The Diverse World of Bugs Within: How the Microbiome affects Transplantation

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Disclosures

The following faculty and planning committee staff have no financial disclosures:

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Maria Brown, MT	CIBMTR
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Learning objectives

At the conclusion of this session, attendees will be able to:

- Define the microbiome and its function
- Describe how HCT can change the microbiome
- Explain the microbiome's role in HCT
- Identify challenges to and opportunities for microbiome research in HCT patients

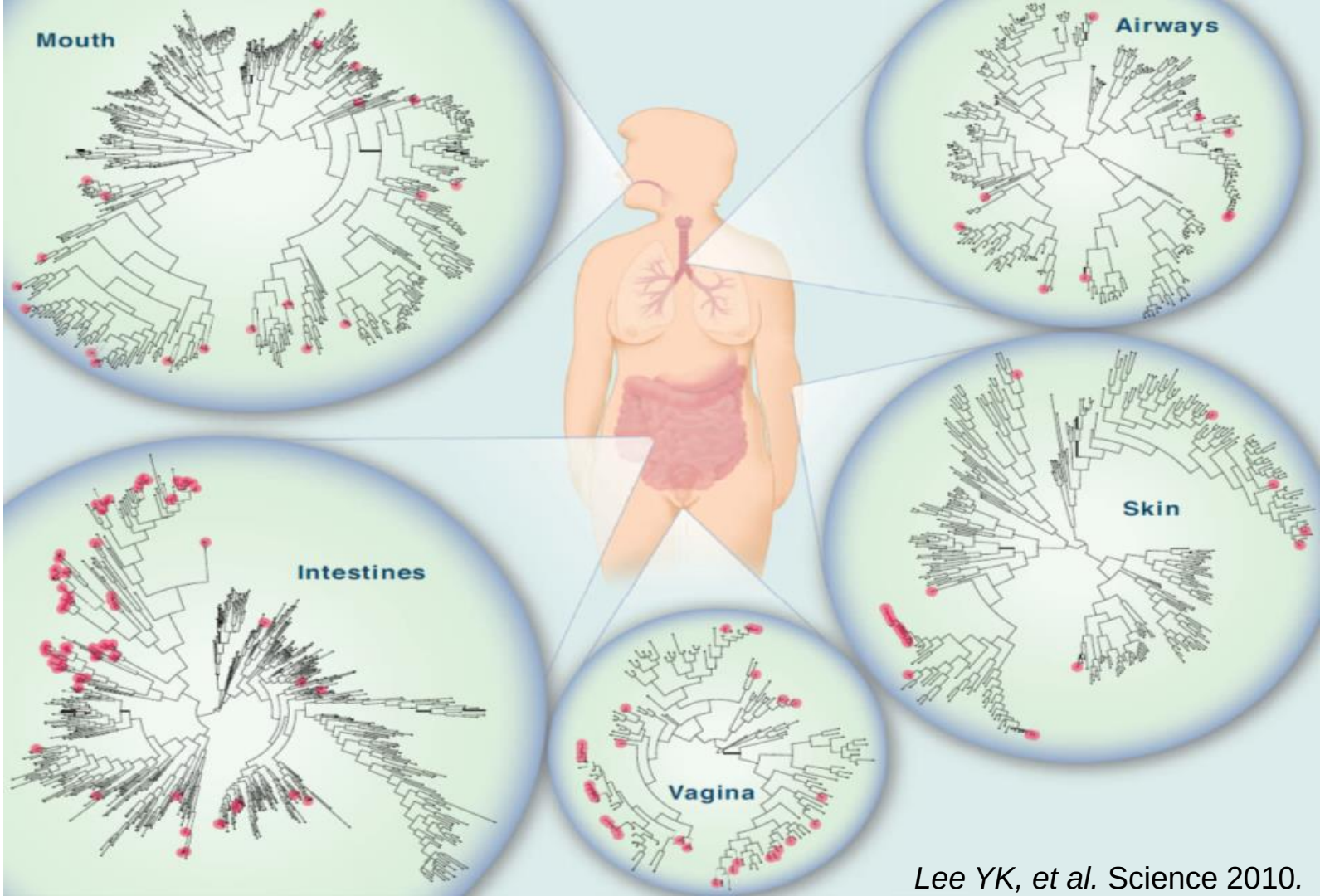
Outline: The Microbiome

1. What it is
2. How we study it
3. How we influence it
4. How it can impact hematopoietic stem cell transplant (HCT) recipients
5. “Microbiome stewardship” in HCT recipients
6. The future of microbiome research

THE HUMAN MICROBIOME

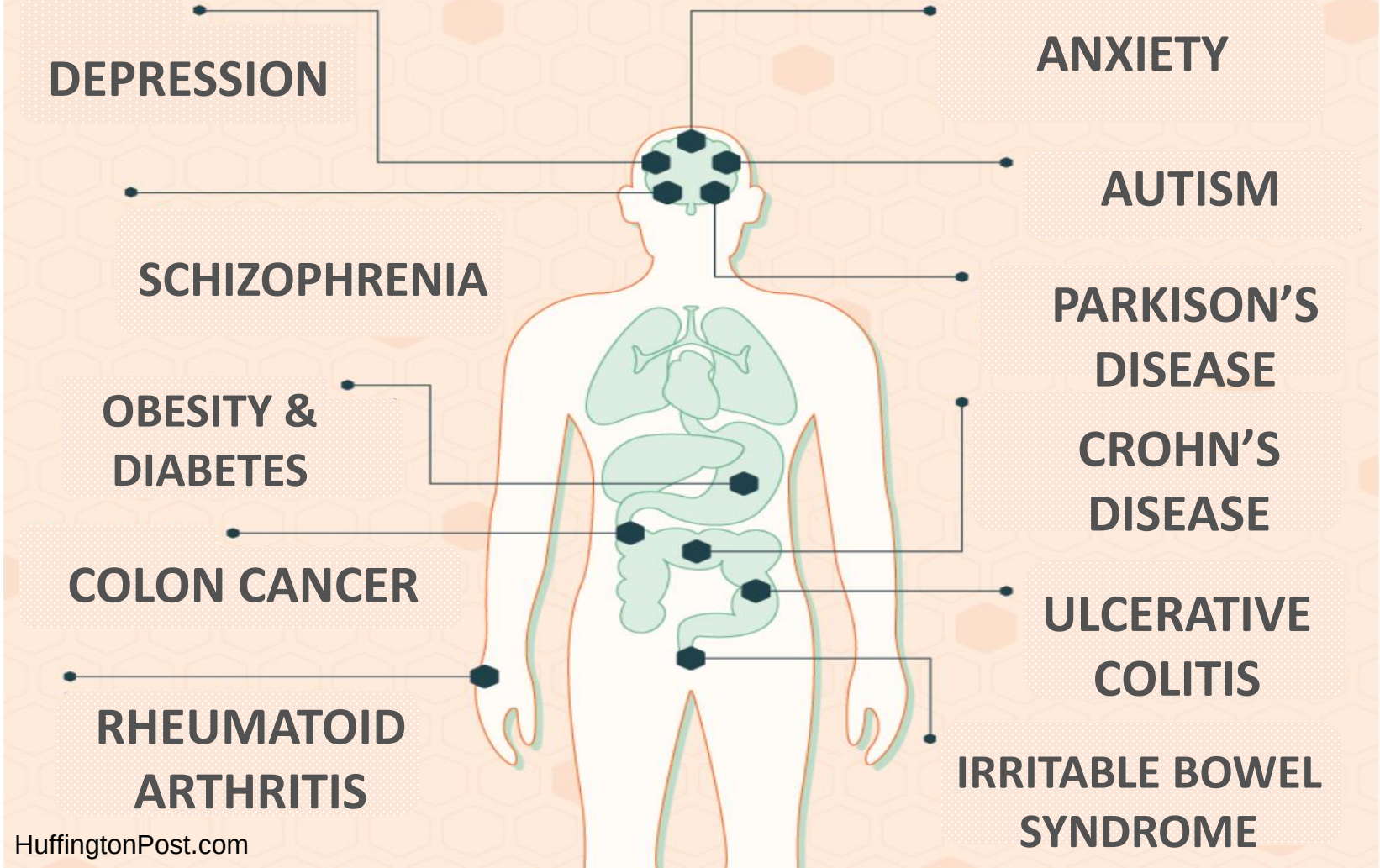
- 100 trillion microbes
- 3% human body mass
- 1-10X microbes : human cells
- 10-100X microbial : human genes
- Largest # microbes: GI tract

Human Microbiome

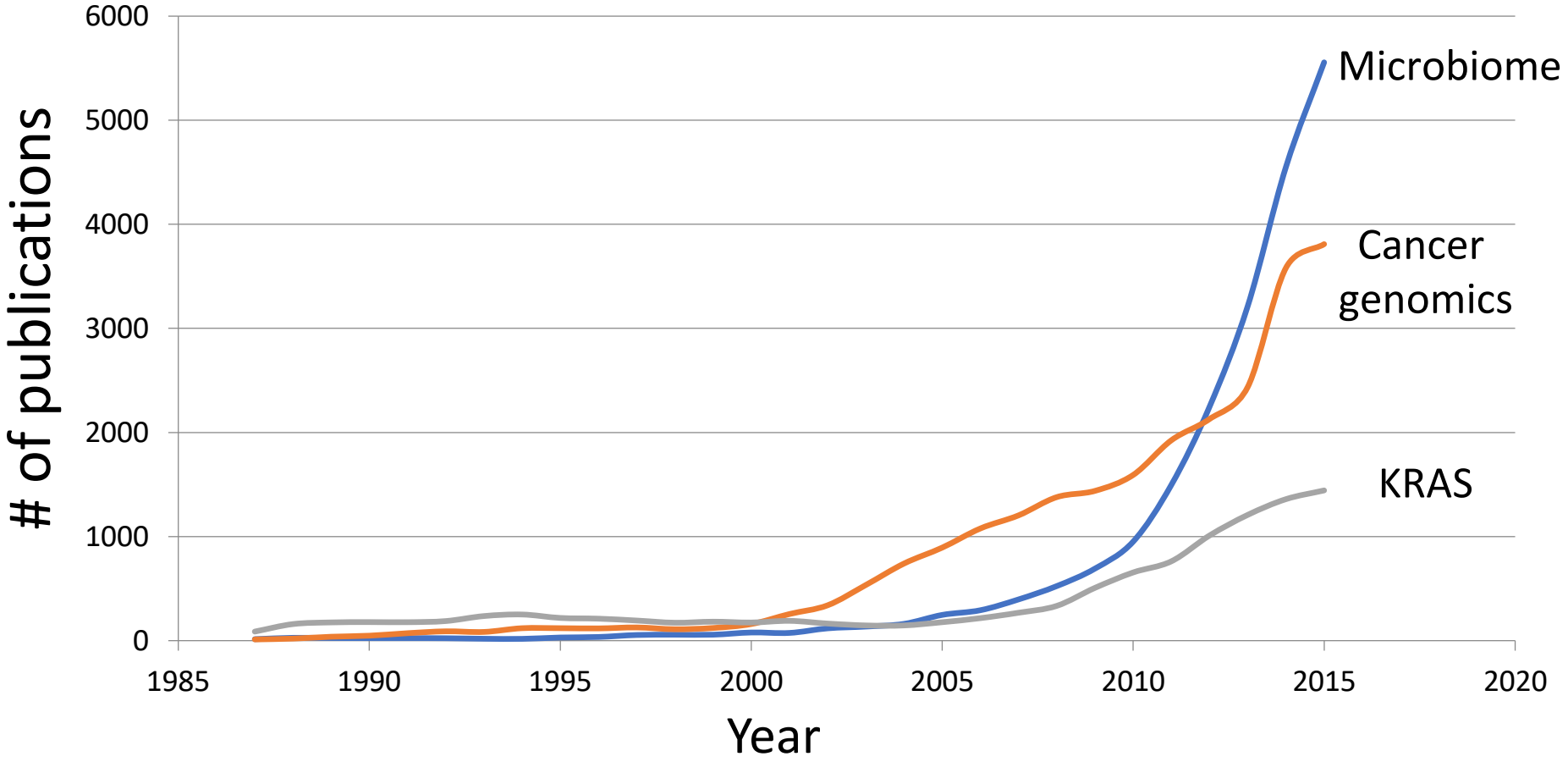


Lee YK, et al. Science 2010.

Microbiome-Disease Associations



A Growing Interest in the Microbiome

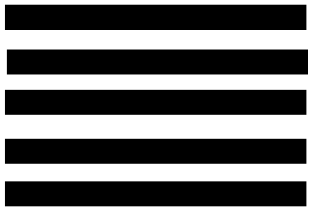


The Evolution of Sequencing to Study the Microbiome

1. Polymerase chain reaction (PCR)

DNA from a single organism isolated from culture

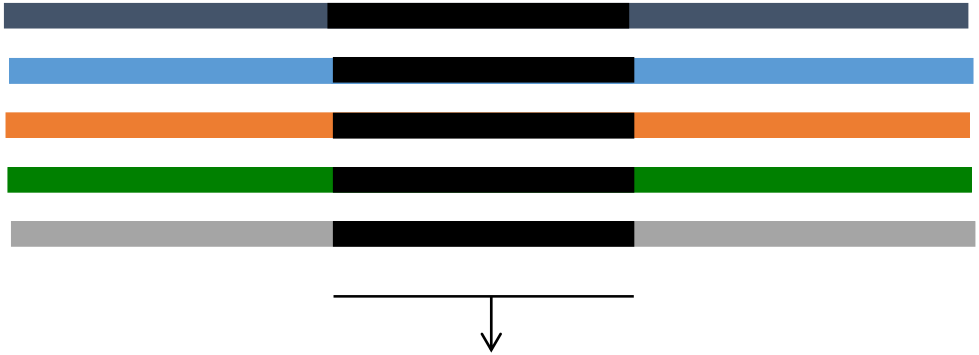
PCR amplification



Sequence of target DNA from one organism

2. 16S Ribosomal RNA Sequencing

Bacterial DNA



PCR of 16S ribosomal RNA gene (only present in bacteria)

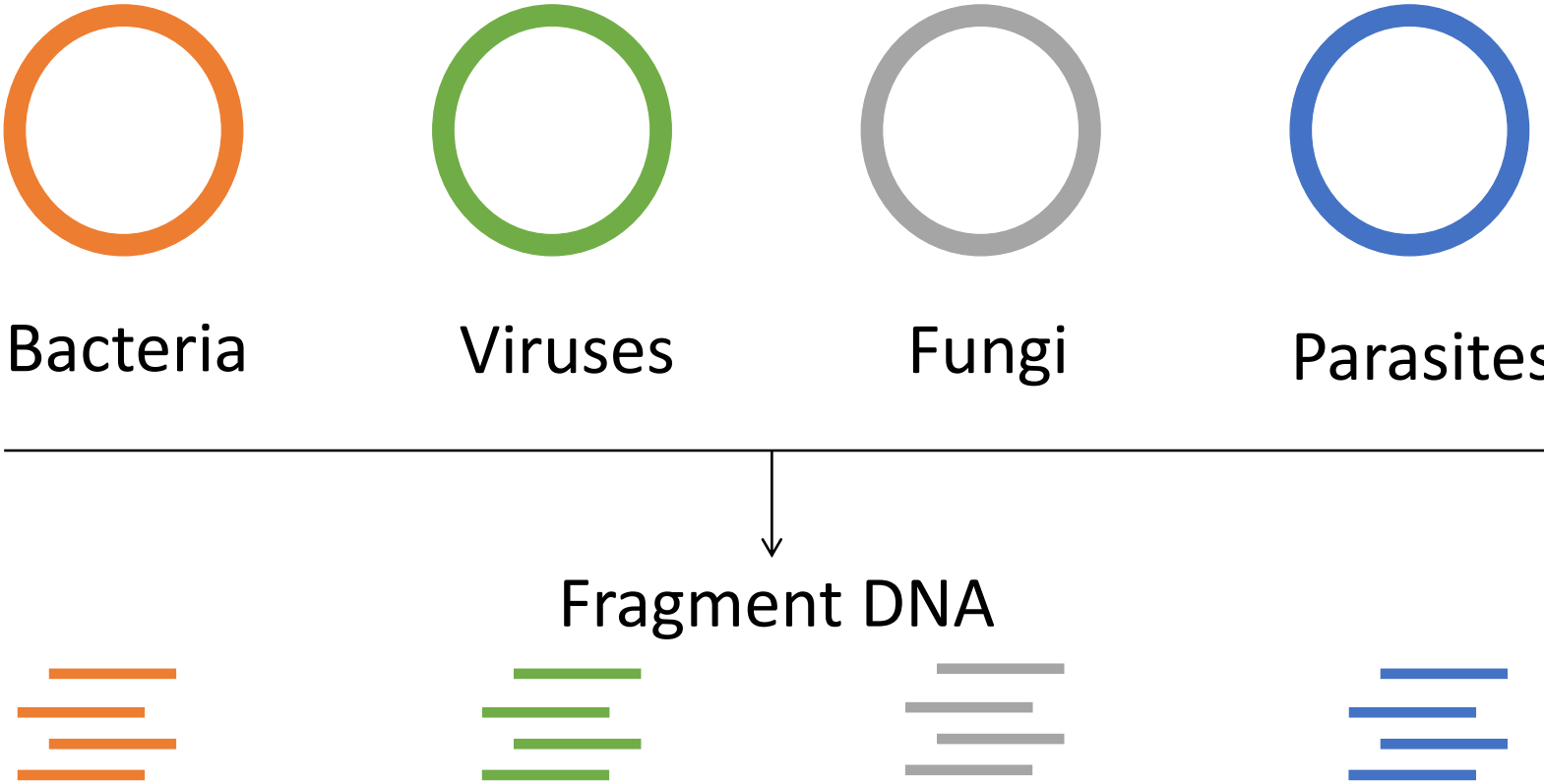
↓

Sequencing and comparison to reference database of bacteria

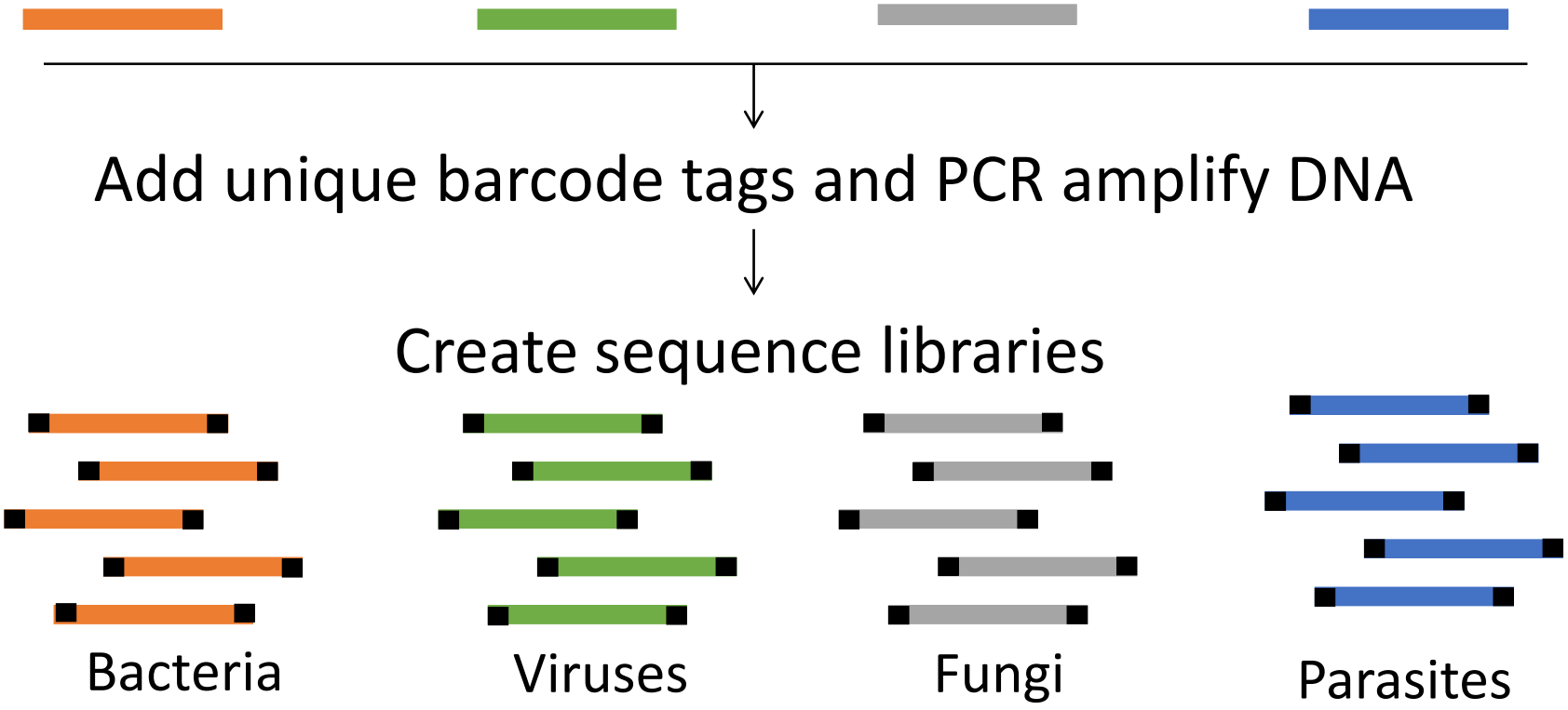
↓

Bacterial microbiome

3. Whole Genome Shotgun Metagenomic Sequencing



3. Whole Genome Shotgun Metagenomic Sequencing



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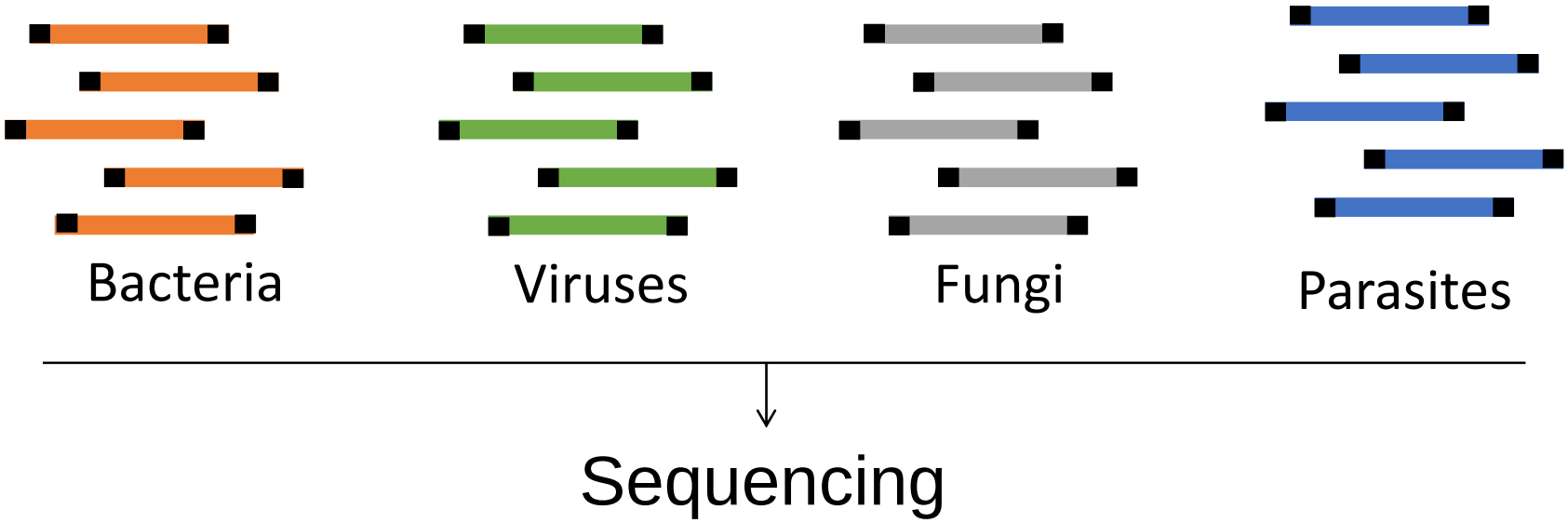
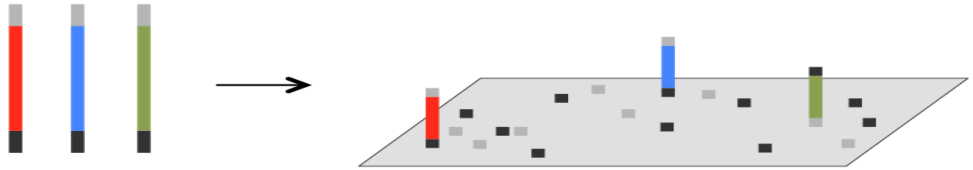


Next-Generation Sequencing



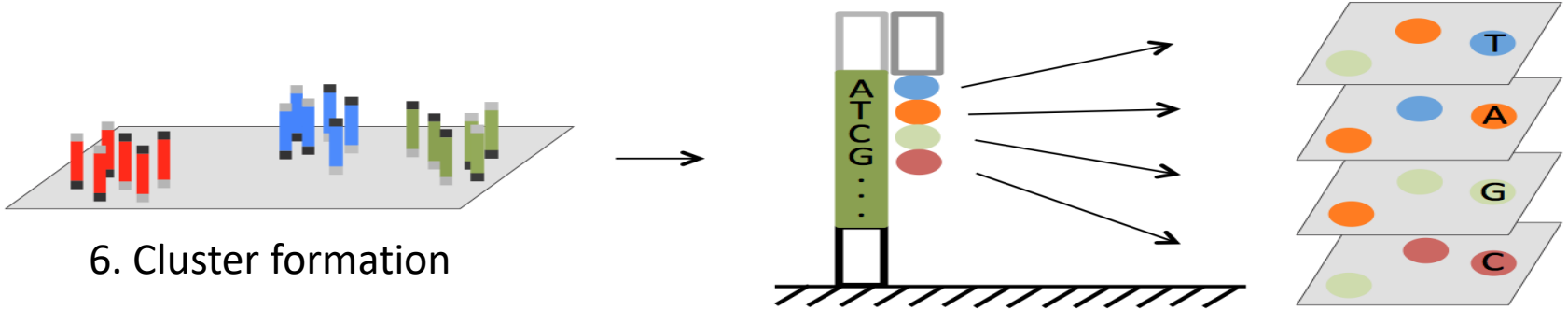
1. DNA Fragments with unique barcodes

2. Attach to flowcell

3. Bind to primers

4. PCR extension

5. Dissociation

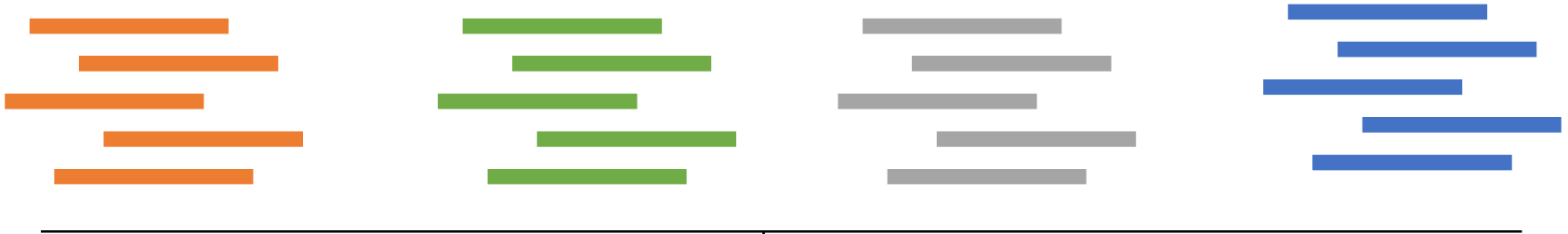


6. Cluster formation

7. Sequencing

8. Signal scanning

3. Whole Genome Shotgun Metagenomic Sequencing



Sequencing

↓

Compare to reference databases of organisms

↓

The microbiome

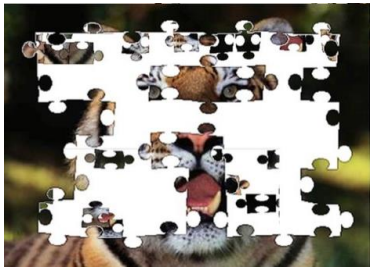
Comparison of Sequencing Techniques

16S sequencing

Gross taxonomic classification

Metagenomic sequencing + limited gene analysis

Higher resolution taxonomic classification



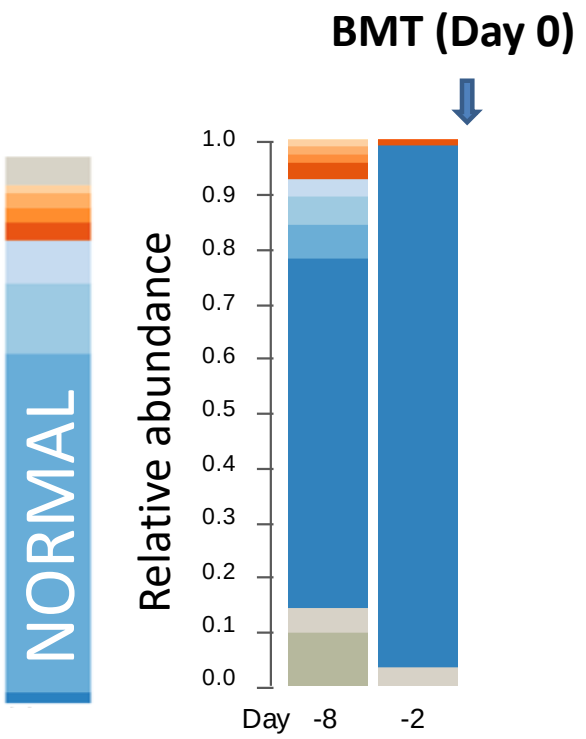
Metagenomic sequencing + whole genome analysis

Species/strain level classification

Non-bacterial data

Metabolic pathways

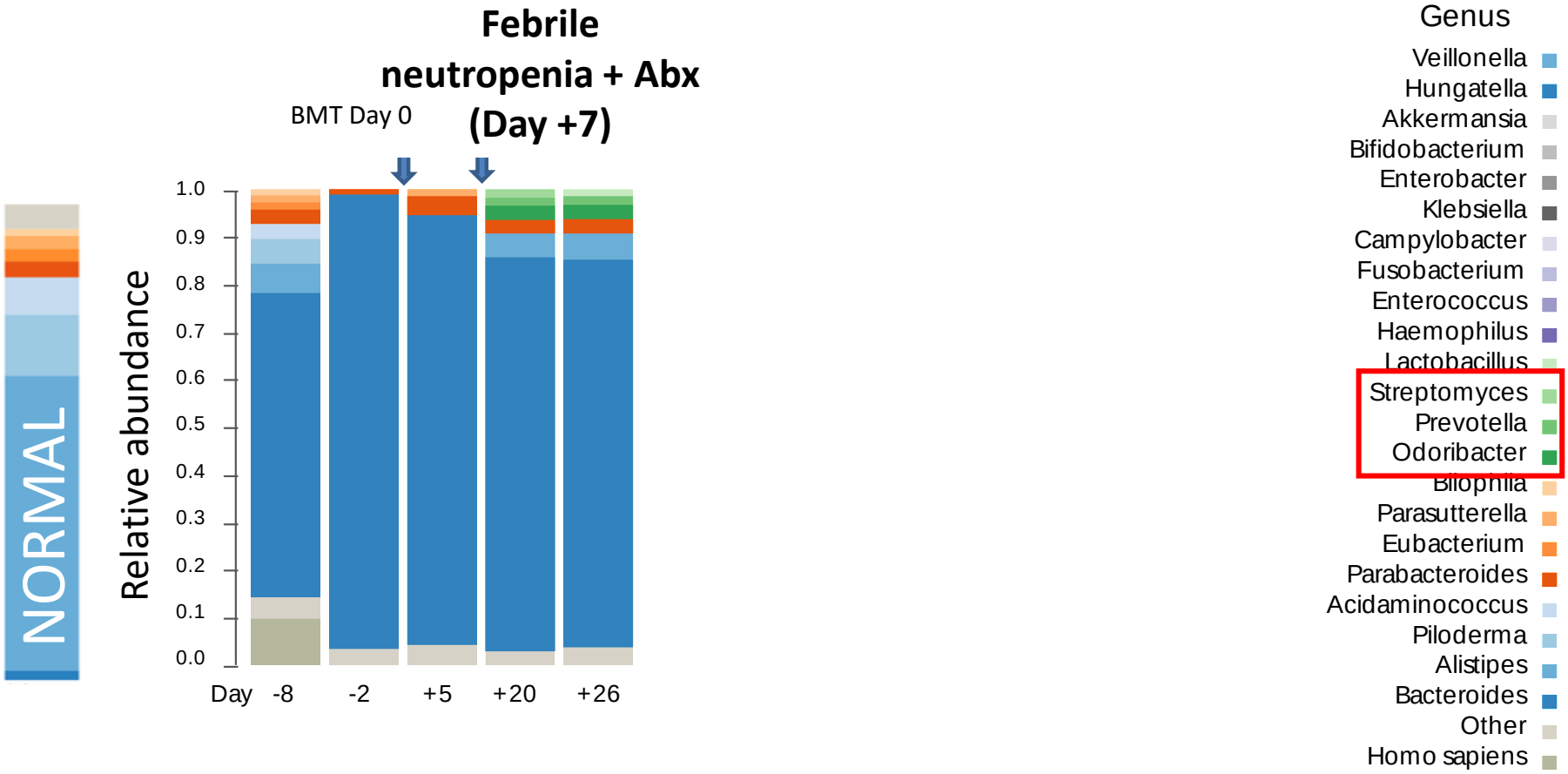
The Microbiome in HCT



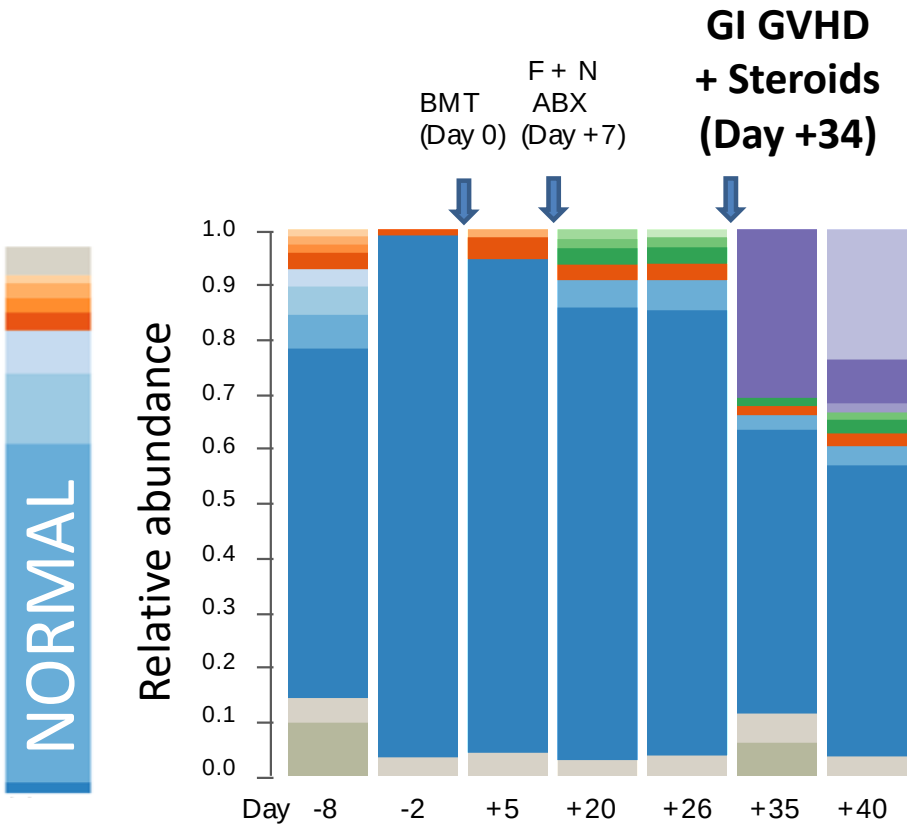
Genus

- Veillonella
- Hungatella
- Akkermansia
- Bifidobacterium
- Enterobacter
- Klebsiella
- Campylobacter
- Fusobacterium
- Enterococcus
- Haemophilus
- Lactobacillus
- Streptomyces
- Prevotella
- Odoribacter
- Bilophila
- Parasutterella
- Eubacterium
- Parabacteroides
- Acidaminococcus
- Piloderma
- Alistipes
- Bacteroides
- Other
- Homo sapiens

The Microbiome in HCT



The Microbiome in HCT



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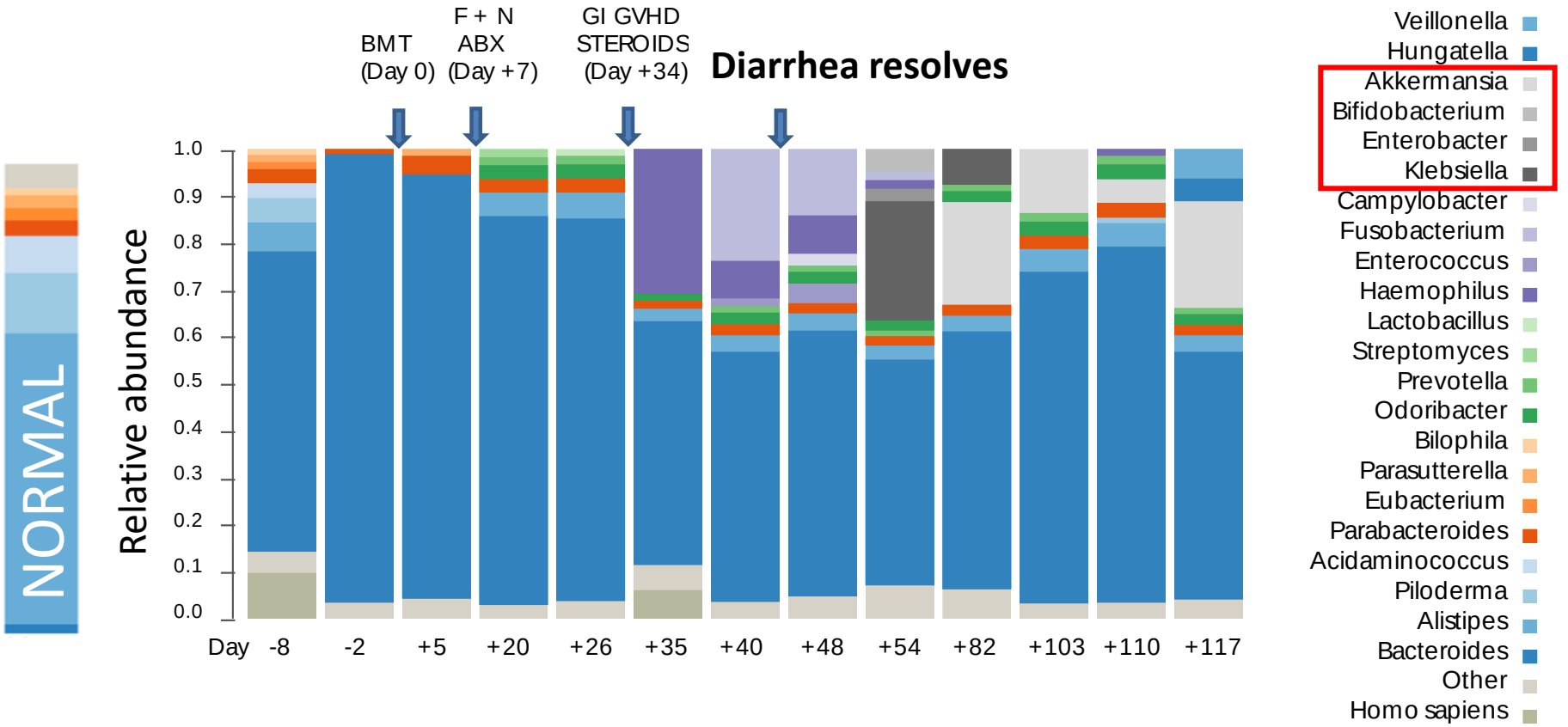
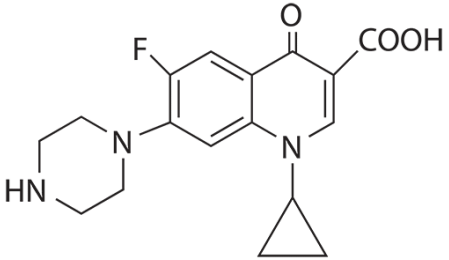
The Microbiome in HCT



How to Impact the Microbiome

Microbiome manipulation

Antibiotics



Antibiotics and the Microbiome

Antibiotics

Before antibiotic treatment

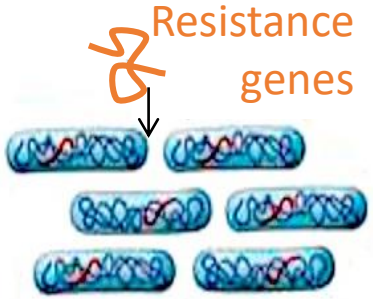
After antibiotic treatment

1. Alteration in population structure

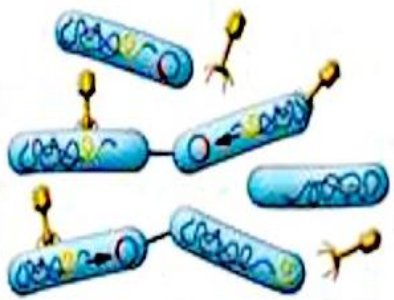
Modi et al. JCI, 2014

Antibiotics and the Microbiome

1. Alteration in population structure



2. Enrichment for resistance

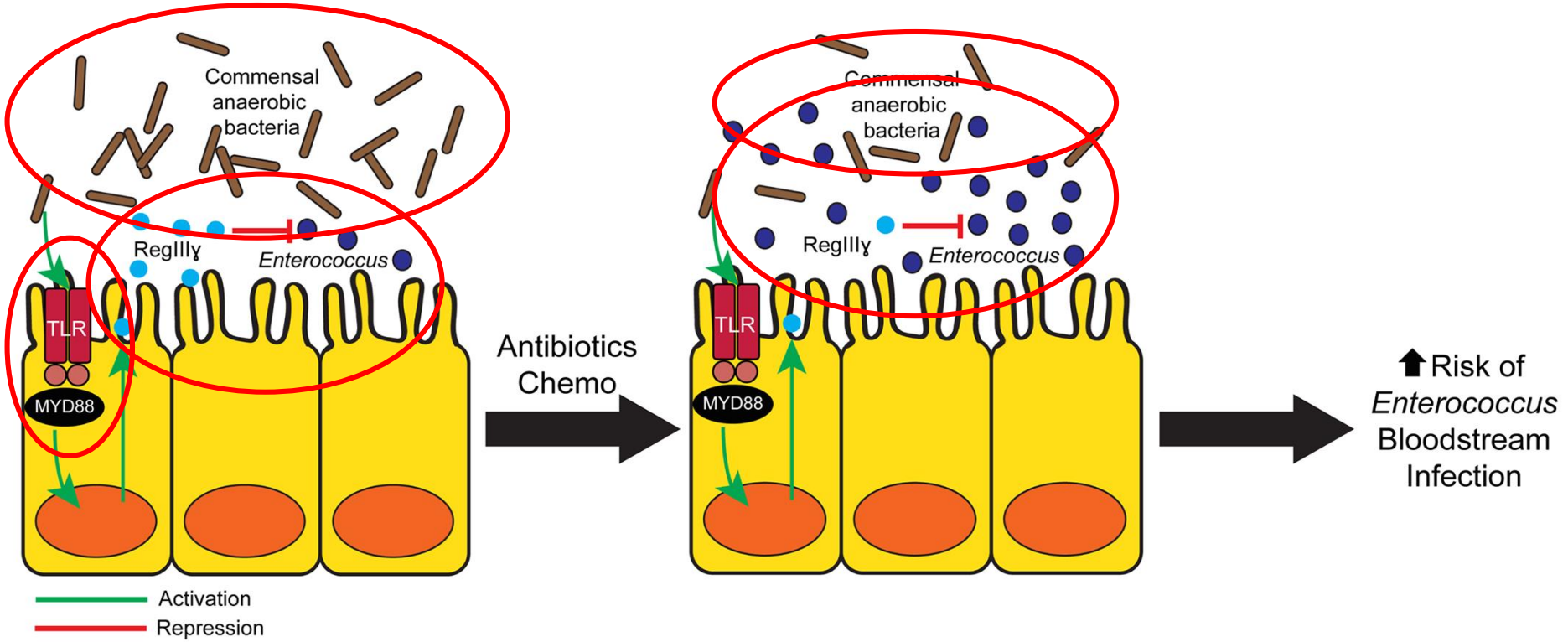


3. Increased mobilization of genetic elements

4. Availability for niche intrusion

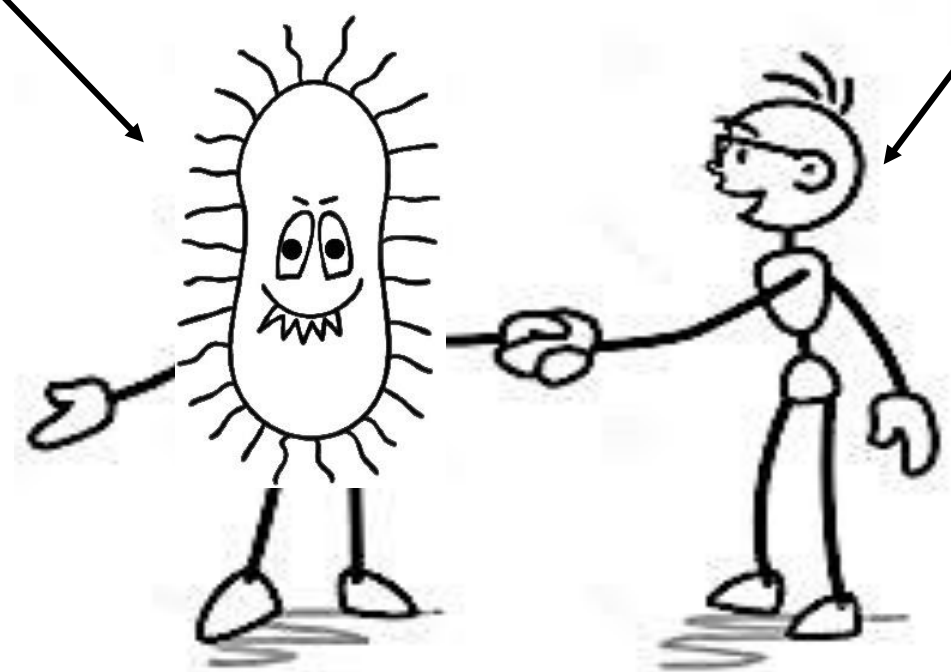
Antibiotics → Decreased resistance to colonization

How Antibiotics Decrease Colonization Resistance



Andrew Koh. PLOS Pathogens, 2017

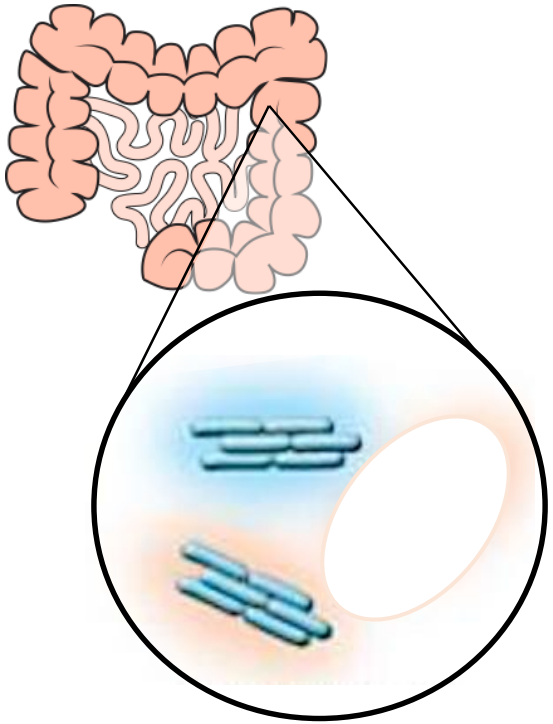
The Microbiome and the Immune System



GO HAND IN HAND

Antibiotics Decrease Colonization Resistance

Metronidazole →



↑ *Enterococcus spp.*

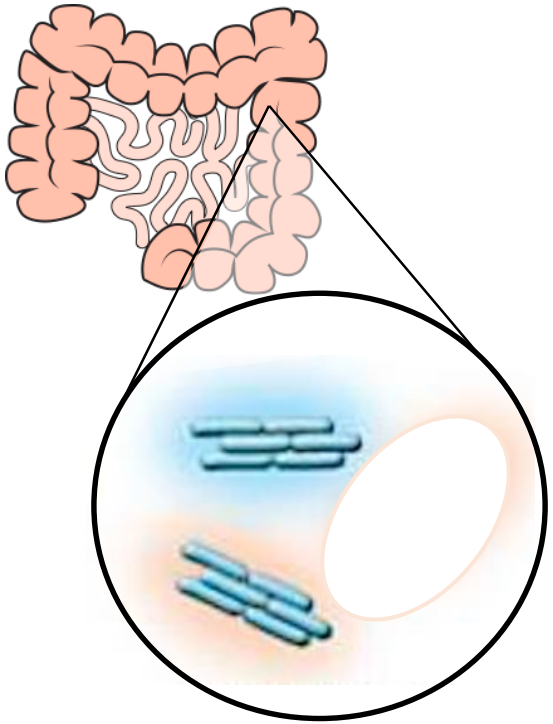
↓

Enterococcus bloodstream infection

Taur et al. CID, 2015

Antibiotics Decrease Colonization Resistance

Fluoroquinolones →



↑

Gram-negative rods (GNRs):

Escherichia coli

Klebsiella pneumoniae

Pseudomonas aeruginosa

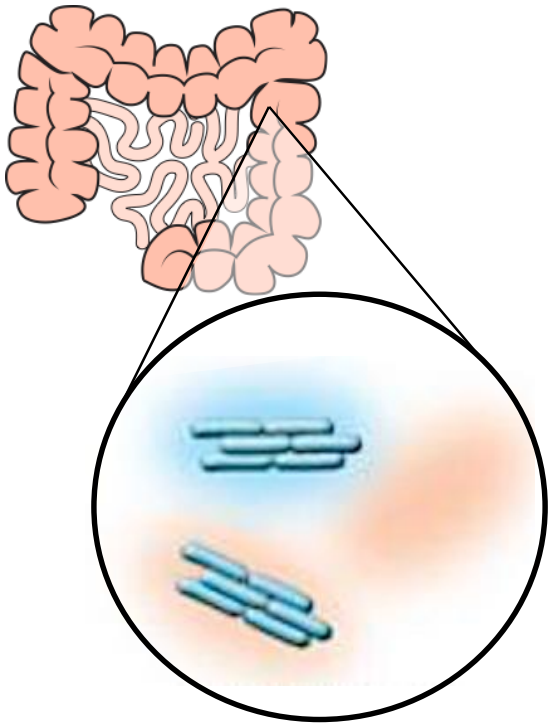
↓

GNR bloodstream infection

Taur et al. CID, 2015

Antibiotics Decrease Colonization Resistance

Fluoroquinolones →



Anaerobes

<u>Gram-negative rods (GNRs)</u>:

Escherichia coli

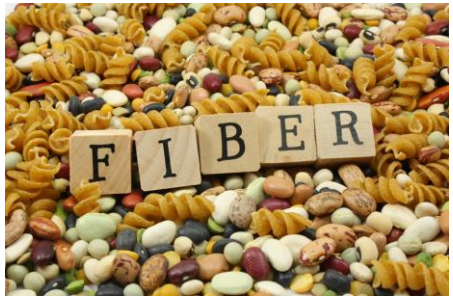
Klebsiella pneumoniae

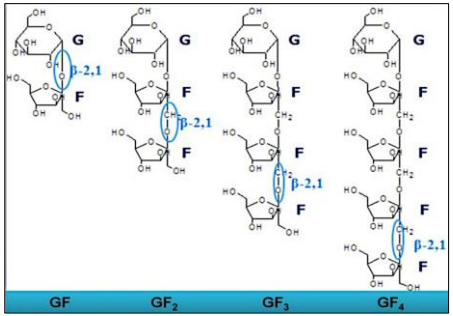
Pseudomonas aeruginosa

Taur et al. CID, 2015

Microbiome manipulation

Prebiotics



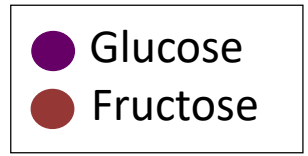


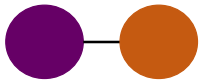
Prebiotics

- Dietary fibers
- Indigestible by humans
- Nutrition for healthy gut bacteria

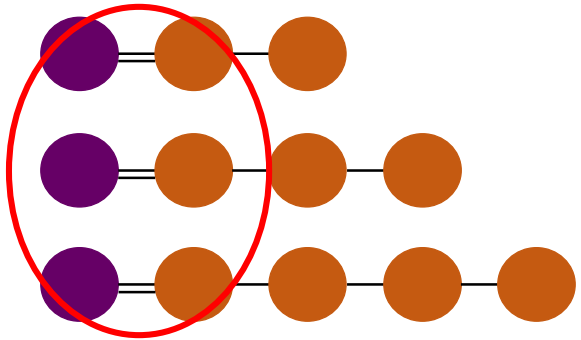
  ↑*Bifidobacterium spp.*

Sugars:

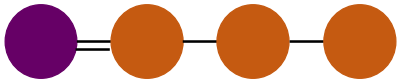




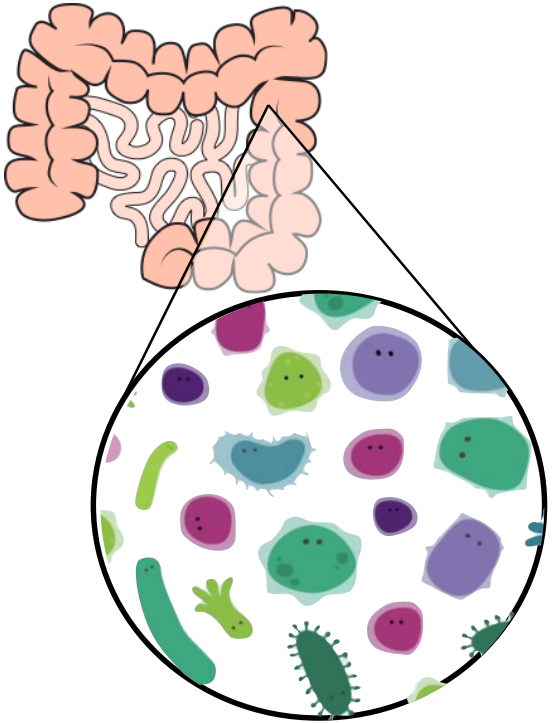
Fructo-oligosaccharides (FOS):



Prebiotics



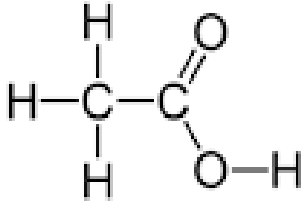
Prebiotic→



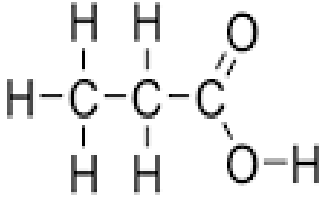
Fermentation

↑Short-chain fatty acids (SCFAs)

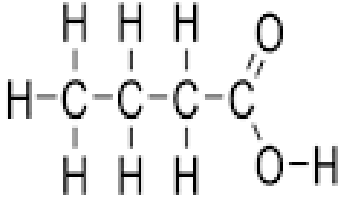
Short-Chain Fatty Acids (SFCAs)



Acetate



Propionate



Butyrate

- Maintain integrity of intestinal mucosa
- Improve intestinal immune regulation

🡻 Pro-inflammatory cytokines & immune cells

🡹 Anti-inflammatory cytokines & immune cells (e.g. T-regs)

Can Prebiotics Benefit HCT Recipients?

- Observational study of 44 HCT recipients

  (Iyama et al. Case Reports in Oncology, 2014)

  - Fructo-oligosaccharides 9g/day (n=22)*
  - Controls (n=22)

- FOS vs. controls

  ↓ Severe diarrhea and mucositis

  ↓ Weight loss

  ↑ Overall survival at day +100 (100% vs. 77%, $p < 0.05$)

* FOS plus glutamine

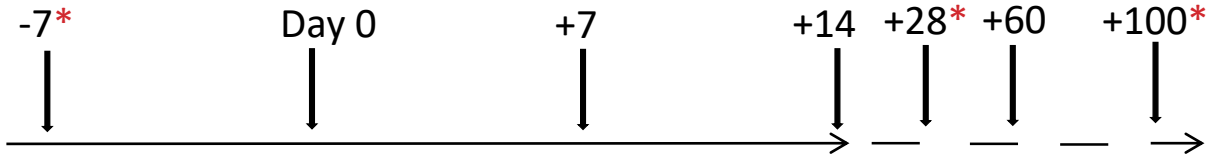
A Single-Arm Dose-Escalation Trial of FOS

Primary Outcome: Maximum tolerated dose
Tolerability = 70% or more of doses taken

Eligibility (n=15)

- ≥ 18 years of age
- Hematologic malignancy
- Allogeneic HCT
- Reduced-intensity conditioning protocol

Stool collection



Treatment (21 days):
FOS 5g, 10g, or 15g per day

* Blood samples

Results of trial to date

FOS dose	5g/day N=5	10g/day N=10	Total N=15
Consumed ≥70% FOS doses*	4 (80%)	6 (60%)	10 (66.7%)
Mean % of doses taken	81.4%	59.1%	80%

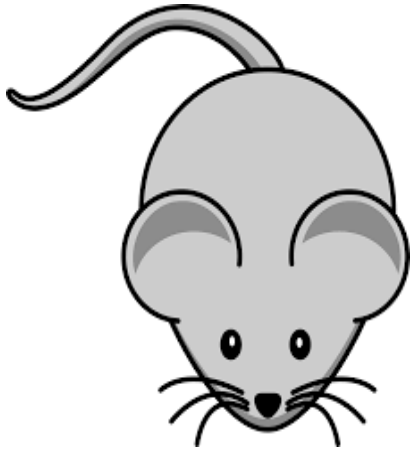
- No serious adverse events
- Minor: flatulence and abdominal discomfort

Microbiome manipulation

Probiotics

Can Probiotics Benefit the Gut Microbiome?

Lactobacillus rhamnosus→



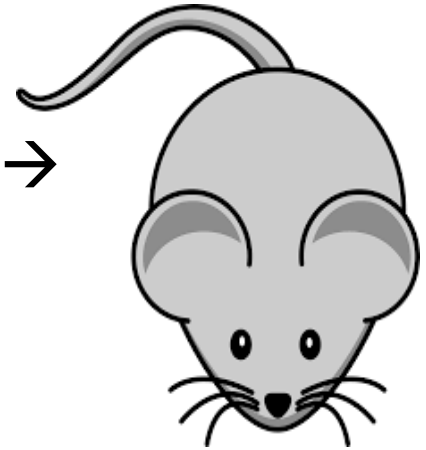
↓ Mortality
↓ GVHD

Allogeneic HCT mouse model

Gerbitz et al. Blood, 2004

Can Probiotics Benefit the Gut Microbiome?

SCFA-producing *Clostridia spp.* →



↓ Mortality
↓ GVHD severity

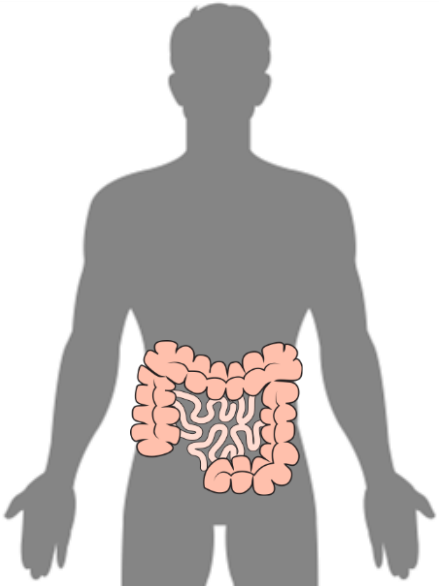
Allogeneic HCT mouse model

Mathewson et al. Nature Immnology, 2016

Simms-Waldrip et al. BBMT, 2017

Can Probiotics Benefit HCT Recipients?

- RCT (n=31)
- *Lactobacillus rhamnosus*→



Allogeneic HCT recipients

<u>No effect:</u>

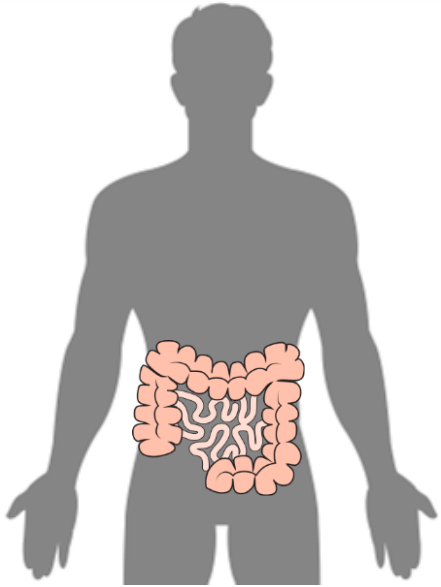
- Mortality
- GVHD
- No adverse events

Gorshein et al. Clinical Transplantation, 2017

Fecal Microbiota Transplant (FMT)

Healthy donor stool →

- Nasogastric tube
- Colonoscopy
- Oral “crapsules”



Recurrent or refra

Clostridium difficile infection (CDI)

<u>In all patients</u>:

- 70-90% resolution of CDI

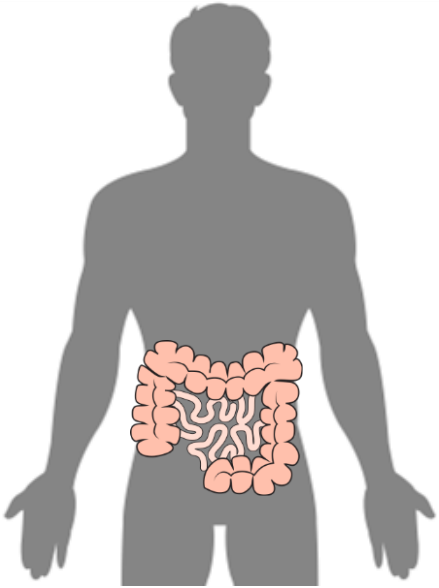
<u>In HCT patients</u>:

- 18 patients published
- 16 of 18 resolved CDI
- No adverse events

Eli Moss, Shannon Falconer et al. PLOS One, 2017

Fecal Microbiota Transplant (FMT)

Healthy donor stool →



Steroid-refractory GI GVHD

Resolution of GI GVHD:

- 5 of 7 published cases
- 2 of 7 partial resolution
- No adverse events

Kakihana et al. Blood, 2016

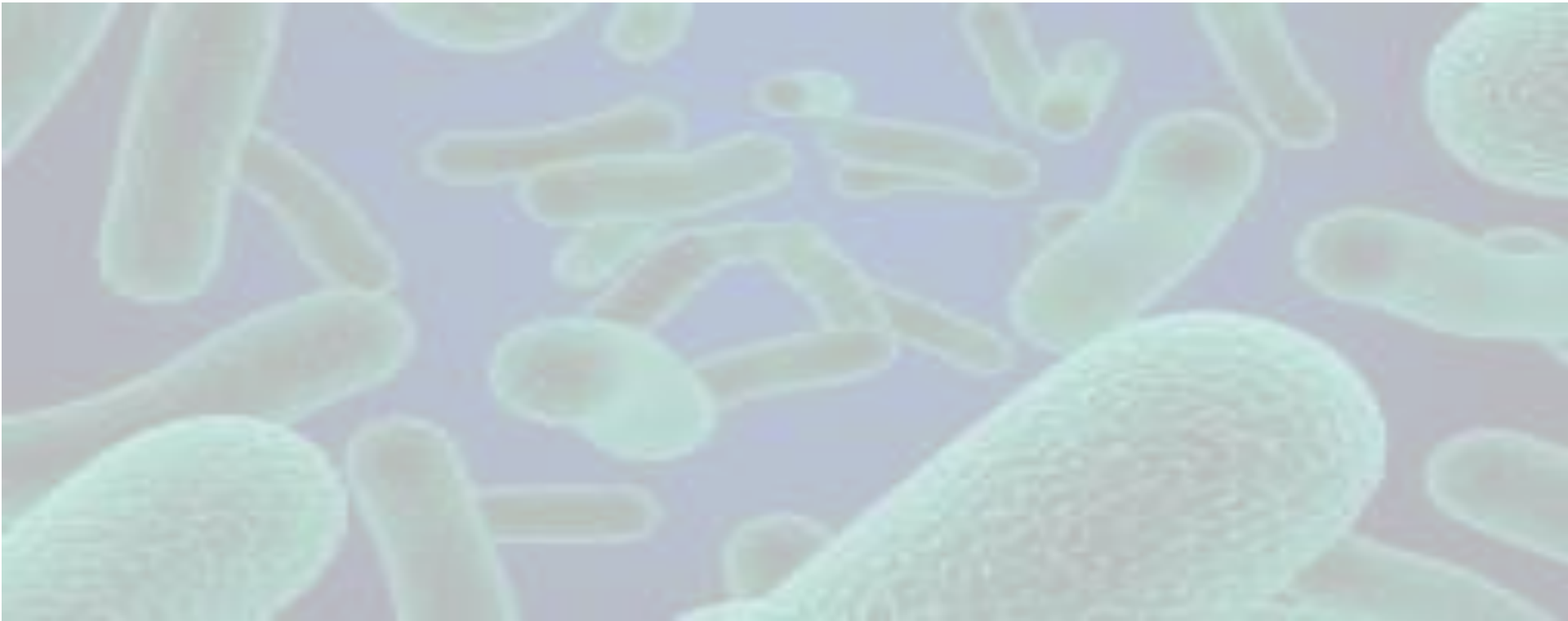
Spindelboeck et al. Haematologica, 2017

How Changes in the Microbiome Can Impact HCT Patients

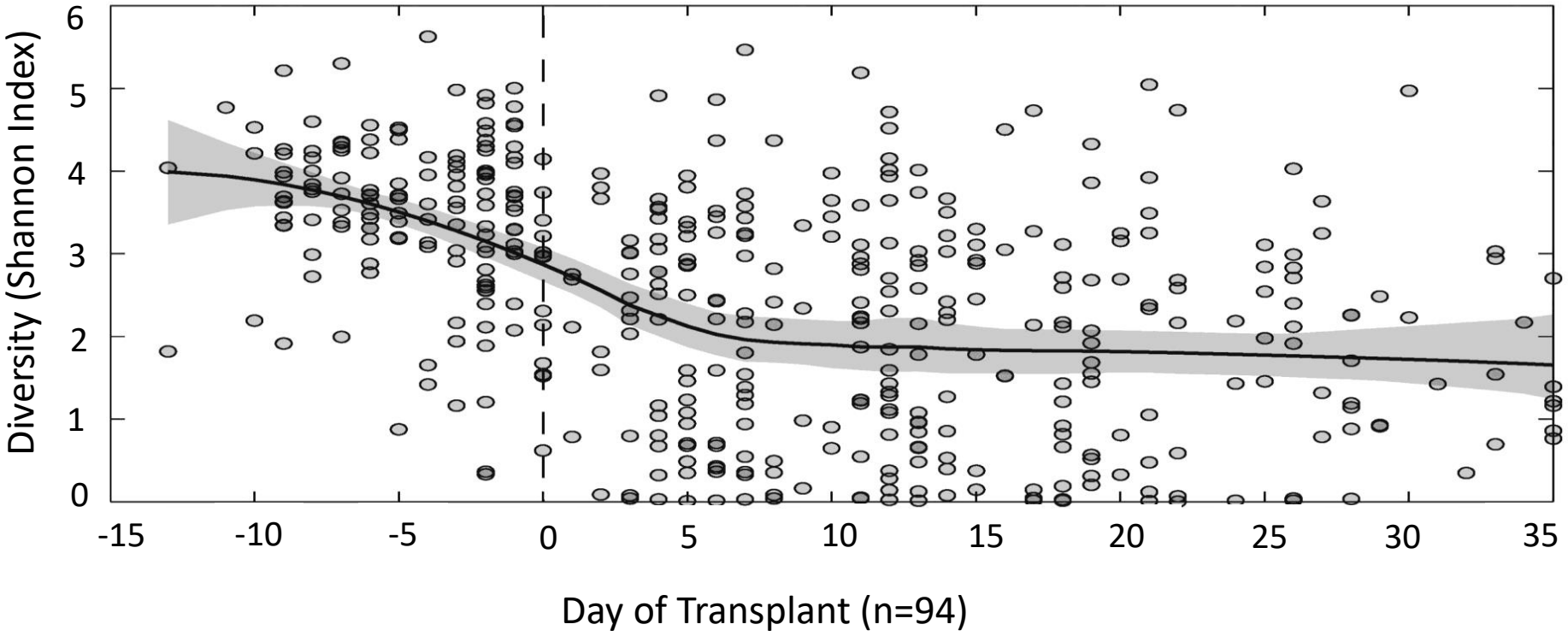
Microbiome Impact:

1. **Mortality**
2. **GVHD**
3. **Relapse**

Microbiome Impact: **Mortality**



Microbiome Diversity Decreases After Allo-HCT

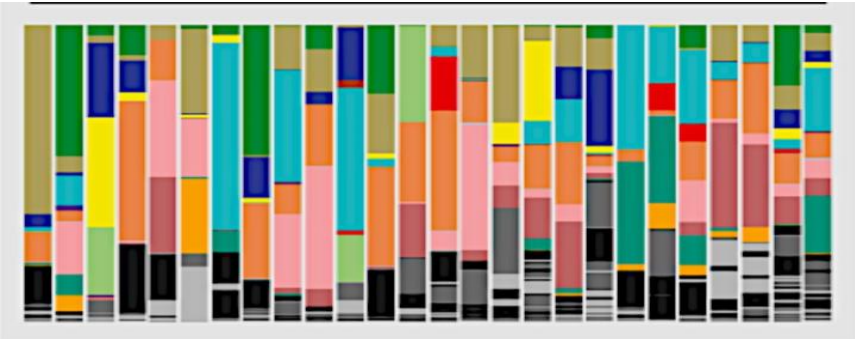


Taur et al. Clin Infect Dis, 2012

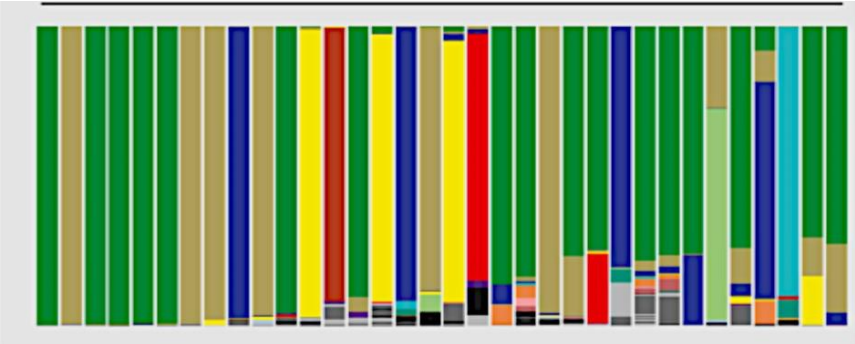
Microbiome Diversity at Engraftment

Relative abundance

High Diversity (Inverse Simpson Index >4)



Low Diversity (Inverse Simpson Index <2)



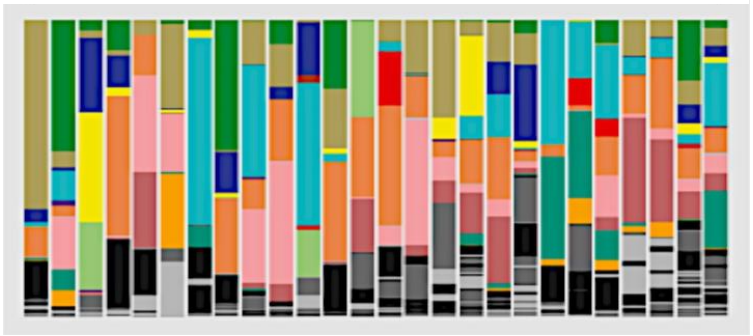
Taxonomy (Genus)

- Enterococcus
- Streptococcus
- Lactobacillus
- Staphylococcus
- Kluyvera
- Bacteroides
- Escherichia
- Lactococcus
- Veillonella
- Clostridium
- Granulicatella
- Blautia
- Ruminococcus
- Parabacteroides
- Alistipes
- Other Bacteria

Taur Y et al. Blood, 2014

Microbiome Diversity

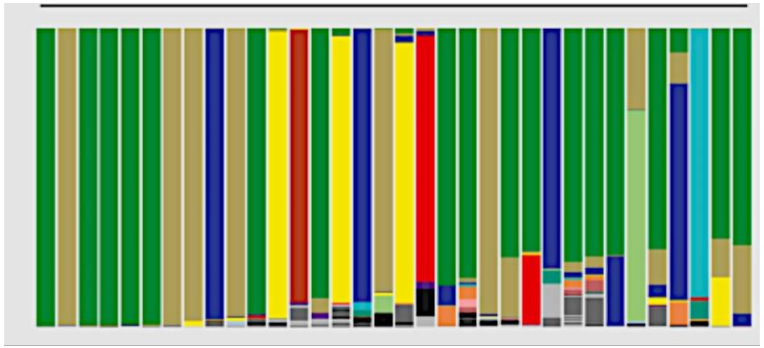
High Diversity



Taur Y et al. Blood, 2014

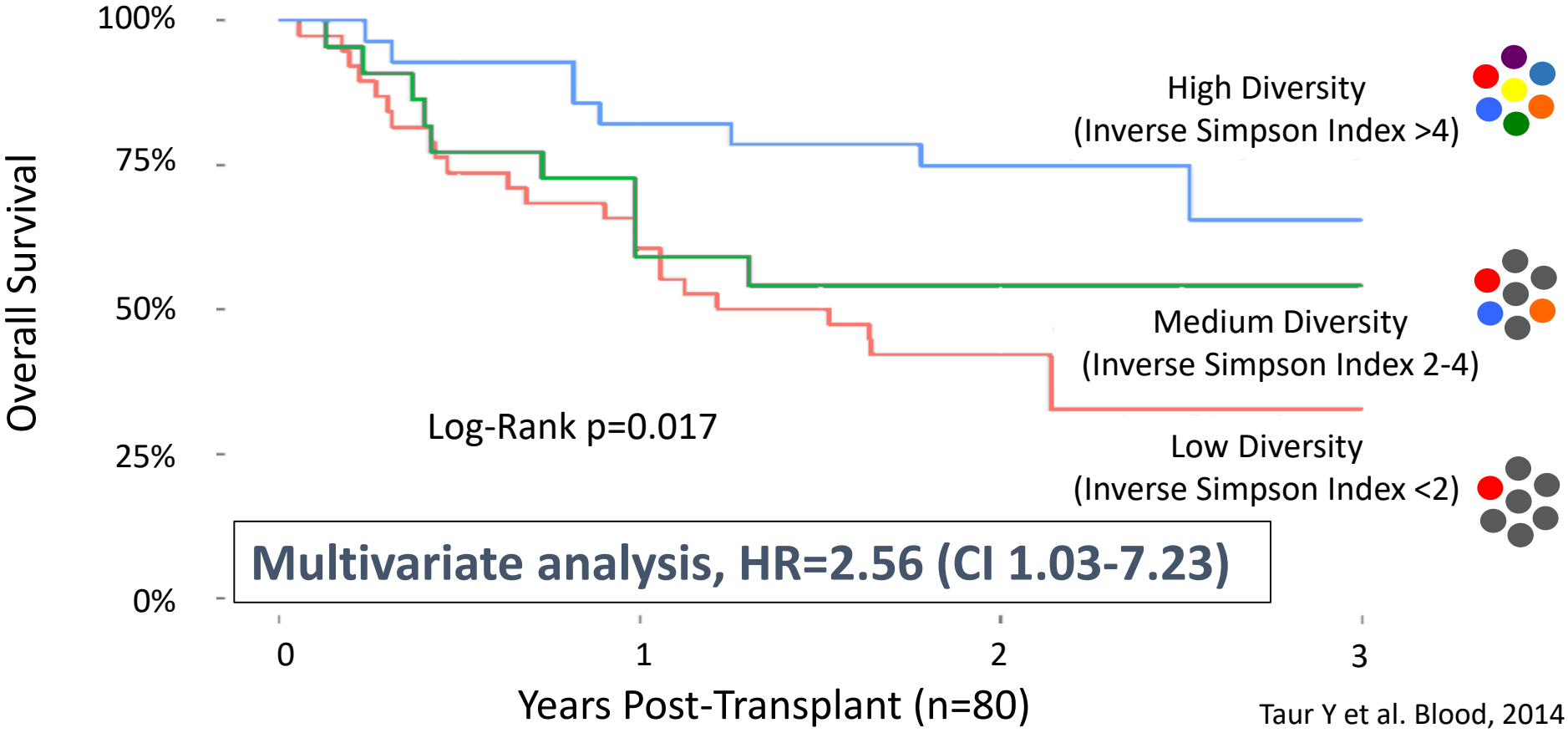
Microbiome Diversity at Engraftment

Low Diversity



Taur Y et al. Blood, 2014

Low Diversity at Engraftment is Associated with Decreased Survival



Taur Y et al. Blood, 2014

Microbiome Impact: **GVHD**

1. **Diversity**

1. **Anaerobic bacteria**

Microbiome Impact: **GVHD**

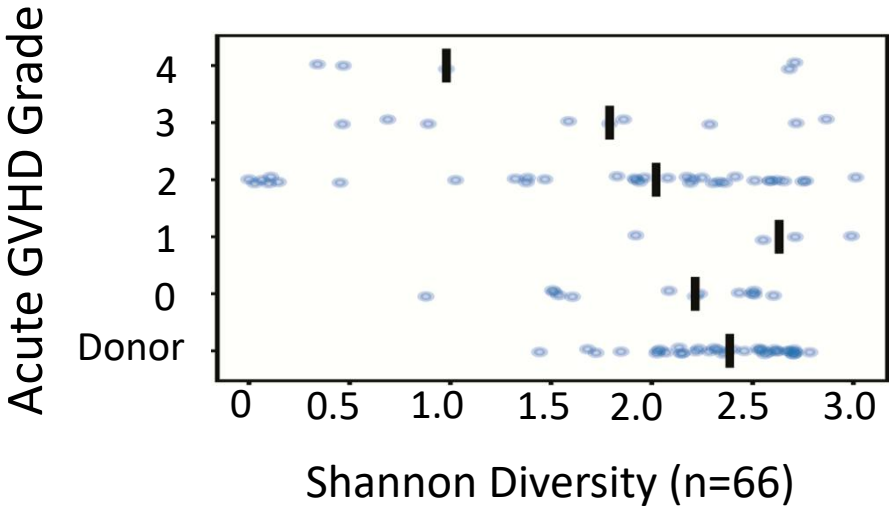
1. **Diversity**

Low Diversity at Engraftment is Associated with GVHD

↓ Stool microbiota diversity at engraftment

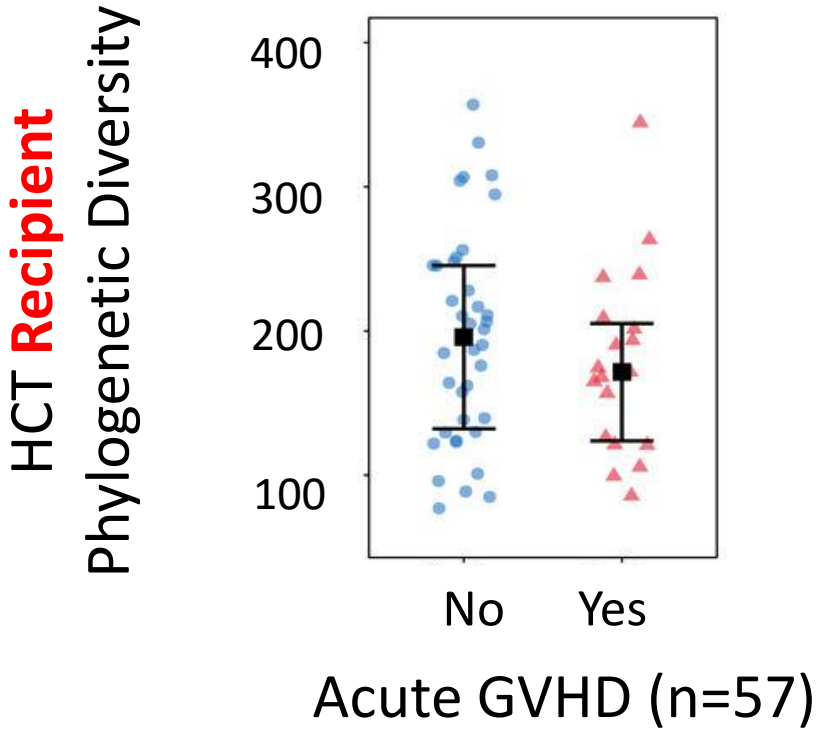
↓

↑ Severe acute GVHD



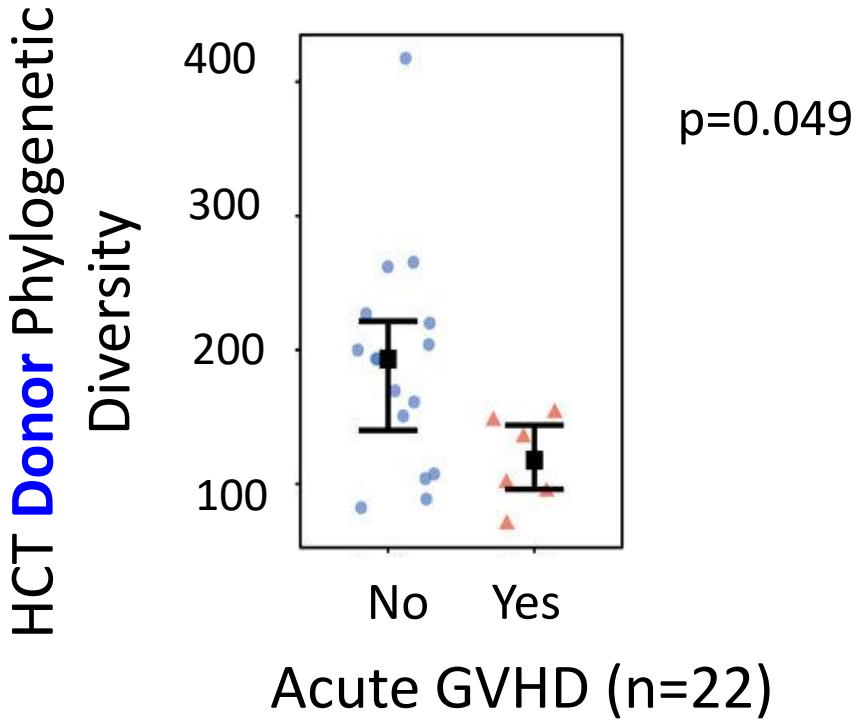
Golob et al. CID, 2017

Diversity Prior to Conditioning is NOT Associated with GVHD



Liu et al. BMT, 2017

Donor Diversity IS Associated with GVHD



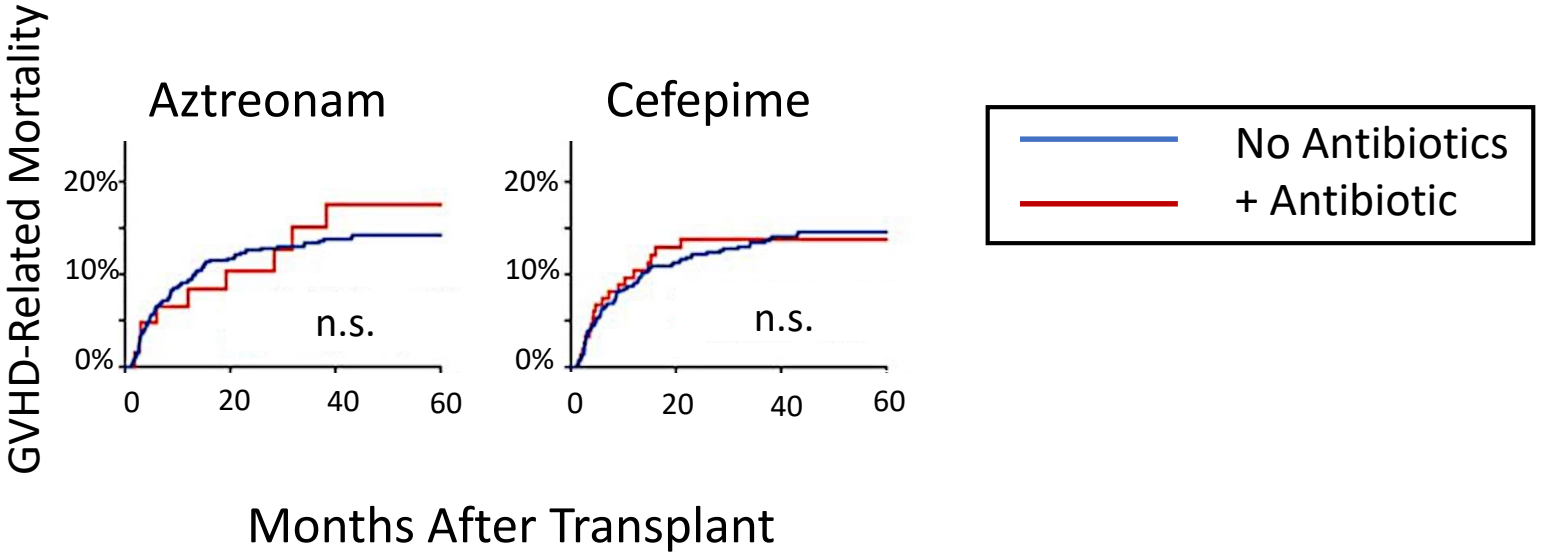
Liu et al. BMT, 2017

Microbiome Impact: **GVHD**

2. Anaerobic bacteria

Decrease in Obligate Anaerobes Associated with GVHD

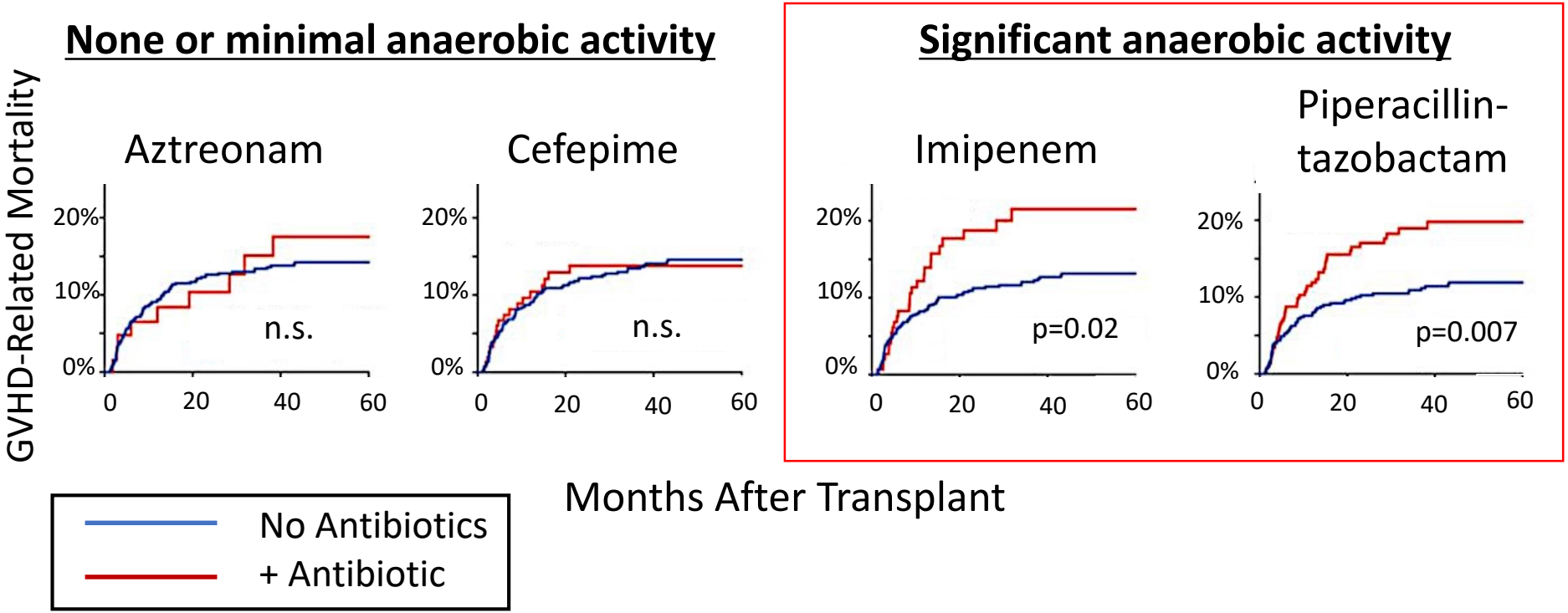
None or minimal anaerobic activity



Shono et al. Science Transl Med, 2016

Decrease in Obligate Anaerobes Associated with GVHD

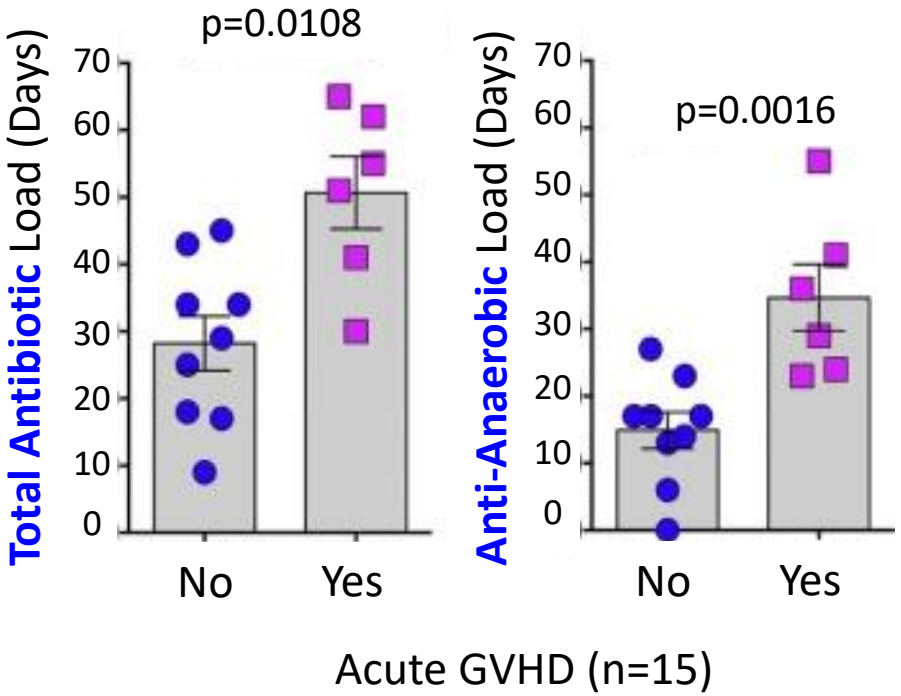
- Anti-anaerobic antibiotics associated with ↑ GVHD-related mortality



Shono et al. Science Transl Med, 2016

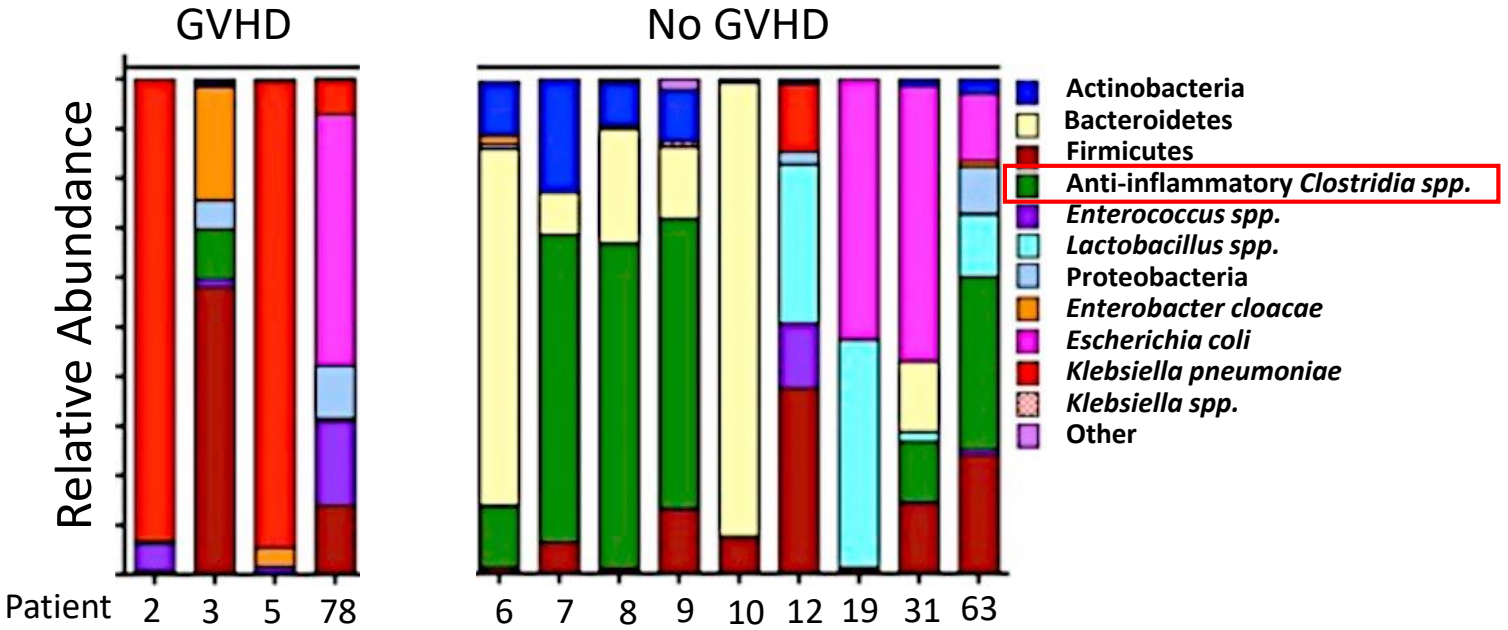
Decrease in Obligate Anaerobes Associated with GVHD

- Anti-anaerobic antibiotic exposure ↑ GVHD
- Greatest association with <u>clindamycin</u>



Simms-Waldrip et al. BBMT, 2017

Decrease in *Clostridia spp*. Associated with GVHD



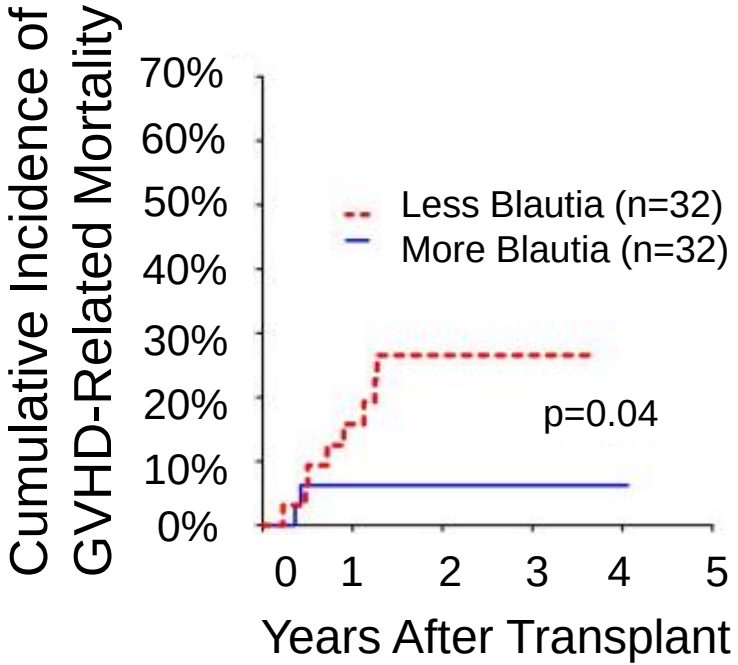
*↑ Anti-inflammatory *Clostridia spp*. → ↓ GVHD

Simms-Waldrip et al. BBMT, 2017

Decrease in *Blautia spp*. Associated with GVHD

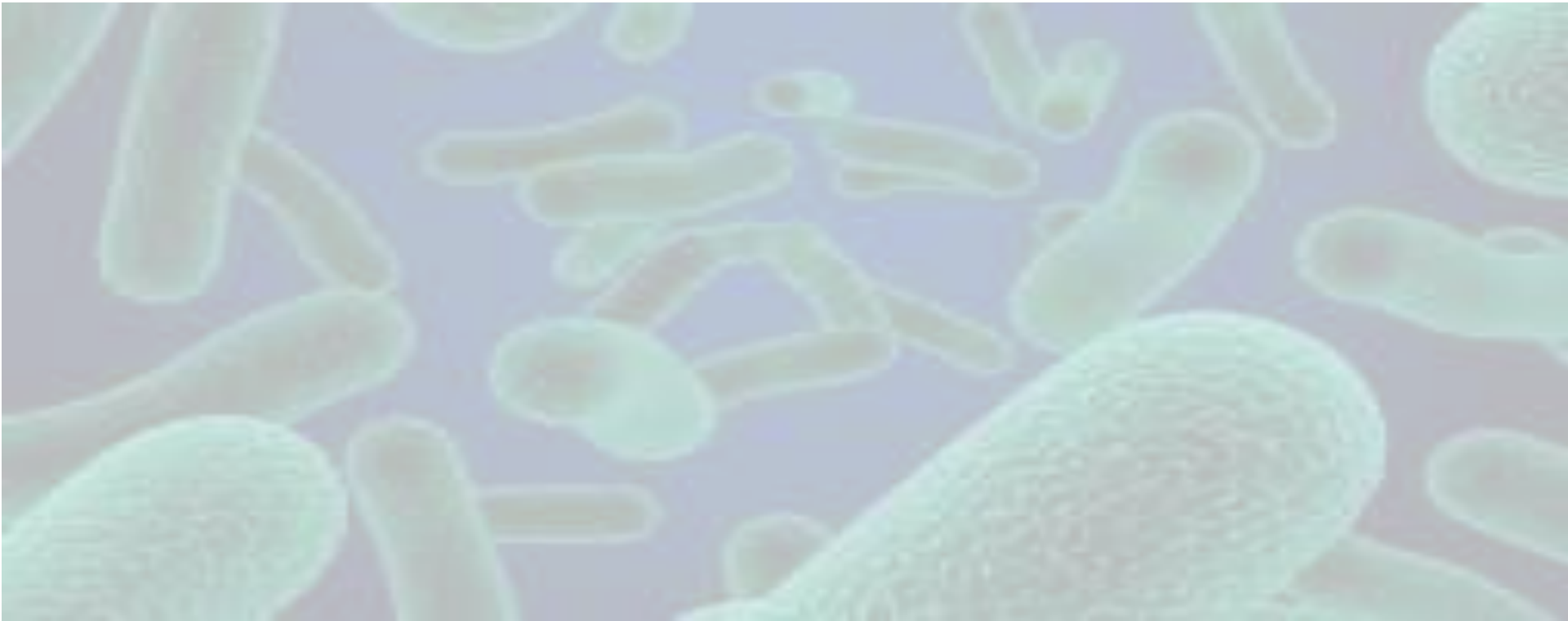
↓ *Blautia spp*. associated with:

- ↑ GVHD-related mortality
- ↑ Overall mortality
- ↑ Treatment-related mortality
- ↑ Relapse-related mortality



Jenq et al. BBMT, 2015

Microbiome Impact: **Relapse**

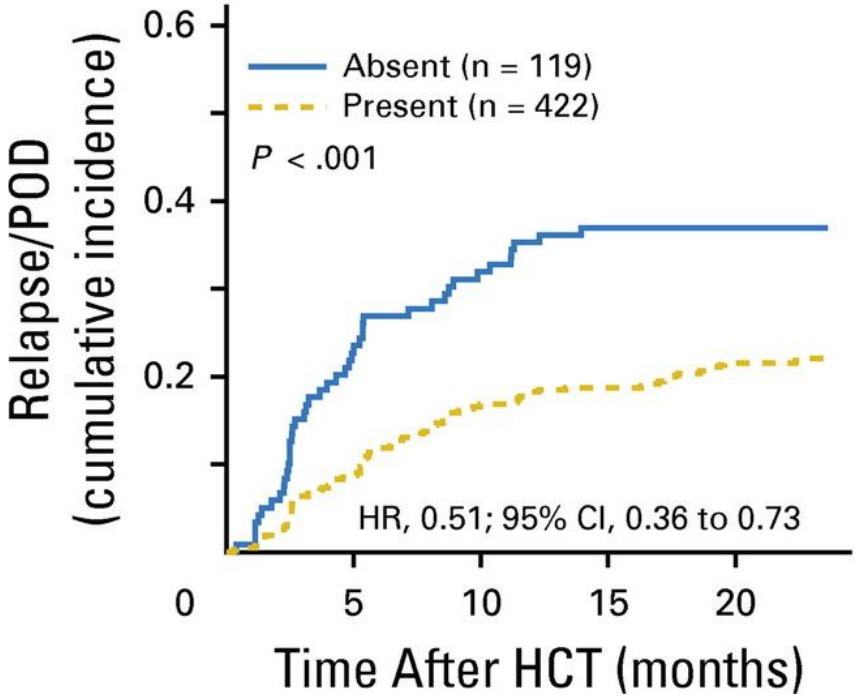


Organisms in the Microbiome are Associated with Relapse

↑ Relative abundance of a bacterial group composed of *Eubacterium limosum*

↓

↓ Relapse of hematologic malignancy



Peled et al. JCO, 2017

Take Home Points: The Microbiome in HCT

1. Complex ecosystem of organisms required for human health

2. Disturbances associated with important clinical outcomes

3. The lack of diversity in the intestinal microbiome is associated with worse outcomes

4. Obligate anaerobic bacteria are associated with colonization resistance and improved outcomes

Microbiome Stewardship in HCT Recipients

- Antibiotics save lives!
- The health cost of antibiotics:
  - To public health → antimicrobial stewardship
    - ↑ Antibiotic resistance

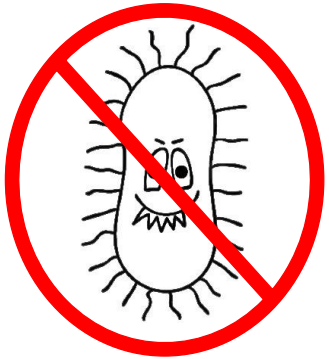
Microbiome Stewardship in HCT Recipients

- Antibiotics save lives!
- The health cost of antibiotics:
  - To public health → antimicrobial stewardship
    - ↑ Antibiotic resistance
  - To individuals → microbiome stewardship
    - ↑ Antibiotic resistance
      +
    - ↑ Infection, GVHD, relapse, mortality

Microbiome Stewardship in Practice

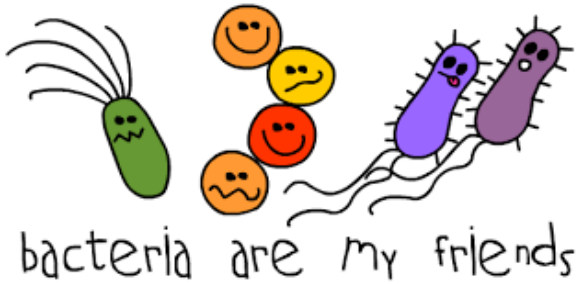
1. Febrile neutropenia

   Anaerobe-killing (pip-tazo, carbapenems)



   vs.

   Anaerobe-sparing (cefepime)



→RCT at MSKCC

Microbiome Stewardship in Practice

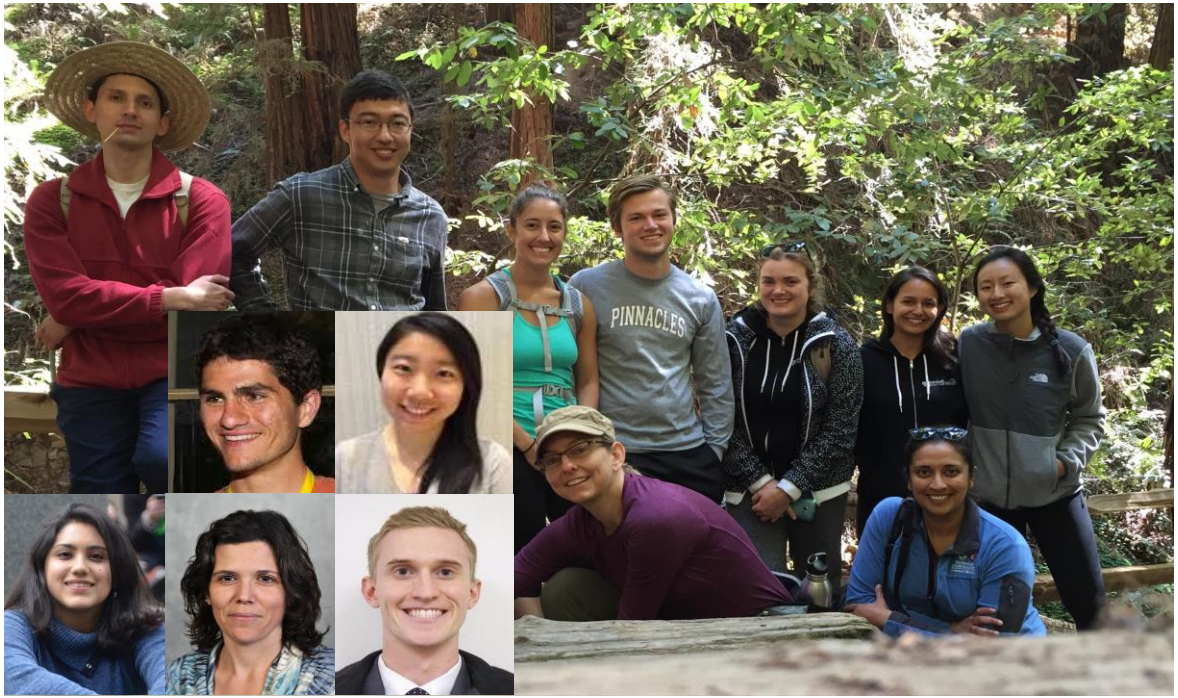
2. Gut decontamination:
   - Rifaximin vs. fluoroquinolones
     - Better preservation of intestinal microbiome
     - Decreased 1-year mortality
   - No antibiotics?

Weber et al. BMT, 2016

Future Areas of Research

Current	Future
Gastrointestinal	Non-GI: skin, eye, lung, vagina
Acute GVHD	Chronic GVHD
16S rRNA sequencing	Metagenomics, metatranscriptomics, metaproteomics
Bacteria	Bacteria, viruses, fungi, parasites
Single-institution	Multi-institutional
Association	Causation

Thank you



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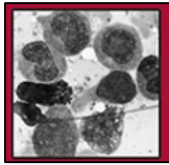
The Stanford Center for Clinical and Translational Research and Education



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Evaluation Reminder

Please complete the Council Meeting 2017 evaluation in order to receive continuing education credits and to provide suggestions for future topics.

We appreciate your feedback!