The "Force" of the Gut Microbiome in Hematologic Malignancies

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Gut microbiome-mediated bile acid metabolism regulates liver cancer via NKT cells

Yun Kit Yeoh, Tao Zuo, Gang Gu, etc.

Gut microbiota composition reflects disease severity and dysfunctional immune responses in patients with COVID-19

Yun Kit Yeoh, Tao Zuo, Gang Gu, etc.

Gut bacteria from multiple sclerosis patients modulate human T cells and exacerbate symptoms in mouse models

Egle Cekanavičiute, Bryan B Yoo, Tessel F Runia, Justine W Debelius, Sneha Singh, etc.
How Can The Microbiome Do All This?

- Hope or Hype?
Outline

• Introduction to the Microbiome
• Connections to Host Immunity as a Mechanism
• Associations with Oncologic Diseases
• Novel Approaches for Therapeutic Manipulation
The gut microbiome

- A community of bacteria, viruses, fungi, and parasites
- They may interact with the host immune system

- Pattern recognition
- Metabolite production
- T-cell activation
- Environment
- Peyers patches
- Secondary LN/spleen

- Cells
- APCs
- Innate lymphoid cells
- Adaptive: Th17, Th1/2

- Antigen mimicry
- Innate priming

Is this a “New Immune Organ” or “The Force of Oncology.... A New Hope?”
Microbiome Endpoints/Targets

• Unclear which are the key features of the gut microbiome to target, not mutually exclusive
  • At what time points?

• Microbiome diversity?
  • Better characterized, associated with survival in variety of oncologic settings/therapies
  • Hard to change
  • Diversity ≠ healthy (at least not necessarily)

• Specific populations?
  • *Eubacterium* sp. -> reduced relapse in myeloma allo-SCT (Peled et al, JCO 2017)
  • *Blautia* sp. -> reduced mortality from GvHD (Jenq et al, BBMT 2015)
  • *Prevotella heparinolytica* -> presence associated with IL-17 driven myeloma progression in mice (Calcinozzo et al, Nat Commun 2018)

• Metabolomics?
  • Provide a readout of the system extending beyond bacterial populations (fungi, viral, epithelial)
  • Short chain fatty acids i.e. butyrate associated with anti-lymphoma activity, regulatory T-cell stimulation

• Endpoint or Process?
  • Does how we reach these endpoints (ie diversity) matter?
  • I.e. antibiotic exposure
Gut Microbiota Diversity

- **Alpha diversity**: measures the observed diversity for a given community
  - Comprised of:
    - Species richness (number)
    - Uniqueness (how many diff)
    - Evenness

- **Beta diversity**
  - How *communities* differ
  - i.e. –populations in a swamp compared to each other
    - Or pre- post- gut microbiota transplant profiles
Mechanism

Li et al, *Cancer Letters*, 2019
Microbiome Mediated Immune Activation

• Metabolites: not an exclusive list
  • Short chain fatty acids
Butyrate: SCFA du jour

- Regulate T-cell populations in the gut
- Potential epigenetic mechanism as a histone deacetylase inhibitor
- Effect differs across cell types and receptors
- G-protein receptor-mediated effects
- HDAC-inhibition effects

Toubai et al, Blood 2022
Short-chain fatty acids can activate effector T-cell populations including CAR-T

Mechanisms:
- HDAC inhibition
- mTOR activation
- GPCR metabolic activity

Lu et al, Nature Communications 2021
Mucosal associated invariant T-cells (MAIT) and Riboflavin

- In human subjects post allogeneic stem cell transplant
- Potential *in vivo* anti-cancer responses, recognize riboflavin metabolites

Microbiome and Immune Cell Activation

• Dendritic cell activation in a murine melanoma model
• Th17 activation in multiple myeloma
Cytotoxic T-cell Activation is Driven by Upstream Dendritic Cell activation from addition of commensal Bifidobacterium sp. in Mouse Melanoma Model

Sivan et al, Science, 2015
Microbiome communities drive Th17 skewing and migration to BM to foster myeloma progression

Calcinoatto et al, Nature Communications, 2018
Microbiome and CLL

Burger et al, NEJM 2020
CLL and the Gut Microbiome

Observations

Clinical
- Median age onset ~72
- Variable clinical presentation
- Geographic disparity

Biological
- Pathogenesis occurs in lymphoid organs
- linked to chronic B-cell receptor signaling
- Microbial antigens can activate BCR
- inflammatory cytokines linked to outcome

Lynch et al, NEJM 2016
DIG-CLL

- Collaboration with ElGamal Lab to profile microbiome
  - Adoptive CLL transfer model
    - Effect of antibiotic ablation on CLL progression
  - Eu-TCL Transgenic Model

- Prospective sampling of CLL patients and cohabitating controls
  - Diet
  - Serum cytokines
  - Microbiome samples
    - BTK inhibit
    - Rai stage 2-4 Untreated
Common Ground Hypothesis

Healthy persons, each with genetic susceptibility to one or more polygenic disorders.

Combination of genetic susceptibility and environmental exposure, resulting in polygenic disorder.

Healthy gut microbiota

Dysbiotic pathobionts

Nonspecific environmental triggering factors, such as chronic infection and unhealthy diet.

Subclinical dysbiotic gut microbiota, intestinal inflammation, and leaky mucosa.

Reproduction of distinct disease phenotype through transplantation of the dysbiotic disease-associated gut microbiota to a genetically susceptible rodent host.

Lynch et al, NEJM 2016
DIG-CLL

Progression Model (CLL vs WT mouse)

Antibiotic-Treated vs Control (CLL transfer)
Eµ-TCL1 Mice Harbor a Unique and Dysbiotic Gut Microbiome

**%CLL cells (blood)**

- **WT**
- **TCL1**

Age (mo) | 4mo | 7mo | 10mo | 12mo
---|---|---|---|---
%CD45+ / CD19+ / CD5+

- **WT B6 mice**
- **Eµ-TCL1 mice**

### Shannon Diversity Index

<table>
<thead>
<tr>
<th>TCL-1</th>
<th>WT</th>
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<tbody>
<tr>
<td>n=51</td>
<td>n=30</td>
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### Abundance

- 4_Clostridiales Butyriciclasticus
- 4_Bacteroidaceae Codenoura
- 4_Bacteroides Faecalibacterium
- 4_Bacteroidales_Bacteroidaceae
- 4_Bacteroidales_Bacteroides
- 4_Bacteroidales_Faecalibacterium
- 4_Bacteroidales_Not subclassified
Antibiotic Ablation Impacts CLL Pathogenesis and Forms Unique Gut Microbial Communities
Antibiotics Produce a Progressively Dysbiotic Microbiome

% CLL cells (blood)

- No ABx
- + ABx

Week Post-Engraftment

* P<0.05
** P<0.01
*** P<0.001

Taxa enriched at phylum level

Ablated-gut (+ ABx)
Intact-gut (water)

Taxa enriched at phylum level

Actinobacteriota
Proteobacteria
Firmicutes

LDA SCORE (log 10)
Clinical Studies

- Alpha diversity as a biomarker
- Microbiome and CAR-T cell therapy in lymphoma
- Microbiome and myeloma
Gut Microbiota Diversity

• Diversity is emerging as a key microbiome trait increasingly linked to outcomes in oncologic disease
  • Allogeneic transplant (Peled et al, NEJM, 2020)
  • Autologous transplant (Khan et al, Blood, 2020)
  • Melanoma and PD-1 inhibition (Gopalakrishnan et al Science 2018)
Why microbial diversity may matter

Cyclophosphamide: Viaud et al revealed that the gut microbiota is involved in the anti-neoplastic activity of cyclophosphamide

- traced to gut translocation of gram-positive species into nearby lymphoid organs, stimulating T-cell responses
- Similar observation for platinum agents
CAR-T and Antibiotics

Smith et al, Nature Medicine, 2022
Autologous Stem Cell Transplantation in Multiple Myeloma

Microbiome diversity at time of engraftment associated with PFS and OS

• What contributes to low diversity at engraftment?
• Why/How does microbial diversity at this timepoint matter?

Autologous Stem Cell Transplant in Multiple Myeloma

- 30 patients with myeloma
- Loss of microbial diversity observed immediately post-transplant
- Suggests this engraftment period is a key timepoint for the microbiome

D'Angelo et al, Leukemia Lymphoma 2022
IV Antibiotic Influence on Post-transplant Diversity

- Alpha diversity at time of engraftment was measured according to IV antibiotic exposure
- IV = cefepime/piperacillin/tazobactam + vancomycin
- Antibiotic exposure had the largest effect on post-transplant gut microbial diversity
- Corroborate findings from El Jurdi et al *BBMT 2019*

**Summary:** microbial diversity is lost in the peri-transplant period and predominantly driven by broad-spectrum abx

D’Angelo et al, Leukemia Lymphoma, 2021
Engraftment Diversity is associated with D+100 response to ASCT

- Response assessed at D+100 per IMWG response criteria
- 29/30 subjects available for response
- Higher diversity was associated with CR/VGPR compared to PR
- * denotes $P < 0.05$ in pairwise comparison to CR and VGPR

Maybe a link is forming -> antibiotic exposure -> microbiome loss during transplant -> impaired response -> reduced PFS -> reduced OS

D'Angelo et al, Leukemia Lymphoma 2022
But questions remain:

- Confounding? -> how do we know the microbiome is indeed an independent trait

- Can we use this data to guide a therapeutic strategy targeting gut microbiota in this setting?
Prebiotics ~ Microbiome ~ Transplant Outcomes

• Antibiotics are necessary for neutropenic fever management
• Main source of neutropenic fever is gut translocation
• Can we target and re-program the gut microbiome to prevent neutropenic fever?

• Hypothesis: prebiotic supplementation peri-transplant can reinforce the integrity of the gut lumen -> reduce the need for antibiotics -> improve diversity
Targeting Gut Microbiota
- Well tolerated
- Pair well with toxic therapies
- Simple to store/deliver
- *Patient interest*
- Target multiple species/niche
- Demonstrated activity in intestinal barrier/SCFA/etc

Van Vliet et al, PLoS Pathogens 2010
What prebiotic?: Resistant Starch

• Traits: inc butyrate, inc probiotic populations (bifidobacterium)
• Improve diversity possibly, possible reduce abx need
• Readily available: Bob’s Red Mill potato starch
• studies in allogeneic transplant confirm feasibility at doses planned here
Prebiotics to Improve Gut Microbiome Diversity After Autologous Stem Cell Transplantation in Multiple Myeloma and Lymphoma: The PRIMAL Trial

Protocol Number: 821-21
Principal Investigator: Christopher D’Angelo, MD
Overall Design: RCT 1:1

<table>
<thead>
<tr>
<th>Enrollment</th>
<th>Randomization</th>
<th>Intervention</th>
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<tbody>
<tr>
<td>Screening: initial ASCT Consult for multiple myeloma relapsed DLBCL</td>
<td>Prebiotic 20g BID (resistant starch)</td>
<td>Pre-Transplant (D0)</td>
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<td>Control 20g BID (maltodextrin)</td>
<td>Day 0: begin Rx, diet survey, stool sample</td>
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<td>Post-transplant (D14-D21)</td>
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<td>D14: Admission + Stem Cell infusion, Diet survey, stool sample</td>
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<td>D21: Neutrophil nadir, stool sample collection</td>
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<td>D26-30: End of study, Cell Engraftment, Final sample collection</td>
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Daily Toxicity Assessment
Primary Objective

- To determine the impact of a prebiotic intervention on gut microbiome diversity post-transplant. The primary endpoint will be a measure of gut microbiome diversity recorded at the time of post-transplant engraftment.
Secondary Objectives:

• **Diet impact**: diversity changes according to dietary intake by DHQ-3 survey, stratified by fiber intake
  - Correlatives with nutritional science group at UNMC/UNL
  - Mariah Jackson, Dr. Heather Rasmussen, and Dr. Corrine Hanson

• **Infectious complications**: neutropenic fever, bacteremia

• **Intestinal permeability**: measured with serial blood testing of serum markers associated with permeability

• **Patient Reported Outcomes**: regarding GI tolerability to the intervention
Study Status

- Open 4/2022
- 13/30 patients recruited
- General observations:
  - Well tolerated in setting of mucositis
  - Significant patient interest re: recruitment
  - Difficulty with diet tool ASA-24
Impact

- The pilot data taken from this study could demonstrate that prebiotic interventions:
  - Are feasible during auto transplant
  - Affect intestinal permeability
  - Impact antibiotic exposure
  - Improve diversity
- Provide a key proof of concept that microbiota directed therapy as an adjunctive measure may help improve therapeutic outcomes in autologous stem cell transplant
Thank you!!!

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