THE GUT MICROBIOME
WHY DOES IT MATTER IN CHEMOTHERAPY-INDUCED GUT TOXICITY?

Professor Rachel Gibson
Conflict of Interest Disclosure
Professor Rachel Gibson, PhD

- Consulting Fees received from Kaleido Biosciences
Changes in the gut microbiome were first reported in 1965.

Over recent years it has become increasingly recognized as a key player in the development of CIGT.
Changes in the gut microbiome were first reported in 1965. Over recent years it has become increasingly recognized as a key player in the development of CIGT.
Changes in the gut microbiome were first reported in 1965

Over recent years it has become increasingly recognized as a key player in the development of CIGT.
Changes in the gut microbiome were first reported in 1965.
Over recent years it has become increasingly recognized as a key player in the development of CIGT.
The microbiome through the gut

**Oral cavity:** Complex ecosystem with moderate numbers of microorganisms. Predominant species; *Streptococcus; Actinomyces* & other obligate anaerobes.

**Stomach:** Relatively low numbers of microorganisms due to highly acidic environment. Most prevalent species is *H. pylori*.

**Small intestine:** Relatively low number of microorganisms; due to presence of oxygen, antimicrobials and acidic environment. Those present are predominantly gram-positive bacteria. Most prevalent species *Lactobacillus & Enterococcus faecalis*.

**Large intestine:** Highest volume of microorganisms, particularly in descending colon. Different populations in the lumen and mucosal regions. Predominant species are the anerobic *Bacteroides & Bifidobacterium*.
Functions of the gut microbiome

The current 5-Phase mucositis model

- Normal epithelium
- Phase 1: Initiation
- Phase 2/3: Messaging, signaling and amplification
- Phase 4: Ulceration
- Phase 5: Healing

The current 5-Phase mucositis model

Involvement of β-Glucuronidase in Intestinal Microflora in the Intestinal Toxicity of the Antitumor Camptothecin Derivative Irinotecan Hydrochloride (CPT-11) in Rats

Kiyoshi Takasuna, Takehiro Hagiwara, Masaaki Hirohashi, Michiyuki Kato, Mamoru Nomura, Eiichi Nagai, Tsuyoshi Yokoi, and Tetsuya Kamataki

Drug Safety Research Laboratory [K. T., T. H., M. K., M. N.] and Medical Product Management and Market Planning [E. N.], Daiichi Pharmaceutical Co., Ltd., 10-13 Kitakasai 1-chome, Edogawa-ku, Tokyo 134, and Division of Drug Metabolism, Faculty of Pharmaceutical Sciences, Hokkaido University, Kita-12, Nishi-6, Kita-ku, Sapporo 060, [T. Y., T. K.], Japan

Early clinical studies of the gut microbiome

Neomycin significantly:

- ameliorated diarrhea in 6 out of 7 patients (85%)
- reduced fecal $\beta$-glucuronidase activity
- decreased fecal concentrations of pharmacologically active metabolite SN-38
Early pre-clinical studies of the gut microbiome

- Stringer and colleagues conducted extensive research on changes between commensal and pathogenic bacteria following chemotherapy

Both clinically and pre-clinically, chemotherapy changes the gastrointestinal bacterial profile.

Figure 2. TaqMan qPCR quantification of bacterial 16S rRNA coding regions showing lower abundance in patients undergoing chemotherapy and antibiotic treatment (P) than healthy controls (C). T₀ samples taken before a single shot of chemotherapy; T₁, 1-2 days after chemotherapy; T₂, 5-9 days after chemotherapy. Asterisk indicates a significant difference at p<0.05.


MODULATION OF THE MICROBIOME
FOR EFFECTIVE SUPPORTIVE CANCER CARE
Lactobacillus-containing probiotics are suggested for the prevention of GI toxicity in patients receiving pelvic radiotherapy (MASCC Guidelines, 2014)\(^1\)

BUT

Very narrow indication  
Widespread applicability is unclear\(^2\)

<table>
<thead>
<tr>
<th>Study or Subgroup</th>
<th>Probiotics</th>
<th>Control</th>
<th>Risk Ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Events</td>
<td>Total</td>
<td>M-H, Random, 95% CI</td>
</tr>
<tr>
<td>Chitpanarux 2010</td>
<td>0</td>
<td>32</td>
<td>0.14 [0.01, 2.58]</td>
</tr>
<tr>
<td>Delia 2007</td>
<td>8</td>
<td>243</td>
<td>0.11 [0.06, 0.23]</td>
</tr>
<tr>
<td>Demers 2014</td>
<td>46</td>
<td>140</td>
<td>1.09 [0.73, 1.62]</td>
</tr>
<tr>
<td>Giralt 2008</td>
<td>20</td>
<td>44</td>
<td>1.24 [0.74, 2.08]</td>
</tr>
<tr>
<td>Lacouture 2016</td>
<td>9</td>
<td>59</td>
<td>1.11 [0.46, 2.67]</td>
</tr>
<tr>
<td>Mego 2015</td>
<td>0</td>
<td>23</td>
<td>0.11 [0.01, 1.95]</td>
</tr>
<tr>
<td>Osterlund 2007</td>
<td>21</td>
<td>97</td>
<td>0.58 [0.35, 0.98]</td>
</tr>
</tbody>
</table>

Total (95% CI) 638 529 100.0% 0.54 [0.25, 1.16]

Heterogeneity: \(\tau^2 = 0.77\); \(\chi^2 = 44.33\), df = 6 (\(P < 0.00001\)); \(I^2 = 86\%\)

Test for overall effect: \(Z = 1.58\) (\(P = 0.11\))

\(^1\) Lalla RV et al (2014) Cancer 120: 1453-1461
Time to take a step back?

• Critical that we now work to comprehensively and critically evaluate the role of the microbiome in CIGT to guide intervention design
  
  o Characterize dynamic shifts in microbiome relative to treatment milestones (diarrhea, barrier dysfunction, infection)
  
  o Identify unique microbial phenotypes at baseline associated with desired response (both treatment efficacy and toxicity)

  ✓ Clinical phenomena drive pre-clinical investigation / design
Can the gut microbiome be used as risk predictor for CIGT?

- Few effective treatments for CIGT
- Risk prediction previously successful
  
  34 patients - 30% with severe CIGT – identified genetic variability in TLR2 & TNFa along with cancer type to be predictive
  
  Specific and sensitive with ROC of 87.3%

Can the gut microbiome also be used as a risk predictor?

The gut microbiome as a risk predictor for CIGT

• Well established chemotherapy causes many changes to the gut microbiome
• Microbiome regulates individual’s risk of CIGT
• Pre-treatment microbial profiling = novel risk stratification method and possibility of identification of patients at high risk of developing CIGT¹

Aim

• To examine the relationship between pre-chemotherapy treatment microbial samples and severity of CIGT

Pre-treatment *Blautia* abundance regulates CIGT risk
Recruitment

Breast and colorectal cancer patients recruited (5-FU-based treatment)\(^1\)

Stool samples collected: before treatment and at day 5 (across a range of chemotherapy cycles)

Microbiome composition assessed by 16S pyrosequencing (Australian Genome Research Foundation)

Clinical case notes to assess diarrhea (NCI CTCAE v5.0\(^2\))


<table>
<thead>
<tr>
<th>Grade 0</th>
<th>Grade 1</th>
<th>Grade 2</th>
<th>Grade 3</th>
<th>Grade 4</th>
</tr>
</thead>
<tbody>
<tr>
<td>No change</td>
<td>Increase of &lt;4 stools per day over baseline</td>
<td>+4-6 stools per 24 h over baseline; IV fluids indicated &lt; 24 h; moderate increase in ostomy output compared to baseline; not interfering with daily living</td>
<td>+7 stools per 24 h over baseline; incontinence; IV fluids 24 h; hospitalization; severe increase in ostomy output compared to baseline; interfering with daily living</td>
<td>Life-threatening consequences (e.g. hemodynamic collapse)</td>
</tr>
<tr>
<td>“Non-toxic”</td>
<td>Excluded</td>
<td>“Toxic”</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Non-toxic (n=8)</td>
<td>Toxic (n=4)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>----------------------</td>
<td>----------------</td>
<td>-------------</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Age</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Median (range)</td>
<td>54.5 (38-72)</td>
<td>61.5 (56-68)</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Sex (n (%))</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Female</td>
<td>4 (50%)</td>
<td>1 (25%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>4 (50%)</td>
<td>3 (75%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Cancer type (n (%))</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Breast</td>
<td>1 (12.5%)</td>
<td>1 (25%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Colon</td>
<td>6 (62.5%)</td>
<td>1 (25%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rectal</td>
<td>1 (12.5%)</td>
<td>2 (50%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Treatment protocol (n (%))</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>FOLFOX</td>
<td>7 (87.5%)</td>
<td>1 (25%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>FEC</td>
<td>1 (12.5%)</td>
<td>1 (25%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Radiation + 5-FU</td>
<td>0 (0%)</td>
<td>2 (50%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Sample treatment cycle</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Median (range)</td>
<td>4 (3-10)</td>
<td>3 (1-4)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

All values P=NS

No demographic differences between non-toxic and toxic patients
Baseline microbiome composition drives treatment response

- *Blautia* is critical in determining CIGT in patients receiving 5-FU-based chemotherapy
Relative abundance of bacterial species

Pre-treatment

Blautia (Phylum = Firmicutes, Class = Clostridia, Family = Lachnospiraceae)

Line shows median, Mann-Whitney test

Relative Abundance

Non-toxic | Toxic

P=0.0081
Take home message:

- The gut microbiome is critical in shaping individual responses to cancer therapy
- It has potential to be further exploited
  - In-depth, longitudinal analysis is required to understand temporal relationship with treatment milestones and outcomes
  - Baseline microbial profiling is likely to play a role in risk prediction
  - Interventions need to be guided by clinical phenomena
Acknowledgements

This study was supported by funds from the Ray and Shirl Norman Cancer Research Trust awarded to RJ Gibson, JM Bowen, JK Coller and DM Keefe and funds from Cure Cancer Australia awarded to RJ Gibson and JM Bowen.

Researcher support was provided by Lions Medical Research Foundation awarded to KR Secombe and Florey Medical Research Foundation PhD Project in Cancer Research Grant awarded to HR Wardill and YZA van Sebille.

Thank you:

A/Professor Joanne Bowen  Professor Richard Logan
Dr Janet Coller  Professor Dorothy Keefe
Ms Kate Secombe  Dr Bronwen Mayo
Dr Hannah Wardill  Mrs Imogen Ball
Dr Ysabella van Sebille  Ms Samantha Korver
Dr Andrea Stringer