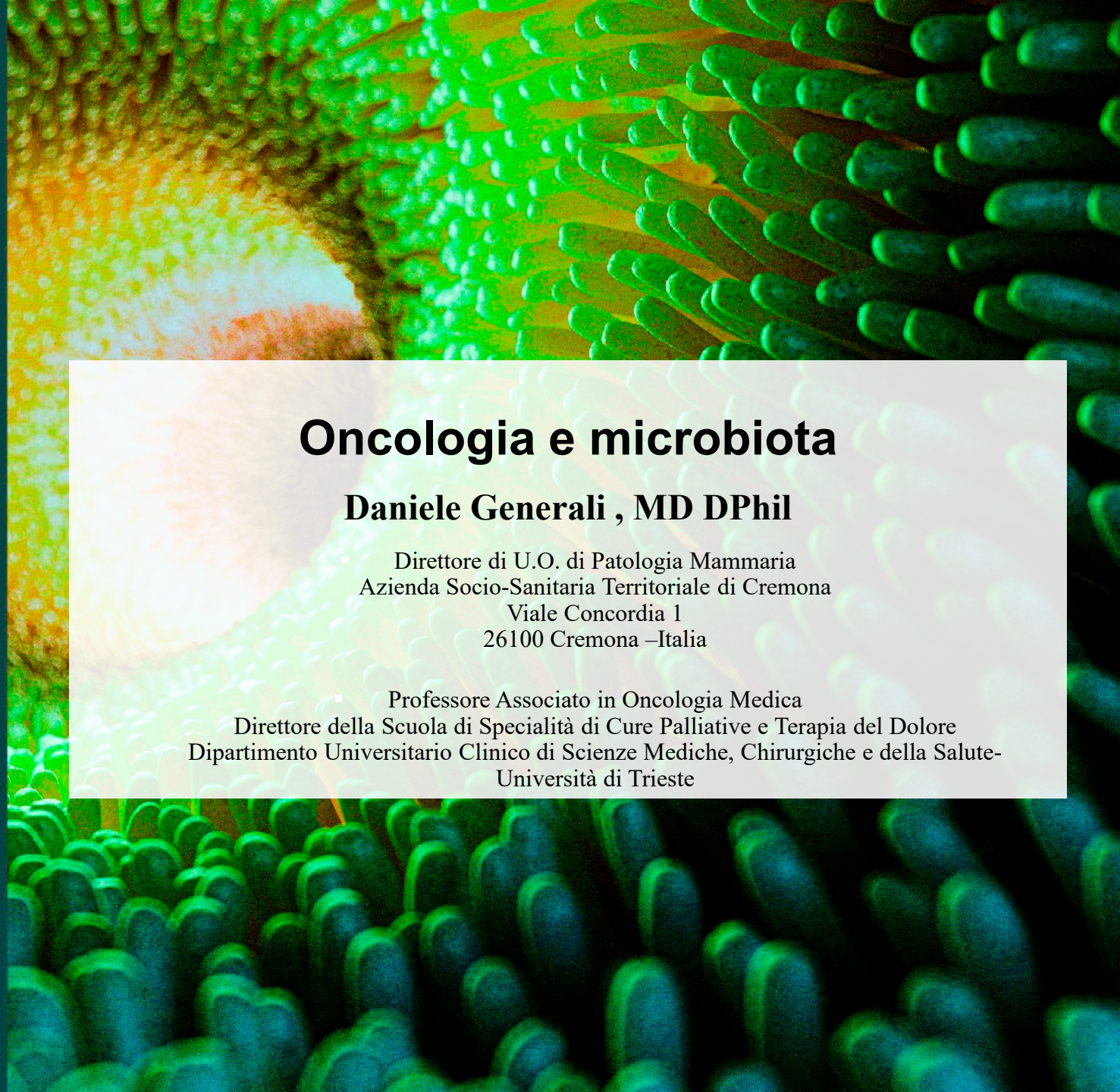
top ten
in gastroenterologia
14^ EDIZIONE
24-25 NOVEMBRE 2023
BERGAMO
HOTEL EXCELSIOR SAN MARCO
Piazza della Repubblica, 6



Oncologia e microbiota

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Disclosure

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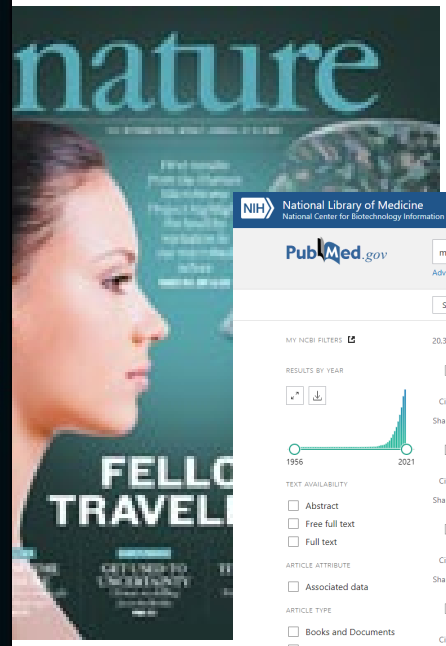
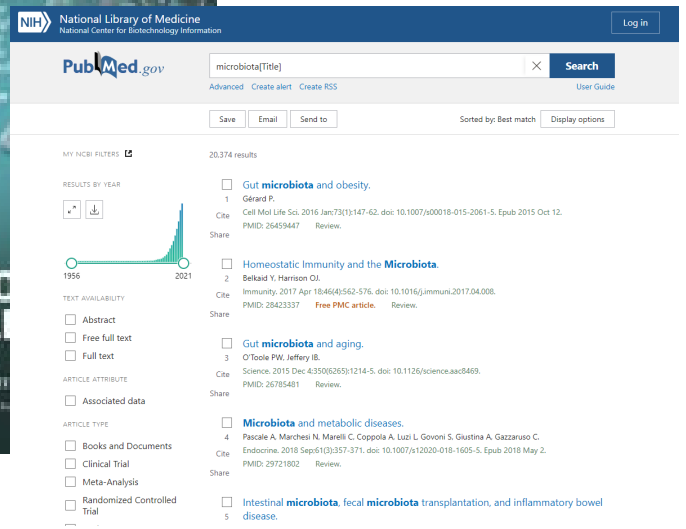
- **Institutional/Research funding: University of Trieste, Novartis, LILT, AstraZeneca, Seagen, Accord**

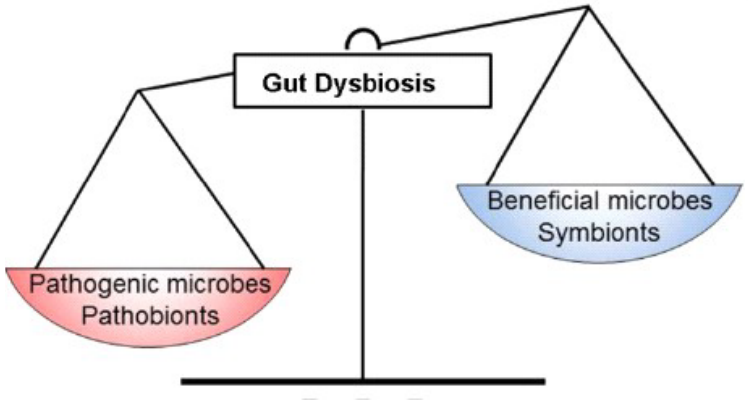
MICROBIOTA REVOLUTION





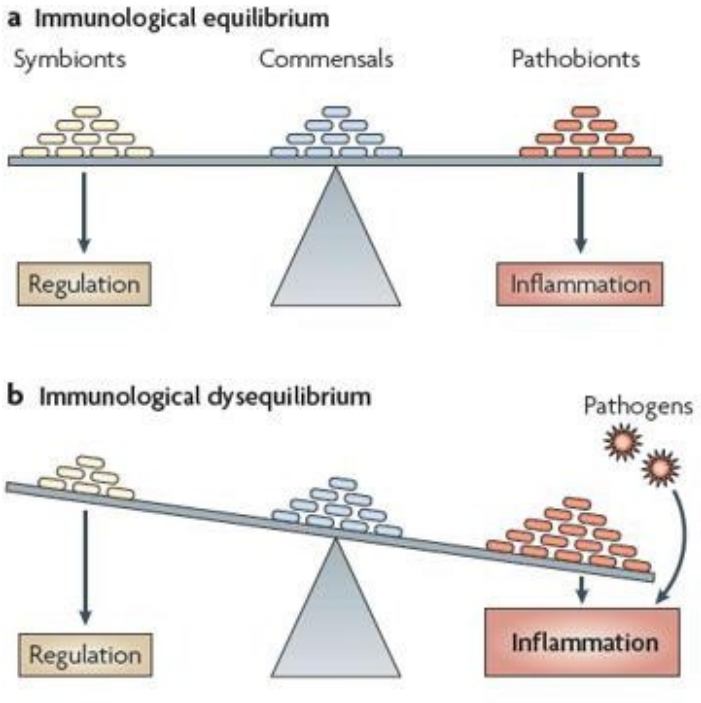
1

INCREASE OF HARMFUL MICROBES
e.g. Escherichia coli

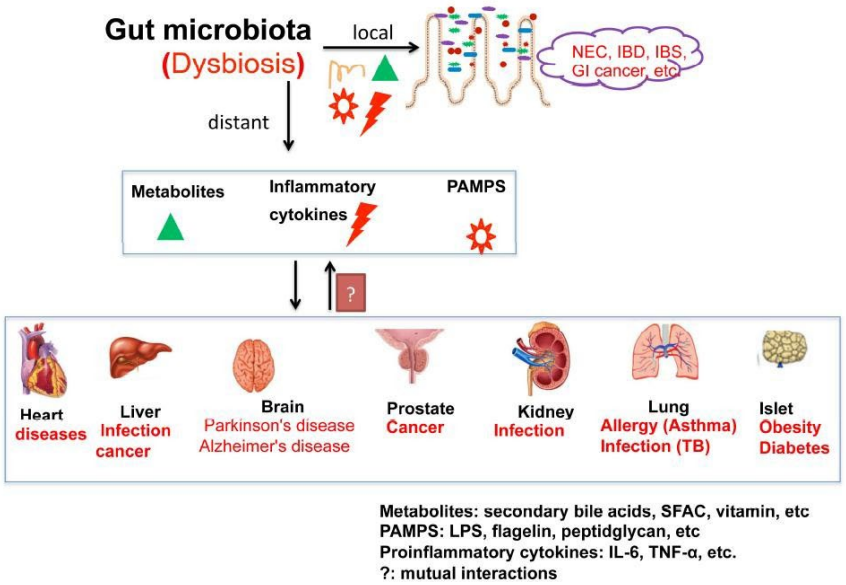


DECREASE OF BENEFICIAL BACTERIA
Firmicutes
Bacteroidetes

Dysbiosis & Disease



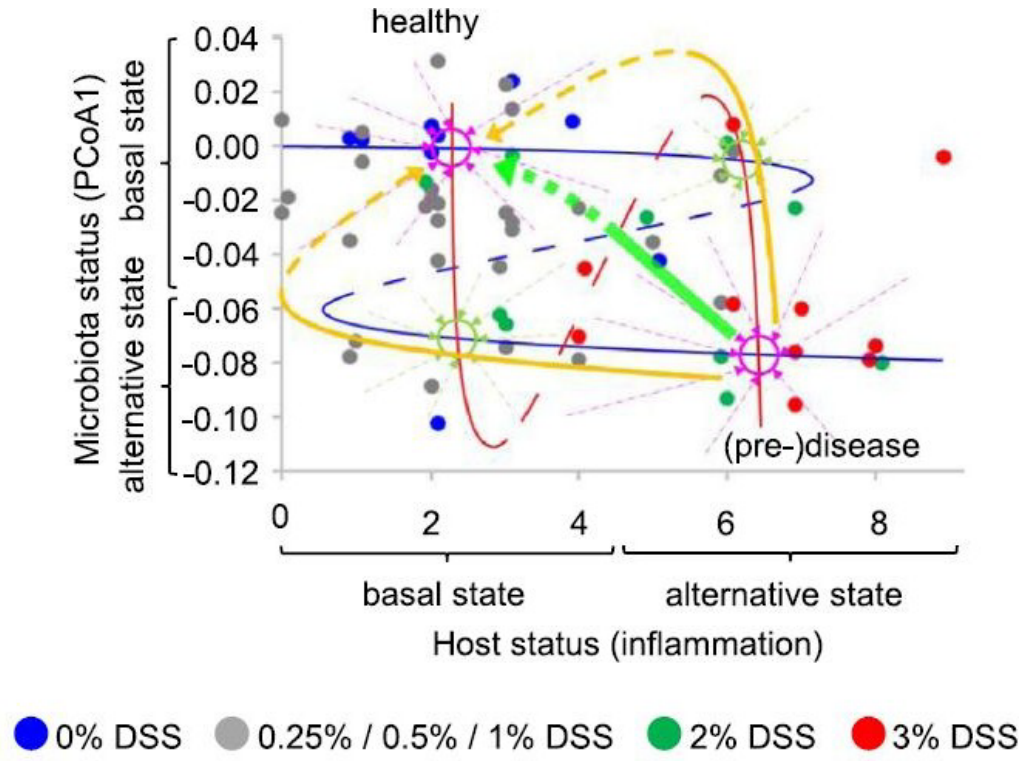
Round and Mazmainan., 2009. Nat Rev. 9:313.



Sun et al., 2014. Genes & Disease. 1:132-139.

Concept of "infection" should be changed for microbiome and disease.

Understanding ecosystem for therapy



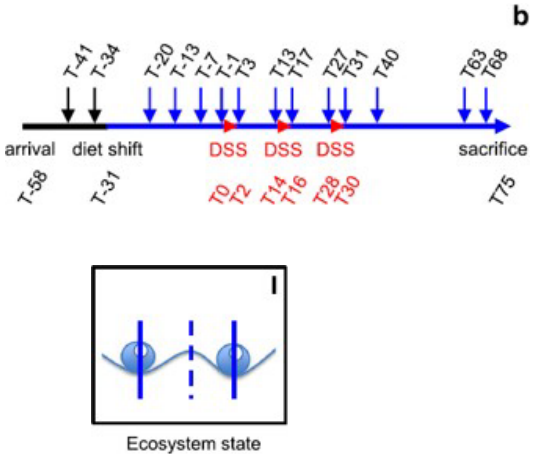
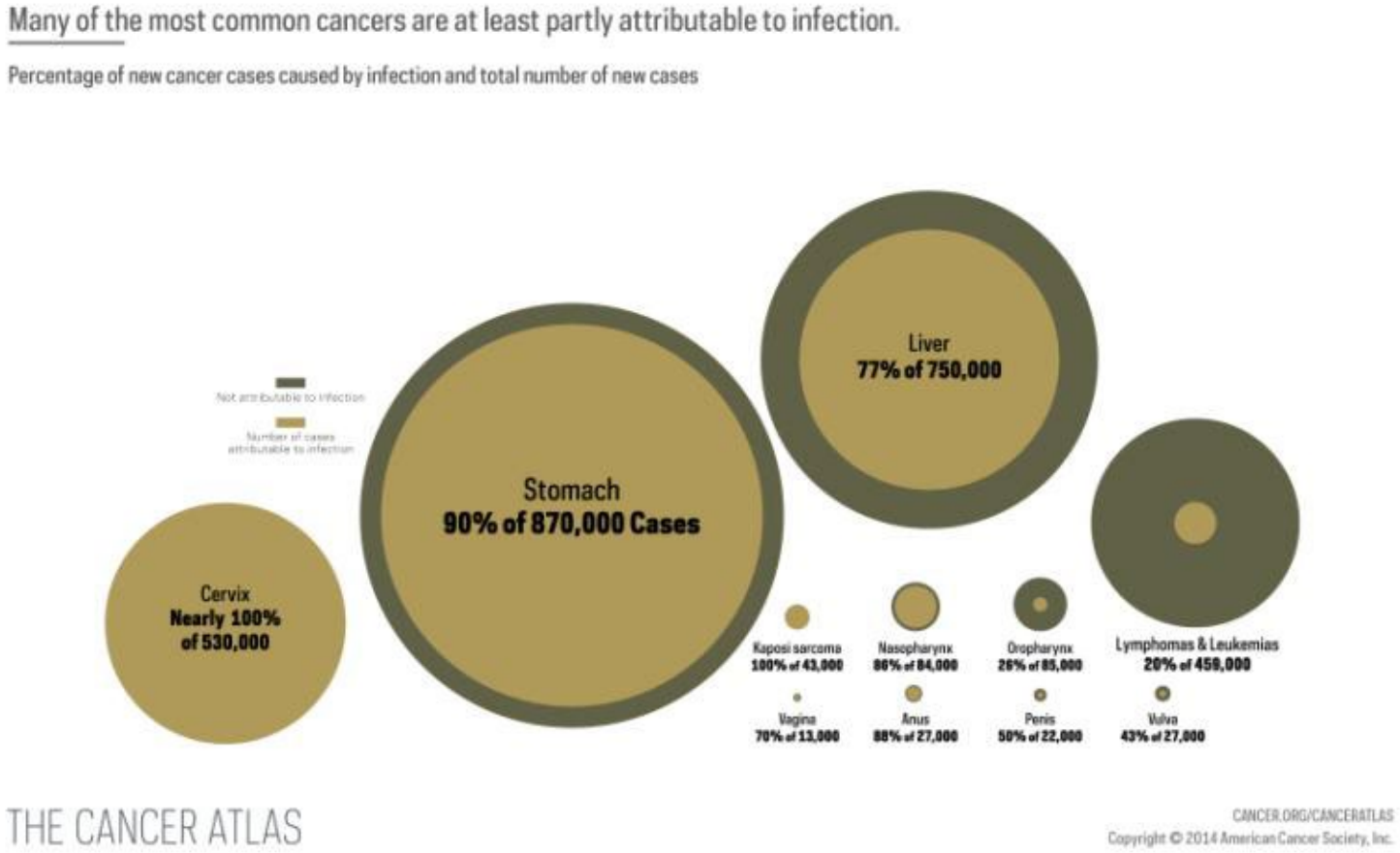


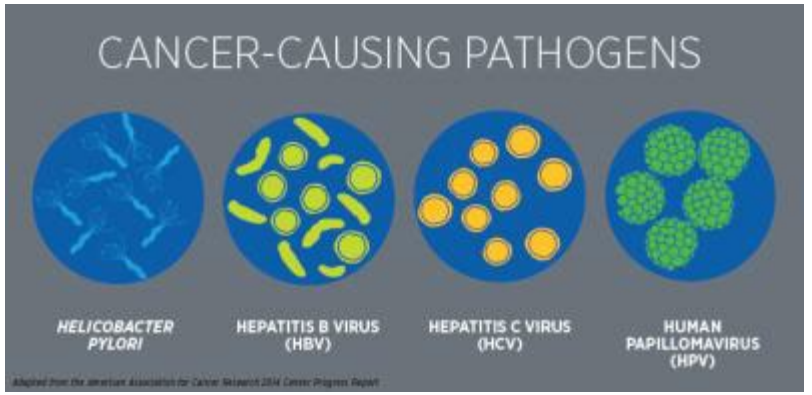
Image from Van de guchete et al., Microbiome. 2020. 8; 153.

Need combination of dedicated treatments and microbiota management

2

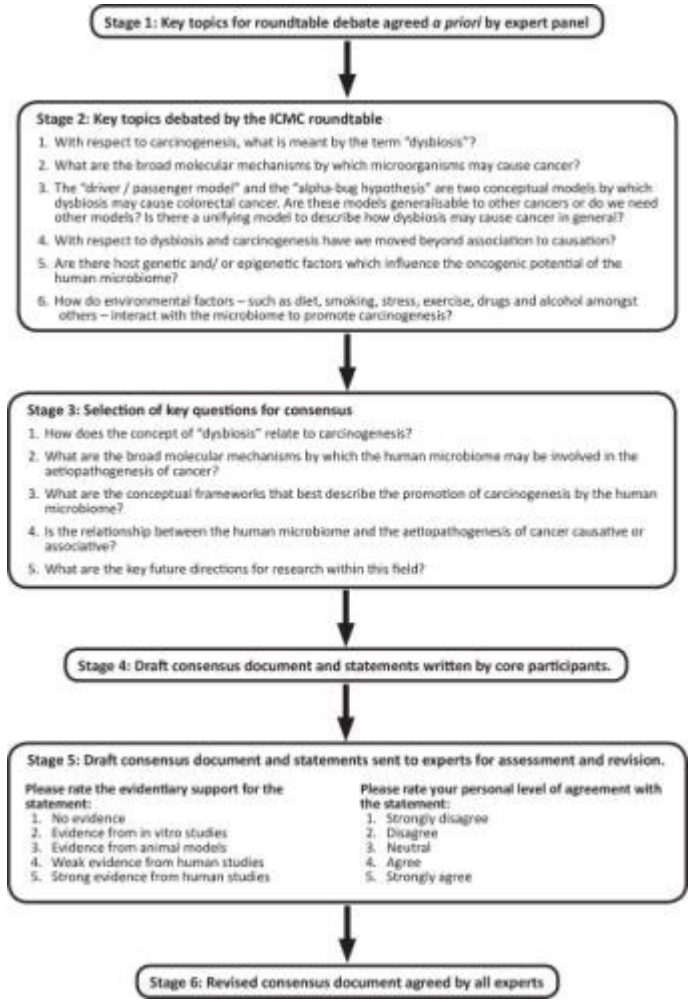
Cancer and microbes





International Cancer Microbiome Consortium consensus statement on the role of the human microbiome in carcinogenesis

- Six key topics agreed a priori by a panel formed by some 18 experts from Canada, China, Europe and the USA (*stage 1*)
- Roundtable discussion centred on the theme of the microbiome and carcinogenesis (*stage 2*)
- Five key questions to be addressed in the consensus statement (*stage 3*)
- Draft statements and supporting discussion in response to each key question (*stage 4*)
- Experts rating the strength of evidentiary support and their personal level of agreement with each statement (*stage 5*)
- Consensus document (*stage 6*)

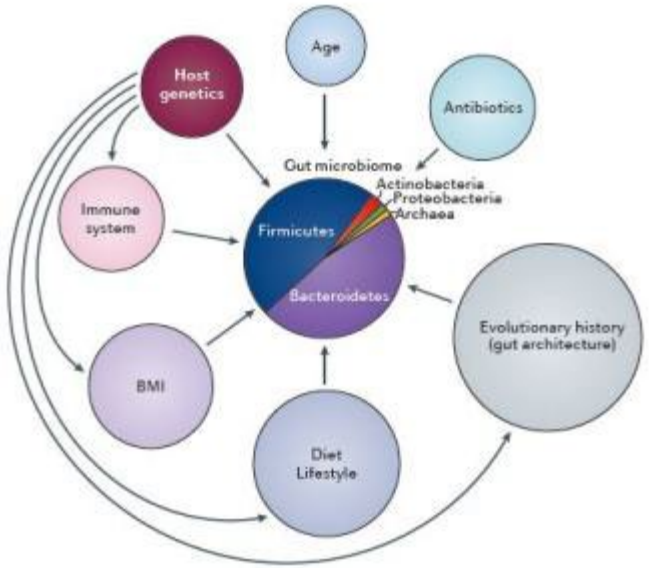


Gut 2019; 68:1624-32

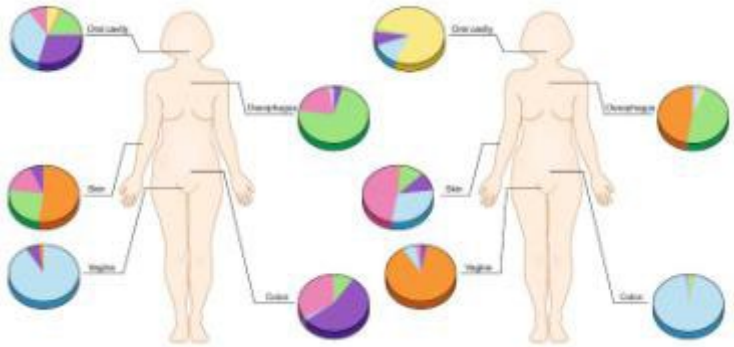
3 How does the concept of dysbiosis relate to carcinogenesis?

Dysbiosis: a *persistent departure* of the host symbiotic microbial ecosystem from the health-associated, homeostatic state, towards a cancer promoting and/or sustaining phenotype.
(weak evidence from human studies)

Does a “normal” microbiome exhist?
(strong evidence from human studies)



Similar ”core microbiome” at phylum level (*Bacteroidetes and Firmicutes*) but different at lower taxonomic leves in appartently healthy individuals.

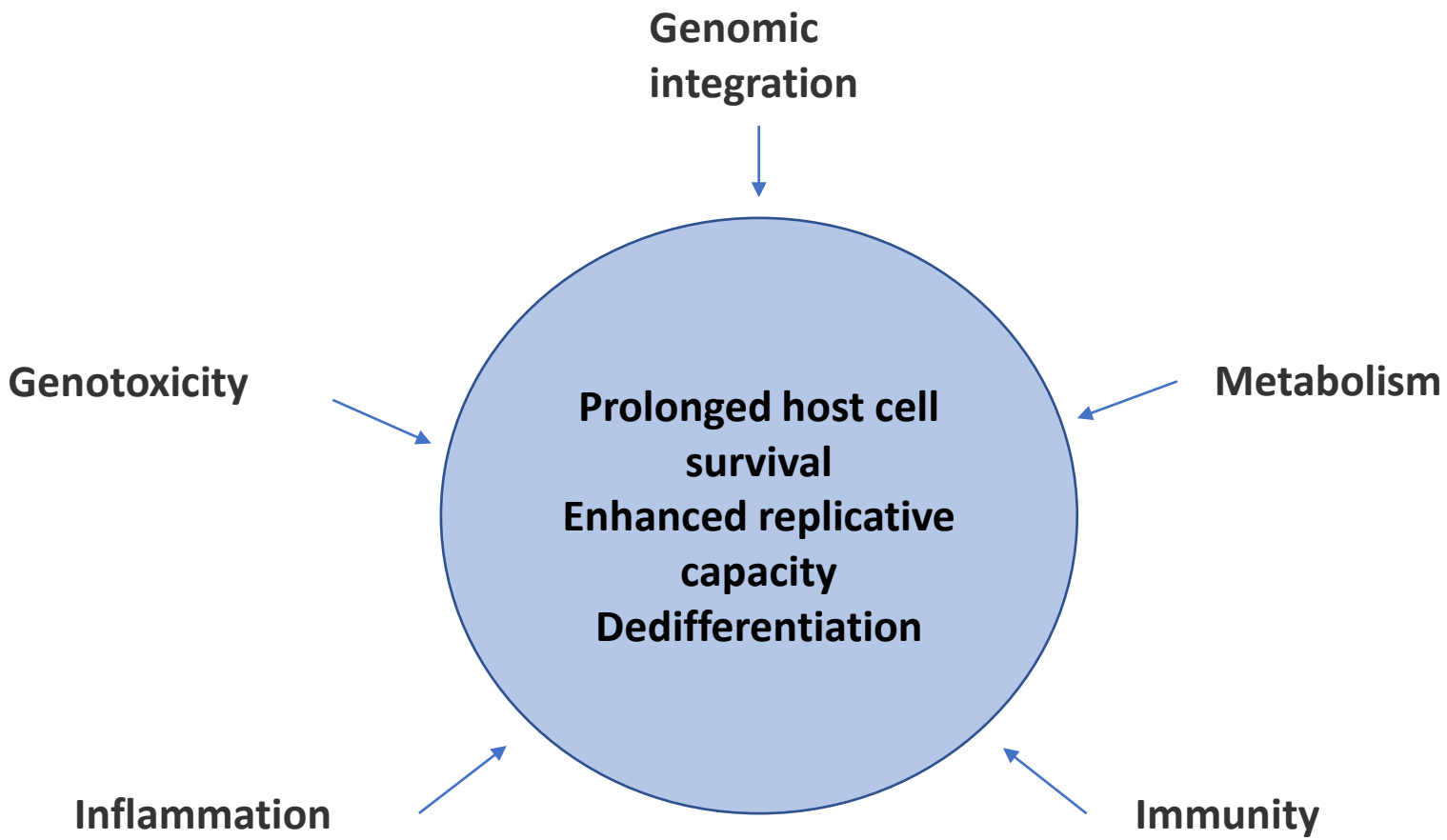


Dysbiosis is specific to the individual, the disease, and the niches.

Brantley Hall *et al*. Nature Rev (2017); Gilbert *et al*. Nature Medicine (2018)

4

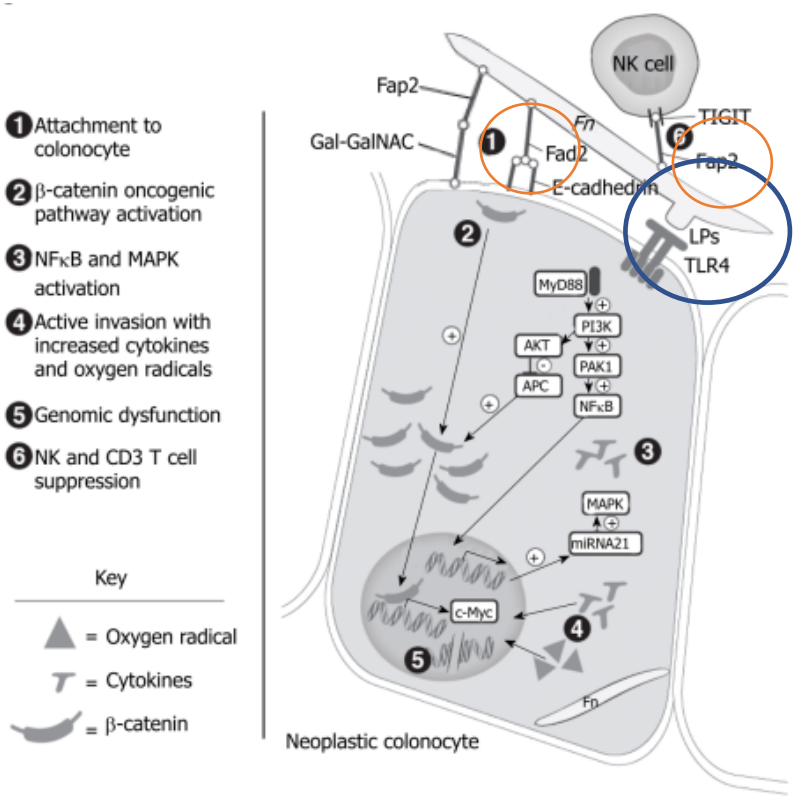
What are the broad molecular mechanisms by which the human microbiome may be involved in the aetiopathogenesis of cancer?



5 What are the broad molecular mechanisms by which the human microbiome may be involved in the aetiopathogenesis of cancer?

Inflammation

(strong evidence from human studies)



Modifiyed from Hussan et al., WJG (2017)

Immunity

(evidence from animal studies/weak evidence from human studies)

Fad2

- Attachment and invasion to colonic epithelia and endotelial cells promoting the release of inflammatory cytokines particularly IL-8, IL-10 and tumor necrosis factor-α (TNF-α) in a proinflammatory microenvironment[2-4]
- Activation of B-catenin pathway through E-cadherin mediated binding[5].
- Increase expression of oncogenic miRNA21 by activating TLR4 signaling to MyD88 which leads to NFKB pathway activation[6].

Fap2

- Adherence to colonic epithelia through host lectin Gal-GalNAc[1]
- Produce an immunosuppressive microenvironment through interaction with TIGIT and attraction of myeloid-derived suppressor cells[7-9]

Unknown

- Inhibit NK cell and T cell function by binding to carcinoembryonic antigen-related cell adhesion molecule (CEACAM) 1[10]

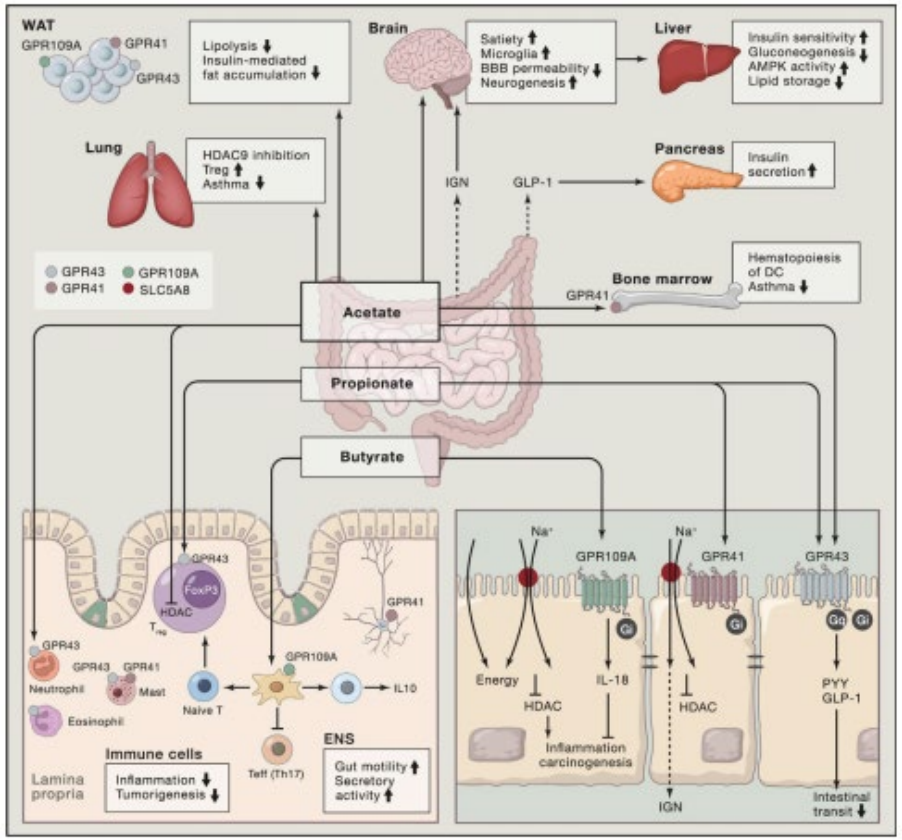
[1]Abed *et al.* Cell Host Microbe (2016); [2]McCoy et *al.* PLoS One (2013); [3]Quah et *al.* Int Endod J (2014); [4]Dharmani *et al.* Infect Immun (2011); [5]Rubinstein *et al.* Cell Host Microbe (2013); [6]Yang *et al.* Gastroenterology (2017); [7]Gur *et al.* Immunity (2015); [8]Kostic *et al.* Cell Host Microbe (2013); [9]Bashir *et al.* Tumour Biol (2016); [10]Gur *et al.* Oncoimmunology (2019)

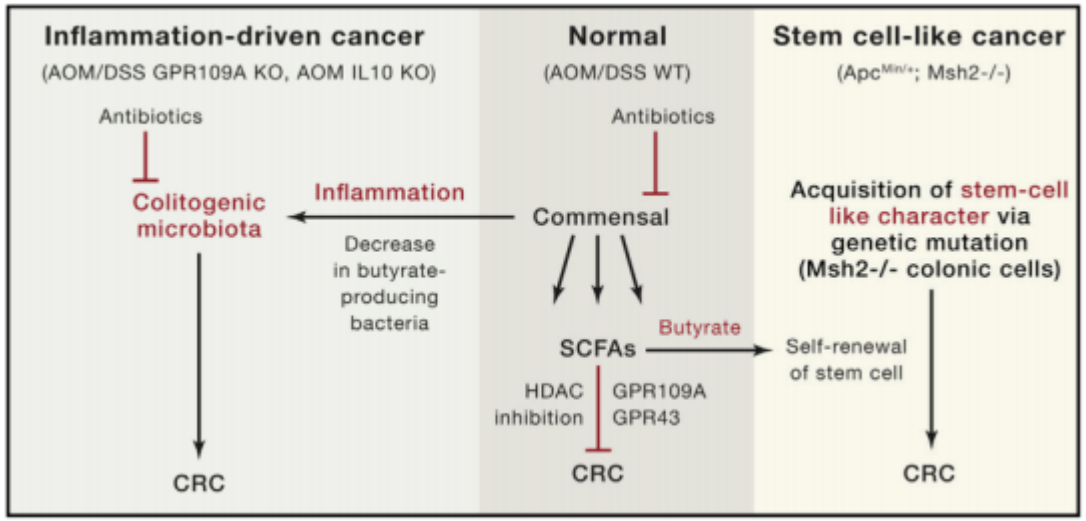
6

What are the broad molecular mechanisms by which the human microbiome may be involved in the aetiopathogenesis of cancer?

Metabolism

(strong evidence from human studies)



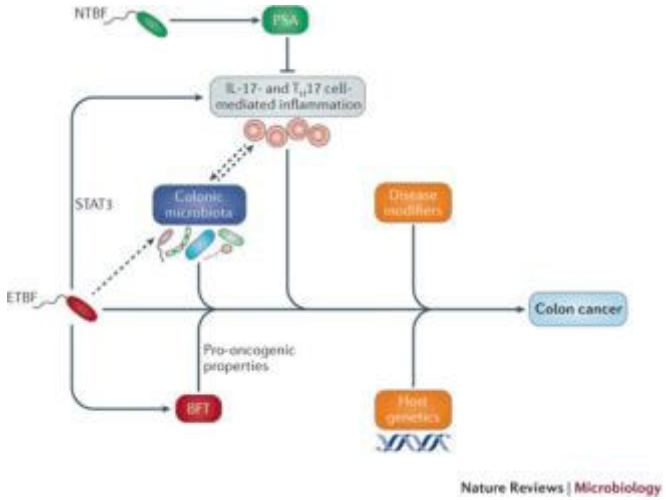


Koh et al, Cell 2019

What are the conceptual frameworks that best describe the promotion of carcinogenesis by the human microbiome?

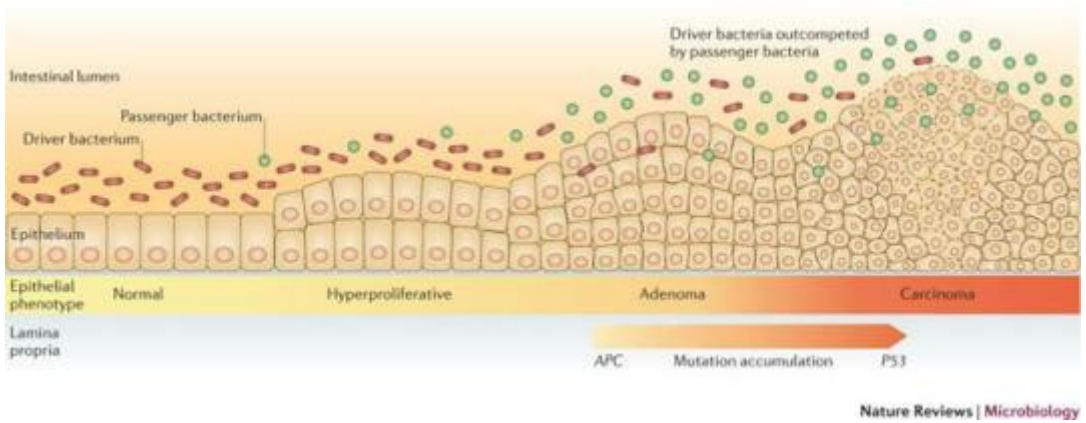
Alpha-bug hypothesis

Sears CL , Pardoll DM. Perspective: alpha-bugs, their microbial partners, and the link to colon cancer. J Infect Dis (2011)



Driver-passenger hypothesis

Tjalsma H et al . A bacterial driver-passenger model for colorectal cancer: beyond the usual suspects. Nat Rev Microbiol 2012



INTERACTOME: carcinogenesis as the outcome of a tripartite multidirectional interaction between the microbiome, the environment and the epigenetically/genetically vulnerable host. *(weak evidence from human studies)*

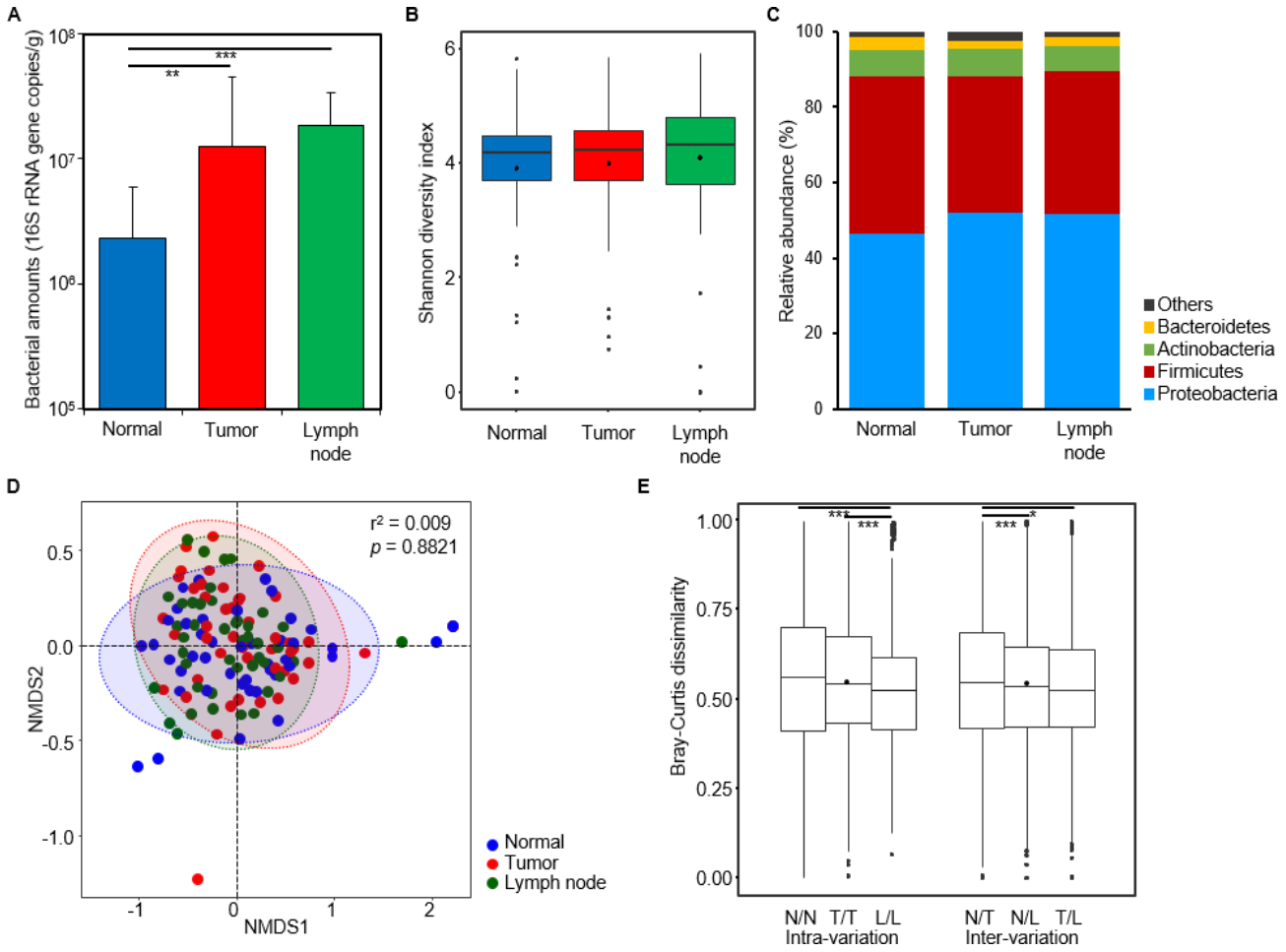
What are the key directions for future research to develop our understanding of the role of the microbiome in carcinogenesis ?

- Large, international longitudinal cohort studies
- Prospective longitudinal sampling
- Increased focus on interventional studies
- Integration with other oncology research
- Standardization and trasparency in reporting microbiome research

Microbiota and patients with breast cancer

- Breast cancer is one of three most common cancers in women.
- Differences in the gut microbiome of patients with breast cancer related to estrogen metabolism.
- Several studies have confirmed the presence of microbiota in breast tissue.
- However, understanding of microbiome in breast tissue in the progression of breast cancer is limited.
- We aimed to determine differences in the microbiota according to tissue types and recurrence in Korean women with breast cancer.

Microbiota difference by tissue type (related to the breast)



Kim et al., 2021. J. Microbiol. Biotech. In press

Fecal microbiota composition is related to response to CDK4/6-inhibitors in metastatic breast cancer: a prospective cross-sectional exploratory study

Background

- The study of fecal microbiota composition is currently a “hot topic” in several diseases (especially in Gastroenterology and Neurology)

- Treatments based on the modification of fecal microbiota (e.g. fecal transplant) have been postulated as a potential strategy for several diseases, including irritable bowel disease, autism spectrum disorders, C. Difficile colitis etc.

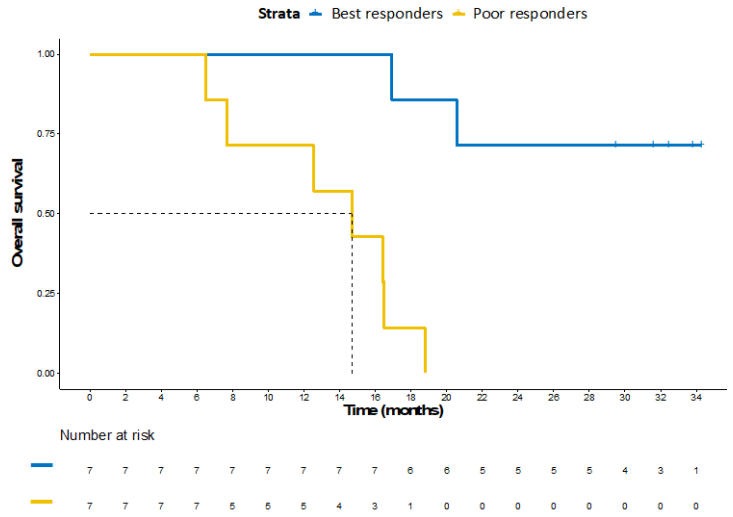
- There is emerging evidence regarding the capability of fecal microbiota to predict treatment response in several tumor types (e.g. to ipilimumab in melanoma, to 5-FU in colorectal cancer etc.) and being directly implied in chemotherapy resistance and development of side effects

- Very little is known regarding fecal microbiome impact on breast cancer treatment efficacy and only preliminary data are currently available

- CDK4/6i+ET are the current 1st-line SoC for HR+/HER2- MBC. Although being very effective, biomarkers predictive of response are needed, to maximize therapeutic benefit and reduce high therapeutic costs

Results: cohort differences and survival analysis

- No significant clinicopathological differences, except for higher BMI in NR (p=0.016) and slightly higher NLR in NR (p=0.026)
- The median follow-up at the time of the analysis was 32.5 months (95%CI: 31.6 –NE)
- Seven (50%) patients were considered as R, while other 7 were considered as NR
- Median PFS and OS for R were not reached at the time of the analysis. For NR, median PFS was 6.2 months (95%CI: 3.8 – NE) and median OS was 14.7 months (95%CI: 7.7 – NE)



- Clinicopathological characteristics and circulating immune cells were not associated with PFS and OS
- Only higher levels of NLR were significantly associated to worse PFS (HR: 4.13, 95%CI: 1.08-15.74; *p*=0.038), with a tendency towards a significantly worse OS (HR: 3.17, 95%CI: 0.87-11.72; *p*=0.081)

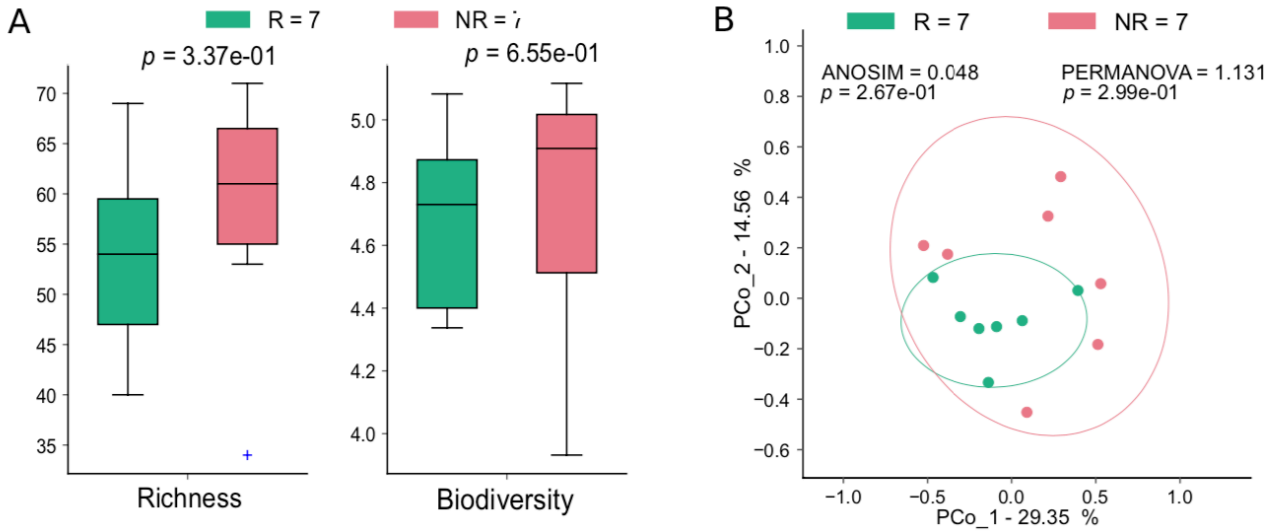
Results: assessment of phyla and species distribution according to response to CDK4/6i

7 Phyla

Phylum	NR	R	P values *
	Mean ± SEM (%)	Mean ± SEM (%)	
Firmicutes	61.75±4.95	55.80±1.92	0.701
Bacteroidota	22.85±3.60	26.61±1.47	0.443
Actinobacteriota	4.14±0.80	11.58±3.35	0.125
Proteobacteria	5.97±2.73	4.53±2.17	1.000
Verrucomicrobiota	2.38±1.69	1.07±0.58	0.891
Desulfobacterota	0.37±0.13	0.38±0.10	1.000
Euryarchaeota	1.53±1.02	0.02±0.02	0.551

Higher relative abundance, though not sig.

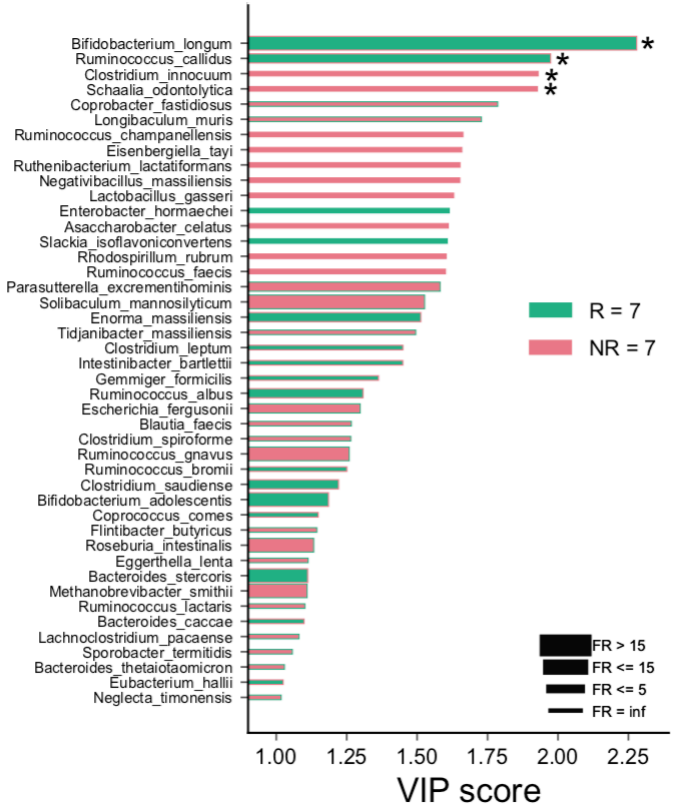
118 Species



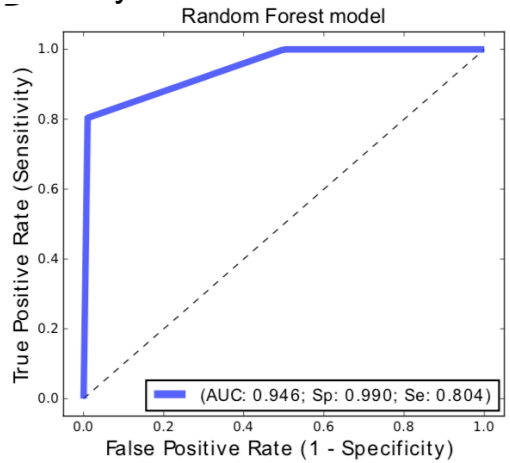
No significant difference between NR and R in alfa and beta diversity

Results: identification of discriminant species

PLS-DA to identify the most discriminant bacterial species among the cohorts



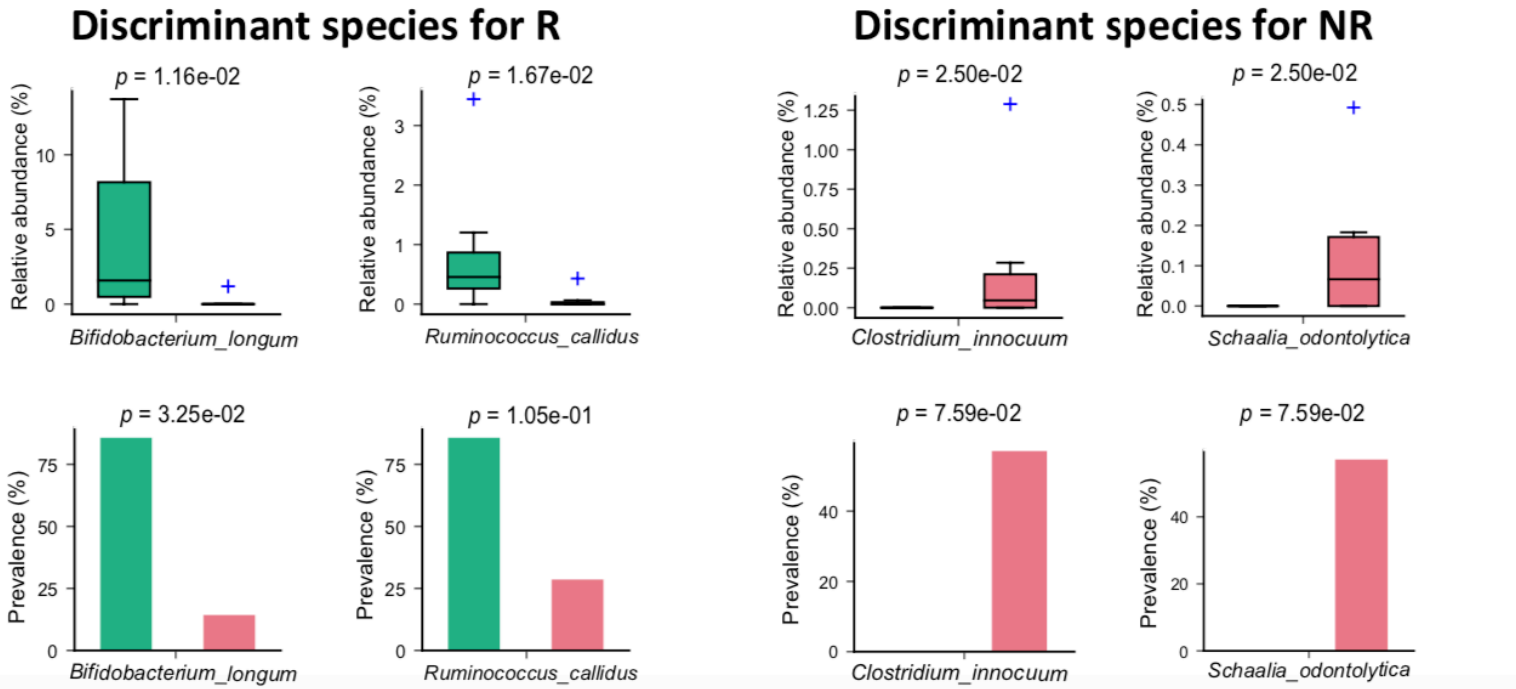
- Discriminant species after PLS-DA in descending order of VIP score (bar length)
- Central bar colors represent the cohort where the highest relative abundance of a species was found
- Edge bar color the cohort where the lowest one was observed
- The thickness of the bars represent the fold ratio (FR) of the highest vs. the lowest relative abundance
- Absent borders indicate mean relative abundance of zero in the compared cohort
- * represent a significant difference between R and NR after Mann–Whitney U test



The 4 differentially distributed species were able to discriminate between NR and R, with an excellent AUC

Results: discriminant species and relative abundance and prevalence

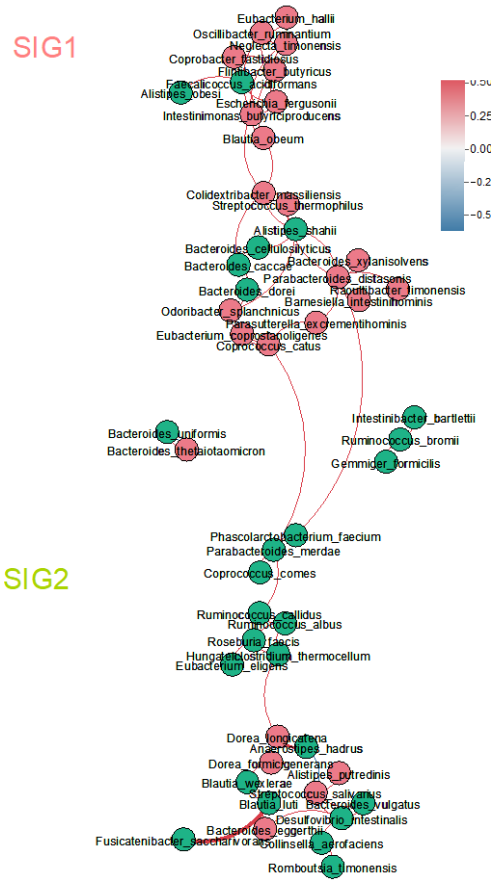
Pairwise analysis of the selected four species depicts significant differences in terms of relative abundance (box plots) and prevalence (bar plots)



Results: network analysis

Figure explanation

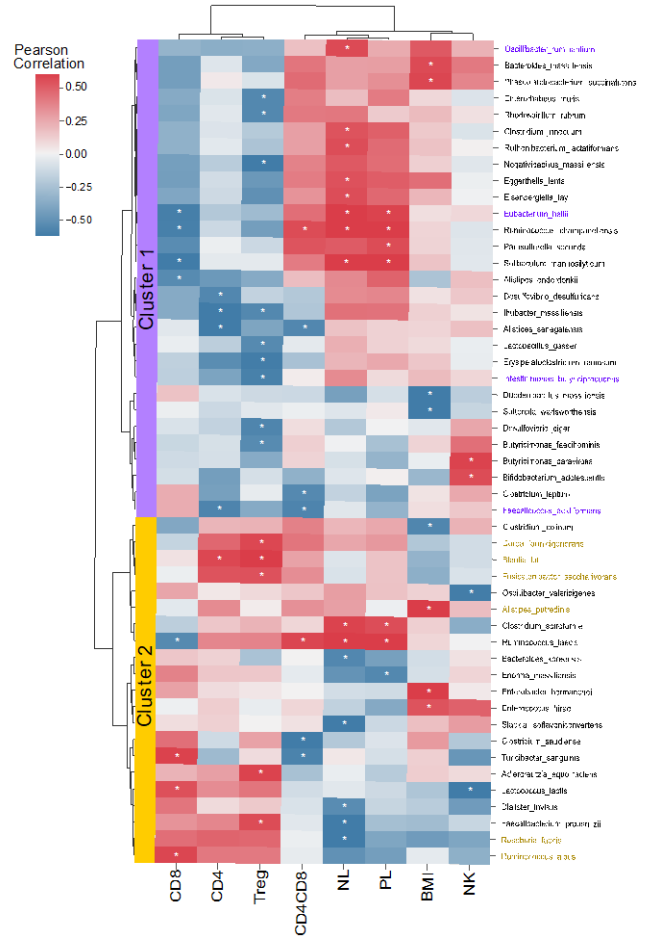
- Network analysis showing communities of bacterial species (species-interacting groups, SIGs) and their positive or negative relative abundances correlation.
- Nodes are colored according to the cohort harboring the higher relative abundance for a definite species, as NR (red) or R (green).
- Edge thickness is inversely proportional to the Pearson p-value after 10% Benjamini–Hochberg two-stages FDR, and it is colored according to positive (red) or negative (blue) Pearson coefficient.



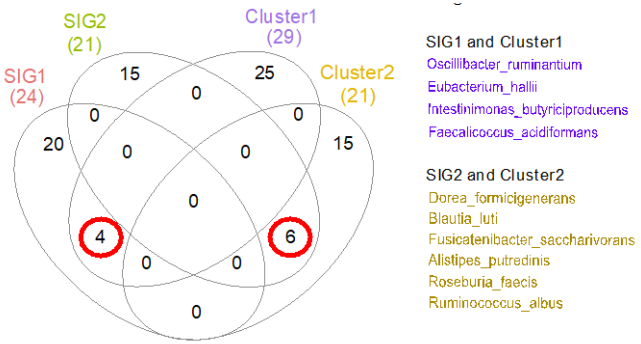
Meaning

- Two major clusters of interacting bacterial species (Species Interacting Groups-SIGs)
- SIG1 group harbored 75% of NR-related species, while a SIG2 group harbored 76% of species with higher relative abundance in R
- This topological distribution was highly significant (p<0.001) → these 2 communities could have an opposite role in responsiveness to CDK4/6i

Results: correlations



- Correlogram of bacterial species and immunological parameters + BMI shows positive (red) or negative (blue) Pearson correlation on bacterial species' abundances
- Significant correlation is marked with an asterisk inside each square: only species or parameters having at least one significant correlation were reported
- Dendrograms on the x and y axes were generated following Bray–Curtis similarity (between sample), evidencing two different clusters for bacterial species (Cluster1 and Cluster2)



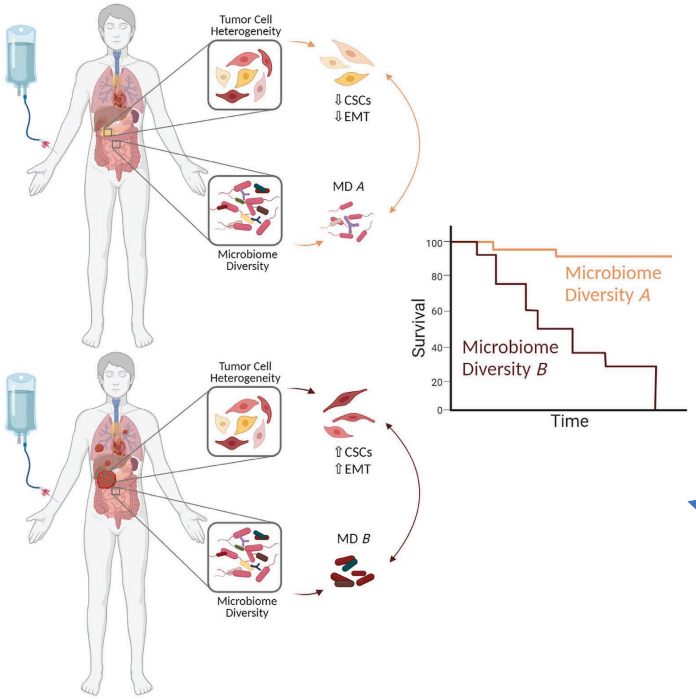
A certain correspondence among SIG1 and Cluster1, and among SIG2 and Cluster2, was observed

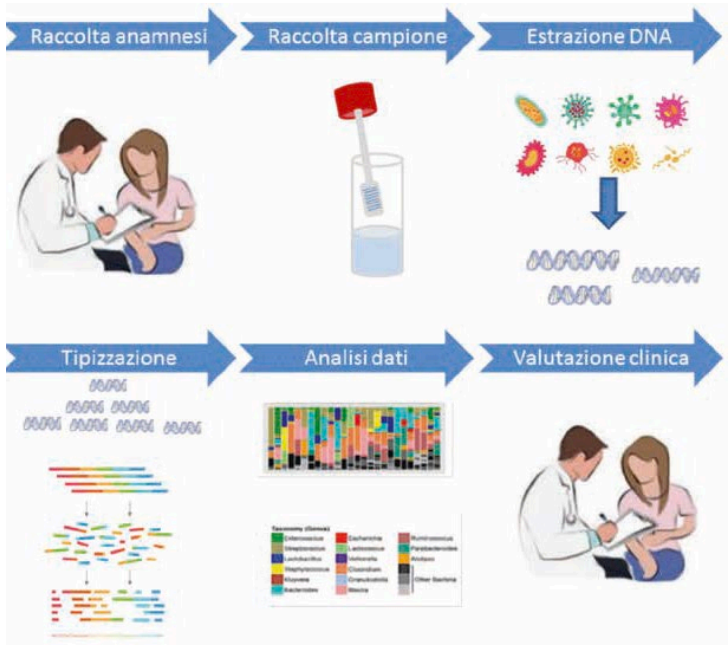
- Among the 4 species evidenced by the VIP plot, only *Clostridium innocuum* showed a positive association with NLR (r=0.53, *p*=0.049) (Cluster1)
- A bunch of species falling within the Cluster1 were positively related to NLR, CD4/CD8 and PLR, and, negatively related to CD8+, CD4+ and Tregs lymphocytes

Conclusions

- Patients experiencing more prolonged responses to CDK4/6-inhibitors-based regimens showed lower basal levels of NLR and lower levels of NLR (higher adaptive immunity activation) showed an association with better prognosis
- Some bacterial species seem to be positively related to NLR, thus probably exerting a negative effect on response to CDK4/6i.
- 1 of those species (*Clostridium innocuum*) showed higher relative abundance and prevalence in NR. On the contrary, species negatively related to NLR, could have a favorable prognostic impact (though no differential abundance was observed)
- A clear and statistically significant differential distribution of fecal bacterial species in SIGs according to response to CDK4/6-inhibitors was observed in the network analysis
- Several members of the *Actinobacteria* phylum, such as *Bifidobacteria*, can be administered *via* probioticsand have been found to increase the efficacy of anti-PD-L1 ICI in breast and other tumors mouse models
- *Bifidobacterium longum* was more abundant in R, compared to NR. If *Actinobacteria* such as *Bifidobacteria* were effectively able to both improve response to CDK4/6-inhibitors and anti-PD-L1 agents, they could be easily provided to patients *via* probiotics as a strategy to boost therapeutic efficacy
- Higher abundance of *Ruminococcus callidus* was also observed in R. Although there is no specific study associating this species with breast cancer, it has been reported to be negatively associated with colorectal cancer
- In general, targeting the fecal microbiota with antibiotics, probiotics, transplants etc. might modulate the reponse to some anti-cancer agents
- Overall, results are limited by the low N. However interesting tendencies should be further explored

Conclusions: Next Future





Summary

•Advancement of sequencing techniques using metagenome has led to deep insights in microbiome studies.

• Understanding the role of microbiome is important in microbiome study with diseases.

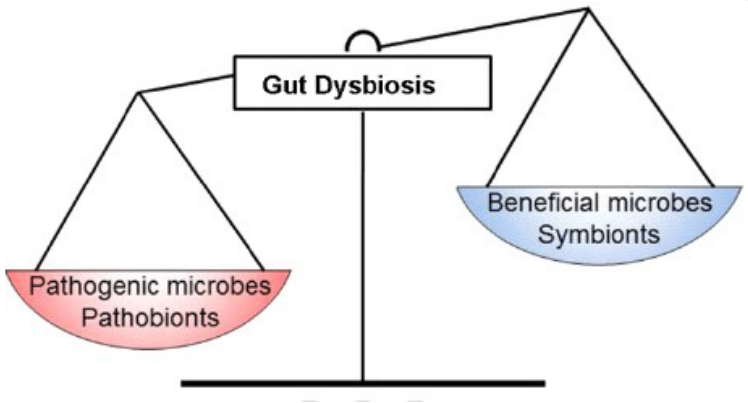
•We should understand the complex interactions between microbiome and host with considering various influencing factors.

•We should understand the complex interactions between microbiome and cancer with the perspective of new tretament or empowered the TT with use in clinical routine for treating cancer

TOP TEN Slides

1

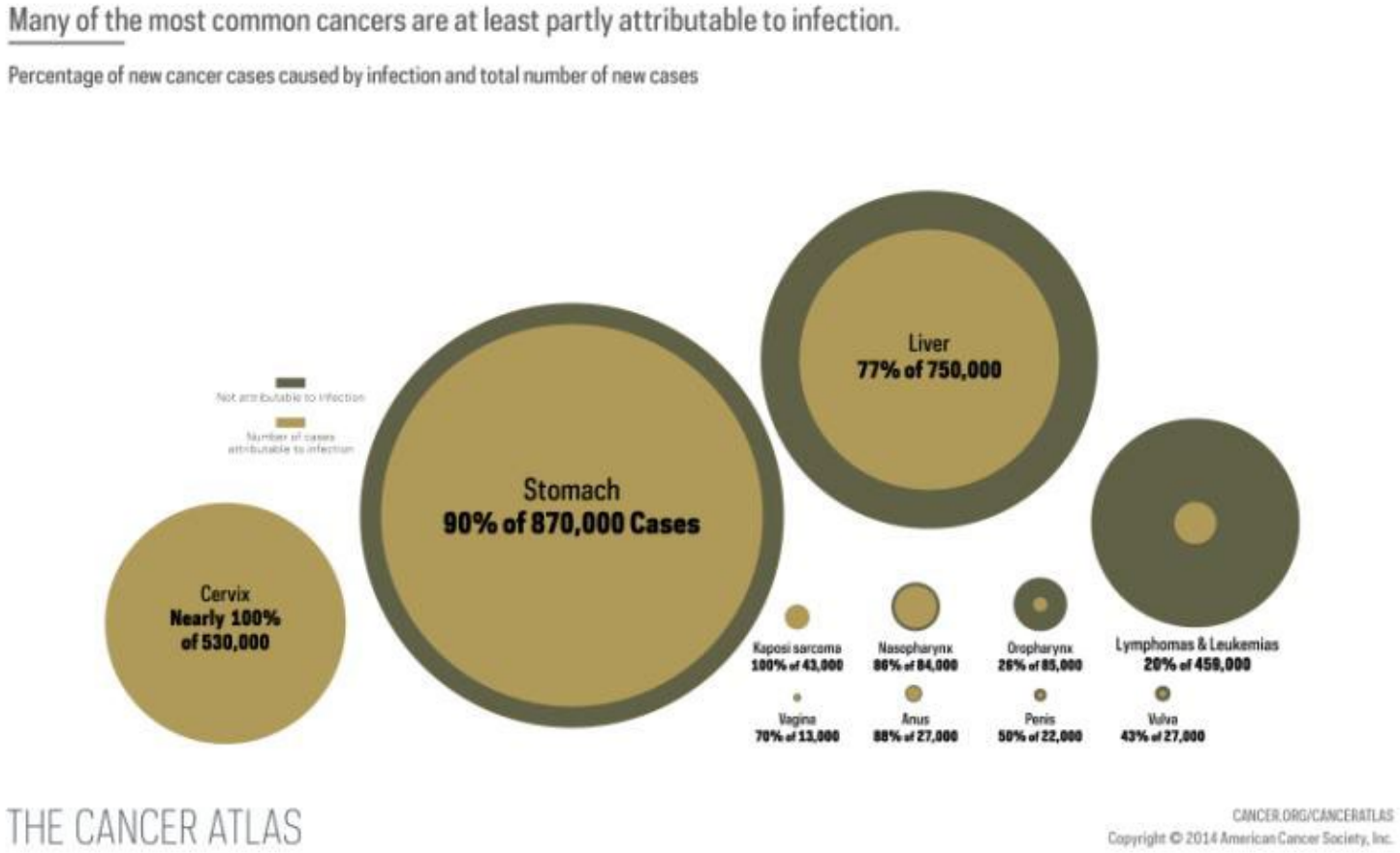
INCREASE OF HARMFUL MICROBES
e.g. Escherichia coli

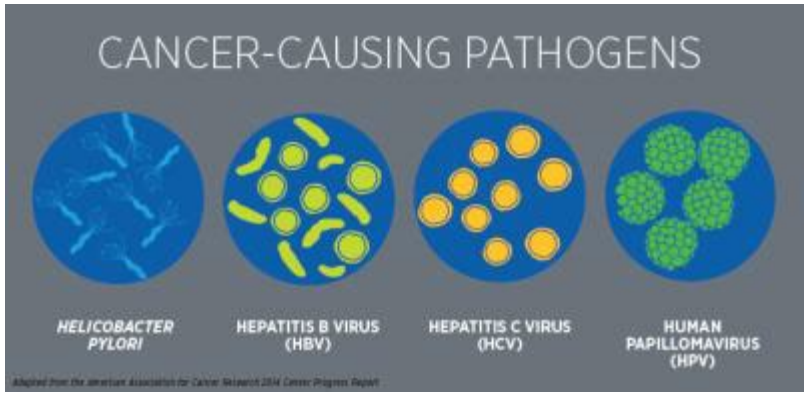


DECREASE OF BENEFICIAL BACTERIA
Firmicutes
Bacteroidetes

2

Cancer and microbes

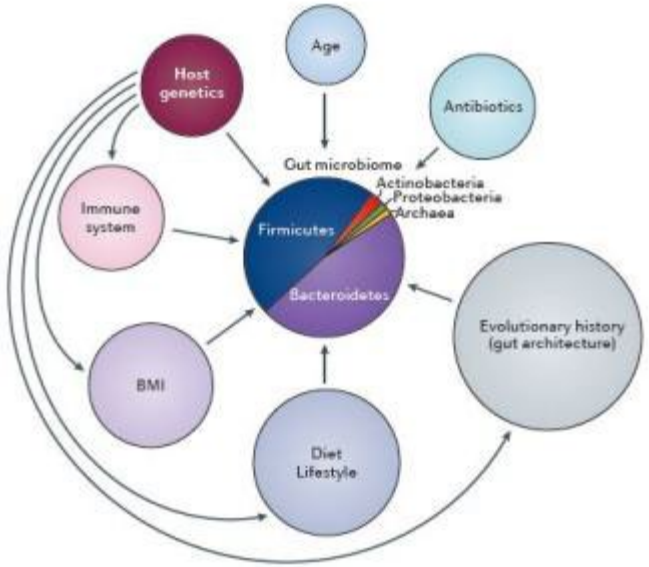




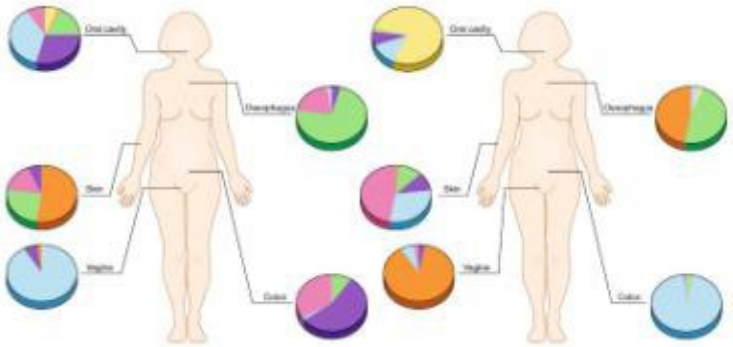
3 How does the concept of dysbiosis relate to carcinogenesis?

Dysbiosis: a *persistent departure* of the host symbiotic microbial ecosystem from the health-associated, homeostatic state, towards a cancer promoting and/or sustaining phenotype.
(weak evidence from human studies)

Does a “normal” microbiome exhist?
(strong evidence from human studies)



Similar ”core microbiome” at phylum level (*Bacteroidetes and Firmicutes*) but different at lower taxonomic leves in appartently healthy individuals.

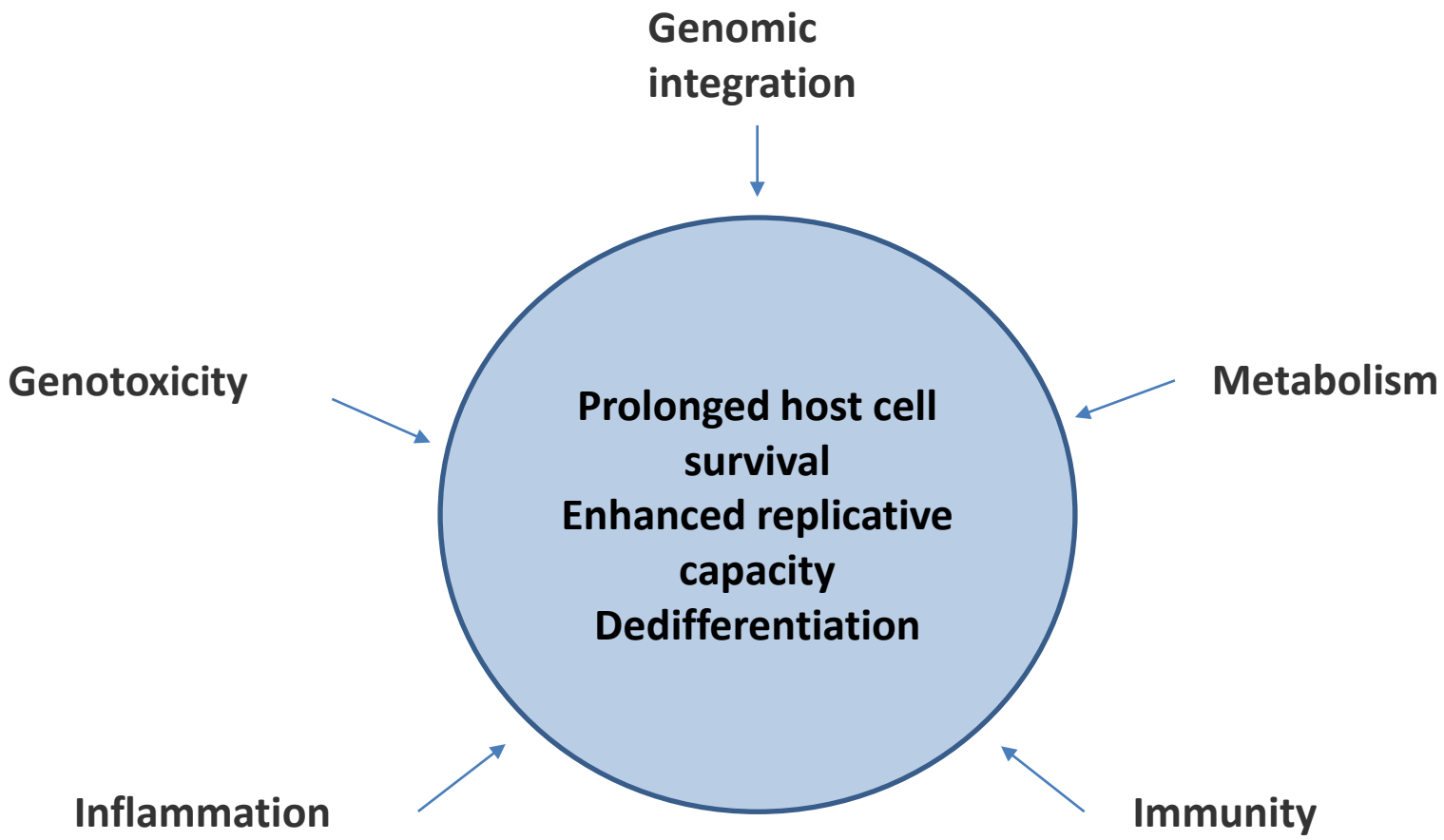


Dysbiosis is specific to the individual, the disease, and the niches.

Brantley Hall *et al*. Nature Rev (2017); Gilbert *et al*. Nature Medicine (2018)

4

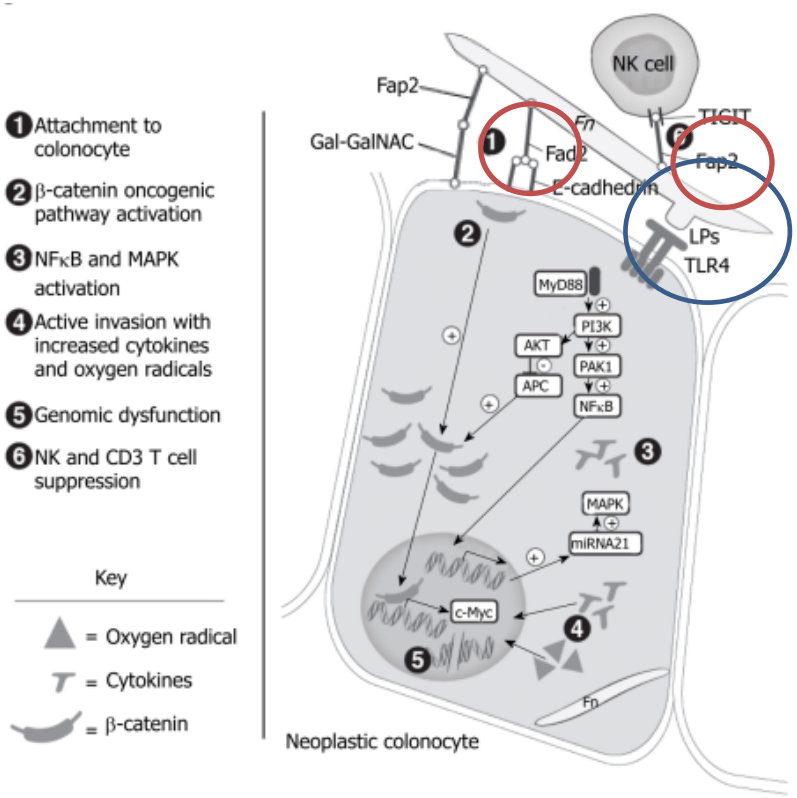
What are the broad molecular mechanisms by which the human microbiome may be involved in the aetiopathogenesis of cancer?



5 What are the broad molecular mechanisms by which the human microbiome may be involved in the aetiopathogenesis of cancer?

Inflammation

(strong evidence from human studies)



Modifiyed from Hussan et al., WJG (2017)

Immunity

(evidence from animal studies/weak evidence from human studies)

Fad2

- Attachment and invasion to colonic epithelia and endotelial cells promoting the release of inflammatory cytokines particularly IL-8, IL-10 and tumor necrosis factor-α (TNF-α) in a proinflammatory microenvironment[2-4]
- Activation of B-catenin pathway through E-cadherin mediated binding[5].
- Increase expression of oncogenic miRNA21 by activating TLR4 signaling to MyD88 which leads to NFKB pathway activation[6].

Fap2

- Adherence to colonic epithelia through host lectin Gal-GalNAc[1]
- Produce an immunosuppressive microenvironment through interaction with TIGIT and attraction of myeloid-derived suppressor cells[7-9]

Unknown

- Inhibit NK cell and T cell function by binding to carcinoembryonic antigen-related cell adhesion molecule (CEACAM) 1[10]

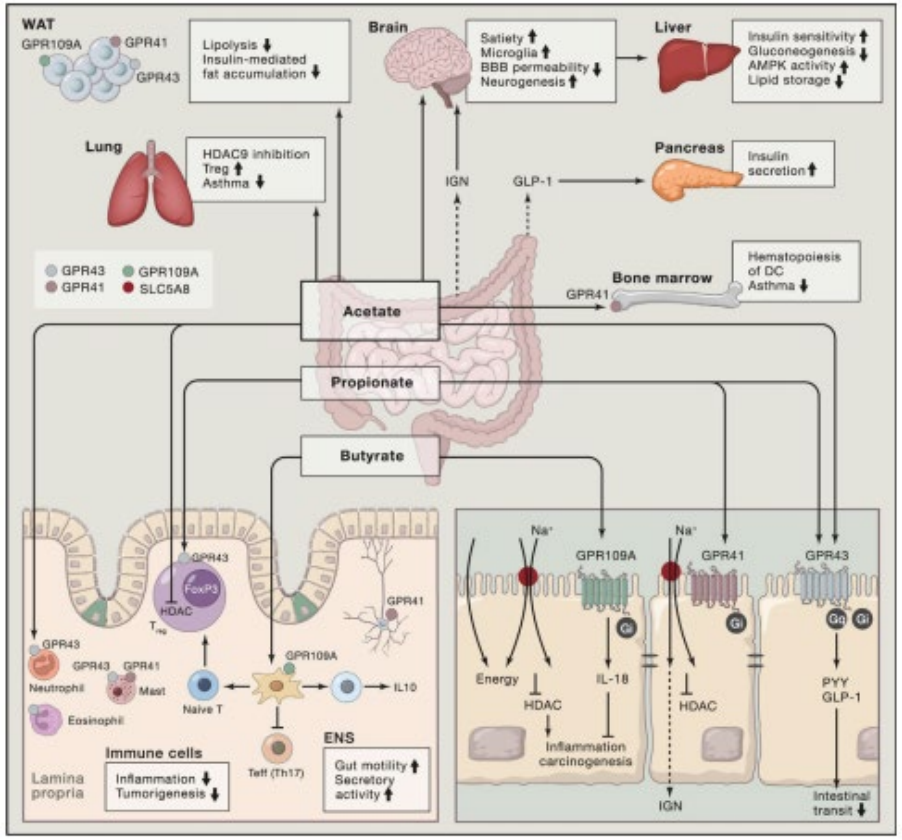
[1]Abed et *al*. Cell Host Microbe (2016); [2]McCoy et *al*. PLoS One (2013); [3]Quah et *al*. Int Endod J (2014); [4]Dharmani *et al*. Infect Immun (2011); [5]Rubinstein *et al*. Cell Host Microbe (2013); [6]Yang *et al*. Gastroenterology (2017); [7]Gur *et al*. Immunity (2015); [8]Kostic *et al*. Cell Host Microbe (2013); [9]Bashir *et al*. Tumour Biol (2016); [10]Gur *et al*. Oncoimmunology (2019)

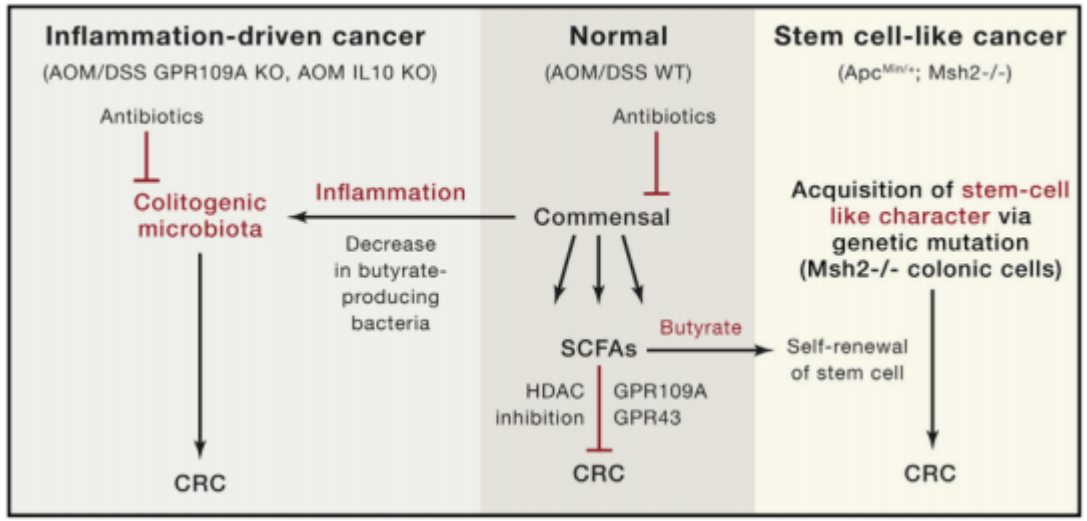
6

What are the broad molecular mechanisms by which the human microbiome may be involved in the aetiopathogenesis of cancer?

Metabolism

(strong evidence from human studies)

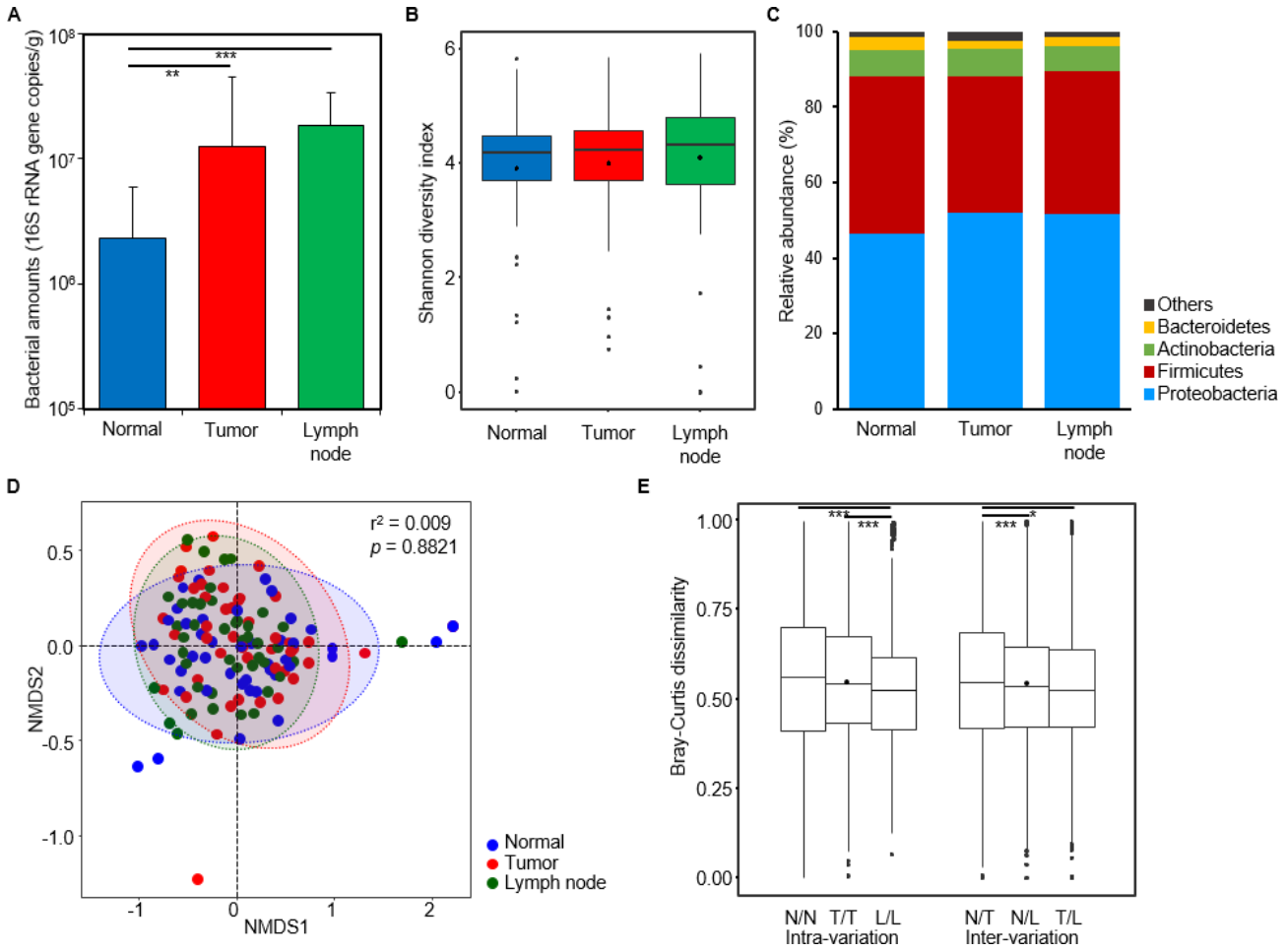




Koh et al, Cell 2019

7

Microbiota difference by tissue type (related to the breast)



Kim et al., 2021. J. Microbiol. Biotech. In press

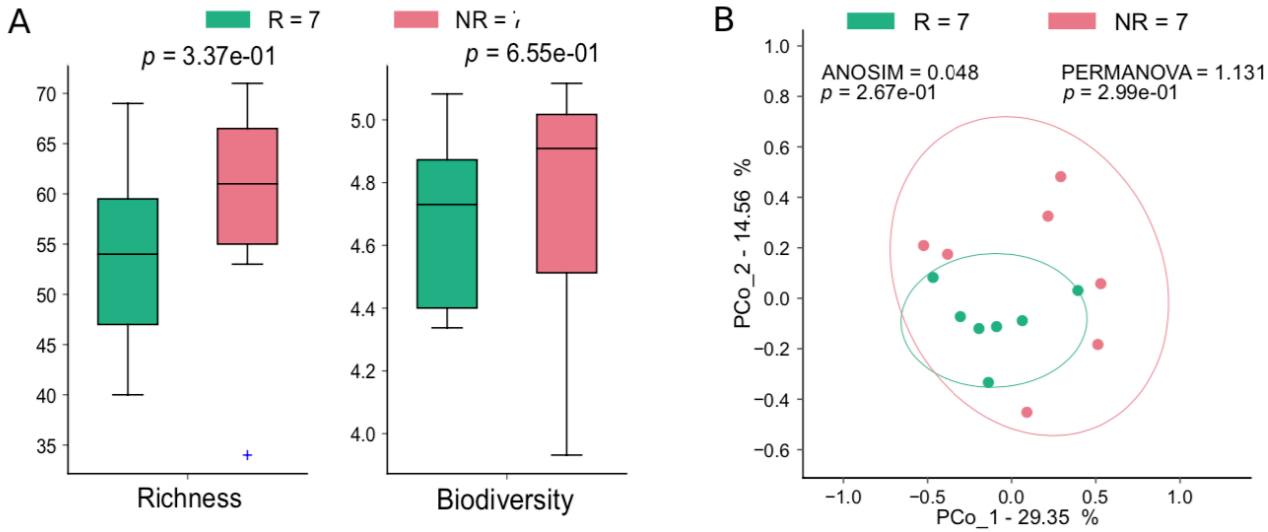
Results: assessment of phyla and species distribution according to response to CDK4/6i

7 Phyla

Phylum	NR Mean ± SEM (%)	R Mean ± SEM (%)	P values *
Firmicutes	61.75±4.95	55.80±1.92	0.701
Bacteroidota	22.85±3.60	26.61±1.47	0.443
Actinobacteriota	4.14±0.80	11.58±3.35	0.125
Proteobacteria	5.97±2.73	4.53±2.17	1.000
Verrucomicrobiota	2.38±1.69	1.07±0.58	0.891
Desulfobacterota	0.37±0.13	0.38±0.10	1.000
Euryarchaeota	1.53±1.02	0.02±0.02	0.551

Higher relative abundance, though not sig.

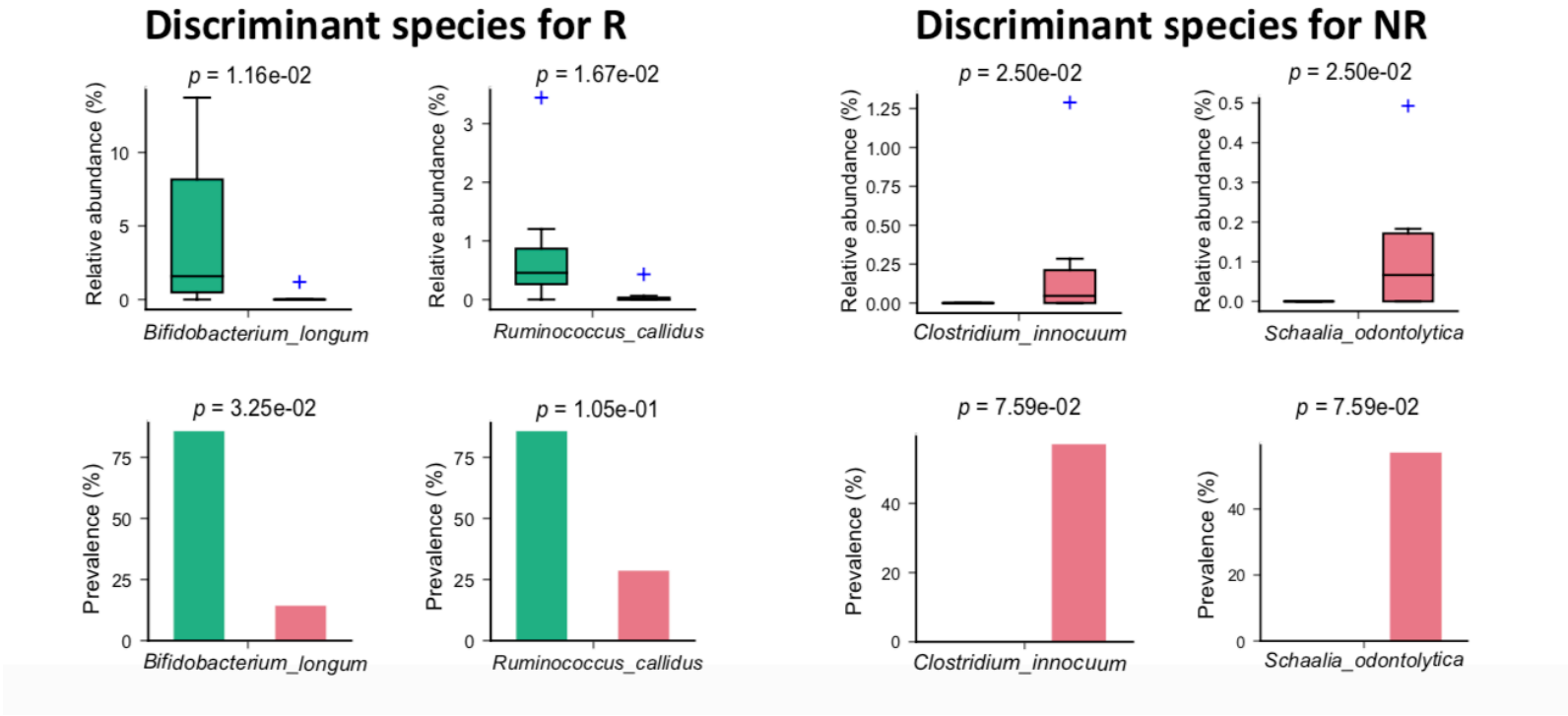
118 Species



No significant difference between NR and R in alfa and beta diversity

Results: discriminant species and relative abundance and prevalence

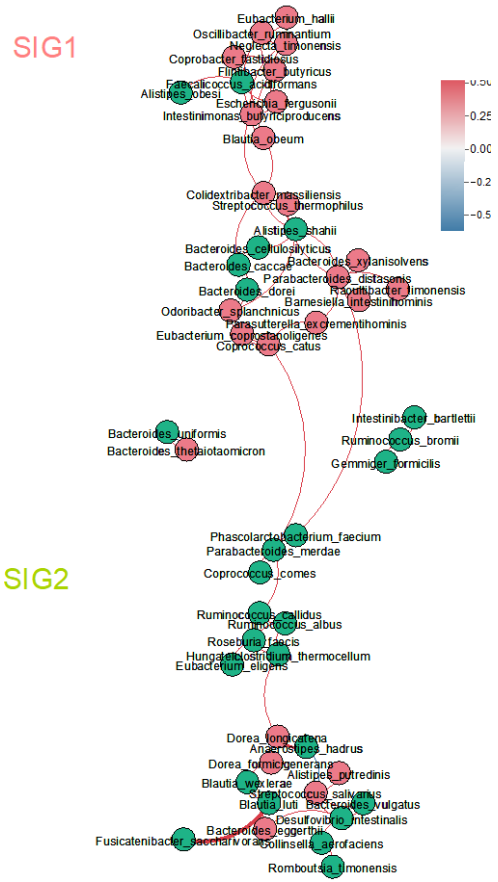
Pairwise analysis of the selected four species depicts significant differences in terms of relative abundance (box plots) and prevalence (bar plots)



Results: network analysis

Figure explanation

- Network analysis showing communities of bacterial species (species-interacting groups, SIGs) and their positive or negative relative abundances correlation.
- Nodes are colored according to the cohort harboring the higher relative abundance for a definite species, as NR (red) or R (green).
- Edge thickness is inversely proportional to the Pearson p-value after 10% Benjamini–Hochberg two-stages FDR, and it is colored according to positive (red) or negative (blue) Pearson coefficient.



Meaning

- Two major clusters of interacting bacterial species (Species Interacting Groups-SIGs)
- SIG1 group harbored 75% of NR-related species, while a SIG2 group harbored 76% of species with higher relative abundance in R
- This topological distribution was highly significant (p<0.001) → these 2 communities could have an opposite role in responsiveness to CDK4/6i