

Microbiota: what reliable support for clinical decisions?

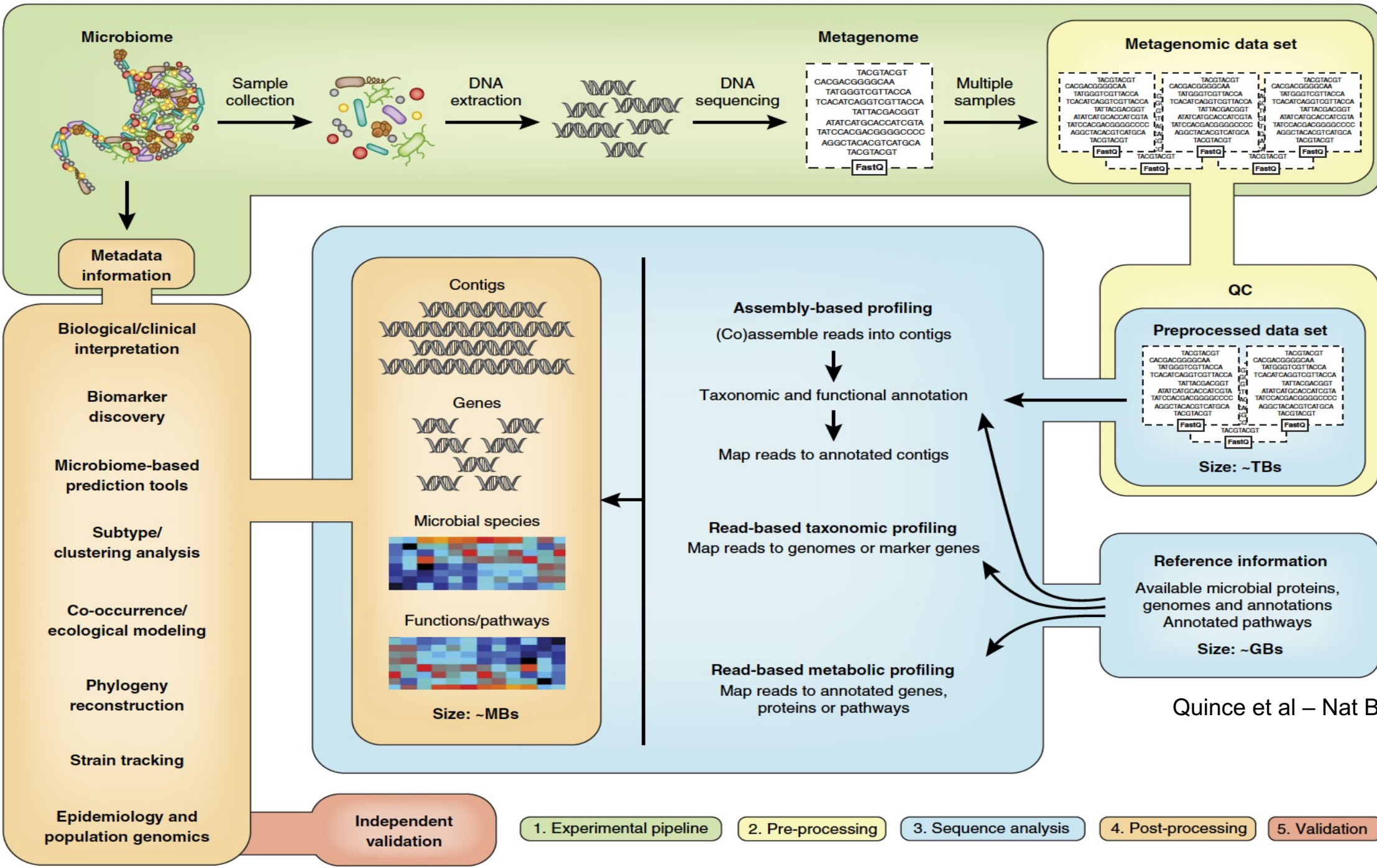
Gianluca Ianiro

Department of Translational Medicine and Surgery, Catholic University of Rome
Gastroenterology Unit, Fondazione Policlinico Universitario "A. Gemelli" IRCCS, Rome, Italy

Email: gianluca.ianiro@unicatt.it

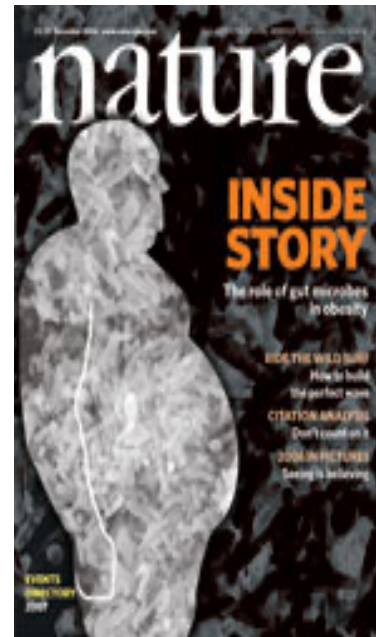
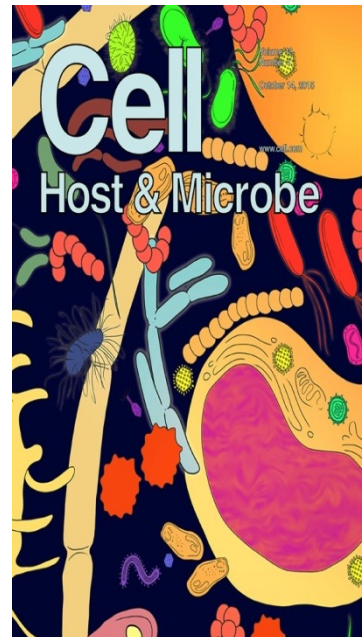
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Quince et al – Nat Biotechnol 2017

MICROBIOTA REVOLUTION



MICROBIOTA

APPLIED AND ENVIRONMENTAL MICROBIOLOGY, July 1996, p. 2273–2278

0099-2240/96/\$04.00+0

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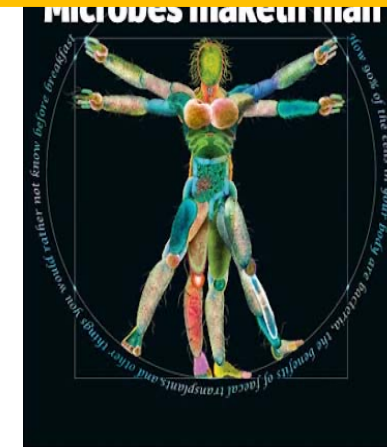
Vol. 62, No. 7

Human Colonic Biota Studied by Ribosomal DNA Sequence Analysis

KENNETH H. WILSON* AND RHONDA B. BLITCHINGTON

Infectious Diseases Section, VA Medical Center, and Duke University Medical Center, Durham, North Carolina

Received 27 June 1995/Accepted 23 February 1996



16s rRNA SEQUENCING

GENE MARKER ANALYSIS:

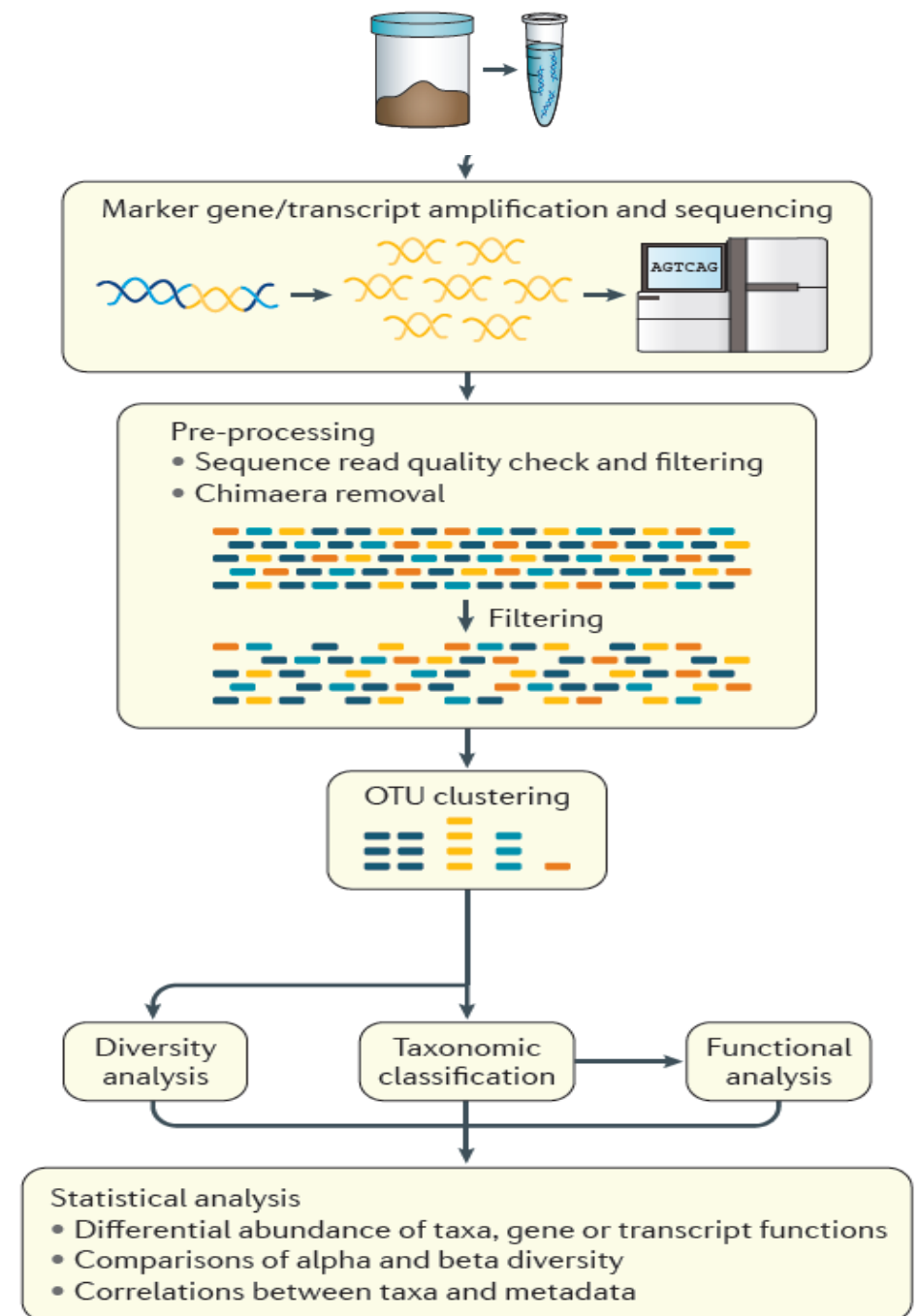
Targeting an amplicon of only one gene

The most commonly used is:

16S ribosomal RNA (rRNA) gene

Reveals “**WHO IS THERE**” in terms of

- **relative abundances** of bacterial taxa
- **alpha diversity** (within-sample diversity; one value per sample)
- **beta diversity** (between-sample diversity; pairwise values for all sample combinations)



16s rRNA SEQUENCING

Advantages

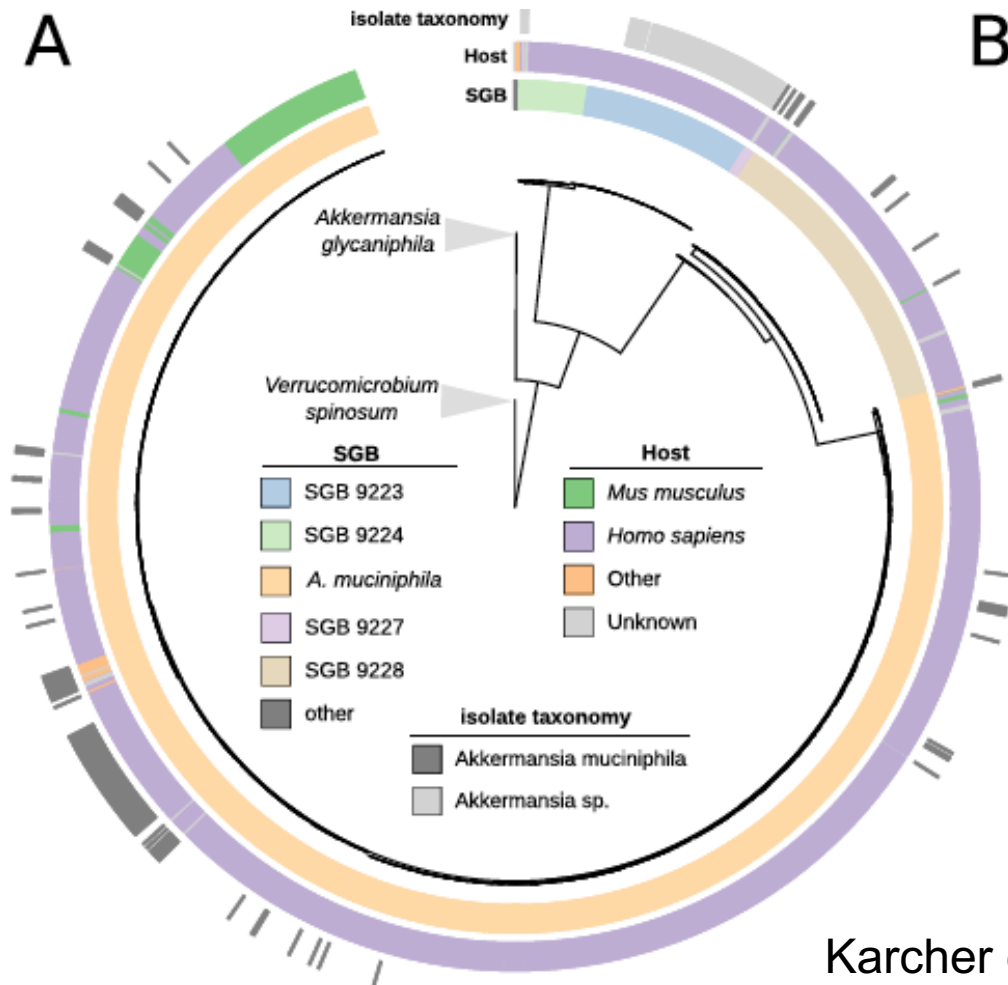
- very convenient for taxonomic classification
- informations on both microbial diversity and composition
- cheap
- many samples being sequenced on one instrument run

Limitations

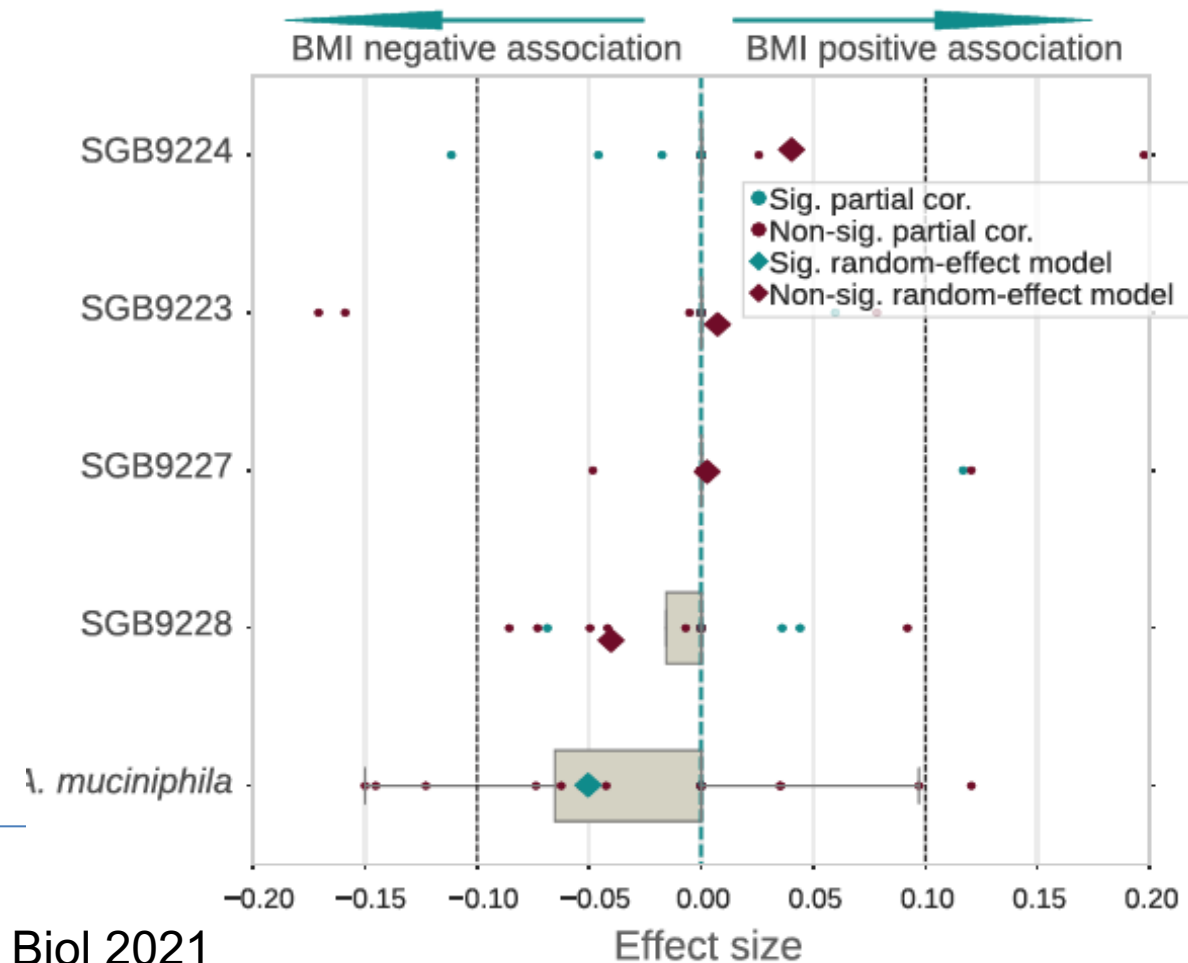
- low-resolution at species level
- issues with varying copy number (multiple copies of the targeted gene)
- difficulty in detecting low-abundant taxa

16s limits may be relevant in clinical practice

Among the 5 species of the genus *Akkermansia*, only *A. muciniphila* is positively associated with low BMI



B



WHOLE GENOME SEQUENCING

Shotgun sequencing

All extracted DNA (whole-metagenome shotgun) is randomly sheered into desired fragment sizes for high-throughput sequencing.

Reveals **“WHAT CAN THEY DO”** in terms of encoded functions of the sequenced microbial DNA.

The **phylogenetic origins** of microorganisms can be determined by comparison with previously annotated genes (similar information derived from 16S rRNA gene sequencing)

Limitations

- Expensive
- Requires specific instruments
- Requires dedicated skilled bioinformatic/biostatistician

MICROBIAL ECOLOGY PARAMETERS

RICHNESS

The number of species per sample is a measure of richness.
The more species present in a sample, the 'richer' the sample
No account of the number of individuals of each species present

EVENESS

Measure of relative abundance of the different species making up the richness of an area.

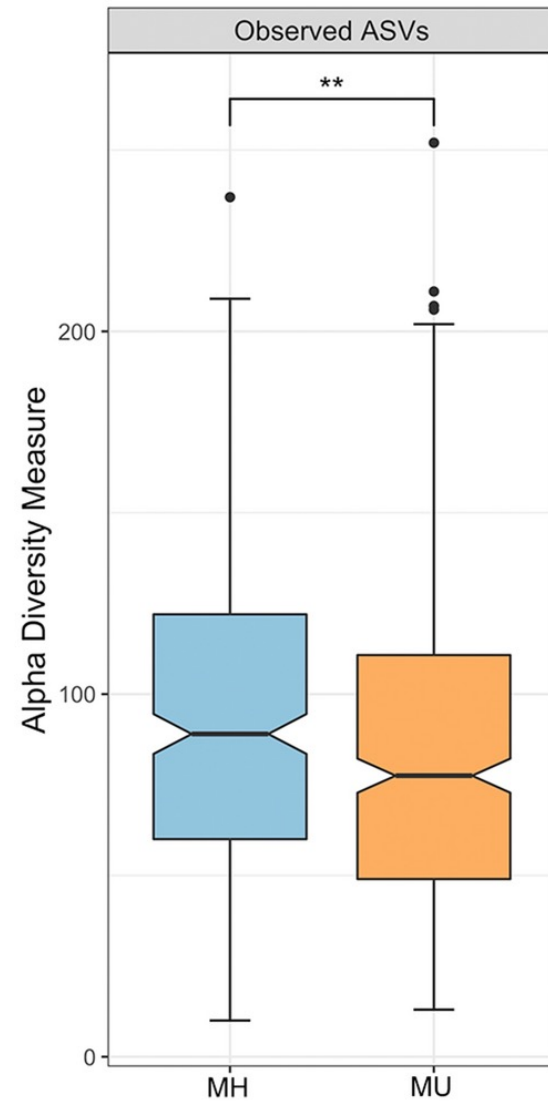
ALPHA DIVERSITY

Within-sample taxonomic diversity

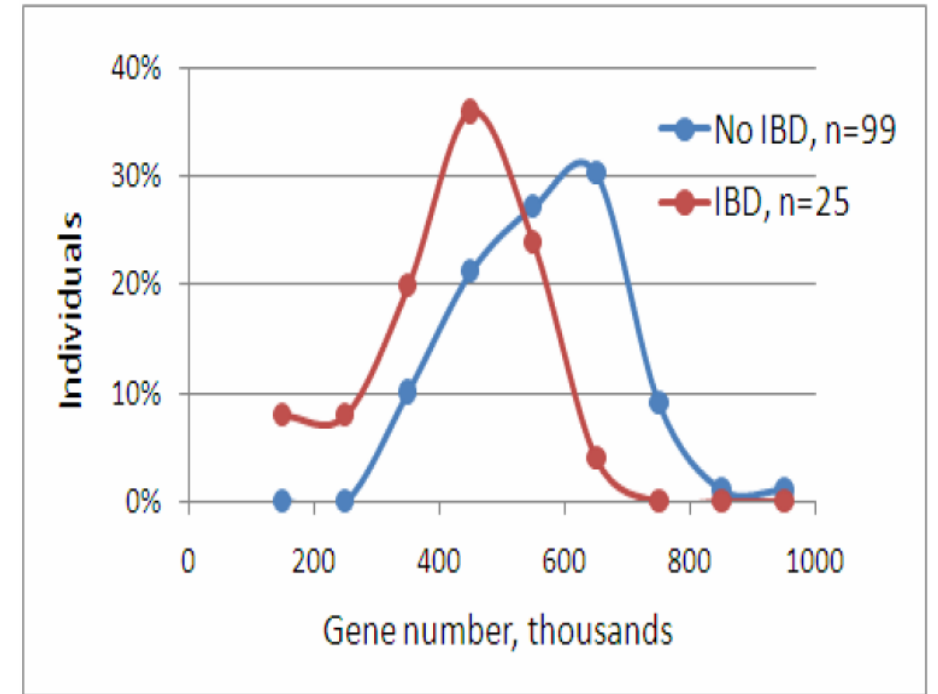
BETA DIVERSITY

Between-sample taxonomic diversity

Microbiome diversity: a marker of human health

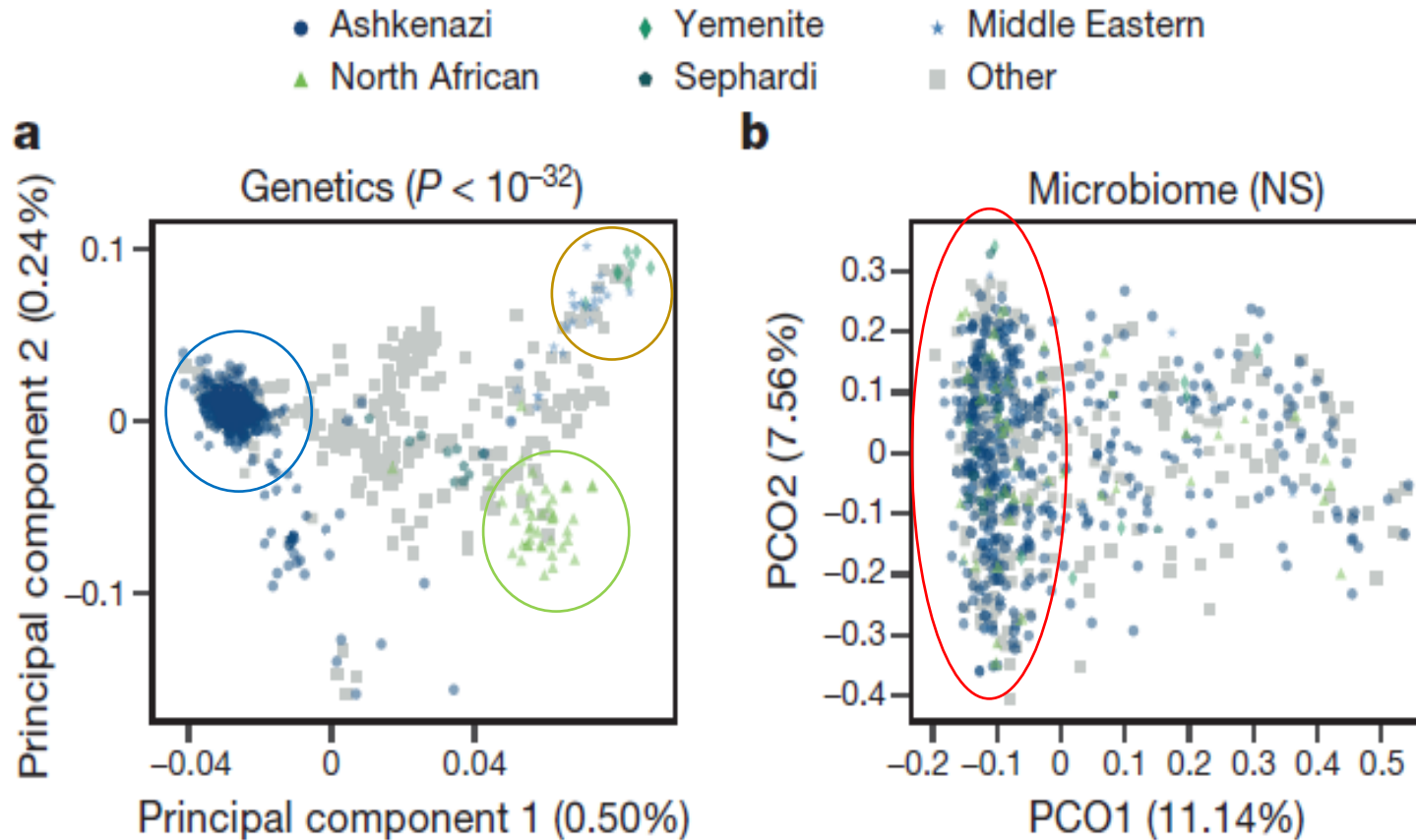


Kim et al – Sci Rep 2020

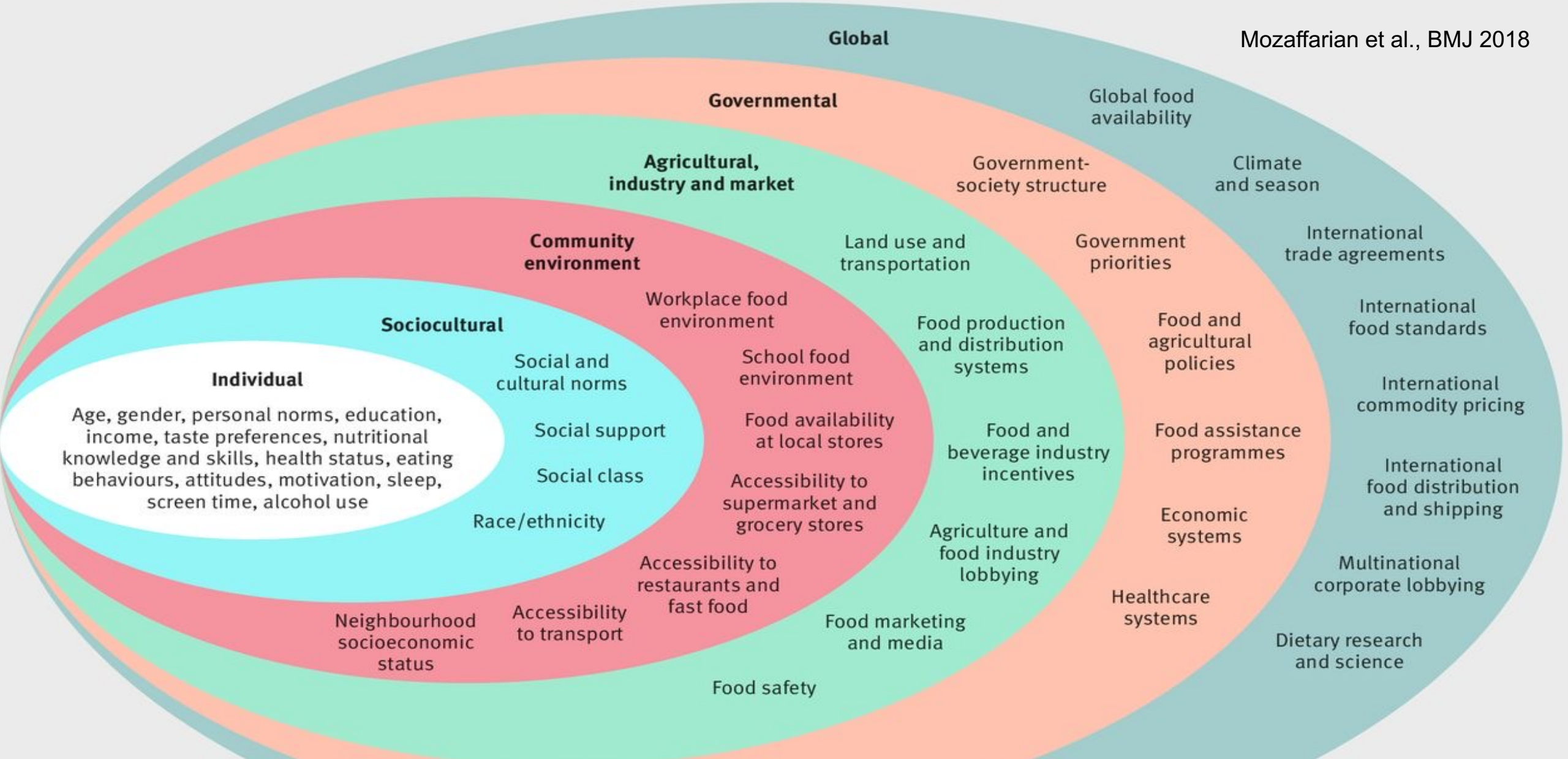


Qin et al – Nature 2010

ENVIRONMENT DOMINATES OVER HOST GENETICS IN SHAPING HUMAN GUT MICROBIOTA



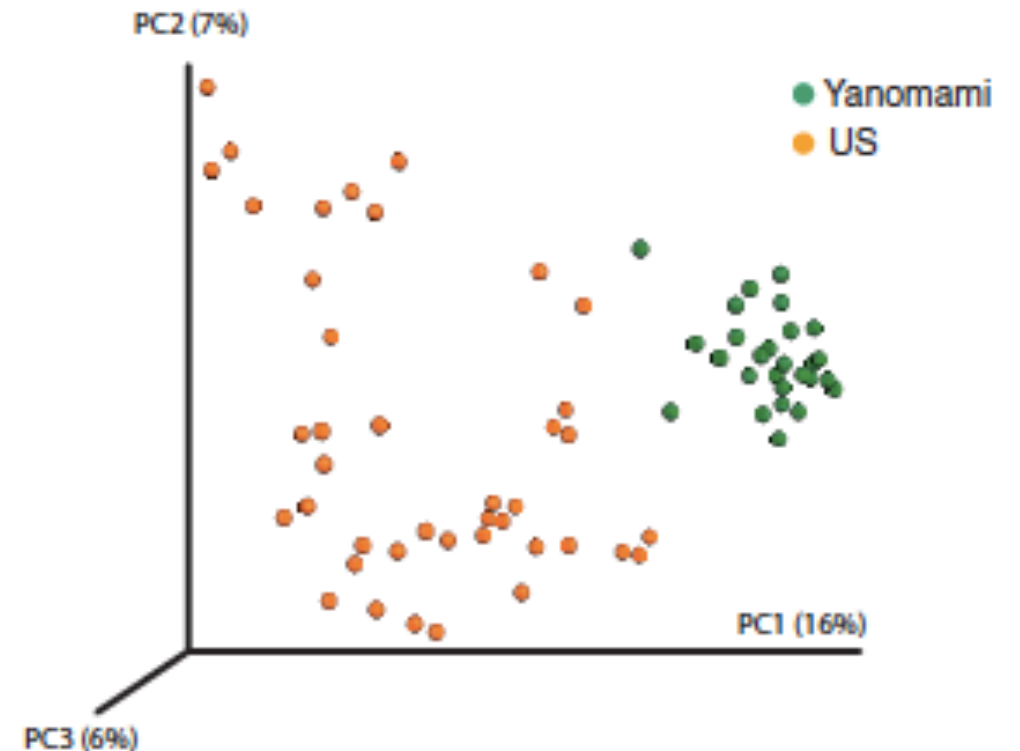
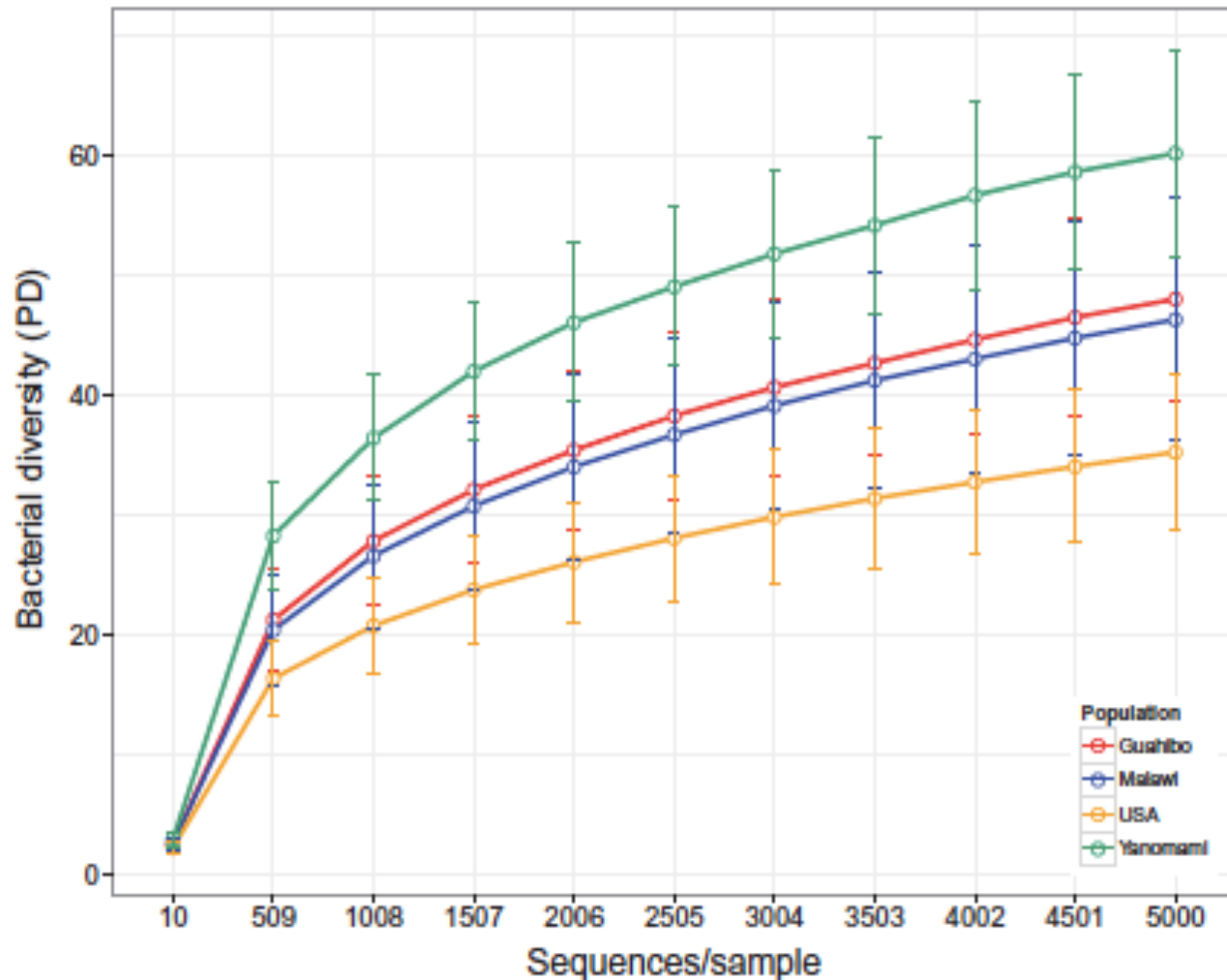
Genotype and microbiome data from **1,046 healthy individuals** with several **distinct ancestral origins** who share a relatively common environment



Microbiome: a permanent hitchhiker

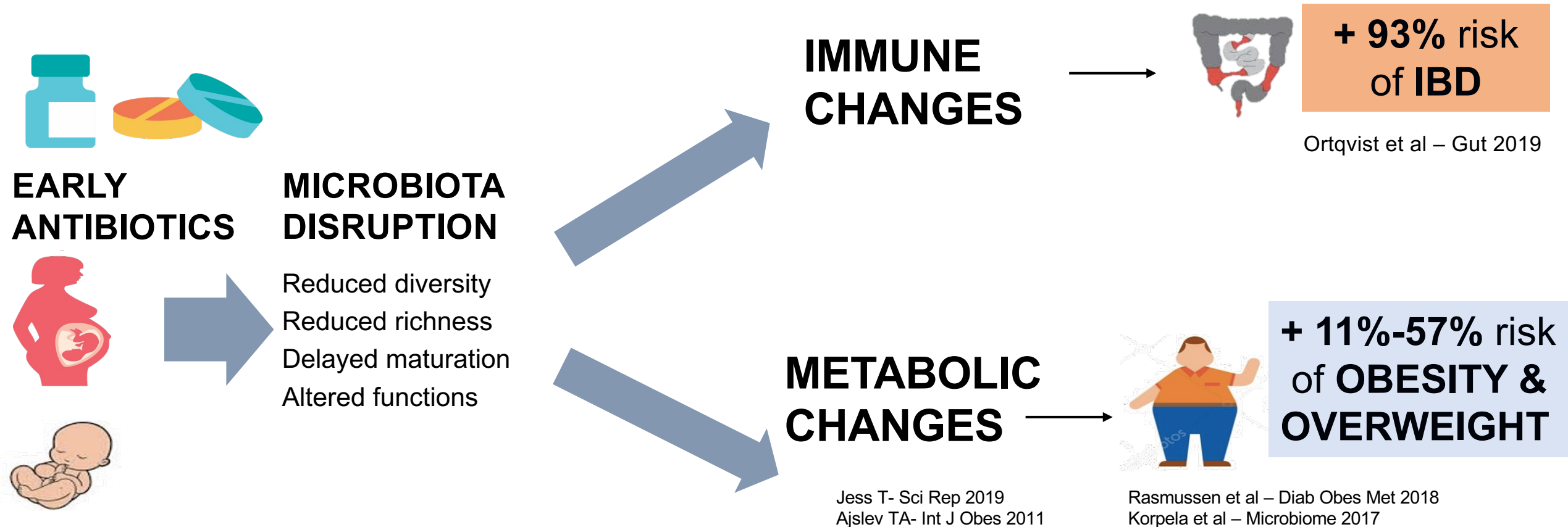
How westernization is influencing human microbial ecology

- Yanomami harbor a microbiome with the **highest diversity of bacteria and genetic functions** ever reported in a human group



Decrease in diversity impairs the microbe-mediated programming of host metabolism and immunity

Antibiotics can drive to decreased microbiome diversity transient alteration of healthy microbiota can drive to long-lasting effects, including **higher risk of VEO-IBD and overweight/obesity**



Environmental risk factors are common to several cancers

- Ageing
- Smoking
- Alcohol
- Diet
- Obesity/Metabolic syndrome/Diabetes
- Chronic inflammation

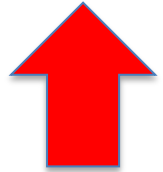
All characterized by severe DYSBIOSIS

Microbiota impairment during most cancer treatments

- Patients receiving cytotoxic and radiation therapy exhibit marked changes in intestinal microbiota



**Bifidobacterium, Clostridium cluster XIVa,
Faecalibacterium prausnitzii**



Enterobacteriaceae and Bacteroides

- These modifications may contribute to the development of mucositis

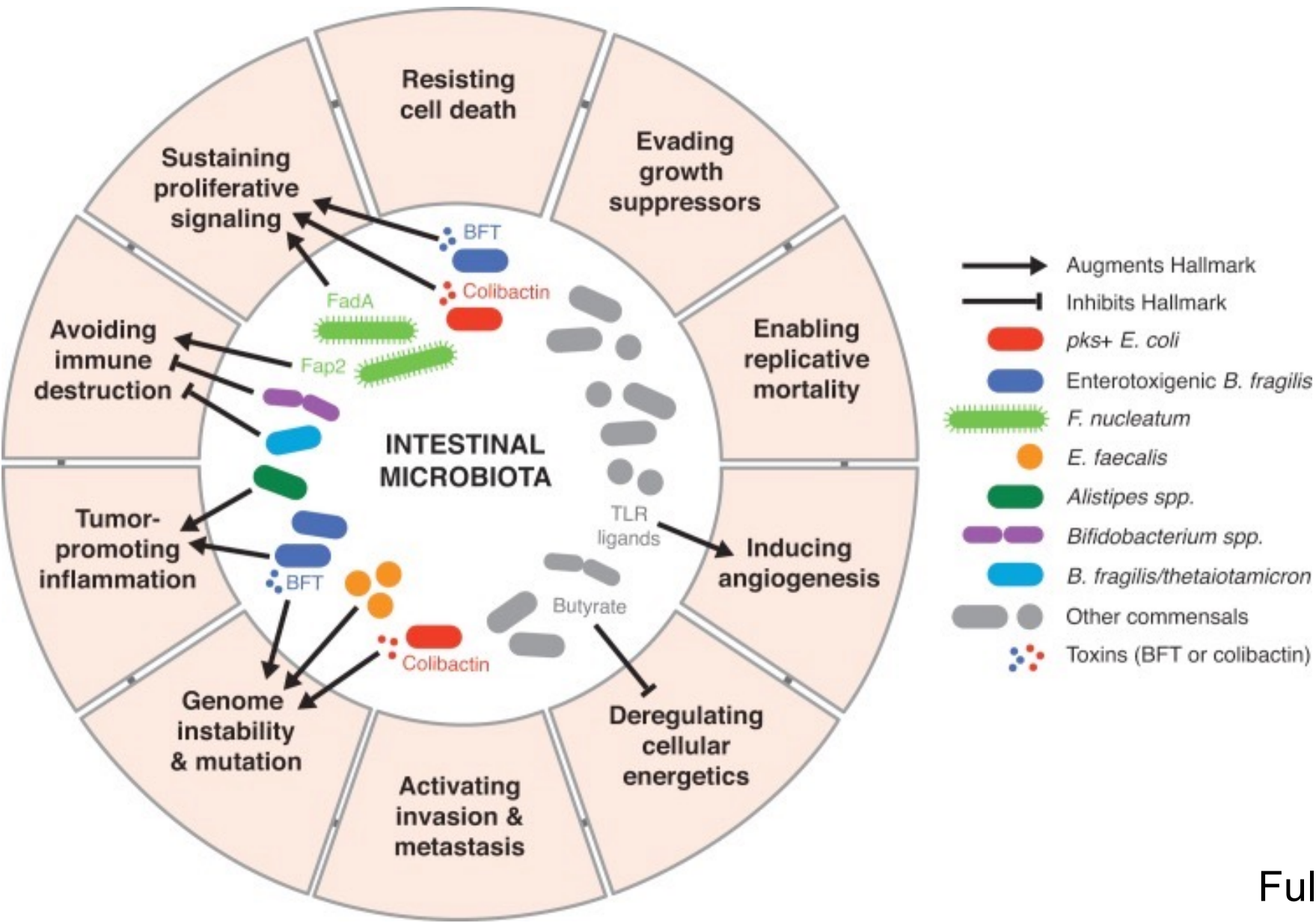
Room for a microbiota-based precision medicine?

- Diagnosis and prognosis of diseases
- Therapeutic correction of dysbiosis

**GUT MICROBIOTA-BASED
PRECISION MEDICINE**

A ROLE FOR A MICROBIOTA-BASED DIAGNOSTIC APPROACH?

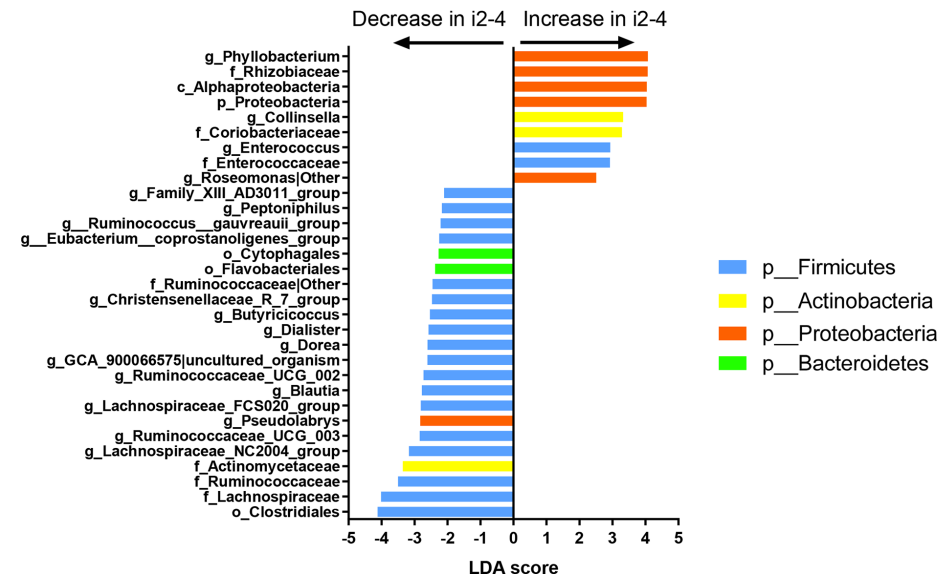
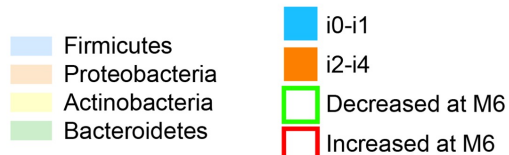
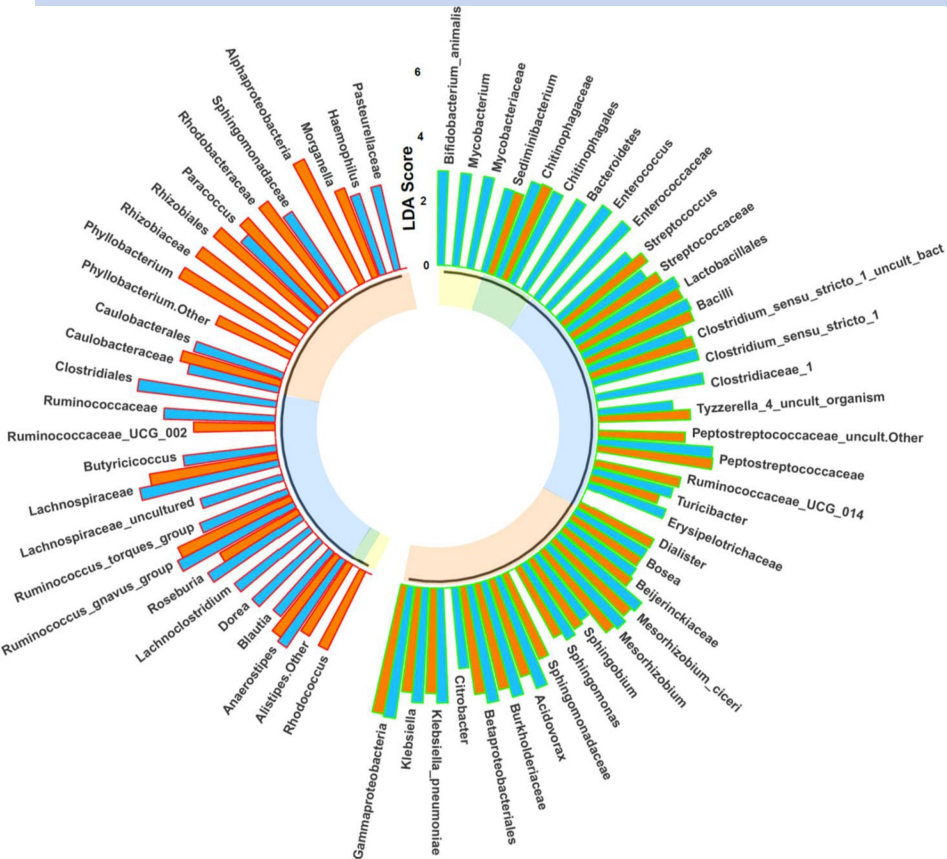
Gut microbiota influences carcinogenesis



Gut microbiome can predict disease course

Post-surgical Crohn's recurrence

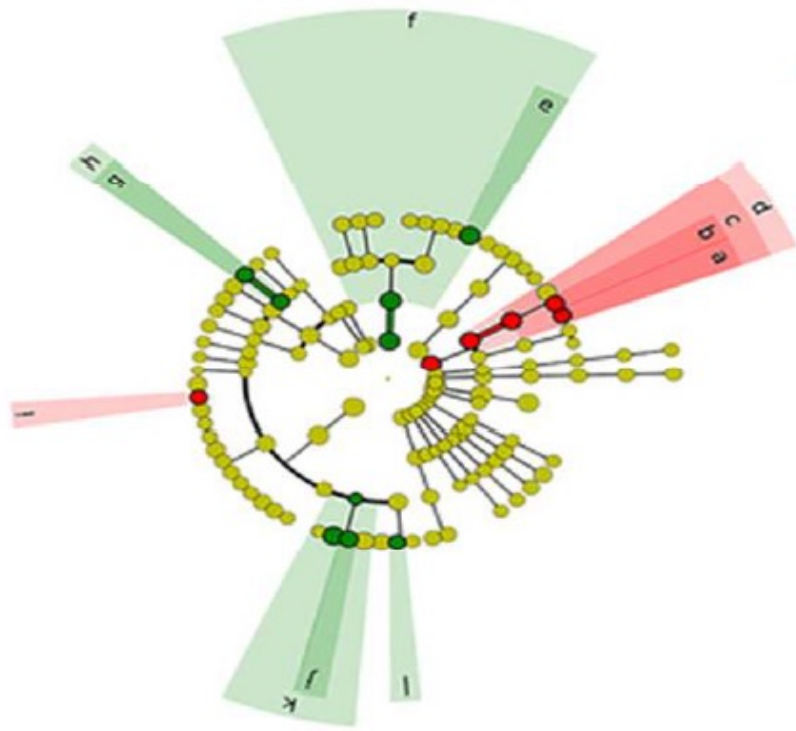
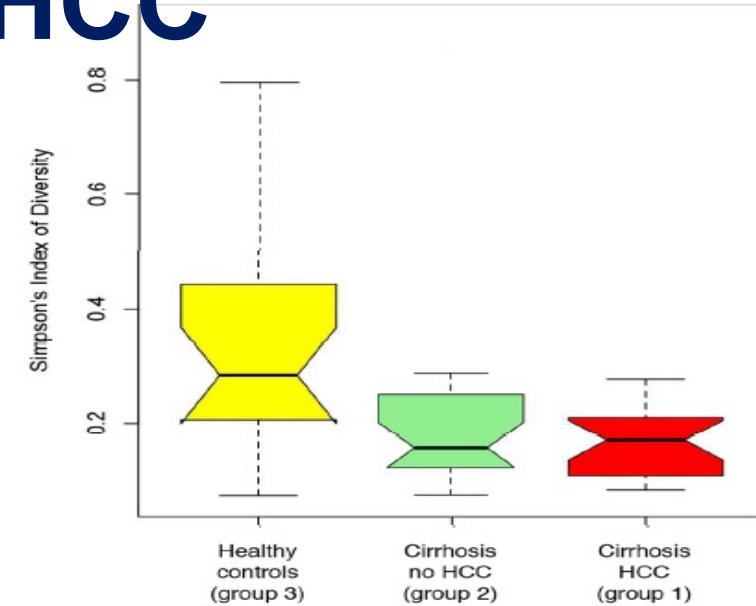
Endoscopic recurrence is associated with strong changes in ileal mucosa-associated microbiota



Gut microbiota at the time of surgery can predict endoscopic recurrence

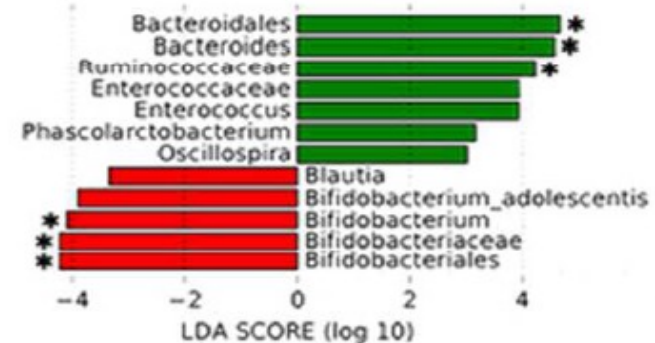
Gut microbiome can predict disease progression

From liver cirrhosis to HCC



■ Cirrhosis no HCC (group 2) **■ Cirrhosis HCC (group 1)**

- a:** Bifidobacterium
- b:** Bifidobacterium_adolescentis
- c:** Bifidobacteriaceae
- d:** Bifidobacteriales
- e:** Bacteroides
- f:** Bacteroidales
- g:** Enterococcus
- h:** Enterococcaceae
- i:** Blautia
- j:** Oscillospira
- k:** Ruminococcaceae
- l:** Phascolarctobacterium

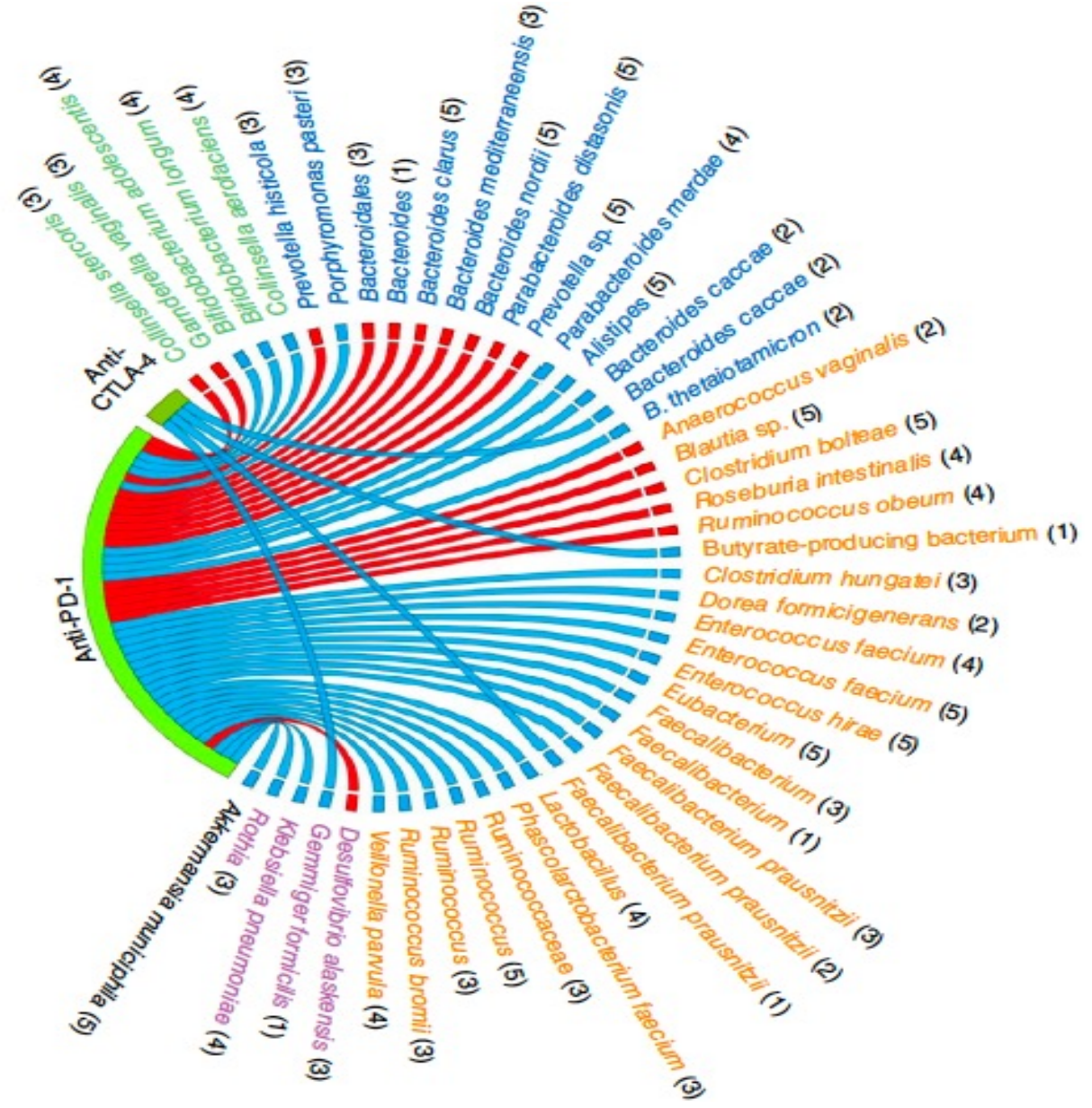
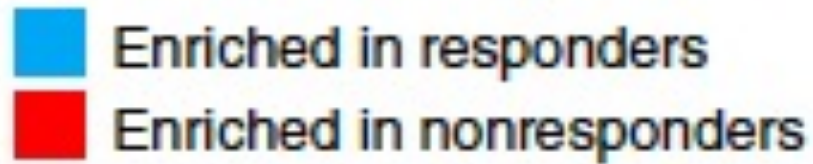


Specific microbial signatures are associated with response to ICIs in epithelial cancers

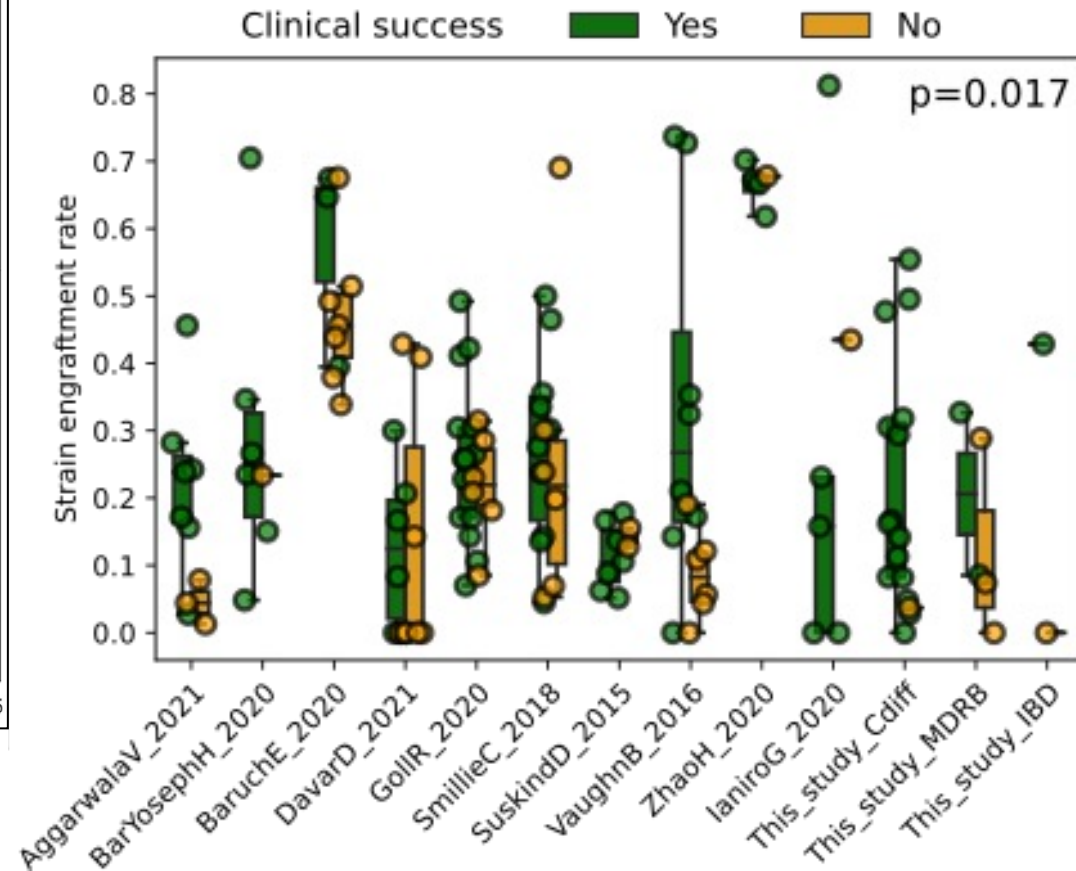
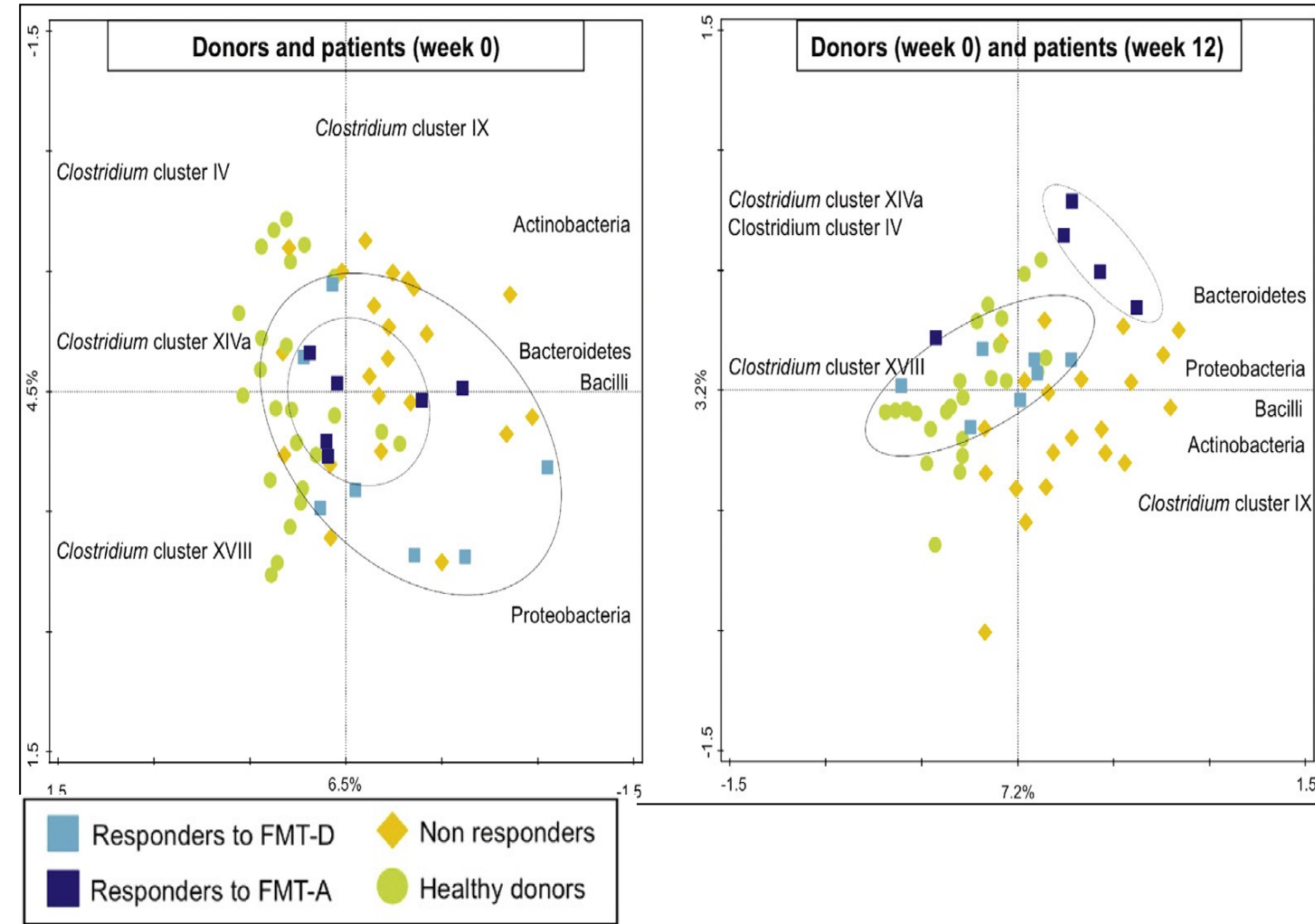
Bacterial phyla involved:



Association with response:



Predicting clinical response to microbiome therapeutics by evaluation of microbial engraftment



The current market of microbiome diagnostics is chaotic and not regulated

NEED FOR:

- **Standardization** (Shipping/storage, sequencing methods, reports)
- Evidence of **association** with clinical response
- **Education** (how to read it)

INTERNATIONAL CONSENSUS CONFERENCE

Report of gut microbiome sequencing in clinical practice

- There are considerable expectations on using the microbiota as diagnostic, prognostic or therapeutic tool to be applied in medicine.
 - However, the **gut microbiome is still a hardly manageable field for most clinicians**
 - In recent years an **increasing number of commercial entities** are offering microbiome diagnostic tests, available directly to patients, **without any common indication/guideline to regulate them.**
- The aim of this consensus report is to **define minimum criteria, indications, pre-testing and sequencing methods, and reporting rules, for commercial microbiome testings.**
 - The final purpose is to **regulate the provision and exploitation of these testings**, avoiding the diffusion of inappropriate diagnostics, and to **promote the knowledge and culture of the human microbiome field.**

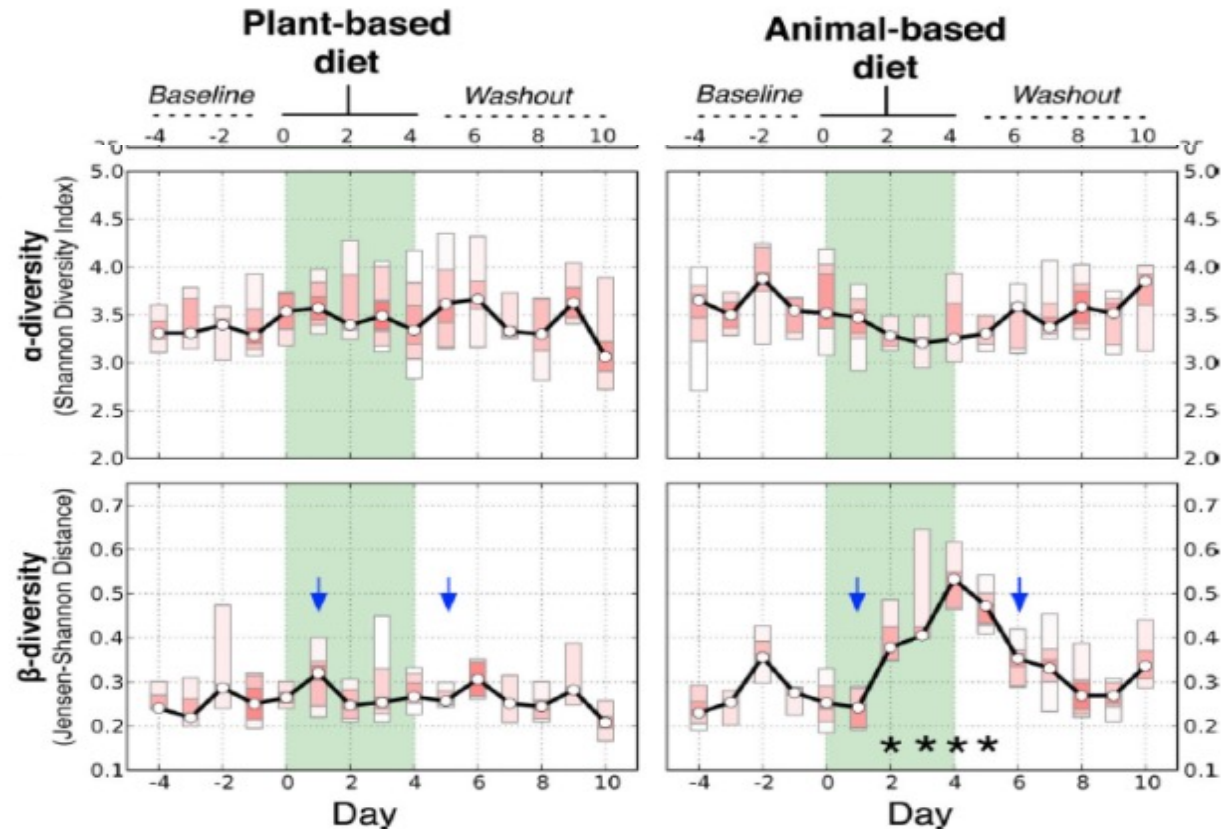
>50 worldwide clinical and non-clinical microbiome experts

**WHICH ROLE FOR A MICROBIOTA-BASED
TREATMENT?**

Diet & Gut microbiota

The short-term modification of diet alters microbial community structure and microbial gene expression

The animal-based diet increases the abundance of bile-tolerant microorganisms (Alistipes, Bilophila, and Bacteroides) and decreases the levels of Firmicutes that metabolize dietary plant polysaccharides (Roseburia, Eubacterium rectale, and Ruminococcus bromii)

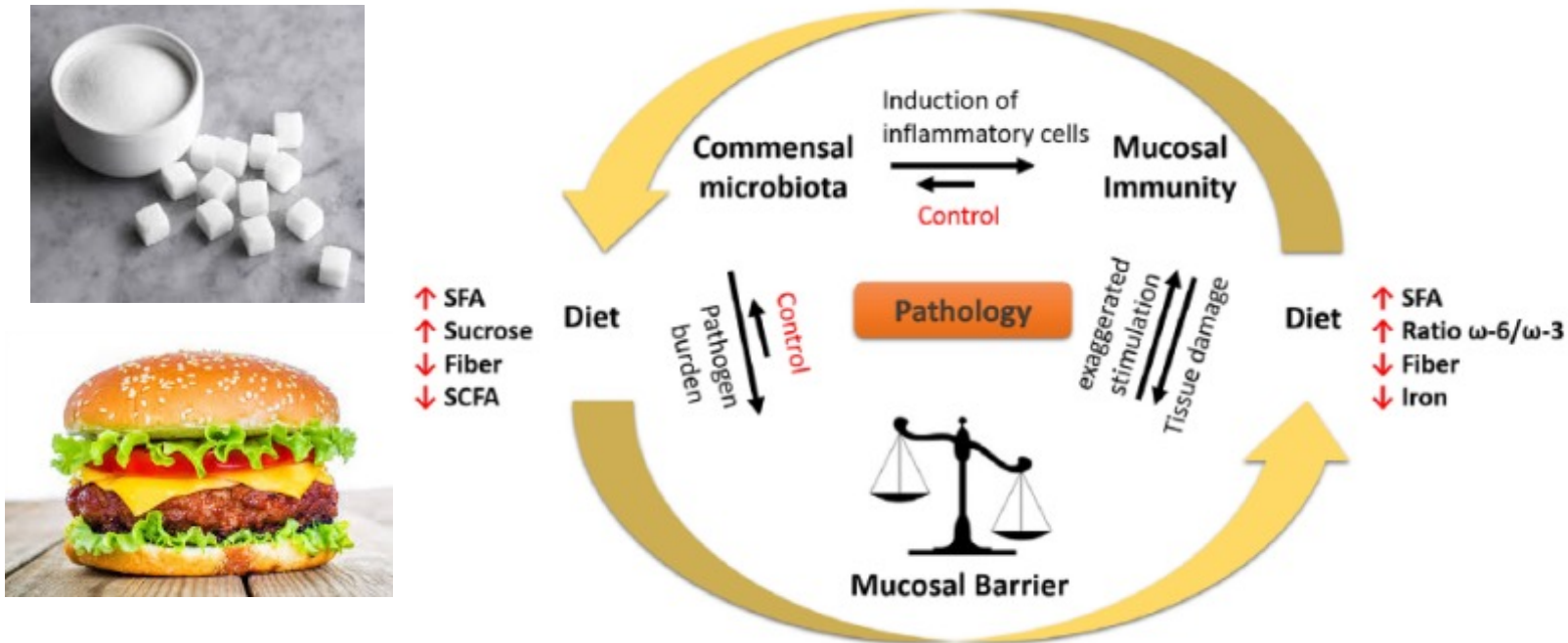


The human gut microbiome can rapidly (as quickly as 24 hours!) switch between herbivorous and carnivorous functional profiles. It may reflect past selective pressures during human evolution.

Dietary microbiome influencers

Western diet

- ★ Western diet is associated with increased risk for IBD, metabolic disease and asthma



High-fat and sucrose-rich diet increases permeability of epithelial barrier

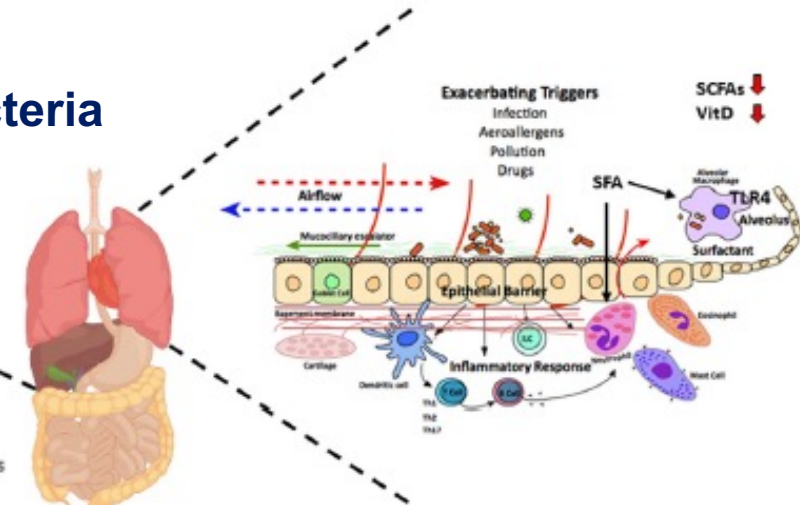
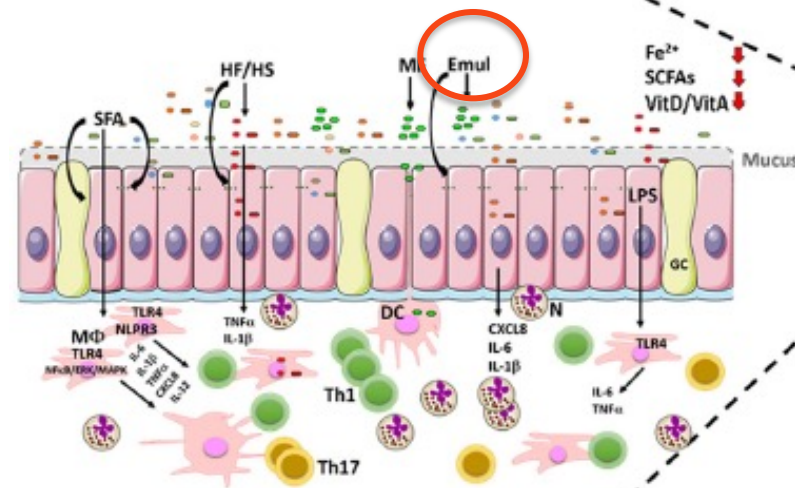
Dietary microbiome influencers

Emulsifiers

- ★ Emulsifiers such as polysorbate-80 and carboxymethylcellulose are richly used in processed foods

Emulsifiers could aggravate colitis:

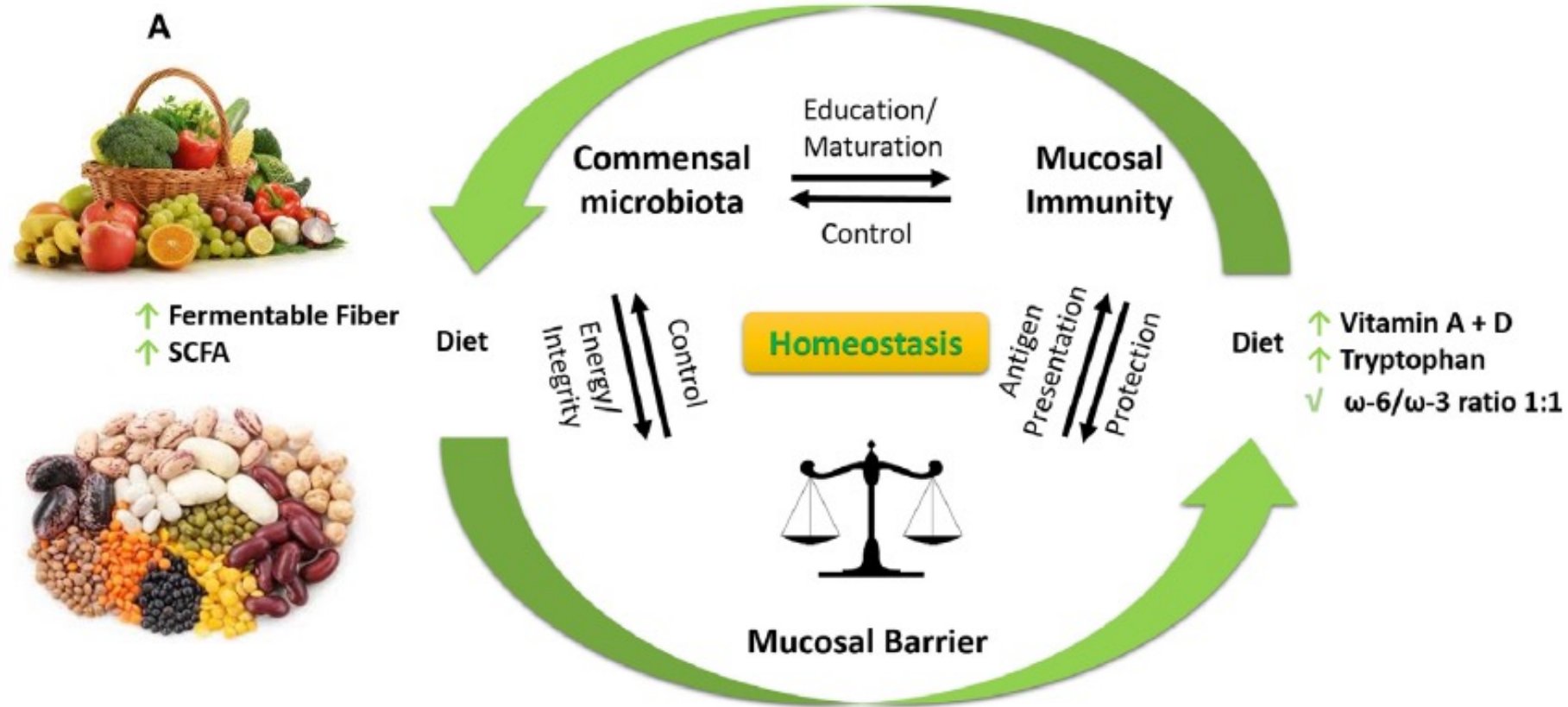
- ↑ gut permeability
- ↓ mucus thickness
- ↑ higher penetration of intestinal bacteria
- alter gut microbiota composition (enrichment in *Bilophila spp.*)



Dietary microbiome influencers

High-fiber diet

- ★ High-fiber diet is associated with reduced risk for IBD, metabolic disease and asthma



Therapeutic pathways of probiotics

ANTIMICROBIAL EFFECT

- Restoration of luminal environment
- Production of antimicrobial molecules
- Inhibition of pathogen adhesion and invasion
- Competition w/ pathogens
- Antitoxin effect

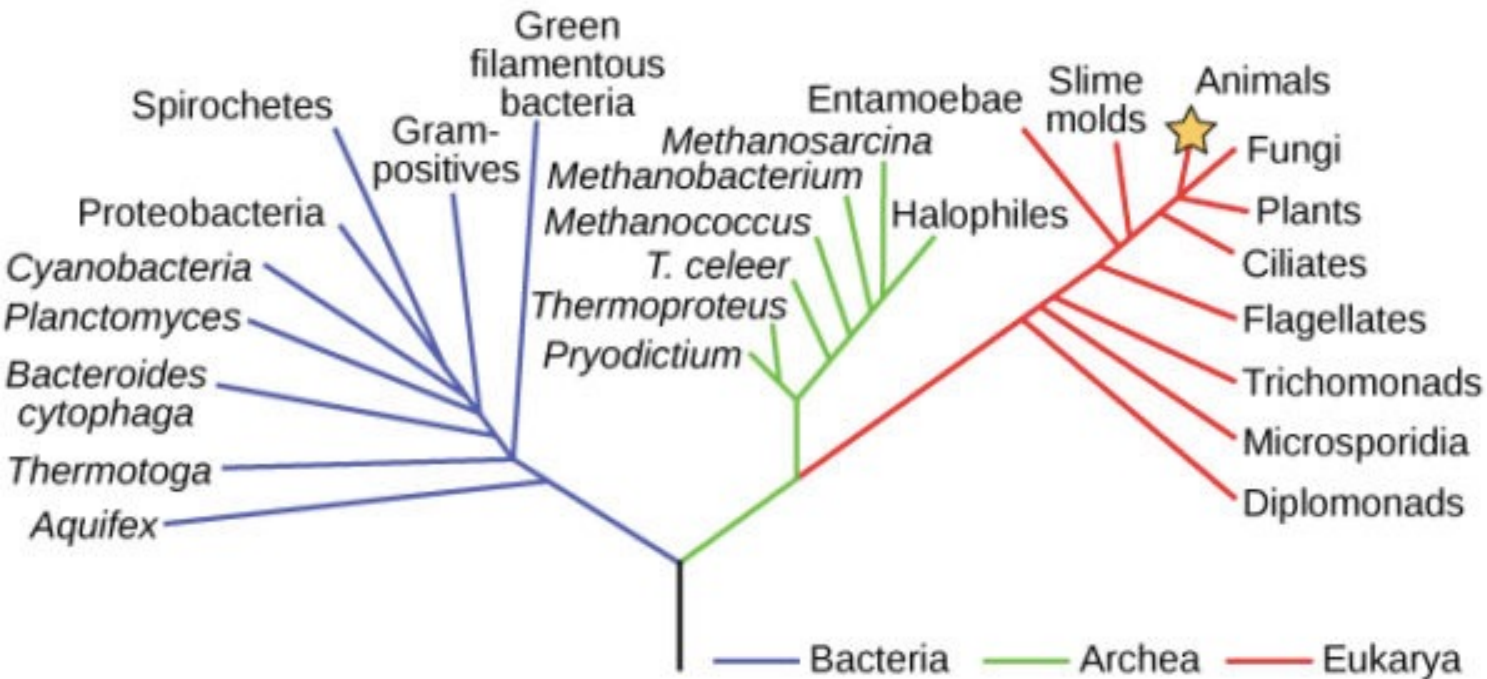
IMMUNE MODULATION

- Inhibition of proinflamm. cytokines
- Increase of anti-inflammatory cytokines
- Priming of host dendritic cells
- Induction of T regs
- Effect on B lymphocytes

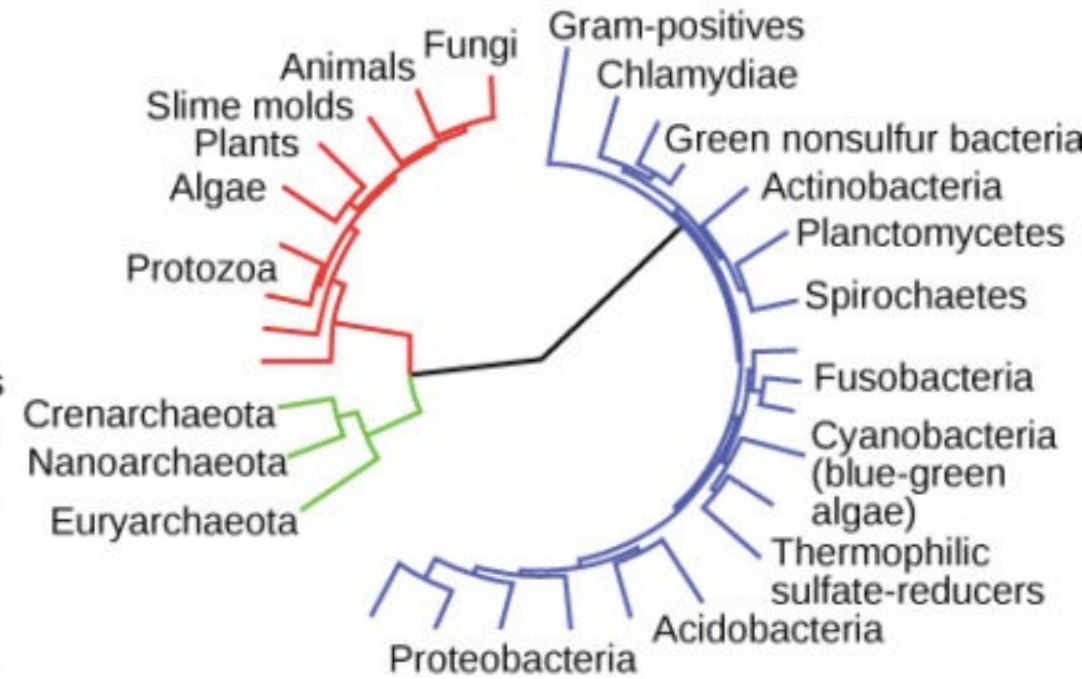
ENHANCEMENT OF GUT BARRIER INTEGRITY

- Strengthening of epithelial barrier (tight junctions)
- Alteration of surface proteins

Phylogenetically, probiotics can be highly different each other



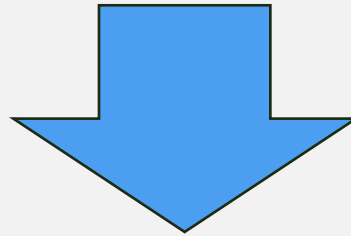
(a) Rooted phylogenetic tree



(b) Unrooted phylogenetic tree

Different action for each Probiotic

Knowledge of micro-organism functions and host genetic modulation by different Species/Strain is crucial



Need for a Specie (Strain)-specific Microbial Therapy

(a) Rooted phylogenetic tree

(b) Unrooted phylogenetic tree

Fecal microbiota transplantation

DONOR SCREENING

Starting questionnaire

To rule out:

- Risk factors for **infect. dis**
- **Drugs** that impair microbiota
- **Diseases** that impair microbiota

Blood & Stool Exams

To exclude transmittable diseases

Questionnaire before donation

To exclude issues risen during screening

INFUSATE PREPARATION

Fresh Material

- To be used **within 6 hours** after defecation
- Manufacturing should be as brief as possible
- **At least 30 g** of faeces should be used
- Feces should be suspended in **saline** with a blender or manual effort & sieved to avoid clogging

Frozen Material

- **At least 30 g of feces** and **150 mL of saline** to be used
- Before freezing, add **glycerol up to 10%**
- Suspensions should be labelled, traceable, stored at **-80°C**
- **Thaw at 37°C** and **infuse within 6 hours** from thawing

FECAL DELIVERY

Bridging atb pre-treatment

Usually vanco 3 days before FMT

Bowel preparation

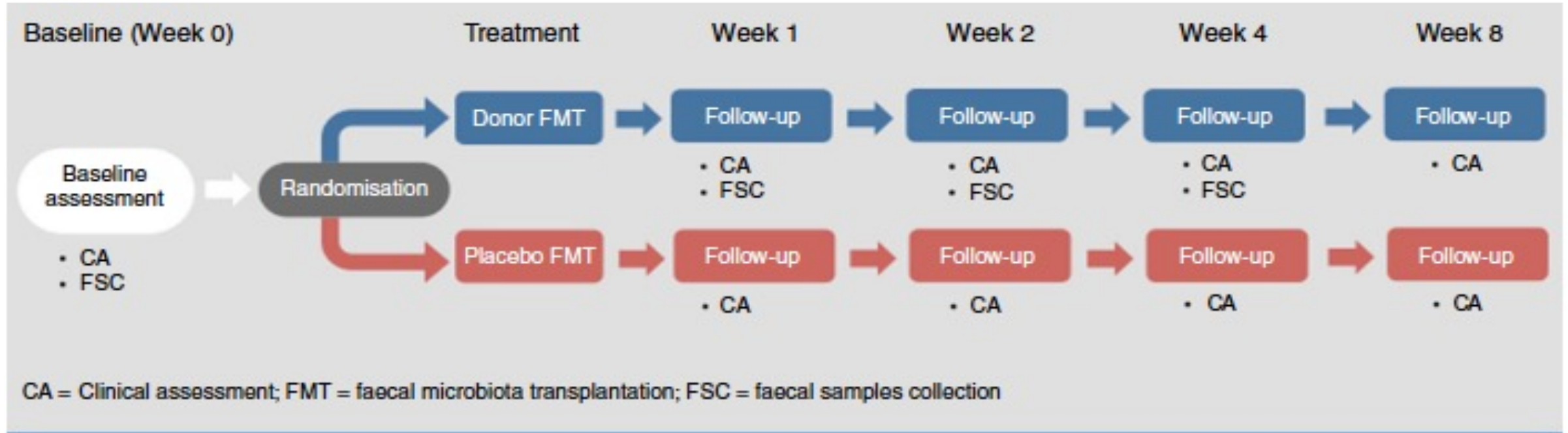
- To remove patient's feces

Routes of delivery

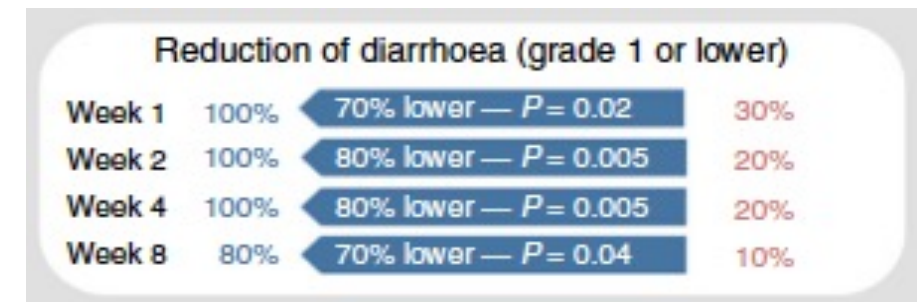
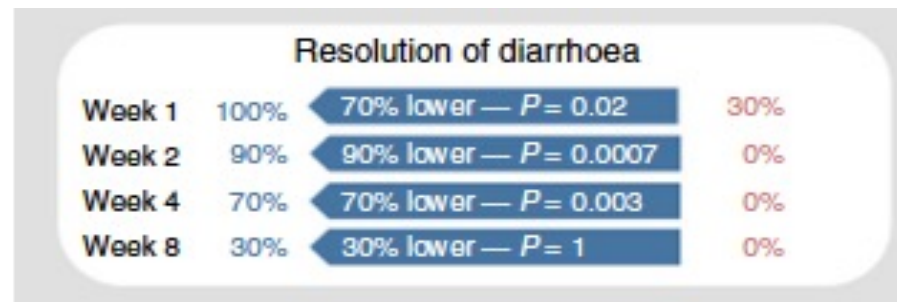
- NJT/NDT
- Capsules
- Colonoscopy
- Enema

FMT may ameliorate cancer therapies-induced diarrhea

RCT of donor FMT vs placebo in 20 pts with advanced RCC under treatment with TKI (pazopanib or sunitinib) and grade ≥ 2 diarrhea not responsive to standard treatments

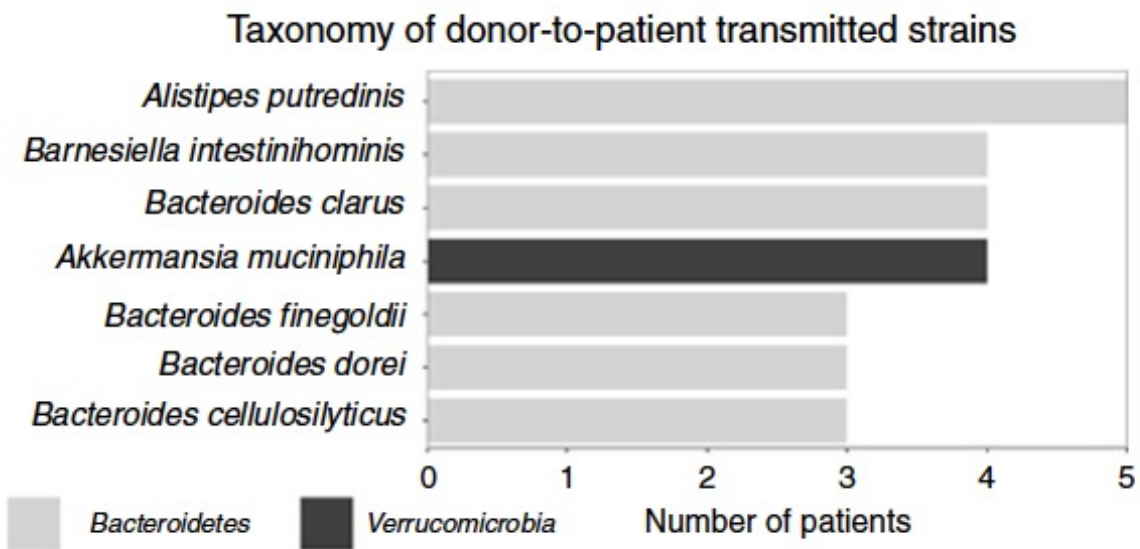


Resolution of diarrhoea at W4 (PE)

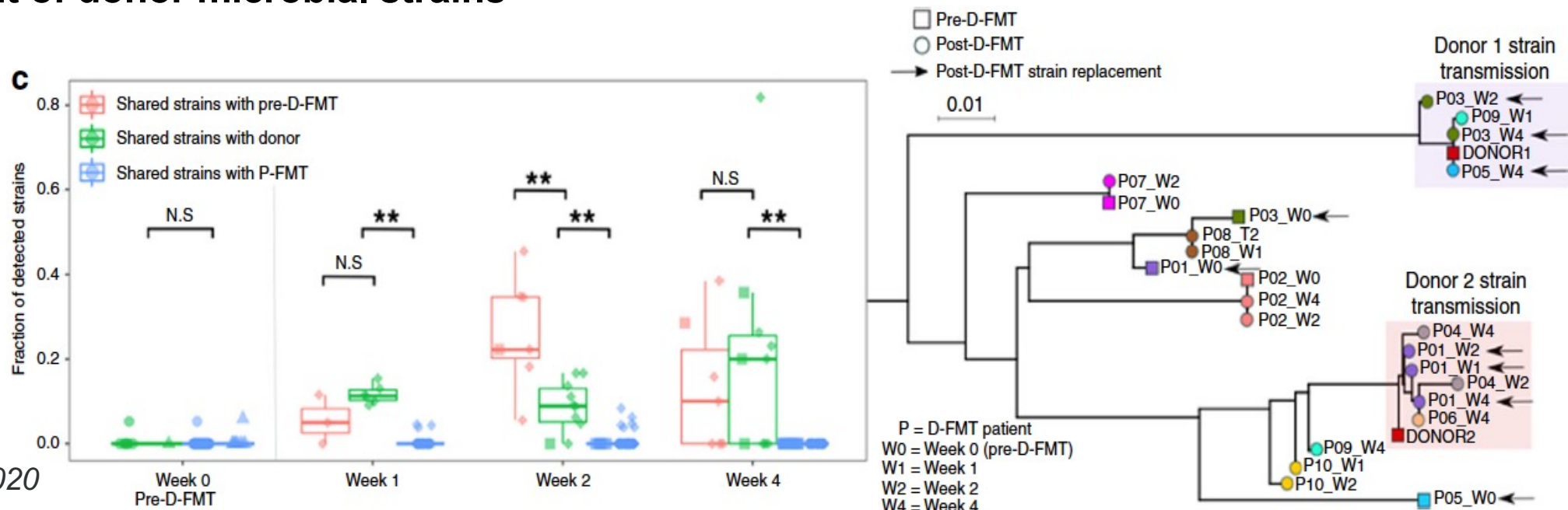


FMT may ameliorate cancer therapies-induced diarrhea

Transfer of beneficial species

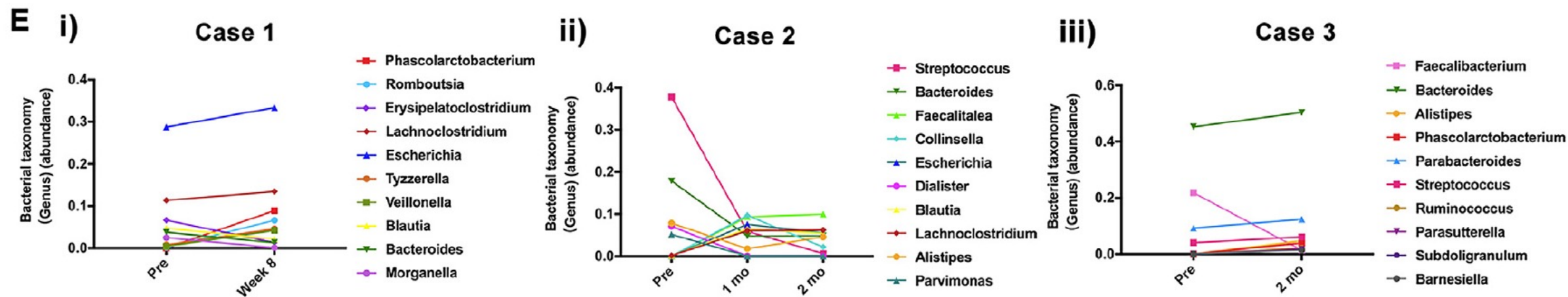


Engraftment of donor microbial strains



FMT for chronic radiation enteritis

- **Five female patients** underwent FMT for CRE
- **Three patients responded** to FMT (improvement in diarrhea, rectal hemorrhage, abdominal/rectal pain and fecal incontinence)
- **No FMT-related death and infectious complications**
- **Increase in microbial diversity and beneficial microbes abundance** in patients after FMT

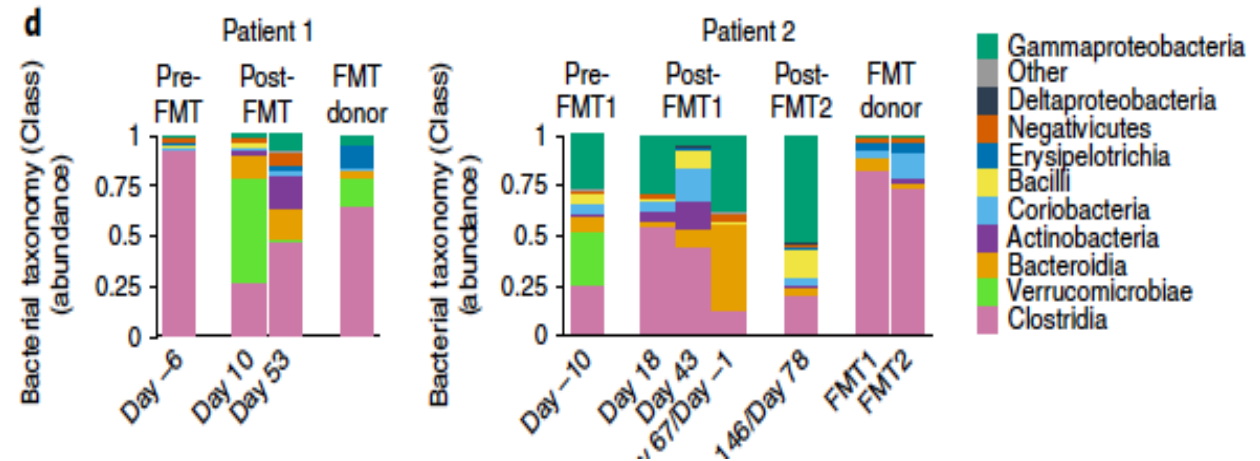
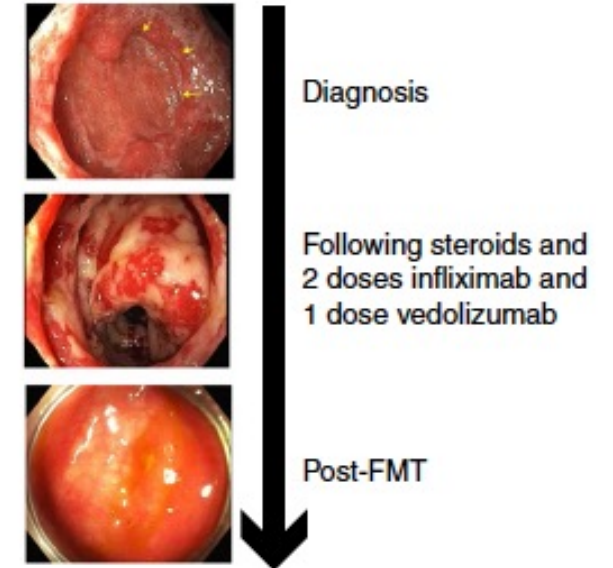


Fecal microbiota transplantation may abrogate immunotherapy-associated colitis

- 2 pts with renal or prostate cancer
- CTLA4+PD1 or CTLA4 alone
- Grade ≥ 2 diarrhea/colitis
- Not responsive to steroids, IFX, VEDO

AFTER FMT

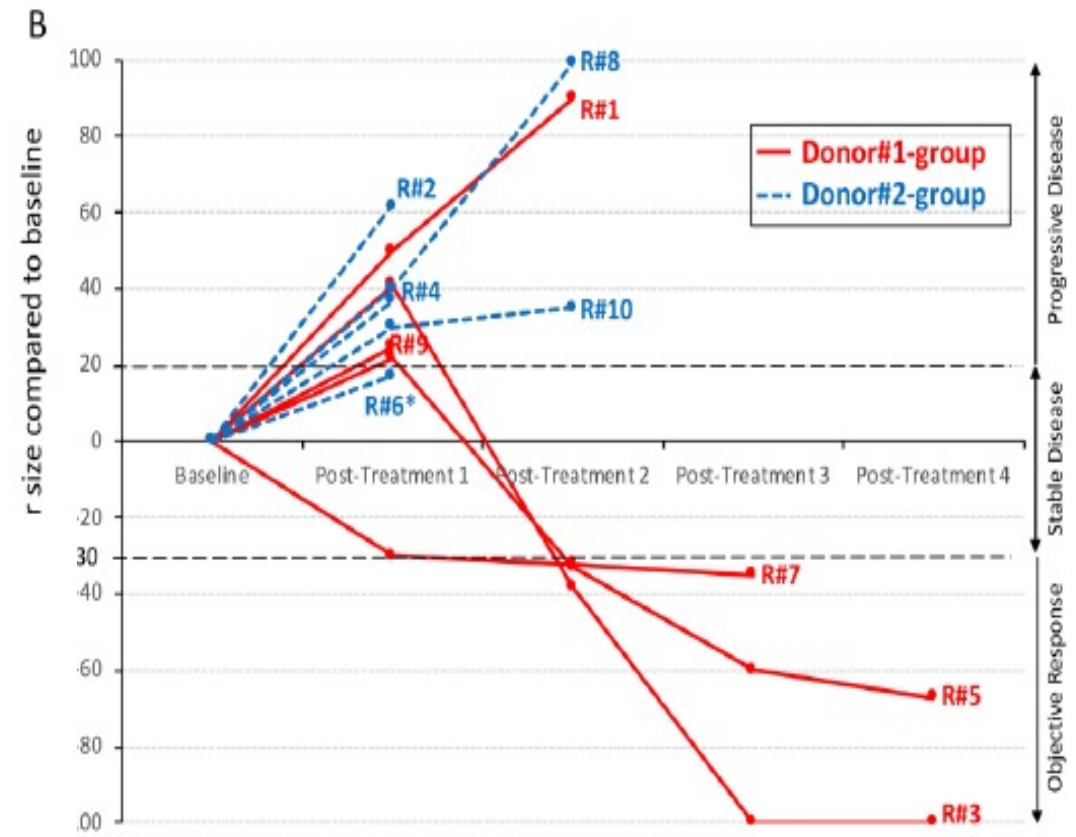
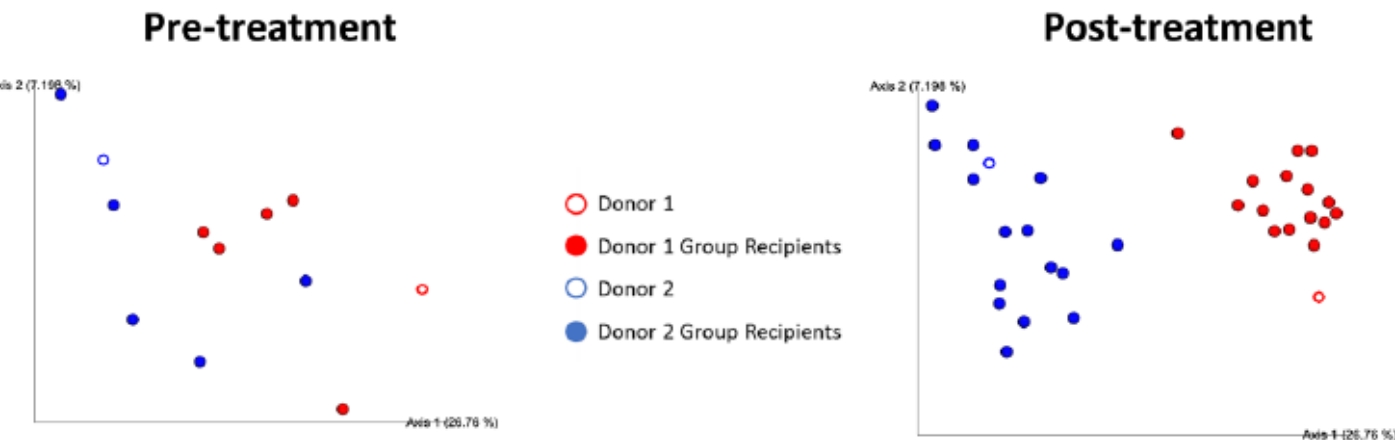
- Improvement of endoscopic appearance
- Reduction in CD8, increase in CD4 T cells
- Increase in Bifidobacteria and Blautia
- Shift toward donor microbiomes



Fecal microbiota transplantation may improve response to ICIs in epithelial cancers

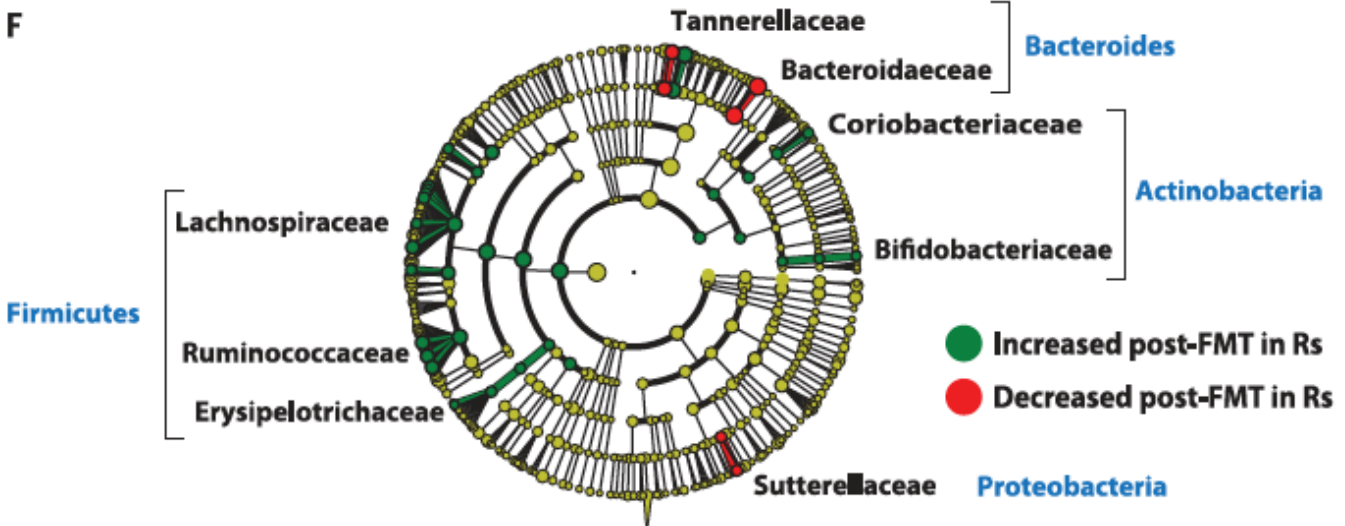
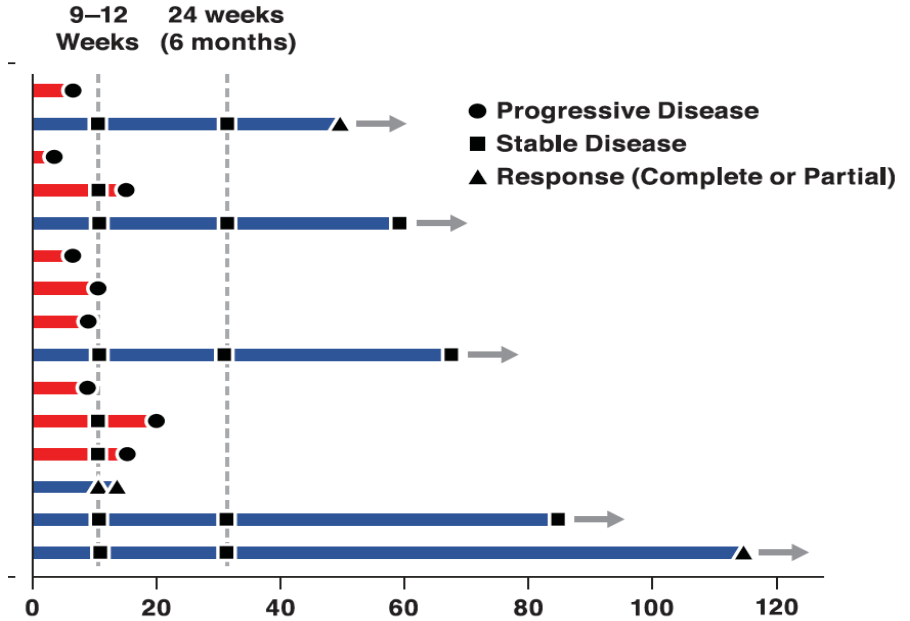
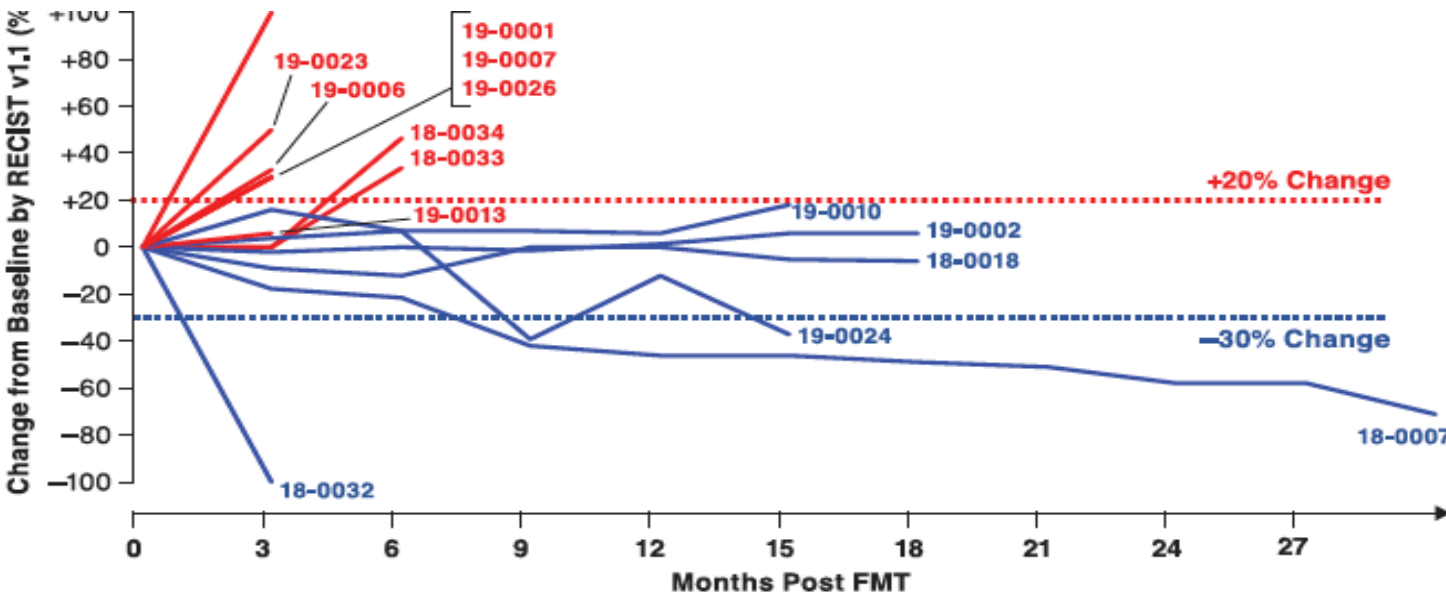
- 10 patients with anti-PD1 refractory melanoma
- 2 donors with complete response
- Antibiotic pre-treatment + colonoscopic FMT + 6 capsules cycles together with nivolumab every 2 weeks

- **Objective response in 3 patients**
- **Favorable changes in immune cell infiltrates and gene expression profiles in both the gut lamina propria and the tumor microenvironment**
- **Satisfactory donor-patient engraftment**



Fecal microbiota transplantation may improve response to ICIs in epithelial cancers

- 16 patients with melanoma in progression
- Single colonoscopic FMT + ICI (pembro)
- 7 donors with partial or total response
- **Clinical benefit in 6 patients**
- **Shift toward beneficial taxa**



Davar et al – Science 2021

The theory-to-practice gap in the world of human microbiome

To date, there is a **gap between research and clinical practice in the microbiome field**

- Most research evidence is not translated into clinical practice
- Communication breakdown between microbiome scientists and clinicians
- There is increasing hype by patients toward a microbiome-based management of diseases (diagnostics and therapeutics)
- Due to this gap, patients are often attracted by strategies and providers that are out of scientific boundaries

Time for a translational figure: the MICROBIOME CLINICIAN
Time for a breakthrough in clinical practice: the MICROBIOME CLINIC

MICROBIOME CLINICIAN

- **Continuous up-to-date on microbiota research**
- **Knowledge of different dysbiotic profiles of GI and extra-GI Disorders**
- **Interpretation of gut microbiota profiling**
- **Application of microbiome research data in clinical practice**
- **Expertise in microbiota modulation (anti-pre-probiotics, FMT)**

MICROBIOME CLINIC

- **Multidisciplinary team** (microbiome clinicians, microbiologists, immunologists, nutritionists, etc.)
- **Availability of microbiota sequencing tools**
- **Availability of stool bank/FMT Centre**
- **Hotspot for microbiota research**
- **Networking and teaching centre**

#microbiomeclinicians