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

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Infectious Disease Fellow at Stanford University
Disclosures

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

<table>
<thead>
<tr>
<th>Name</th>
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<tbody>
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<td>NMDP</td>
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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
Microbiome-Disease Associations

- Depression
- Schizophrenia
- Anxiety
- Autism
- Parkinson’s Disease
- Crohn’s Disease
- Ulcerative Colitis
- Irritable Bowel Syndrome
- Obesity & Diabetes
- Colon Cancer
- Rheumatoid Arthritis
A Growing Interest in the Microbiome

# of publications

Year


Microbiome
Cancer genomics
KRAS
The Evolution of Sequencing to Study the Microbiome
1. Polymerase chain reaction (PCR)

DNA from a single organism isolated from culture

Primers

PCR amplification

Sequence of target DNA from one organism
2. 16S Ribosomal RNA Sequencing

Bacterial DNA

\[ \downarrow \]

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

\[ \downarrow \]

Sequencing and comparison to reference database of bacteria

\[ \downarrow \]

Bacterial microbiome
3. Whole Genome Shotgun Metagenomic Sequencing

- Bacteria
- Viruses
- Fungi
- Parasites

Fragment DNA
3. Whole Genome Shotgun Metagenomic Sequencing

Add unique barcode tags and PCR amplify DNA

Create sequence libraries

Bacteria

Viruses

Fungi

Parasites
3. Whole Genome Shotgun Metagenomic Sequencing

- Bacteria
- Viruses
- Fungi
- Parasites

Sequencing
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
- Streptomycyes
- Prevotella
- Odoribacter
- Bilophila
- Parasutterella
- Eubacterium
- Parabacteroides
- Acidaminococcus
- Piloderma
- Alistipes
- Bacteroides
- Other
- Homo sapiens

Relative abundance

BMT (Day 0)

Day -8 -2
The Microbiome in HCT

Febrile neutropenia + Abx (Day +7)

Relative abundance

Day -8  -2  +5  +20  +26

BMT Day 0

Genus

Veillonella
Hungatella
Akkermansia
Bifidobacterium
Enterobacter
Klebsiella
Campylobacter
Fusobacterium
Enterococcus
Haemophilus
Lactobacillus
Streptomyces
Prevotella
Odoribacter
Biophila
Parasutterella
Eubacterium
Parabacteroides
Acidaminococcus
Piloderma
Alistipes
Bacteroides
Other
Homo sapiens
The Microbiome in HCT

Relative abundance

Day -8 Day -2 Day +5 Day +20 Day +26 Day +35 Day +40

BMT (Day 0)
F + N ABX (Day +7)
GI GVHD + Steroids (Day +34)

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

Diarrhea resolves

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

Genus

Day 0
Day +7
Day +34
How to Impact the Microbiome
Antibiotics and the Microbiome

Before antibiotic treatment

1. Alteration in population structure
2. Enrichment for resistance
3. Increased mobilization of genetic elements
4. Availability for niche intrusion

Resistance genes

Modi et al. JCI, 2014

After antibiotic treatment

1. Alteration in population structure
Antibiotics and the Microbiome

Antibiotics

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 $\rightarrow$

↑ 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

Gram-negative rods (GNRs):
- *Escherichia coli*
- *Klebsiella pneumoniae*
- *Pseudomonas aeruginosa*

Taur et al. CID, 2015
Prebiotics
Prebiotics

- Dietary fibers
- Indigestible by humans
- Nutrition for healthy gut bacteria
  \[\uparrow Bifidobacterium \text{ spp.}\]

Sugars:

- Glucose
- Fructose

Fructo-oligosaccharides (FOS):
Prebiotics

Prebiotic → Fermentation → Short-chain fatty acids (SCFAs)
Short-Chain Fatty Acids (SFCAs)

- Maintain integrity of intestinal mucosa
- Improve intestinal immune regulation
  - **↓** Pro-inflammatory cytokines & immune cells
  - **↑** Anti-inflammatory cytokines & immune cells (e.g. T-reggs)

**Acetate**

**Propionate**

**Butyrate**
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**
-7* → Day 0 → +7

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

* Blood samples
Results of trial to date

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

<table>
<thead>
<tr>
<th>FOS dose</th>
<th>5g/day N=5</th>
<th>10g/day N=10</th>
<th>Total N=15</th>
</tr>
</thead>
<tbody>
<tr>
<td>Consumed ≥70% FOS doses*</td>
<td>4 (80%)</td>
<td>6 (60%)</td>
<td>10 (66.7%)</td>
</tr>
<tr>
<td>Mean % of doses taken</td>
<td>81.4%</td>
<td>59.1%</td>
<td>80%</td>
</tr>
</tbody>
</table>
Microbiome manipulation

Probiotics
Can Probiotics Benefit the Gut Microbiome?

Lactobacillus rhamnosus → Allogeneic HCT mouse model

→ Mortality
→ GVHD

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 Immunology, 2016  
Simms-Waldrip et al. BBMT, 2017
Can Probiotics Benefit HCT Recipients?

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

**No effect:**
- Mortality
- GVHD
- No adverse events

Allogeneic HCT recipients

Gorshein et al. Clinical Transplantation, 2017
Fecal Microbiota Transplant (FMT)

Healthy donor stool →
- Nasogastric tube
- Colonoscopy
- Oral “crapsules”

In all patients:
- 70-90% resolution of CDI

In HCT patients:
- 18 patients published
- 16 of 18 resolved CDI
- No adverse events

Recurrent or refractory *Clostridium difficile* infection (CDI)

Eli Moss, Shannon Falconer et al. PLOS One, 2017
Fecal Microbiota Transplant (FMT)

Healthy donor stool → Resolution of GI GVHD:
- 5 of 7 published cases
- 2 of 7 partial resolution
- No adverse events

Steroid-refractory GI GVHD

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

Diversity (Shannon Index) vs. Day of Transplant (n=94)

Microbiome Diversity at Engraftment

High Diversity (Inverse Simpson Index >4)

Low Diversity (Inverse Simpson Index <2)

Relative abundance

Microbiome Diversity

High Diversity

The Amazon Rainforest

Microbiome Diversity at Engraftment

Low Diversity

Low Diversity at Engraftment is Associated with Decreased Survival

Multivariate analysis, HR=2.56 (CI 1.03-7.23)

Overall Survival

Log-Rank p=0.017

High Diversity
(Inverse Simpson Index >4)

Medium Diversity
(Inverse Simpson Index 2-4)

Low Diversity
(Inverse Simpson Index <2)

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

Acute GVHD (n=22)

HCT Donor Phylogenetic Diversity

No

Yes

p=0.049

Liu et al. BMT, 2017
Microbiome Impact: GVHD

2. Anaerobic bacteria
Decrease in Obligate Anaerobes Associated with GVHD

None or minimal anaerobic activity

<table>
<thead>
<tr>
<th>Antibiotic</th>
<th>GVHD-Related Mortality</th>
<th>Months After Transplant</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aztreonam</td>
<td>0% n.s.</td>
<td>0 20 40 60</td>
</tr>
<tr>
<td>Cefepime</td>
<td>0% n.s.</td>
<td>0 20 40 60</td>
</tr>
</tbody>
</table>

Decrease in Obligate Anaerobes Associated with GVHD

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

### None or minimal anaerobic activity

- Aztreonam
- Cefepime

### Significant anaerobic activity

- Imipenem
- Piperacillin-tazobactam

**GVHD-Related Mortality**

<table>
<thead>
<tr>
<th>Antibiotic</th>
<th>None or minimal anaerobic activity</th>
<th>Significant anaerobic activity</th>
</tr>
</thead>
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<tr>
<td>Aztreonam</td>
<td>No Antibiotics</td>
<td>Imipenem</td>
</tr>
<tr>
<td>Cefepime</td>
<td>n.s.</td>
<td>p=0.02</td>
</tr>
<tr>
<td>Piperacillin-tazobactam</td>
<td>+ Antibiotic</td>
<td>p=0.007</td>
</tr>
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</table>

*Months After Transplant*

Decrease in Obligate Anaerobes Associated with GVHD

- Anti-anaerobic antibiotic exposure \(\uparrow\) GVHD
- Greatest association with clindamycin

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

\[ \downarrow \text{*Blautia* spp. associated with:} \]

\[ \uparrow \text{GVHD-related mortality} \]
\[ \uparrow \text{Overall mortality} \]
\[ \uparrow \text{Treatment-related mortality} \]
\[ \uparrow \text{Relapse-related mortality} \]

Cumulative Incidence of GVHD-Related Mortality

- Less *Blautia* (n=32)
- More *Blautia* (n=32)

\[ p=0.04 \]

Years After Transplant

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

![Graph showing the association between microbiome and relapse](image)

- Absent (n = 119)
- Present (n = 422)

$P < .001$

HR, 0.51; 95% CI, 0.36 to 0.73

Peled et al. JCO, 2017
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
• Antibiotics save lives!
• The health cost of antibiotics:
  • To public health $\rightarrow$ antimicrobial stewardship
    $\uparrow$ Antibiotic resistance
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
1. Febrile neutropenia
   Anaerobe-killing (pip-tazo, carbapenems)

   vs.

   Anaerobe-sparing (cefepime)

→ RCT at MSKCC
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

<table>
<thead>
<tr>
<th>Current</th>
<th>Future</th>
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</thead>
<tbody>
<tr>
<td>Gastrointestinal</td>
<td>Non-GI: skin, eye, lung, vagina</td>
</tr>
<tr>
<td>Acute GVHD</td>
<td>Chronic GVHD</td>
</tr>
<tr>
<td>16S rRNA sequencing</td>
<td>Metagenomics, metatranscriptomics, metaproteomics</td>
</tr>
<tr>
<td>Bacteria</td>
<td>Bacteria, viruses, fungi, parasites</td>
</tr>
<tr>
<td>Single-institution</td>
<td>Multi-institutional</td>
</tr>
<tr>
<td>Association</td>
<td>Causation</td>
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</table>
Thank you

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Jessica Ribado
Hila Sberro
Fiona Tamburini
Katia Tkachenko
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!