MRO. ART
INNOVATIVE TREATMENTS AND PATIENTS COMPLIANCE
31° RESIDENTIAL COURSE

Innovation: Microbiome

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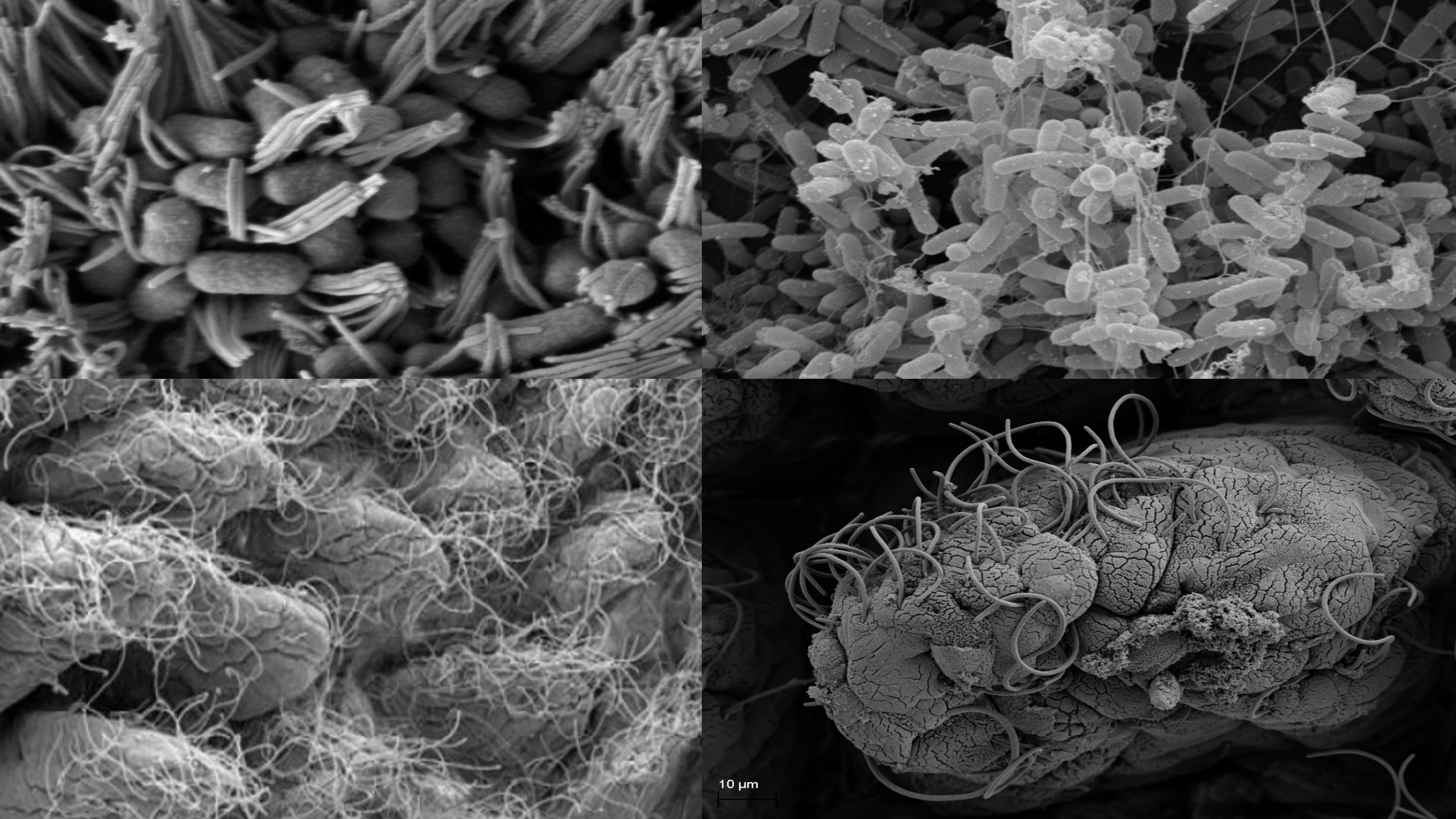
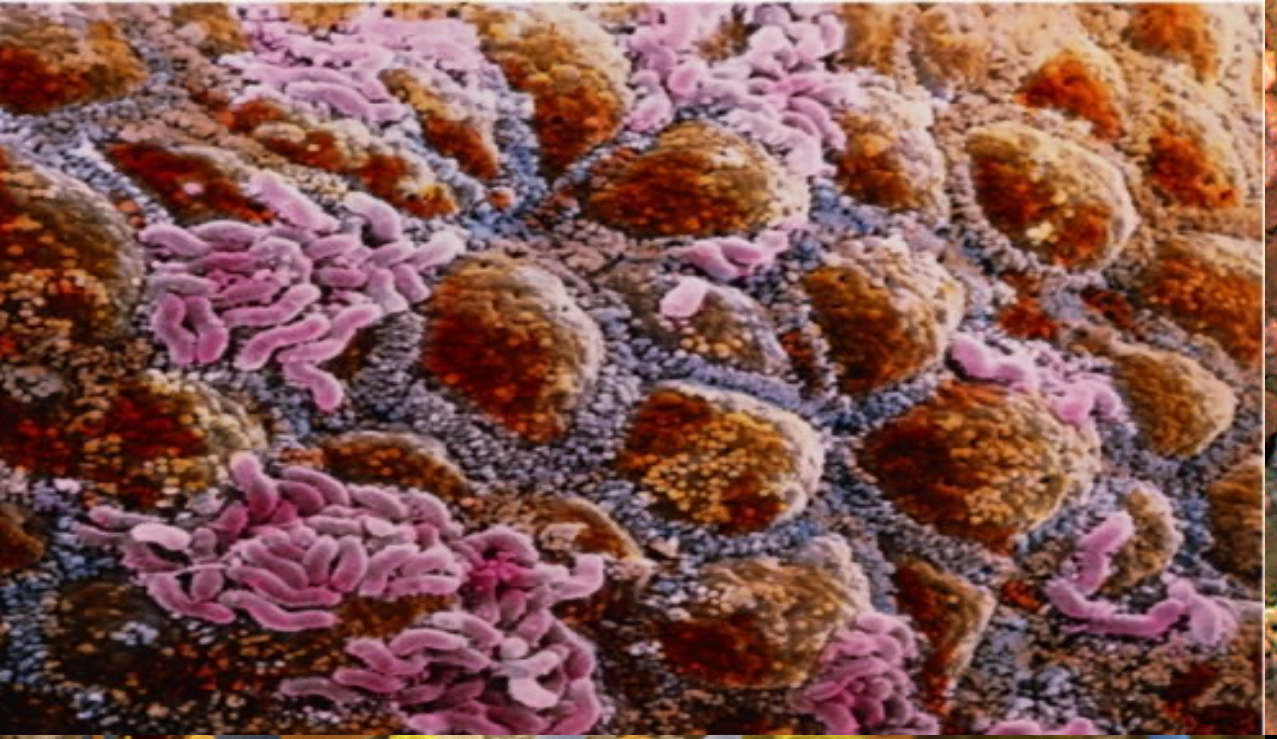
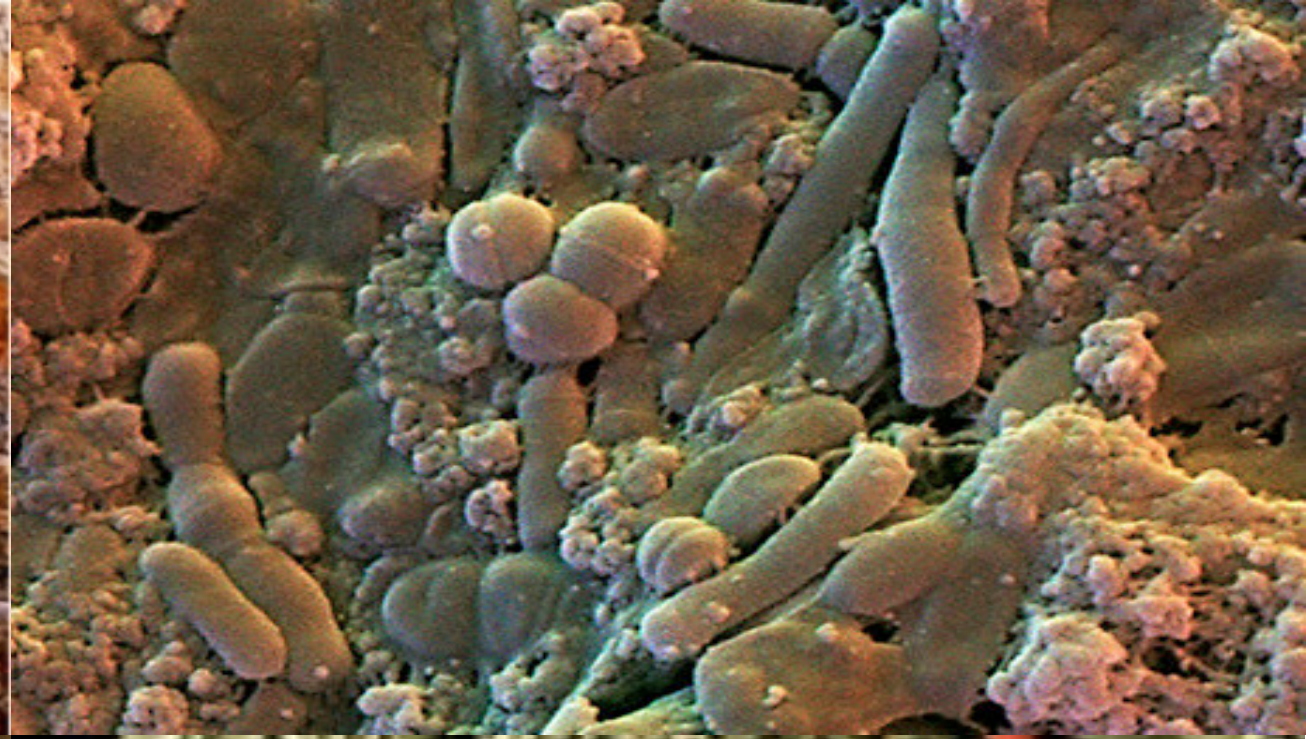
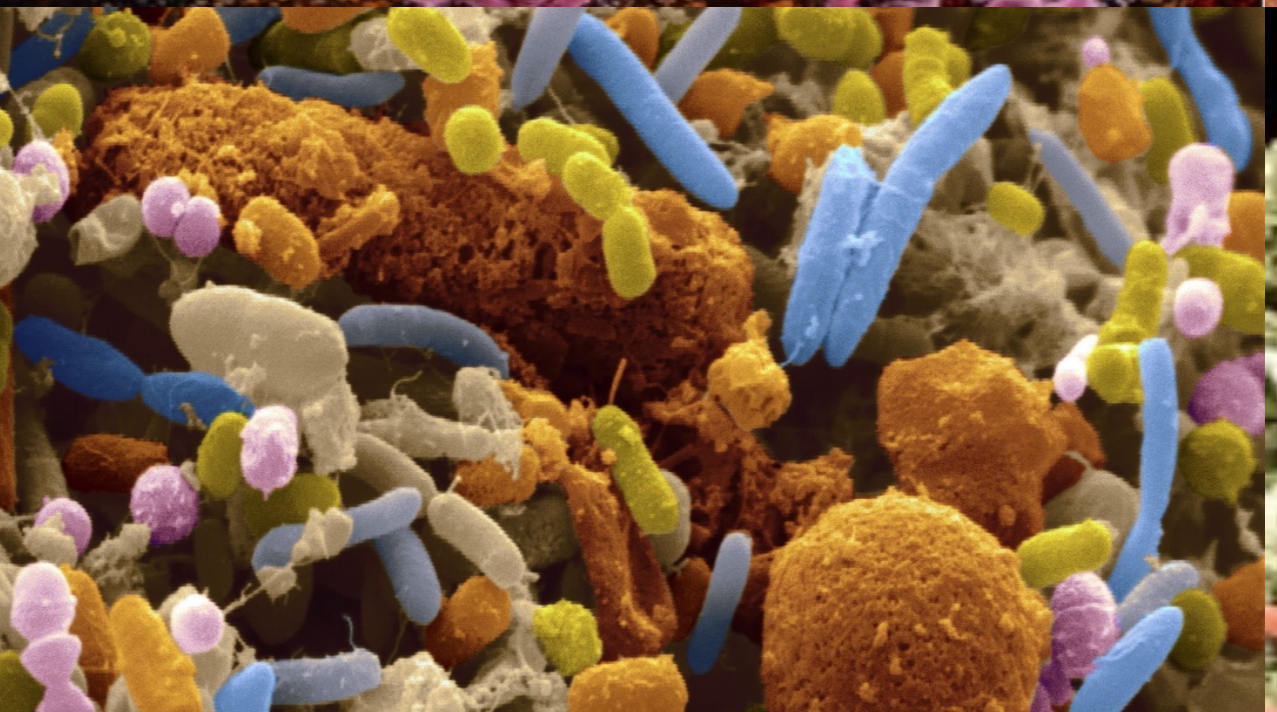
CEMAD
Centro Malattie Apparato Digerente
Digestive Disease Center


10 µm


ANATOMO-MICROBIOLOGICAL BARRIER

THE -OMICS REVOLUTION

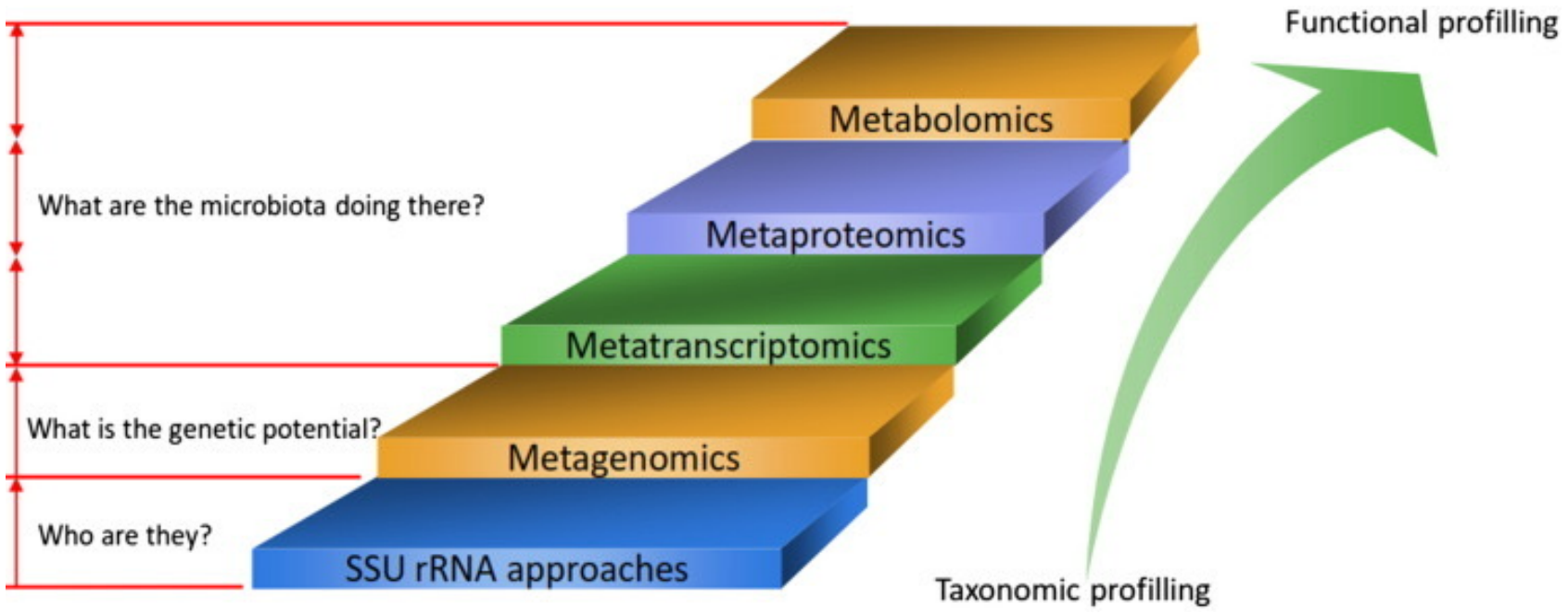
MICROBIOMICS: microbiota collective coding capacity

METAGENOMICS: experimentally determined dataset from shotgun sequencing the genomes of microorganisms in a particular sample.

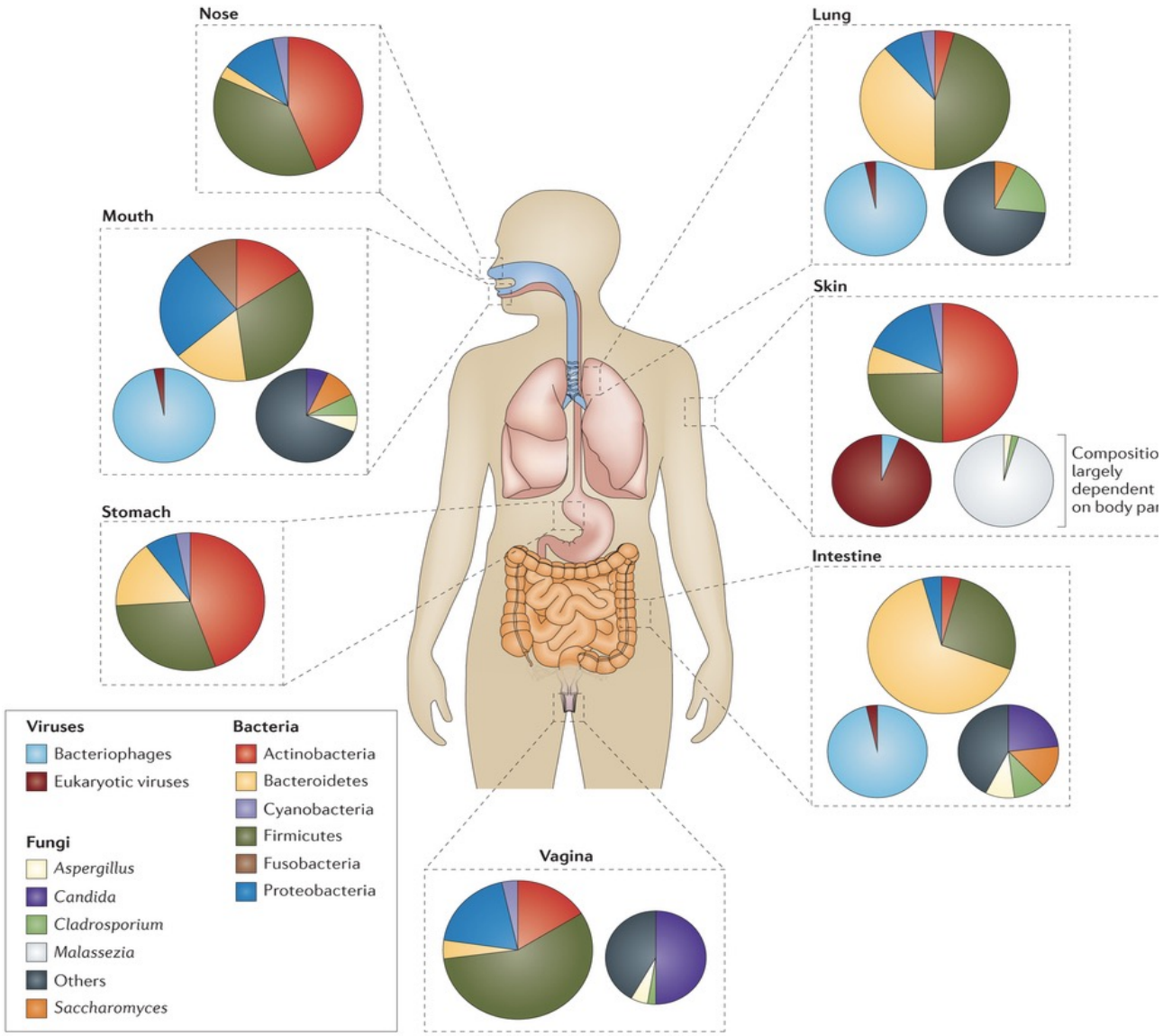
METATRANSCRIPTOMICS: shotgun sequencing of reverse-transcribed RNA transcripts

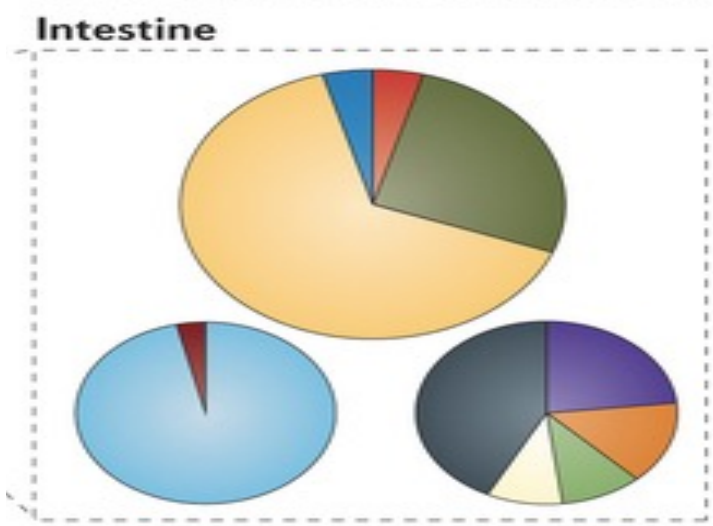
METAPROTEOMICS: the quantification of protein or peptide levels

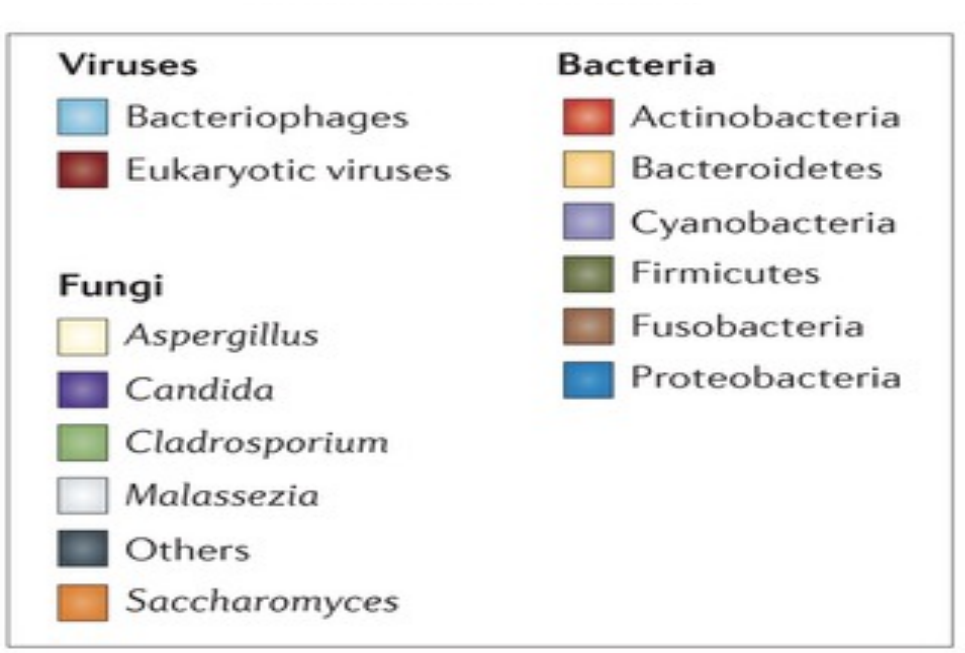
METABOLOMICS: investigation of small-molecule metabolites



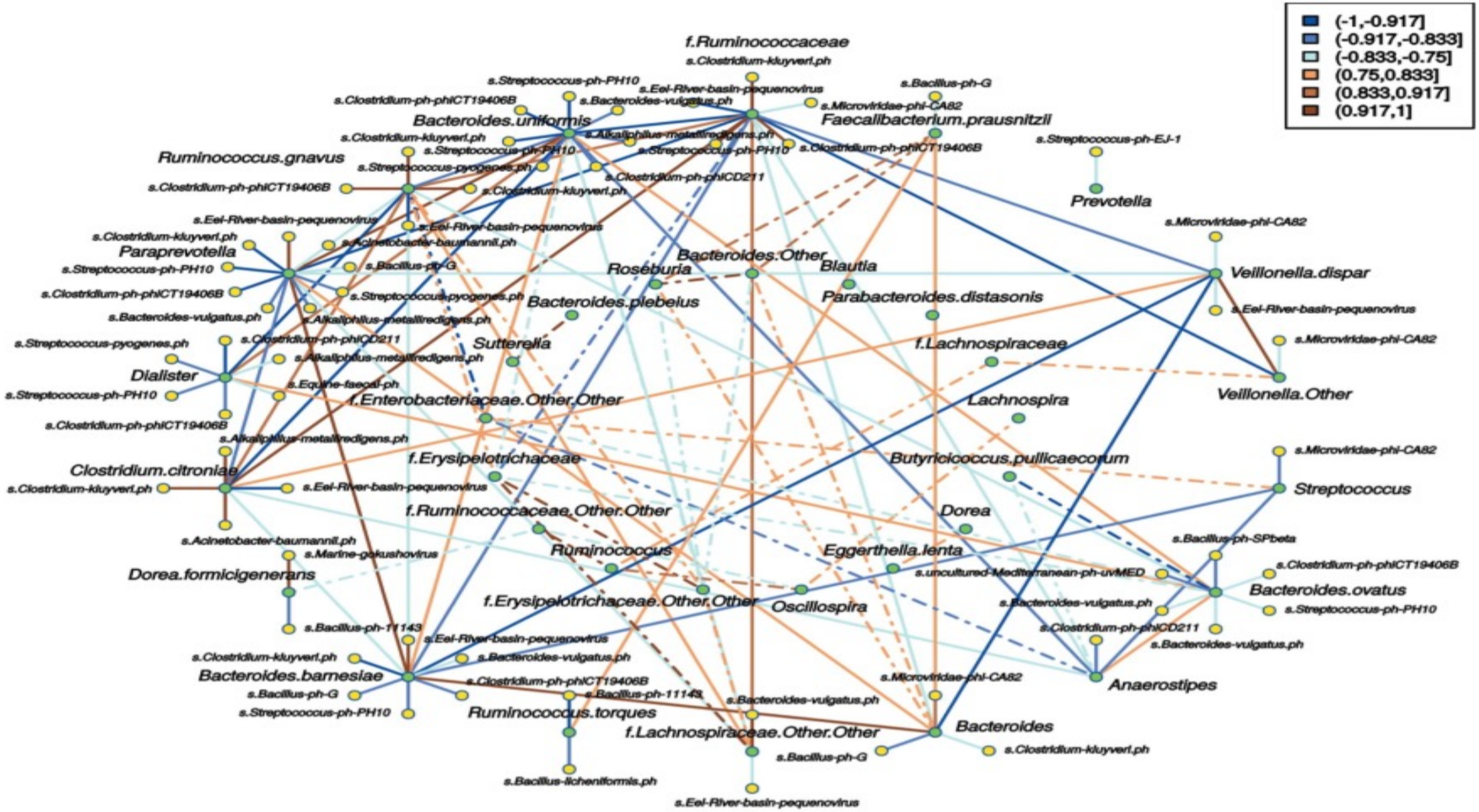
Human microbiota composition







Microbial correlation networkorking between relative abundance of *bacterial, yeast and bacteriophage*-matching reads



EUBIOSIS

EU= good; BIOS= life

Eubiosis is the healthy relationship among commensal MICROBES of the gut

COMPOSITION

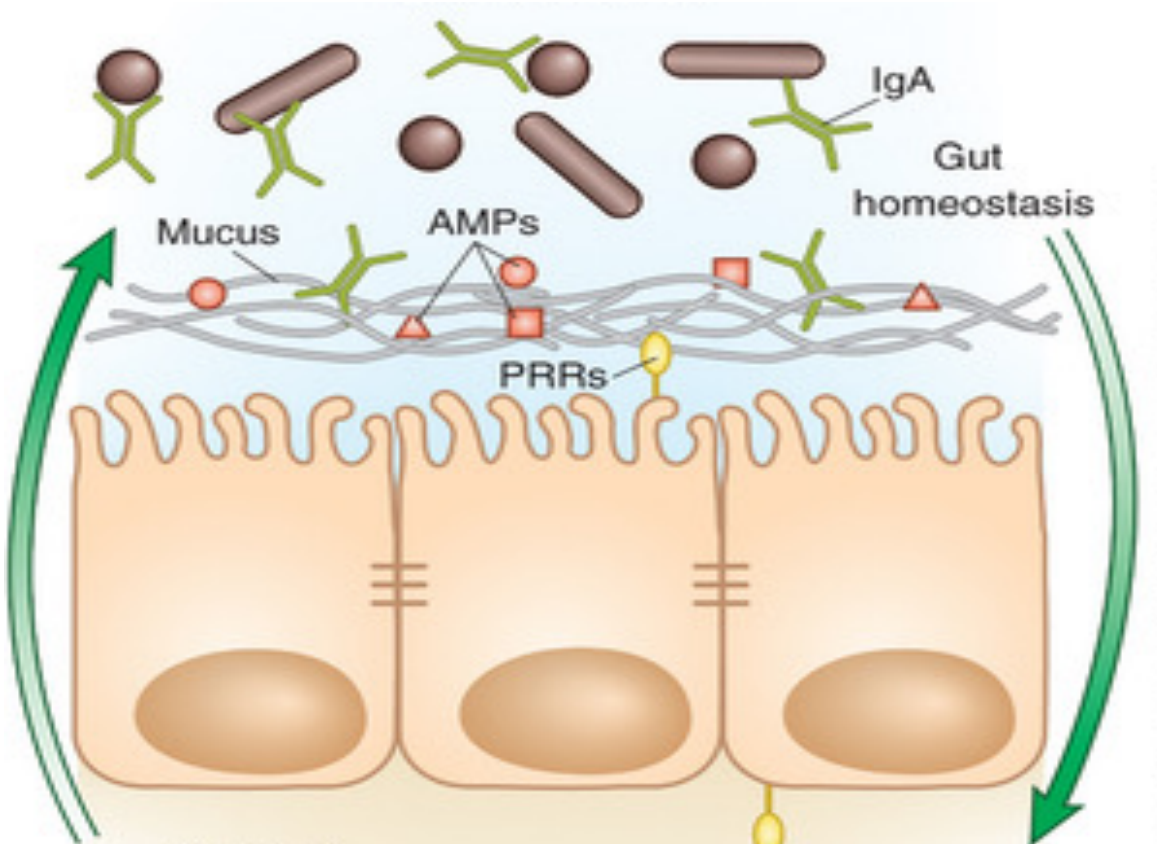
- **Diversity**
- **Richness**
- **Relative Abundance**

FUNCTION

- **Microbiota's effect on host health**

FUNCTIONS OF GUT MICROBIOTA ON HOST HEALTH

- Immunocompetence/Tolerance
- Barrier effect
- Synthesis
- Metabolism
- Drug metabolism
- Behavior conditioning

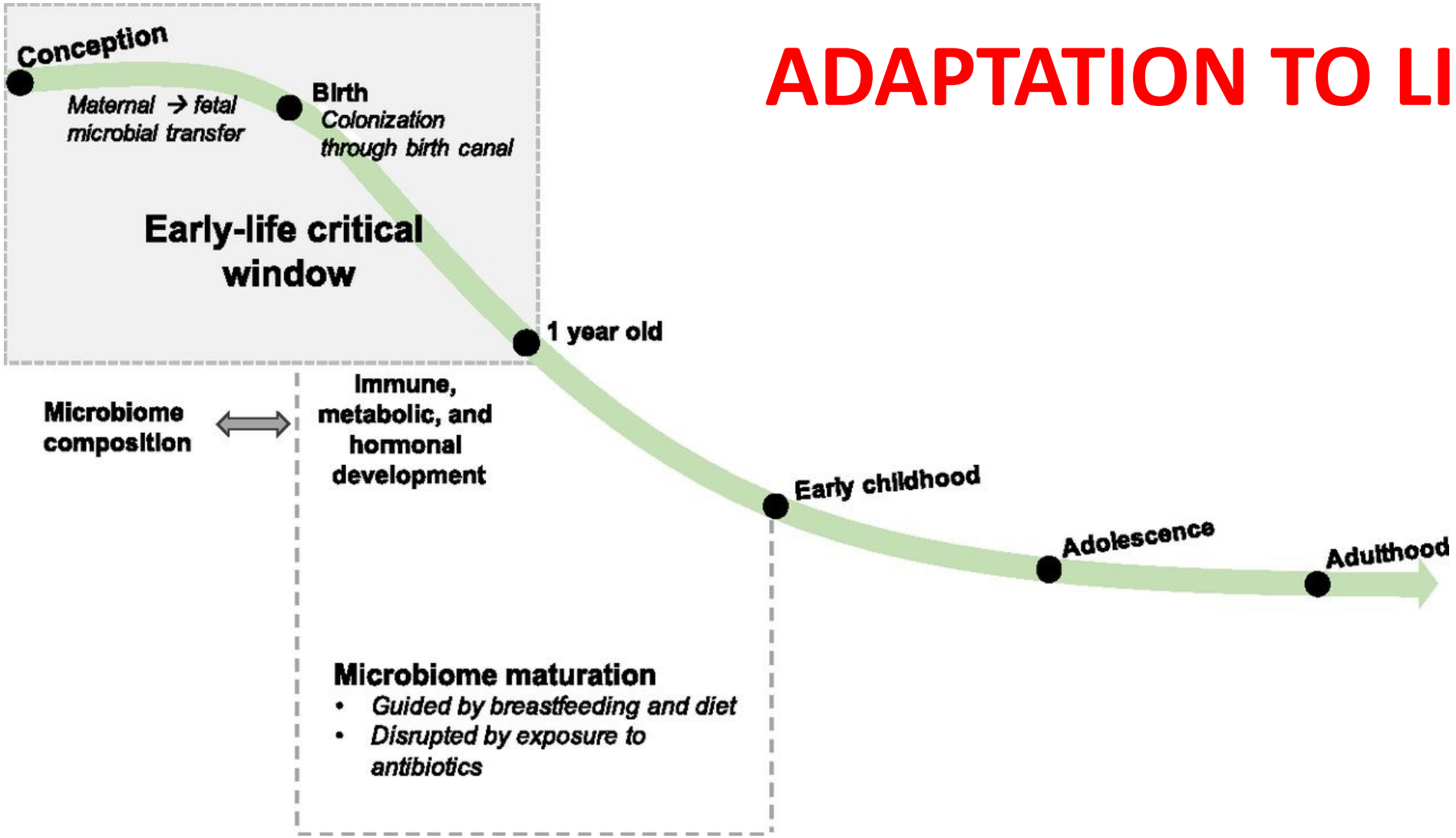


HOST-MICROBIAL INTERACTION

The microbial genome is the variable part of our genome that makes possible human adaptation to external perturbations *(ie diet, starvation, overfeeding, food preservatives, antibiotics, stress, violence..)*

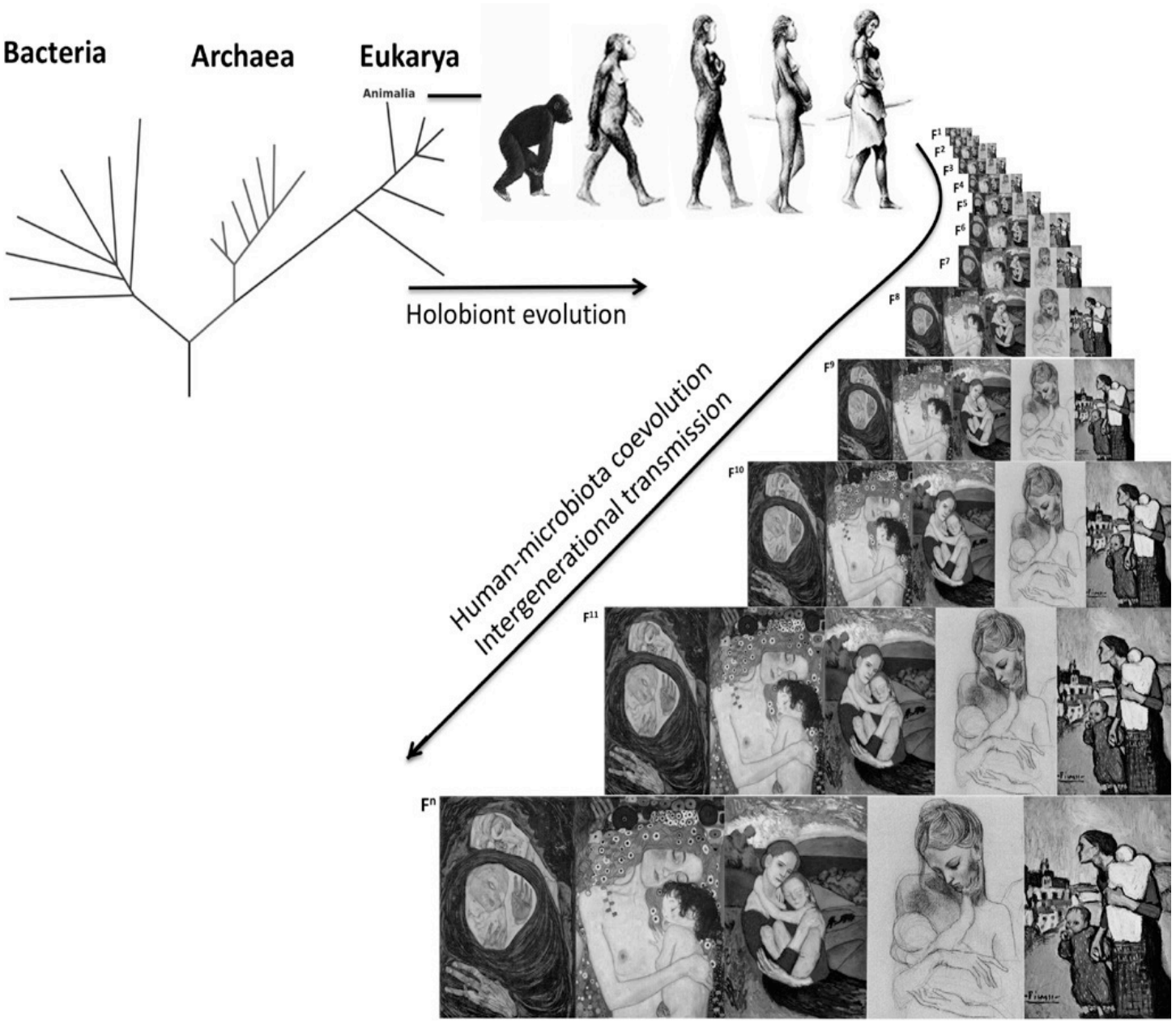
Past selective pressures during human evolution

ADAPTATION TO LIFE



*Any **stressor** in this phase = long term effects*

Milani C et al. Microbiol and Mol Biol Rev 2017



In stable condition The microbiota has been transferred throughout generations of humans with **MATRILINEAL VERTICAL LINE**

Vertical humans trasmission led to ***conservation of phylogenetic signal*** in human microbiota comunities

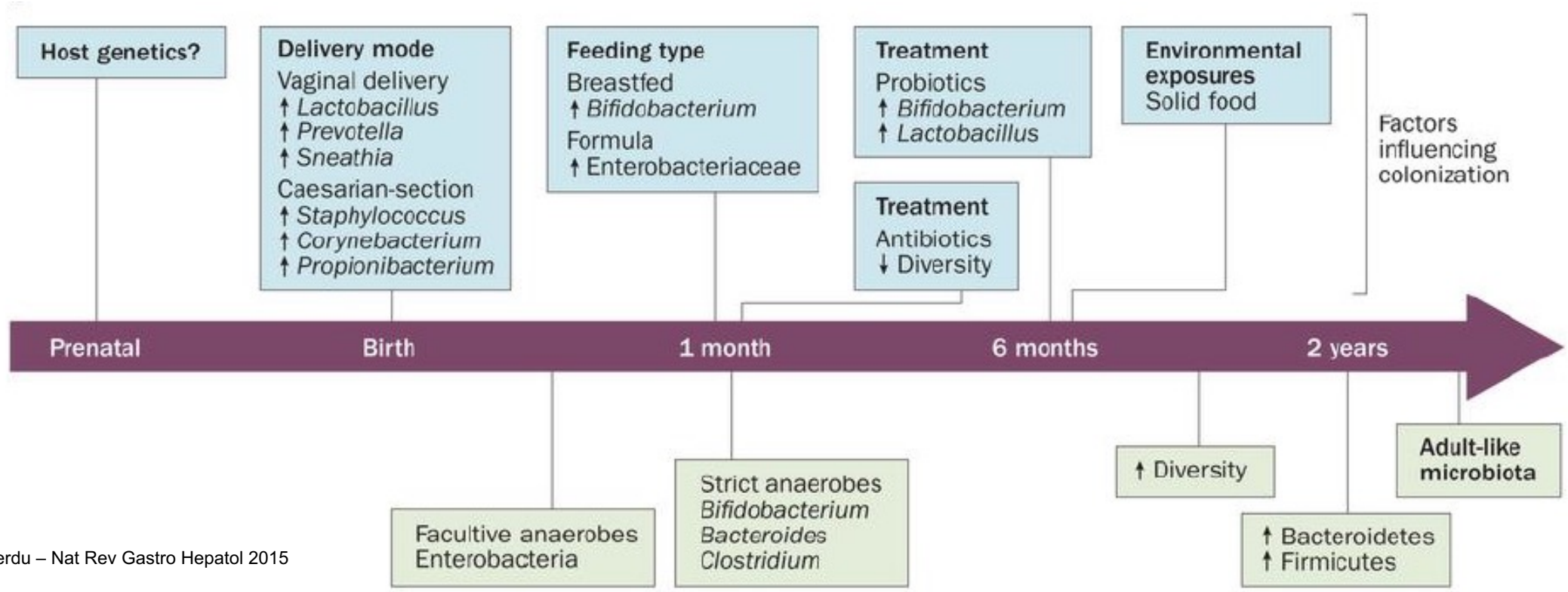
Dominguez-Bello MG,Gut, 2019

Native CORE microbiota

An early programming with life long-effects develops during weaning (first 24 months of life)

Koenig JE et al, PNAS 2010

Early determinants of microbiota composition

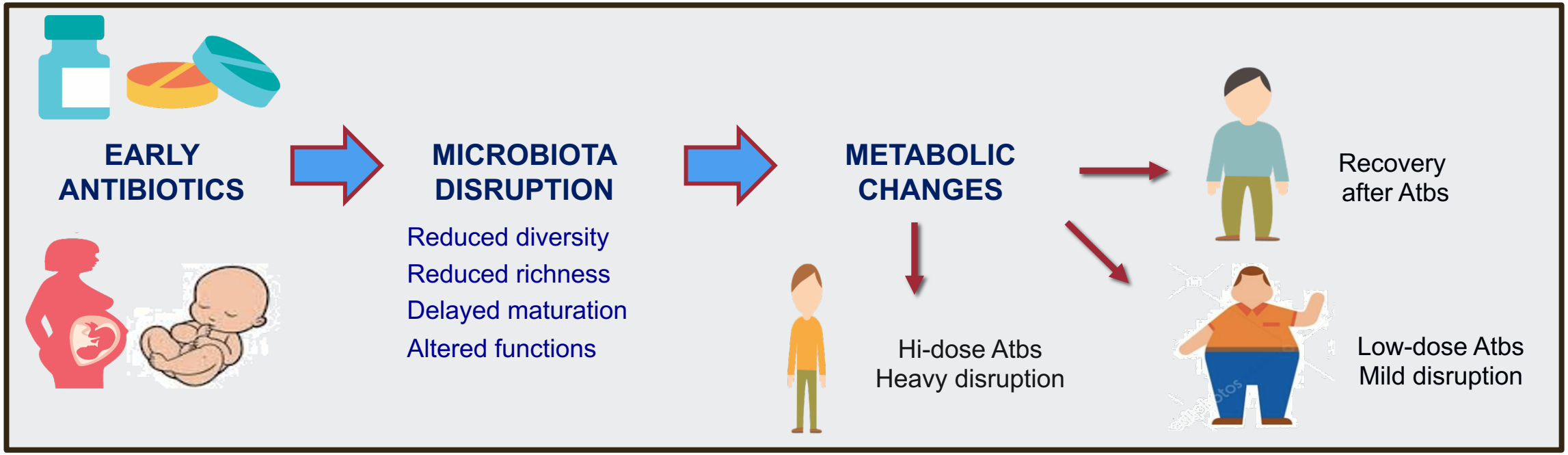


Verdu – Nat Rev Gastro Hepatol 2015

Early life is the key period for microbe-mediated programming of host metabolism

- There is a **critical window** (early life) where even transient alteration of healthy microbiota can drive to long-lasting effects

Cox et al – Cell 2014 Cox et al - Nat Rev Endocrin 2015



Impact of diet in shaping gut microbiota revealed by a comparative study in children from Europe and rural Africa

Carlotta De Filippo[a], Duccio Cavalieri[a], Monica Di Paola[b], Matteo Ramazzotti[c], Jean Baptiste Poullet[d], Sebastien Massart[d], Silvia Collini[b], Giuseppe Pieraccini[e], and Paolo Lionetti[b,1]

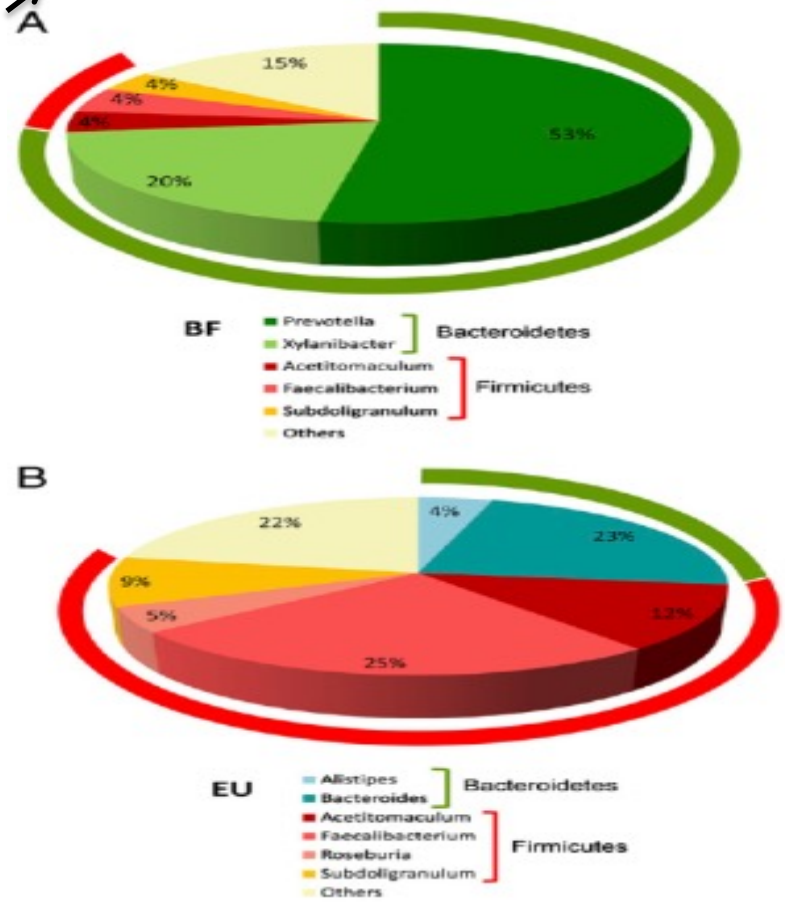
Resistant starch: millet and sorghum

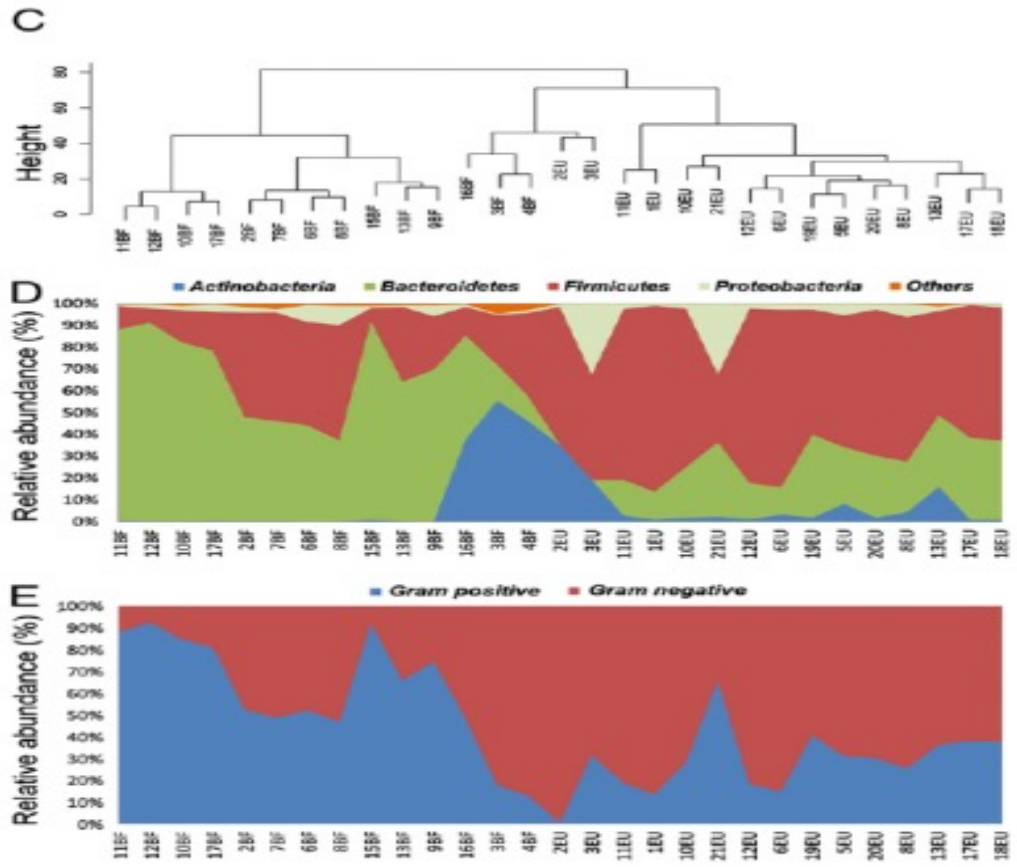
Burkina Faso
Bacteroidetes >Firmicutes

Prevotella

Europe
Firmicutes >Bacteroidetes

Bacteroides

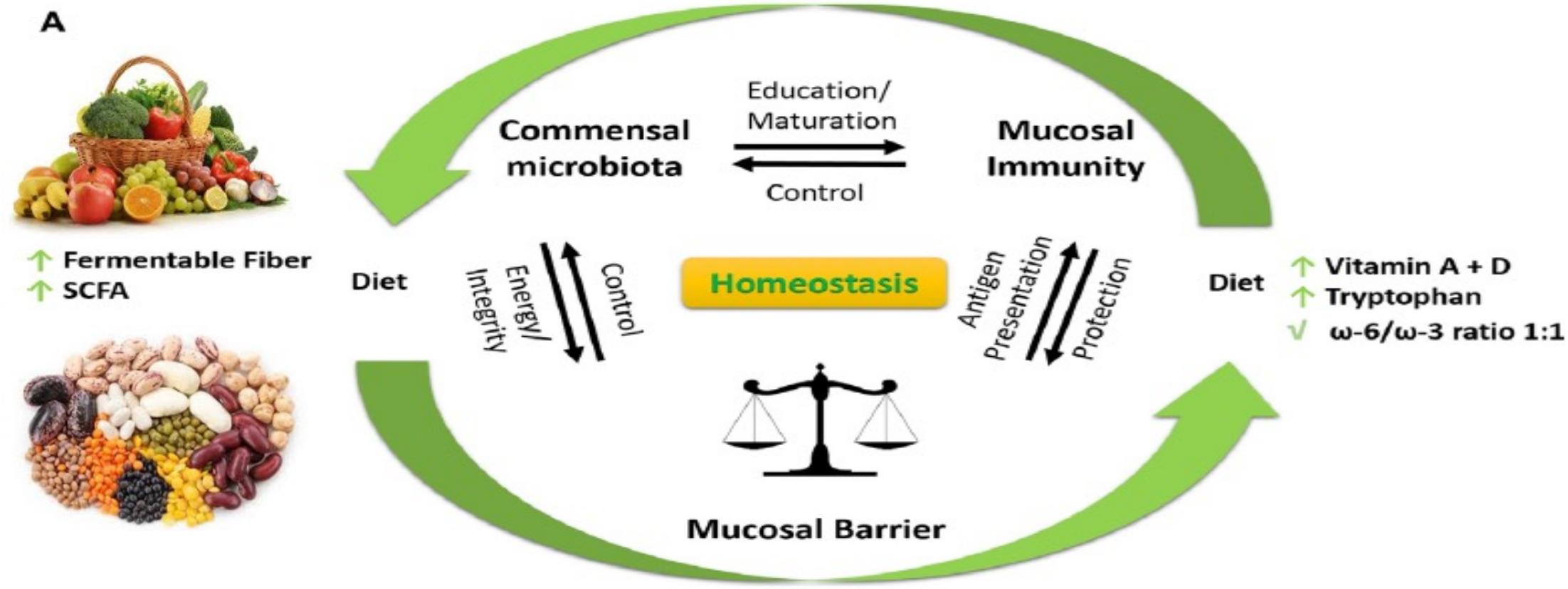




De Filippo et al, PNAS 2010

Microbiota influencers
High-fiber diet

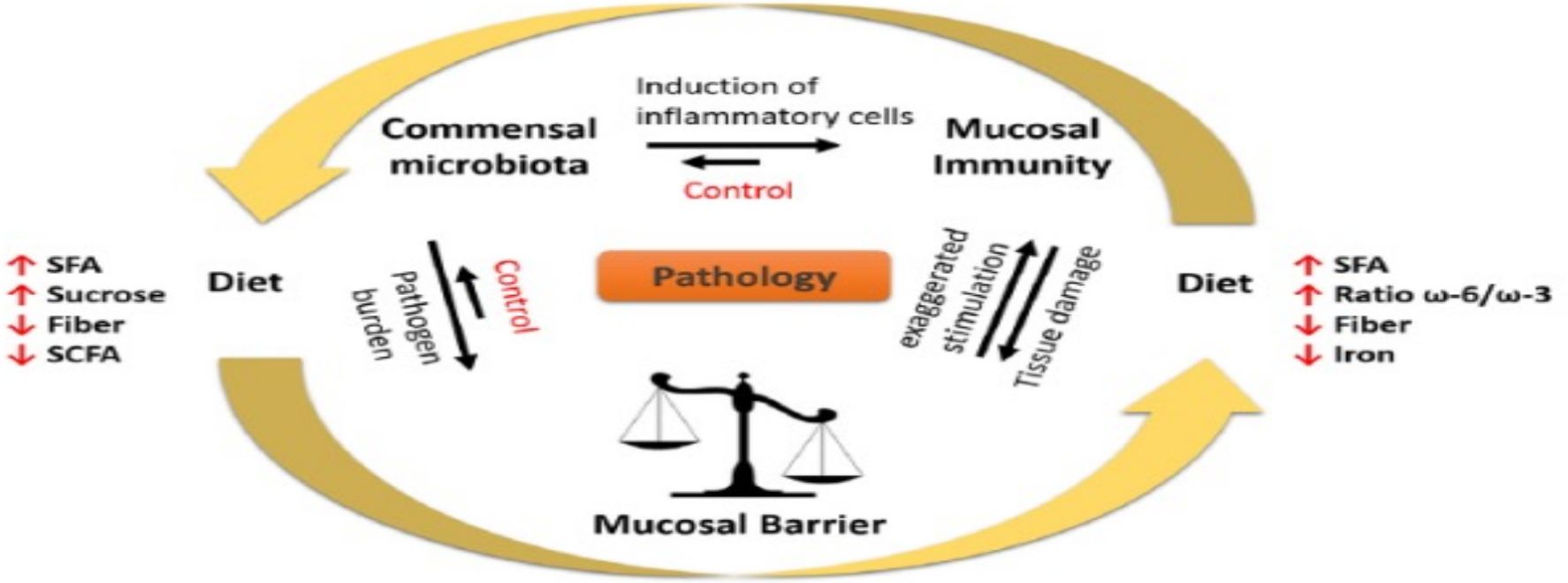
★ High-fiber diet is associated with reduced risk for IBD, metabolic disease and asthma



Statovci et al, Front Immunol. 2017

Microbiota 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

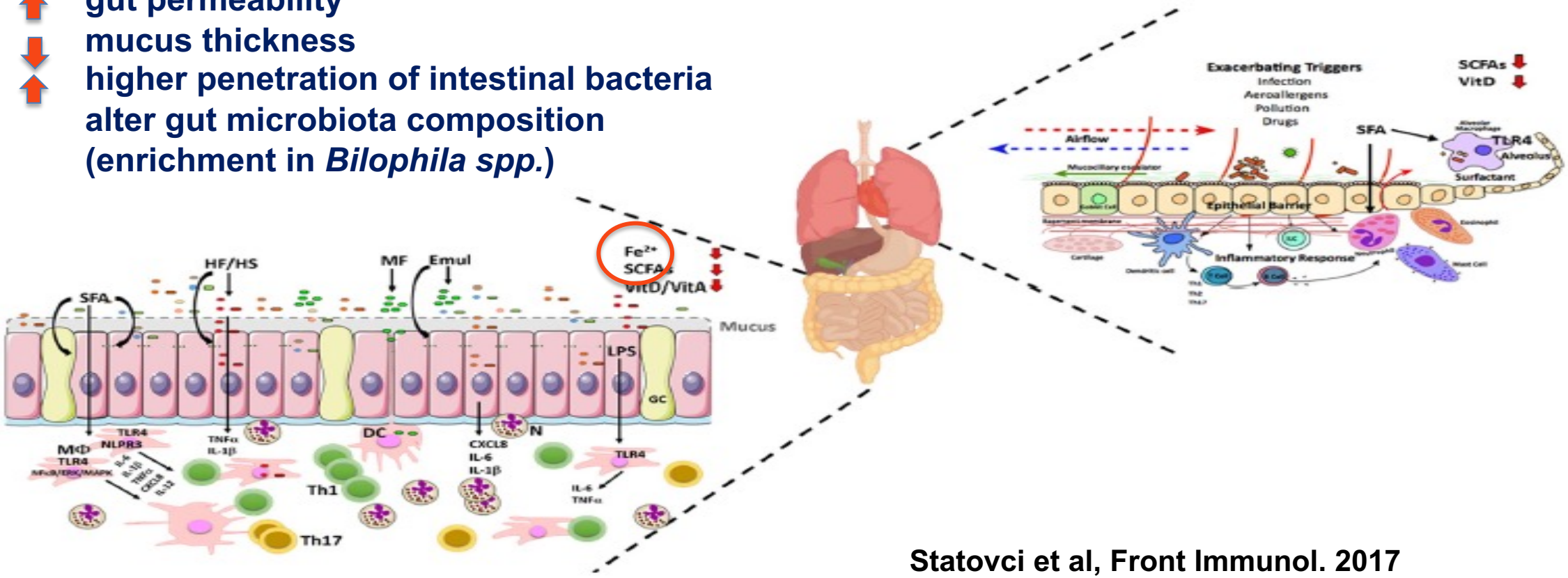
Statovci et al, Front Immunol. 2017

Microbiota influencers
Emulsifiers

Emulsifiers such as **polysorbate-80** and **carboxymethylcellulose are 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.*)



Statovci et al, Front Immunol. 2017

MICROBIOTA ASSOCIATED DISEASES

- *Gatrointestinal, lung, genito-urinary tract infections*
- *Irritable Bowel Syndrome*
- *Inflammatory Bowel Disorders*
- *Diverticulosis*
- *Celiac disease and Malabsorption*
- *Food Intolerance/Allergy*
- *Gastrointestinal Cancers*
- *Liver diseases*
- *Pancreatic diseases*
- *Obesity, Diabetes and Metabolic Syndrome*
- *Nephrological, Gynecological, Urological, Oncological, Rheumatological/autoimmune, Cardiovascular, Neurological (Parkinson, Alzheimer, MS..), Psichiatric disorders (schizofrenia, anxiety/depression, autism..)*

ENTEROPATHOGENETIC SYNDROMES

THE IMPACT OF MICROBIOTA ON DIGESTIVE AND EXTRADIGESTIVE DISORDERS

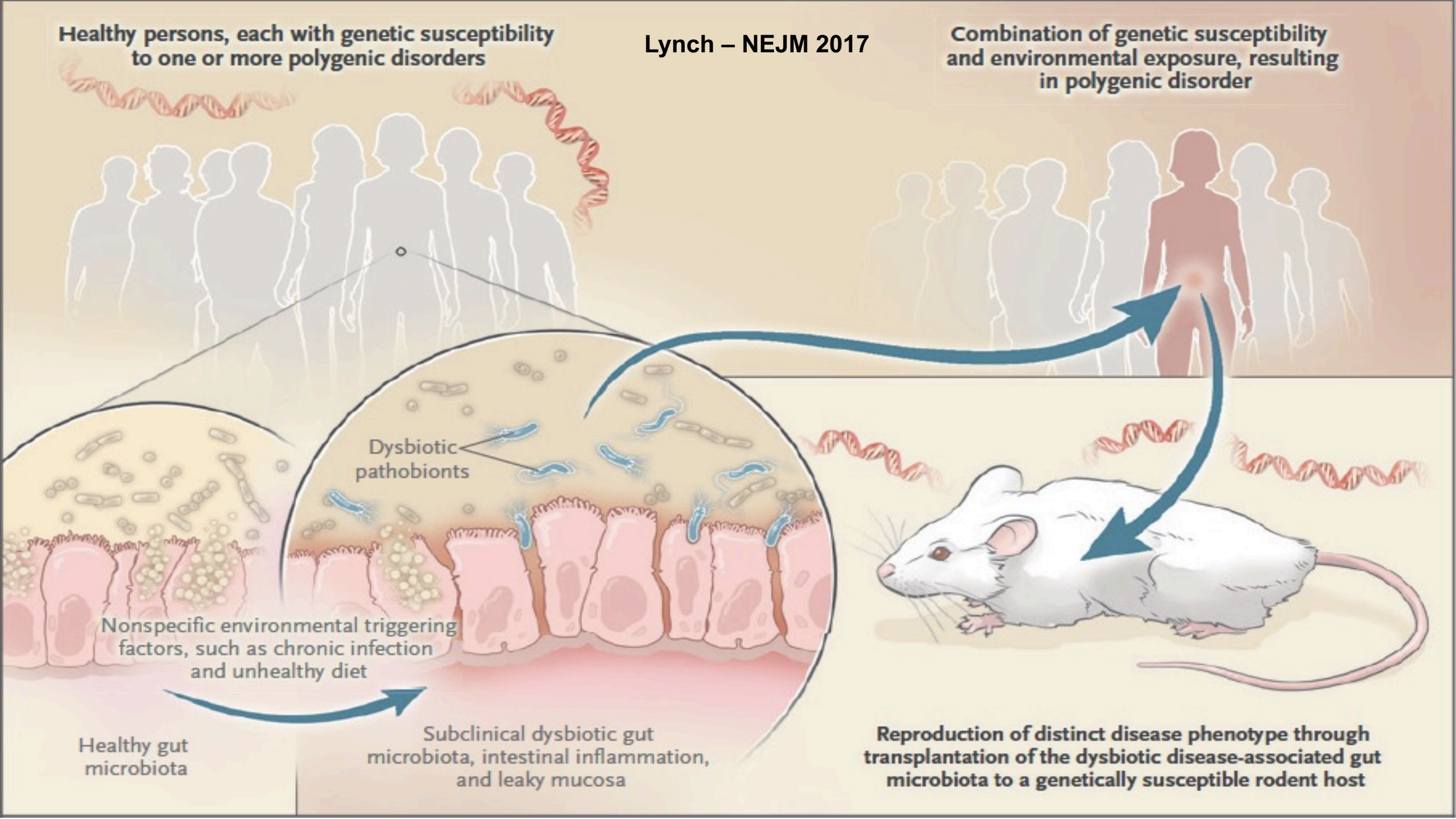
DEVELOPMENT

PROGRESSION

RESPONSE TO THERAPY

THE IMPACT OF MICROBIOTA ON DIGESTIVE AND EXTRADIGESTIVE DISORDERS

DEVELOPMENT


Healthy persons, each with genetic susceptibility to one or more polygenic disorders
Lynch – NEJM 2017
Combination of genetic susceptibility and environmental exposure, resulting in polygenic disorder
Dysbiotic pathobionts
Nonspecific environmental triggering factors, such as chronic infection and unhealthy diet
Healthy gut microbiota
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

Microbiota transmits Colitic phenotype

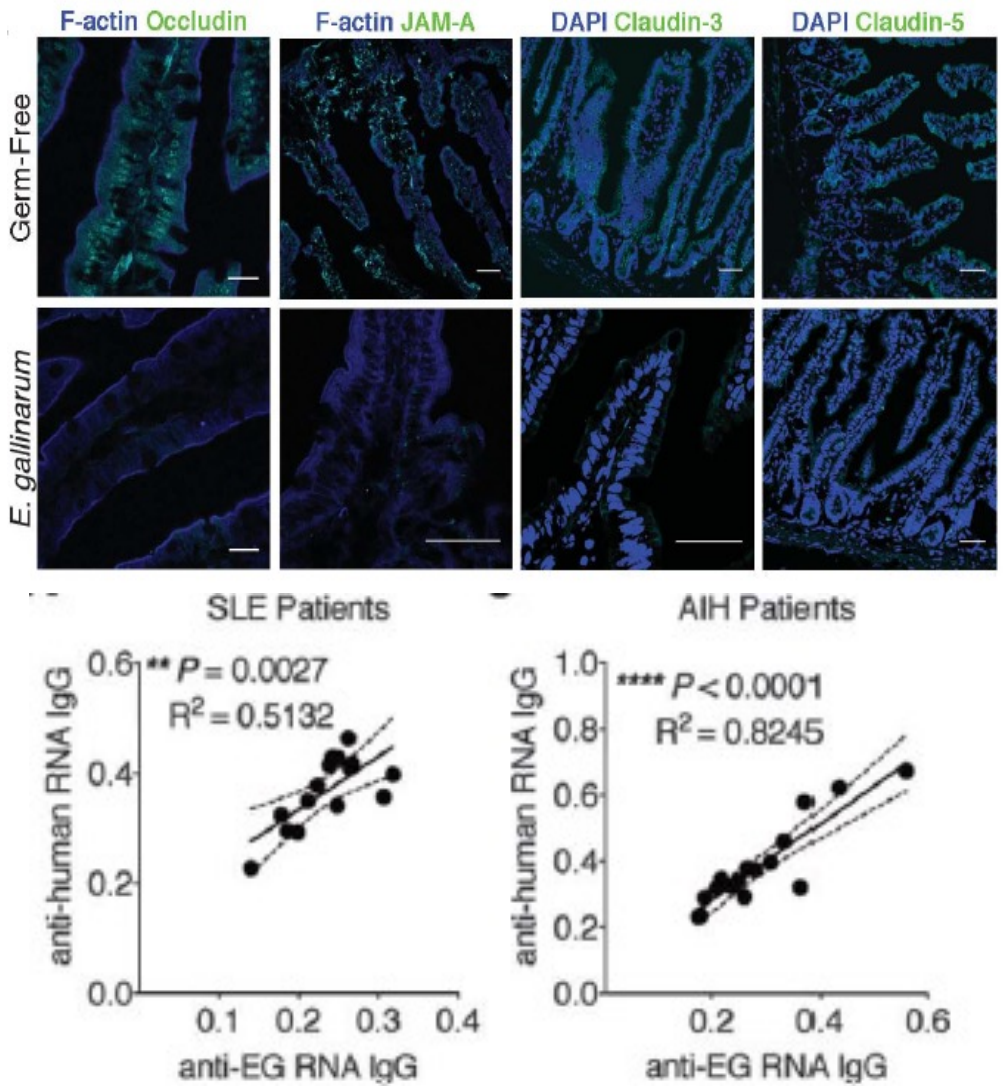
Garrett, Cell 2007;131(1):33-45

TRUC mice, deficient for Tbet and Rag

Colitic phenotype could be transmitted vertically to progeny of affected parents and horizontally to unrelated animals

Microbiota transmits an Autoimmune Hepatitis phenotype

Vieira et al – Science 2018

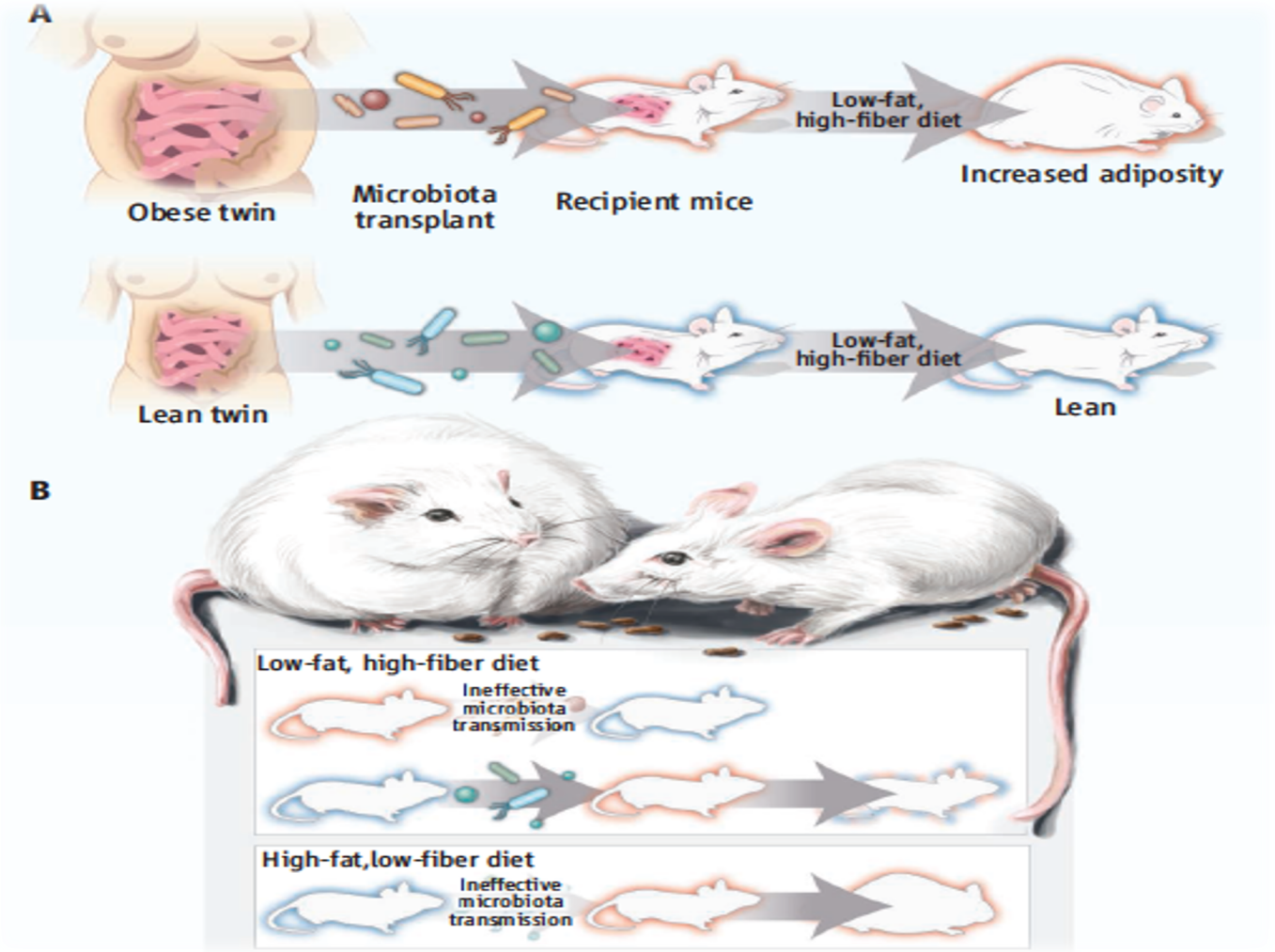


- **Translocation of a gut pathobiont**, E. gallinarum, to the liver and other tissues **triggers autoimmune responses** in a **mouse model** of genetic background predisposing to autoimmunity.
- **Antibiotics** suppressed growth of E. gallinarum in tissues, and **eliminated pathogenic autoantibodies and Tcells**
- Cocultures with human hepatocytes **from patients with autoimmune hepatitis and SLE** replicated the murine findings

Microbiota transmits an Obesity phenotype

Ridaura et al. Science 2013, 341 (6150)

Fecal microbiota from 4 human female twin pairs discordant for obesity



TRANSFERRED INTO THE INTESTINES OF GERM-FREE MICE

(Ob) twin + mice = ↑adiposity

(Ln) twin + mice = ↓ adiposity

TRANSMISSIBILITY OF INTESTINAL MICROBES AND ADIPOSITY PHENOTYPE ARE TIGHTLY LINKED

COHOUSING

(Ob) twin transplanted mice +

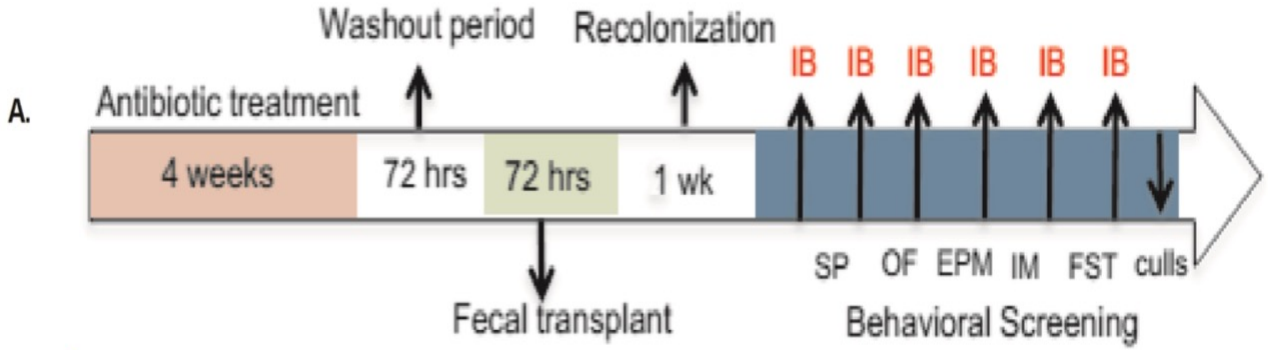
(Ln) twin transplanted mice =

(Ob) mice became LEAN

(Ln) mice remain LEAN

Microbiota transmits a Depressive phenotype

Kelly, J Psychiatric Research 2016, Kelly Nature 2019



Microbiota depleted antibiotic rat model

FMT from depressed patients to rats with a depleted gut microbiota (after antibiotics treatment)

DEPRESSIVE PHENOTYPE

Anhedonia

Anxiety-like behaviuors

- Increased kynurenine/tryptophane ratio

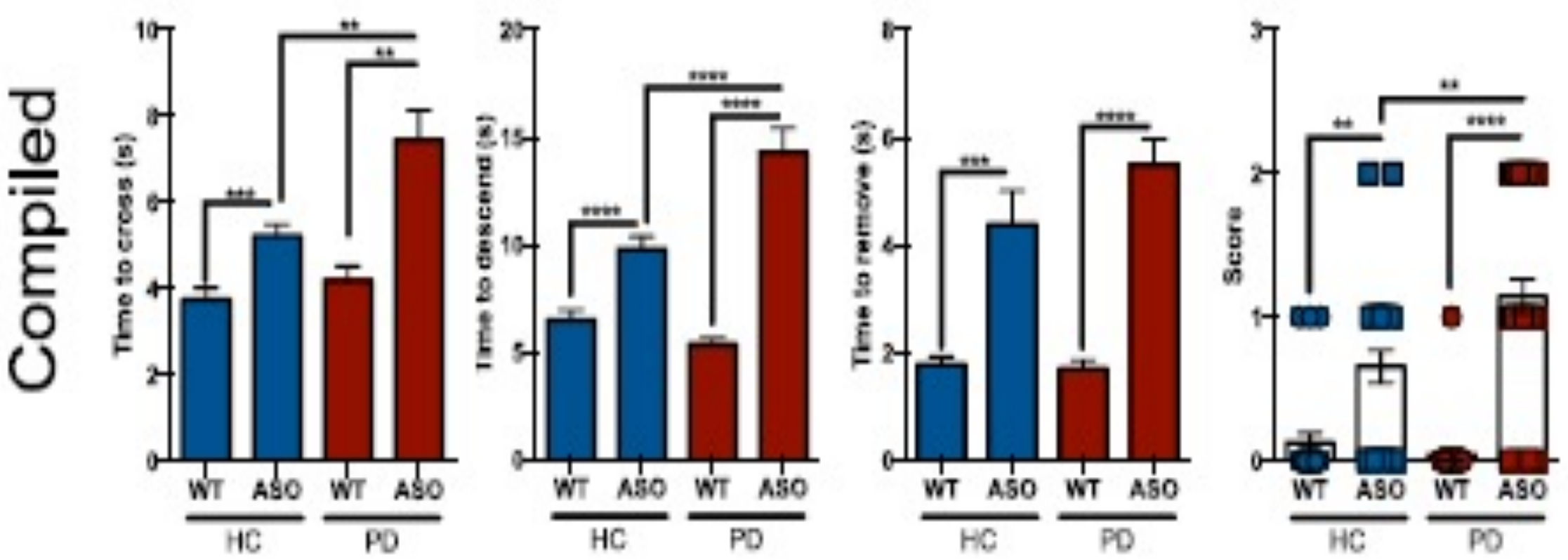
- Proinflammatory profile

IMMUNE MEDIATED MECHANISM for the development of the depressive phenotype in rats

Microbiota transmits a Parkinson phenotype

Sampson et al, Cell 2016

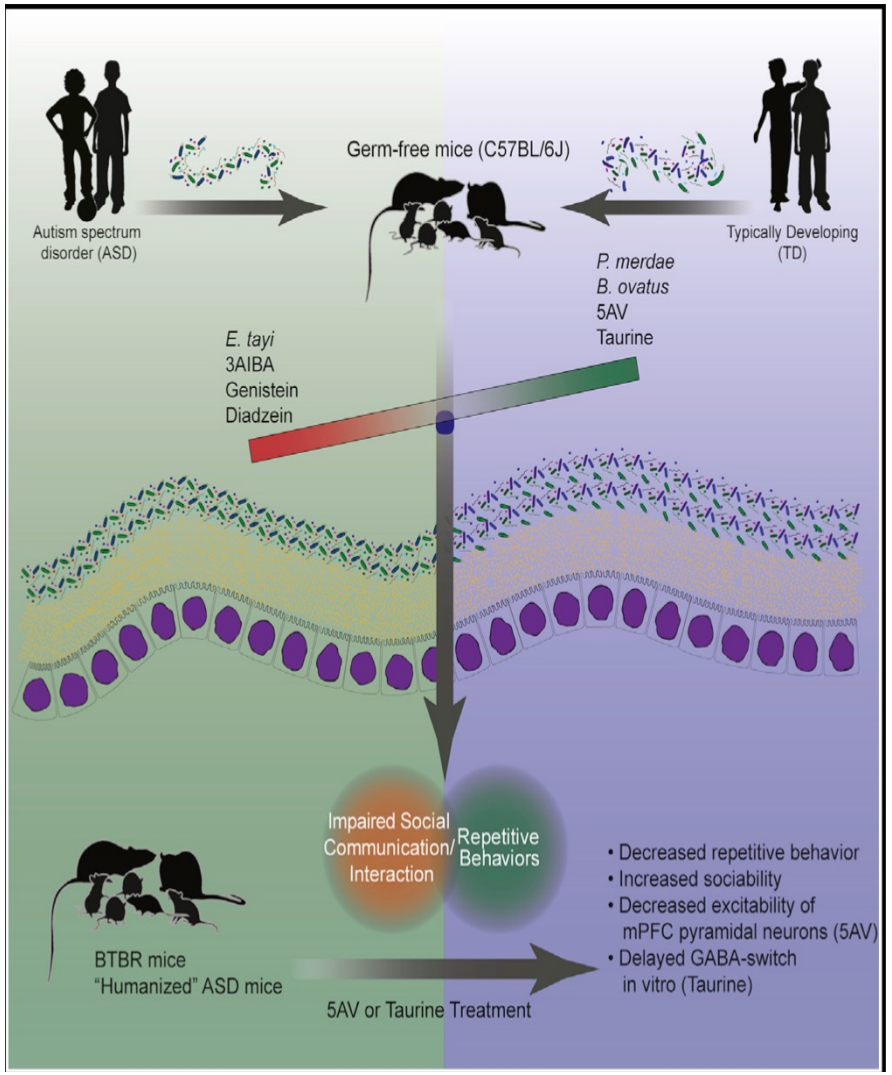
Microbiota from Parkinson patients induces increased aSyn- Mediated Motor Deficits in mice

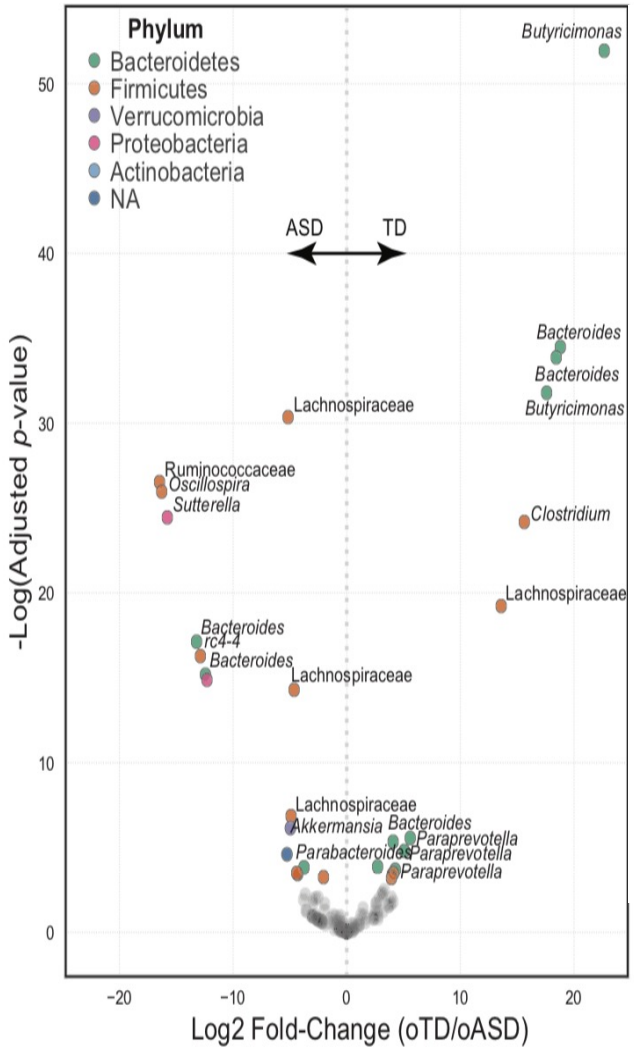


Sampson et al. Cell 2016

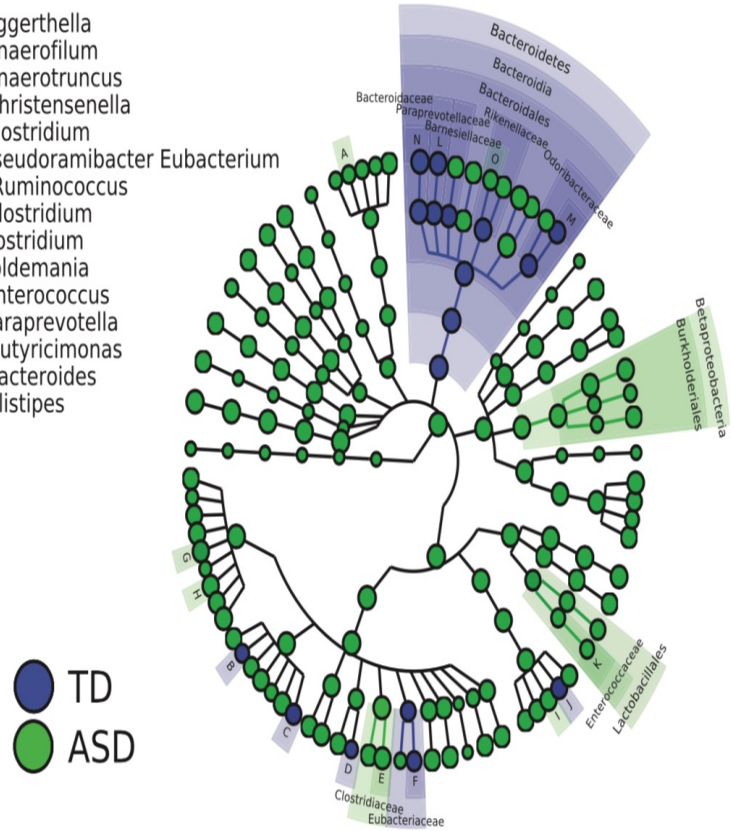
Microbiota transmits an Autistic Spectrum Disorders phenotype

Sharon, 2019, Cell





A:Eggerthella
B:Anaerofilum
C:Anaerotruncus
D:Christensenella
E:Clostridium
F:Pseudoramibacter Eubacterium
G: Ruminococcus
H:Clostridium
I:Clostridium
J:Holdemania
K:Enterococcus
L:Paraprevotella
M:Butyricimonas
N:Bacteroides
O:Alistipes

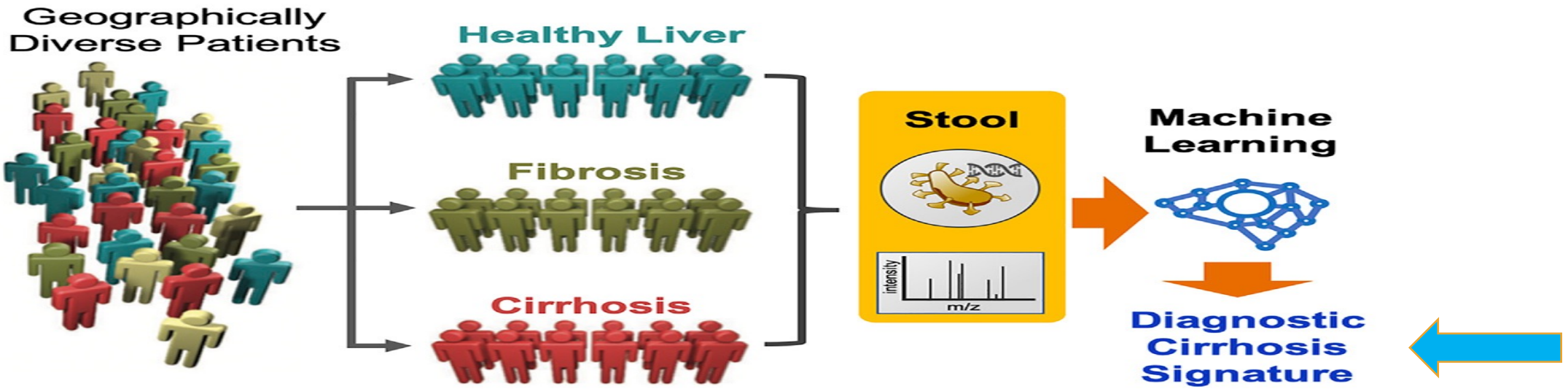


THE IMPACT OF MICROBIOTA ON DIGESTIVE AND EXTRADIGESTIVE DISORDERS

PROGRESSION

MICROBIOME SIGNATURE can predict progression from NAFLD to liver cirrhosis

Diagnostic signatures for fibrosis from stool metagenomic and metabolomic profiling that, when combined with serum AST levels, distinguishes cirrhosis in mixed fibrosis cohort.

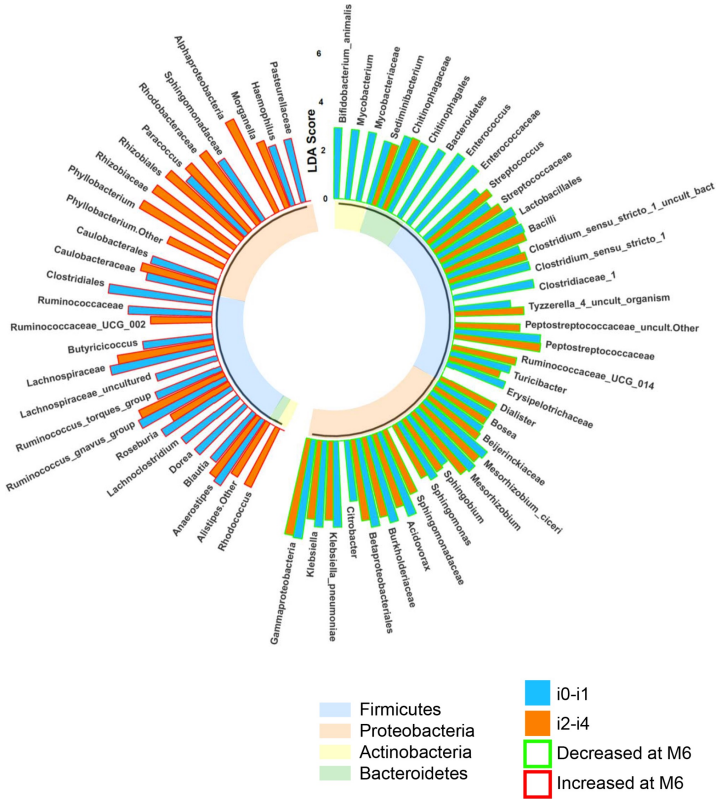


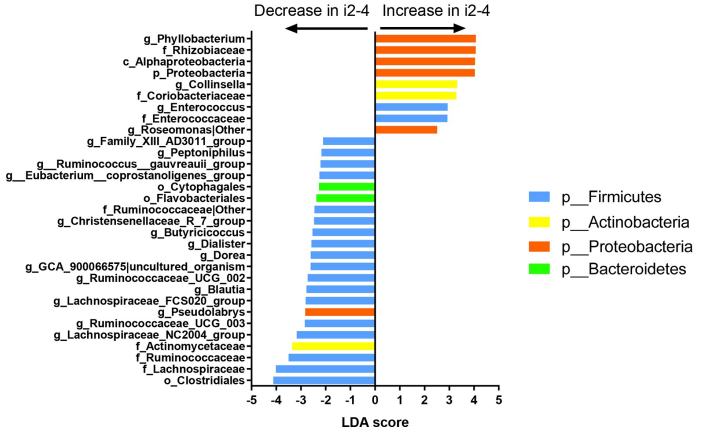
This combination signature was validated in racially and geographically independent cohorts

Oh et al – Cell Metab 2020

MICROBIOME SIGNATURE can predict post-surgical Crohn's recurrence

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

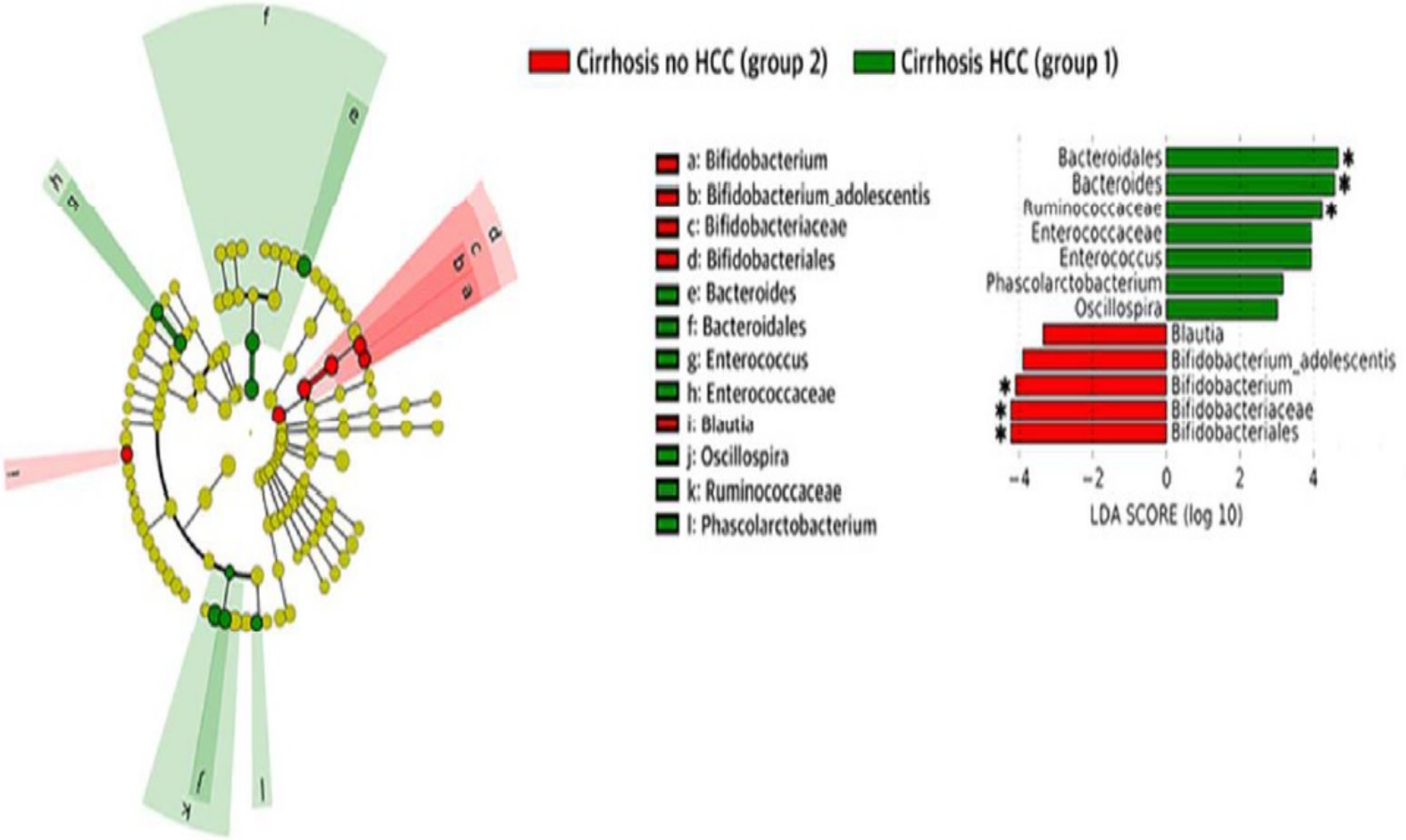


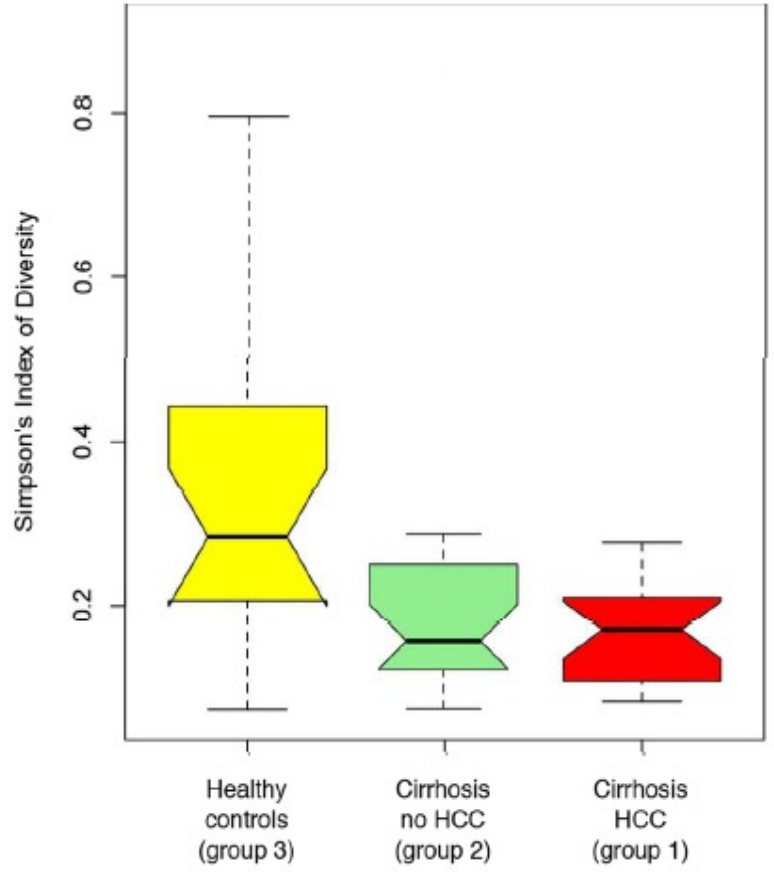


Gut microbiota at the time of surgery can predict endoscopic recurrence

Sokol et al – Gut 2020

MICROBIOME SIGNATURE can predict progression from liver cirrhosis to HCC

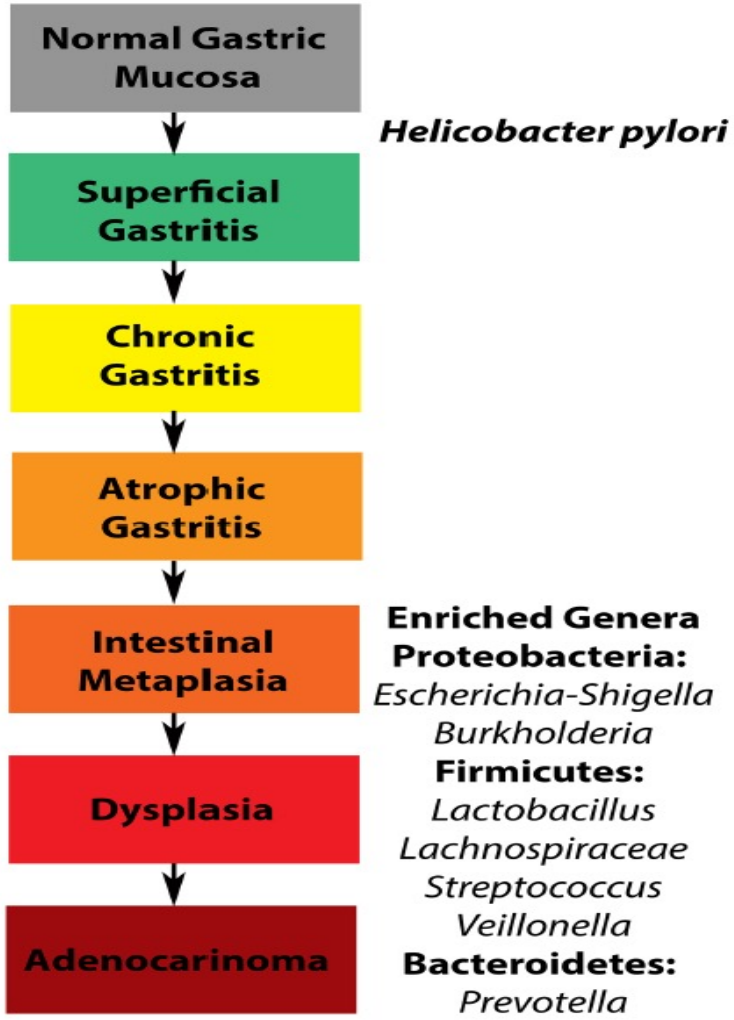


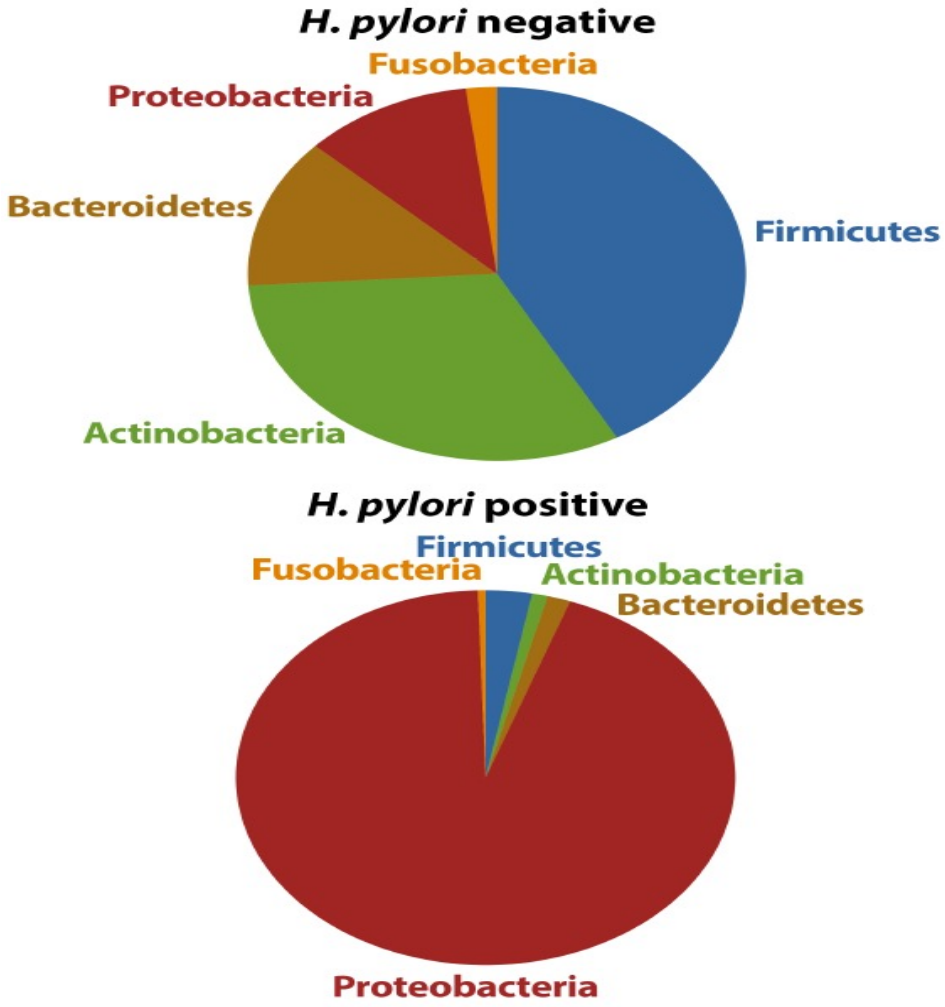


Ponziani, Hepatology 2018

MICROBIOME SIGNATURE can predict progression from H. pylori gastritis to Gastric Cancer

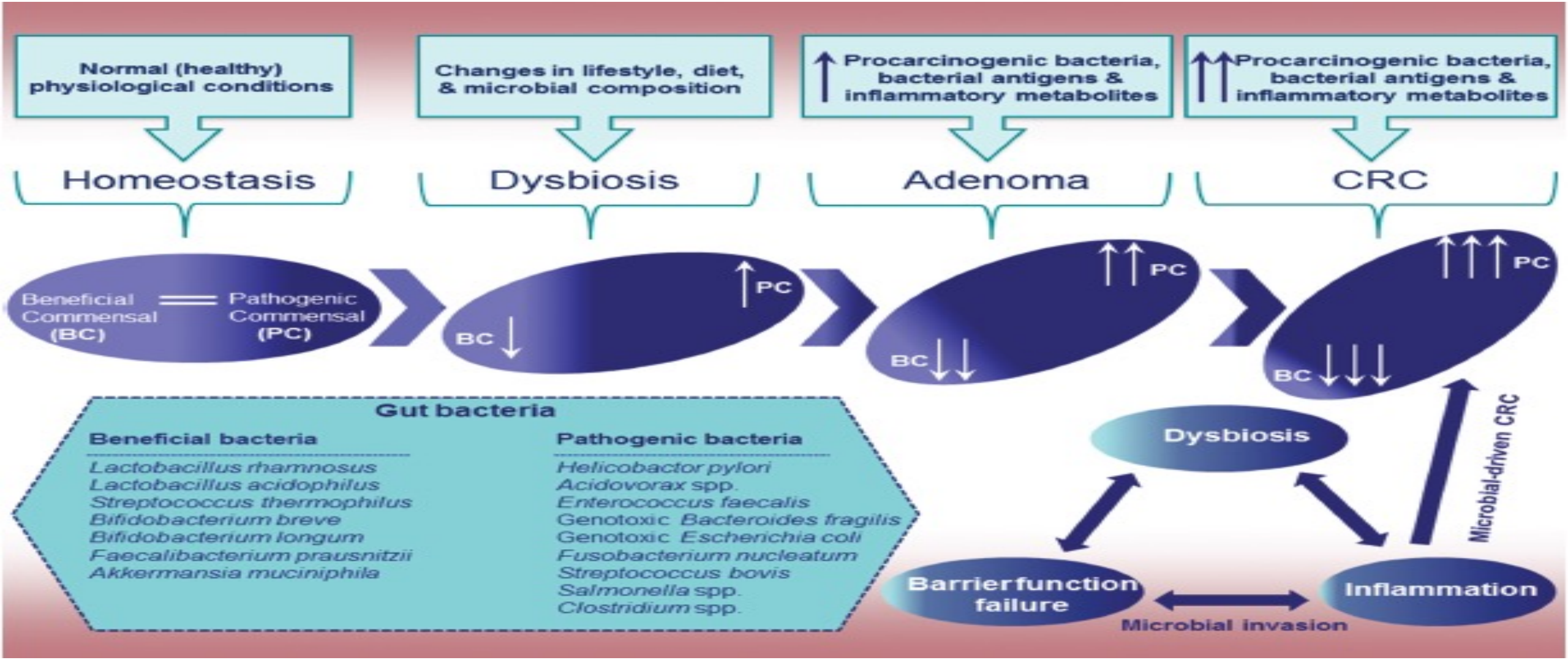
Alterations in the gastric microbiota following *Helicobacter pylori* infection





Noto, PLoS Pathog2017

MICROBIOME SIGNATURE can predict progression from adenoma to COLORECTAL CANCER



Dulal - Cancer J 2014

THE IMPACT OF MICROBIOTA ON DIGESTIVE AND EXTRADIGESTIVE DISORDERS

RESPONSE TO THERAPY

MICROBIOME SIGNATURE predicts response to low-FODMAP diet

67 patients with IBS randomised to traditional IBS or low FODMAPs diets for 4 weeks.

- Responders to low FODMAP diet were discriminated from non-responders based on their **microbiota profiles**

- **Bacterial abundance** tended to be **higher in nonresponders**

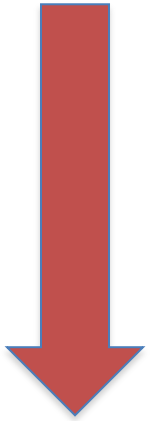
NONRESPONDERS VS RESPONDERS

Bacteroides stercoris

Pseudomonas

Acinetobacter

Desulfitispora



Streptococcus

Dorea

Ruminococcus gnavus

Bennet, Gut 2018

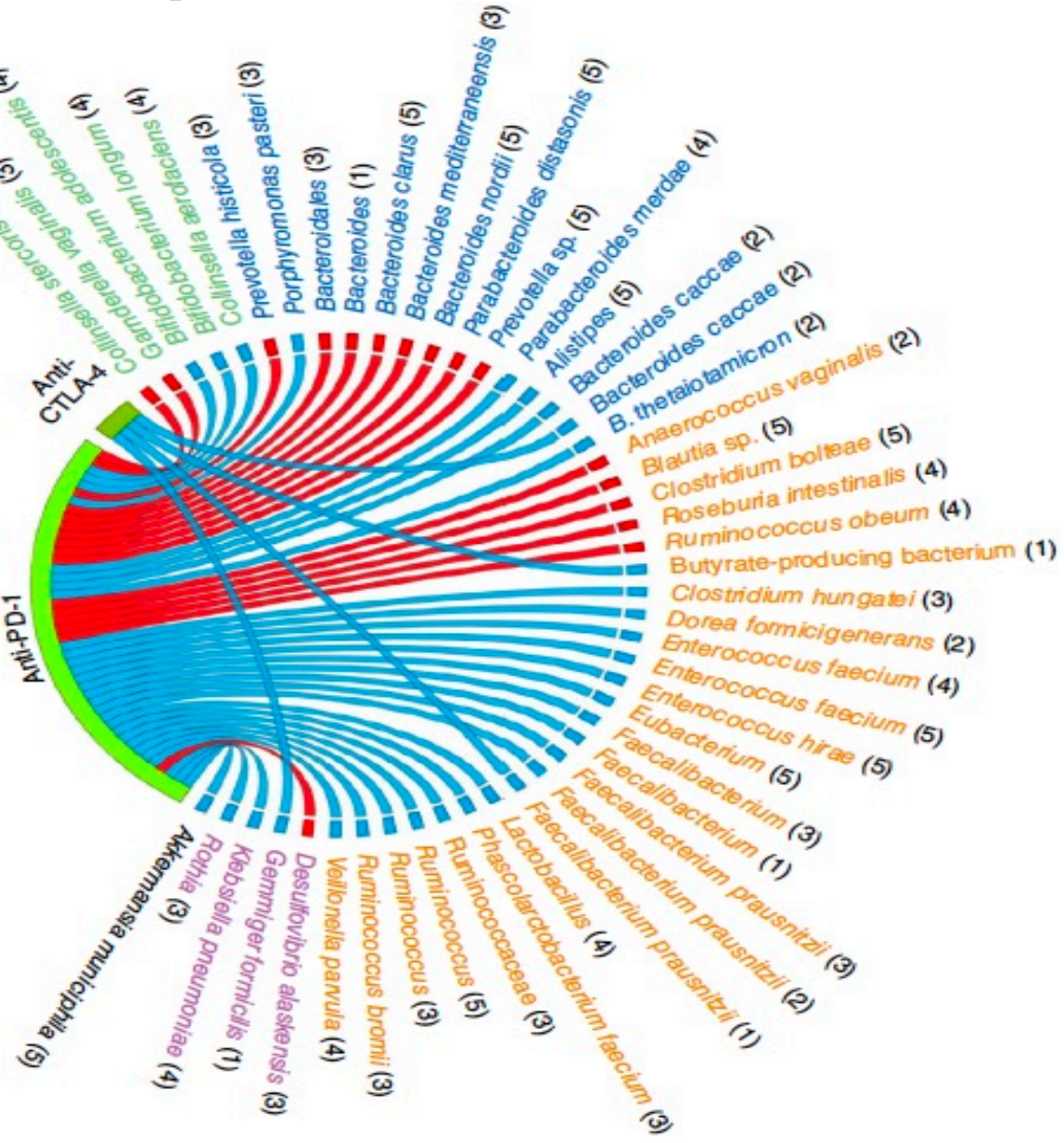
MICROBIOME SIGNATURES are associated with clinical response to IMMUNOTHERAPY in Epithelial Cancers

Bacterial phyla involved:

- Actinobacteria
- Bacteroidetes
- Firmicutes
- Proteobacteria
- Verrucomicrobia

Association with response:

- Enriched in responders
- Enriched in nonresponders



Ma et al, Frontiers Micro 2019;
Routy et al, Science 2018;
Gopalakrishnan et al, Science 2017

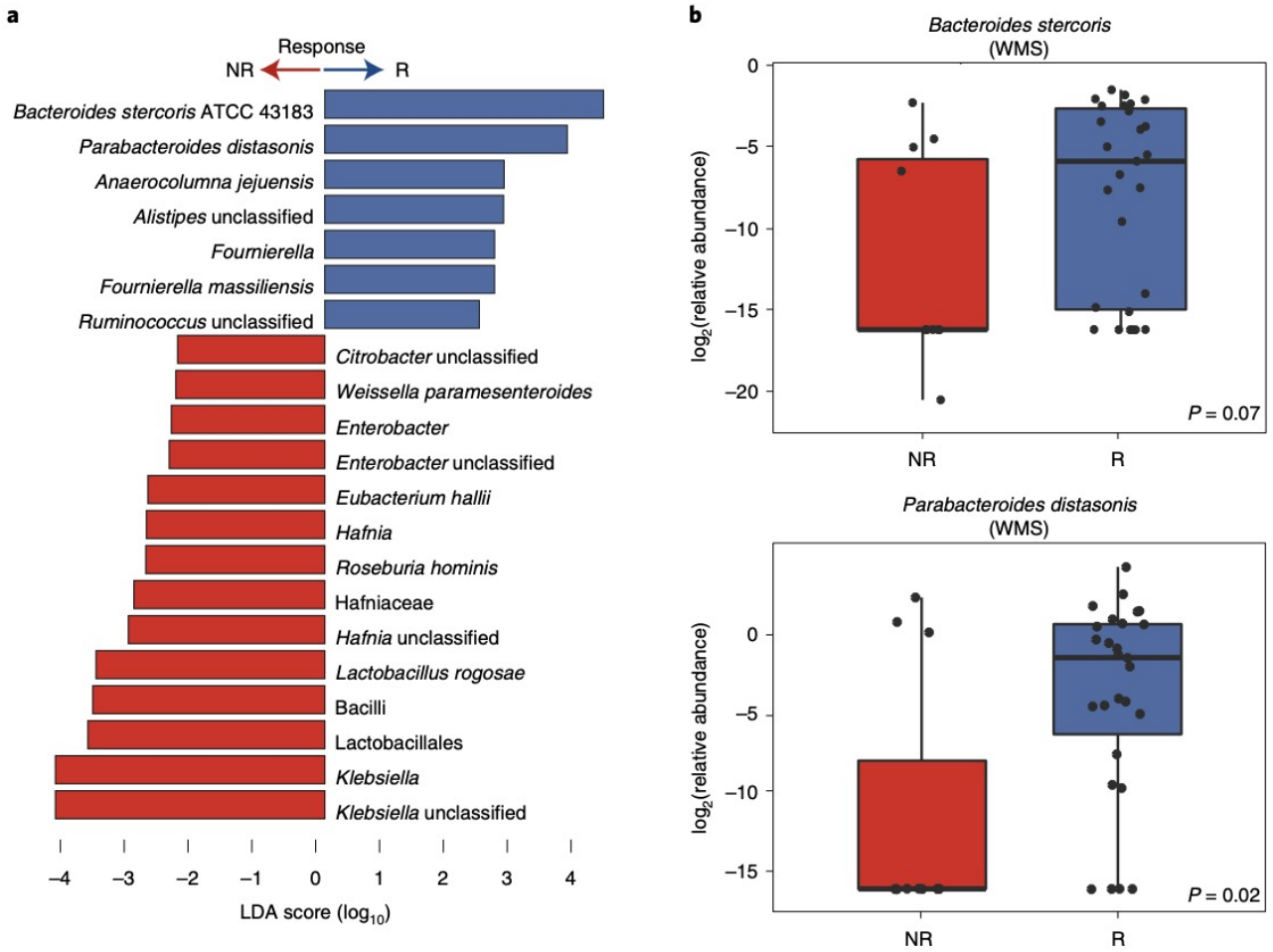
MICROBIOME SIGNATURE are associated with clinical response and toxicity to combined CTLA-4 and PD-1 blockade

- Higher abundance of **Bacteroides intestinalis** in patients with toxicity
- Upregulation of mucosal IL-1β in patient samples of colitis and in pre-clinical models

- Taxa enriched in non-responders included **Klebsiella aerogenes** and **Lactobacillus rogosae**

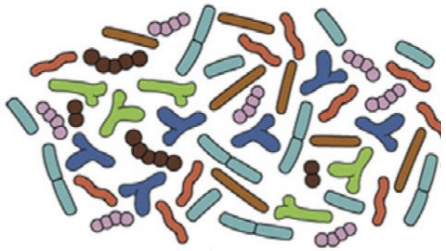
- Taxa enriched in responders included B. stercoris (P=0.07) and **P. distasonis** (P=0.024)

Andrews et al – Nat Med 2021



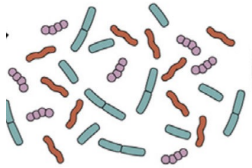
Strong rationale for a Microbiota modulation

Healthy microbiota



Diet & Lifestyle
Drugs
Systemic disorders
Stressful events

Dysbiosis (Loss of symbiosis)



Quantity

Quality

Diseases

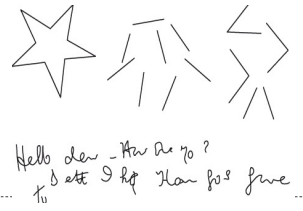
GI infections

Metabolic disorders

IBD

IBS

Hepatic Encefalopathy

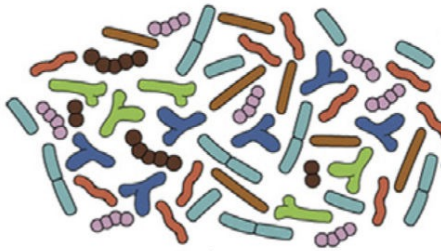


Healthy individual

Diet
Prebiotics
Probiotics
Antibiotics
FMT

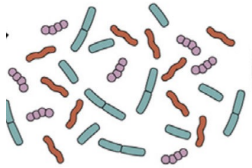
Strong rationale for a Microbiota modulation

Healthy microbiota



Diet & Lifestyle
Drugs
Systemic disorders
Stressful events

Dysbiosis (Loss of symbiosis)



Quantity

Quality

Diseases

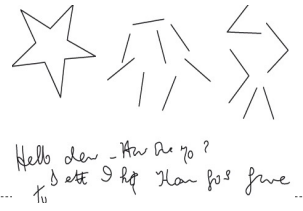
GI infections

Metabolic disorders

IBD

IBS

Hepatic Encefalopathy



Healthy individual

MICROBIOTA TRANSPLANTATION

FMT: A SUCCESS STORY

FMT has rapidly become an established treatment option to manage rCDI

First reported application of FMT for pseudomembranous colitis

Eiseman B, et al., Surgery 1958

- **FMT more effective than vancomycin** for rCDI
- **FMT more effective than fidaxomicin** for rCDI
- **FMT increases survival** in patients with rCDI
- **A synthetic microbiota consortium** is effective against rCDI

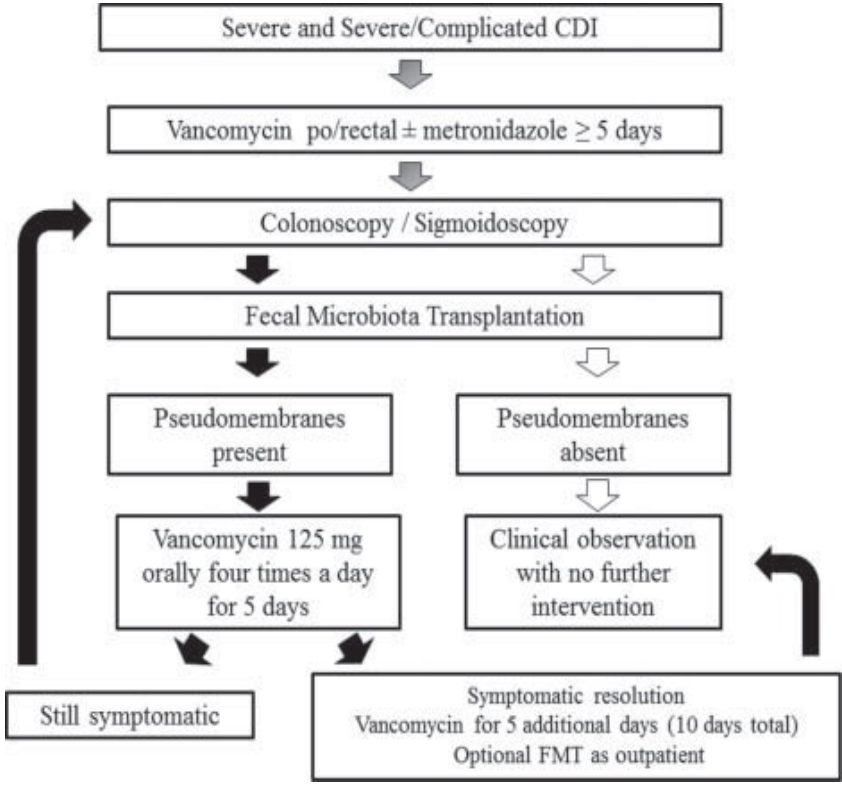
van Nood E, et al. N Engl J Med 2013;
Cammarota et al. Aliment Pharmacol Ther 2015;
Hvas et al. Gastroenterology 2018;
Ianiro et al. Ann Intern Med 2019;
Orenstein et al. Clin Infect Dis 2016;

- **FMT** in AGA/IDSA/ESCMID **guidelines of CDI**
- First **Consensus Conference on FMT**
- First **Consensus Conference on stool banking**

Surawicz C, et al. Am J Gastroenterol 2013;
Debast et al – Clin Microbiol Infect 2014;
Cammarota G, et al. Gut 2017;
McDonald et al. Clin Infect Dis 2018;
Cammarota et al. Gut 2019;

FMT is effective in treating severe CDI

- FMT was shown to be effective in treating **severe CDI** and **pseudomembranous colitis**
- Repeat FMT appears to be the **keystone** of a successful FMT protocol to treat **severe CDI** and **pseudomembranous colitis**



+ 30%

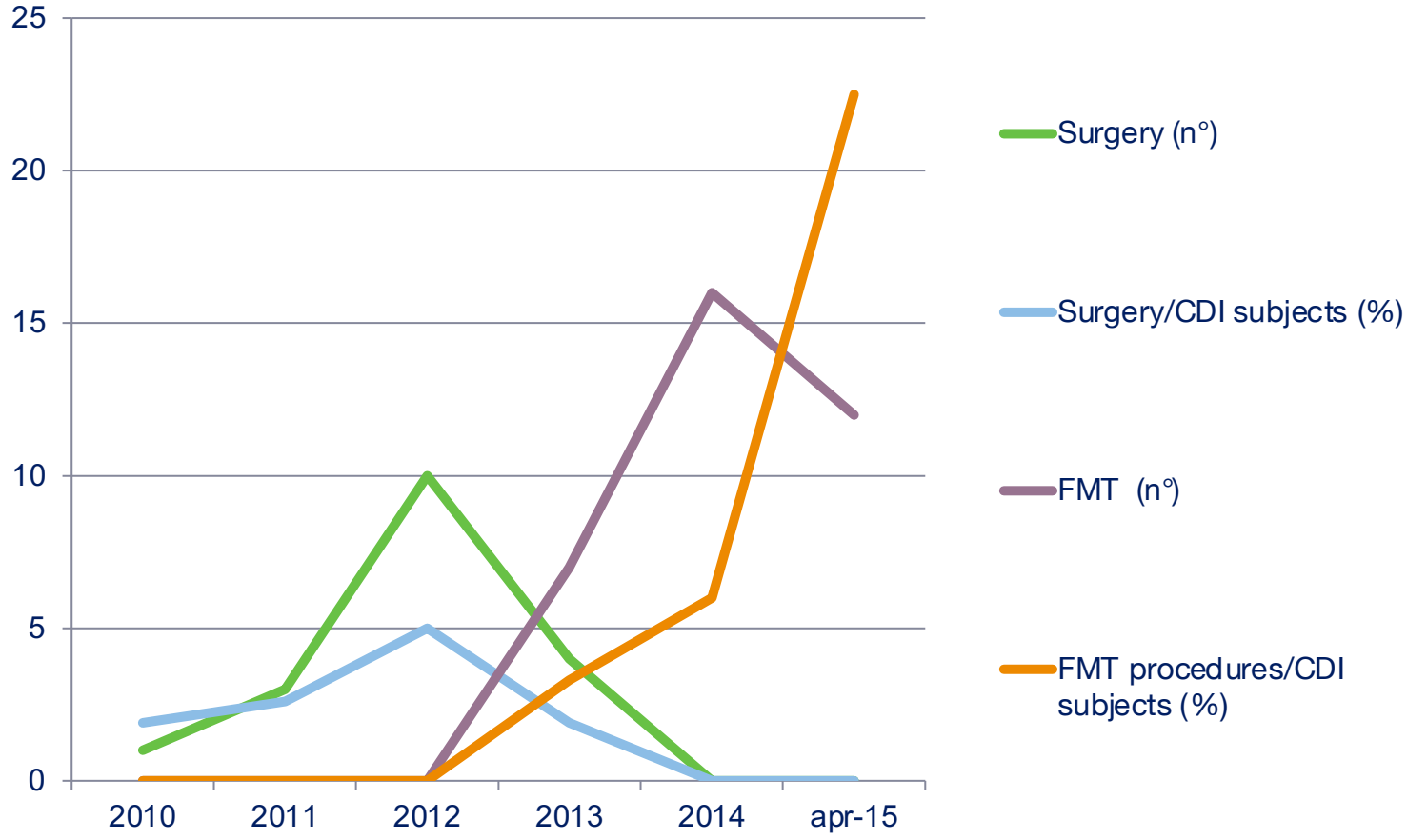
success rate

FMT-S	V	V + BP	V + BP	FI	V × 14 d			
FMT-M	V	V + BP	V + BP	FI	V	V	V+FI	V in all subjects × further 11 d Additional FI every 3 d in subjects with PMC

Cammarota, Gasbarrini et al – AP&T 2015; Lee, Allen et al – JAMA 2016; Weingarden, Cani' et al – J Cin Gastroenterol 2013;; Fischer, Loyer, et al – AP&T 2015; Ianiro, Gasbarrini et al – AP&T 2018;

FMT cuts the need for C. difficile-related surgery

- Retrospective review of **901 pts with CDI**
- **No more surgery after the establishment of a FMT service**
- Relevant **decrease in CDI-related mortality** (surgical pts: 83%; FMT pts: 6%)



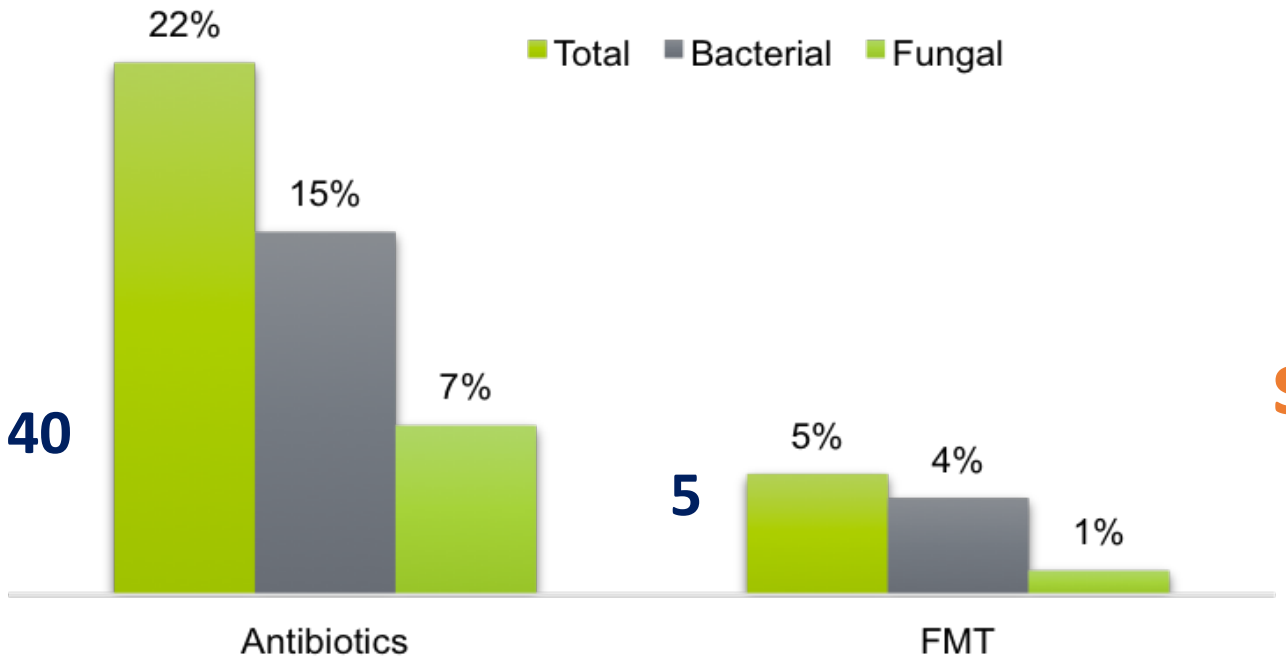
Cammarota, Ianiro, Gasbarrini et al – Ann Intern Med 2015

FMT decreases sepsis rates and increases survival in rCDI

Observational cohort, 290 hospitalized pts (181 atb, 109 FMT)

4x BloodStream infection rates

Sepsis occurrence at day 90

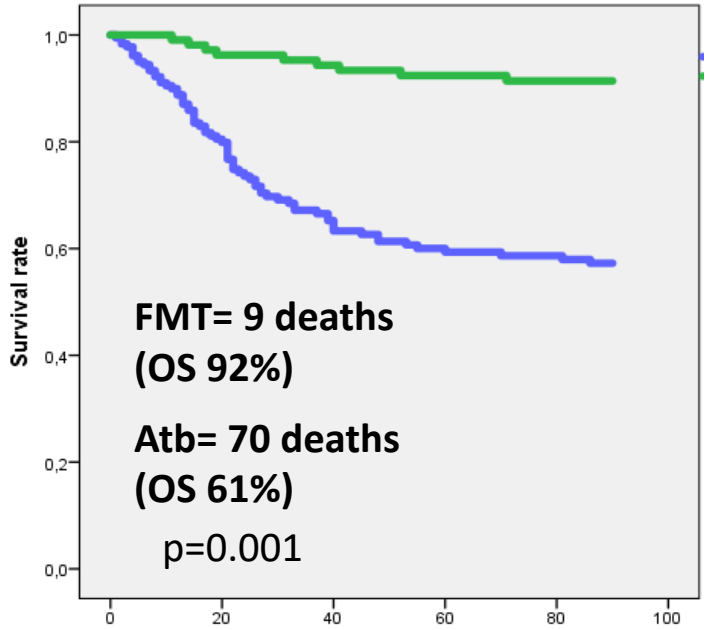


50% Length of stay

29.7 d (Atb) vs 13.3 d (FMT) p<0.001

31% Overall survival

90-day OS



Ianiro, Cammarota, Gasbarrini et al, Ann Intern Med 2019

FMT for *C. difficile*: European consensus conference

FMT for recurrent *Clostridium difficile* infection

Statement: **FMT is recommended as a highly effective and safe treatment option for both mild and severe rCDI**. Its implementation in clinical practice is recommended

Quality of evidence: high **Strength of recommendation:** strong

FMT for the first episode of *Clostridium difficile* infection

Statement: There is **insufficient evidence to recommend FMT as a treatment for the first episode of CDI.** Additional studies are needed to determine if FMT could have an advantage over antibiotics for this indication

Quality of evidence: low **Strength of recommendation:** weak

FMT for refractory *Clostridium difficile* infection

Statement: FMT can be considered as a **treatment option for refractory CDI**

Quality of evidence: high **Strength of recommendation:** strong

Cammarota, Ianiro, Gasbarrini et al – Gut – 2017

FMT: REALLY A SUCCESS STORY?

FMT has partially been lost in translation (from research to clinical practice)

Evidence for different indications of FMT in 2021

	Metanalyses	RCTs	Open label trials	Case series/reports	Efficacy data	Used in clinical practice
C. difficile infection	+++	+++++	++++	++++	Outstanding	YES
Ulcerative colitis	+	+++	+++	+++	Promising	NO
Hepatic encefalopathy		+		+	Quite promising	NO
Metabolic syndrome		+++		+	Quite promising	NO
Crohn's disease		+	++	+	Poor	NO
IBS	+	++++	++	+	Quite promising	NO
Multi-resistant infections		+	++	++	Quite promising	NO
Autism			+	+	Poor	NO
GVHD				+	Poor	NO
Chemotherapy-dependent diarrhea		+	+	+	Quite promising	NO

How to evolve FMT from fecal microbiota transplantation to future microbiota therapeutics?

EXPLORE NEW INDICATIONS

GUARANTEE SAFETY

STANDARDIZE & DISSEMINATE

IMPROVE WORKING PROTOCOLS

NECESSARY MINDSHIFTS

How to evolve FMT from fecal microbiota transplantation to future microbiota therapeutics?

EXPLORE NEW INDICATIONS

New indications beyond *C. difficile?*

Other multi-drug resistant pathogens

BURDEN OF ANTIBIOTIC RESISTANCE

- Nearly **700.000 deaths/year** worldwide
- €1.5 billion per year in EU
- **Up to $55 billion/year** in the **US**
- **Up to $100 trillion** (£63.68 trillion) by **2050**

https://www.reactgroup.org

Successful case series/case reports on:

- Methicillin-resistant Staphylococcus aureus (MRSA) Enterocolitis
- Vancomycin-resistant Enterococcus (VRE)
- K. pneumoniae MBL(+)
- Escherichia coli ESBL(+)

Wei et al – BMC Infect Dis 2015
Stripling et al – Open Forum Infect Dis 2015
Bilinsky et al- Arch Immunol Ther Exp 2016

One open-label trial:

- 20 participants, median of 2 strains of ARB
- FMT by nasoduodenal tube
- Complete ARB decolonization in 15 of 20 patients (75%)
- No severe adverse events

Bilinsky et al – Clin Infect Dis 2017

Ulcerative colitis: not there yet

4 RCTs

- **Clinical remission 28%** vs 9% placebo (OR 3.67- 95%CI 1.82-7.39, P<0.01)
- **Endoscopic remission 14%** vs 5% placebo (OR 2.89 – 95%CI 1.07-6.74, P=0.04)

14 cohort studies

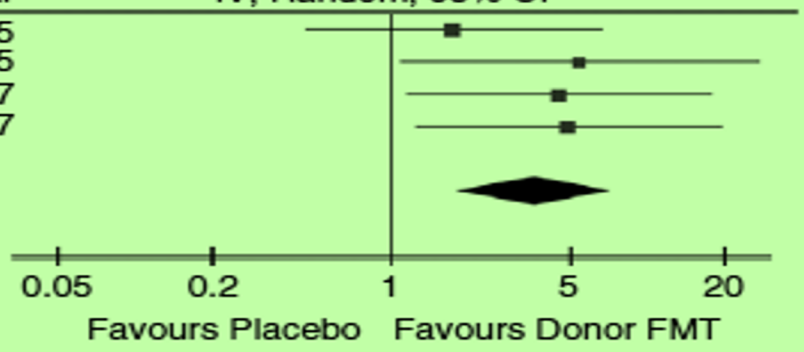
- **Clinical remission 24%**

Marked differences between FMT working protocols

Costello et al – AP&T 2017

Study or Subgroup	Donor transplant Events	Total	Placebo Events	Total	Weight	Odds Ratio IV, Random, 95% CI	Year
Rossen 2015	7	23	5	25	28.2%	1.75 (0.47, 6.57)	2015
Moayyedi 2015	9	38	2	37	19.0%	5.43 (1.09, 27.15)	2015
Paramsothy 2017	11	41	3	40	26.5%	4.52 (1.16, 17.70)	2017
Costello 2017	12	38	3	35	26.4%	4.92 (1.25, 19.31)	2017
Total (95% CI)		140		137	100.0%	3.67 (1.82, 7.39)	
Total events	39		13				

Heterogeneity: $\tau^2 = 0.00$; $\chi^2 = 1.70$, df = 3 (P=.64); $I^2 = 0\%$
Test for overall effect: $Z = 3.63$ (P=.0003)



We are still far from finding a magic bullet

Comparison of RCTs

Authors (Year)	*Moayyedi 2015*	*Rossen 2015*	*Paramsothy 2017*	*Costello 2019*
People (number)	70	37	85	73
Comparator	Water	Autologous stools	Water	Autologous stool
FMT protocol and route	1 infusion per week for 6 weeks by enema	2 infusions in 3 weeks by naso-duodenal tube	1 infusion by colonoscopy followed by 5 enemas per week for 8 weeks	1 infusion by colonoscopy followed by 2 enemas in one week
Faecal infusates	Fresh, frozen, aerobiosis, single donor	Fresh, aerobiosis, single donor	Frozen, aerobiosis, multiple (3-7) donors	Frozen anaerobiosisi, multiple (3-4) donors
Primary outcome	Remission (Mayo score <3 plus endoscopic score of 0) at week 7	Remission (SCCAI≤2) plus 1 point decrease in endoscopic Mayo score at week 12	Steroid-free clinical remission with endoscopic remission or response at week 8	Steroid-free clinical remission at week 8
Results (primary outcome)	24% FMT group vs 5% placebo group (p=0.03)	30.4% FMT group vs 20% placebo group (p=0.51)	27% donor FMT group vs 8% autologous FMT group (p=0.021)	32% donor FMT group vs 9% autologous FMT group (p=0.03)

Cammarota, Gasbarrini & Ianiro – Nat Rev Gastro Hep 2019

Metabolic syndrome: One-hit wonder or rising star?

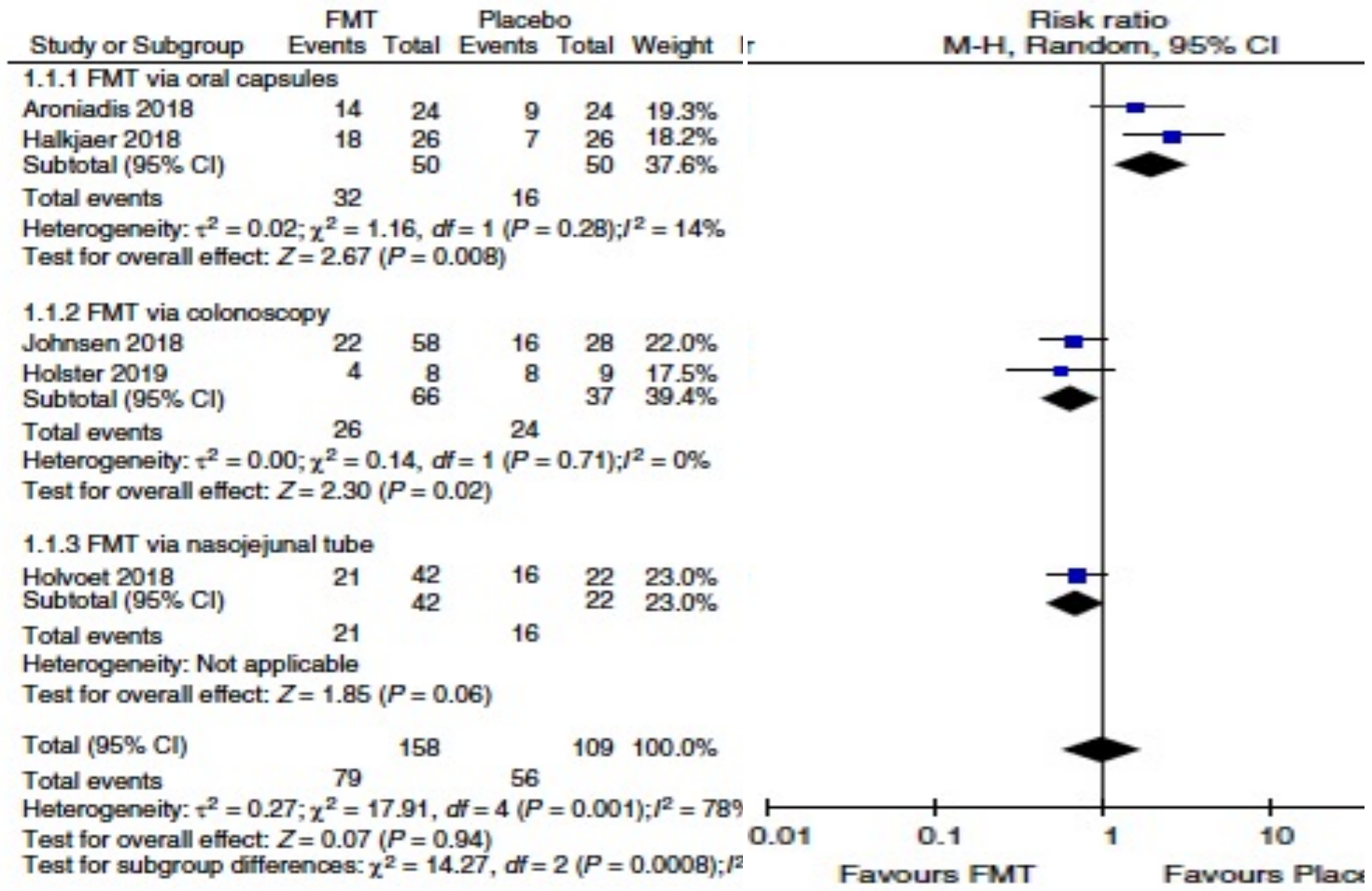
	Vrieze et al – 2012	Kootte et al - 2017
Design	RCT (donor vs autologous feces)	RCT (donor vs autologous feces)
Population	18 treatment-naive males w/ MetS	44 treatment-naive males w/ MetS
Donors	Lean male donors	Lean male donors
Route	Nasoduodenal tube	Nasoduodenal tube
Infusions	Single infusion	2 infusions in some donor-FMT pts
Follow-up	6 weeks	6 weeks and 18 weeks
Main results (donor vs autol. FMT)	• Improvement of **peripherical insulin sensitivity** • Increase in **microbiota diversity** • Increase of **R. intestinalis** abund.	*6-wk follow-up* • Improvement of **peripherical insulin sensitivity** and **HbcA1** • No increase in microbiota diversity • Increase in **A. muciniphila** abund. *18-wk follow-up* • **No differences** between groups

Vrieze et al – Gastroenterology 2012- Kootte et al – Cell Metabolism 2017

Irritable Bowel Syndrome: still constipated?

- **Metanalysis of five RCTs, 267 patients (92.2% IBS-D or IBS-M, 7.8% IBS-C)**

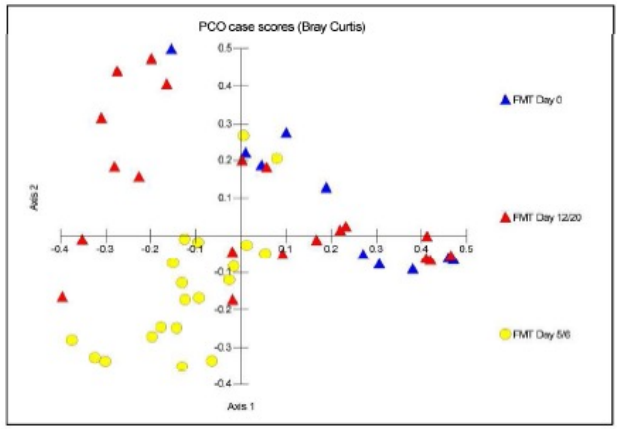
- **RR of IBS symptoms not improving was 0.98 (95% CI 0.58-1.66).**
- **Placebo capsules superior to capsules containing donor stool (RR = 1.96; 95% CI 1.19-3.20).**
- **FMT from donor stool delivered via colonoscopy was superior to autologous stool (RR = 0.63; 95% CI 0.43-0.93).**



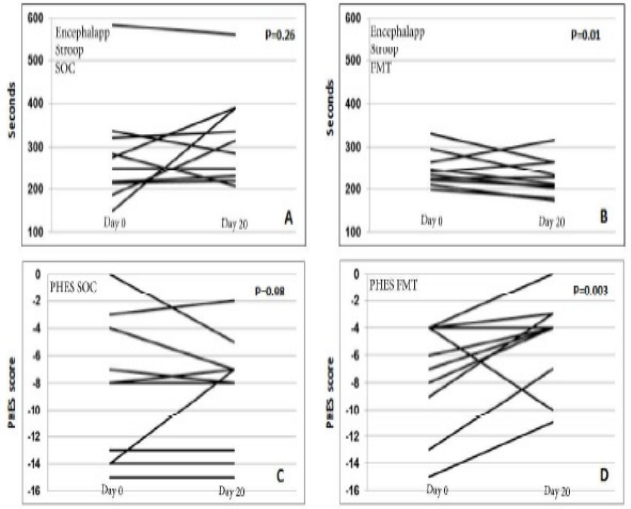
Ianiro, Cammarota, Gasbarrini et al – Aliment Pharmacol Ther 2019

Hepatic encephalopathy: following the eubiotic concept

- Pilot RCT
- **20 cirrhotic patients with recurrent HE on standard-of-care** (SOC) were randomized to SOC or FMT (5-days of broad-spectrum antibiotic pre-treatment then a single FMT enema)



- FMT plus antibiotic pre-treatment was well tolerated
- **5 SOC and no FMT participants developed further HE (p=0.03)**
- **Cognition improved only in the FMT group**
- FMT increased **microbiota diversity** and **beneficial taxa**



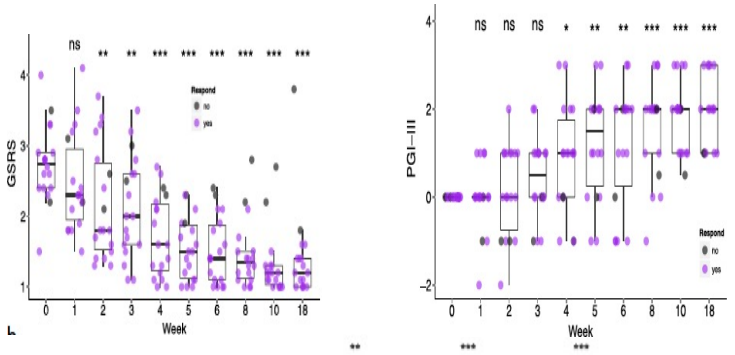
Bajaj et al – Hepatology 2017

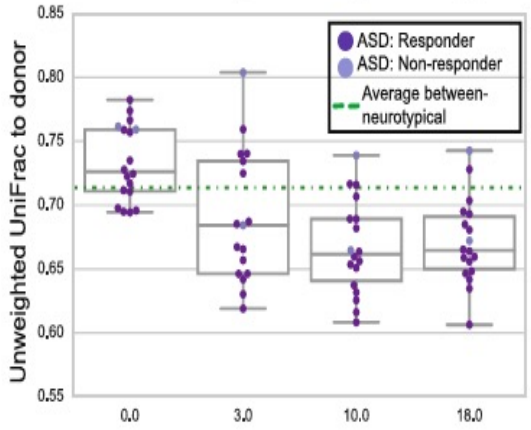
Autistic spectrum disorders: a strong rationale

- Open-label clinical trial – 18 children with ASD
- 2-wk antibiotic treatment, bowel cleanse, and then repeated FMT for 7–8 weeks

Outcomes

- **80% reduction of GI symptoms at GSRS** after treatment for 8 weeks after treatment
- **Significant improvement of behavioral ASD symptoms** for **8 weeks** after treatment





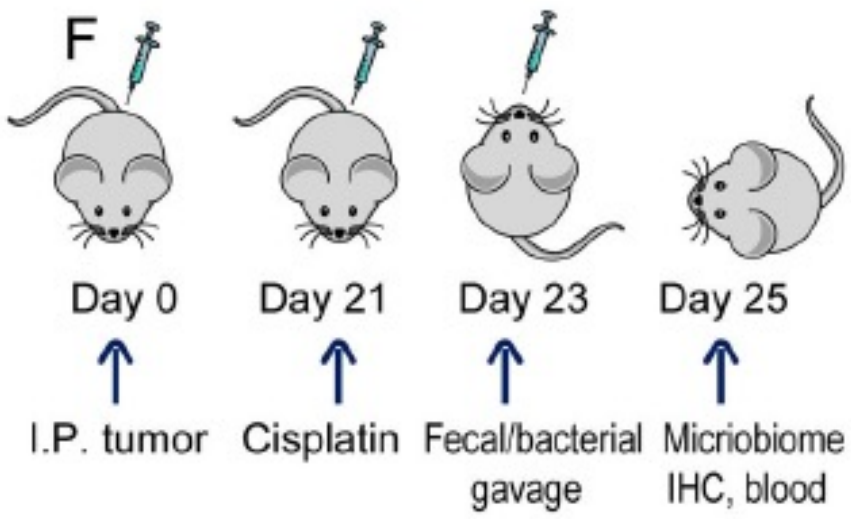
Microbiological findings

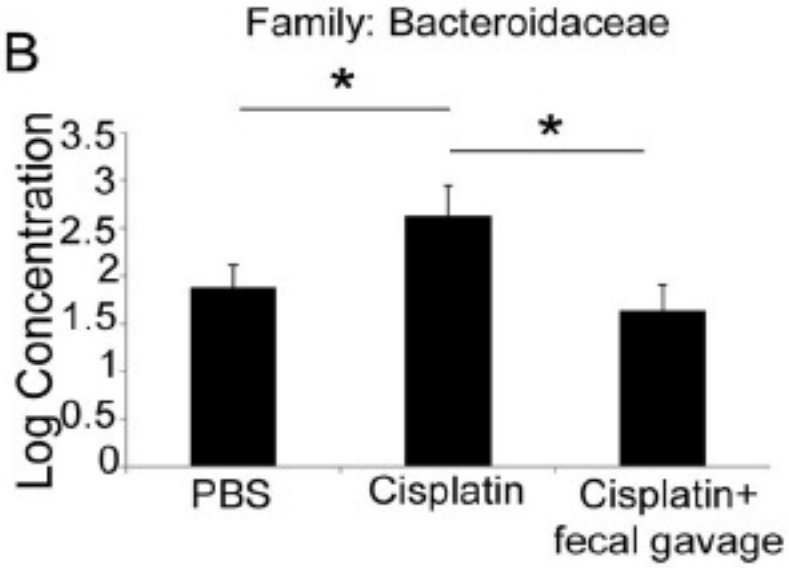
- Successful partial **engraftment of donor microbiota**
- **Bacterial diversity** and Bifidobacterium, Prevotella, and Desulfovibrio abundance increased following FMT

Kang et al – Microbiome 2017

FMT in Oncology: a revolution in progress

FMT reverses cisplatin-induced dysbiosis in mice

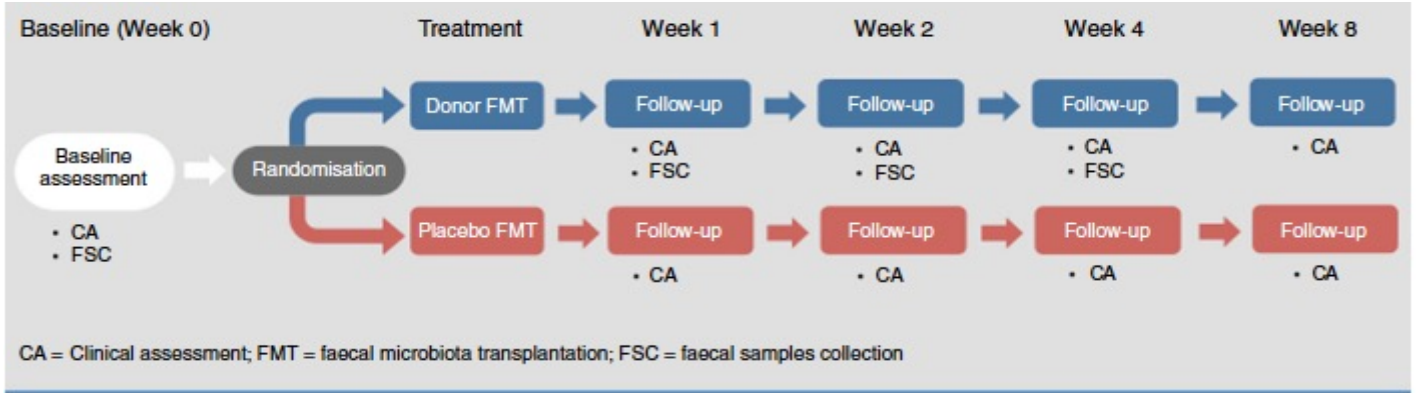




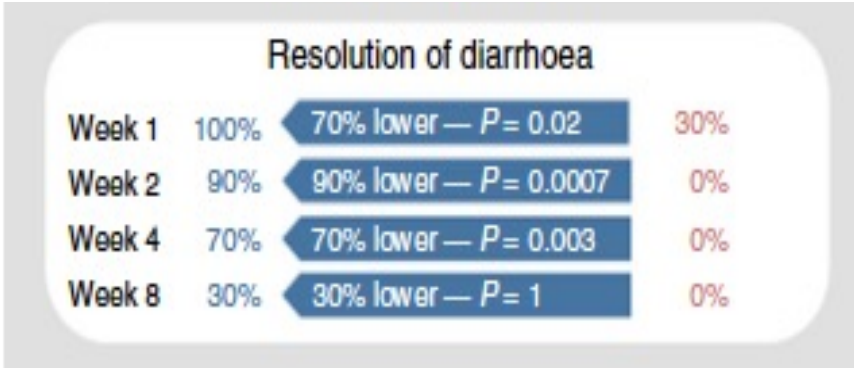
Perales-Puchalt, J Leukoc Biol. 2018

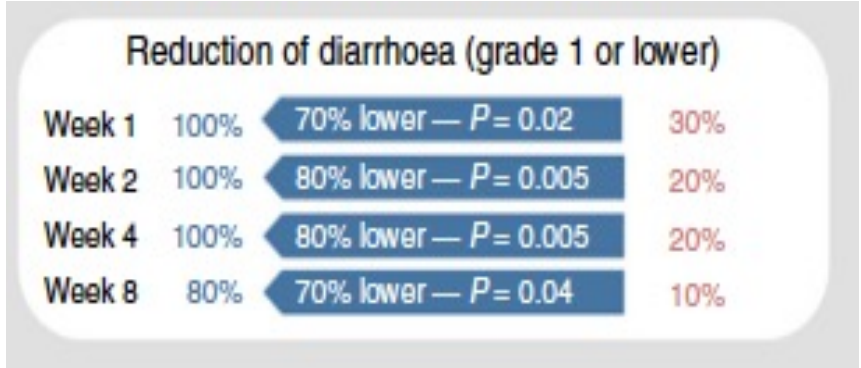
FMT ameliorates cancer therapies-induced diarrhea

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

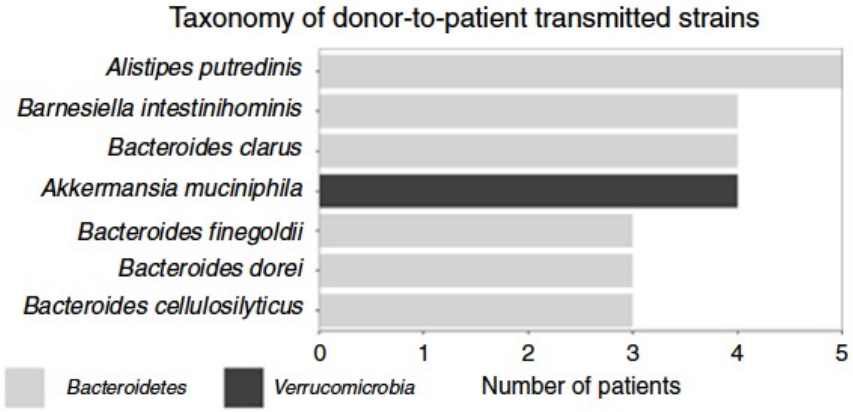


Resolution of diarrhoea at W4 (PE)





Transfer of beneficial species

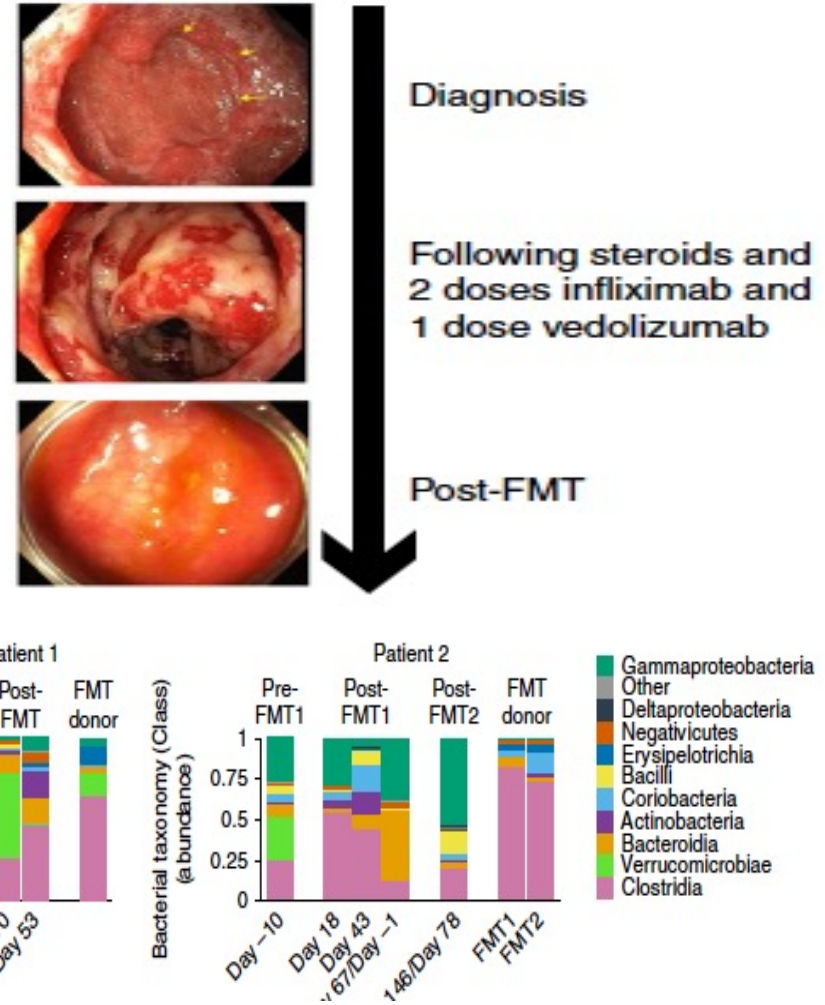


Ianiro, Gasbarrini et al – Nat Comm 2020

FMT 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

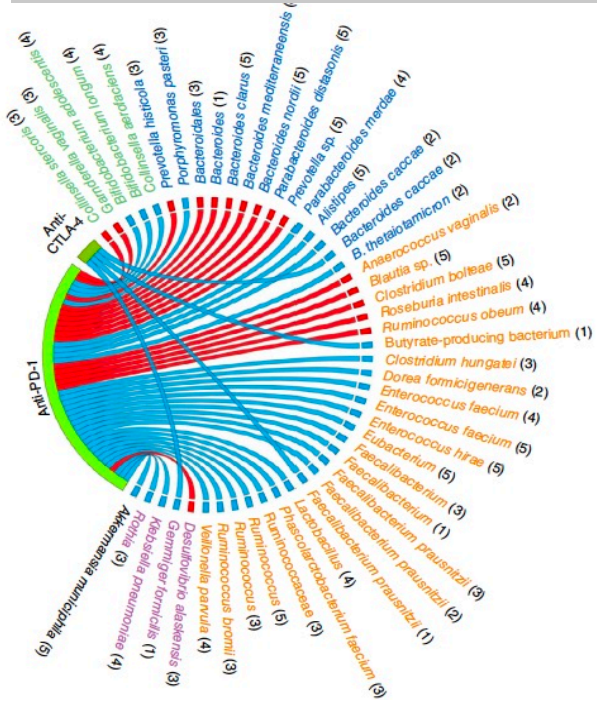
AFTER FMT
- **Improvement of endoscopic appearance**
- **Reduction in CD8, increase in CD4 T cells**
- **Increase in Bifidobacteria and Blautia**
- **Shift toward donor microbiomes**

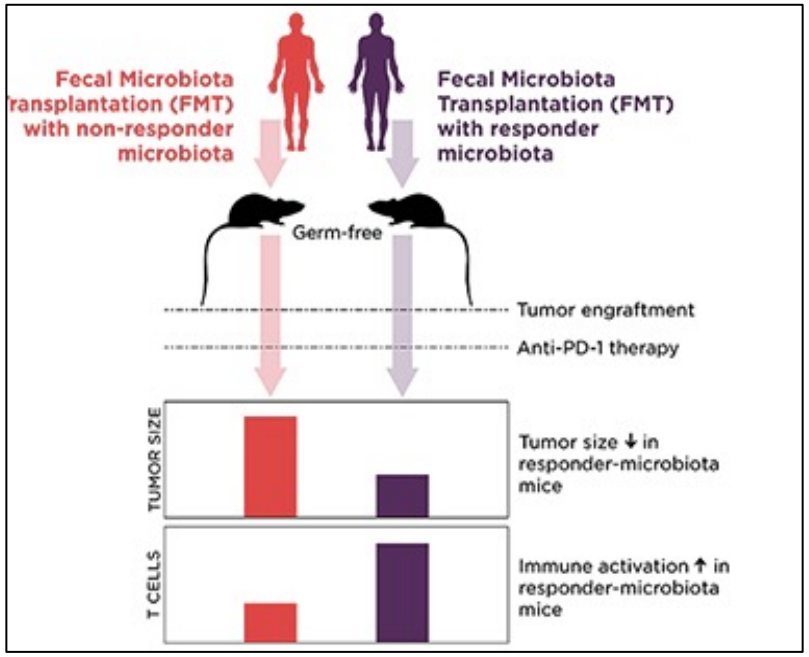


Wang et al, Nat Med 2018

FMT improves efficacy of IMMUNOTHERAPIES in epithelial cancers

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





Active FMT trials in humans

Country	Cancer Type
Canada	Melanoma
USA	Melanoma
Israel	Melanoma
Italy	Renal cell carcinoma

Bacterial phyla involved:
- Actinobacteria
- Bacteroidetes
- Firmicutes
- Proteobacteria
- Verrucomicrobia

Association with response:
- Enriched in responders
- Enriched in nonresponders

Ma et al, Frontiers Micro 2019; Routy et al, Science 2018; Gopalakrishnan et al, Science 2017

How to evolve FMT from fecal microbiota transplantation to future microbiota therapeutics?

GUARANTEE SAFETY

- Safety in the short term
- Safety in the long term
- What does it happen in the COVID-19 era?

Is FMT safe in the short term?

FDA In Brief: FDA warns about potential risk of serious infections caused by multi-drug resistant organisms related to the investigational use of Fecal Microbiota for Transplantation

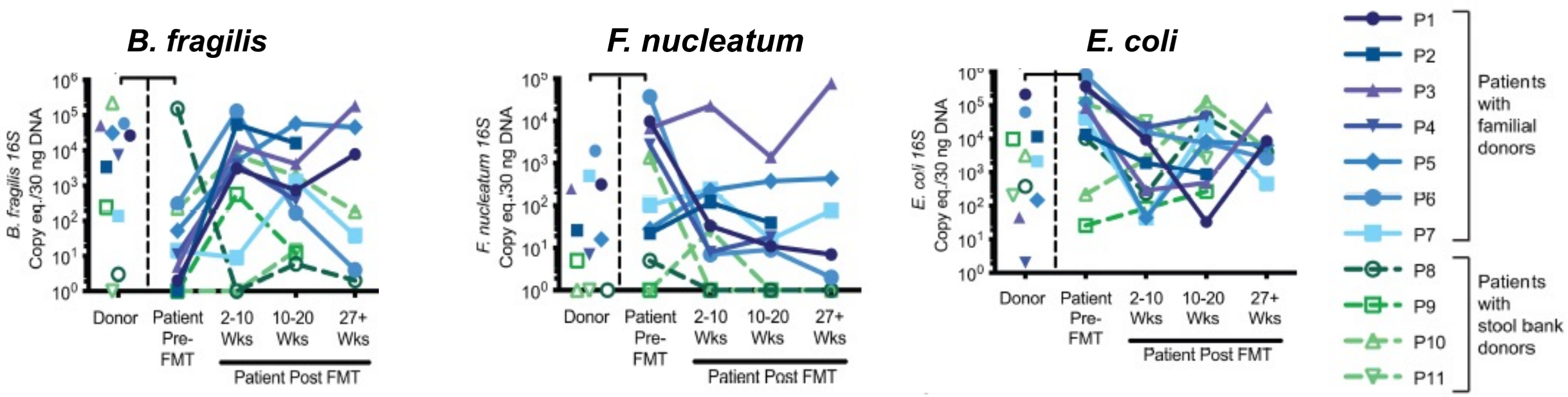
- **On June 13, 2019, the FDA issued a safety alert concerning the risk of serious adverse reactions due to transmission of MDRO by FMT**
- This was in response to transmission of an ESBL producing *Escherichia coli* strain from a feces donor to two immunocompromised recipients, with one death. For reasons not specified, the donor had not been screened for MDRO
- **The FDA now requires inclusion of MDRO screening into all active and future FMT-based study protocols**

- **All major stool banks have implemented screening protocols to detect MDRO, without SAEs** (>45000 FMTs by OpenBiome since 2012)
- **Adherence to standard screening protocols** used by major stool banks worldwide **could have prevented these incidences**

Kujiper, Ianiro, Cammarota, Gasbarrini et al – Lancet Infect Dis 2019

Is FMT safe in the long term?

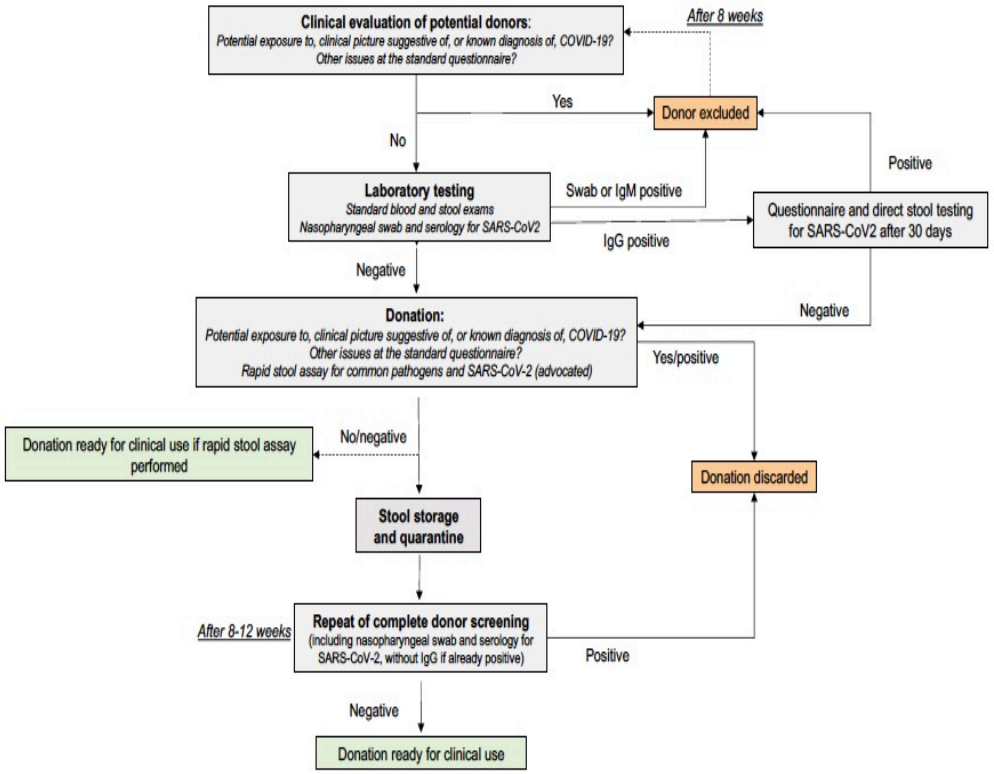
- FMT transferred procarcinogenic microbiota in 11 rCDI pediatric patients
- This did not happen when using stool bank donors
- This effect was reversed by another FMT by donors negative for this signature

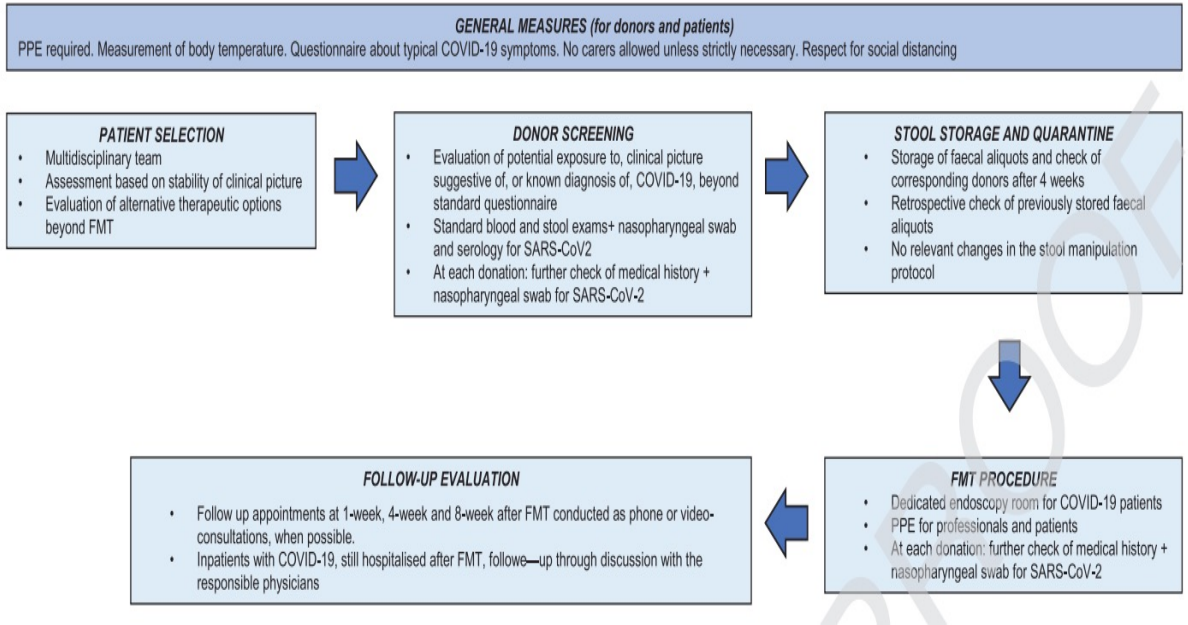


Drewes et al, JCI Insight 2019

Can we perform FMT in the COVID-19 time?

- Several clinical activities have been reduced during the COVID-19 pandemic
- SARS-CoV-2 can be potentially transmitted by feces
- However, CDI still represents a clinical priority
- In the time of the pandemic, FMT centres and stool banks are required to adopt a workflow that continues to ensure reliable patient access to FMT while maintaining safety and quality





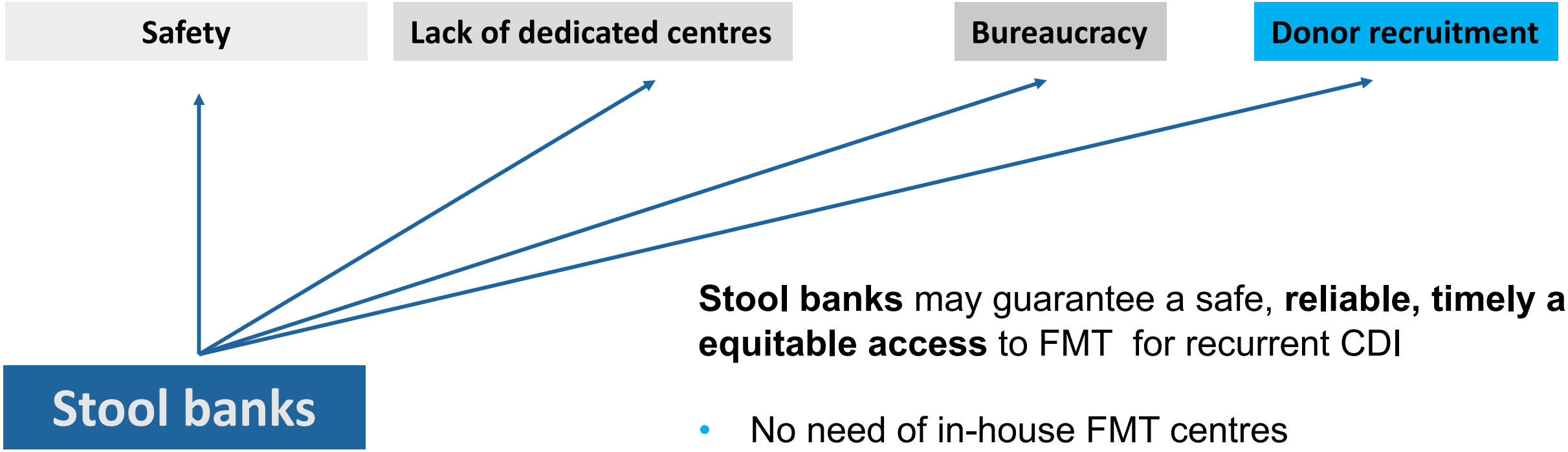
Ianiro, Cammarota, Gasbarrini et al – Lancet Gastro Hep 2020; Ianiro, Cammarota, Gasbarrini et al – Gut 2020; Ianiro, Cammarota, Gasbarrini et al – DLD 2020

How to evolve FMT from fecal microbiota transplantation to future microbiota therapeutics?

STANDARDIZATION

- Stool banks

Potential solutions to overcome barriers to dissemination



Stool banks may guarantee a safe, **reliable, timely and equitable access** to FMT for recurrent CDI

- No need of in-house FMT centres
- High quality control
- No matter of donor shortages
- Clear traceability and organization

Cammarota, Ianiro, Gasbarrini, et al – Gut 2019;

Keller, Ianiro, Cammarota, Gasbarrini et al – UEG J 2020

The ROME II STOOL BANK-FMT Consensus report

Preparation and storage of faeces

- Biosafety level 2 to prepare feces
- Clear traceability of all processes
- Storage for max 2 y

Services & clients

Minimum criteria to release feces to recipient centres (not patients)

Registries & monitoring of outcomes

Registries are mandatory to assure traceability and check for AEs

Evolving role of FMT in clinical practice

No evidence to go outside Cdiff in clinical practice

How to evolve FMT from fecal microbiota transplantation to future microbiota therapeutics?

IMPROVE WORKING PROTOCOLS

- How to improve efficacy of FMT?
- New techniques
- Beyond the gut

FMT: How to improve it?

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

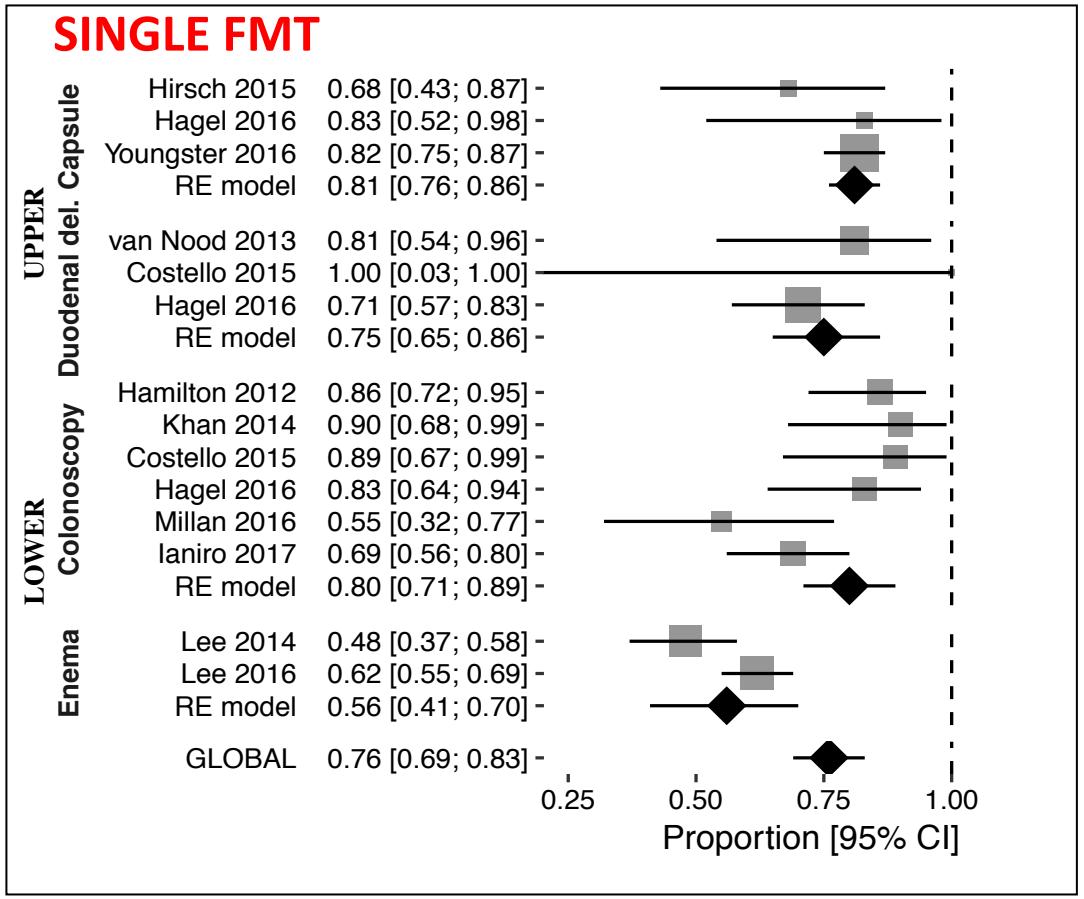
Routes of delivery
- NJT/NDT
- Capsules
- Colonoscopy
- Enema

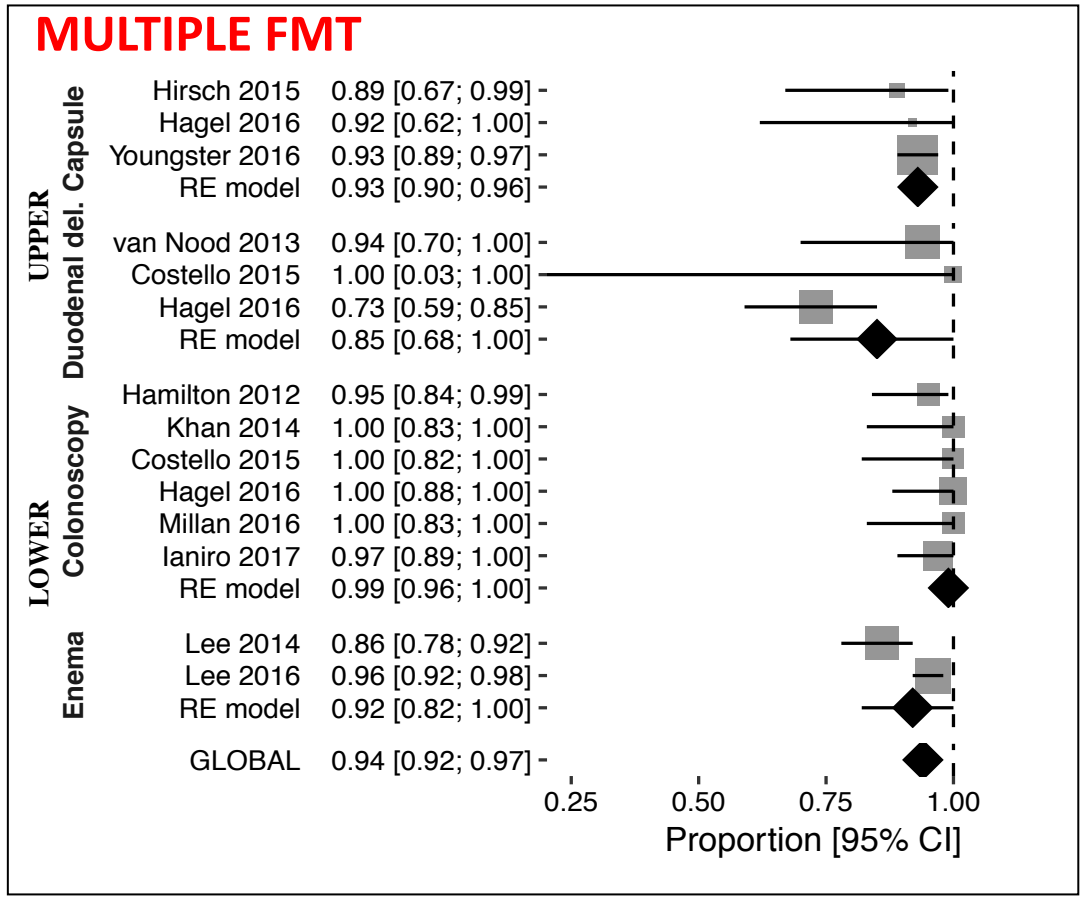
Cammarota, Ianiro, Gasbarrini et al – Gut – 2017

How to improve FMT efficacy?

Protocol predictors: number of infusions

Metanalysis of 15 studies with meta-regression





Ianiro, Cammarota, Gasbarrini et al, UEG Journal 2018

How to improve FMT efficacy?

Protocol predictors: routes of delivery and fecal amount

Metanalysis of 15 studies with meta-regression

META-REGRESSION ANALYSIS

ROUTES OF DELIVERY

- **Colonoscopy** associated with **higher efficacy rates (p= 0.006)**
- **Enema** associated with **lower efficacy rates after single infusion** (p= 0.019)

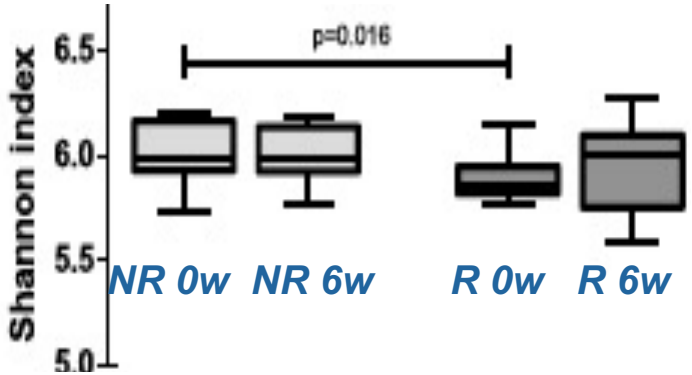
FECAL AMOUNT

Faecal amount ≤50 g associated with **lower efficacy rates after single infusion** (p= 0.006)

Ianiro, Cammarota, Gasbarrini et al, UEG Journal 2018

How to improve FMT efficacy?
Protocol predictors: microbial predictors

- **MetS** pts with **lower baseline microbial diversity** are more likely to **benefit** from lean donor FMT(p=0.016)

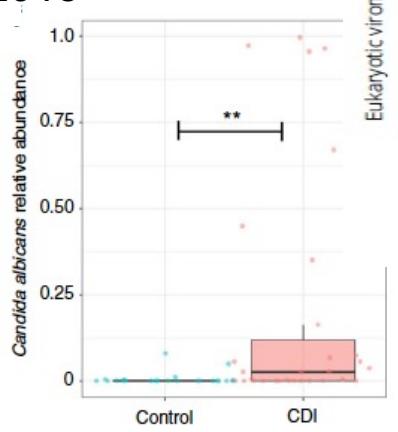


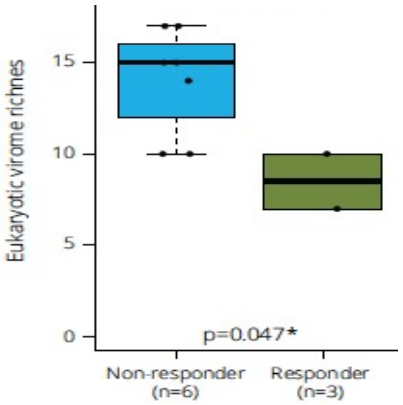
Kootte et al – Cell Metab 2017

- **Diversity increase after FMT** is a marker of **response** in UC

Rossen et al – Gastroenterology 2015

- **Donor-derived Bacteria, Fungi and Viruses are associated with FMT response**



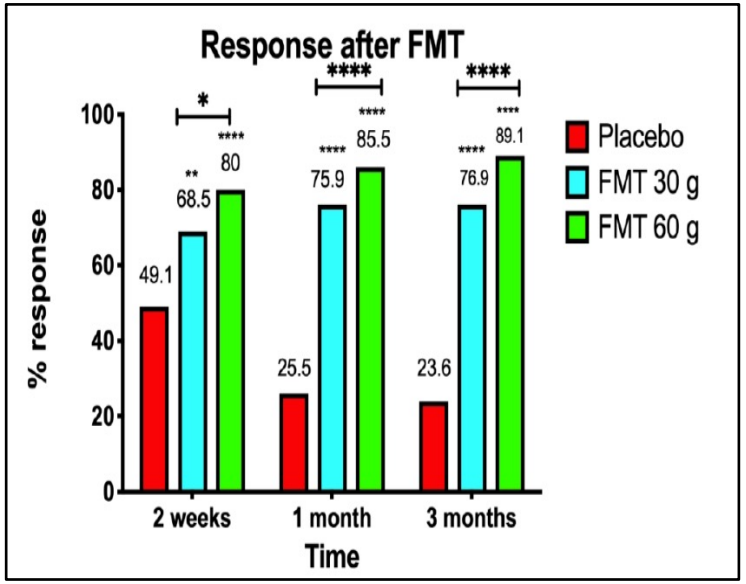


Kump - APT 2017; Moayyedi- Gastro 2015; Kang - Microbiome 2017; Kakihana - Blood 2016;

Conceição-Neto et al – Gut 2017
Zuo et al – Nat Comm 2018
Zuo et al – Gut 2017

FMT success in IBS: a matter of quantity and quality of microbes

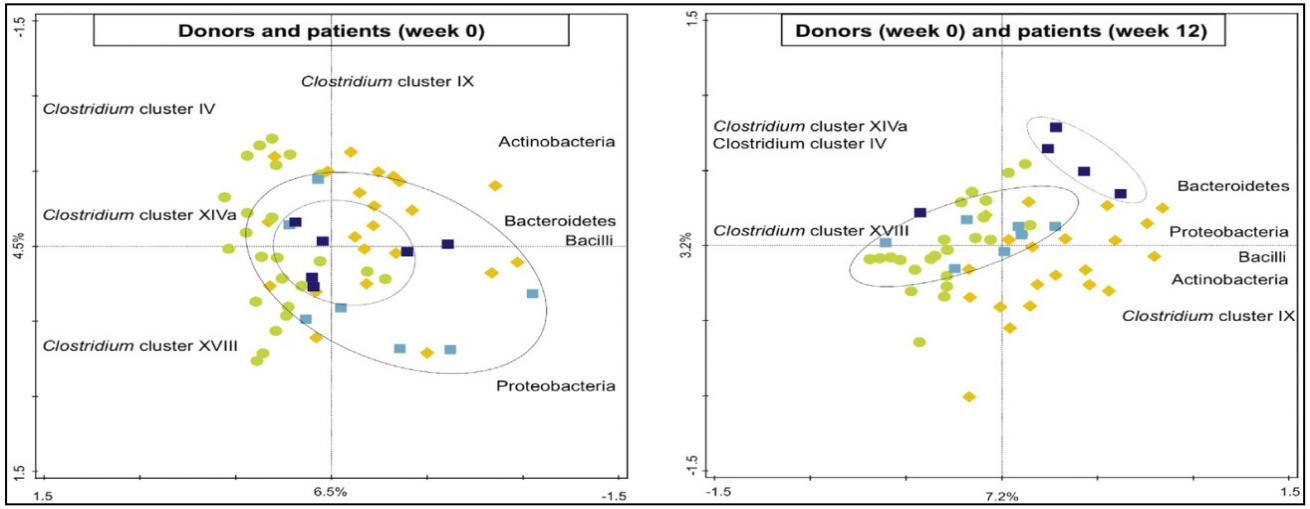
- **RCT** of **164 patients with IBS** (all subtypes)
- Single donor FMT vs placebo (autolgous feces), **upper GI delivery**, **two dosages** (30 g and 60 g)
- **Single super-donor:** healthy, drug-free, lean, athletic, young male, born through vaginal delivery, breastfed, with a history of only three antibiotic courses in his life, eating a healthy diet, with a **favourable microbiota profile** (richer in Lactobacilli, Lachnospiraceae and Verrucomicrobia, and lower in Shigella and Escherichia spp)
- Primary endpoint: **reduction of IBS symptoms at 3 amonths**
- **Responses occurred in 23.6%, 75.9% (P<0.0001), and 89.1% (P<0.00001) of the patients who received placebo, 30-g FMT, and 60-g FMT**
- Significant improvements in fatigue and the quality of life, and changes of microbiome profiles, in the FMT group
- **>80 % of patients mantained response after 12 months (either 30 or 60 gr)**

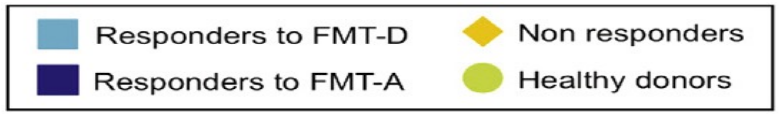


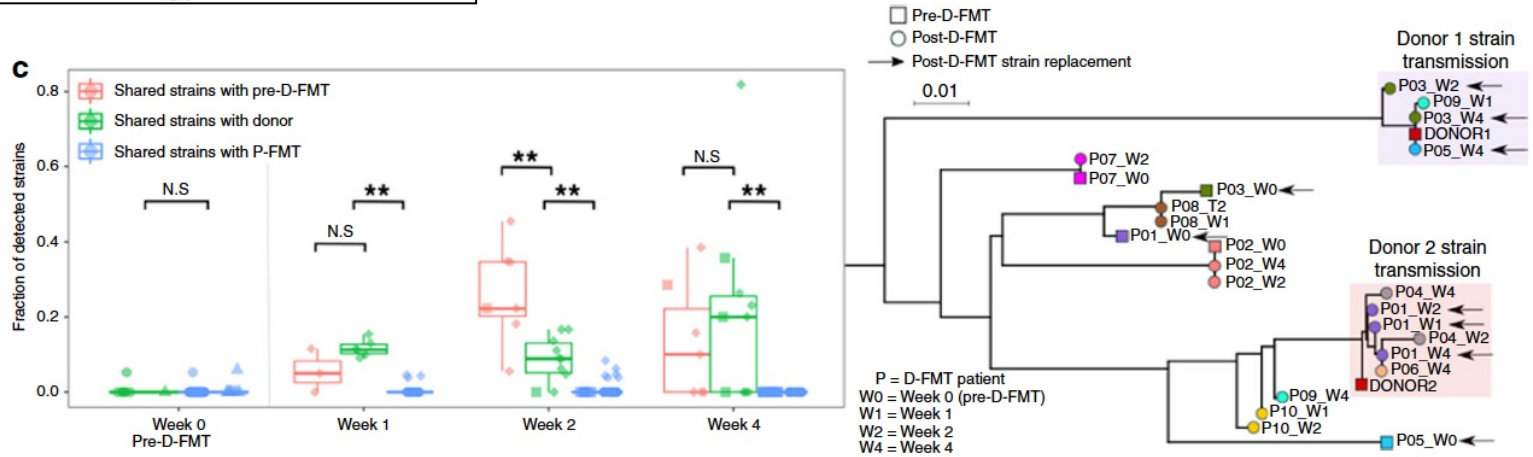
El Salhy et al- Gut 2020: Barbara & Ianiro, Gut 2020; El Salhy et al; Neurogas Motil 2021

FMT: the key role of engraftment

Recipient-donor engraftment is the key for therapeutic success in UC and other chronic disorders







Ianiro et al – Nat Comm 2020;
Kootte et al – Cell Metabolism 2017;
Moayyedi et al – Gastroenterology 2015;
Rossen et al – Gastroenterology 2015

FMT: as easy as swallowing a pill?

Capsule FMT has been being used since 2014 to treat CDI, with success

Year	1° author	Design	Sample	Feces/capsule	Single course	CDI Cure rate
2014	Youngster	Prospective	20	1.6 g (mean)	30 capsules	**70%** (single course); **90%** (multiple courses)
2015	Hirsch	Retrospective	19	2.3 g (mean	8-12 capsules	**68%** (single course); **89%** (multiple courses)
2016	Hagel	Retrospective	12	NR	NR	**83%** (single course); **92%** (multiple courses)
2016	Youngster	Prospective	180	1.6 g (mean)	30 capsules	**82%** (single course); **94%** (multiple courses)
2017	Staley	Prospective	49	NR	Different n°	**88%** (single course)
2017	Kao	Non-inferiority RCT	57 caps. 59 colon	80-100 g per treatment	40 capsules	96% (single course): not inferior to colonoscopy

- Capsule FMT restored bacterial diversity and resolved dysbiosis
- Shifts in the fecal microbiome were incremental rather than immediate

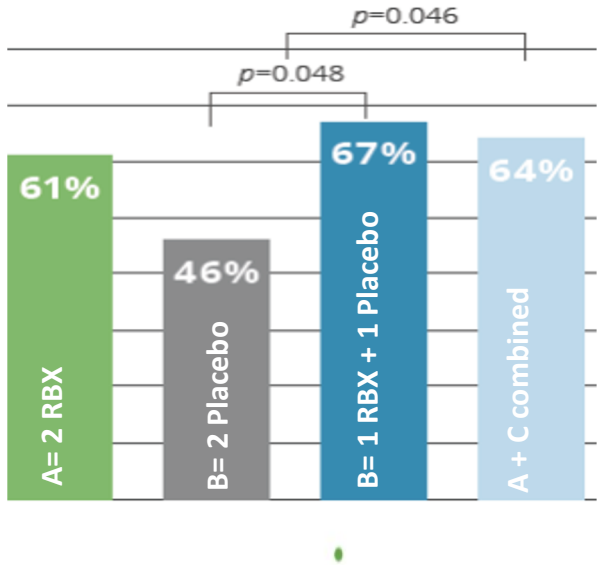
Capsule FMT may boost **dissemination of FMT** and ease sustained **cure of chronic disorders** (e.g. UC) through repeated treatment sessions

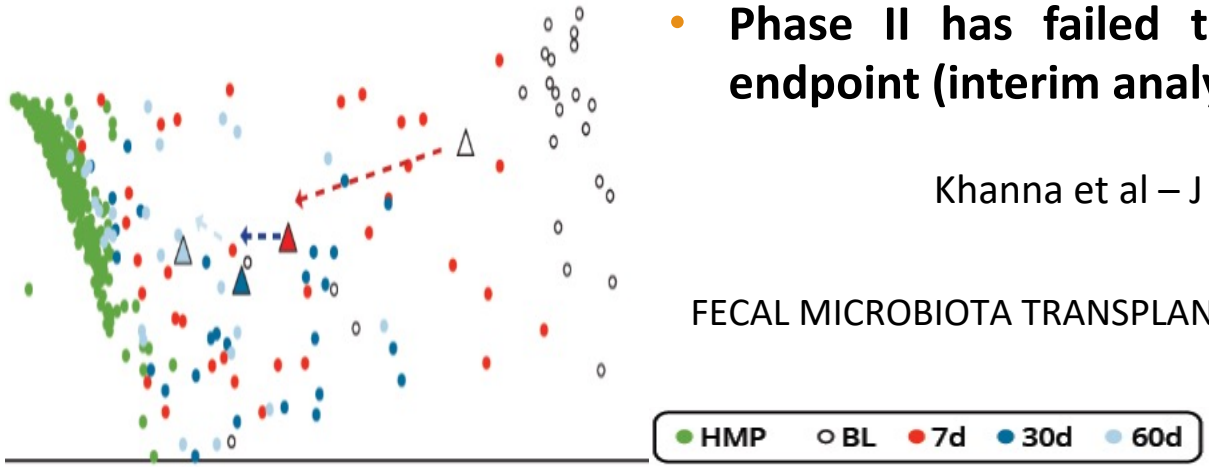
Staley et al – Gut microbes 2017

FMT 2.0 – Microbiota suspensions from industry

RBX2660

- **87.1%** cure of rCDI + no SAE – pilot study 31 pts
- **Significant benefit of a single** (**67%** rCDI cure rates vs placebo 46%), but **not of 2 RBX doses – 89.2% cumulative** cure rate after open-label treatment of all failures - **127 pts (RCT)**
- Patients' microbiota **shifts towards donor biomes** after treatment





SER-109

- **86.7%** cure of rCDI - pilot study, 30 pts
- Rapid **microbiota diversification**, with **durable engraftment of spores** (both with 1 or 2 SER109 doses)
- No treatment-related SAEs
- **Phase II has failed the primary endpoint (interim analysis)**

Khanna et al – J Infect Dis 2016

FECAL MICROBIOTA TRANSPLANTATION

Orenstein et al – Clin Infect Dis 2016
Dubberke et al – Open Forum Infect Dis 2016

Orenstein et al – UEG Week 2016;
Blount et al – ASM Congress 2017

How to evolve FMT from fecal microbiota transplantation to future microbiota therapeutics?

NECESSARY MINDSHIFTS

Some mandatory mindset shifts are needed to go outside CDI

- **Chronic disease are lifelong**, and patients need effective and safe therapies not only to induce remission but also to maintain it
- The poor rate of donor–recipient microbial engraftment — which is associated with clinical outcomes — achieved by a single faecal infusion suggests that **FMT is unlikely to act as a one-time treatment**

FMT should be considered as a **chronic treatment to be integrated among other options**

- **Specific donor microbial signatures** are known to influence response to FMT
- They are hardly reproducible, especially if FMT should be repeated over time

Microbiome sequencing cannot remain outside clinical practice in the future

Fine-tuned/Tailored synthetic microbiome consortia will be used together with FMT in the management of patients

Cammarota, Ianiro & Gasbarrini– Nat Rev Gastro Hep 2019

To date, there is a **gap between microbiome basic scientists and clinicians** involved in dysbiosis-related disorders

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, nutricians, etc.)
- **Availability of microbiota sequencing tools**
- **Availability of stool bank/FMT Centre**
- **Hotspot for microbiota research**
- **Networking and teaching centre**

Beyond the gut: Vaginal Microbiome Transplant

- VMT to cure intractable bacterial vaginosis in 5 patients
- **Long-term remission in 4 of them**

Lev-Sagie et al – Nat Med 2019