Progetto Ematologia Romagna

MICROBIOMA INTESTINALE E PATOLOGIE METABOLICHE E NEOPLASTICHE

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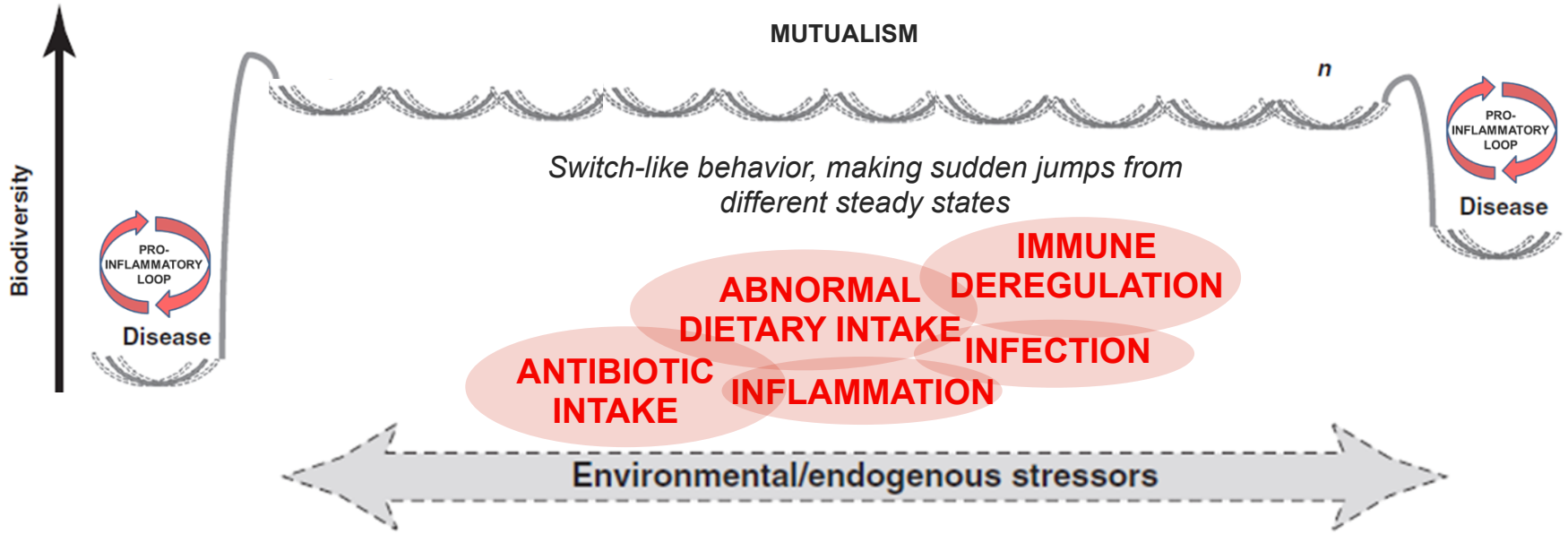
MUTUALISM INTERRUPTION - DYSBIOSIS



RUPTURE OF THE MICROBIOTA-HOST MUTUALISTIC RELATIONSHIP AND COMPROMISED HOST ENERGY BALANCE AND IMMUNE HOMEOSTASIS

A PARTIAL LIST OF DISEASES AND THEIR LINKS TO THE MICROBIOME

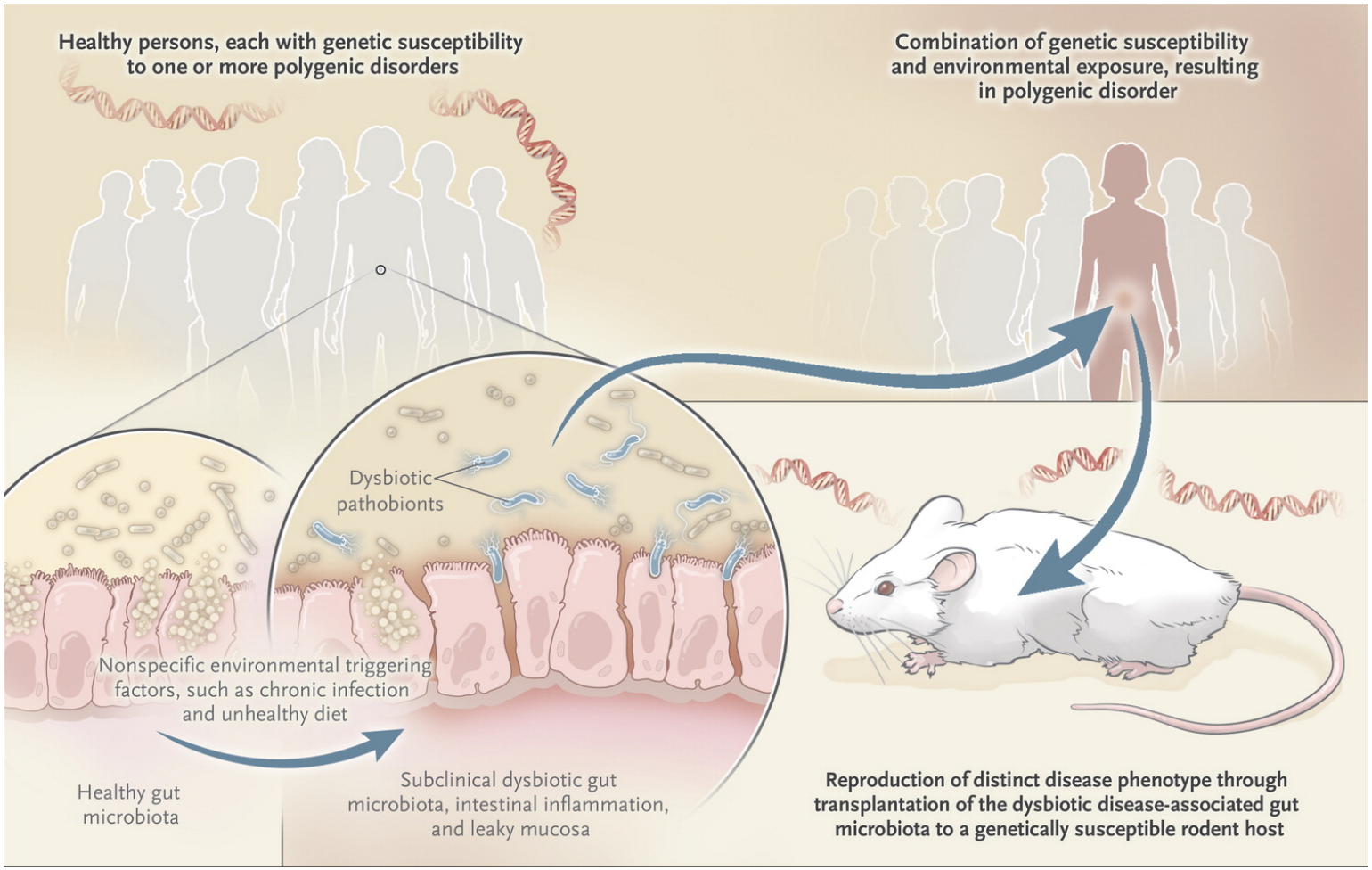
Disease	Description and microbiome link
Acne vulgaris	This skin disorder is mediated by specific *Propionibacterium acnes* strains, together with the vitamin B_{12} pathway in addition to other pathways.
Acute anorexia	Anorexia patients have lower gut alpha diversity. Molecular mimicry of microbial metabolites may contribute to autoantibody production.
Addiction	In a mouse model of addition, antibiotic treatment increased addictive behavior in animals receiving low-dose opioids.
Alcoholic liver disease	Alcoholic liver disease is characterized by intestinal dysbiosis, bacterial overgrowth, and increased gut permeability.
Asthma and allergies	Dust on traditional farms stimulates the immune response and protects against asthma and allergies.
Atherosclerosis	There are suggested links with the translocation of oral microbes into atherosclerotic plaques. Microbially mediated lipid metabolism in the gut may affect the formation of plaques as well.
Atopic dermatitis	Skin inflammation is driven by *Staphylococcus aureus* dominance (with genetic predisposition).
Autism	Differences in gut microbial communities have been observed between children with autism and neurotypical controls; however, there are some inconsistencies. Maternally produced microbial metabolites lead to an autism phenotype in mice.
Bacterial vaginosis	Bacterial vaginosis is characterized by deviation from a low-pH, *Lactobacillus*-dominated community to a higher-pH, more diverse microbial community.
Cardiovascular disease	Diet and the gut microbiome are linked with trimethylamine-*N*-oxide levels in plasma and cardiovascular disease risk (with genetic predisposition).
Chronic skin wounds	*Staphylococcus aureus*, *Pseudomonas aeruginosa*, and other bacteria play a role in pathogenesis in chronic wounds.
Clostridium difficile–associated diarrhea	*C. difficile*–associated diarrhea is a typical example of a change in the gut microbiome leading to an enduring disease state.
Colorectal cancer	Pathogenic microorganisms can potentially initiate and facilitate the development of colorectal cancer.
Cystic fibrosis	Cystic fibrosis is characterized by chronic lung infections, commonly with hypermutable *Pseudomonas aeruginosa* strains.
Dental caries	Dental caries are associated with increased phylogenetic diversity and overabundance of *Prevotella* taxa.
Depression	Transplantation of microbiota from individuals suffering from major depressive disorder into germ-free mice induced depression symptoms in the mice. These symptoms are associated with alterations in carbohydrate metabolism in the microbiome and hippocampus.
Diabetes, type 1	In mouse models, the microbiome is required for the development of diabetes, although low-dose antibiotics increase susceptibility. Changes in microbial development mark the progression to disease but predate the clinical presentation.
Diabetes, type 2	The blood of type 2 diabetes patients has reduced levels of bacterial lipopolysaccharide.
Inflammatory bowel disease	Gut inflammation disease is driven by genetic, environmental, and altered microbial factors. Adherent enterobacteria may promote initial ulceration events.
Irritable bowel syndrome	Patients with irritable bowel syndrome show mucosal and luminal gut microbial changes, although the causal effect is unproven.
Malnutrition	An altered gut microbiome is strongly linked with childhood malnutrition.
Multiple sclerosis	Gut microbiota changes may be related to autoimmunity and the pathology of multiple sclerosis.
Obesity (metabolic disease)	The gut microbiomes of obese individuals show an increased capacity to harvest energy from the diet.
Osteoporosis	The gut microbiome has both direct and indirect effects on deregulated bone remodeling.
Parkinson's disease	The microbiome can promote Parkinson's disease progression in genetically susceptible individuals.
Rheumatoid arthritis	Rheumatoid arthritis patients show altered gut and oral microbiomes. They also have increased translocation of oral bacteria in the gut, which treatment partially corrects.

Knight *et al.*, Annu Rev Genomics Hum Genet. 2017

AN ALTERED MICROBIOME IS THE CAUSE OF A DISEASE STATE OR A CONSEQUENCE ??

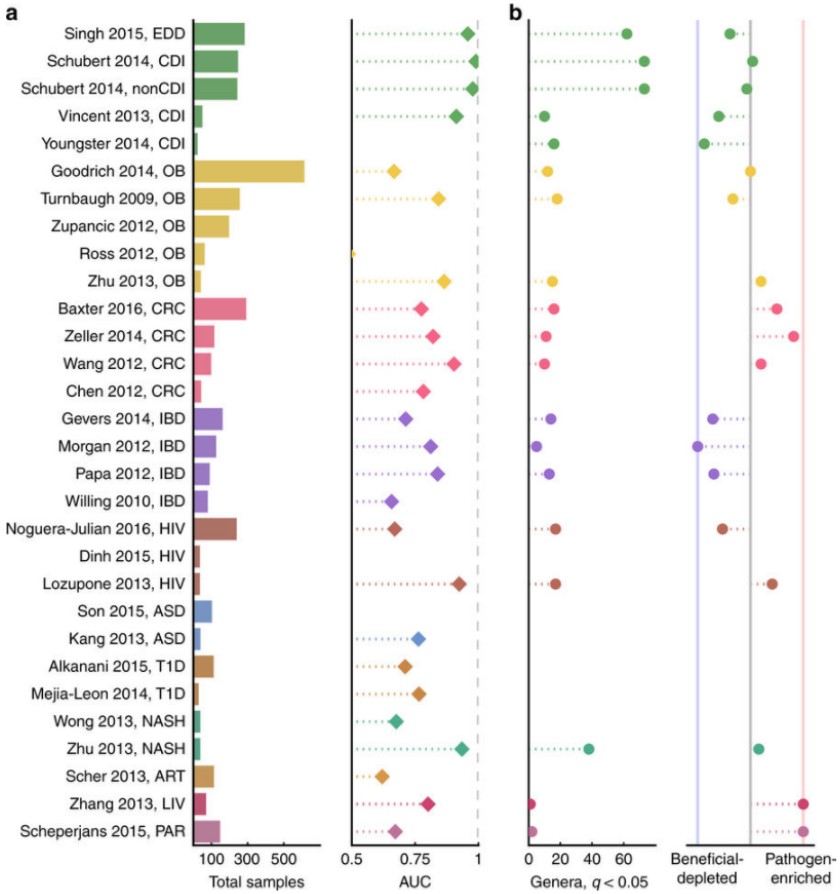
THE CHICKEN OR THE EGG: A "COMMON GROUND" HYPOTHESIS

Lynch and Pedersen, N Engl J Med. 2016



META-ANALYSIS OF GUT MICROBIOME STUDIES IDENTIFIES DISEASE-SPECIFIC AND SHARED RESPONSES

Duvallet *et al.*, Nat Commun. 2017



1. **LOSS OF BENEFICIAL MICROBES**
   (e.g., butyrate-producing Clostridiales)
   → *probiotics replacing missing taxa*

2. **ENRICHMENT OF PATHOGENS**
   ~~(e.g.~~, *Fusobacterium*)
   narrow-spectrum antimicrobials

3. **BROAD RESTRUCTURING**
   ~~(e.g.~~, diarrhoea)
   faecal microbiota transplantation

Modulation of the gut microbiota dysbiosis in T2D patients by a macrobiotic diet

Candela M, Biagi E, Soverini M, Consolandi C, Quercia S, Severgnini M, Peano C, Turroni S, *et al.,* Br J Nutr 2016;116:80-93.

40 overweight/obese subjects affected by T2D, randomized, controlled, open-label 21-day trial

(BMI: 27-45; Age: 40-77)

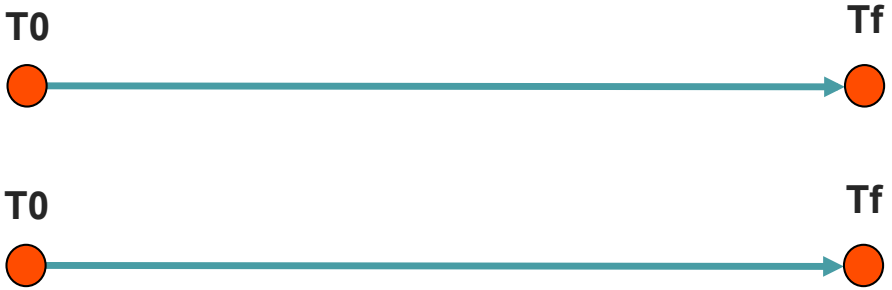
21 subjects **Macrobiotic diet**

72% carbohydrate, 18% fat, 10% protein, 30 g/1000 kcal fiber

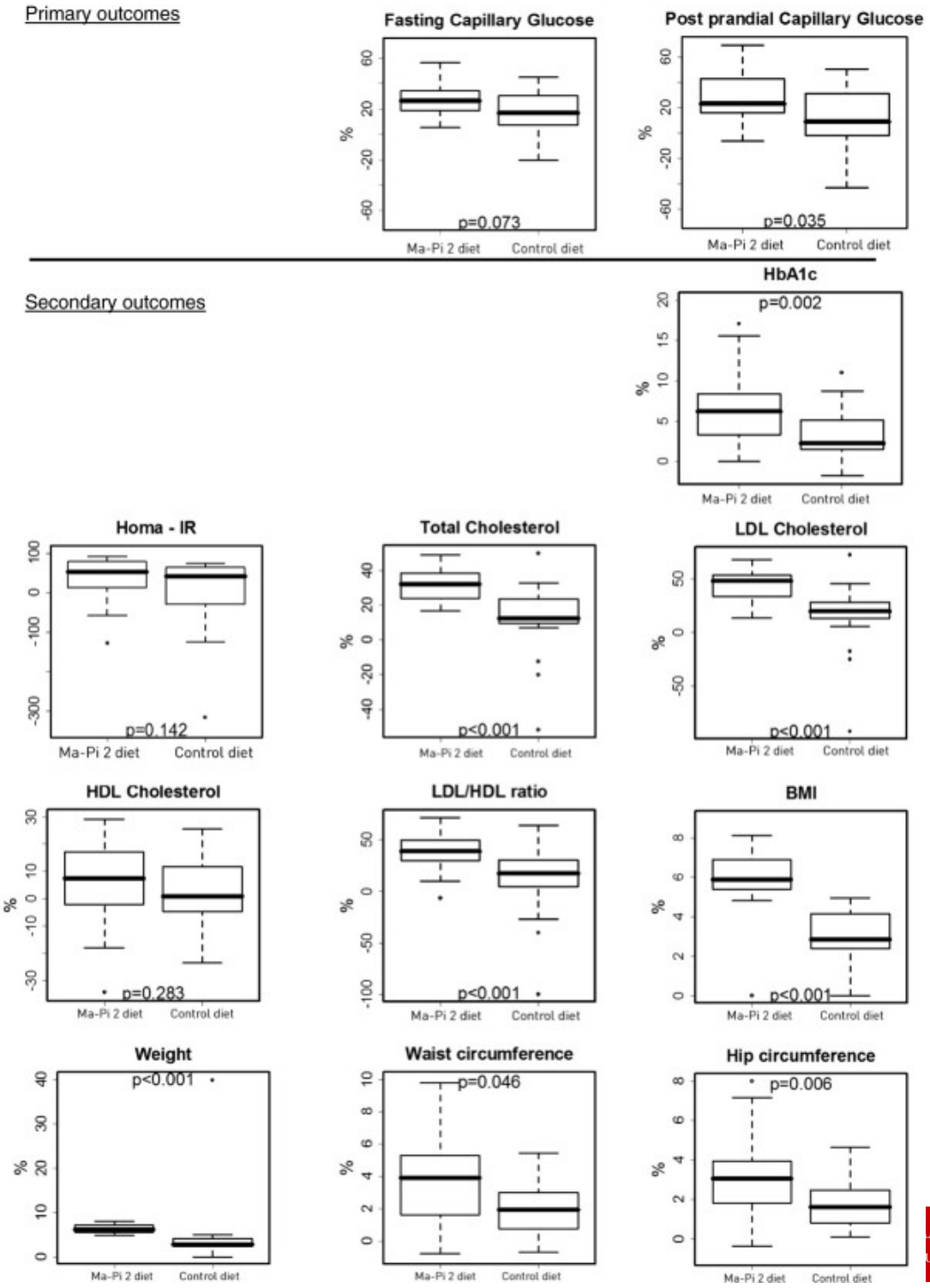
19 subjects **CTR**

standard Italian diet for T2D according to AMD-SID

50% carbohydrate, 30% fat, 20% protein, ≥20 g/1000 kcal fiber



13 NORMAL WEIGHT HEALTHY CONTROLS



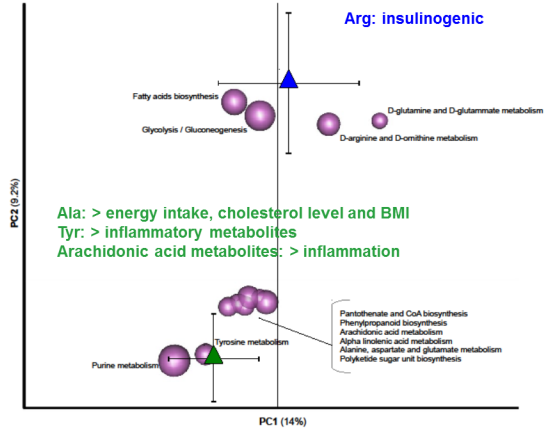
Significantly greater reduction in the **PRIMARY OUTCOMES** FBG and PPBG, as well as in the **SECONDARY OUTCOMES** HbA1c, insulin resistance, total cholesterol, LDL cholesterol and LDL/HDL ratio, BMI, body weight, waist and hip circumference in patients receiving macrobiotic vs control diet

BOTH DIETS: reduced plasma TNF-α levels

MACROBIOTIC DIET: significant reduction in plasma levels of CRP and IL-6

DYSBIOTIC MICROBIAL COMMUNITY IN T2D

Candela *et al.,* Br J Nutr. 2016

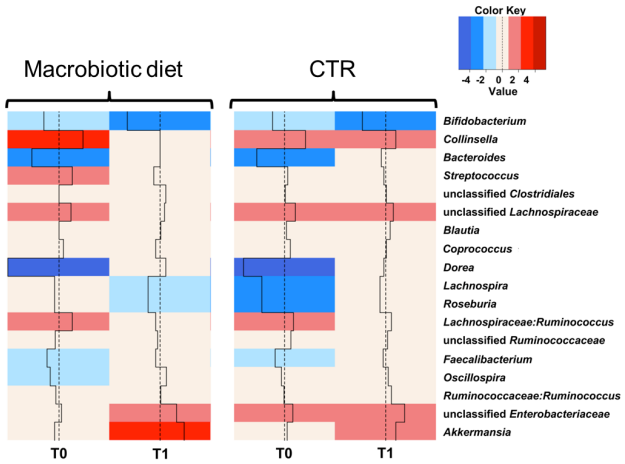


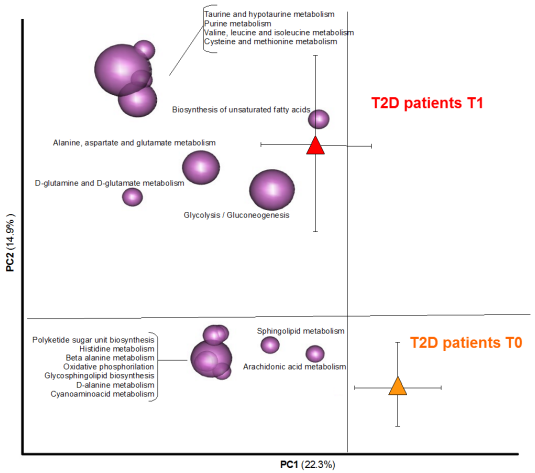
T2D patients (T0)
Healthy controls

- ✓ Significant **REDUCTION OF DIVERSITY**
- ✓ Enrichment in several pro-inflammatory components, «**PATHOBIONTS**» (*Enterobacteriaceae*, *Collinsella*, *Streptococcus*)
- ✓ **DEPLETION OF HEALTH-PROMOTING SCFA PRODUCERS** (*Lachnospiraceae*, *Faecalibacterium*, *Bacteroides*, *Prevotella*)
- ✓ **DE-REGULATION IN PATHWAYS** involved in the metabolism of amino acids, lipids and secondary metabolites

IMPACT OF NUTRITIONAL INTERVENTIONS ON THE MICROBIOTA OF T2D PATIENTS

Candela *et al.,* Br J Nutr. 2016





BOTH DIETS: increased diversity, recovery of a balanced health-promoting community of fibrolytic SCFA producers (*Bacteroides, Dorea, Faecalibacterium),* and *Akkermansia*

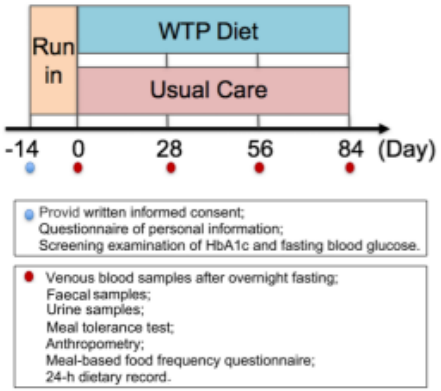
ONLY MACROBIOTIC DIET:
reduction of pro-inflammatory components (*Collinsella, Streptococcus*) and decrease of markers of functional dysbioses (oxidative phosphorylation, glycosphingolipid biosynthesis), increase of functions involved in the biosynthesis of metabolites, including unsaturated fatty acids

RECOVERY OF METABOLIC CONTROL

Gut bacteria selectively promoted by dietary fibers alleviate type 2 diabetes, the open-label, parallel-group GUT2D study

Zhao *et al.*, Science. 2018

A



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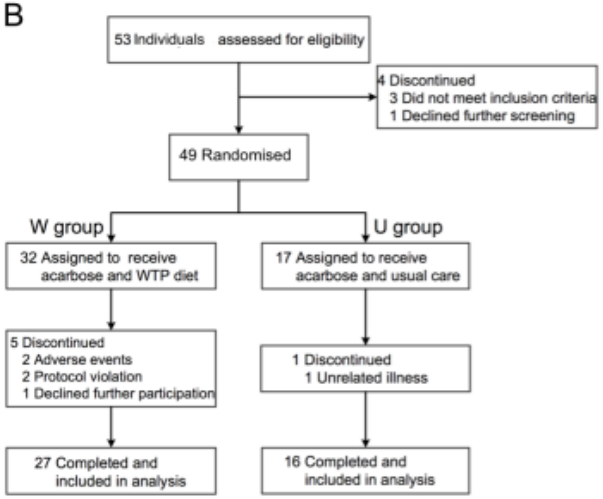


The GUT2D study

Control group, U
16 patients receiving the usual care (education and dietary recommendations based on the 2013 Chinese Diabetes Society guidelines for T2DM)

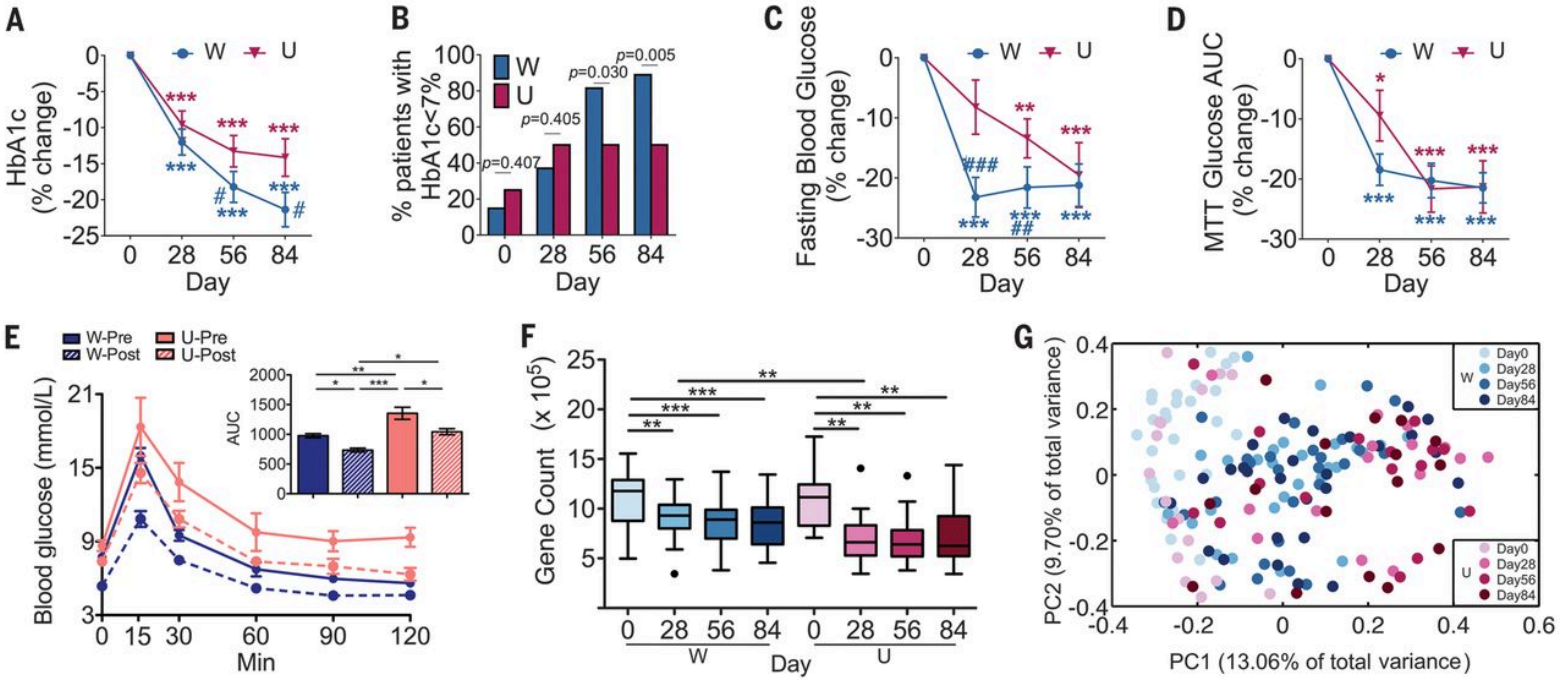
Treatment group, W
27 patients receiving a high-fiber diet (whole grains, traditional Chinese medicinal foods and prebiotics)

Table S2 Daily energy and macronutrient intake before and during the dietary intervention [a].

Group	Daily intake	Day 0	Day 84
W (*N*=24)	Total Energy (kcal)	1924.93±129.67	1874.87±71.10
	Fat (g)	63.48±4.57	58.32±4.04
	Fat %	31.03±1.86	27.54±1.07
	Protein (g)	81.52±5.90	74.58±3.67
	Protein %	16.94±0.63	15.88±0.49
	Total carbohydrate (g)	268.77±25.67	282.72±9.63
	Total carbohydrate %	52.03±2.16	56.58±1.09
	Total fiber (g)	12.12±1.24	37.10±1.90$^{***\#\#\#}$
	Soluble fiber (g)	4.59±0.47	14.61±0.69$^{***\#\#\#}$
U (*N*=14)	Total Energy (kcal)	2063.54±161.42	1954.48±142.80
	Fat (g)	70.44±8.30	62.41±5.14
	Fat %	30.70±2.39	29.16±1.57
	Protein (g)	87.31±9.14	79.32±9.00
	Protein %	16.65±0.88	15.76±0.86
	Total carbohydrate (g)	285.53±24.85	284.94±21.45
	Total carbohydrate %	52.65±2.44	55.08±1.63
	Total fiber (g)	15.43±2.43	16.06±1.95
	Soluble fiber (g)	5.85±0.92	6.09±0.74

Gut bacteria selectively promoted by dietary fibers alleviate type 2 diabetes

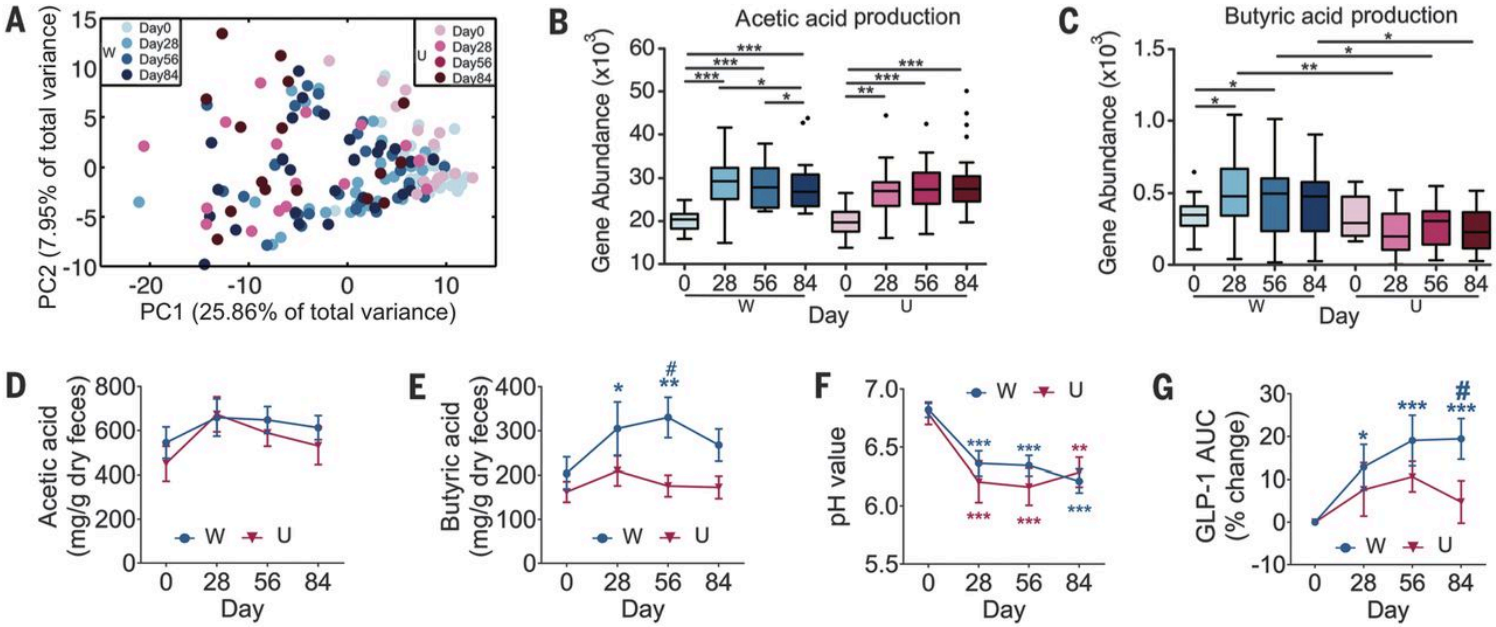
Zhao *et al.*, Science. 2018



A high-fiber diet improves glucose homeostasis (HbA1c, % pts with adequate glycemic control, FBG, MTT glucose AUC, oral glucose tolerance test) and alters the gut microbiota (gene richness, structure) in T2DM

Gut bacteria selectively promoted by dietary fibers alleviate type 2 diabetes

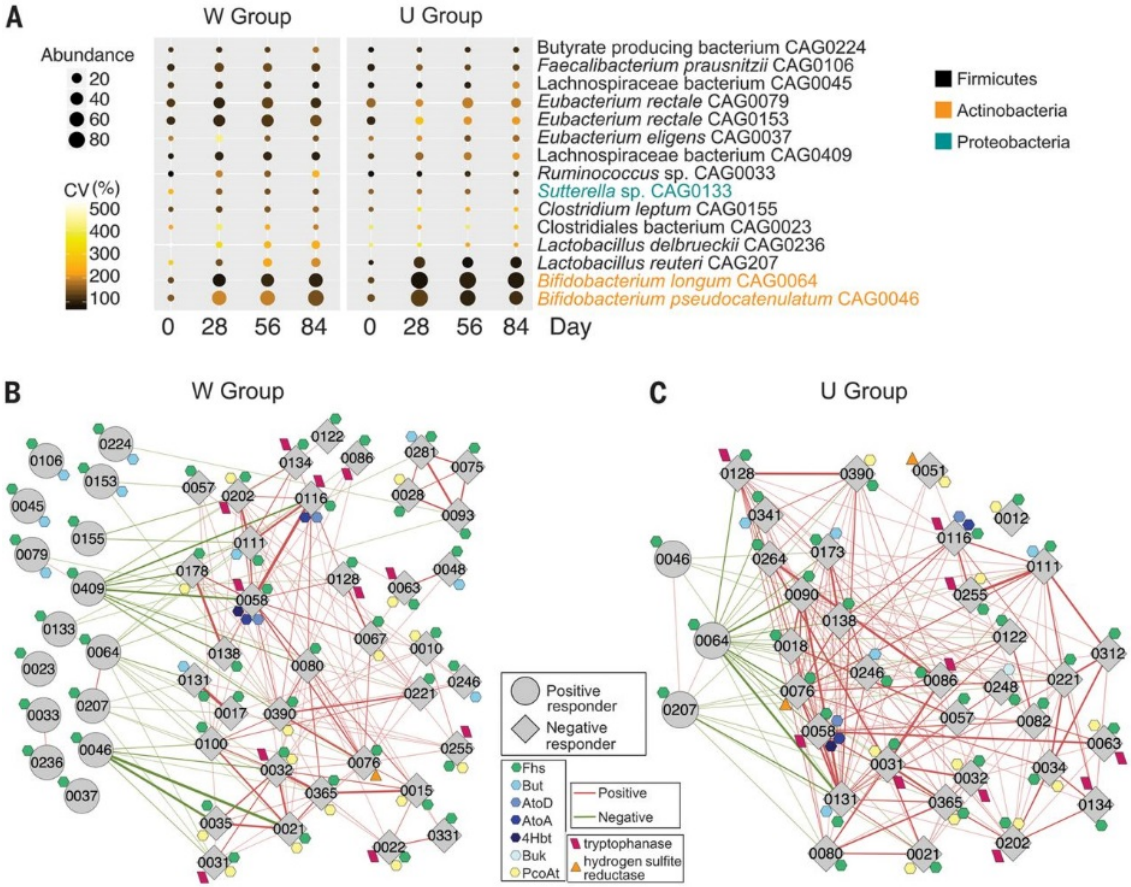
Zhao *et al.*, Science. 2018



A high-fiber diet alters gut bacterial fermentation of carbohydrates (CAZy family genes, genes encoding key enzymes for acetic/butyric acid production, fecal level of metabolites, pH, GLP-1, PYY) in T2DM

Gut bacteria selectively promoted by dietary fibers alleviate type 2 diabetes

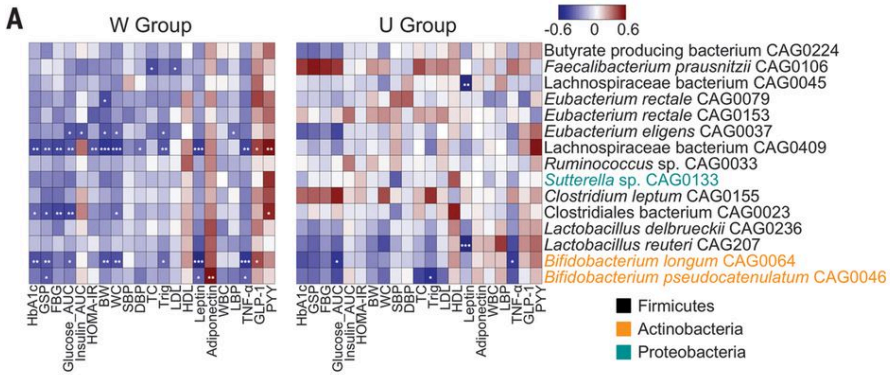
Zhao *et al.*, Science. 2018

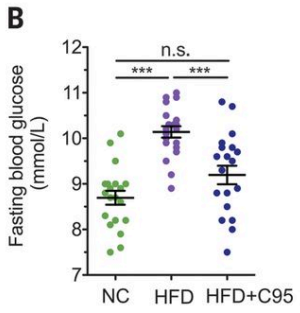


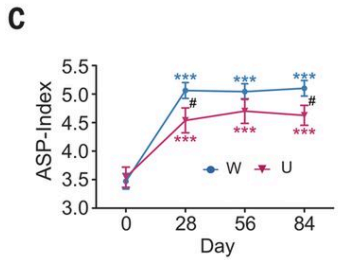
A high-fiber diet selectively promotes a group of SCFA producers as the major active producers (*Faecalibacterium*, *Bifidobacterium pseudocatenulatum*), with diminished proportions of producers of metabolically detrimental compounds (hydrogen sulfide)

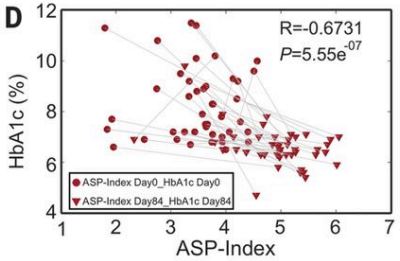
Gut bacteria selectively promoted by dietary fibers alleviate type 2 diabetes

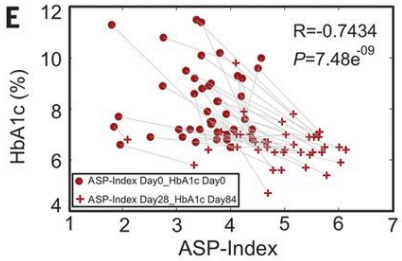
Zhao *et al.*, Science. 2018











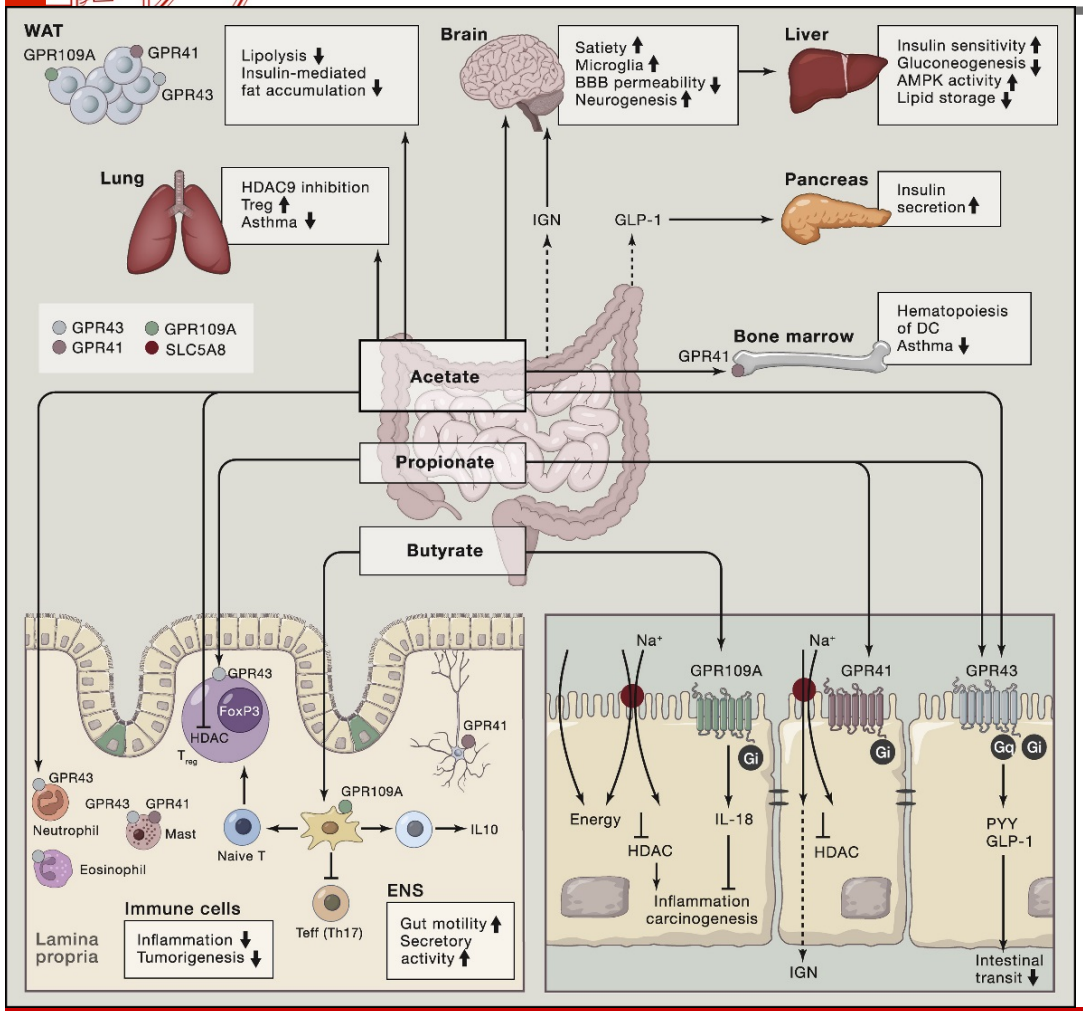
The group of active SCFA producers correlates with metabolic outcomes in T2DM

ASP index, as a measure of effectiveness of microbiome-targeted dietary interventions

TARGETED PROMOTION OF ACTIVE SCFA PRODUCERS AS “ECOSYSTEM SERVICE” PROVIDERS VIA PERSONALIZED NUTRITION, AS A NOVEL ECOLOGICAL APPROACH FOR MANIPULATING THE GUT MICROBIOTA TO MANAGE T2DM AND POTENTIALLY OTHER DYSBIOSIS-RELATED DISEASES

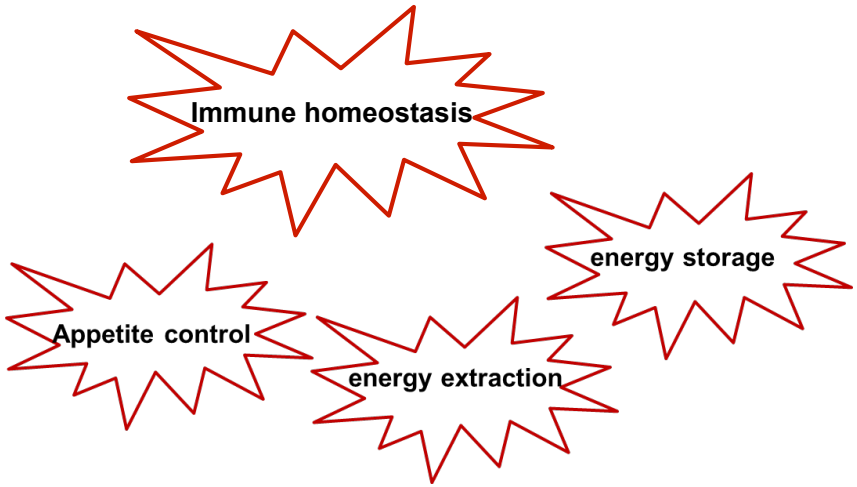
SCFA, MICROBIAL METABOLITES WITH A KEY MULTIFACTORIAL ROLE IN HOST PHYSIOLOGY

Koh *et al.*, Cell. 2016



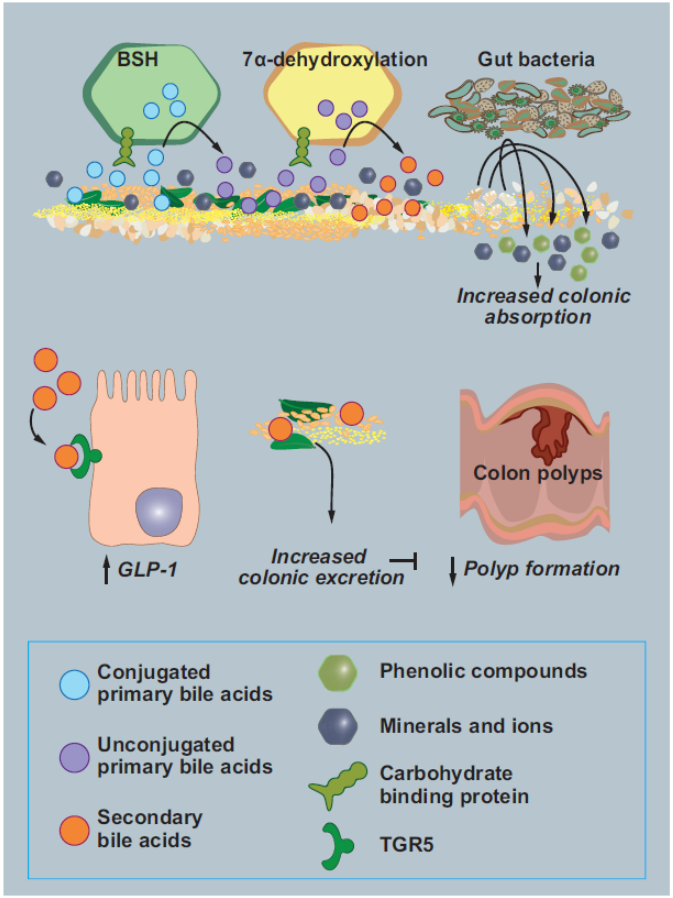
SCFAs as Signaling Molecules

- *HDAC inhibitors*
- *GPCR ligands*



SCFA-INDEPENDENT EFFECT OF DIETARY FIBERS

Makki *et al.*, Cell Host Microbe. 2018

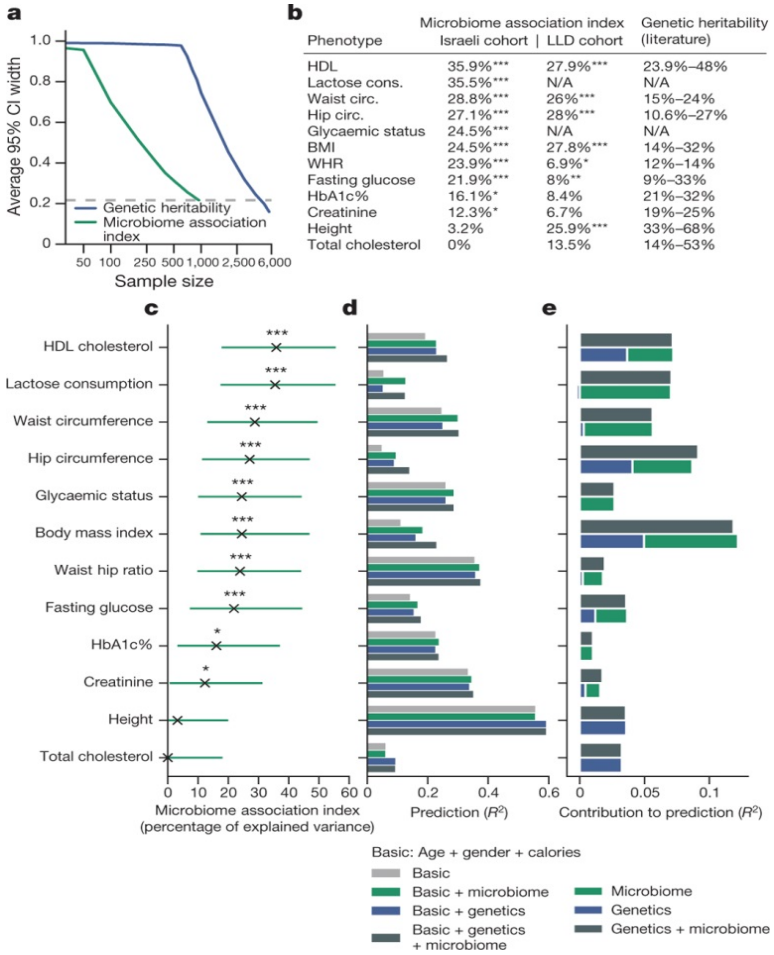


Microbial metabolism of fibers has additional effects:

- **FERULIC ACID** (antioxidant and anti-inflammatory properties, anti-diabetic effects)
- **MICRO- AND MACRO-NUTRIENTS** (anti-microbial action, improved metabolic health parameters)
- **REGULATION OF BILE ACID LEVELS** (by preventing the accumulation of toxic bile acids or increasing the disposal of bile acids that can activate TGR5 to increase GLP-1 secretion)

ENVIRONMENT DOMINATES OVER HOST GENETICS IN SHAPING HUMAN GUT MICROBIOTA

Rothschild *et al*., Nature. 2018



Phenotype	Microbiome association index: Israeli cohort	Microbiome association index: LLD cohort	Genetic heritability (literature)
HDL	35.9%***	27.9%***	23.9%–48%
Lactose cons.	35.5%***	N/A	N/A
Waist circ.	28.8%***	26%***	15%–24%
Hip circ.	27.1%***	28%***	10.6%–27%
Glycaemic status	24.5%***	N/A	N/A
BMI	24.5%***	27.8%***	14%–32%
WHR	23.9%***	6.9%*	12%–14%
Fasting glucose	21.9%***	8%**	9%–33%
HbA1c%	16.1%*	8.4%	21%–32%
Creatinine	12.3%*	6.7%	19%–25%
Height	3.2%	25.9%***	33%–68%
Total cholesterol	0%	13.5%	14%–53%

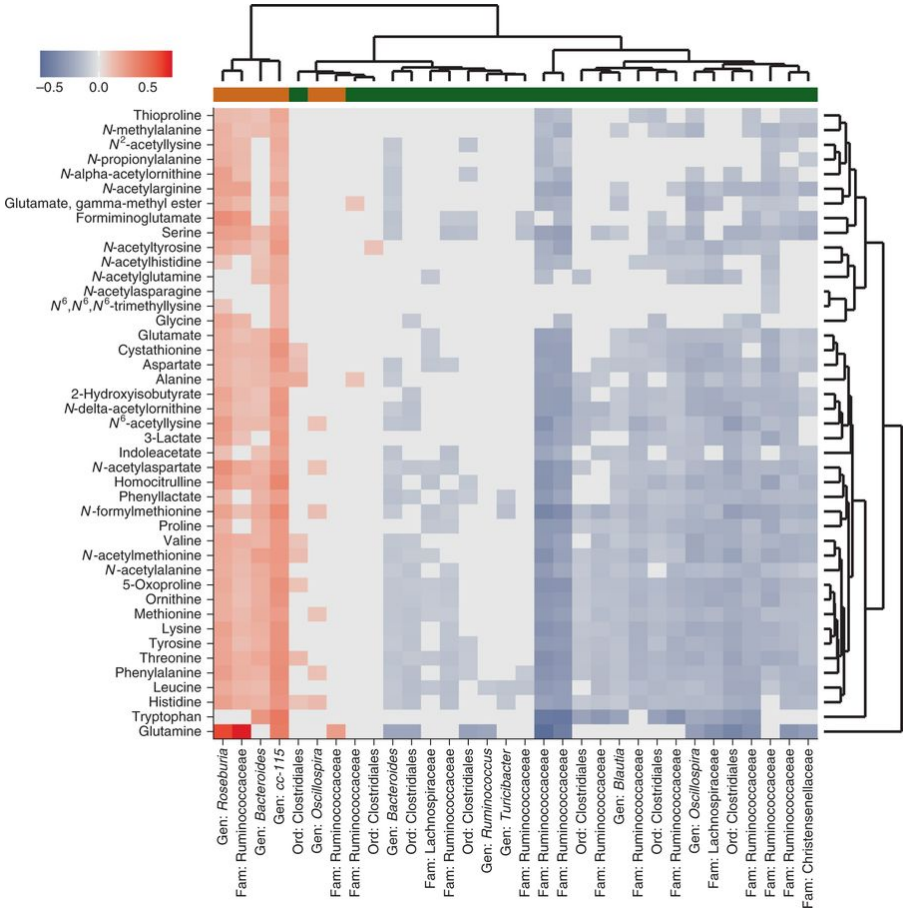
BASED ON A NEWLY DEFINED "**MICROBIOME-ASSOCIATION INDEX**", THE GUT MICROBIOME CAN BE USED TO INFER A SIGNIFICANT FRACTION OF THE VARIANCE OF SEVERAL HUMAN PHENOTYPES:

- HDL cholesterol
- Lactose consumption
- Waist and hip circumference (ratio)
- Glycaemia status & fasting glucose
- BMI

OVER 20% OF THE INTER-PERSON MICROBIOME VARIABILITY IS ASSOCIATED WITH FACTORS RELATED TO DIET, DRUGS AND ANTHROPOMETRIC MEASUREMENTS

THE FECAL METABOLOME AS A FUNCTIONAL READOUT OF THE GUT MICROBIOME – VISCERAL-FAT MASS

Zierer *et al*., Nat Genet. 2018



<u>The fecal metabolome is strongly associated with visceral-fat mass</u>

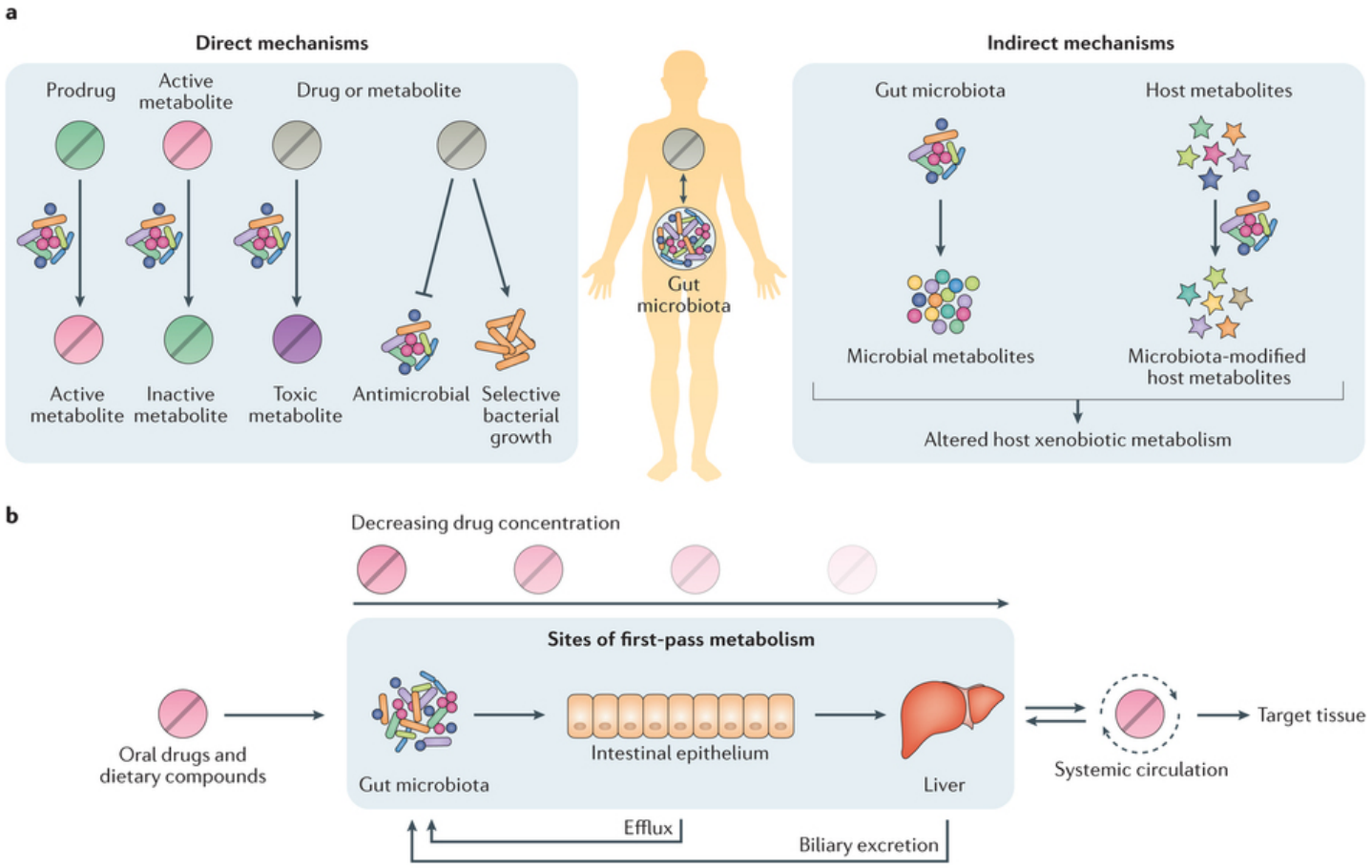
102 associations including 43 amino acids, but also fatty acids – arachidonate -, nucleotides, sugars and vitamins (all positive)

BMI: association with 5 fecal lipids (arachidonate), the hemoglobin metabolite bilirubin and two unknown metabolites

THE FECAL METABOLOME AS AN INTERMEDIATE PHENOTYPE PROMOTING MICROBIAL EFFECTS ON THE HOST AND VICE VERSA

THE MICROBIAL PHARMACISTS WITHIN US: A METAGENOMIC VIEW OF XENOBIOTIC METABOLISM

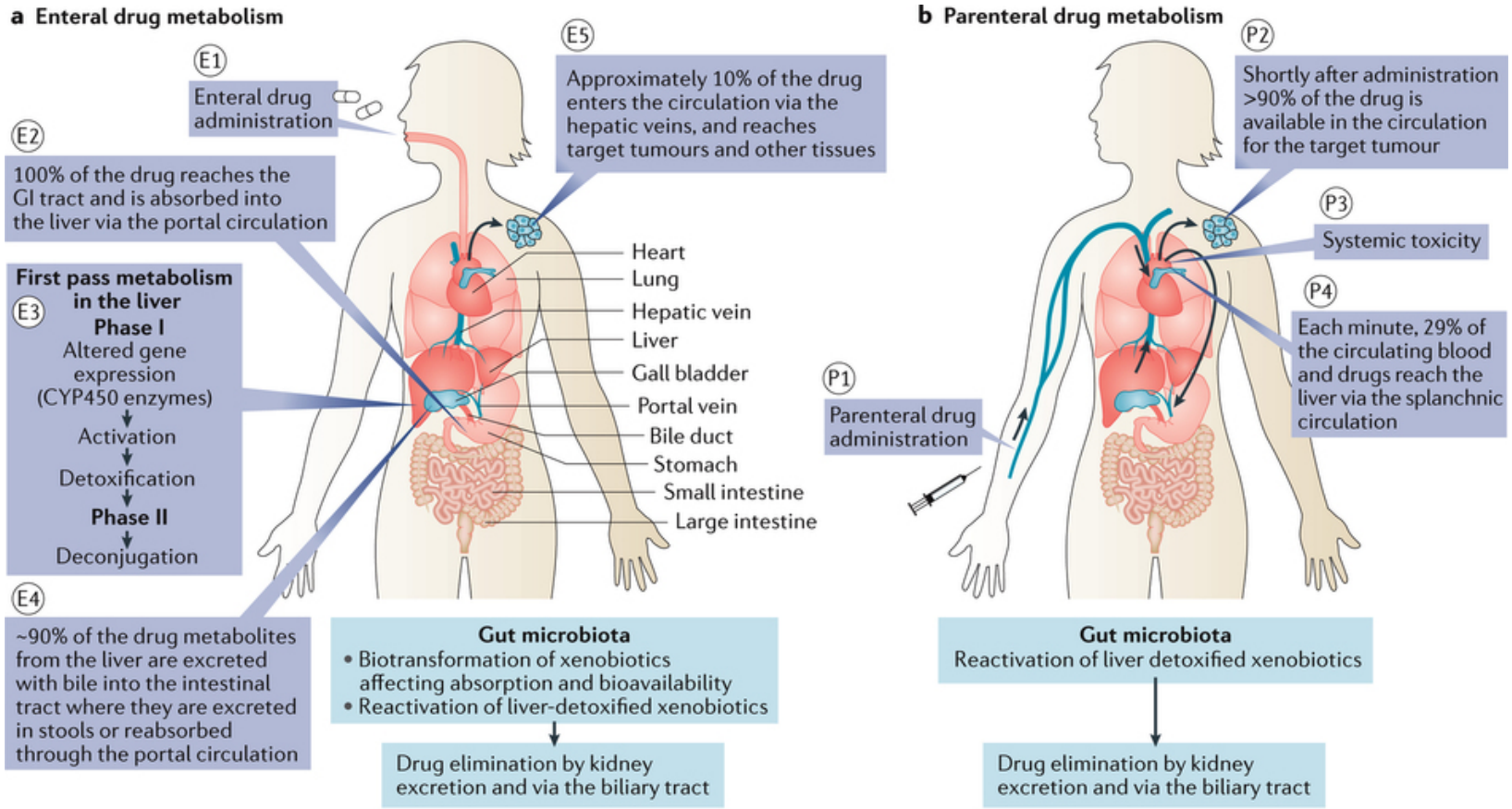
Spanogiannopoulos *et al.*, Nat Rev Microbiol. 2016



Nature Reviews | Microbiology

MICROBIOTA: A KEY ORCHESTRATOR OF CANCER CHEMO, RADIO AND IMMUNOTHERAPY

Roy and Trinchieri, Nat Rev Cancer. 2017



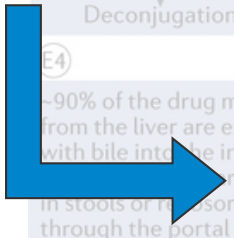
Nature Reviews | Cancer

MICROBIOTA: A KEY ORCHESTRATOR OF CANCER CHEMO, RADIO AND IMMUNOTHERAPY

Roy and Trinchieri, Nat Rev Cancer. 2017

IRINOTECAN (intravenous chemotherapeutic drug used for CRC treatment)

- is transformed into its active form SN-38 by liver and small intestine tissue carboxylesterase and detoxified in the liver by host UDP-glucuronosyltransferases into inactive SN-38-G before being secreted into the gut
- in the gut can be reconverted by bacterial beta-glucuronidases into active SN-38, with significant intestinal toxicity and diarrhoea

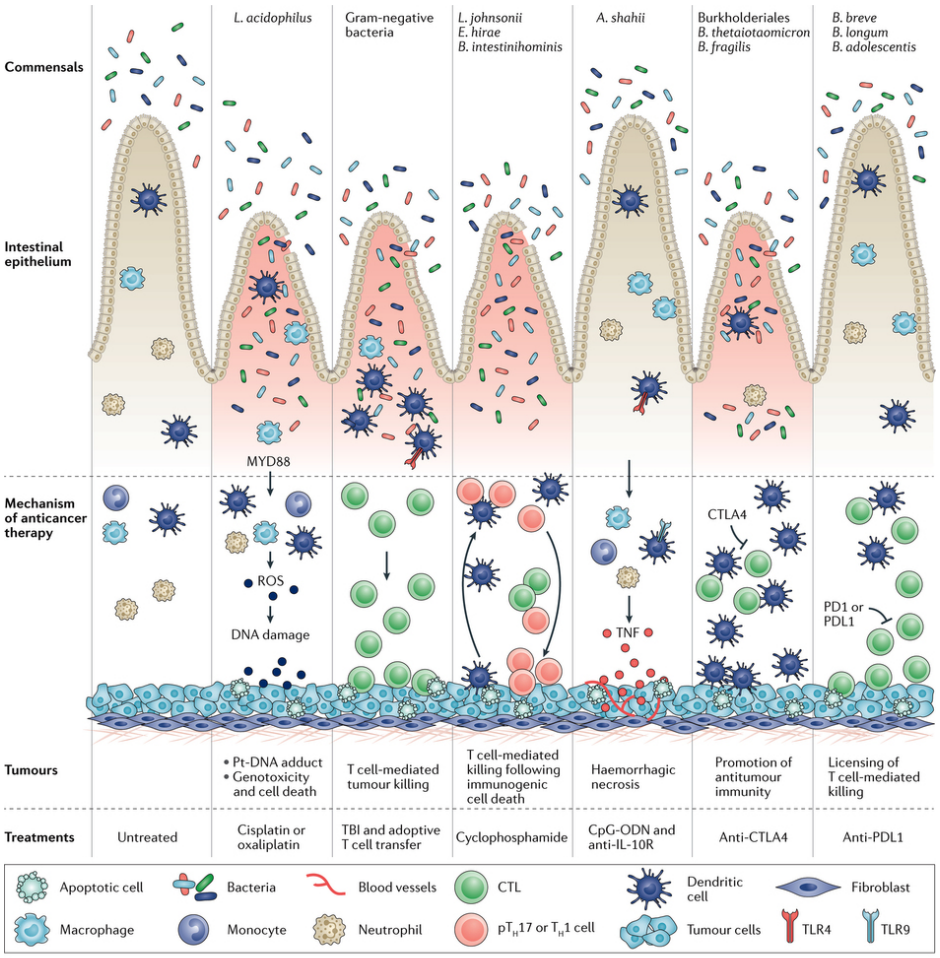


Antibiotic treatment and/or use of an inhibitor specific for bacterial beta-glucuronidases effectively treat intestinal inflammation in animal models

Nature Reviews | Cancer

BACTERIAL SPECIES AFFECTING MECHANISMS OF GUT-ASSOCIATED TOXICITY AND TUMOUR CLEARANCE

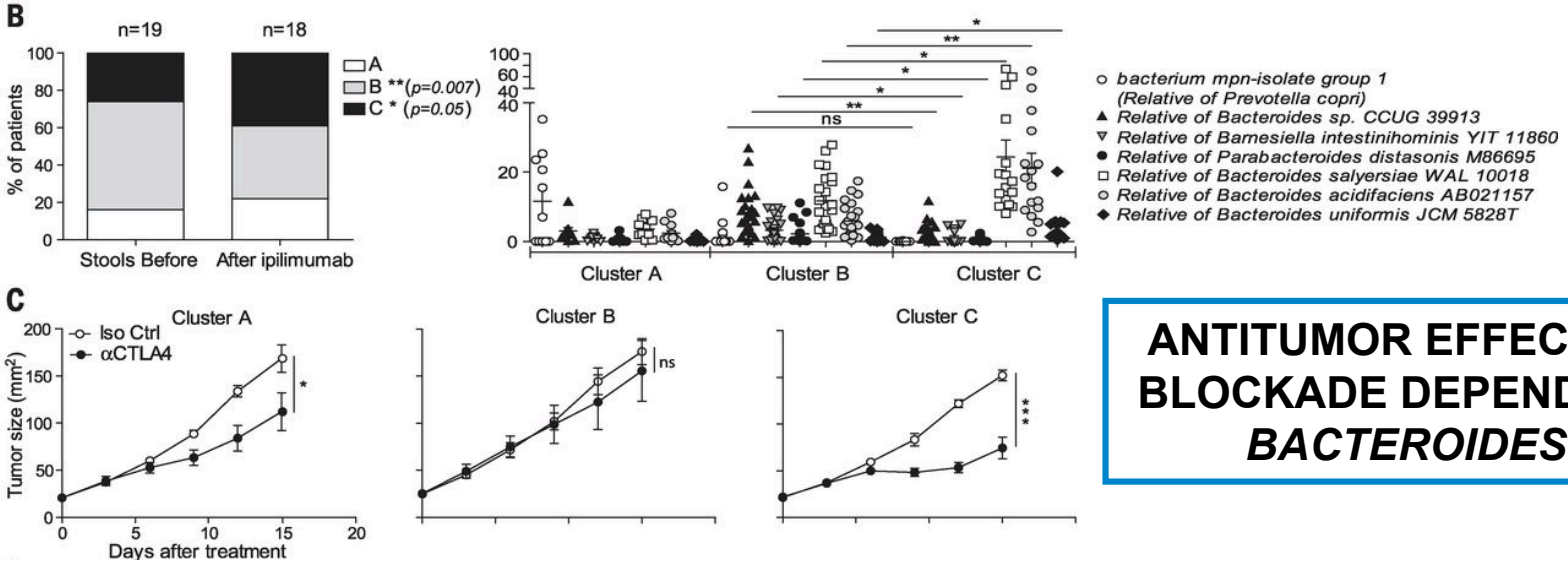
Roy and Trinchieri, Nat Rev Cancer. 2017



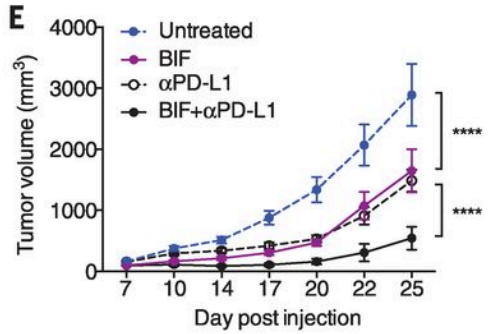
The gut microbiota may impact on anticancer activity, by activating innate immune cells and initiating local and systemic inflammation

IMMUNE CHECKPOINT INHIBITORS AND THE GUT MICROBIOTA: ANTI-CTLA4 AND ANTI-PD-1/PD-L1

Sivan *et al.*, Science. 2015; Vétizou *et al.*, Science. 2015



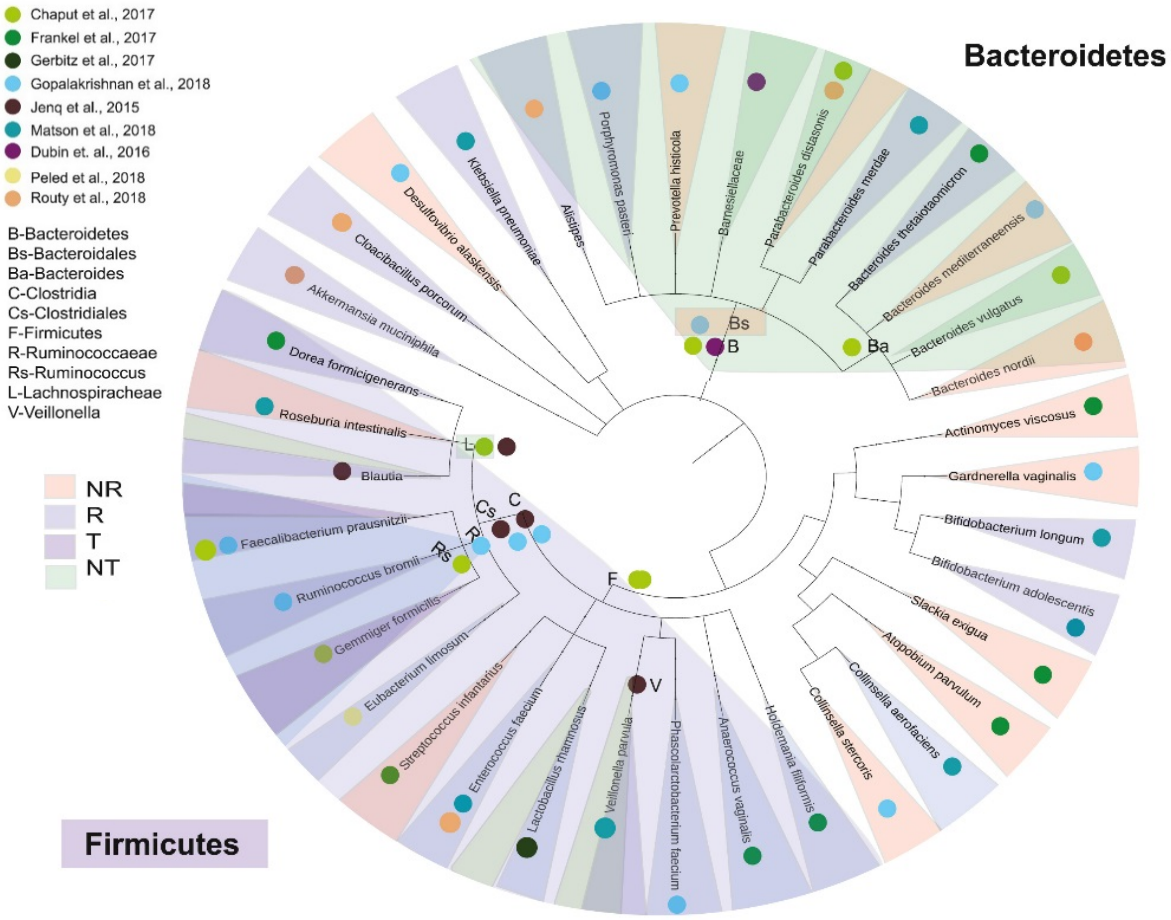
ANTITUMOR EFFECTS OF CTLA-4 BLOCKADE DEPEND ON DISTINCT *BACTEROIDES* SPECIES



COMMENSAL *BIFIDOBACTERIUM* PROMOTES ANTITUMOUR IMMUNITY AND FACILITATES ANTI-PD-L1 EFFICACY

THE GUT MICROBIOME STRUCTURE IS PREDICTIVE OF RESPONSE TO IMMUNOTHERAPY

Gopalakrishnan *et al.*, Cancer Cell. 2018



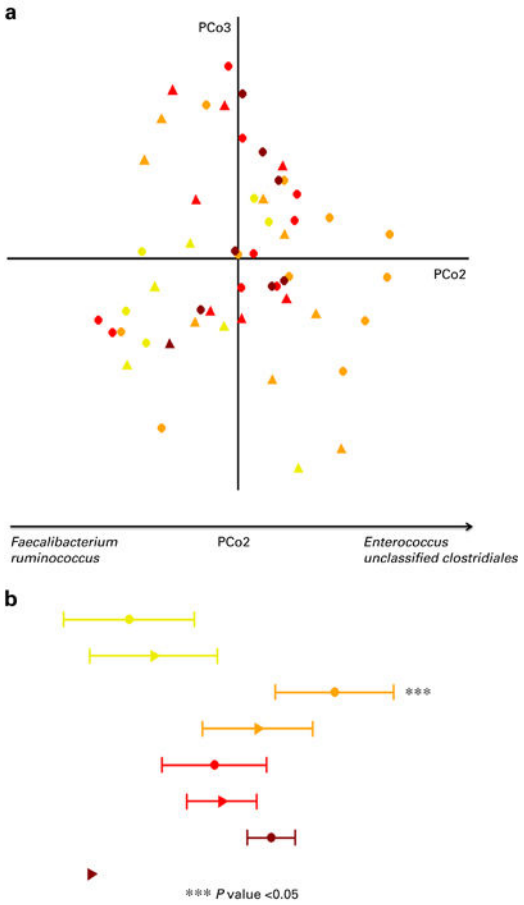
A more "favourable" gut microbiome:

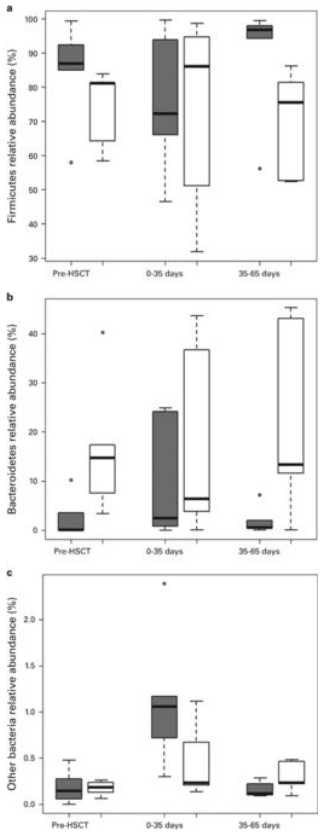
- **higher diversity**
- **higher relative abundance of health-associated microbes (*Ruminococcaceae* and *Lachnospiraceae*)**

Enhanced systemic and anti-tumour immune responses and improved effector T cell function

GUT MICROBIOTA TRAJECTORY IN PEDIATRIC PATIENTS UNDERGOING HSCT

Biagi E, Zama D, Nastasi C, Consolandi C, Fiori J, Rampelli S, Turroni S, Centanni M, Severgnini M, Peano C, de Bellis G, Basaglia G, Gotti R, Masetti R, Pession A, Brigidi P, Candela M. Bone Marrow Transplant. 2015 Jul;50(7):992-8.



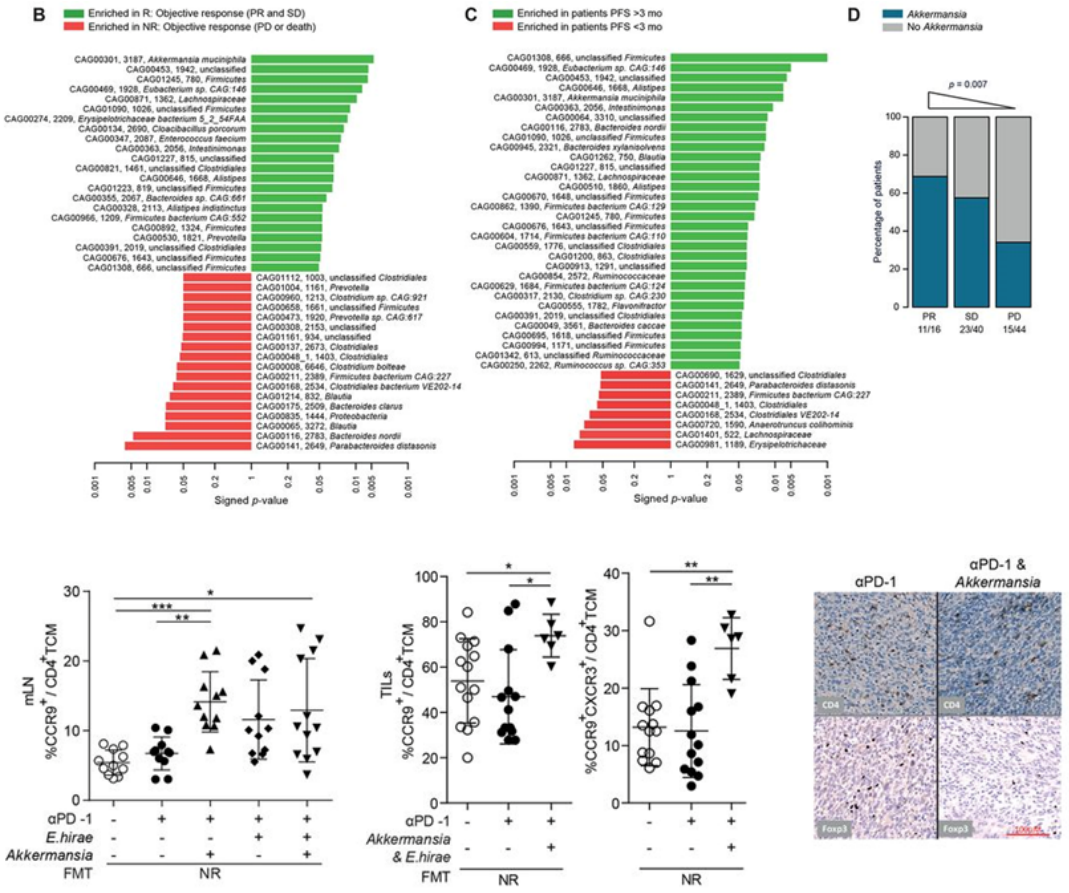


- **Disruption of the existing state of equilibrium of the gut microbiota post-HSCT** (loss of biodiversity and stability)
- **GVHD is associated with gut microbiota signatures prior to HSCT**

MANIPULATING THE GUT MICROBIAL ECOSYSTEM TOWARDS A MORE "FAVOURABLE" GUT MICROBIOME CONFIGURATION

MANIPULATING THE GUT MICROBIAL ECOSYSTEM TO CIRCUMVENT PRIMARY RESISTANCE

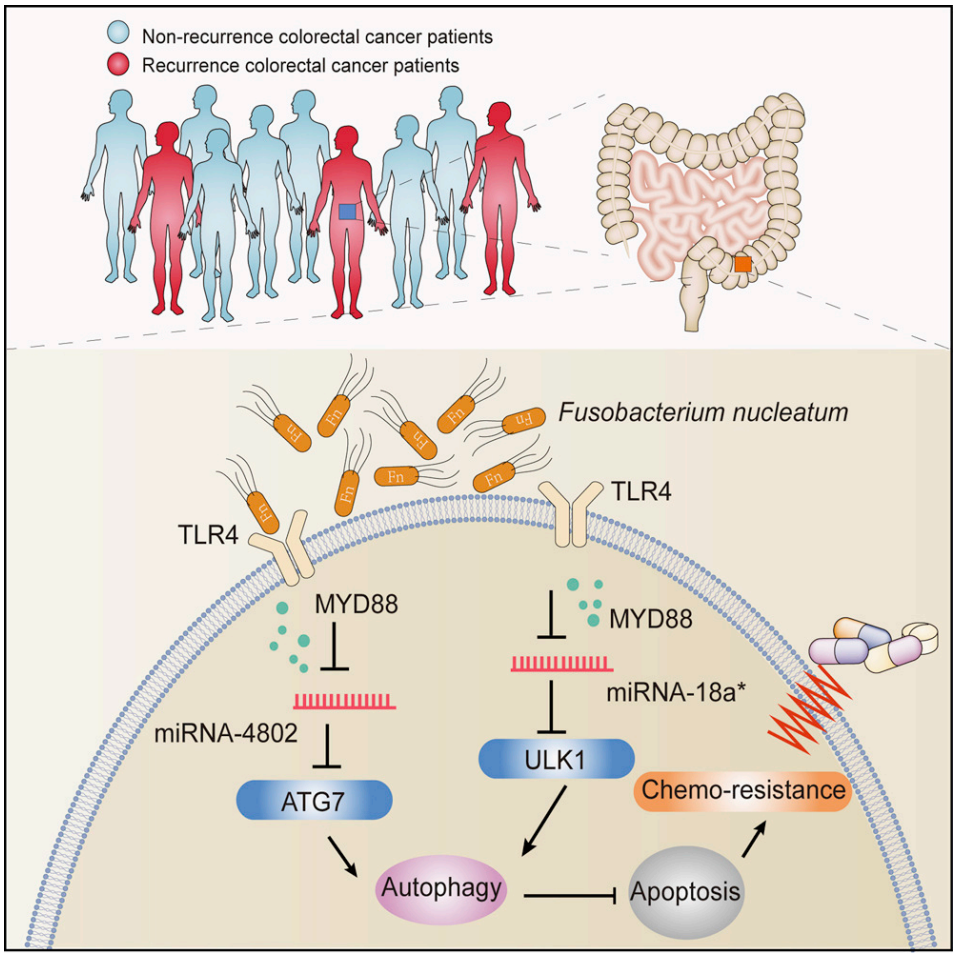
Routy *et al.*, Science. 2018



1. *Akkermansia muciniphila* was significantly associated with favourable clinical outcome
2. Oral supplementation with *A. muciniphila* post-FMT with NR faeces in mice restored the efficacy of PD-1 blockade in an IL-12-dependent manner

MANIPULATING THE GUT MICROBIAL ECOSYSTEM TO CIRCUMVENT RECURRENCE OF CRC

Yu *et al.*, Cell. 2017



- ***Fusobacterium nucleatum*** **is abundant in CRC tissues in patients with recurrence post chemotherapy**
- ***F. nucleatum*** **promotes CRC resistance to chemotherapy by modulating autophagy**
- **Measuring and targeting *F. nucleatum* (antibiotic or autophagy inhibitor) may be useful for patient prognosis and management**

MODULATION OF THE GUT MICROBIOTA AS AN IMPORTANT ADJUNCT TO CURRENT ANTI-CANCER THERAPIES

Gopalakrishnan *et al.*, Cancer Cell. 2018

Table 1. Manipulation of the Gut Microbiome to Enhance Responses to Cancer Immunotherapy

Trial Number	Patient Population	Intervention	Outcome(s)	Status
NCT02843425	all cancer patients treated at MDACC	addition of ½ cup beans per day to regular diet in a crossover design	**primary:** change in fecal microbiome profile from baseline (via 16S profiling)	open and recruiting (MDACC)
NCT02079662	stages II and III breast cancer patients treated at MDACC ages 18+	randomized intensive lifestyle change (diet, exercise, psychosocial)	**primary:** disease-free survival (DFS) **secondary:** change in fecal and oral microbiome (via 16S profiling)	open and recruiting (MDACC)
NCT01895530	CRC patients ages 18+ undergoing elective CRC resection	randomized probiotic (*S. Boulardii*) administration	**primary:** cytokine expression in colonic mucosa (via qPCR) **secondary:** post-operative complications	completed (Consoli et al., 2016)
NCT03072641	CRC patients ages 18+	randomized probiotic (ProBion Clinica *B. lactis* BI-04, *L. acidophilus* NCFM + Inulin) administration	**primary:** change in fecal and tumor microbiota from baseline **secondary:** changes in epigenetic patterns of tumor tissue from baseline	completed (Hibberd et al., 2017)
NCT03358511	post-menopausal breast cancer patients stages I–III	single-arm probiotic (Primal Defense Ultra multi-strain probiotic formula) administration	**primary:** change in mean number of CD8+ cells from baseline	open and recruiting (Mayo Clinic)
NCT02928523	acute myeloid leukemia patients ages 18–65 treated with intensive chemo and antibiotics	single-arm autologous FMT (frozen inoculum)	**primary:** diversity of the gut microbiome, multi-drug-resistant bacteria eradication **secondary:** signature of dysbiosis of gut microbiome	ongoing, closed to recruiting (France)
NCT03353402	metastatic melanoma patients ages 18+ who previously failed standard therapies	single-arm FMT (colonoscopy or gastroscopy) from patient donors who responded to immunotherapy	**primary:** safety (AEs associated with FMT), engraftment of FMT **secondary:** changes in immune cell populations and activity, objective response rate	open and recruiting (Israel)

MODULATION OF THE GUT MICROBIOTA AS AN IMPORTANT ADJUNCT TO CURRENT ANTI-CANCER THERAPIES

Gopalakrishnan *et al.*, Cancer Cell. 2018

Several ongoing and planned clinical trials will investigate the therapeutic potential of manipulation of the gut microbiota directly in cancer patients, by:

- **DIET**: favourable safety profile, cost and accessibility of dietary interventions, a simple and safe opportunity for assessing the implications of microbiota and downstream immune manipulation in cancer patients
- **ADMINISTRATION OF BACTERIAL CONSORTIA OR "DESIGNER PROBIOTICS"**: a more feasible method of microbial manipulation in the clinical setting
- **FMT**: the most direct means to manipulate the microbiota

GUT MICROBIOTA-DERIVED MOLECULES IN ANTI-CANCER THERAPY

NITROGENOUS AND SULFUR METABOLITES, pro-inflammatory and pro-carcinogenic (Windey *et al.*, Mol Nutr Food Res. 2012)

SECONDARY BILE ACIDS, with DNA-damaging and hence carcinogenic effects, regulating liver cancer via NKT cells (Ma *et al.*, Science. 2018)

UNFAVOURABLE

SCFAs, anti-tumor activity in inflammation-driven cancers (but the butyrate paradox remains) (Koh *et al.*, Cell. 2016)

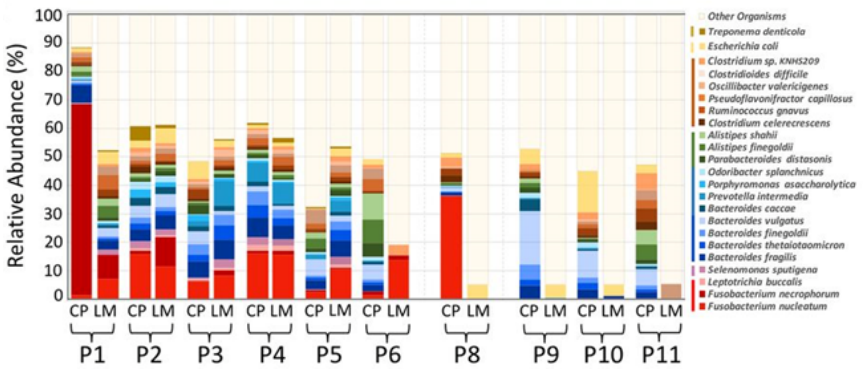
POLYAMINES, especially spermidine that augments anticancer immunosurveillance (Pietrocola *et al.*, Cancer Cell. 2016)

MYROSINASE, to transform host-ingested glucosinolates to sulphoraphane (Ho *et al.*, Nat Biomed Eng. 2018)

FAVOURABLE

TUMOUR BACTERIA: INTRINSIC AND ESSENTIAL COMPONENTS OF THE CANCER MICROENVIRONMENT

Bullman *et al.*, Science. 2017; Geller *et al.*, Science. 2017



Intra-tumour bacteria can play critical roles in mediating chemoresistance