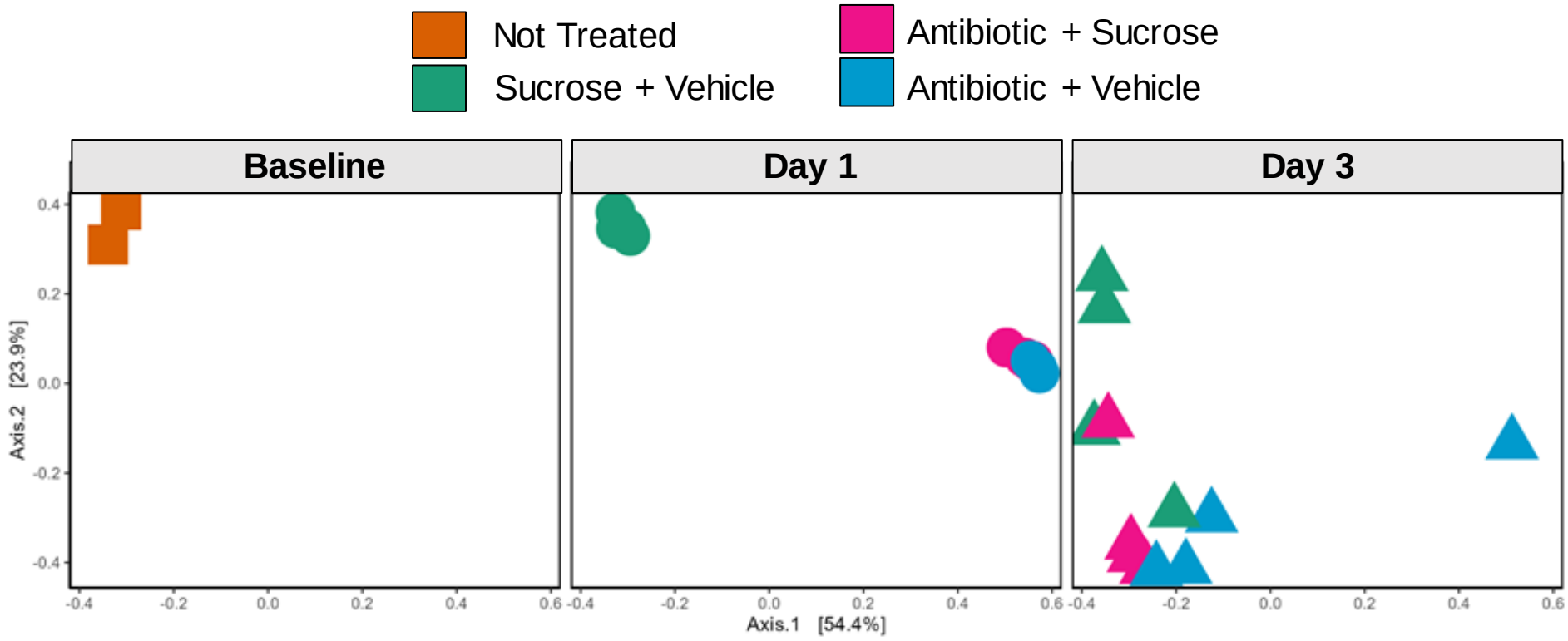


No-fiber background diet



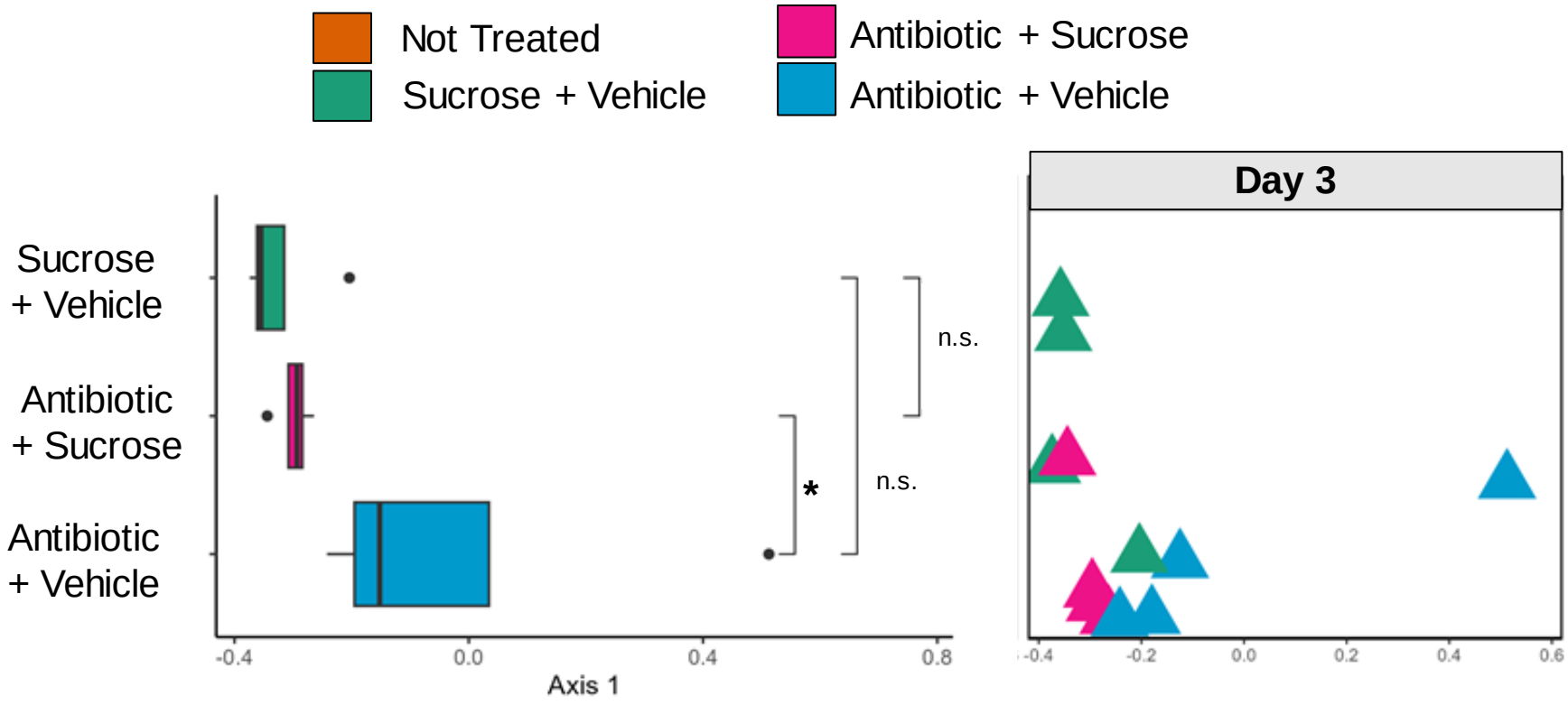
Comparable chow intake

Shallow shotgun sequencing indicates antibiotics dominate the day 1 composition, but by day 3 sucrose effects are discernable



N = 26 Mice
Mice are singly housed.
Taxonomic abundances assessed via MetaPhlAn 3.
PCoA based on Bray-Curtis dissimilarity

Shallow shotgun sequencing indicates antibiotics dominate the day 1 composition, but by day 3 sucrose effects are discernable



N = 26 Mice
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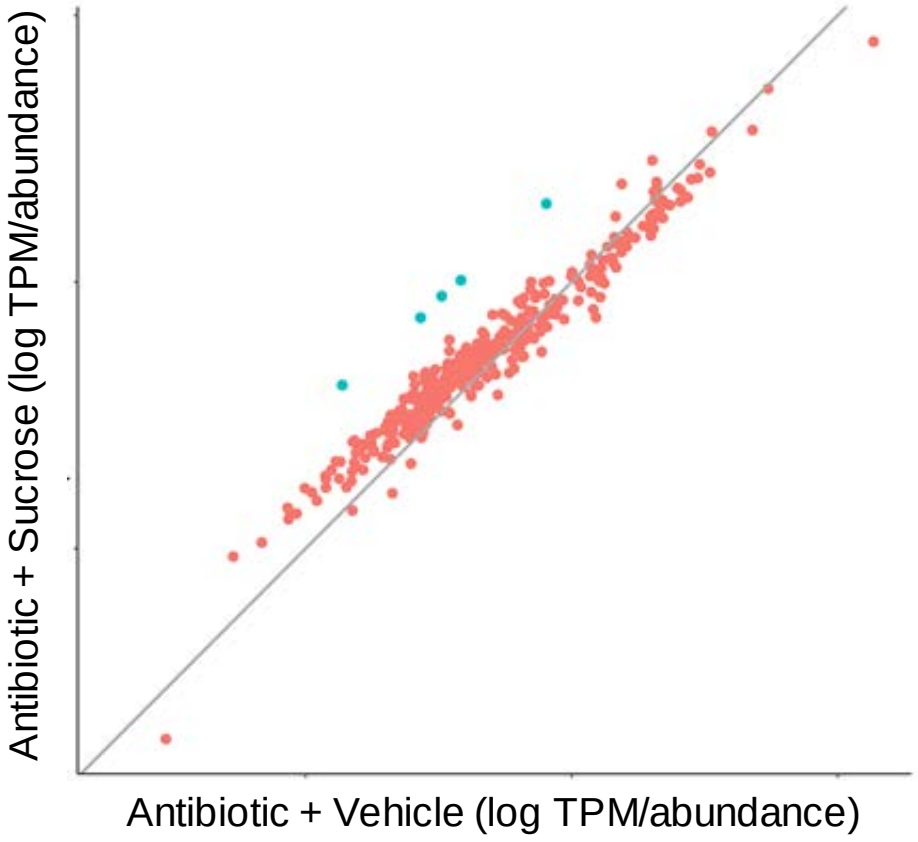
PCoA based on Bray-Curtis dissimilarity

Enterococcus Kegg Orthologs Shows Increase Expression of Sugar Metabolism Orthologs Under Sucrose Diet (Day 1)



Fold Change

< 1

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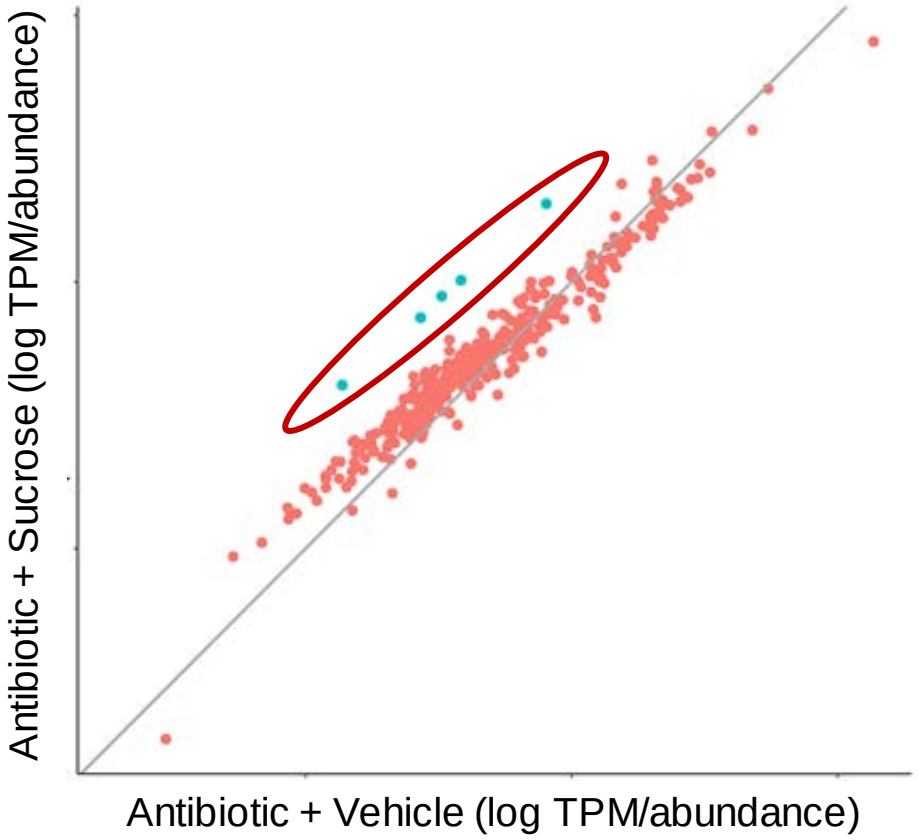
Enterococcus Kegg Orthologs Shows Increase Expression of Sugar Metabolism Orthologs Under Sucrose Diet (Day 1)



Fold Change

< 1

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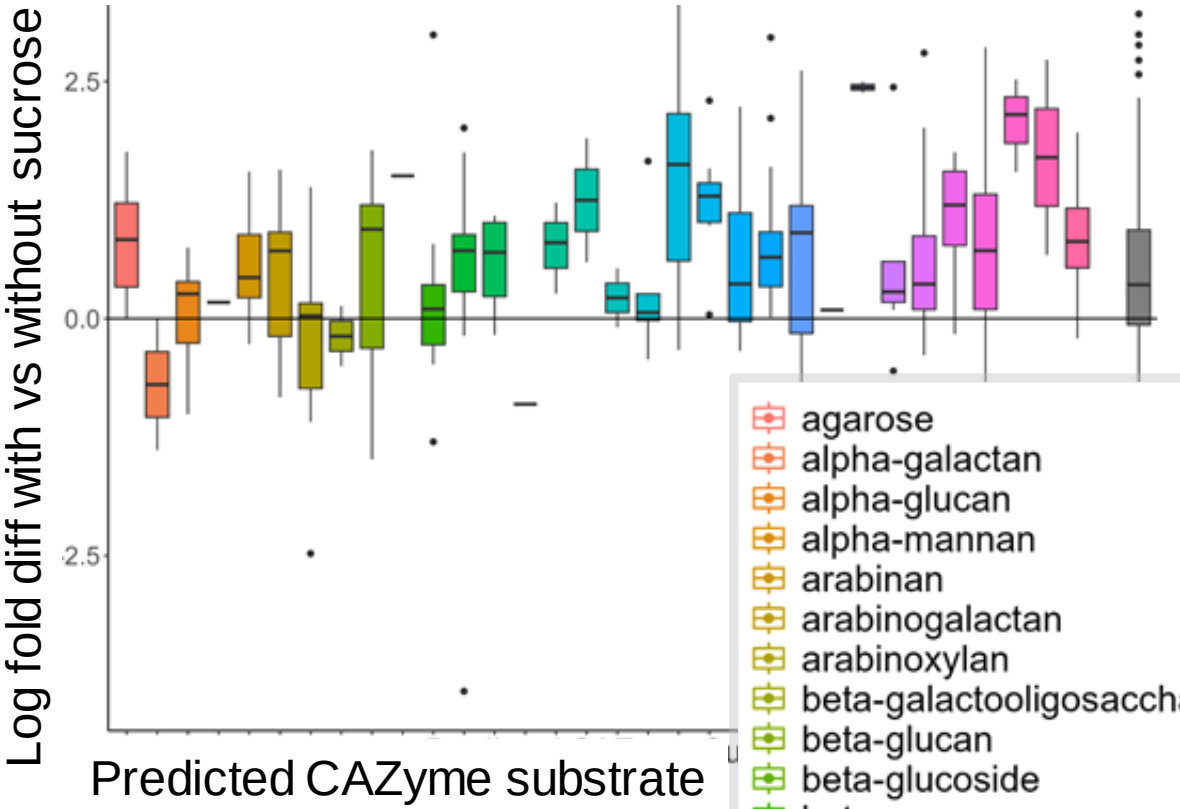
Orthologs Associated with the uptake and metabolism of simple sugars and sugar alcohols: tagatose, mannitol, ribose.

Sucrose induces upregulation of many classes of carbohydrate-active enzymes (CAZyme).



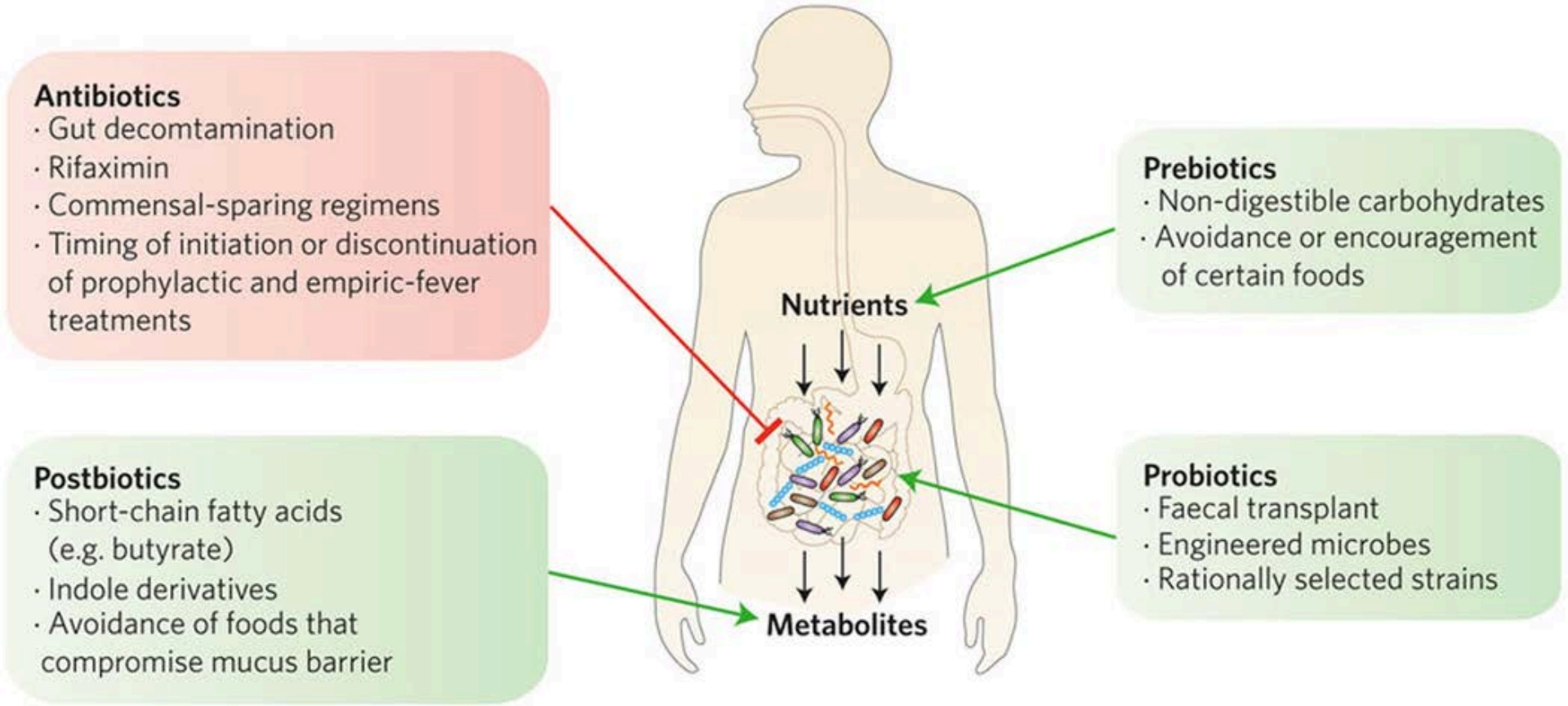
- agarose
- alpha-galactan
- alpha-glucan
- alpha-mannan
- arabinan
- arabinogalactan
- arabinoxylan
- beta-galactooligosaccharide
- beta-glucan
- beta-glucoside
- beta-mannan
- capsule polysaccharide synthesis
- cellobiose
- cellulose
- chitin
- fructan
- fucose
- galactan
- galactomannan
- glycogen
- glycosaminoglycan
- host glycan
- human milk oligosaccharide
- levoglucosan
- melibiose
- mucin
- pectin
- raffinose
- starch
- trehalose
- X
- xylan
- xyloglucan
- NA

CAZyme families and substrates predicted with run_dbcan based on RNA/DNA ratios from day 3 cecal contents

Diet & Microbiome injury: Conclusions

- Calorie & fiber intake are positively associated with fecal microbiota diversity and *Blautia*, and inversely with *Enterococcus*
- Ecological metrics are a useful approach to analyzing complex diet data
- Food alpha diversity declines during transplant in a conditioning-intensity-specific fashion
- Consumption of foods enriched in simple sugars in the previous 2 days are associated with lower fecal diversity
- sucrose can exacerbate antibiotic-induced pathobiont expansion
- Are the dietary recommendations we give to allo-HCT patients (e.g. Boost, Ensure, smoothies) correct?
- Is avoiding sugar intake while on antibiotics, in general, a way to mitigate abx-induced dysbiosis?

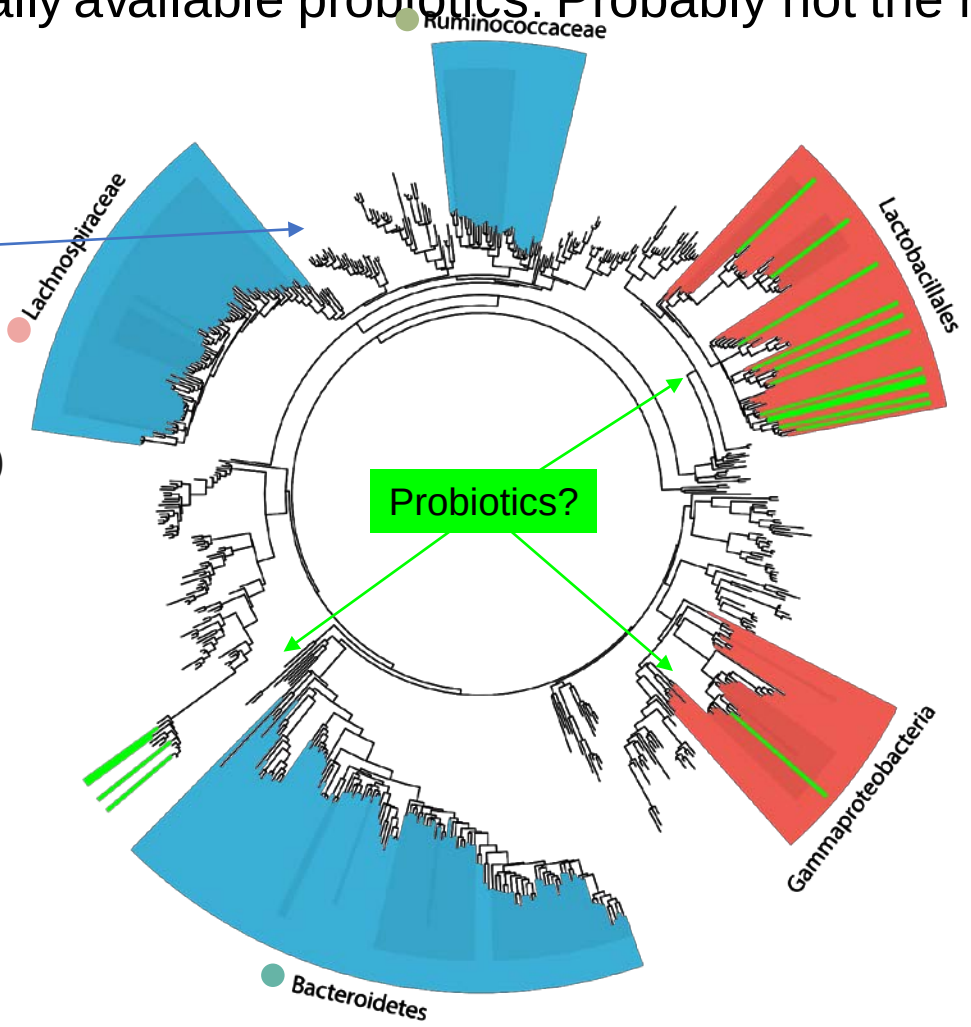
Approaches to Manipulating Microbiota-Host Interactions



Peled *et al*., *Nature Microbiology* 2016

Current Commercially available probiotics: Probably not the right approach

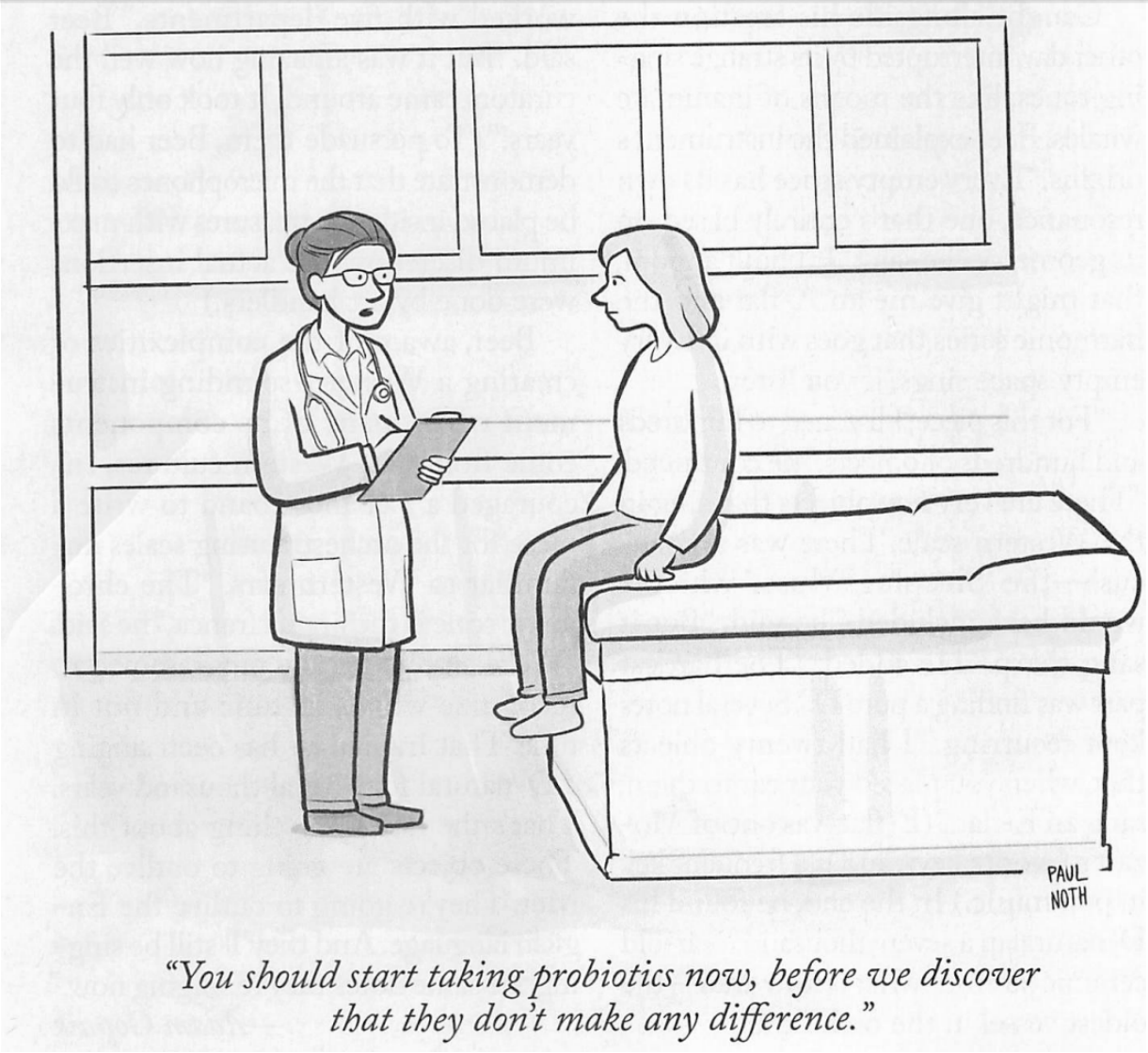
Clostridium butyricum (CBM588) improves ICI responses in RCC (Dizman *Nat Med* 2022)



CBM588 in Improving Clinical Outcomes in Patients Who Have Undergone Donor Hematopoietic Stem Cell Transplant NCT03922035, Karamajeet Singh Sandhu & Ryotaro Nakamura

Probiotic strains shown: *L. acidophilus, L. reuteri, L. rhamnosus, L. casei, L. delbrueckii, L. fermentum, L. gasseri, L. johnsonii, L. paracasei, L. plantarum, L. salivarius, B. cereus, B. coagulans, B. subtilis, B. adolescentis, B. animalis, B. bifidum, B. breve, B. longum, E. durans, E. coli (Nissle), L. lactis, L. mesenteroides, P. acidilactici, S. thermophilus*

Commercially available probiotics are probably not the answer

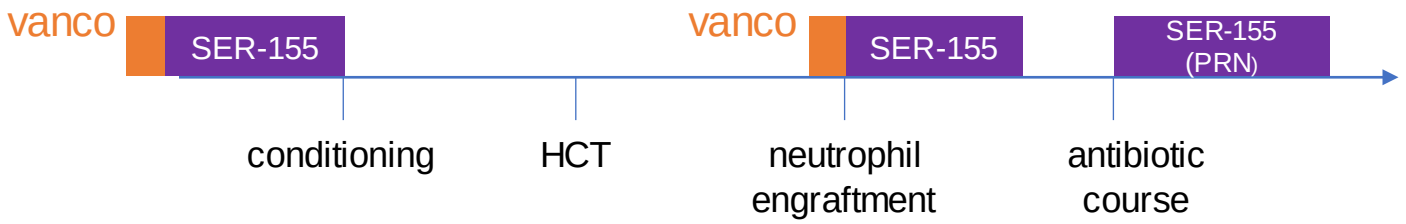


"You should start taking probiotics now, before we discover that they don't make any difference."

New Yorker 8/19/19

Can a rationally designed probiotic preserve gut microbiome integrity in allo-HCT?

Phase 1b multicenter trial of SER-155 (Seres Therapeutics)



Oral combination of 16 fermented strains, selected rationally for

- colonization resistance against VRE and CRE
- improved gut barrier function
- reduction in gut inflammation and local immune activation
- selection of strains & intervention timepoints informed by Taur *Blood* 2014, Peled *JCO* 2017, Peled *NEJM* 2020.

~10-patient safety lead-in → 20+20 placebo-randomized in allo-HCT

MSKCC (Doris Ponce), U. of Chicago (Satya Kosuri), Mayo (Nandita Khera), MGH (Zacharia DeFilipp), Fred Hutch (David Fredricks), others…

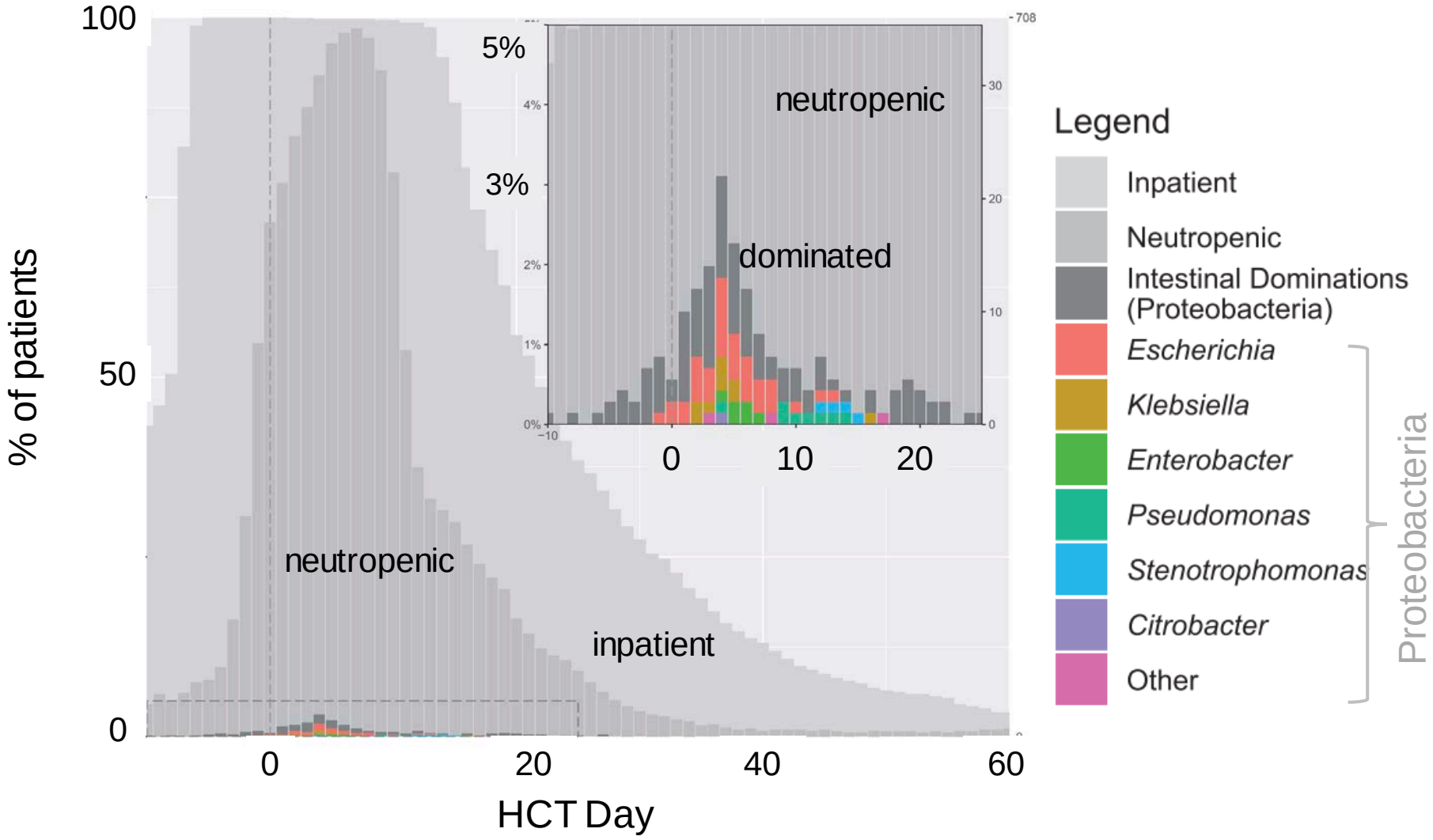
Oral vancomycin microbiome 'conditioning'

Primary outcome: safety & strain engraftment ("PK" and "PD")

Secondary outcomes: F&N, BSI, GVHD

NCT04995653

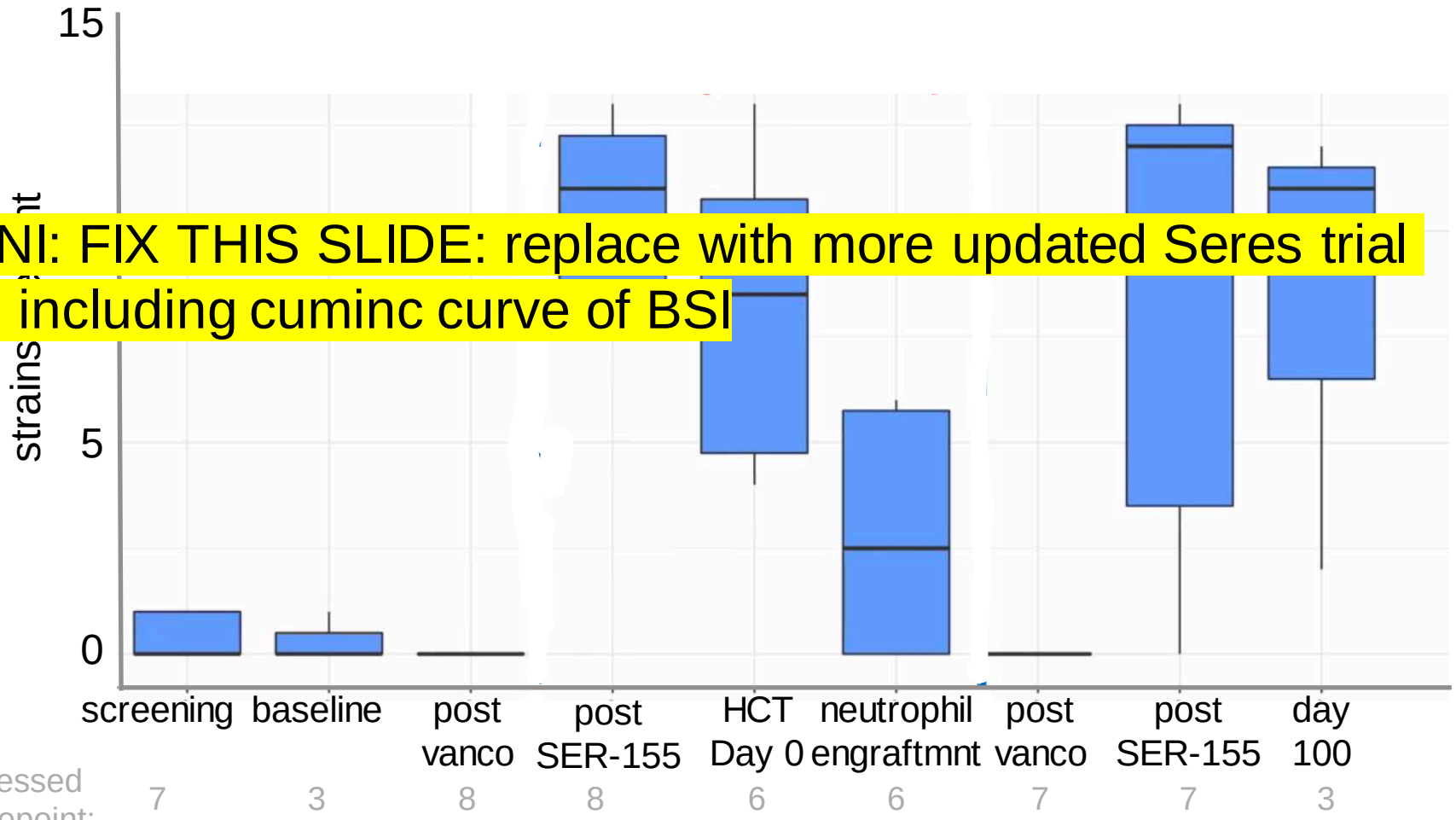
Intestinal domination coincides with bloodstream infection



Stoma *Clin Infect Dis* 2021
Tamburini… Bhatt *Nature Med* 2018

A portion of the 16 strains in SER-155 exhibited sustained engraftment

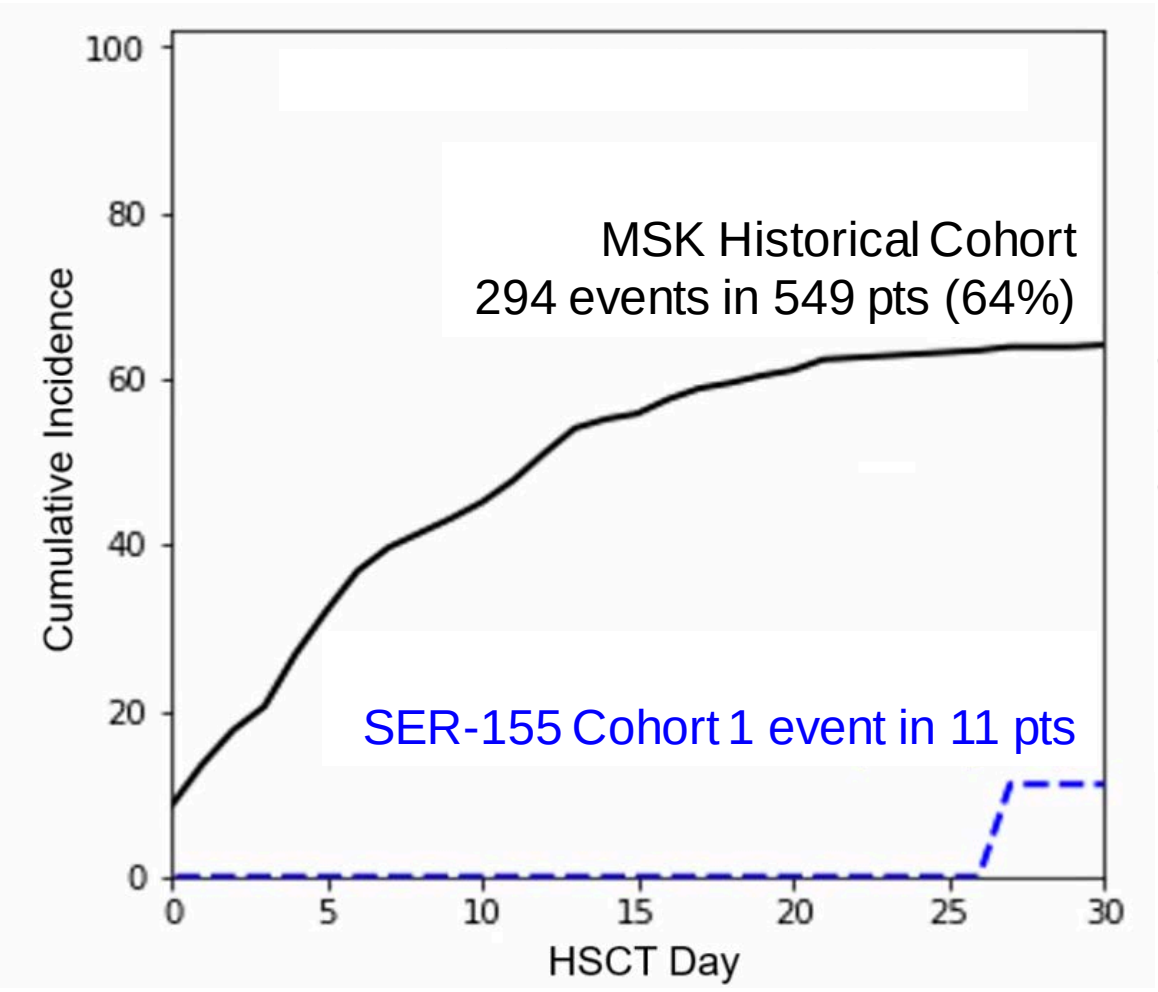
TSONI: FIX THIS SLIDE: replace with more updated Seres trial data, including cuminc curve of BSI



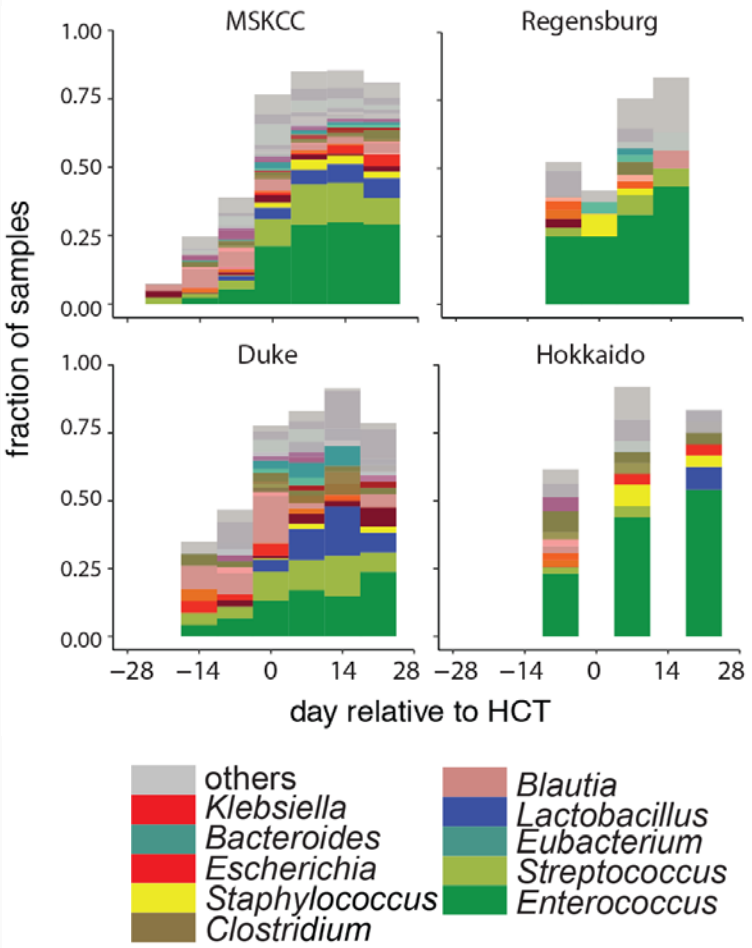
Cohort 1

PLEASE DO NOT PHOTO OR POST

Incidence of domination by pathobionts* was strikingly lower in SER-155 recipients than historical controls



Historical Cohorts



*Enterococcaceae, Enterobacteriaceae, Streptococcaceae, Staphylococcaeae

SER-155 reduced incidence of bloodstream infection in allo-HCT

$p = 0.04$

Bloodstream infections from Day 0 to Day 100 (# patients)	SER-155 n=20 n (%)	Placebo n=14 n (%)
Subjects with confirmed BSI	2 (10.0%)	6 (42.9%)
95% confidence interval	(1.2, 31.7)	(17.7, 71.1)

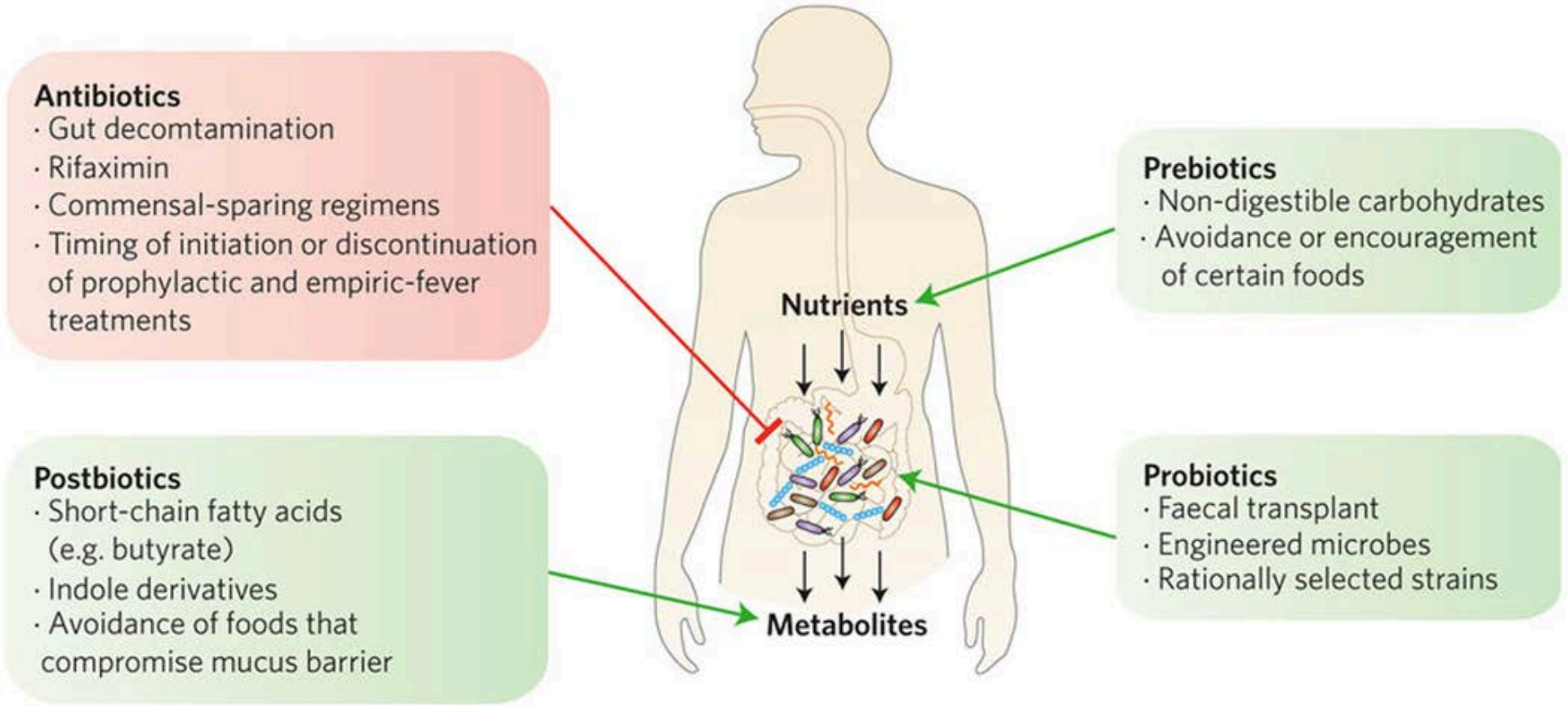
Finegoldia magna
E. coli/Strep mitis

E. coli
Enterococcus faceium/Staph haemolyticus/Candida krusei
Staph aureus
Staph haemolyticus
Pseudomonas aeruginosa
E. coli

Oral combination of 16 fermented strains, selected rationally for
- colonization resistance against VRE and CRE
- improved gut barrier function
- reduction in gut inflammation and local immune activation

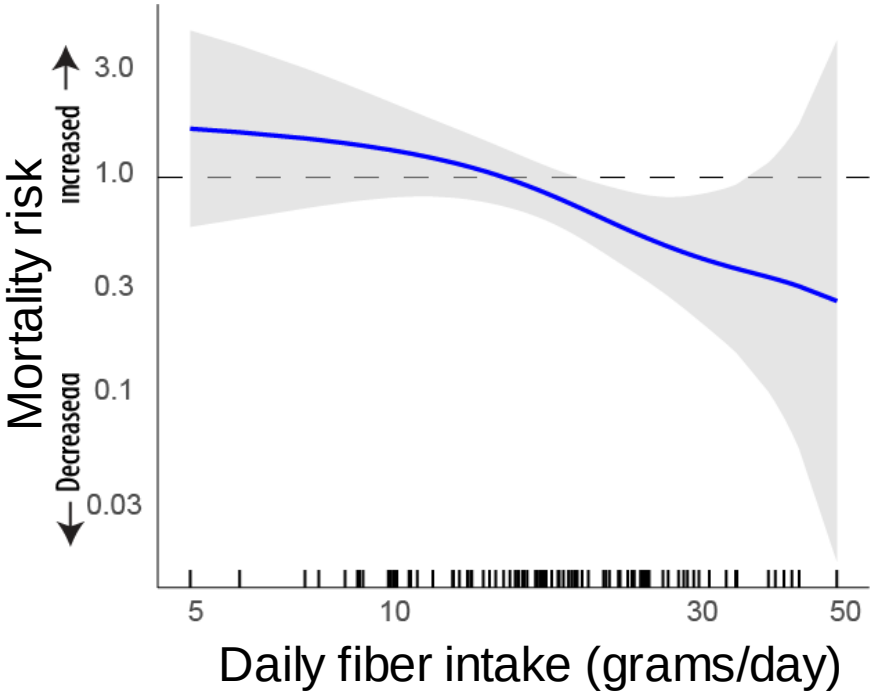
- Met primary endpoints (safety and strain engraftment)
- Modified intention to treat population
- Seres Therapeutics, Doris Ponce, NCT04995653

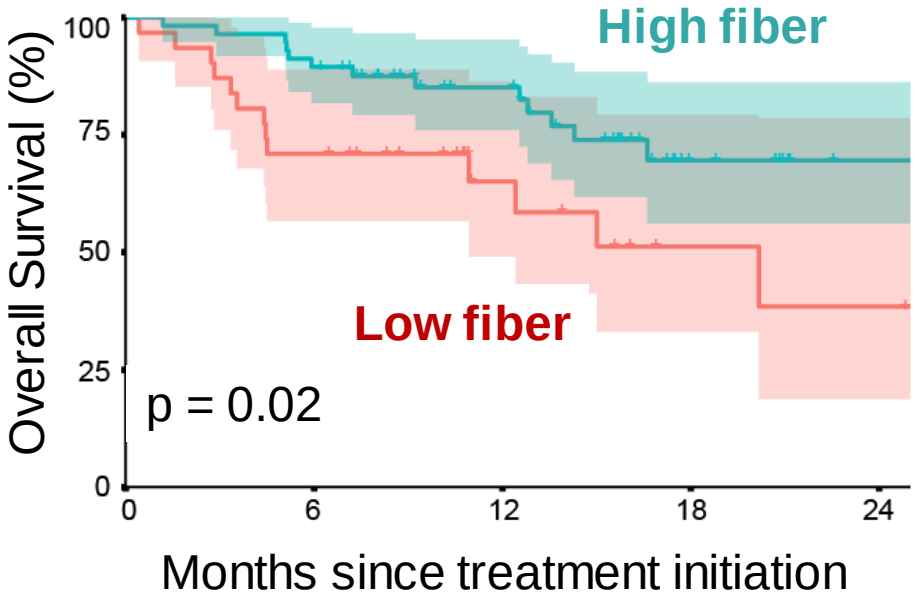
Approaches to Manipulating Microbiota-Host Interactions



Peled *et al*., *Nature Microbiology* 2016

Fiber consumption predicts lower mortality risk in immunotherapy-treated genitourinary cancer

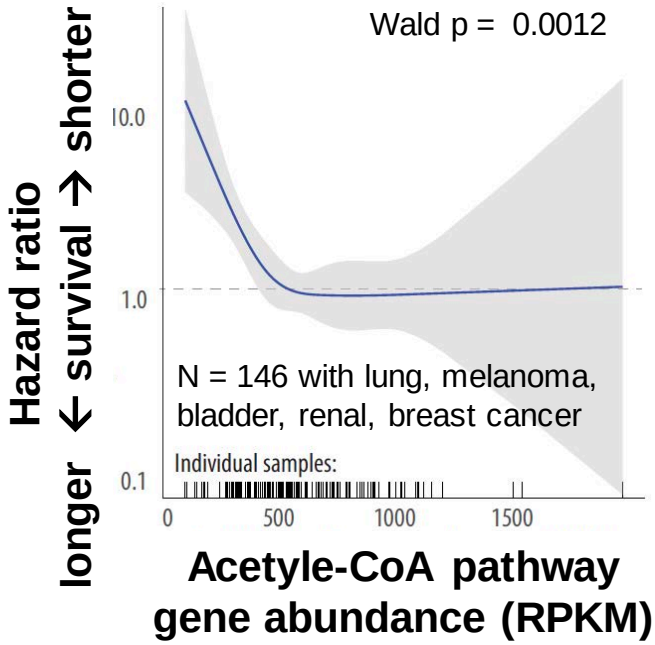


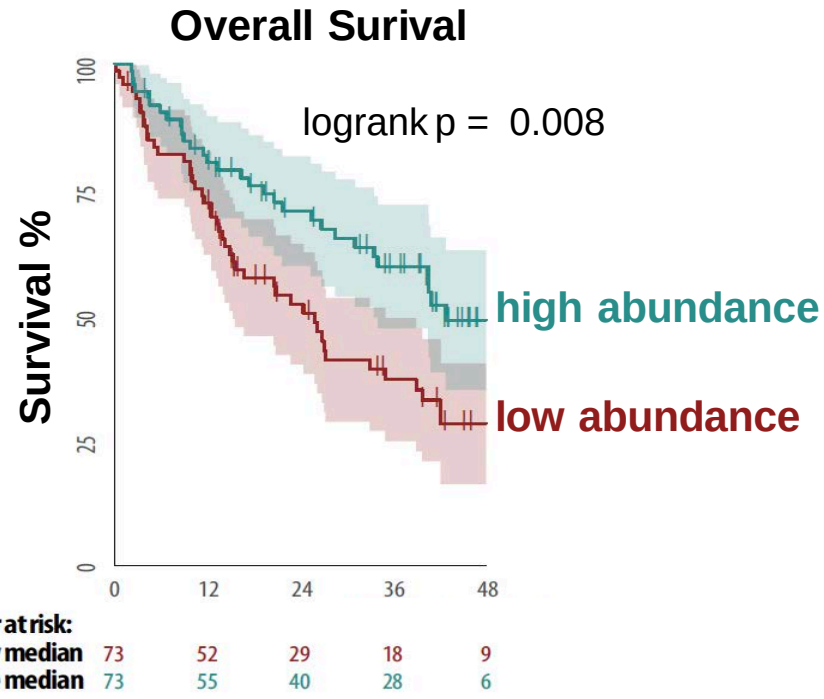


N = 88 patients with renal and bladder cancer treated with checkpoint-blockade drugs

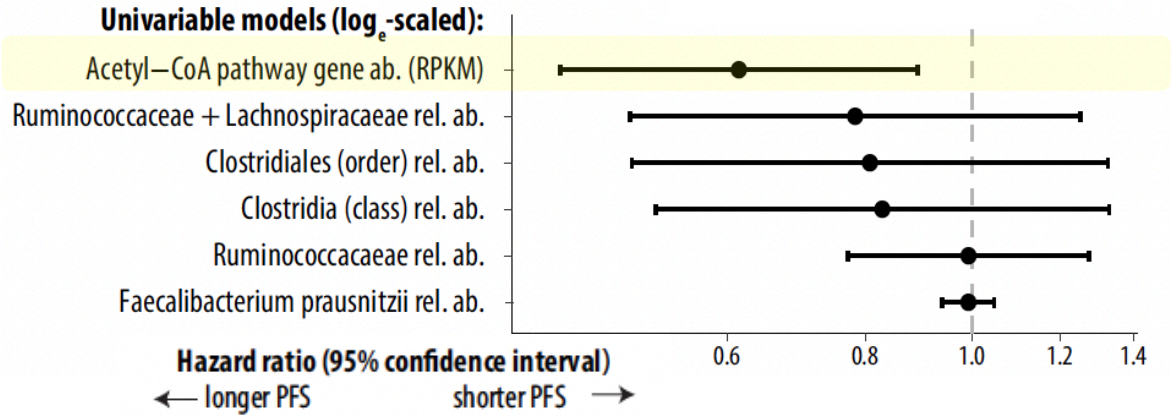
Josh Fein & Brenan Guercio; unpublished

Abundance of butyrate-production genes in the the Acetyl-CoA subpathway predicts overall survival following ICI



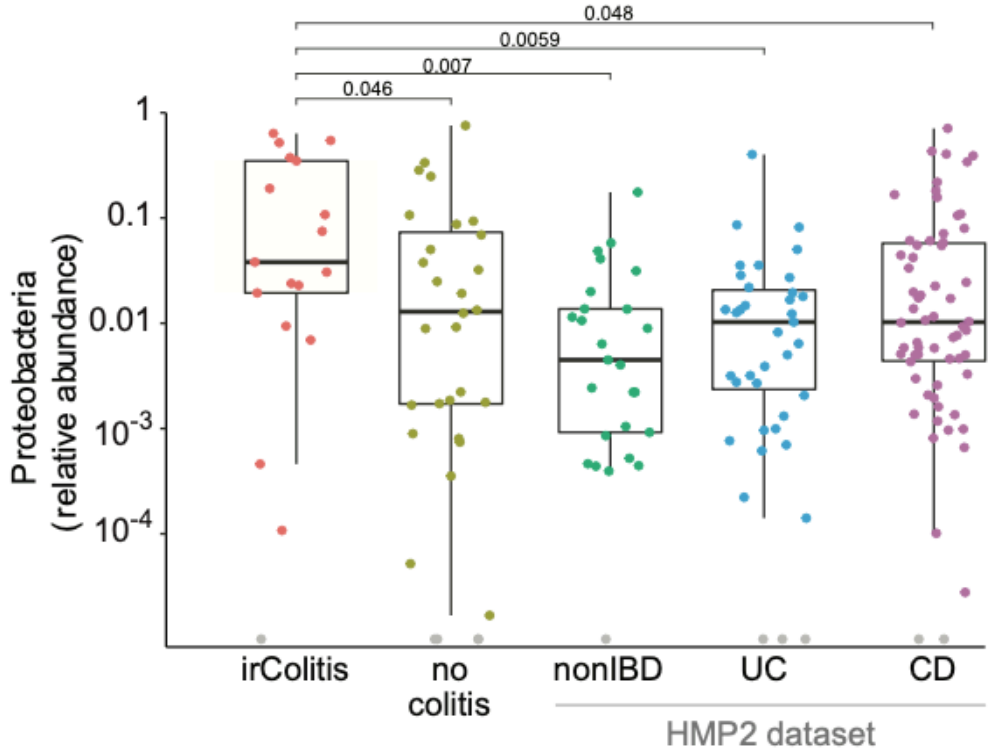


Re-analysis of melanoma cohort from Spencer…Wargo *Science* 2021



Josh Fein & Brenan Guercio; unpublished

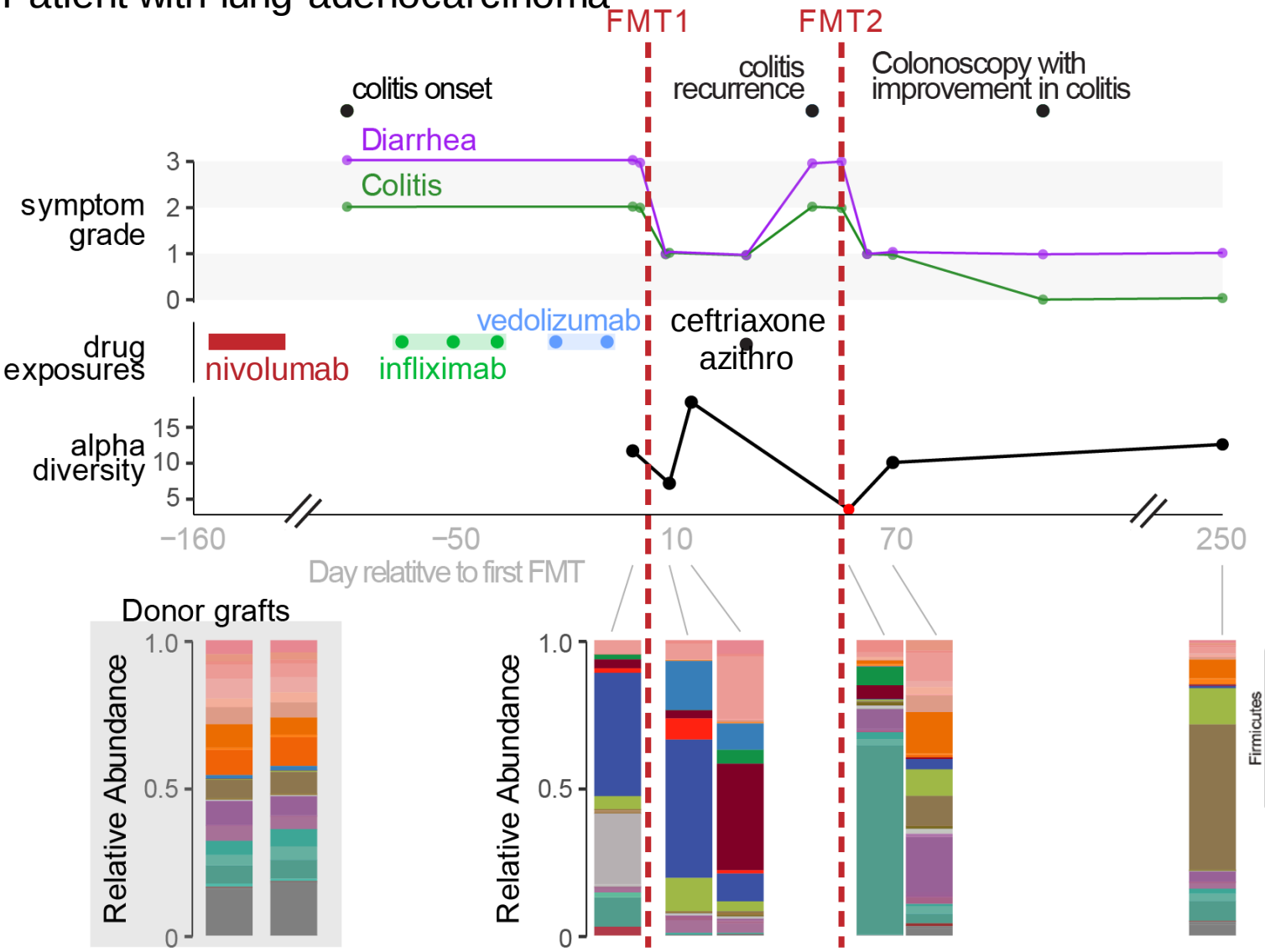
At onset of checkpoint colitis, fecal microbiota composition is enriched for Proteobacteria



Elkrief… Faleck*, Peled* *Cancer Immunology Research* 2024

FMT may ameliorate checkpoint colitis

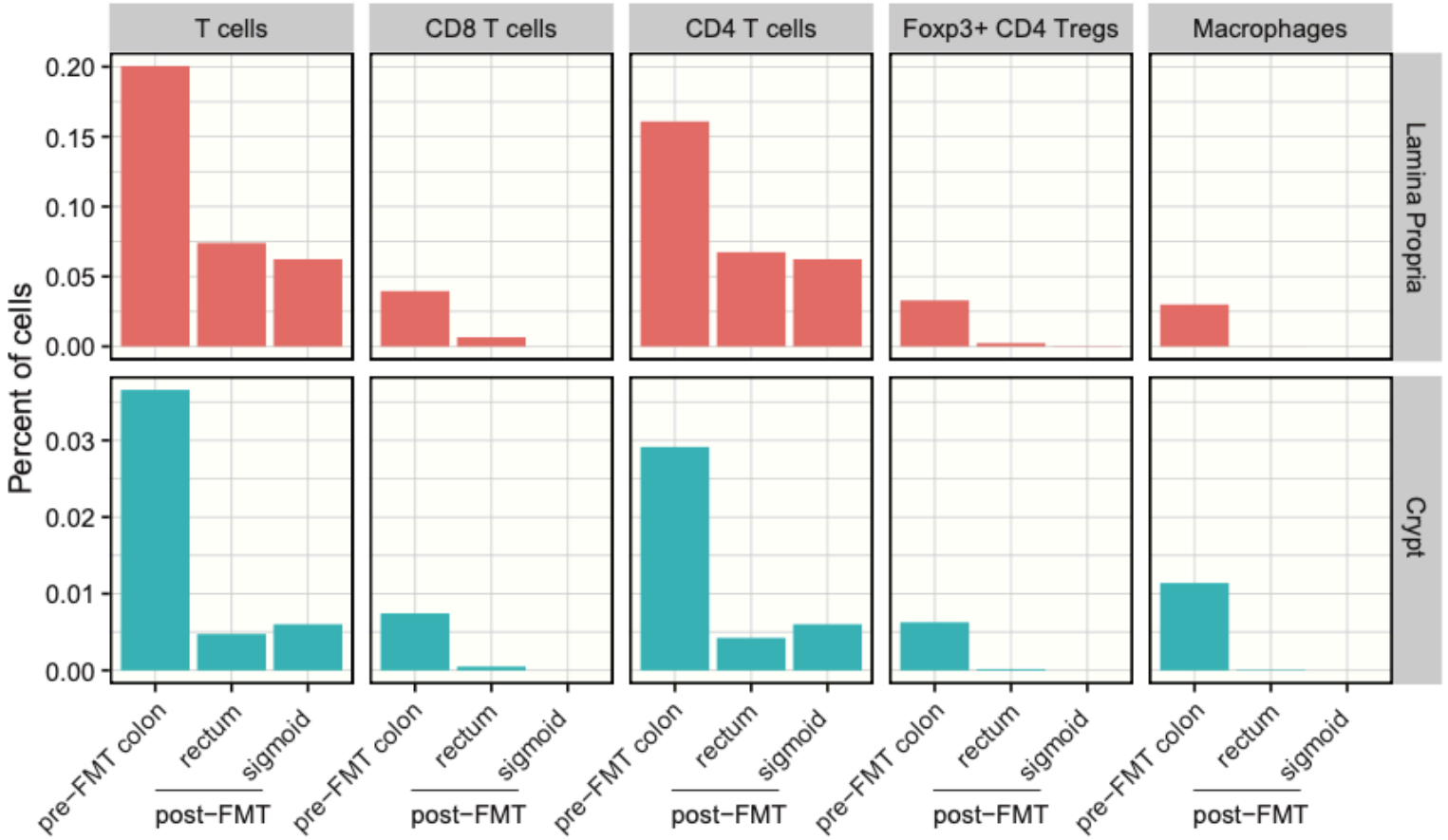
Patient with lung adenocarcinoma



Similar observation by Wang *Nature Med* 2018

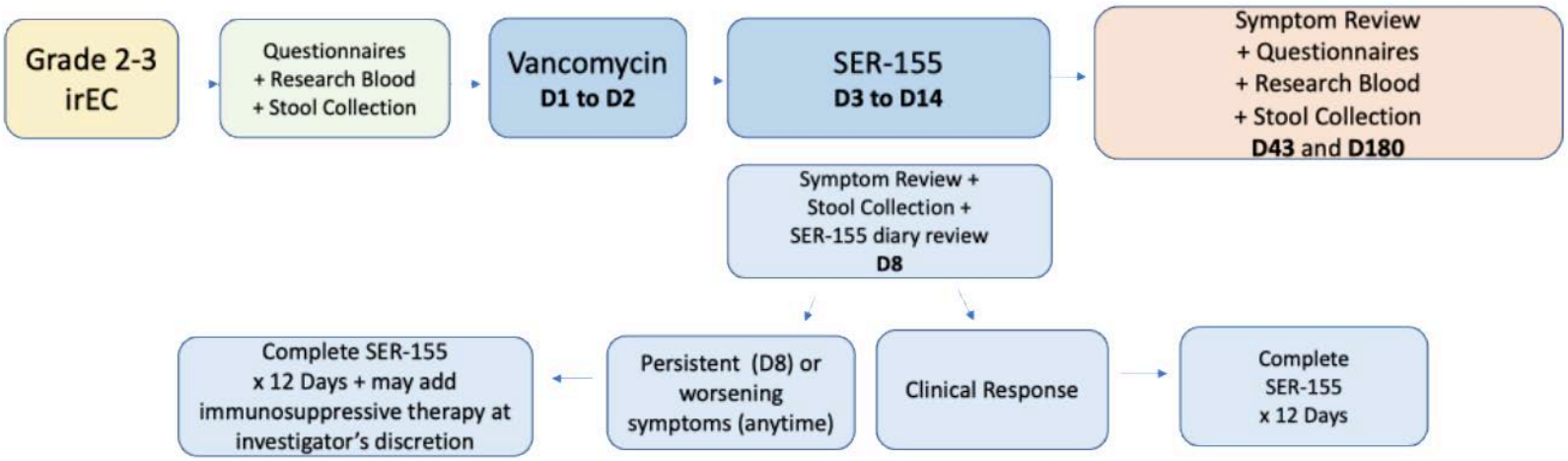
Elkrief… Faleck*, Peled* *Cancer Immunology Research* 2024

Clearance of immune infiltrate accompanied clinical improvement following FMT for irColitis



Elkrief… Faleck*, Peled* *Cancer Immunology Research* 2024

Pilot Clinical Trial: Can a microbiome therpauetic attenuate checkpoint-blockade colitis?



TSONI: FIX THIS SLIDE: shorten the objectives, add Luoma data and add citations to Mimi Wang's data, and add NCT identifier

Primary Objective: 1. To evaluate the safety and tolerability of SER-155 for treatment of irEC 1. Safety Endpoint: 1. Proportion of patients with treatment-related adverse events of special interest, i.e. blood stream infection 2. Proportion of patients with treatment-related adverse events.

Secondary Objectives:

1. To assess the preliminary efficacy of SER-155 in the treatment of patients with grade 2-3 diarrhea from irEC a. Primary Efficacy Endpoint: Proportion of patients with immunosuppressive-free clinical response of irEC at day 15, defined as at least one grade decrease in diarrhea symptoms without the use of immunosuppressive therapy
2. 2. To assess the engraftment of SER-155 bacterial strains in the gastrointestinal microbiome of patients with irEC a. Engraft Endpoints: Number of detectable SER-155 strains at days 15 and 43

NCT06801067

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Roni Shouval
Angel Dai
Tobias Hohl
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U of Minnesota: Dan Knights & Abigail Johnson
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Fred Hutch: Kate Markey

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V Foundation
Riney Foundation, Seres Therapeutics

Peled Lab is recruiting

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