

# Microbiota and its role for diagnostic, prediction, prevention and therapy: where we are?

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PD Dr. med. Christian Schulz

Medical Department 2

LMU-Hospital Munich, Germany

## Financial disclosures

- Scientific support: DZIF, bayresq.net, Else- Kröner- Stiftung
- Advisory honorary: Sanofi, Janssen Cilag, Luvos, Tillotts, Alfasigma, Astellas, Richen, Juvisce
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- Project support: Luvos, Imevax, Menarini

# Microbiota research – Status quo



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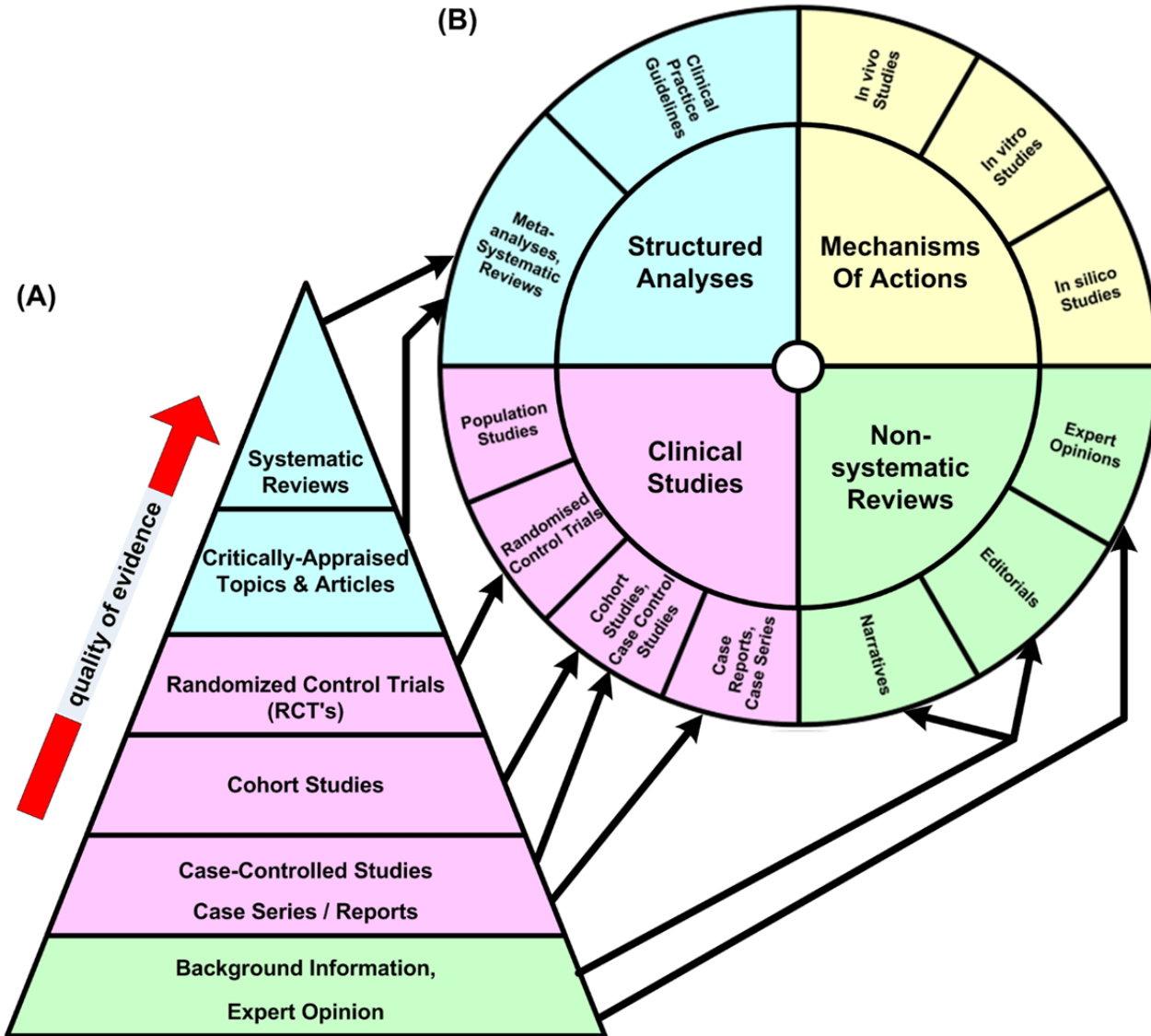


**Trials based  
on  
Associations**

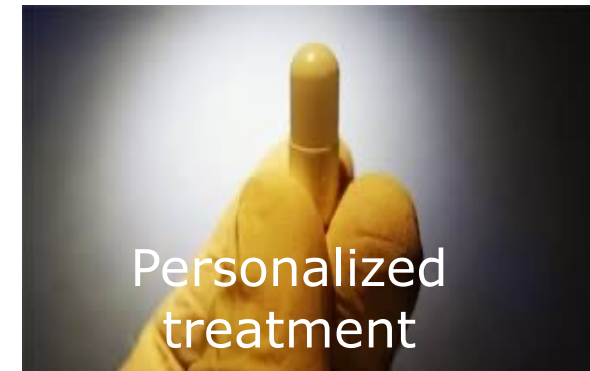
**Interventional  
studies**

**Functional  
Analyses**

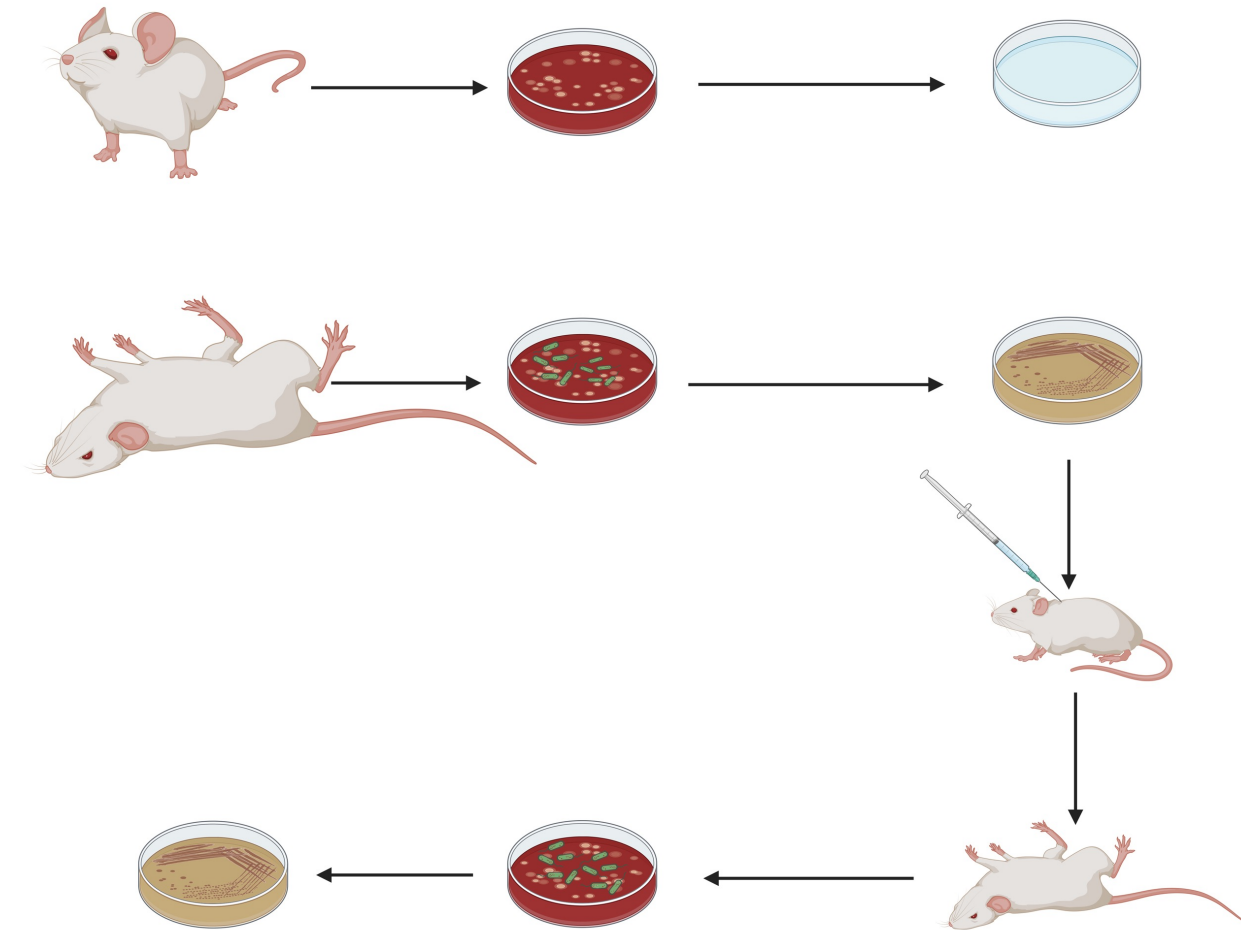
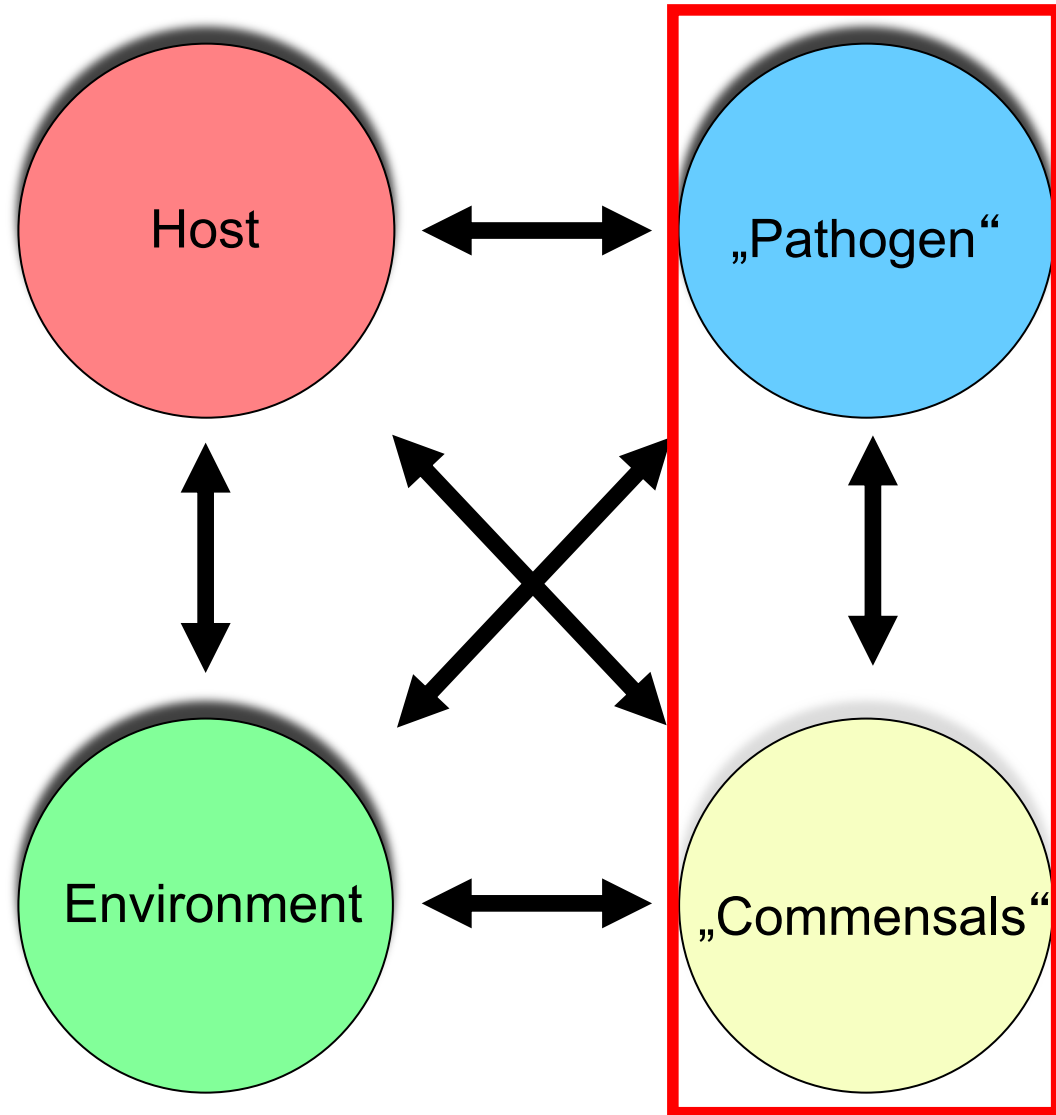
# To overcome associations



Association  
Intervention  
Functional analyses

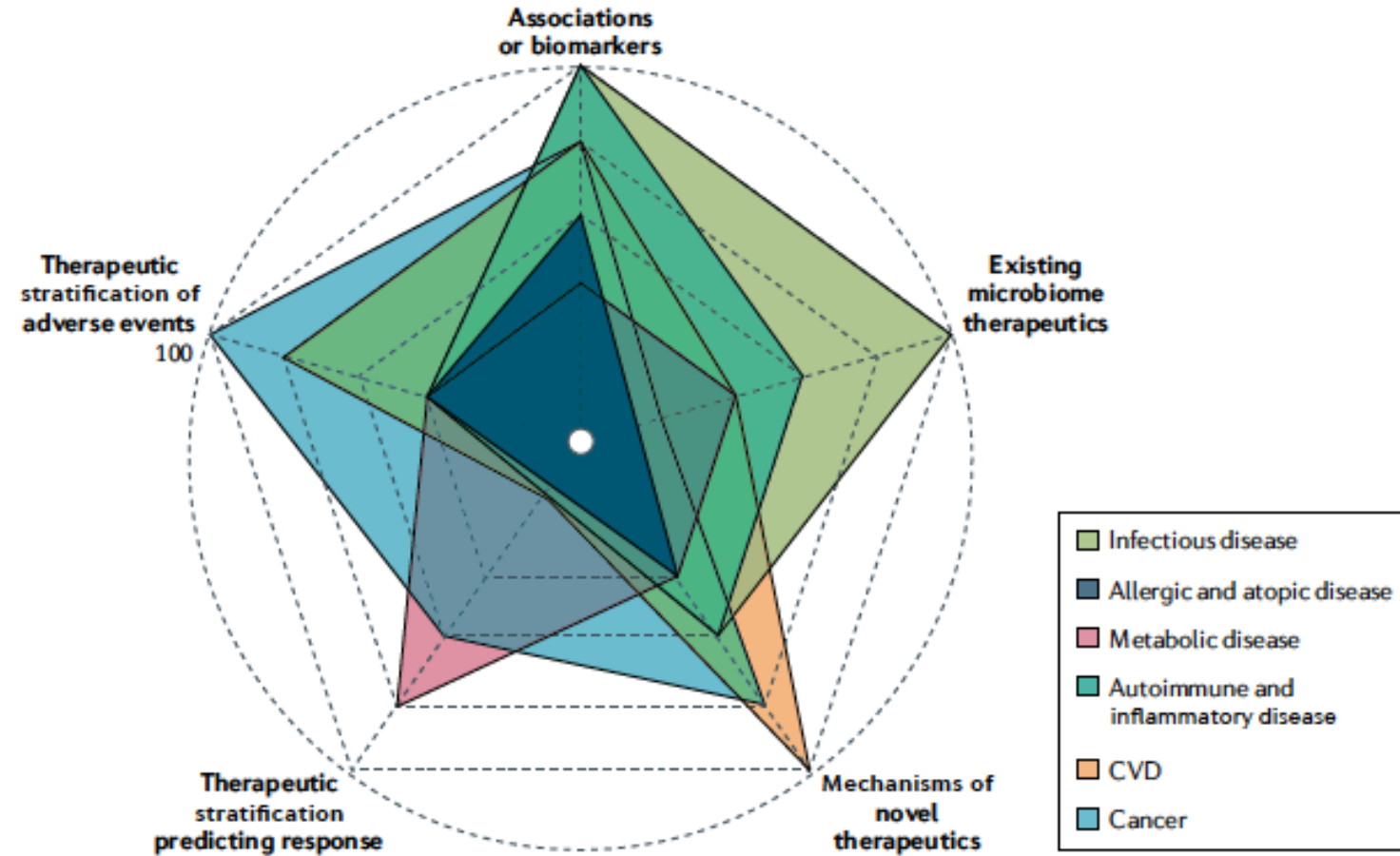
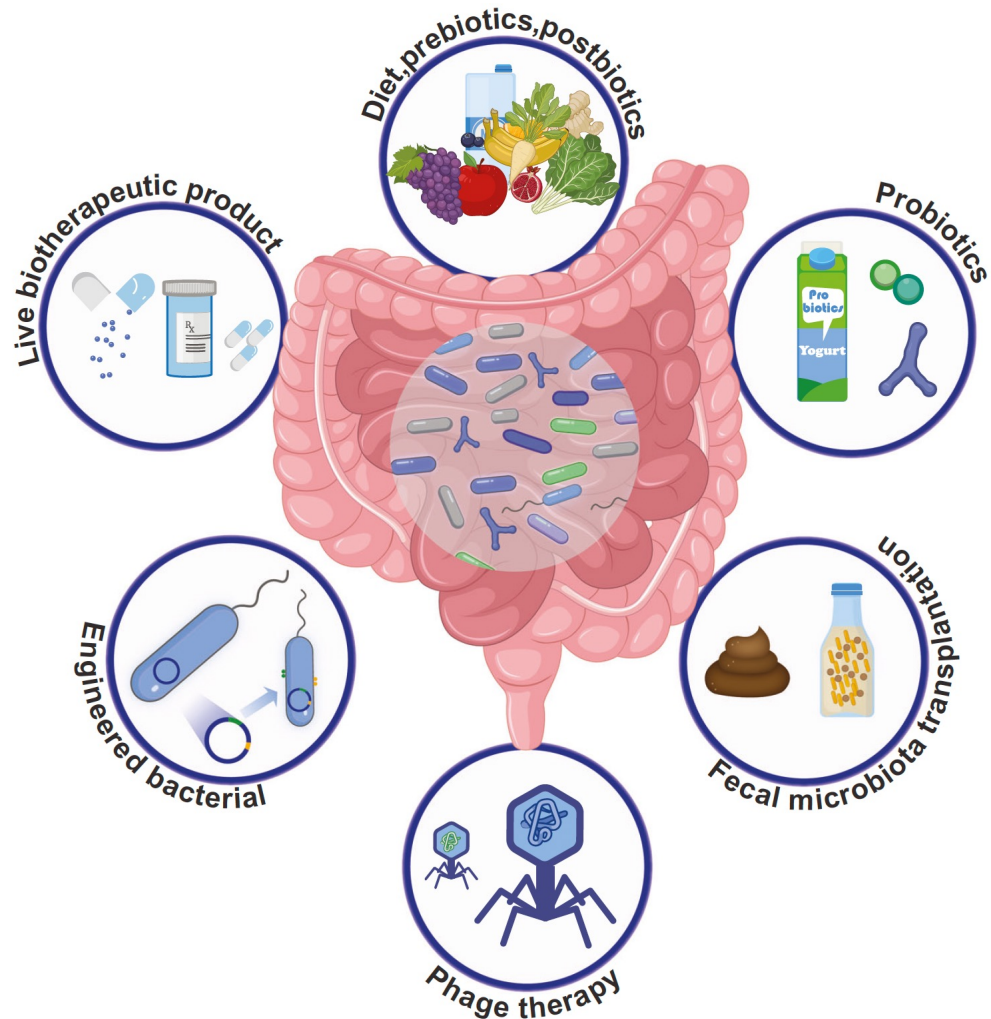


# Why focussing on Microbiota analysis in Science and Clinical routine?

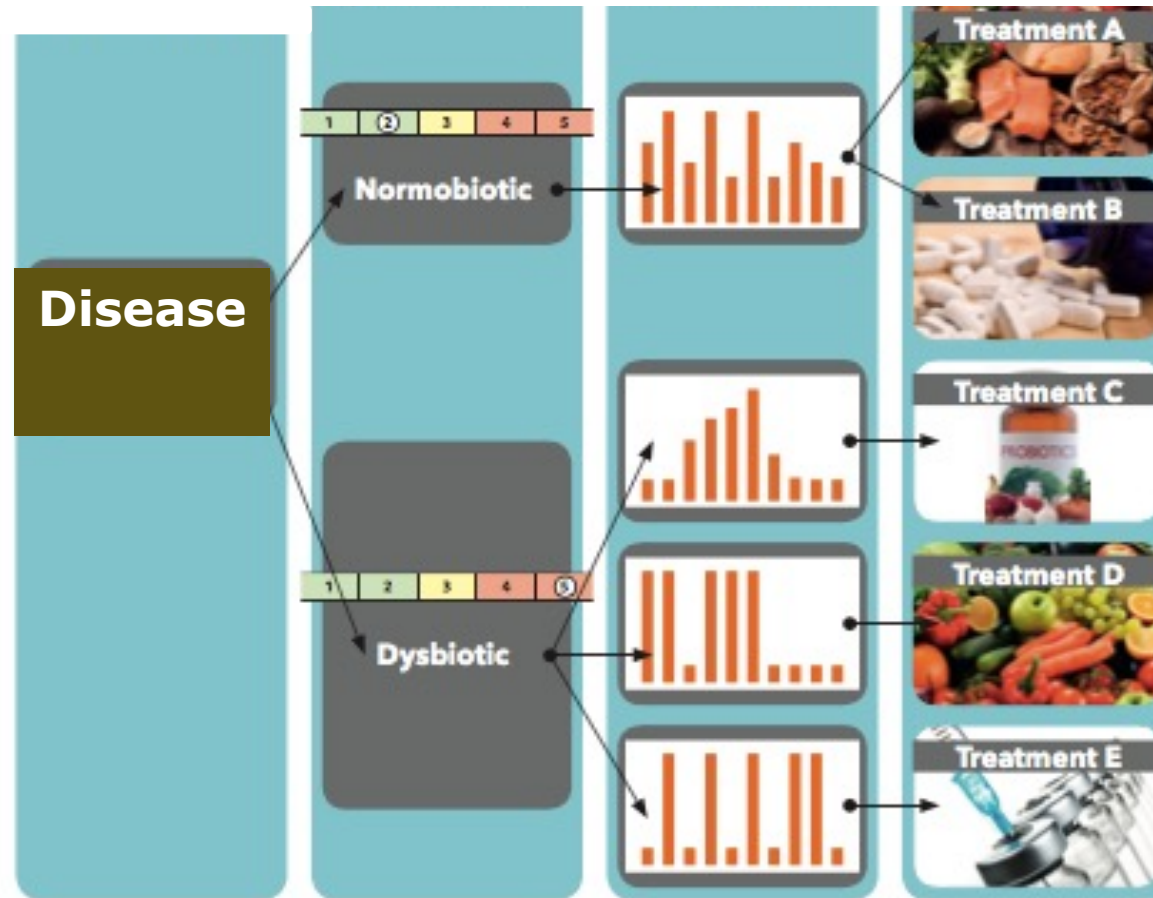


Koch- Henle- Loefflersches Theory

# How to modify microbial communities?



# Potential Aim





Technology	Advantages	Limitations
qPCR/dPCR	<ul style="list-style-type: none"> <li>• <b>Very specific</b></li> <li>• Quantitative PCR</li> <li>• Wide dynamic range</li> </ul>	<ul style="list-style-type: none"> <li>• <b>Limited number of bacteria</b></li> </ul>
Microarrays	<ul style="list-style-type: none"> <li>• Established procedures</li> <li>• <b>Very high multiplex capacity</b></li> <li>• Easy adaptable to integrated systems</li> </ul>	<ul style="list-style-type: none"> <li>• <b>Challenges with unspecific binding</b></li> <li>• Additional computational analysis to interpret signals</li> <li>• Not standardized procedures</li> </ul>
Next-generation sequencing	<ul style="list-style-type: none"> <li>• <b>Possible to discover all bacteria present</b></li> <li>• Detailed information</li> </ul>	<ul style="list-style-type: none"> <li>• <b>Extensive computational analysis to interpret signals</b></li> <li>• <u>Not standardized procedures</u></li> </ul>
Culture	<ul style="list-style-type: none"> <li>• <b>Possible to analyze resistances, function, interactions</b></li> <li>• metabolomic analyzes</li> </ul>	<ul style="list-style-type: none"> <li>• <b>Only minority of bacteria is cultivatable</b></li> <li>• Time and resources consuming</li> </ul>
Culturomics	<ul style="list-style-type: none"> <li>• <b>Isolating unknown bacteria</b> might influence corrections of bacterial communities and study microbial networks in vitro</li> </ul>	<ul style="list-style-type: none"> <li>• <b>Cost- and time consuming approach</b></li> <li>• Enables culture of around 23% of the known bacterial species</li> </ul>

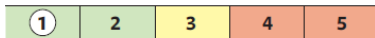
# Where we are in 2024?

- Pooled reference values of selected bacteria of interest
- Interpretations are based on association trials
- Measures recommended are based on empiric assumptions

**Status quo:**  
 offered analyses by private laboratories  
 promising insights in health and disease to supporting preventional measures

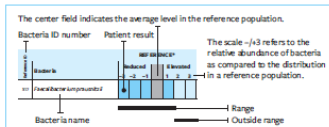
### DYSBIOSIS INDICATOR

A Dysbiosis Index above 2 indicates a microbiota profile that differs from the reference population\*. Dysbiosis Index 3 indicates a mild dysbiosis.



### BACTERIA PROFILE

A selection of bacteria with high clinical relevance is presented in the bacteria profile summary. See page 2 for the full bacteria profile.



### BACTERIA PROFILE SUMMARY

Bacteria	REFERENCE*					
	Reduced			Elevated		
	-3	-2	-1	1	2	3
103 Bifidobacterium spp.				●		
201 Alistipes				●		
203 Bacteroides fragilis				●		
317 Faecalibacterium prausnitzii				●		
320 Lactobacillus spp.				●		
324 Ruminococcus gnavus				●		
326 Streptococcus salivarius ssp. thermophilus & S. sanguinis				●		
500 Proteobacteria				●		
504 Shigella spp. & Escherichia spp.				●		
701 Akkermansia muciniphila				●		

### FULL BACTERIA PROFILE

SAMPLE ID: GA10116\_B Collection date: 2017-02-02 Date received: 2017-02-15 Report date: 2017-02-28

Actinobacteria	REFERENCE*					
	Reduced			Elevated		
	-3	-2	-1	1	2	3
100 Actinobacteria				●		
101 Actinomycetales				●		
103 Bifidobacterium spp.				●		

Bacteroidetes	REFERENCE*					
	Reduced			Elevated		
	-3	-2	-1	1	2	3
201 Alistipes				●		
202 Alistipes onderdonkii				●		
203 Bacteroides fragilis				●		
206 Bacteroides spp. & Prevotella spp.				●		
207 Bacteroides stercoris				●		
208 Bacteroides zooglyphiformans				●		
209 Parabacteroides johnsonii				●		
210 Parabacteroides spp.				●		

Firmicutes	REFERENCE*					
	Reduced			Elevated		
	-3	-2	-1	1	2	3
300 Firmicutes				●		
302 Bacilli				●		
304 Catenibacterium mitsuokai				●		
305 Clostridia				●		
307 Clostridium sp.				●		
310 Dialister invisus				●		
311 Dialister invisus & Megaspheera micronuciformis				●		
312 Dorea spp.				●		
313 Eubacterium biliforme				●		

Firmicutes cont.	REFERENCE*					
	Reduced			Elevated		
	-3	-2	-1	1	2	3
314 Eubacterium hallii				●		
315 Eubacterium rectale				●		
316 Eubacterium siraeum				●		
317 Faecalibacterium prausnitzii				●		
318 Lachnospiraceae				●		
319 Lactobacillus ruminis & Pediococcus acidilactici				●		
320 Lactobacillus spp.				●		
322 Phascolarctobacterium sp.				●		
323 Ruminococcus albus & R. bromii				●		
324 Ruminococcus gnavus				●		
325 Streptococcus agalactiae & Eubacterium rectale				●		
326 Streptococcus salivarius ssp. thermophilus & S. sanguinis				●		
327 Streptococcus salivarius ssp. thermophilus				●		
328 Streptococcus spp.				●		
330 Veillonella spp.				●		

Proteobacteria	REFERENCE*					
	Reduced			Elevated		
	-3	-2	-1	1	2	3
500 Proteobacteria				●		
504 Shigella spp. & Escherichia spp.				●		

Tenericutes	REFERENCE*					
	Reduced			Elevated		
	-3	-2	-1	1	2	3
601 Mycoplasma hominis				●		

Verrucomicrobia	REFERENCE*					
	Reduced			Elevated		
	-3	-2	-1	1	2	3
701 Akkermansia muciniphila				●		

# Potential needs of Microbiota analyses for clinical applications

- Biomarker to distinguish health and disease (and pre-stages)
- Biomarker for certain (rare) diseases
- Stratification of patients into specific treatment groups (so called *personalized treatment*)
- Prediction of treatment response
- Prediction of course of disease
- Modification of microbial communities to improve treatment response and –in best case- healing

# CHALLENGES NEED TO BE SOLVED

# CHALLENGE #1: DEEP OF ANALYSIS

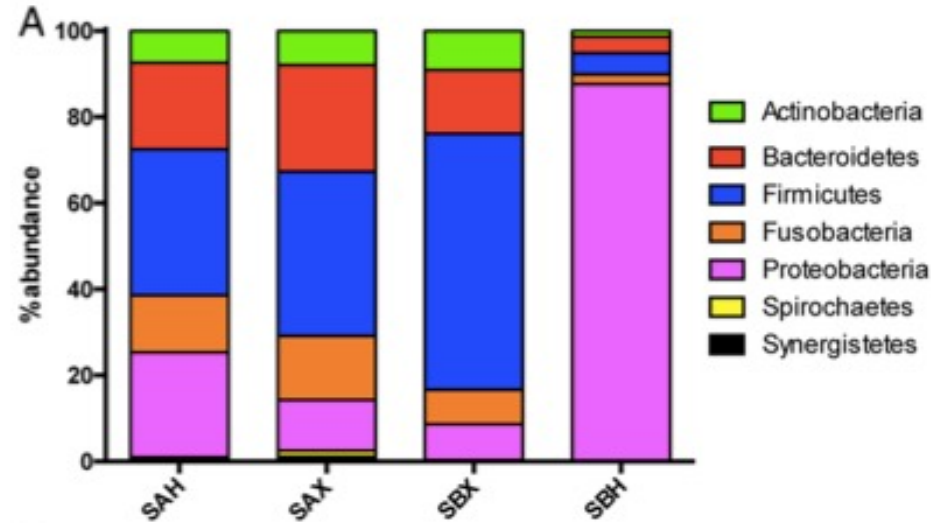
## Bacterial Taxonomy

- Domain
- Phylum
- Class
- Order
- Family
- Genus
- Species

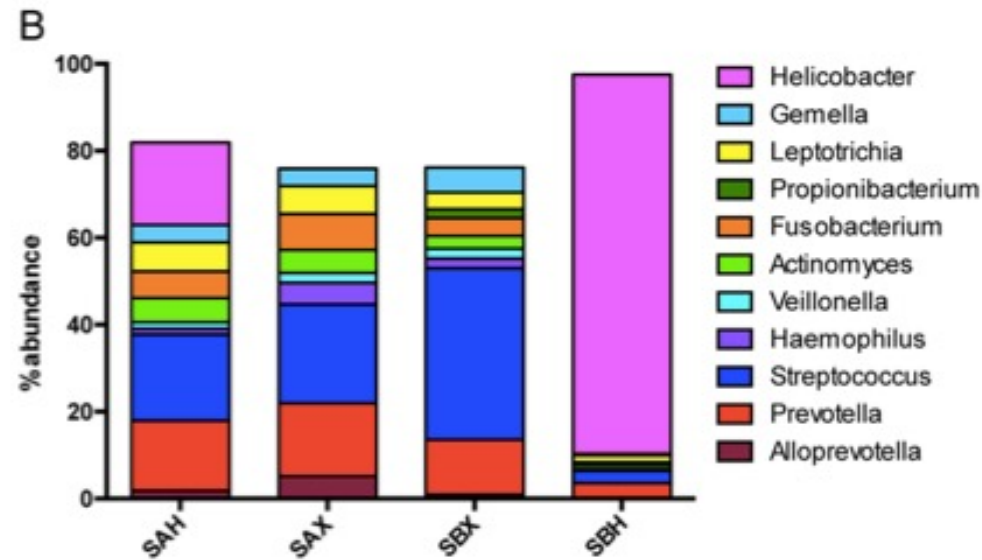
- Clostridioides difficile*
- *Bacteria*
  - *Firmicutes*
  - *Clostridia*
  - *Clostridiales*
  - *Clostridiaceae*
  - *Clostridioides*
  - *Clostridioides difficile*



# CHALLENGE #1: DEEP OF ANALYSIS

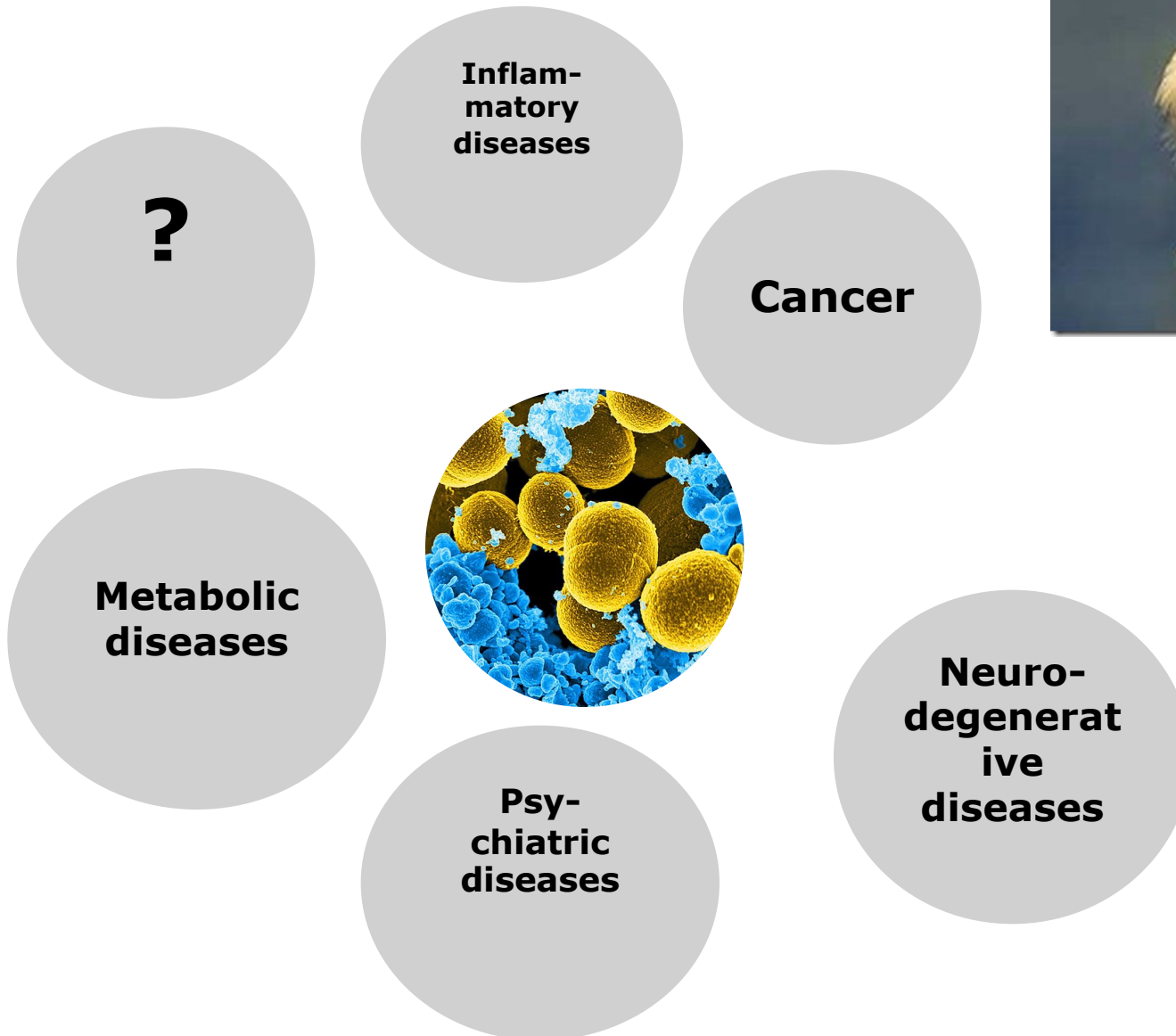


Phylum level

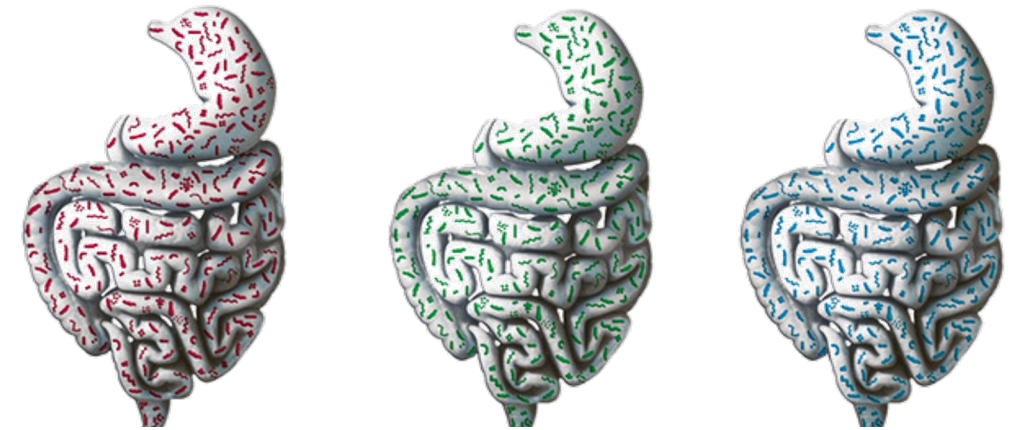
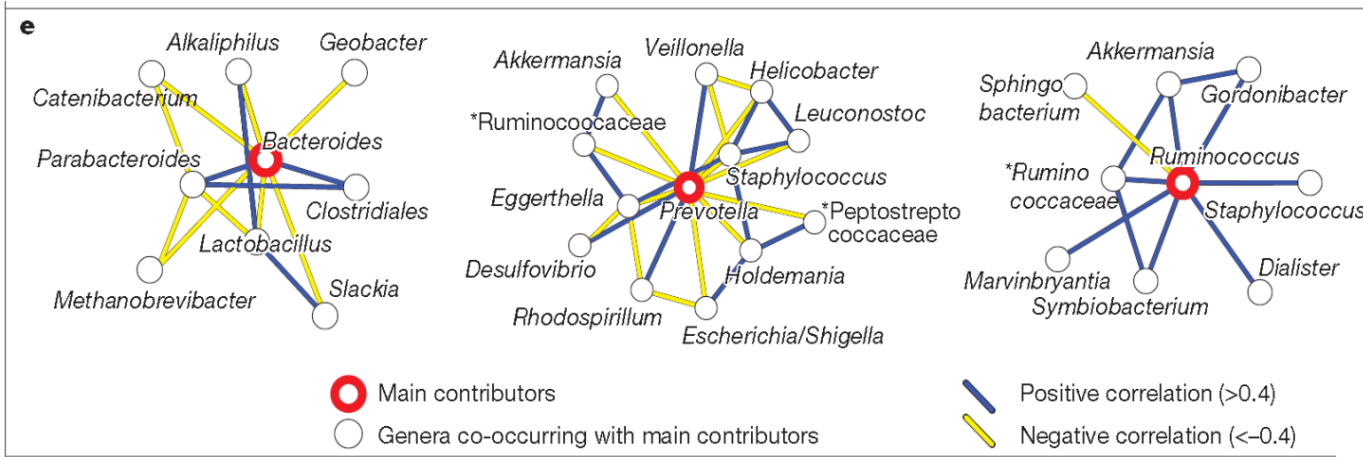


Genus level

# Challenge #2: Chicken- egg- Dilemma

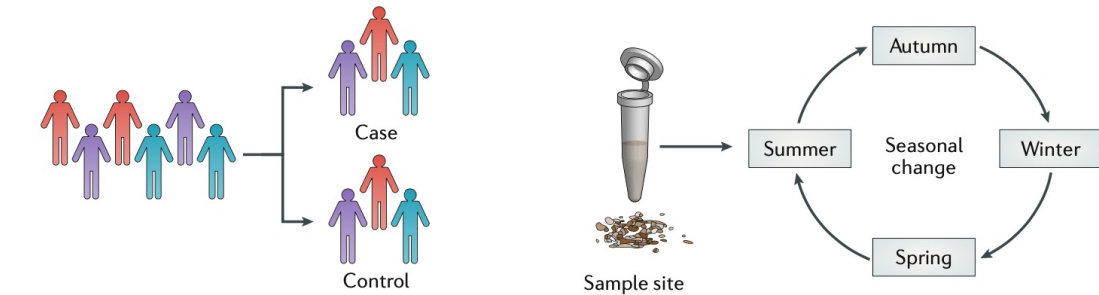
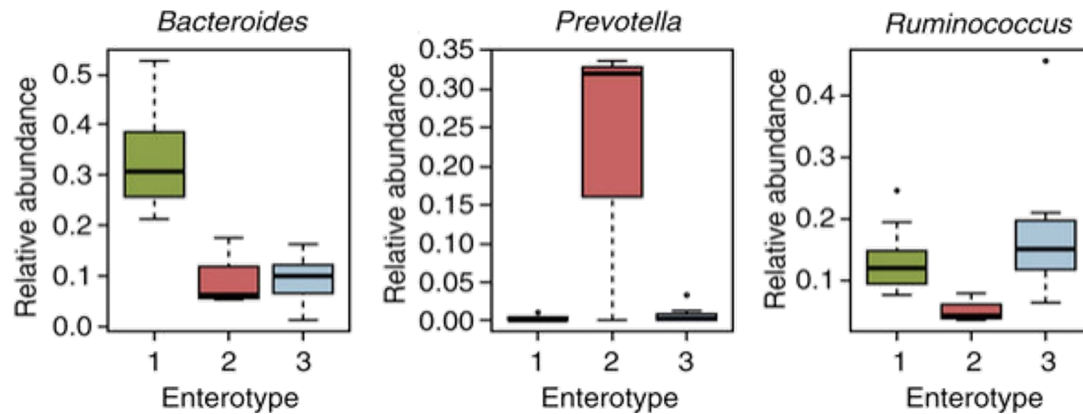


# Challenge #3: Reference values



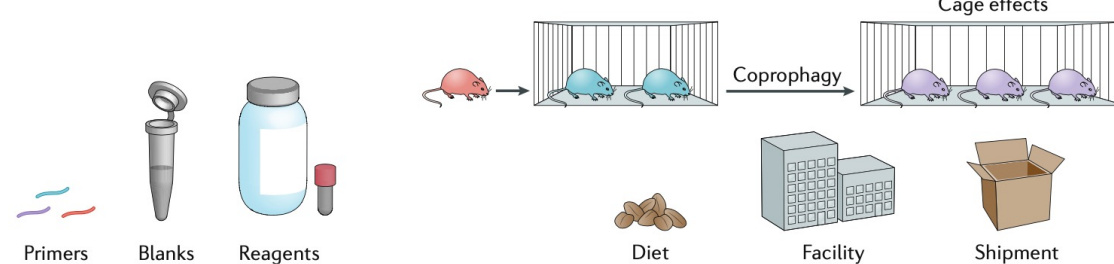
**a** Confounder controls Age, gender, diet and lifestyle

**b** Longitudinal sampling



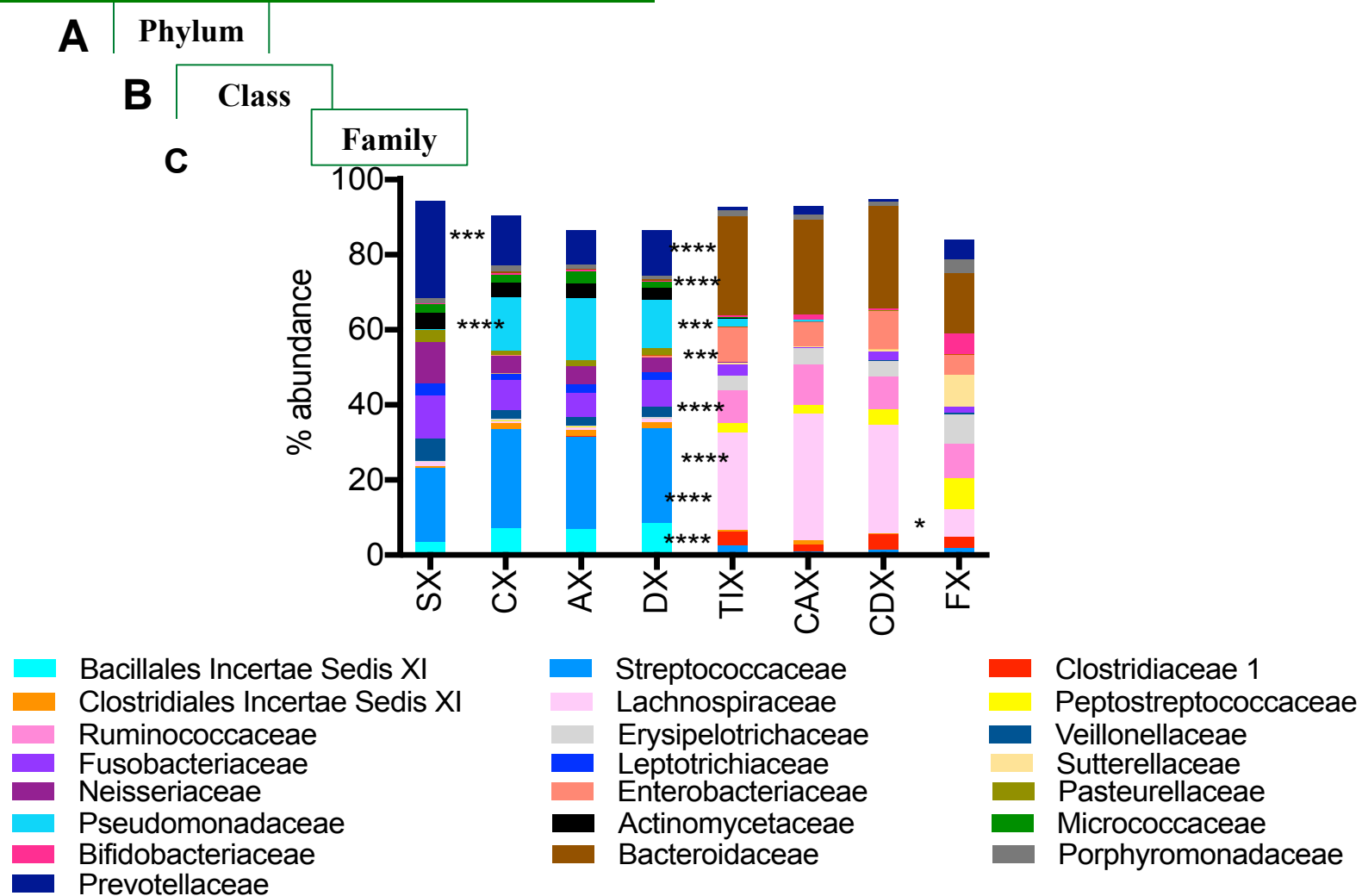
**c** Technical variation

**d** Animal models

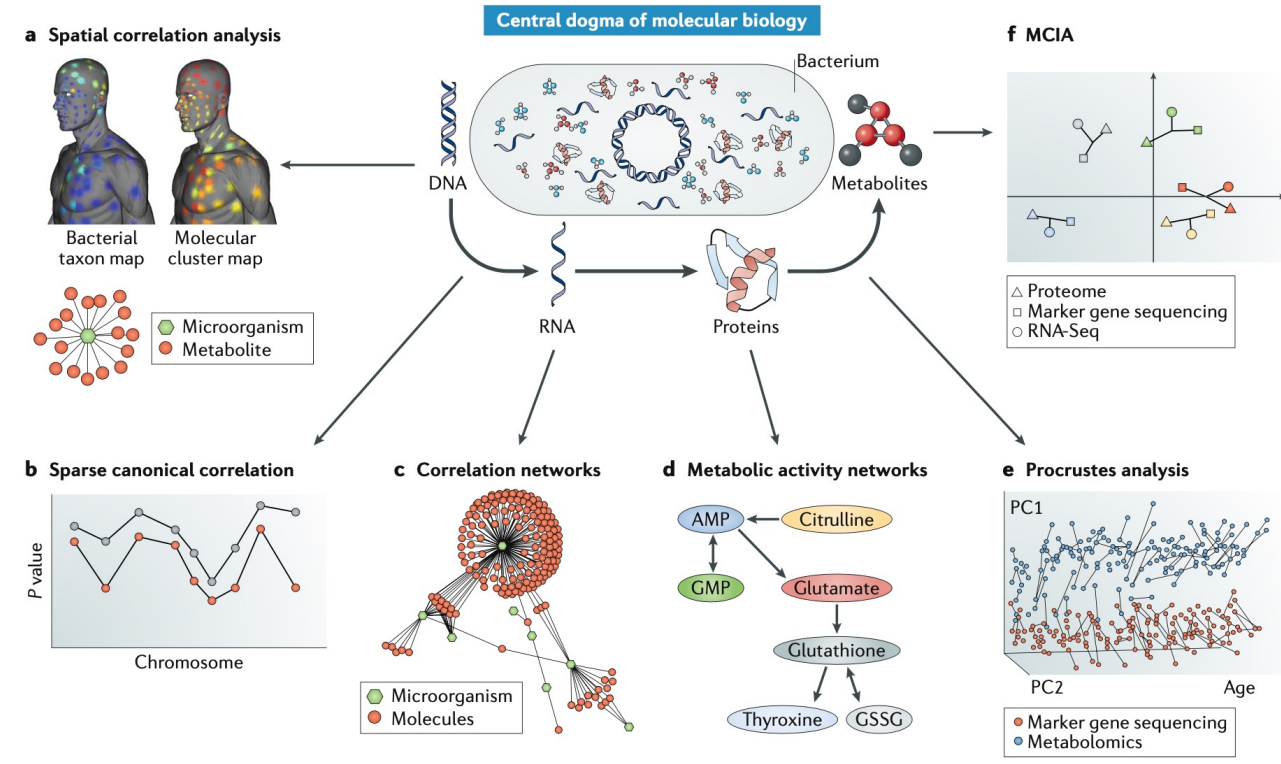
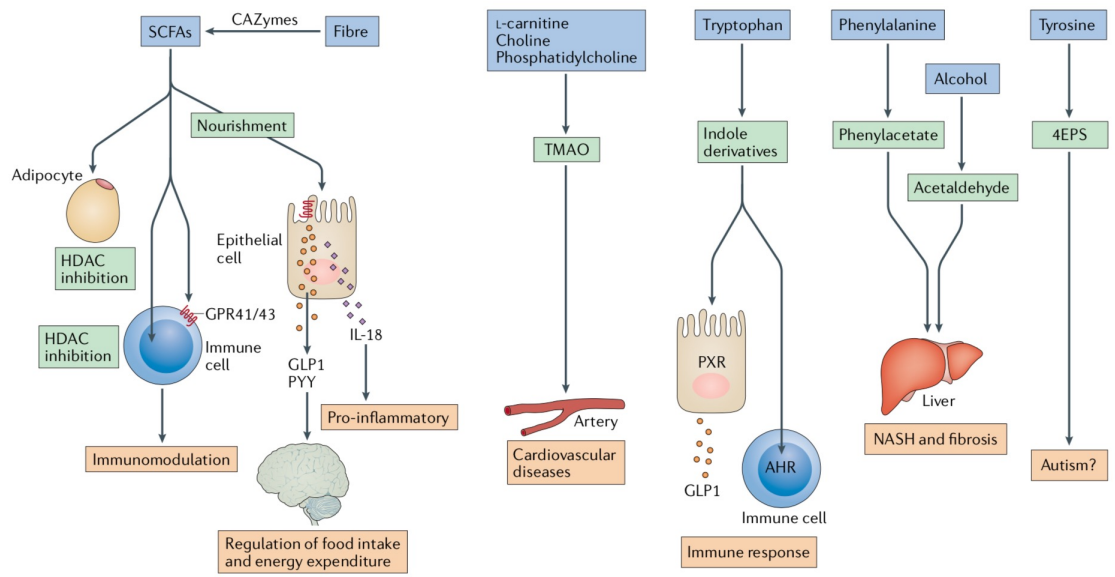




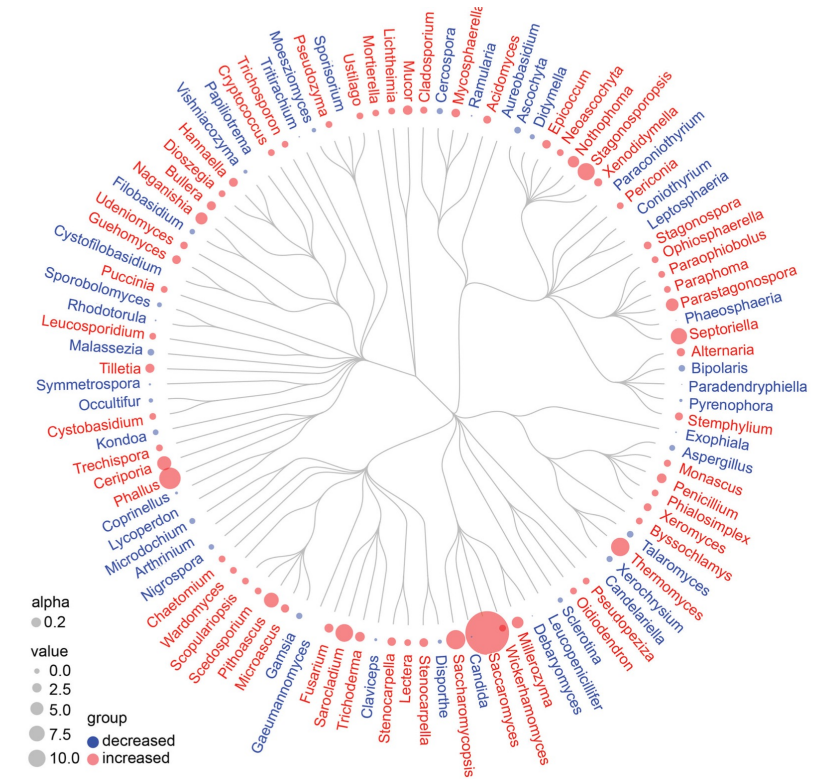
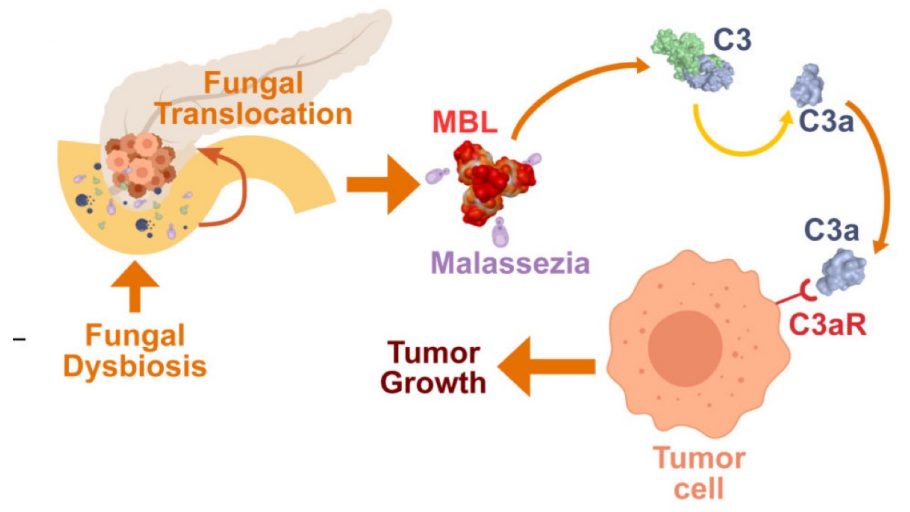
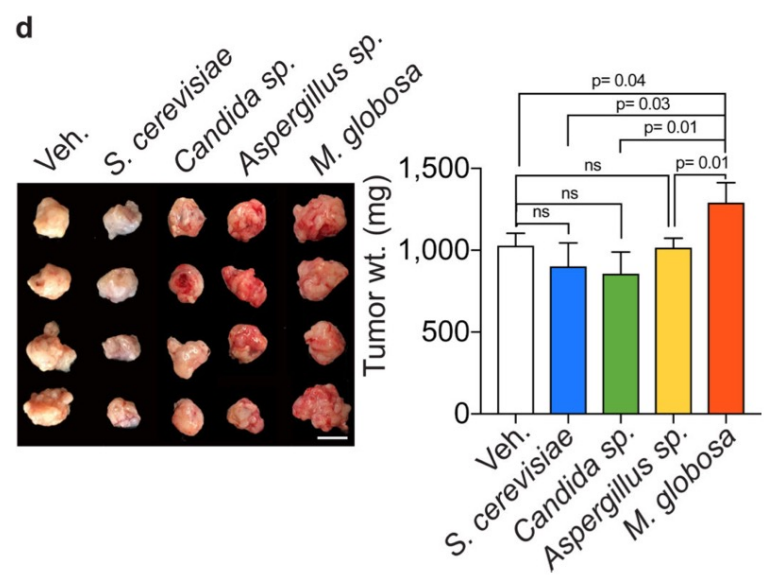
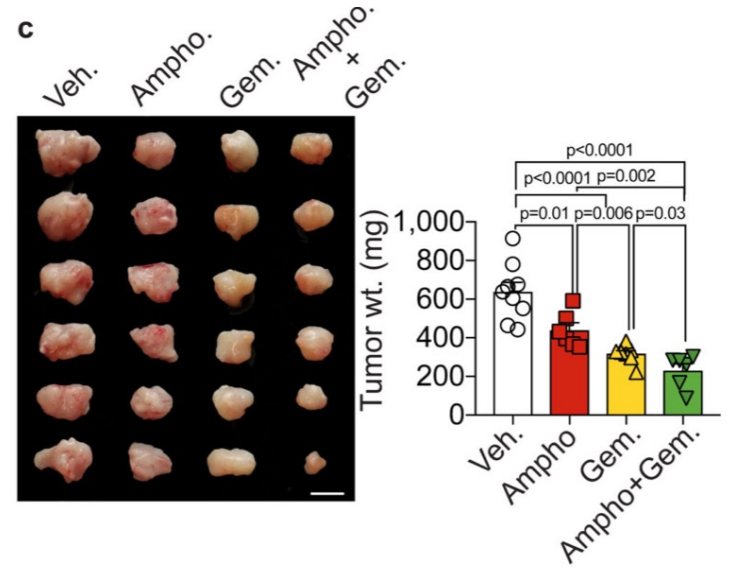
# CHALLENGE #4: FECAL MICROBIOTA DO NOT REPRESENT A SPECIFIC ECOLOGICAL NICHE



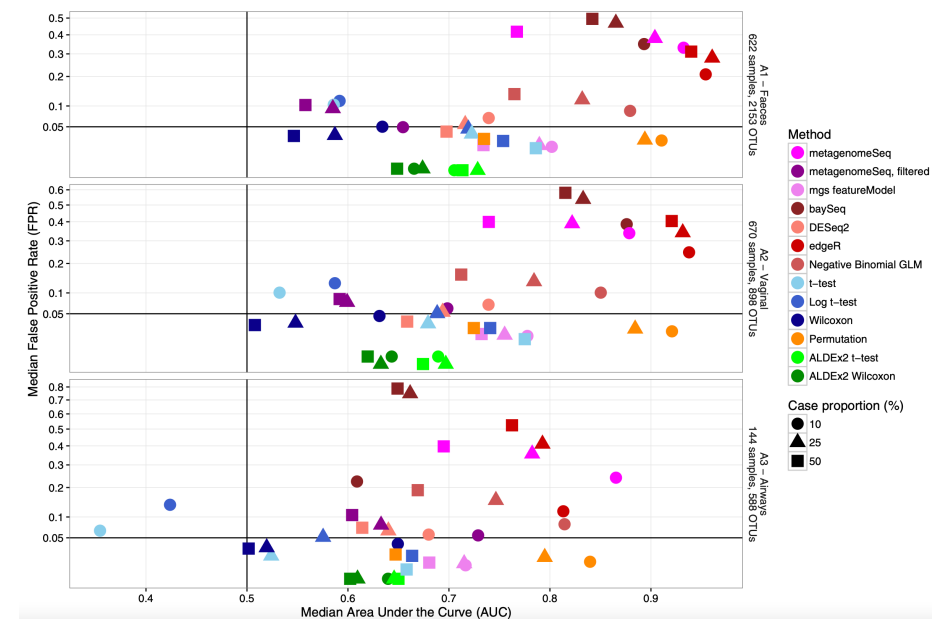
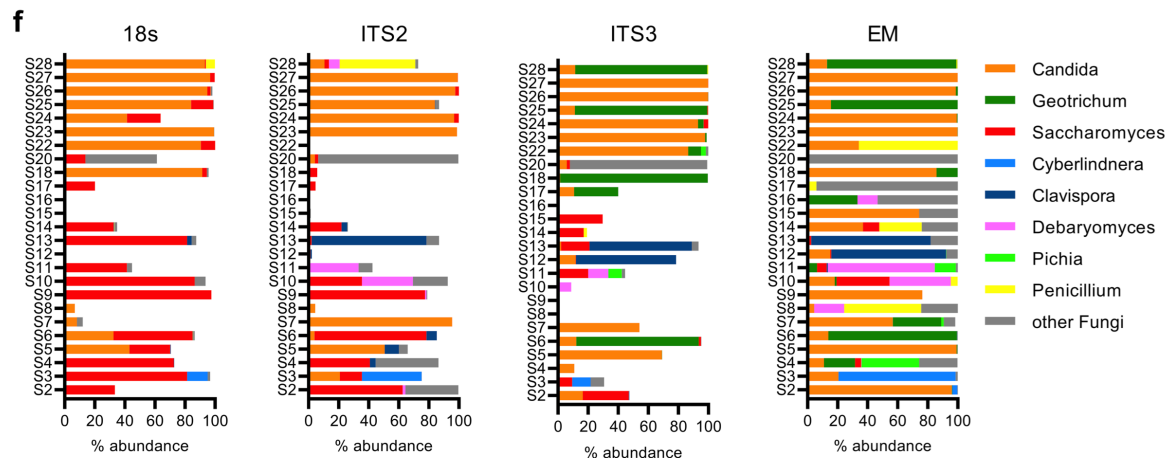
# CHALLENGE #5: BEEING THERE DOES NOT REFLECT DYSFUNCTION-



# CHALLENGE #6: MICROBIOTA ≠ BACTERIA



# CHALLENGE #7: NO STANDARDIZED METHODS



Genera	27F-338R (V1-V2)	27F-534R (V1-V3)	341F-785R (V3-V4)	515F-806R (V4)	515F-944R (V4-V5)	939F-1378R (V6-V8)	1115F-1492R (V7-V9)
<i>Acetatifactor</i>	o	o	o	o	-	-	o
<i>Actinomyces</i>	-	o	-	-	x	x	-
<i>Akkermansia</i>	x	x	o	o	-	-	-
<i>Alistipes</i>	-	-	-	-	x	x	o
<i>Atopobium</i>	-	-	-	-	-	-	o
<i>Bacillus</i>	o	o	o	o	+	-	o
<i>Bacteroides</i>	o	+	+	+	+	x	x
<i>Bifidobacterium</i>	-	-	+	+	o	x	+
<i>Cellulosimicrobium</i>	-	-	-	-	o	x	x
<i>Citrobacter</i>	o	o	o	-	x	x	x
<i>Clostridium XVIII</i>	o	o	o	-	+	+	o
<i>Collinsella</i>	o	o	+	+	-	-	o
<i>Eggerthella</i>	-	o	o	+	+	x	+
<i>Enterobacter</i>	x	o	-	x	x	x	x
<i>Enterococcus</i>	o	o	o	o	o	o	+
<i>Enterohabdus</i>	o	o	o	-	-	-	-
<i>Escherichial/Shigella</i>	o	o	o	o	o	o	x
<i>Flavonifractor</i>	-	-	-	-	x	x	x
<i>Klebsiella</i>	o	o	o	o	x	x	o
<i>Lactobacillus</i>	-	x	x	o	o	o	o
<i>Listeria</i>	+	+	o	o	o	o	o
<i>Microbacterium</i>	-	-	-	o	x	x	+
<i>Oscillibacter</i>	-	-	-	o	x	x	x
<i>Parabacteroides</i>	-	-	-	-	x	x	-
<i>Prevotella</i>	-	-	o	o	+	x	o
<i>Pseudomonas</i>	-	-	-	o	o	x	o
<i>Ruminococcus</i>	x	x	x	x	x	x	o
<i>Salmonella</i>	+	+	o	+	x	x	o
<i>Staphylococcus</i>	+	o	+	o	-	+	o
Database	RDP	Silva	RDP	Silva	RDP	Silva	RDP
	Silva	RDP	Silva	RDP	Silva	RDP	Silva

Different methods (e.g. primers, sequenced regions, storage of samples, biostatistical analysis...) underestimate significant differences in results published.

# Optimistic outlook

# Status quo: Fecal microbiota transplantation

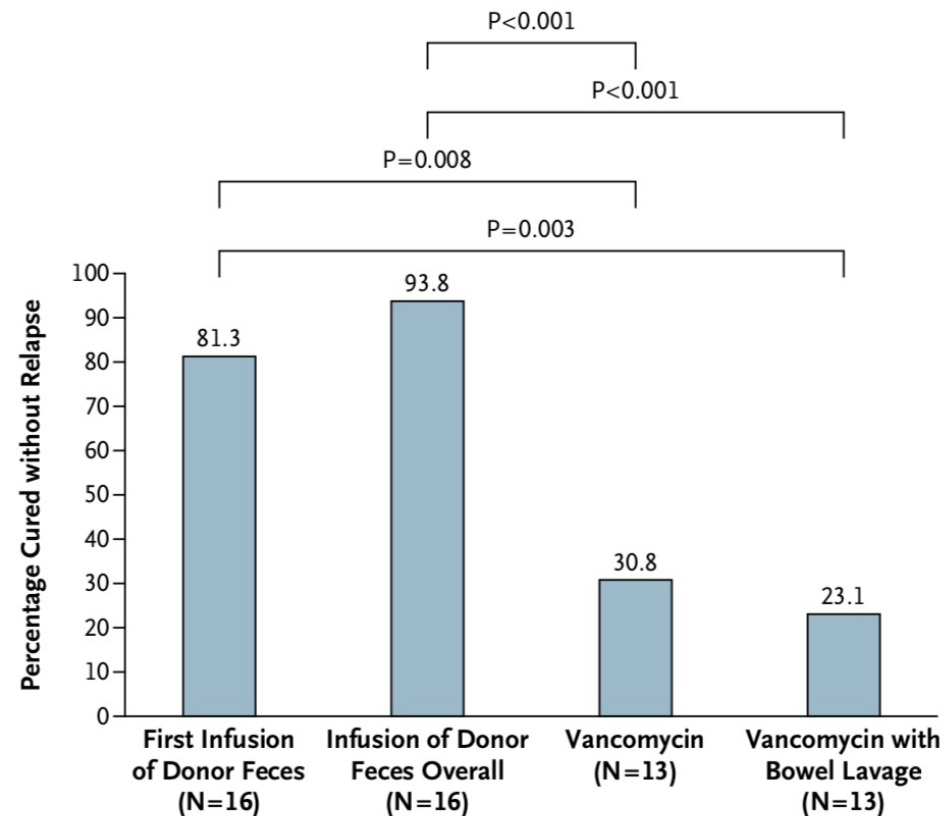
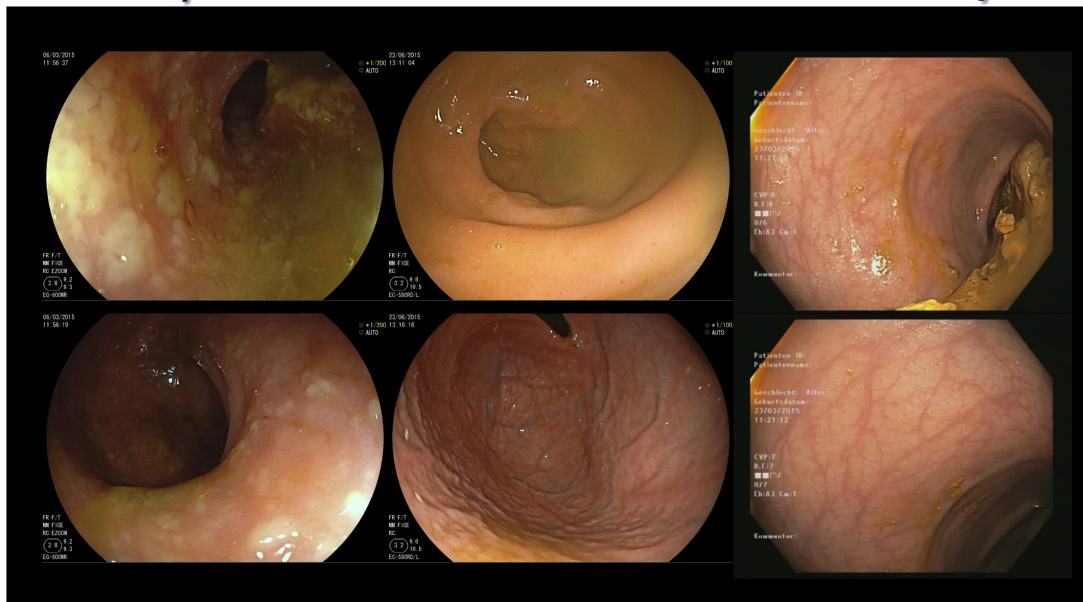
The NEW ENGLAND JOURNAL of MEDICINE

ESTABLISHED IN 1812 JANUARY 31, 2013 VOL. 368 NO. 5

Duodenal Infusion of Donor Feces for Recurrent *Clostridium difficile*

Els van Nood, M.D., Anne Vrieze, M.D., Max Nieuwdorp, M.D., Ph.D., Susana Fuentes, Ph.D., Erwin G. Zoetendal, Ph.D., Willem M. de Vos, Ph.D., Caroline E. Visser, M.D., Ph.D., Ed J. Kuijper, M.D., Ph.D., Joep F.W.M. Bartelsman, M.D., Jan G.P. Tijssen, Ph.D., Peter Speelman, M.D., Ph.D., Marcel G.W. Dijkgraaf, Ph.D., and Josbert J. Keller, M.D., Ph.D.

CDI/PMC FMT 14 days



**Figure 2.** Rates of Cure without Relapse for Recurrent *Clostridium difficile* Infection.

Shown are the proportions of patients who were cured by the infusion of donor feces (first infusion and overall results), by standard vancomycin therapy, and by standard vancomycin therapy plus bowel lavage.

# Microbial modification using commercially collected bacterial communities– RBX 2660

RBX2660 bacterial community from donor feces

Phase 3, double-blind, Placebo-controlled

Pat. > 2 episods of CDI treatment with RBX2660 (n = 180) or placebo (n = 87)

Approved by FDA

## EFFICACY

**70.6%**  
RBX2660

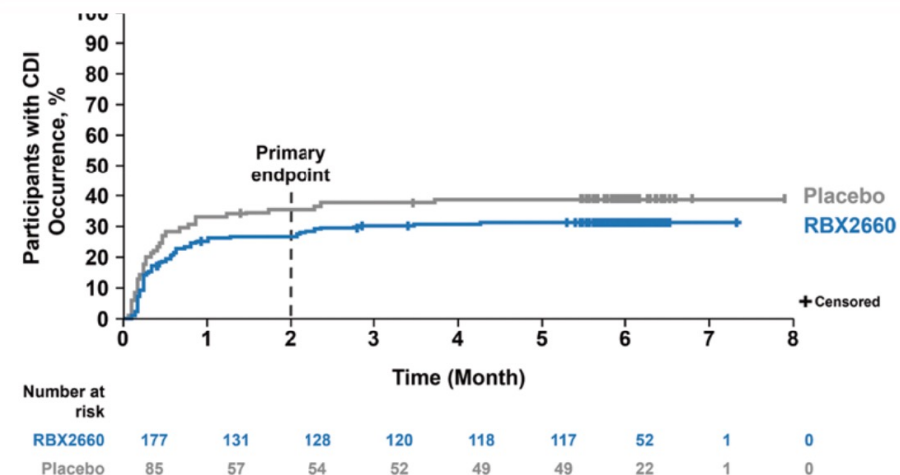
### 13.1%-point Treatment Difference

- Treatment success based on a Bayesian analysis integrating data from phase 2b study

**57.5%**  
Placebo

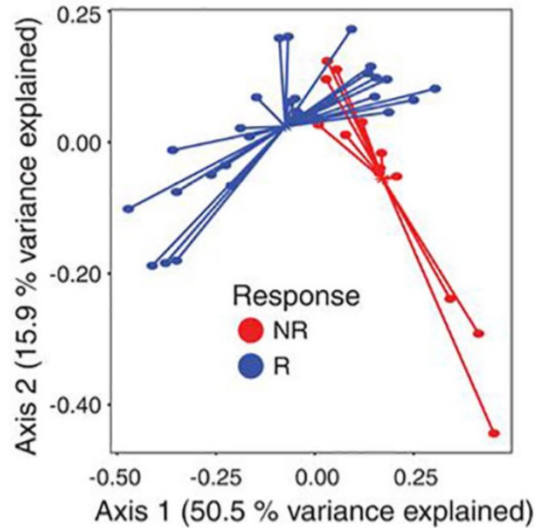
- **92.1%** of patients with success at 8 weeks remained free of CDI recurrence for 6 months

Posterior probability of superiority of 0.991

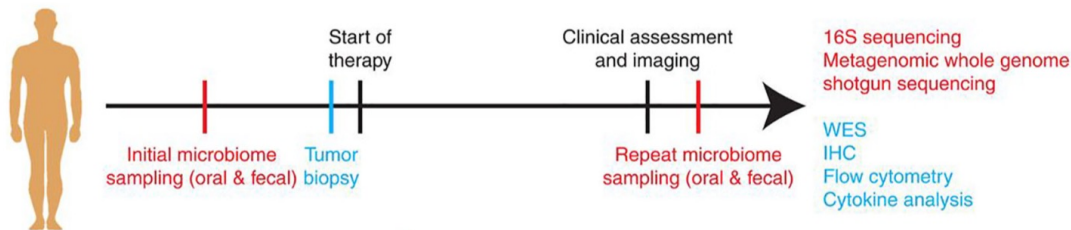
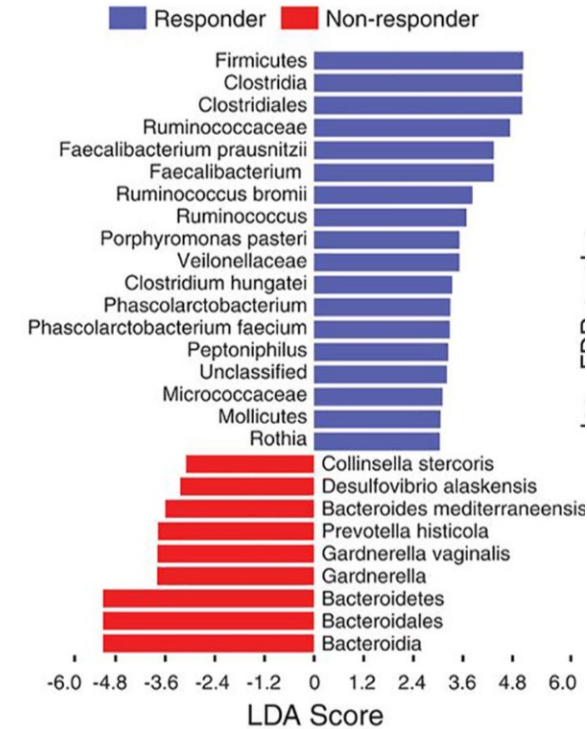


# Gut Microbiota as predictor of therapy-response

## Gut microbiome modulates response to anti-PD-1 immunotherapy in melanoma patients



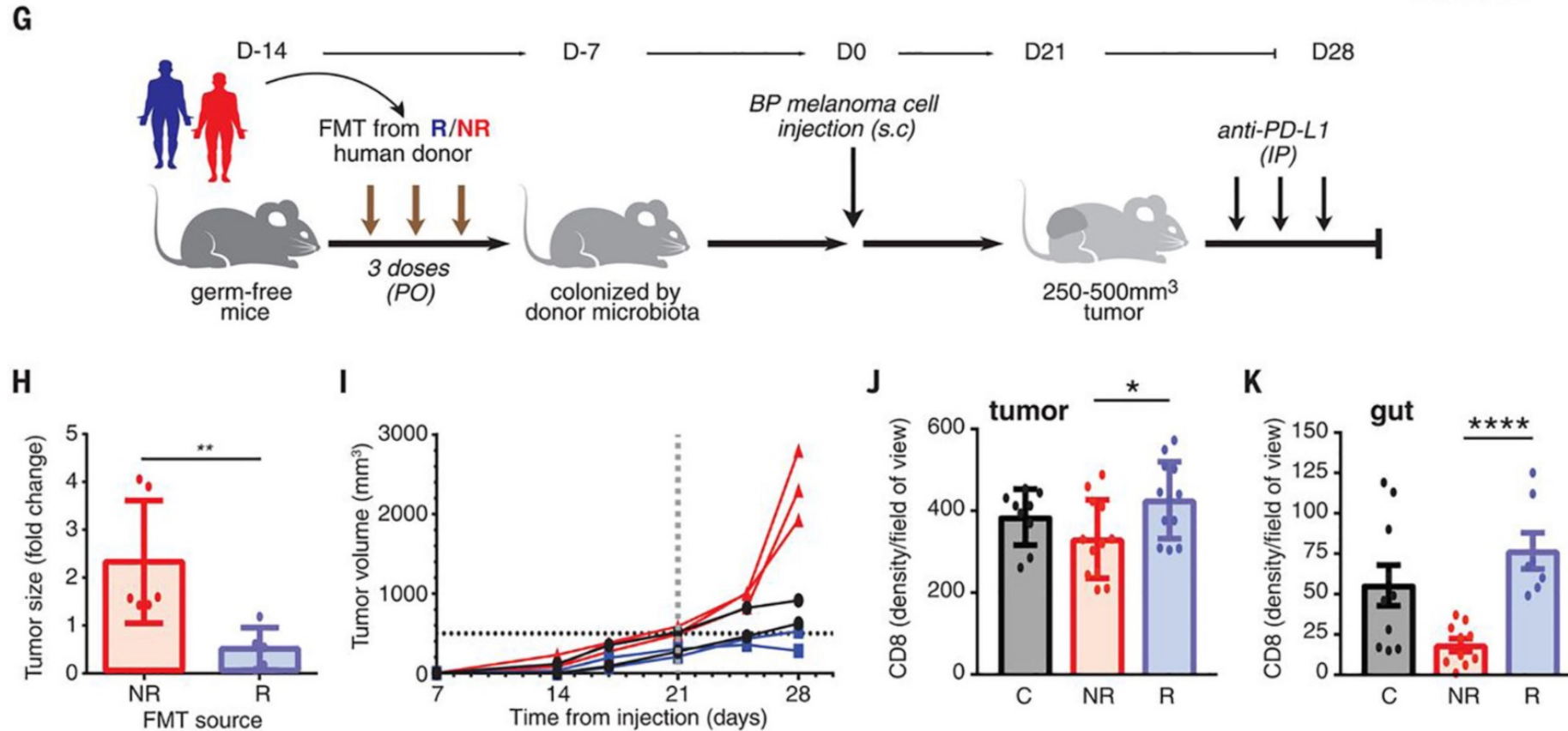
- 112 pts suffering from metastatic malignant melanoma undergoing anti PD1- therapy
- Sequencing of oral and gut microbiome



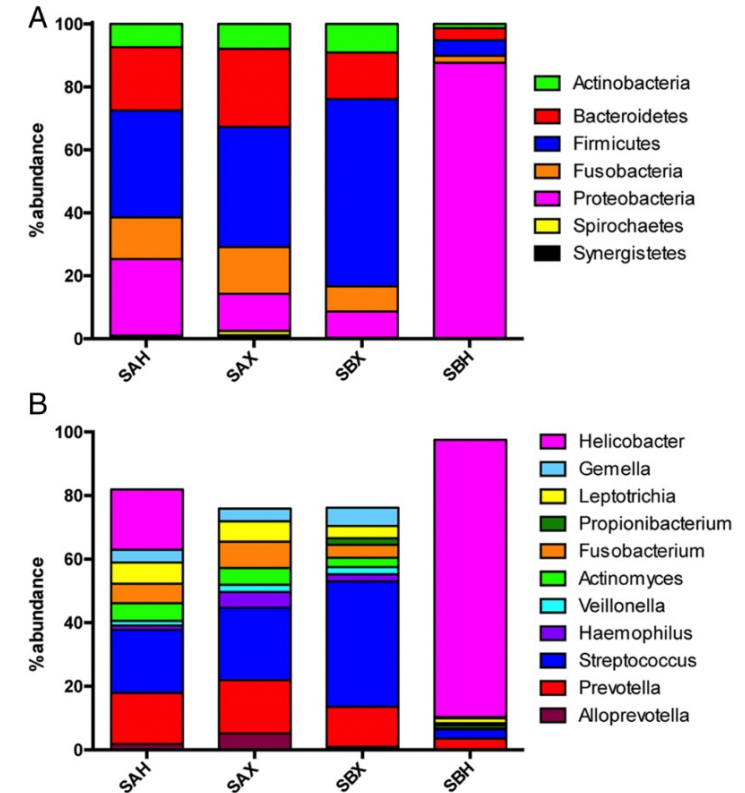
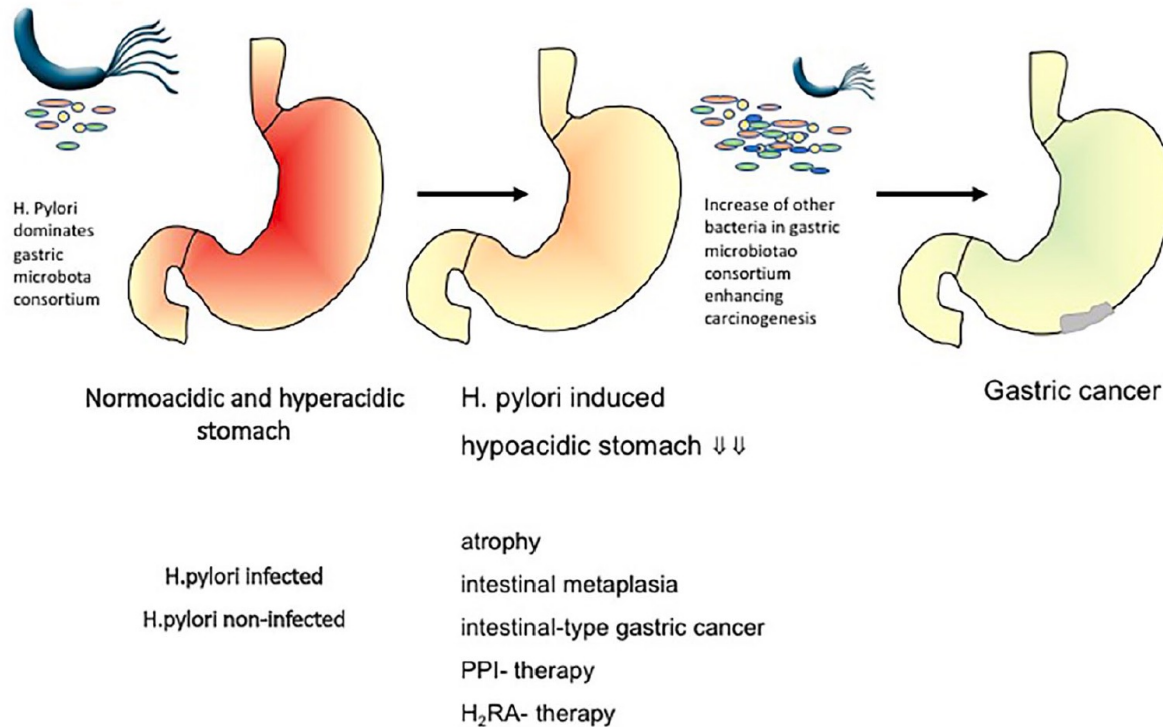


# Gut Microbiota as effector of therapy-response

## Gut microbiome modulates response to anti-PD-1 immunotherapy in melanoma patients



# Pathogen – commensal interplay in the human stomach

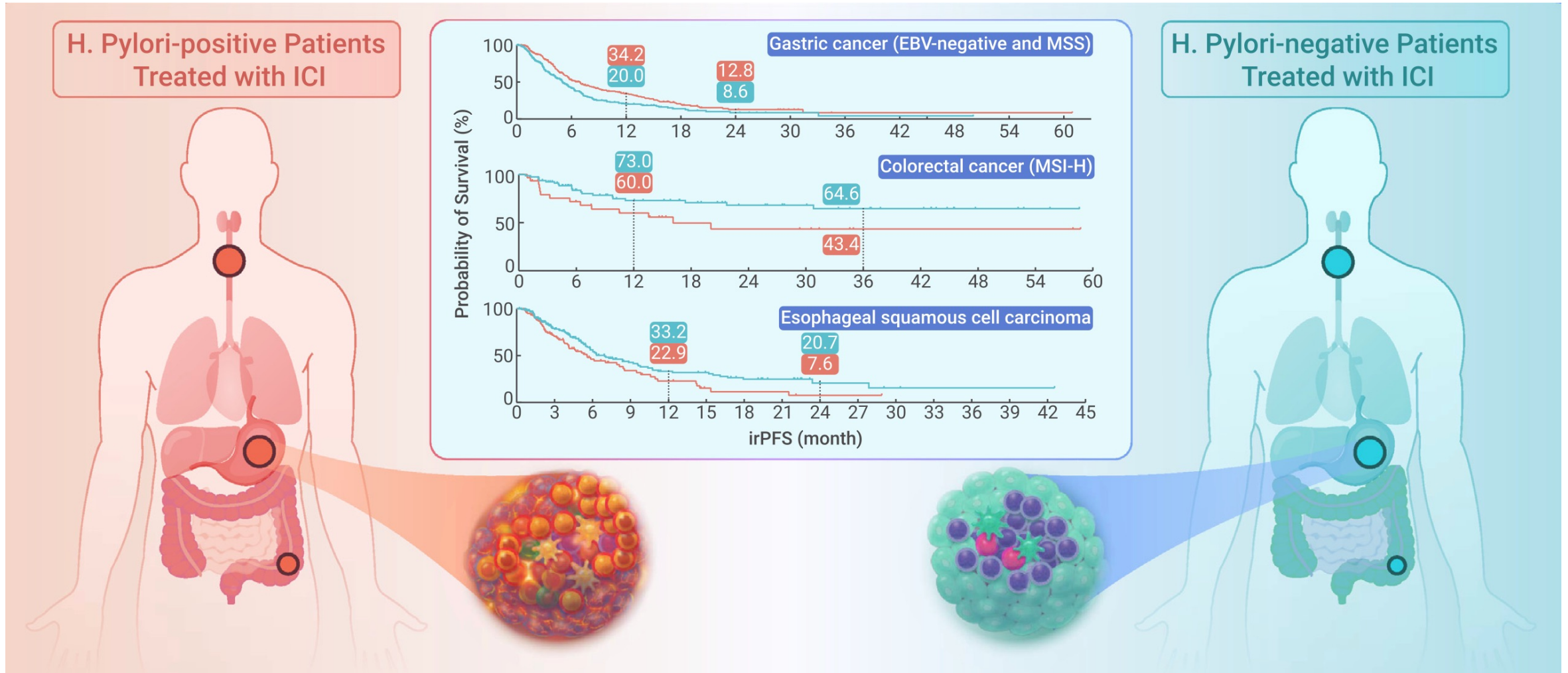


**Figure 6** Differences in bacterial community structures between stomach aspirates and biopsies in individuals infected with and without *Helicobacter pylori*. (A) Relative mean abundance of phyla and (B) relative mean abundance of genera in stomach aspirates of individuals infected with (SAH) or without *H. pylori* (SAX) as well as in stomach biopsies of non-infected (SBX) or infected individuals (SBH).

Distinct bacterial communities related to *H.pylori* status not only in the human stomach but even in adjacent niches.

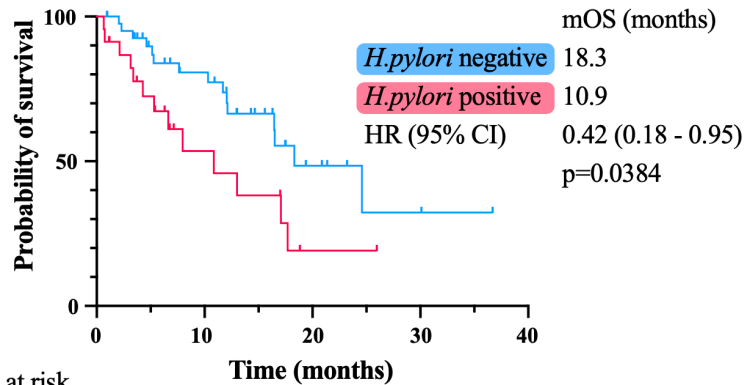
Physiological conditions (e.g. pH value) influence bacterial communities.

# Pathogens and commensals are predictive for response on oncological treatment



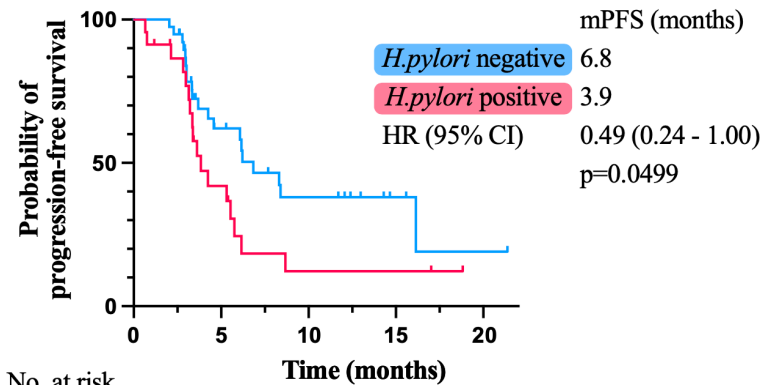
# Pathogens and commensals are predictive for response on oncological treatment

Overall survival: ICI-based regimen



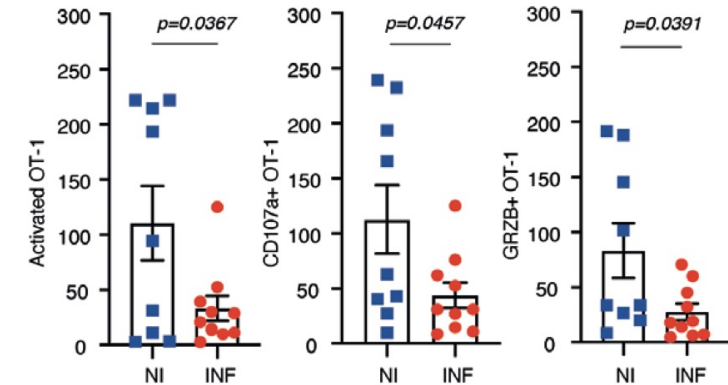
No. at risk	0	10	20	30	40
Negative	41	24	6	2	0
Positive	23	7	1	0	0

Progression-free survival: ICI-based regimen



No. at risk	0	5	10	15	20
Negative	41	17	9	3	1
Positive	23	8	2	2	0

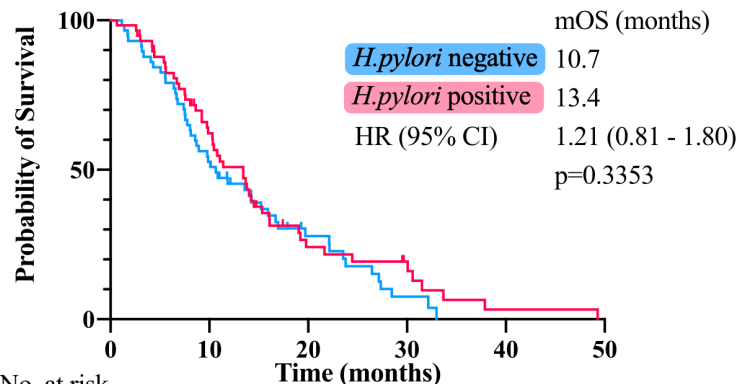
Tumour (number of cells per mm<sup>3</sup>)



Velin D et al, GUT 2022

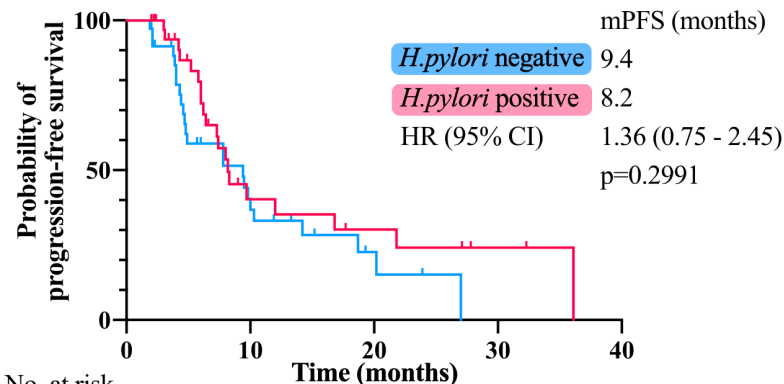
- *Helicobacter pylori* Infection is associated with decreased response on CPI treatment (reduced OS und PFS).

Overall survival: Sorafenib-based therapy



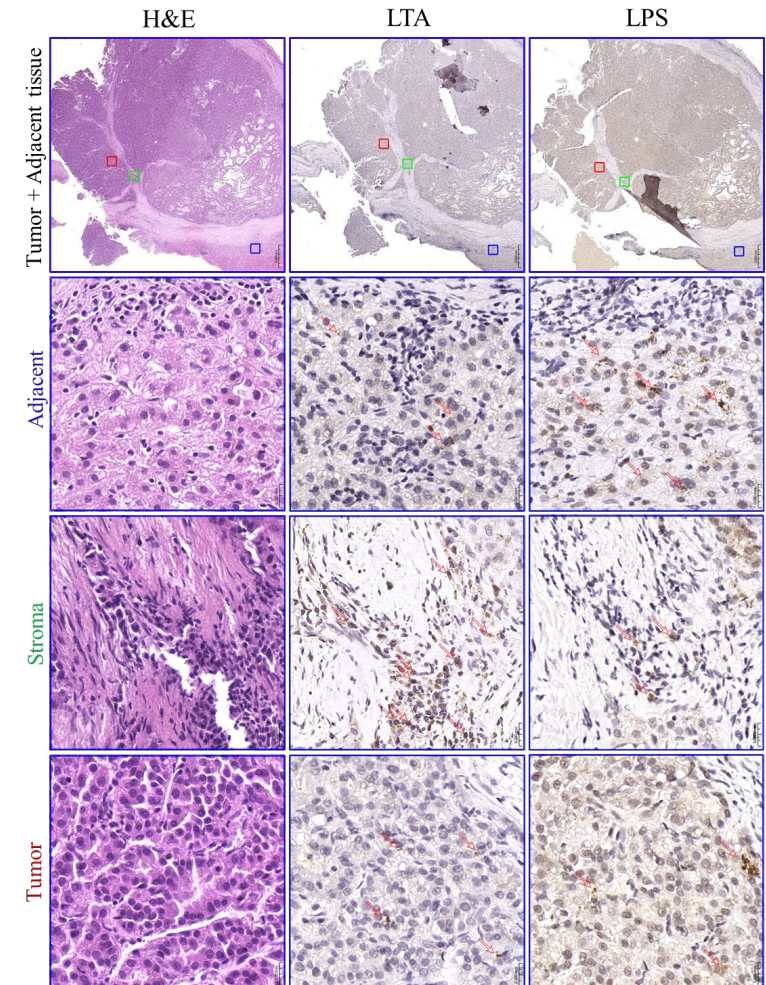
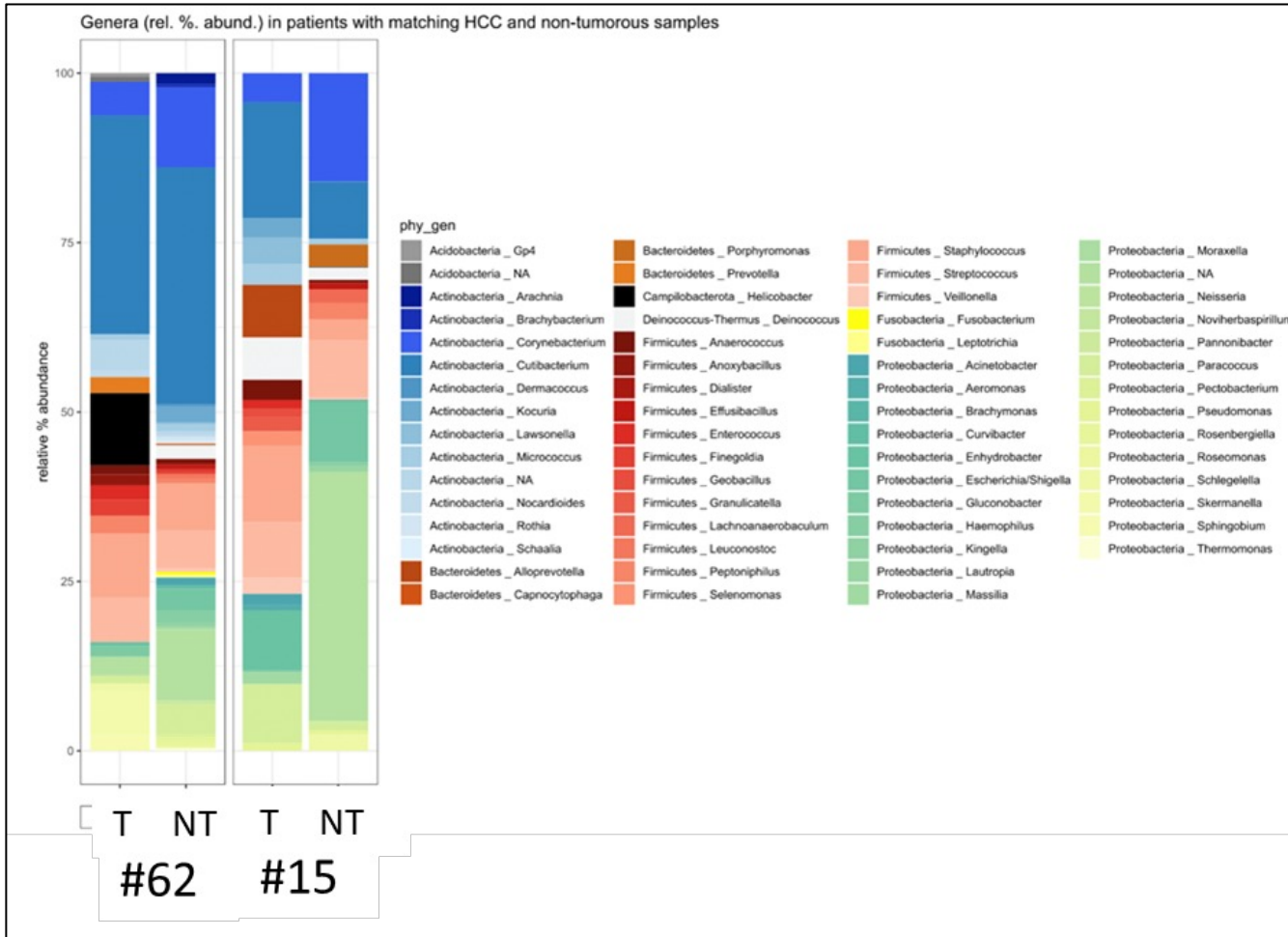
No. at risk	0	10	20	30	40	50
Negative	58	30	11	2	0	0
Positive	58	33	10	6	1	0

Progression-free survival: Sorafenib-based therapy



No. at risk	0	10	20	30	40
Negative	36	11	3	0	0
Positive	35	8	5	2	0

# Tumor-associated microbial communities in HCC and adjacent tissue

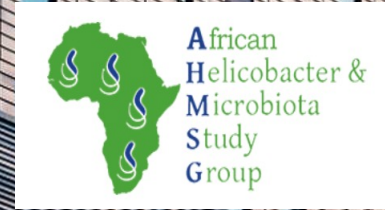
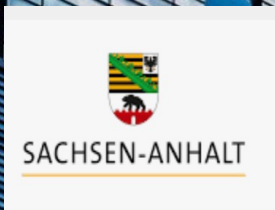


- Distinct microbial signatures in tumor- and adjacent tissue
- Higher abundance of Campilobacterota in responders (OS > 16 Mon)
- Lower abundance of Proteobacteria in non- responders (OS < 16 Mon)

# Potential needs of Microbiota analyses for clinical applications

- Biomarker to distinguish health and disease (and pre-stages) 
- Biomarker for certain (rare) diseases 
- Stratification of patients into specific treatment groups (so called *personalized treatment*) 
- Prediction of treatment response 
- Prediction of course of disease 
- Modification of microbial communities to improve treatment response and –in best case- healing 

- Promising results open a broad spectrum of applications in clinical routine (e.g. oncology, metabolic disorders, prevention)
- Questions to solve to overcome the unselected interventions we are currently offering.
- To reach the aim of “personalized treatment“ methodological standardization and deeper insights in functional pathways are needed.



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