The Center for Translational Microbiome Research (CTMR): An Open Academic Research Collaboration between Ferring Pharmaceuticals and the Karolinska Institutet

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The Microbiome is Changing Perspectives on Human Health and Disease

• One of the most densely populated microbial ecosystems on Earth
• 100 trillion cells (10 x host cells ~2 kg)
• 3,000,000 encoded genes: complement host’s metabolic pathways
• 4 dominant phyla; ~1000 species; ~10000 strains (“the microbiome“)
• Most (approximately 80%) have not yet been cultivated
• Great impact on our health!

(When) will it change our therapeutic practices?
New Technologies Enabling Microbiome Research

- **FERRING RESEARCH INSTITUTE**
  - GC profiling and fractionation
  - 
  - **Proteomics**
  - Cloning & sequencing
  - PCR 16S rRNA genes
  - Microbiomics
  - Metagenomics
  - T-RFLP
  - Metabolomics
  - Feces
  - Biopsies

**NGS sequencing**
Modern Sequencing & Computational Technology Is Driving Microbiome Research

2005
0.5 Gb / run
0.5 Gb / day
240 days / human genome

2015
1800 Gb / run
600 Gb / day
1800 human genomes per year
Figure 1  Journal articles and INPADOC (International Patent Documentation Center) families, including patent and patent applications referencing the terms microbiome, microbiota, gut flora, or gut microflora. Note: Before 2006, most references are to the term ‘microbiota,’ which had historically been used in the field of applied microbiology (e.g., soil microbes). Source: Web of Science; Thomson Innovation Research (US Patent Grants, US Patent Application, World Patent Application, European Patent Grants; European Patent Applications).

Primary Literature Search by Year
Microbiota and Inflammatory GI Disease

Pubmed: (microbiome or microbiota or microflora or dysbiosis or commensal or probiotic) AND (IBD or esophagitis or oesophagitis or gastritis or pancreatitis or cholangitis or steatohepatitis or enterocolitis or crohn* or colitis or celiac or coeliac or diverticulitis or pouchitis)

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2005: Science 308:1635 human intestine bacteriome paper
2011: >1 paper/day inflection point
2014: 254/793 = reviews
2015: Cell 160:447 human fecal virome paper

Total = 5165**

** Total @ Jun 2015; includes 1729 reviews
A bacterial nudge to T-cell function

The epithelial cells that line the intestine have been found to sense tight attachment of bacteria, and to respond by producing proteins that shape the effector functions of the immune system’s T\(_\text{H} 17\) cells.

**Figure 1 | Bacterial attachment to the intestinal surface shapes T\(_\text{H} 17\)-cell function.**

- **a.** Segmented filamentous bacteria (SFB; coloured green) attach tightly to the epithelial cells that line the outer surface of the intestine in mice.
- **b.** Atarashi *et al.*\(^1\) provide evidence that this tight attachment of SFB to the intestinal epithelium shapes the function of T\(_\text{H} 17\) cells of the immune system by inducing epithelial-cell expression of the protein serum amyloid A (SAA).

Sano *et al.*\(^2\) show that SAA spurs production of the cell-signalling molecule IL-17 by differentiated T\(_\text{H} 17\) cells. The authors also unravel a regulatory circuit, involving other subepithelial immune cells, that governs epithelial Saa expression in this context. The circuit includes IL-23 (probably produced by dendritic cells in response to bacterial signals), which stimulates innate lymphoid cells to produce IL-22. IL-22 then induces Saa expression in epithelial cells.
Significant Investment in Microbiome R&D

- NIH Human Microbiome Project
- National Microbiome Initiative (May 2016)
- VC funding raised to date in 2016 = $617M
- Rate of VC funding of microbiome companies growing faster than overall VC funding

Under the Microscope
Investors pour into microbiome companies

Venture capital investment, in millions

$700  600  500  400  300  200  100  0

Total amount raised by year (left scale)

Change (right scale)

Year to date

Sources: Dow Jones VentureSource; Securities and Exchange Commission; the companies
Great Enthusiasm Around the Microbiome...

But......

Can we sort out the hope from the hype?
# Recent Noteworthy Clinical Studies of Microbiomes in GI Inflammatory Disease

<table>
<thead>
<tr>
<th>GIT Disease</th>
<th>Reference</th>
<th>Notables</th>
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<tbody>
<tr>
<td>Esophagitis - Eosinophilic</td>
<td>PLoSOne 2015 10:e0128346</td>
<td>Esophageal mucus bacteriomes of n=37 patients</td>
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<tr>
<td>Esophagitis - Eosinophilic</td>
<td>Microbiome 2015 3:23</td>
<td>Longitudinal study of oral &amp; esophageal bacteriomes in n=33 patients</td>
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<tr>
<td>Gastritis - Chronic</td>
<td>Helicobacter 2014 19:407</td>
<td>Gastric mucosa bacteriomes of n=31 patients</td>
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<tr>
<td>Pancreatitits - Acute</td>
<td>Pancreas 2015 PMID:25931253</td>
<td>Fecal bacteria vs plasma LPS &amp; cytokines for n=76 patients</td>
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<td>Cholangitis - PSC</td>
<td>JHepatol 2012 57:366</td>
<td>Bile bacteriomes vs FUT2 genotype of n=39 patients</td>
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<tr>
<td>Steatohepatitis - NASH</td>
<td>Hepatology 2013 57:601</td>
<td>Fecal bacteriomes of n=22 patients vs n=25 obese controls</td>
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<tr>
<td>Celiac - Adult</td>
<td>InflamBowelDis 2013 19:934</td>
<td>Duodenal mucosa bacteriomes of n=33 patients</td>
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<tr>
<td>Celiac - Pediatric</td>
<td>Gut 2015 64:406</td>
<td>Fecal bacteriomes of n=22 HLA-DQ2+ vs control infants</td>
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<td>Celiac - Pediatric</td>
<td>AppEnviroMicro 2014 80:3416</td>
<td>Saliva bacteriomes &amp; metabolomes of n=13 children</td>
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<td>Enterocolitis - GVHD</td>
<td>BBMT 2015 PMID:25977230</td>
<td>Fecal bacteriomes of n=64 patients implicate Blautia</td>
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<td>Enterocolitis - GVHD</td>
<td>PLoSOne 2014 9:e105706</td>
<td>Clinical trial of total GIT decontamination to prevent GVHD</td>
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<td>Enterocolitis - Hirschspring</td>
<td>PLoSOne 2015 10:e0124172</td>
<td>Fecal bacteria &amp; fungi in n=9 patients implicate Candida</td>
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<td>Enterocolitis - Necrotizing</td>
<td>ClinInfectDis 2015 60:389</td>
<td>Longitudinal study of fecal bacteriomes of n=12 neonates</td>
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<td>Crohn’s - Adult</td>
<td>Cell 2015 160:447</td>
<td>Fecal viromes of n=43 adults; RNA viruses not studied</td>
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<tr>
<td>Crohn’s - Adult</td>
<td>JClinGastroenter 2014 48:513</td>
<td>Fecal fungi vs mucosal inflammation in n=12 patients</td>
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<tr>
<td>Crohn’s - Pediatric</td>
<td>CellHostMicrobe 2014 15:382</td>
<td>Ileal, rectal &amp; fecal bacteriomes of n=447 children pre-Tx</td>
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<tr>
<td>Crohn’s - Pediatric</td>
<td>JClinInvest 2014 124:3617</td>
<td>Ileal mucosa bacteriomes + host transcriptomes of n=240</td>
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<td>Microscopic colitis</td>
<td>Gut 2015 PMID:25841239</td>
<td>Fecal bacteriomes of n=10 adults implicate Akkermansia</td>
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<td>Ulcerative colitis - Adult</td>
<td>Cell 2015 160:447</td>
<td>Fecal viromes of n=65 patients; RNA viruses not studied</td>
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<td>Ulcerative colitis - Pediatric</td>
<td>InflamBowelDis 2012 18:1799</td>
<td>Fecal bacteriomes of n=27 children vs steroid responses</td>
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<td>Diverticulitis</td>
<td>EJC MID 2014 33:1927</td>
<td>Fecal bacteriomes of n=31 cases implicate Proteobacteria</td>
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<td>Pouchitis</td>
<td>Gastroent 2015 PMID:26026389</td>
<td>Fecal bacteriomes of n=131 show antibiotics worsen dysbiosis</td>
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<tr>
<td>Pouchitis</td>
<td>GenomeBiol 2015 16:67</td>
<td>Pouch mucosa bacteriomes + host transcriptomes of n=96</td>
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* Selections incorporate assessment of study design, data quality & clinical significance; studies of <10 patients generally excluded.
Notable Concerns with Microbiome-Related Studies & Target Analyses

• Bacterial bias in current microbiome studies yields under representation of targets with validated viral/fungal/parasitic disease impacts

• Many established GIT pathogens remain under-studied in terms of their microbiome impacts before and after therapy/intervention

• Is presence of certain bacteria in the gut an effect, rather than a cause, of pathology?
  – Can this be elucidated in animals?
  – Does this lead to bias & limit scope to find the best targets?
What are the gaps we need to address so that we can identify the best drug targets?

• What represents the ‘normal flora’?
  – Is this determinable at the population level?
  – Is inter- /intra-person variability manageable?

• How do we determine what is significant?
  – Expression/abundance vs. presence of microbiota signals
  – Microbiome products/metabolites may be more important?

• Need to account for host genetics in the context of microflora phenotype?
  – Genetic and environmental factors may render the host unable to clear certain organisms or propagate a phenotype?
What are the gaps we need to address so that we can identify the best drug targets? (cont.)

• Are animal model suitably predictive?
  – Contrasts in the human vs mouse microbiome
  – Limitation of germ-free mice, etc.

• Culturing certain microbial species is problematic
  – As a result it may inhibit identification of targets

• How will we monitor positive therapeutic intervention
  – What are the most appropriate methodologies, etc.?

• Targets amenable to biopharmaceutical development to be more successful than bacteria-based strategies?
Ferring’s Involvement in Microbiome Research

Ferring Pharmaceuticals moves forward with early stage development of bacteriophage therapy for inflammatory bowel disease

SAINT-PREX, Switzerland--(BUSINESS WIRE)--Ferring Pharmaceuticals announced today that it will collaborate with Baltimore-based Intralytix, Inc. in the latest phase of its early stage development programme for a bacteriophage-based therapy for inflammatory bowel disease (IBD).

Press releases
WEDNESDAY, 27 JANUARY 2016

Ferring Pharmaceuticals and Karolinska Institutet sign collaboration agreement on the development of a Human Microbiome Translational Research Programme
Evidence-based Medicine And Regulatory Requirements

Will microbiome research change the probiotics business?

Current situation
• Tradition and experience
• No medical claims
• Lower regulatory bar:
  • Food, food additives, devices
    Generally Regarded As Safe (GRAS)

vs.

Future situation?
• Evidence-based medicine
• Medical claims
• Reimbursement
• NDA/BLA track
Opportunities For Therapeutic Intervention And Prevention

- Bacteria with direct impacts on human physiology and/or pathophysiology
- Bacterial products and metabolites
- Modulation of the microflora by phages
- Microflora and its products/metabolites as biomarkers
Center for Translational Microbiome Research (CTMR)

- TA focus where Ferring has extensive expertise
  - Reproductive Health and Gastroenterology
- Karolinska Institutet’s human microbiome expertise
- Science for Life Laboratory (SciLifeLab)
  - Metabolomics → Swedish Metabolomics Centre (SMC)
  - Next-generation sequencing
CTMR Overview (cont.)

- An ideal setting for study of complex microbiological communities in well-defined human material
  - Clinical epidemiology
  - Swedish population-based registries
  - Clinical Genomics
  - Clinical Microbiology
  - Clinical Networks
CTMR
Current Areas of Focus

• Gut and vaginal Microbiome
  – baseline/what is normal?

• Pediatric gastroenterology / NEC

• Inflammatory bowel disease

• Vaginal microbiome in reproductive health and preterm birth
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