Gut microbiome may hold key insights for drug discovery and development

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Microbiome: Collection of microorganisms that live within and on all living things

There are 1:1 microbial cells to human cells in our bodies, the majority of which reside in the gut

Gut microbes are essential for:

- Producing essential vitamins and metabolites
- Breakdown and processing of drugs
- Protecting against disease-causing pathogens
- Educating and regulating the gut and systemic immune system

Individual’s microbial communities are shaped by:

- Lifestyle, Diet
- Immune system
- History of previous infections
- Medications (antibiotics and chemotherapy)
Extensive impact of non-antibiotic drugs on human gut bacteria

- Screened more than 1,000 marketed drugs against 40 representative gut bacterial strains
- 24% of drugs with human targets inhibited the growth of at least one strain in vitro
- These effects are concordant with existing human cohort studies that have shown affects of:
  - antidiabetics (metformin)
  - proton pump inhibitors (PPIs)
  - nonsteroidal anti-inflammatory drugs
  - atypical antipsychotics
- Species with higher relative abundance across healthy individuals were significantly more susceptible to human-targeted drugs (P = 0.0012)

Maier et al. Nature 2018
Human gut microbes metabolize xenobiotics, including pharmaceuticals

- Gut microorganisms can directly alter the chemical structure of compounds, thus modifying their
  - Pharmaokinetic properties
  - Prodrug activation
  - Side effects
  - Loss of efficacy
- Microbes utilize hydrolytic and reductive reactions to metabolize xenobiotics, while host enzymes use oxidative and conjugative chemistry.
- There is still a knowledge gap in the association of microbial modifications with specific organisms and genes.

Pharmaceuticals transformed by microbiome:
- Sulfasalazine: IBD
- Sulindac: anti-inflammatory
- Loperamide: anti-diarrheal
- Levodopa: Parkinsons
- Digoxin: blood pressure and antiarrhythmic
- Tenofovor: HIV
- Irinotecan: Chemotherapy agent

Koppel et al., Science 356, 1246 (2017)
In steady state, gut microbiome composition is also mostly stable over time while microbial expression is more dynamic

- Gut microbiota dynamics in two subjects over 1 year
- Time series show overall microbial communities to be stable for months.
- Rare events (travel, infection) can rapidly and dynamically impact microbiota dynamics
- David et al Genome Biology 2014

- 308 individuals' microbial community structure (species) and metagenomic functional potential were more self-similar (red boxes) over time than between subjects (blue boxes)
- Short: 24-72 hours
- Long: 6 months
- Mehta et al Nature Microbiology 2018
Across geographies, gut bacterial communities are strongly shaped by lifestyle.

- PC1, delineates the gut of westernized populations (right) from less westernized ones.

- PC2 is based on age. Babies have distinct microbial communities from those of adults.

Smits et al. Science 2017
Gut microorganisms are at the intersection of several diseases

- Cardiovascular disease
- Liver disease
- Appetite disorders
- Parkinson disease
- Alzheimer disease
- Neurodegenerative diseases
- Multiple sclerosis
- Anxiety
- Depression
- Autism
- Stress
- Addiction
- Diabetes
- Insulin resistance
- Obesity
- C. difficile
- IBD
- Ulcerative colitis
- Cancer
- Low-grade inflammation
- Arthritis
- Allergy
- Eczema

Numerous factors are influencing the complex gearing:
- Lifestyle
- Food
- Immune priming
- Host metabolic signalling
The microbiome is a rapidly growing area of interest in biotech

- Venture capitalists have invested millions of dollars in companies investigating the microbiome as a drug target.
- Many companies initially prioritized *C. difficile* infection and IBD but are rapidly moving into the CI space.
- 1,000s of people have donated stool samples to promote citizen science efforts.
An individual’s response to cancer immunotherapy may be impacted by the gut microbiome

- While immunotherapy has transformed cancer care, it remains partially effective with only a subset of patients responding to treatment.
- Variability in the genetics, tumor microenvironment, stage of disease, and other host related factors may determine response to immunotherapy.
- The gut microbiome is a newly appreciated determinant of treatment outcome and important component of personalized healthcare.
Last year:
Gut microbes are associated with response to αPD-L1 in preclinical models

- Baseline growth of subcutaneous tumors was variable between genetically identical mice with different microbiomes.
- Both groups demonstrate αPD-L1 effect, but overall response is a reflection of different immunological set point.
- Differences disappeared when JAX microbes were given to TAC mice.

*Figure adapted from Sivian et al. Nature 2015
**Correlation between gut microbiome composition and adverse effects of α-CTLA4 therapy**

- The family **Bacteroidaceae** is enriched in stool from colitis-free patients compared to those who progressed to colitis.

**Dubin et al. Nature Communications 2016**
Antibiotic treatment prior to and during CI treatment compromises clinical efficacy

- **NSCLC+RCC+UC**
  - Overall survival (%)
  - Median OS
    - No ATB: 20.6 mo
    - ATB: 11.5 mo
    - \( p < 0.001 \)
    - \( n = 249 \)

- **Progression-free survival**
  - \( n = 100 \) patients
  - \( p < 0.001 \)

- **Months**
  - 0 6 12 18 24 30 36 42 48
  - 69 41 17 6 1 1 0 0 0

- **Time to event (months)**
  - 0 24 48 72 96

- **Antibiotic treatment prior to and during CI treatment**
  - **28%** were prescribed ATB
    - \( \beta \)-lactam+/- inhibitors, fluoroquinolones or macrolides
    - Within 2 months before or 1 month after first aPD-1/PD-L1 treatment
  - ATB taken orally for common indications (dental, urinary, and pulmonary infections)
  - No major statistical differences in baseline clinical characteristics between ATB-treated and untreated patients
  - Routy et al. Science 2017

- **Same is true for chemotherapy**
  - Retrospective analysis: CLL and lymphoma patients receiving antibiotics had lower OS and PFS to chemo
  - Pflug et al., Oncoimmunology 2016.
Gut microbes are associated with response to αPD-1 in 3 patient cohorts

- Responders have more diverse bacterial communities than non-responders.
- Responders and non-responders to αPD-1 have differential gut microbial communities.
  - Taxonomic signatures were variable across studies
  - Variability could be due to A) technical differences in collection, sequencing, and analytical methods B) geographical differences

Stool from responder patients but not non-responders is sufficient to restore αPD-1 efficacy in germ-free mice

- Germ free and ATB treated mice do not respond to αPD-1
- In 3 unique tumor models, response to αPD-1/PD-L1 was restored when mice were given FMT from responder patients, but not non-responders.
- All studies demonstrated altered intra-tumoral immune profiles following FMT.
- The mechanism underlying gut microbes effecting anti-tumor immunity remain largely unknown.

Matson et al. Science 2018

Routy et al. Science 2017
Microbiome-immunotherapy clinical trials are scheduled to begin this year.

In addition, stool collections have been implemented across many Cancer Immunotherapy trials.

FMT = Fecal Microbiota Transplant
Mullard Nature Reviews Drug Discovery 2018

### Table: Sponsors and collaborators

<table>
<thead>
<tr>
<th>Sponsors and collaborators</th>
<th>Checkpoint inhibitor</th>
<th>Microbiome intervention</th>
<th>Indication</th>
<th>Status</th>
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<tbody>
<tr>
<td>University of Pittsburg and Merck&amp;Co.</td>
<td>Pembro</td>
<td>FMT from responders</td>
<td>Melanoma</td>
<td>March 2018</td>
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<tr>
<td>Evelo Biosciences</td>
<td>Undisclosed</td>
<td>Single Bifido strain</td>
<td>Melanoma, CRC, renal</td>
<td>Q2 2018</td>
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<tr>
<td>Parker Institute, Seres Therapeutics, MD Anderson</td>
<td>αPD-1</td>
<td>FMT from responders, SER401 (spore based bacterial consortium)</td>
<td>Melanoma</td>
<td>2018</td>
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<td>Vedanta Biosciences</td>
<td>Undisclosed</td>
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Mullard Nature Reviews Drug Discovery 2018

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Additional stool samples with allow the community to validate published findings and discover greater mechanistic insight.

Biobanked stool specimens can be processed for:
- Metagenomic shotgun sequencing to characterize the microbes present
- Targeted metabolomic assays

This will power discoveries in:
- **Efficacy**: What baseline microbes/metabolites correlate with response to therapy?
- **Safety**: How does immunotherapy alter the microbiome? Do particular shifts contribute to the development of adverse side effects, i.e. colitis?
- **New Targets/Therapeutic Approaches**: Defining MOA of bacterial metabolites will reveal new targets, druggable via conventional means.
Microbiome research provides broad-ranging opportunities for drug discovery and development

- Variable microbiome signatures may determine response to therapy, risk of adverse effects, and other key phenotypes

- Defining MOA of bacterial metabolites will reveal new targets, druggable via conventional means (e.g., bile acid receptors)

- Unique source of small molecules (e.g., bile acid metabolites) that can be mimicked for drug development

- Multiple companies developing bacterial strains or bacterial consortia as strategy for modifying microbiome
Intestinal bacteria regulate barrier functions, whole-body metabolism, and systemic immunity via secretion of cytokines/chemokines, metabolites, antimicrobial and neuropeptides.

Zitvogel et al. Science 2018
Routy et al. Science 2017
Gopalakrishnan et al. Science 2017
Matson et al. Science 2018

Intra-tumoral bacteria can promote tumorigenesis and chemoresistance via biofilms, toxins, drug degrading enzymes, and miRNAs.

Dejea et al. PNAS 2014
Kostic et al. Cell Host Microbe 2013
Yu et al. Cell 2017
Gellet et al. Science 2017
Dejea et al. Science 2018
Intra-tumoral bacteria can mediate tumor resistance to the chemotherapeutic drug gemcitabine

- A, B. Of 113 human PDACs tested, 86 (76%) were positive for bacteria, mainly Gammaproteobactia.

- C. Gammaproteobactia that express the long form of the enzyme cytidine deaminase (CDD\textsubscript{L}) can metabolize gemcitabine to an inactive form.

- Gemcitabine is commonly used to treat pancreatic ductal adenocarcinoma (PDAC).

- 14/15 (93%) of bacteria cultured from PDAC tumors rendered cell lines fully resistant to gemcitabine.
Executive summary

• The microbiome represents a cornerstone of personalized healthcare

• The gut microbiome has been shown to play important roles in cancer immunotherapy efficacy and safety

• Microbiome-immunotherapy clinical trials will begin this year

• Intra-tumoral bacteria may impact tumorigenesis and treatment efficacy
Additional Microbiome Resources and Reviews

• The Invisible Universe Of The Human Microbiome by NPR
• Ed Young's The Ins and Outs of FMT
• The Human Gut Microbiome: From Association to Modulation
• Oncologists tap the microbiome in bid to improve immunotherapy outcomes
• The microbiome in cancer immunotherapy: Diagnostic tools and therapeutic strategies