Because you care about CONSUMERS' HEALTH

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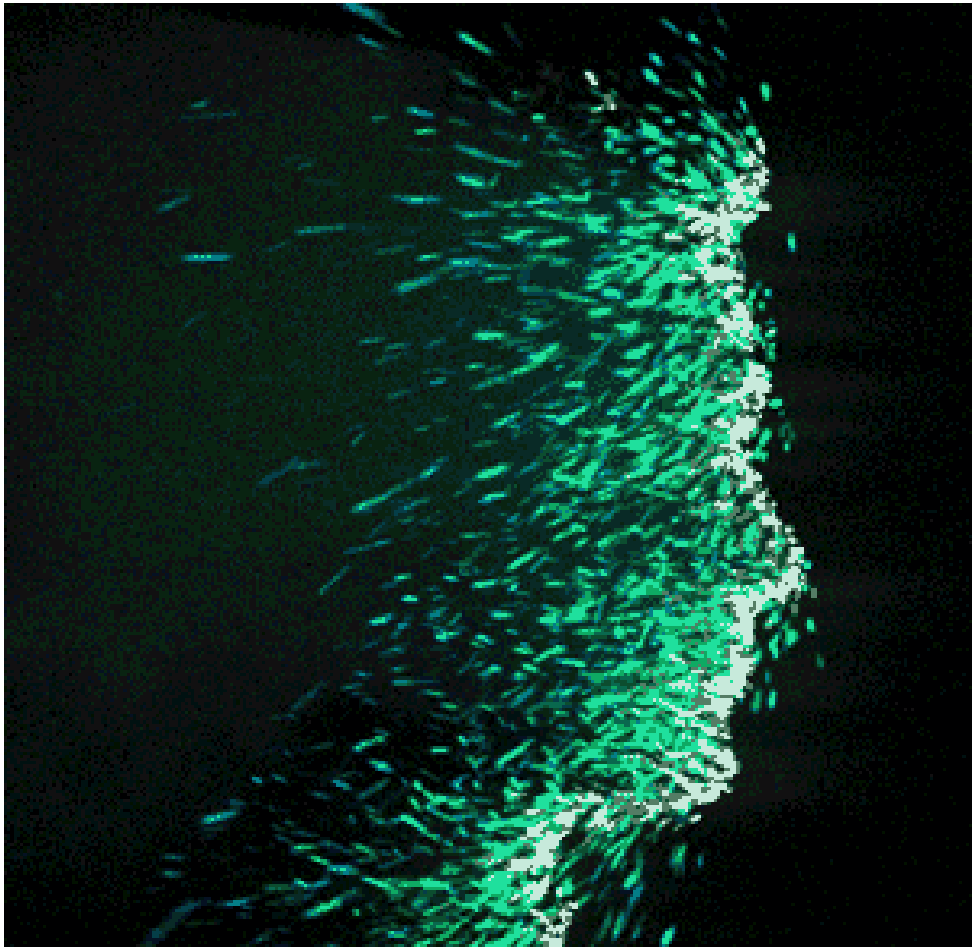
Présente

SFT
Société Française de Toxicologie

BIOFORTIS INNOVATION SERVICES

MERIEUX NutriSciences

Paris, 4-5 Novembre 2019



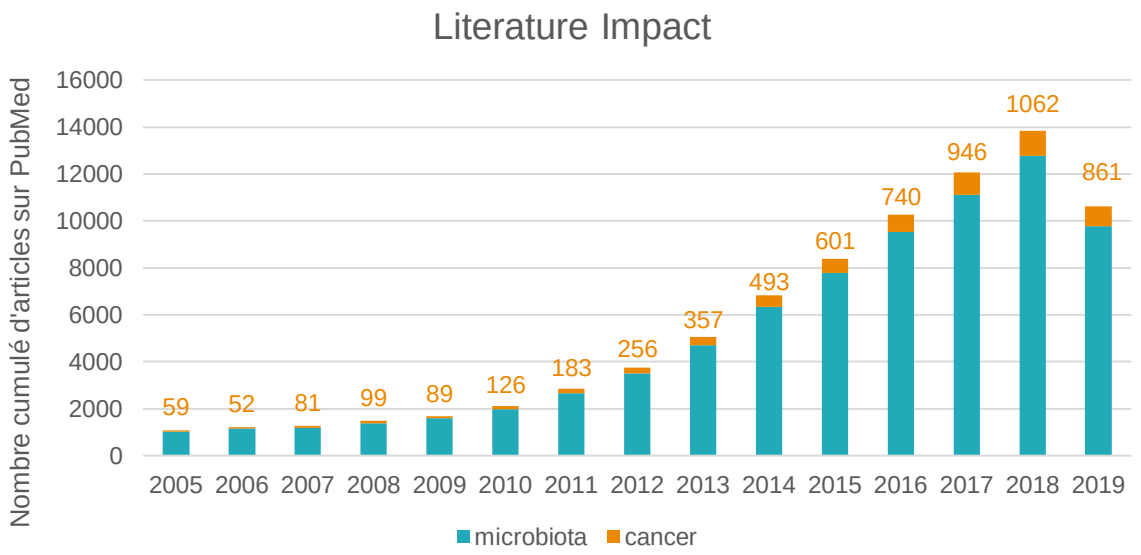
“Prise en compte du microbiote dans la toxicologie de demain”

CONTENT

- The Human Microbiota
- Chemical Effects on Bacteria
- Bacterial Effects on Chemicals
- Tools for Toxicological Assessment
- Contribution of Biofortis
- Take Home Message

Microbiome Acceleration

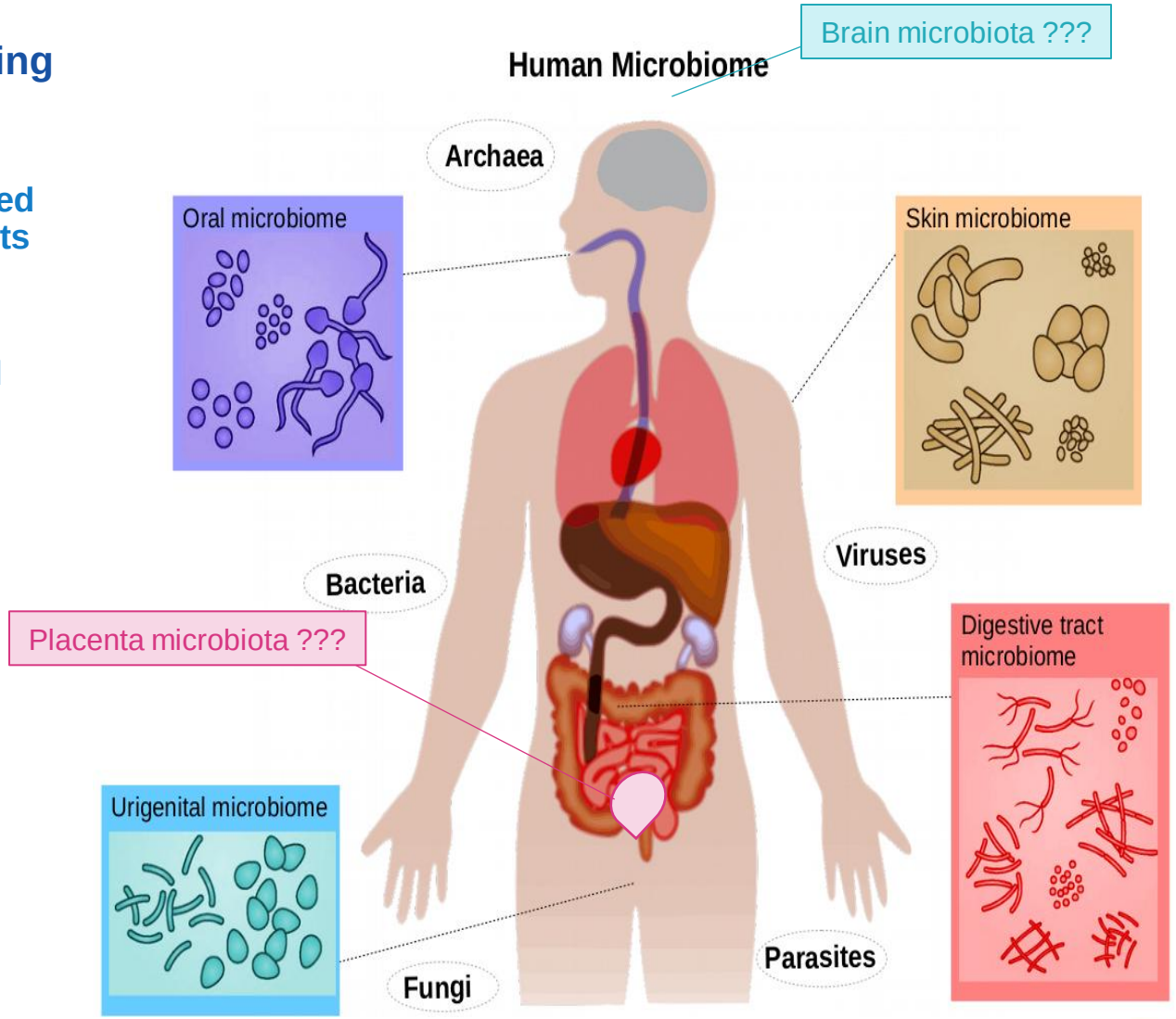
- Increasing number of scientific communications and clinical trials
- Major advances in sequencing and computational biology
- Progress from associations to causality



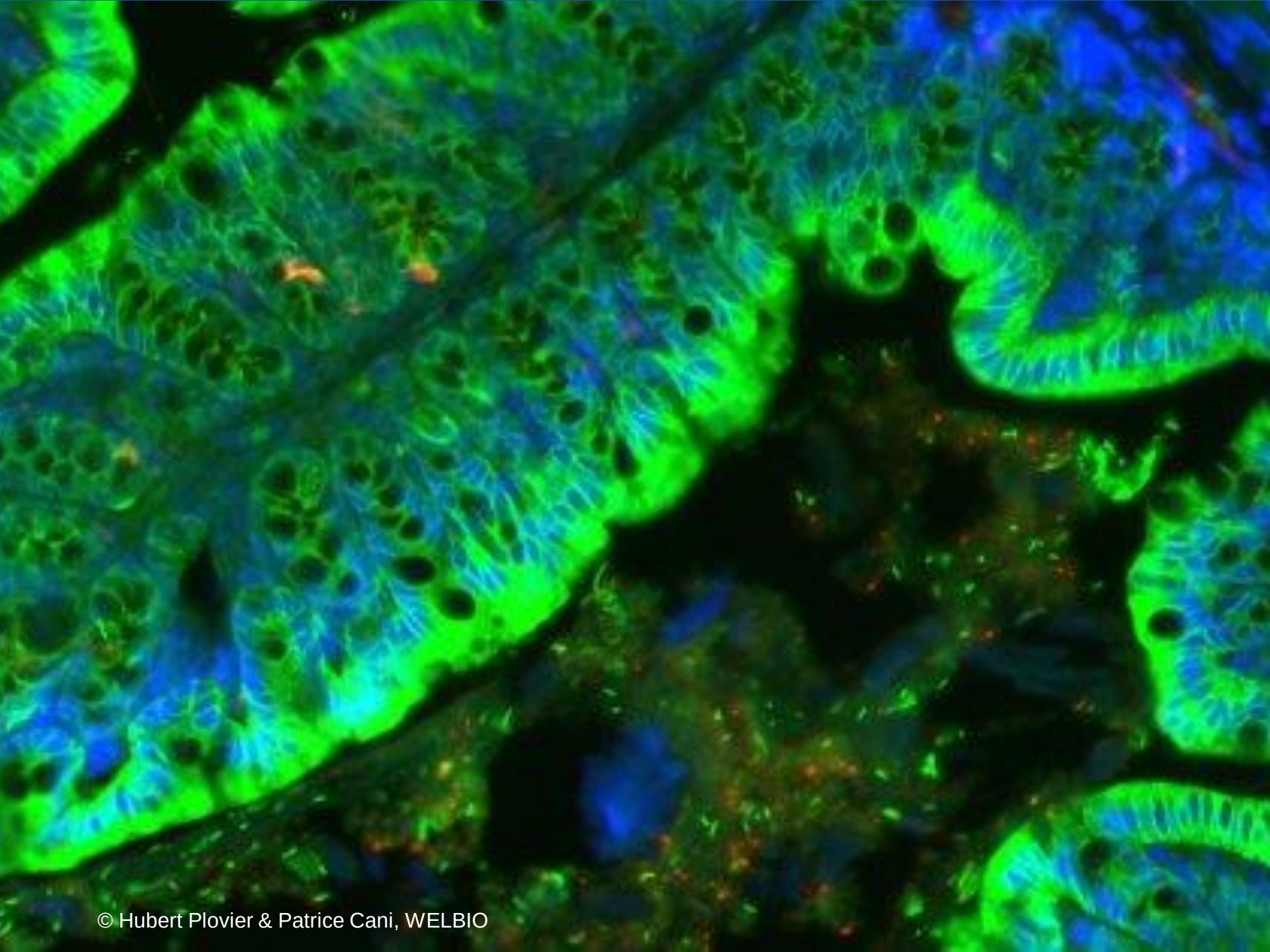
October

The Human Microbiota

- **39 trillions of microbes living commensally in several tissues**
  - **Some microbes associated with known health benefits « symbionts »**
  - **Some potentially harmful microbes « pathobionts »**
  - **E.g. gut 10^{12} to 10^{14} microorganisms**



http://www.whatisbiotechnology.org 2019

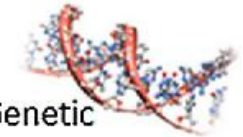


© Hubert Plovier & Patrice Cani, WELBIO

Bacteria in amniotic fluid

Delivery procedure

breast-fed



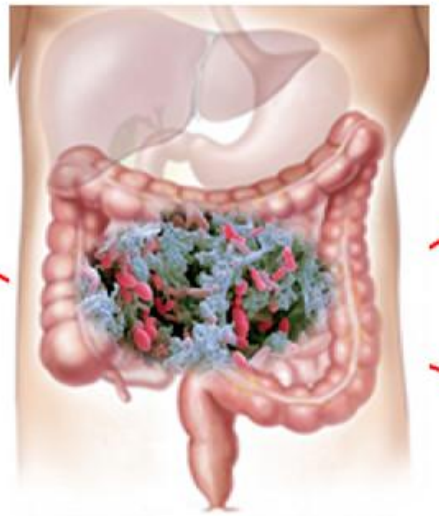
Genetic background

Age

Antibiotics

Diet

Lifestyle



Metabolic functions

- Amino acids synthesis
- Dietary fats absorption
- Fat-soluble vitamins absorption
- Calories removal
- Production of SCFAs
- Composition of bile acid
- Lipid energy metabolism
- Activation of glucose homeostasis

Protective funcions

- Prevention of pathogens colonization
- Dietary fats absorption
- Fat-soluble vitamims absorption
- Innate and adaptative immunity
- Colonization resistance

Stuctural functions

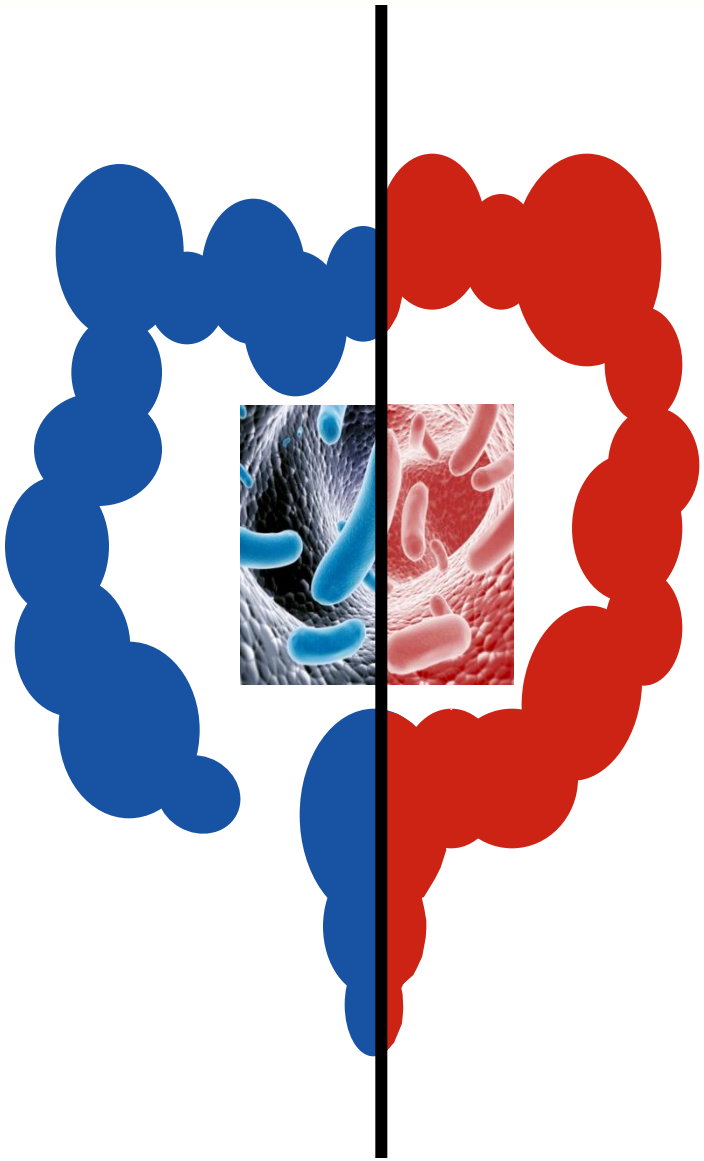
- Intestinal architecture regulation
- Gut permeability regulation
- Immune system and barrier function

Blandino 2016

Gut Microbiota in Health & Disease

Main functions

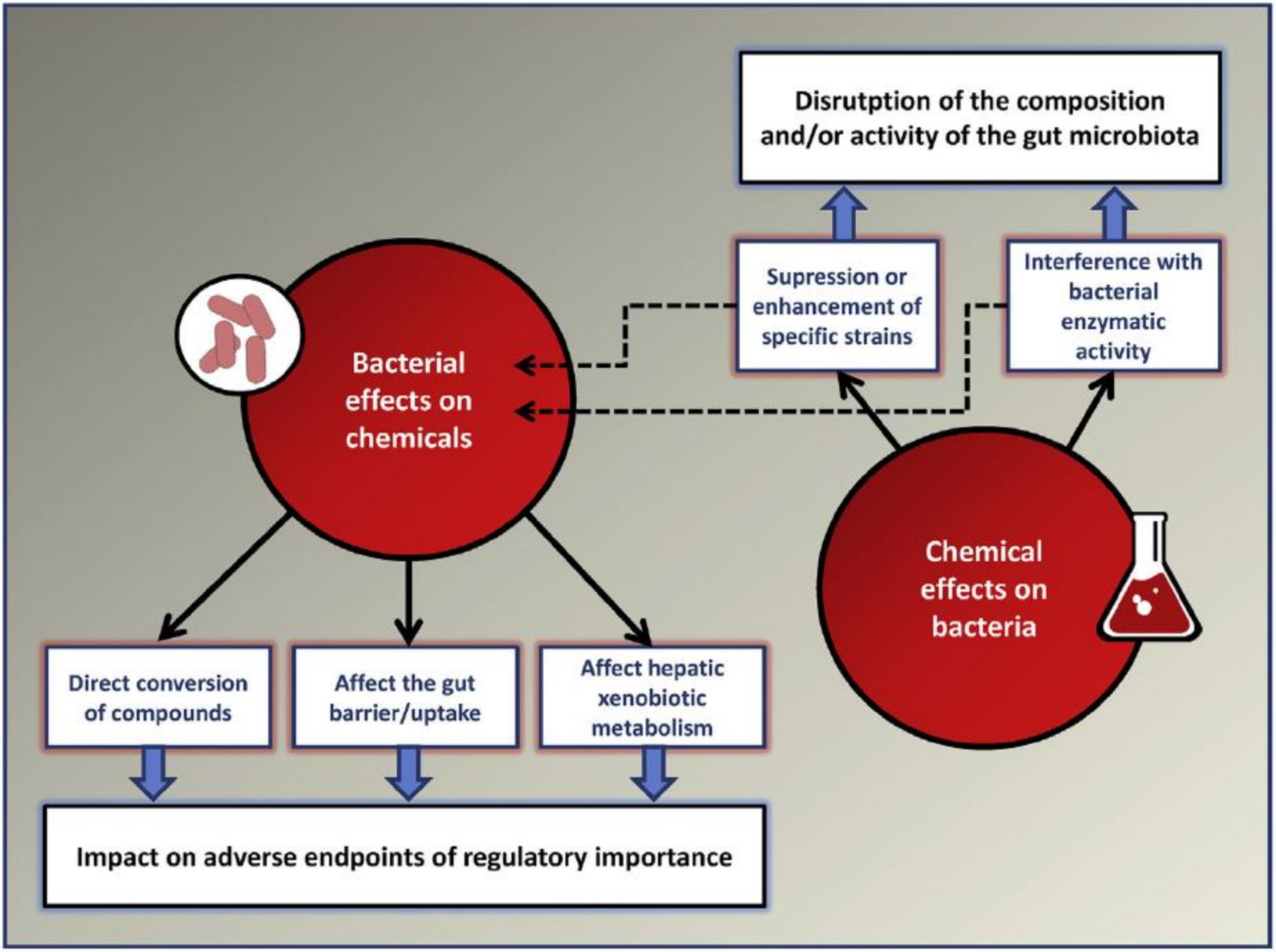
- Immunity
  - Development
  - Functions
- Direct protection against pathogens
- Digestion
- Metabolism
  - Development
  - Functions
- Brain
  - Development
  - Functions



Alteration

- Metabolic disorders
- Brain disorders
- Inflammatory & Immune disorders
- Cancer

Cindy Pensec slide



Rask Licht and Iain Bahl 2018

Chemical Effects on Bacteria

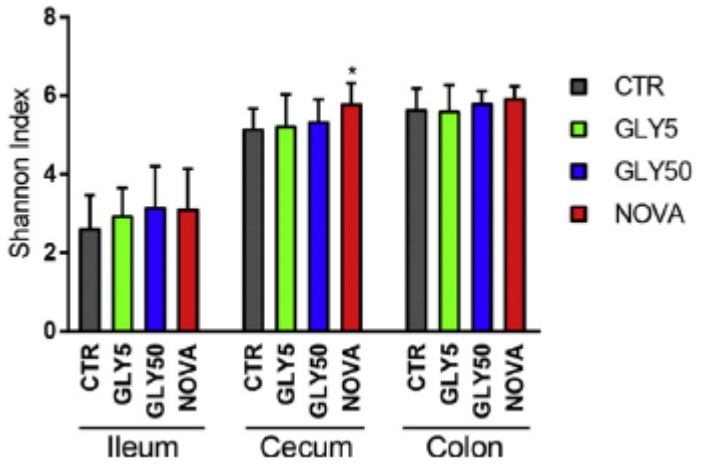
Glyphosate Exposure

in vitro

Species	MIC (mg/mL) BHI
Bifidobacterium adolensis DSM 20083	10
Bifidobacterium bifidum DSM 20456	10
Bifidobacterium breve DSM 20091	10
Bifidobacterium longum subsp. infantis DSM 20088	10
Bifidobacterium animalis DSM 10140	10
Bifidobacterium animalis lactis BL-04	10
Clostridium perfringens CCUG 1795	10
Clostridium leptum DSM 753	10
Clostridium nexile DSM 1787	10
Enterococcus faecalis ATCC 29212	80
Enterococcus faecalis DSM 2570	80
Lactobacillus johnsonii DSM 10533	20
Lactobacillus planetarum DSM 20174	40
Lactobacillus reuteri DSM 20016	40
Lactobacillus rhamnosus ATCC 53103	40
Bacteroides uniformis DSM 6597	5
Bacteroides vulgatus DSM 1447	5
Bacteroides ovatus DSM 1896	10
Bacteroides fragiles DSM 2151	5
Escherichia coli ATCC 25922	80
Escherichia coli DSM 18039	80
Akkermansia muciniphila DSM 22959	20

in vivo

In vivo effects on intestinal bacteria
4-week old male Sprague Dawley rats
2 weeks exposure
16S rRNA gene sequencing



= Intestinal bacteria may be differently affected by glyphosate*

= No effects on alpha diversity

Nielsen *et al.* 2017

* Glyfonova 450 plus

PCB Exposure in Mice

In vivo effects on intestinal bacteria
11 months old male C57BL/6 Charles River
150µM/kg ⇔plasma level 5µM
n=6/group
PhyloChip Arrays -affymetrix

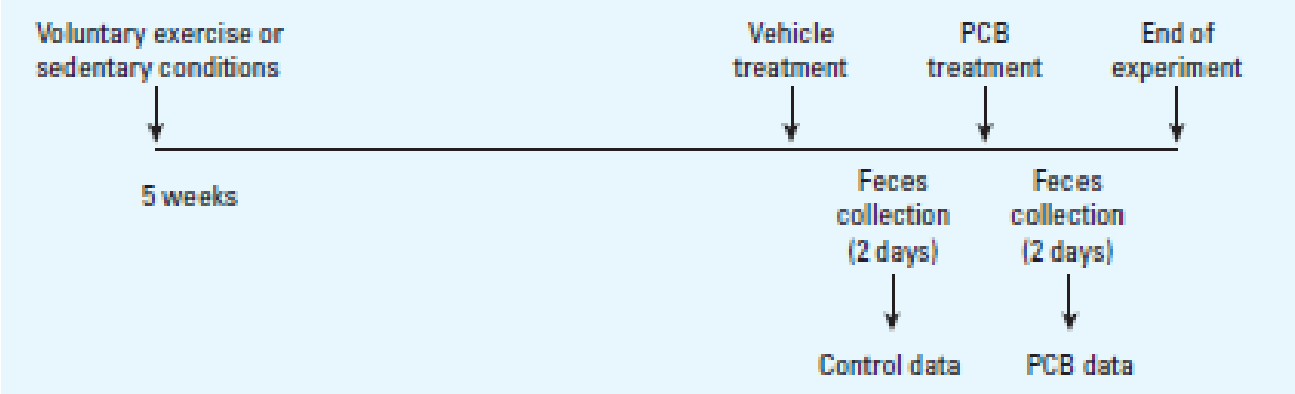


Figure 1. Experimental design indicating treatment and sampling times.

= PCB exposure decreases the abundance of the gut microbiota

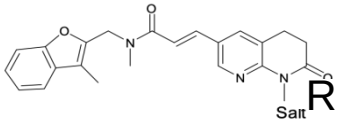
= Exercise attenuates PCB-induces alterations of gut microbiota composition

Choi *et al.* 2013

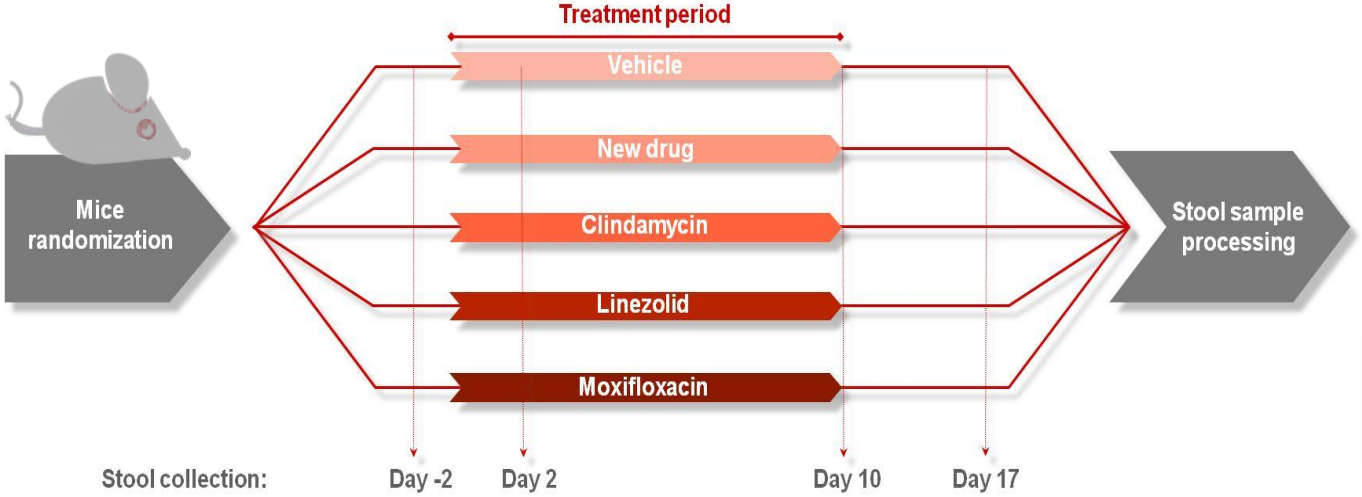
Antimicrobial Drug Candidate

- Does a new drug targeting *Staphylococcus aureus* disturb the gut microbiota?

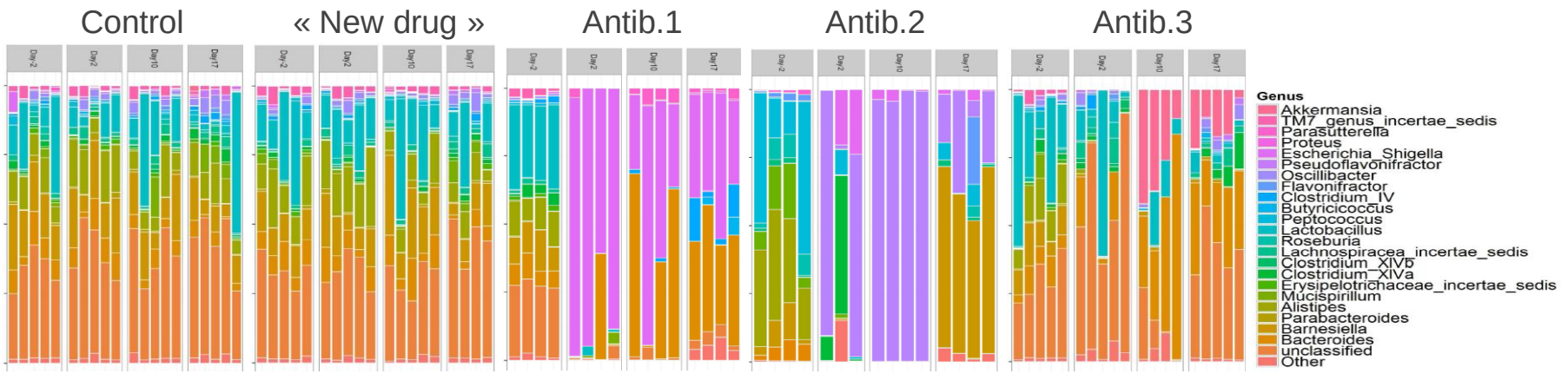
Debio 1450 (prodrug)

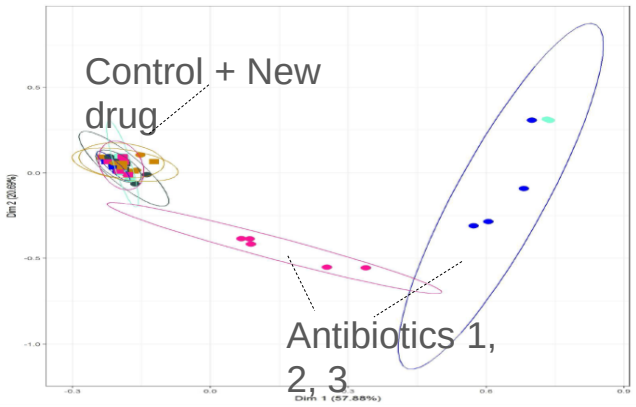


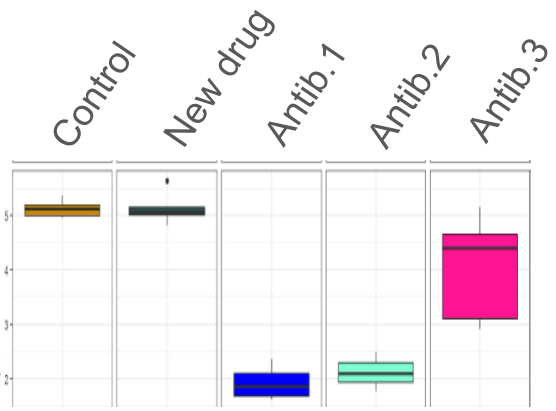
Study design



Antimicrobial Drug Candidate : Results







No observed side effect onto composition compared to reference antibiotherapies

+ No impact on Diversity

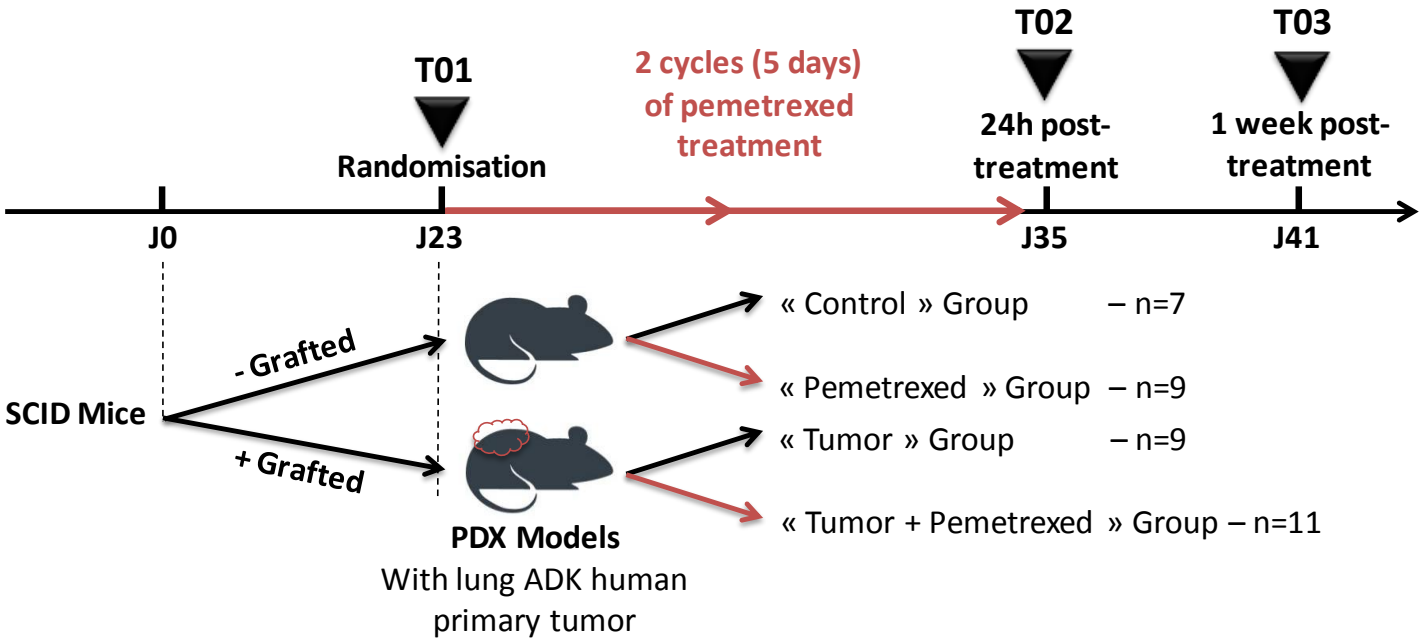
Kuehn J, Wittke F, Barbier M, Le Fresne S, Carton T, Leuillet S, Le Vacon F, Vuagniaux G. . Microbiome R&D and Business Collaboration Forum: Europe; 2017 April 3-4; Amsterdam

Toxicity of Chemotherapy on Microbiota

Mechanism	Chemotherapy	Effect
Translocation	Doxorubicin	Commensal bacteria cross the intestinal barrier to enter secondary lymphoid organs
Immunomodulation	Cyclophosphamide	Gram+ commensals mediate accumulation of Th17 and Th1-cell response
Enzymatic degradation	CPT11 (Irinotecan)	Bacterial beta-glucuronidase cleaves glucuronide from inactive metabolite, releasing active metabolite(SN-38) in the gut
Reduced diversity	BEAM Carmustine, etoposide, cytarabine and melphalan combination	Chemotherapy associated with increase in bacteria associated with colitis

Adapted from Alexander *et al.*, 2017

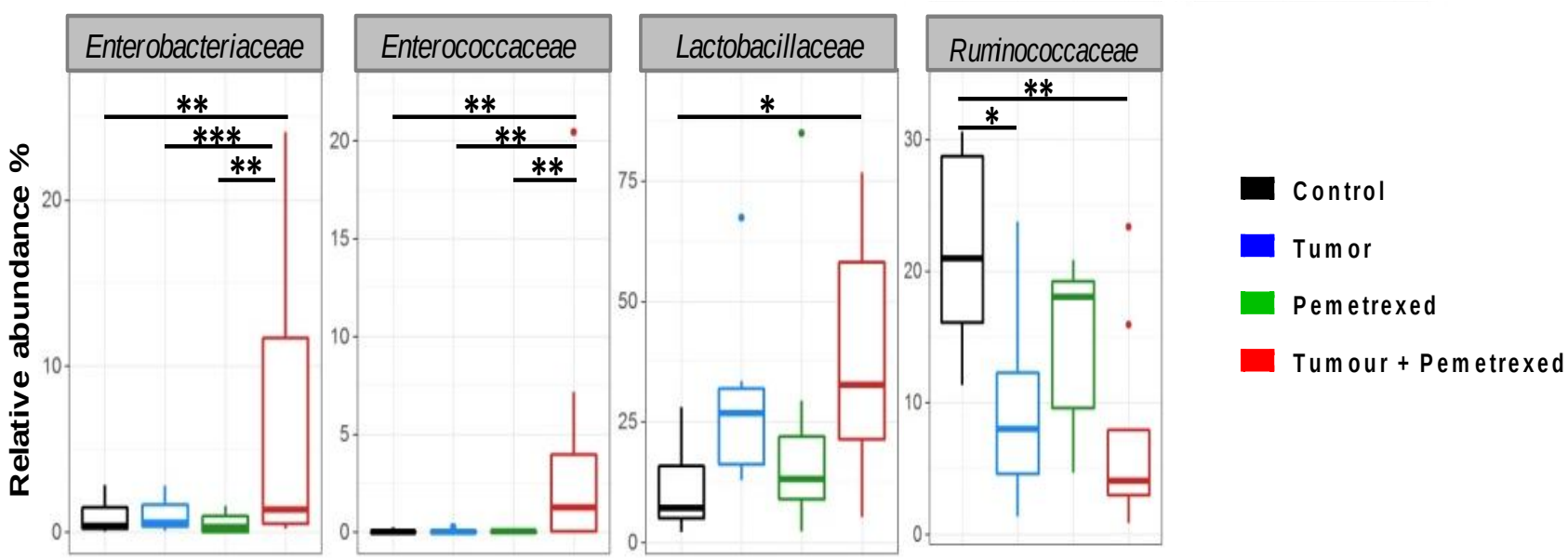
Impact of Pemetrexed on the Gut Microbiome in PDX Models of Lung Cancer



Impact of Pemetrexed on the Gut Microbiome in PDX Models of Lung Cancer

BIOFORTIS INNOVATION SERVICES

16S rRNA gene sequencing

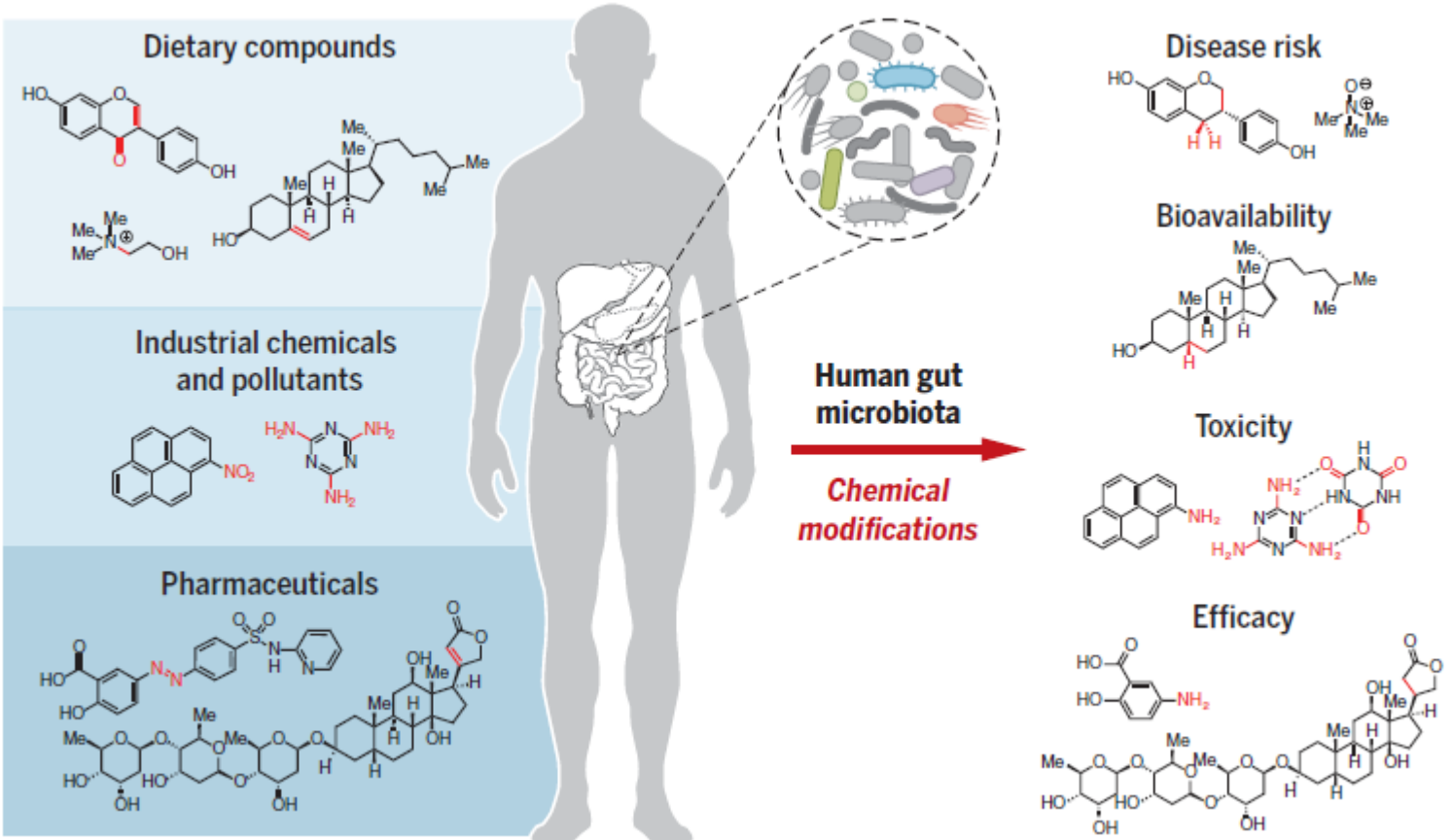


IMODI
INNOVATIVE MODELS INITIATIVE AGAINST CANCER

Pensec *et al.* AACR Boston 2019

Bacterial Effects on Chemicals

Human Gut Microbiota Metabolize Xenobiotics



Koppel *et al.* 2017

Gut Microbiota and Chemical Contaminants

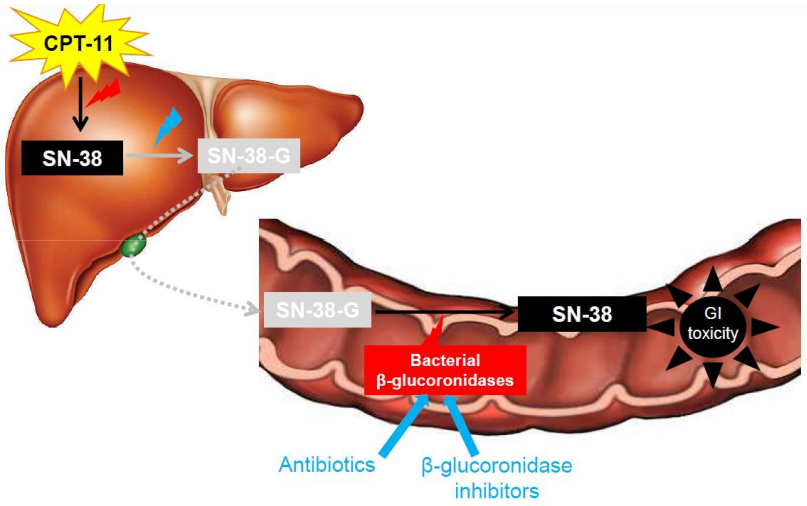
Types of chemicals with evidence that gut microbial metabolism affects toxicity :

- Azo compounds : textile dyes, food colorings, drugs
- Micropollutants : mycotoxins, heavy metals, polycyclic aromatic hydrocarbons, pesticides…
- Process Induced Toxicants: acrylamide, N-nitrosamines …

Koppel *et al.* 2017

Gut Microbiota and Drugs

- Anti-Inflammatory ex Sulfasalazine
- Central Nervous System targeted drugs
- Cancer Chemotherapy ex Irinotecan
- Cancer Immunotherapy ex ICI

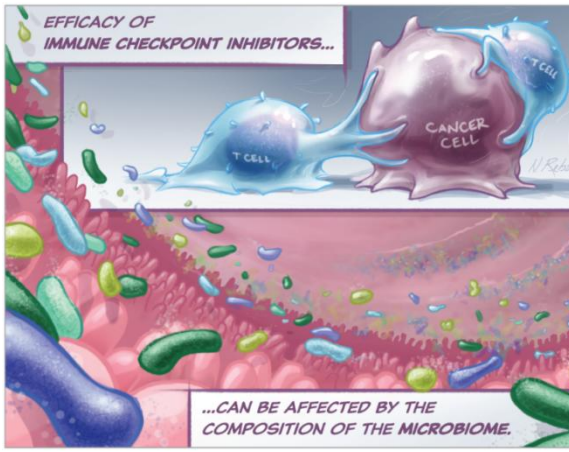


Fujita & Sparreboom 2010, Wallace BD *et al* 2015

“ Pharmacomicrobiomics”
ex : Microbiota & ICI

- Microbiota effect on pharmacokinetics
- Microbiota effect on anti-cancer immunity (Pharmacodynamics)

Immunoregulatory or effector immune functions



Hampton 2018

- Microbiota effect on local tissue integrity

Barrier function (mucus synthesis, cell junctions, xenobiotics transporters?)

- Drug toxicity on the microbiota

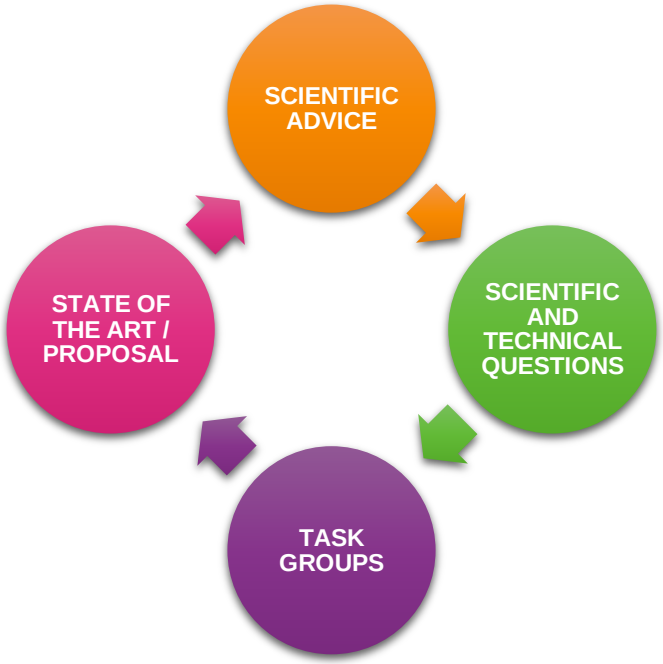
Δgut barrier, killing

Poutahidis T. et al 2014, Spor et al 2011, Swidsinski A et al 2016, Zitvogel et al 2016, Sivan et al 2015, Longman & Littman 2015, Kumar A et al 2018, Tilg et al 2018

Pharmabiotic Research Institute (PRI)

Pharmabiotic Research Institute

- A neutral, financially-independent, non-profit association.
- A unique collaborative approach to improve market access and provide technical and regulatory intelligence for its members in the microbiota-based product industry.



PRI Safety Task Group

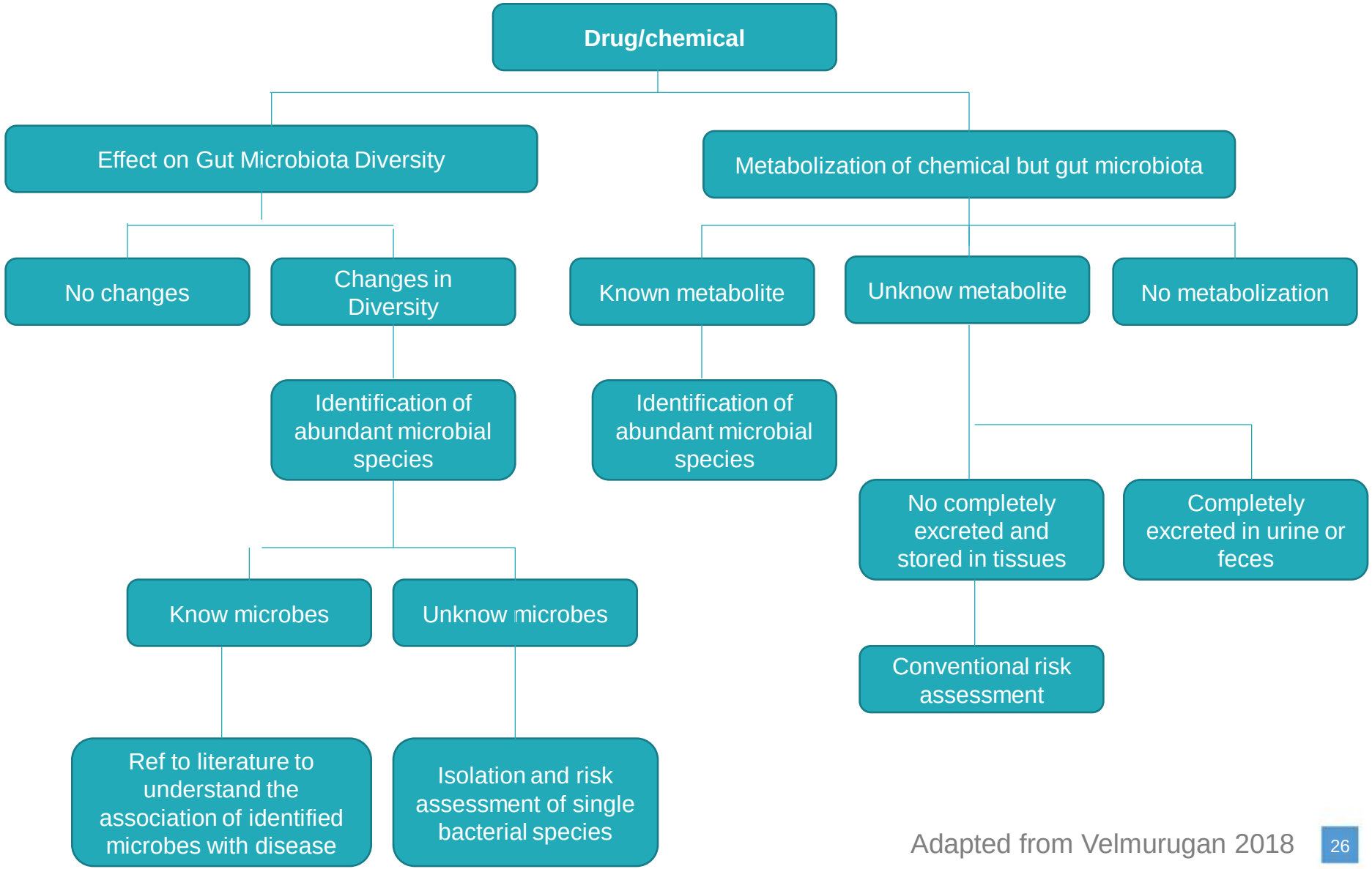
- **Challenges in the development of microbiota-based products**
  - Absence of regulatory framework
  - Very unusual mechanism of action compared to drugs and other bio-agents
  - No proper animal model

- **PRI Safety Task group**
  - Mismatch with present regulatory framework
  - State-of-the-art in microbiota-based product toxicity assessment
  - Roadmap for future regulatory guidelines on safety evaluation of these products

Dr Sidonie N. Lavergne, DVM, PhD
Pharmacotoxicologue
Research Partnership Manager
Biofortis Mérieux NutriSciences

Tools for Toxicological Risk Assessment

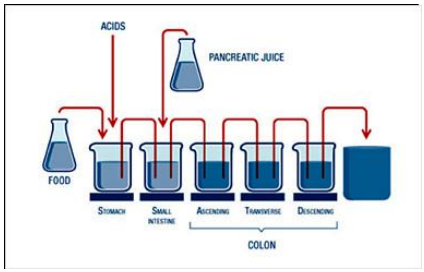
Work flow for toxicological risk assessment of gut microbiota for a drug and chemical



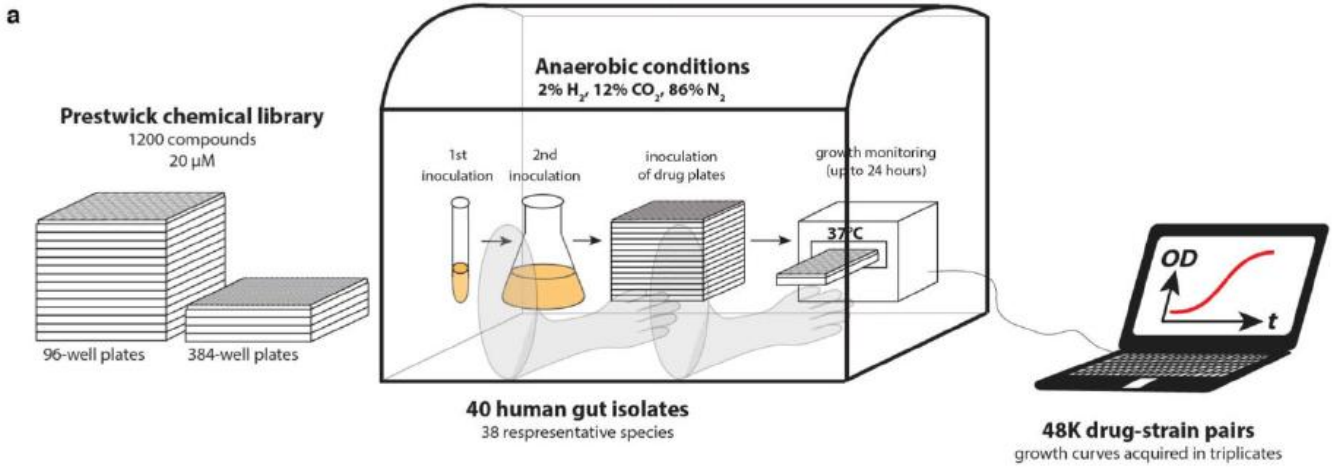
Adapted from Velmurugan 2018

In Vitro Models

- The Stimulator of Intestinal Microbial Ecosystem : SHIME – ProDigest

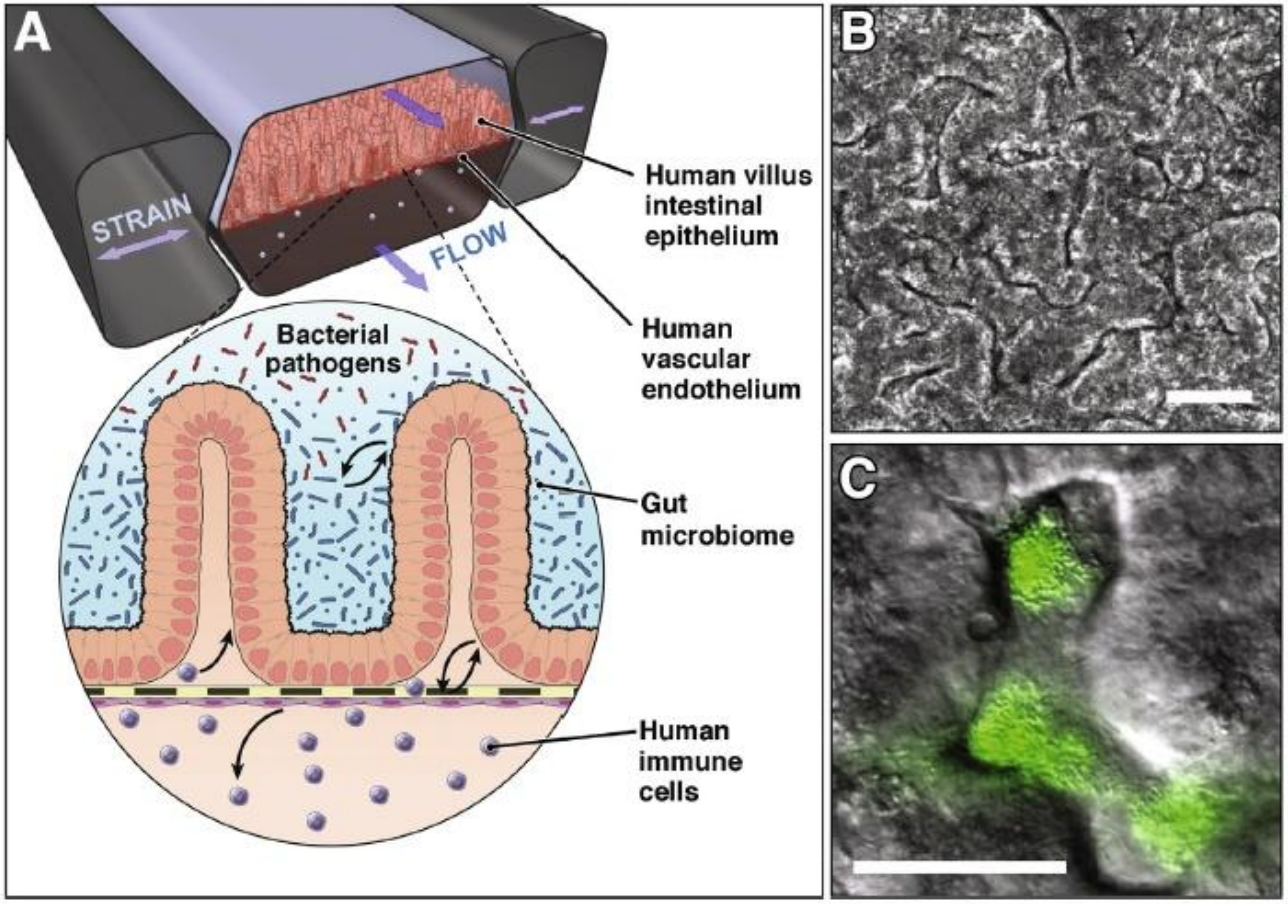


- High-throughput drug screen on gut bacterial species



Maier et al. 2018

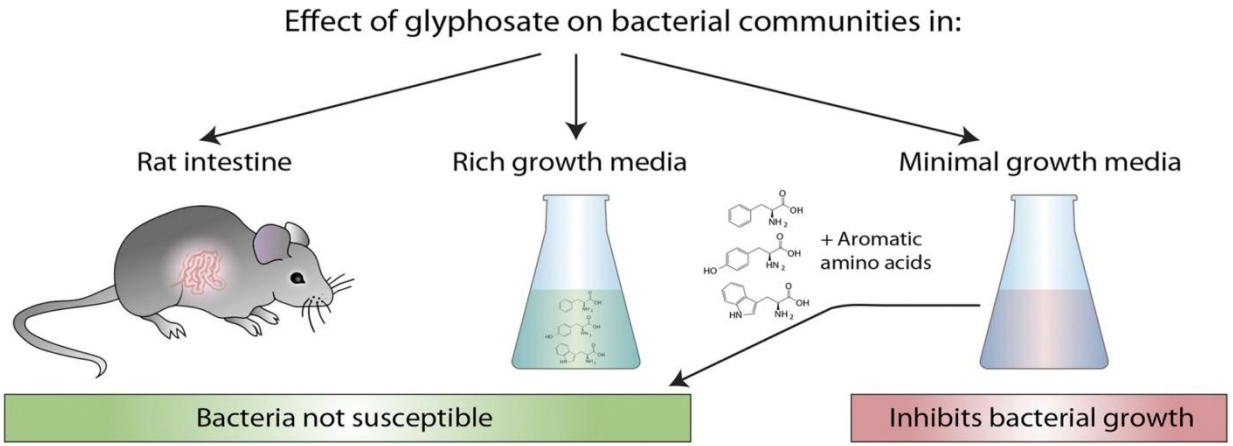
Microfluidic Organ Chip models of human Intestine



Bein et al. 2017

Single bacteria or or whole microbiota
The chemical can be mixed with flow liquid at desired C° and its effect on both microbial cells and host cells can be studied simultaneously

Importance of Employing Animal Models



« Choose animal models with a high bacterial diversity (wild)
Request animals with standardized microbiota »

HMA Mice, zebrafish, *Caenorhabditis elegans, Drosophila melanogaster*

Rask Licht and Lain Bahl 2018

Contribution of Biofortis

Our Mission & Customers

Biofortis' strategy : **Combine the microbiome services, the central lab and the clinical trial activities to create a unique partner for Food and Pharma companies**

- Customers are :
  - Food and ingredient companies
  - Pharma and food supplements companies
  - Research consortia and partnerships

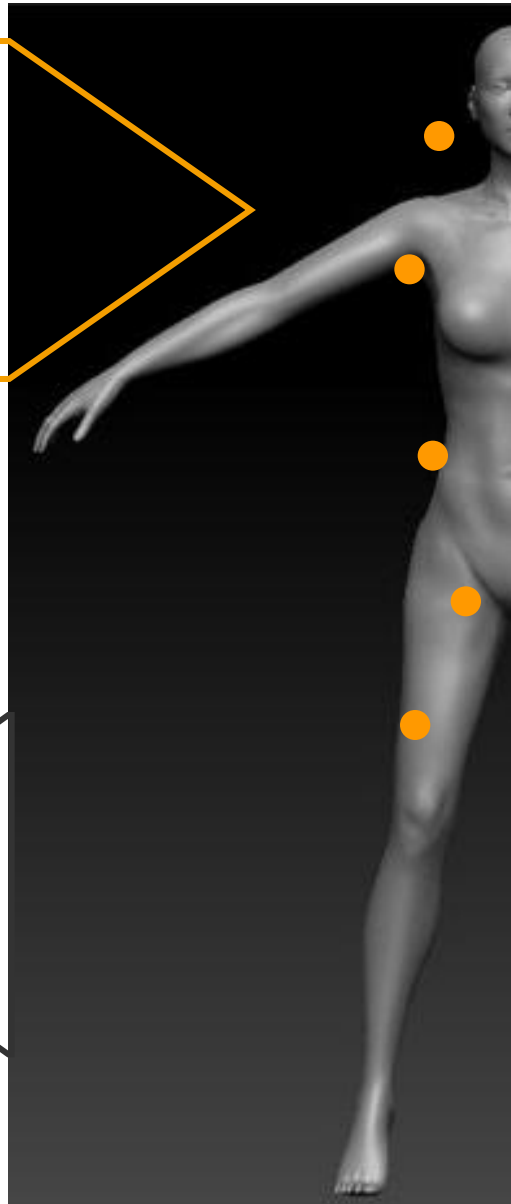
  (innovative projects in Personalized Medicine)
  - Biotech companies (new drugs or biomarkers)
  - Cosmetic companies ("Skin microbiota")

Microbiome Sequencing

Drugs

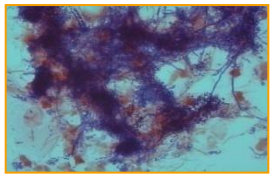
Food Supplements

Cosmetics…

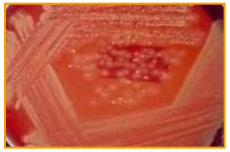


Gut

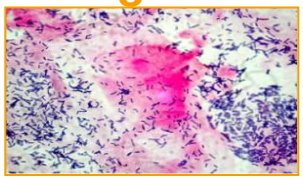
Oral



Skin/Axillary

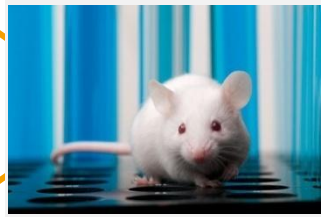


Vaginal



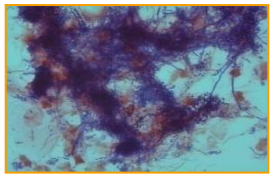
Microbiome Sequencing

Drugs, Food Supplements, Cosmetics…



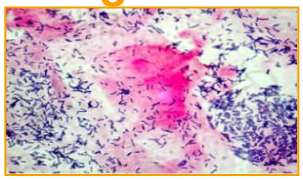
Gut

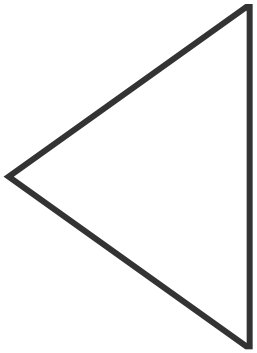
Oral



Biopsy

Vaginal





Take Home Message

- Chemical can impact the Gut Microbiota
- By altering the chemical structures of ingested compounds, the gut microbiota mediate the effects of diet, pollutants and drugs on host physiology in multiple ways
- Next challenges in identifying the organisms, genes and enzymes involved in metabolic processes
- Microbiome Standardization

« Consideration of the Gut Microbiota

as a new Parameter in Risk Assessment ! »

Thank you for attention

FULLY INTEGRATED MICROBIOME SERVICES