

# OPTIMIZING IMMUNOTHERAPY IN mCRC

## THE HOST MICROBIOME-IMMUNITY CROSSTALK

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Rambam Health Care Campus,  
Rappaport Faculty of Medicine, Technion  
Haifa



# DECLARATION OF INTERESTS

Prof. Irit Ben-Aharon MD, PhD

Astra Zeneca – Invited speaker

MERCK– Invited speaker

MSD – Invited speaker

BMS – Invited speaker

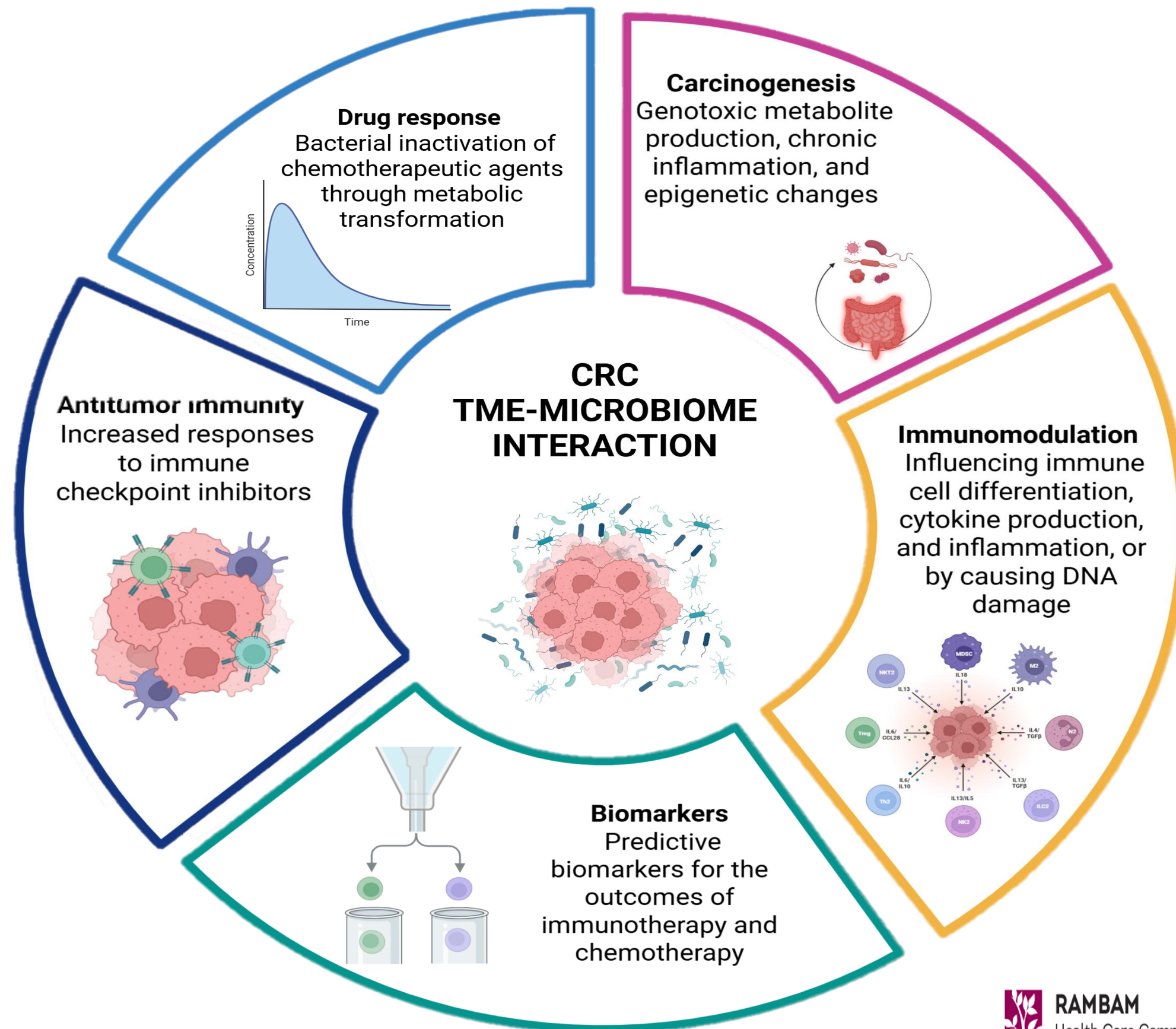
Medison – Invited speaker

Astellas – Advisory Board

BeiGene - Advisory Board

**Editor role:** Editor in Chief – ESMO Gastrointestinal Oncology

# The microbiome had been implicated in several pillars of CRC pathophysiology and treatment



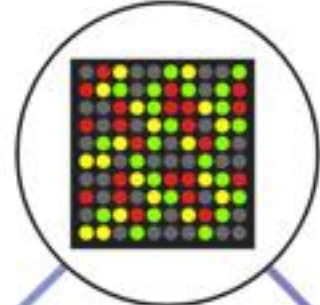
Initial discovery of an association between the human microbiome and cancer



2001



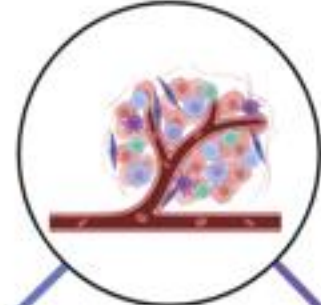
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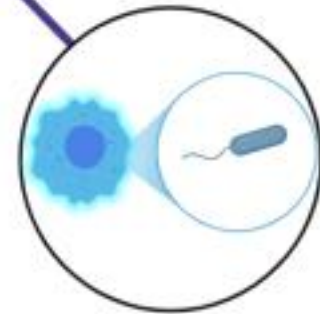
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2019



2022

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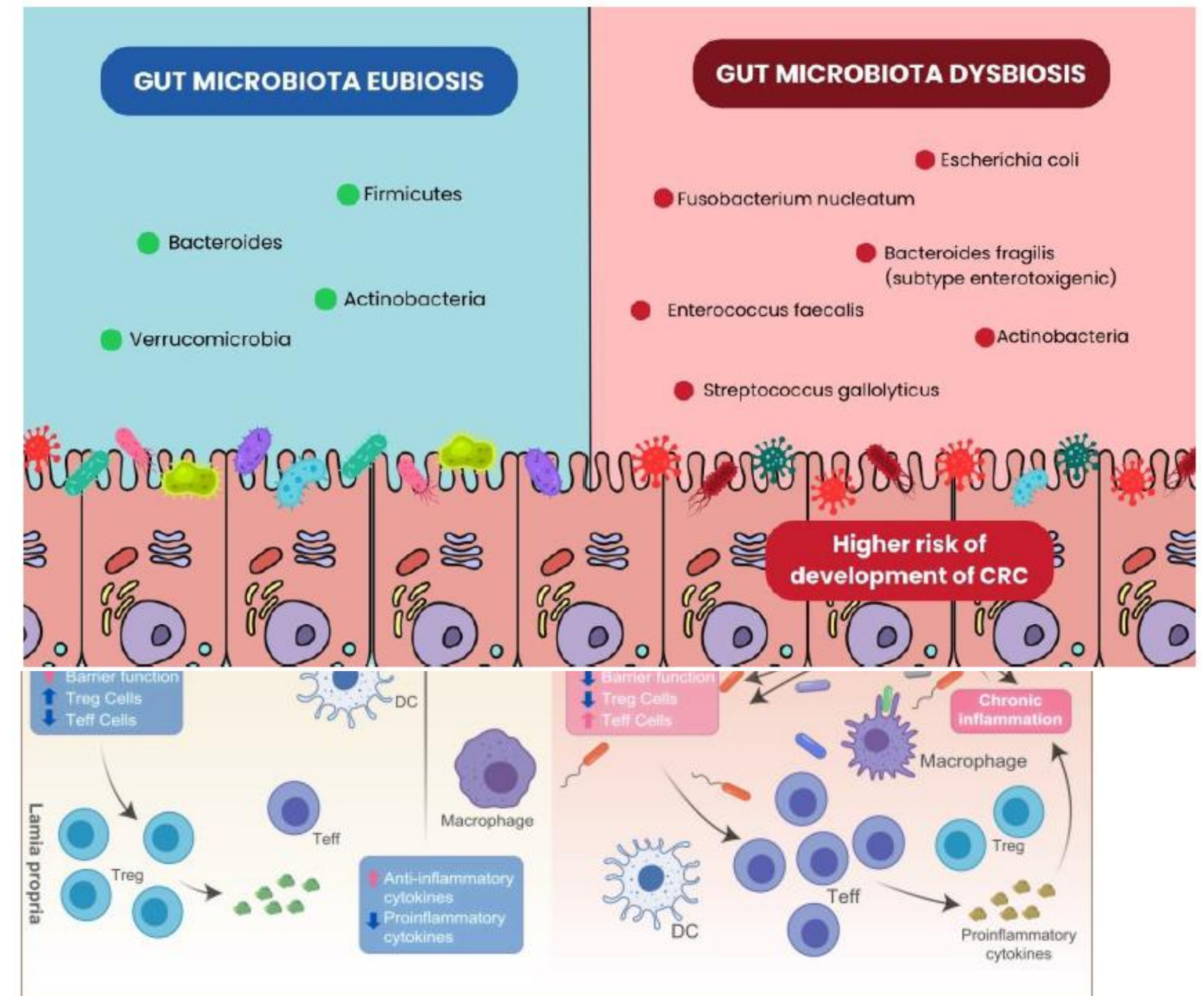
ICI modulation; Fecal microbiota transplantation in cancer management

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Intracellular bacteria identified as drivers of breast cancer metastasis

# Gut dysbiosis and disease state

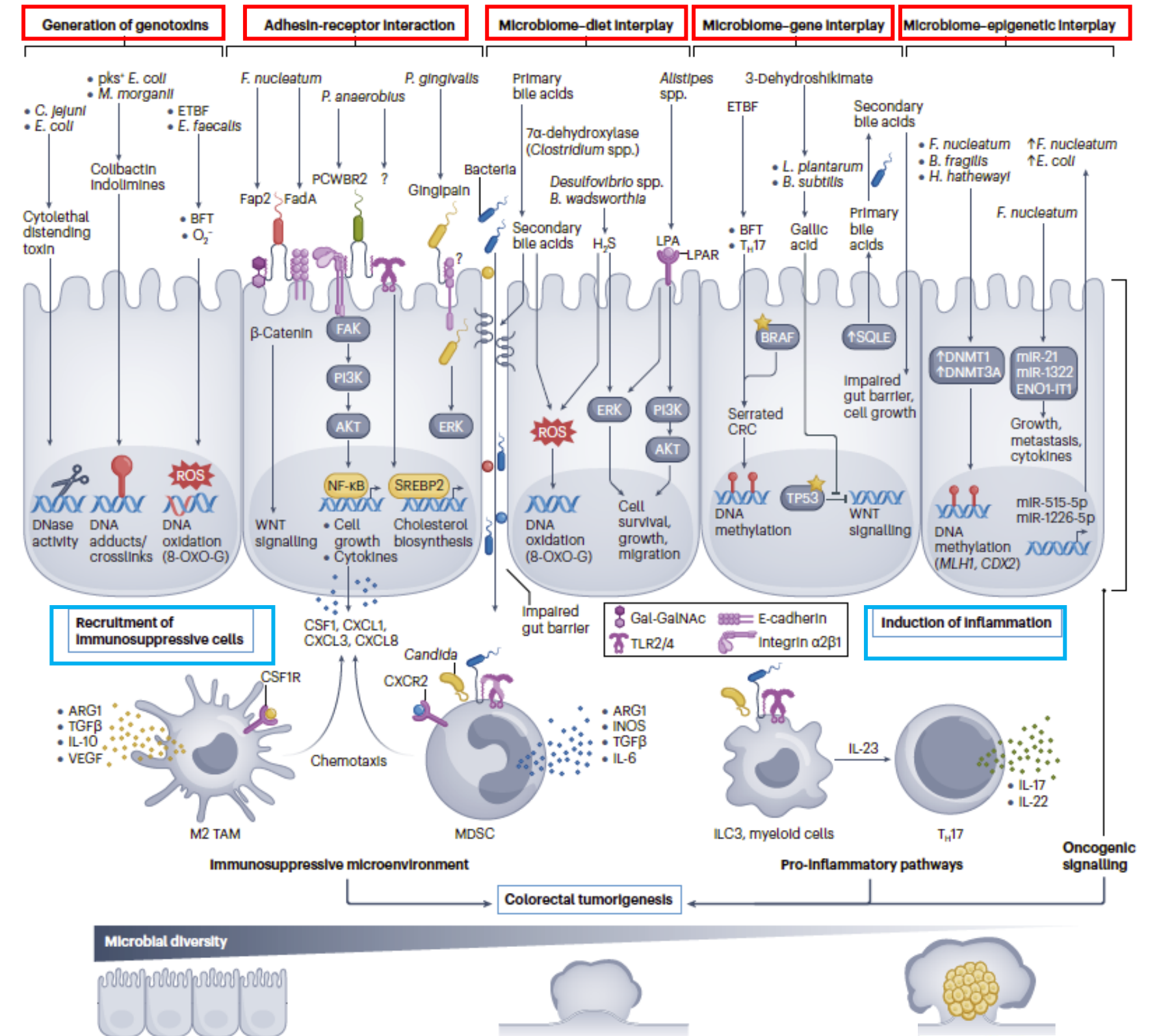
- **Dysbiosis**, low diversity of the associated microbiota, and their variability: a so-called “pathobiome” state.
- Large-scale multicohort analysis of the CRC metagenome has unraveled consistent enrichment of a ‘core microbiome’ with pro-tumorigenic functions, consistent with the definition of dysbiosis



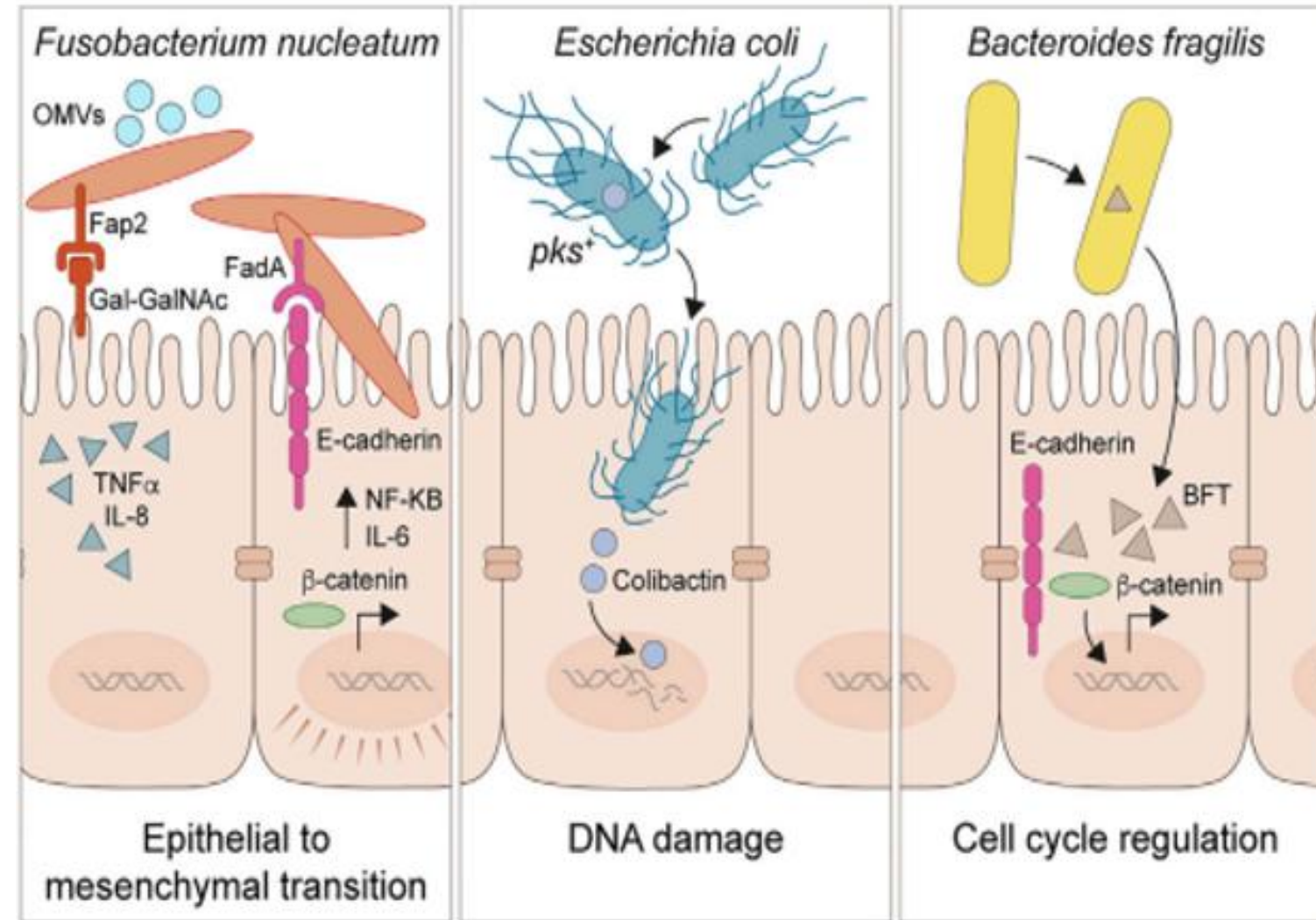
Hou et al, Sug Transduct Target Ther, 2022  
Cintoni et al., Microorganisms 2025

# Gut microbial dysbiosis contributes to the development of CRC

- Pathobionts present in the gut through a diverse range of molecular mechanisms :
- These interactions culminate in an increased incidence of tumor-promoting genetic alterations and tumor cell proliferation and foster a repressed immune microenvironment that enables immune evasion, leading to the establishment of CRC.



# Microbiota-associated mechanisms involved in the pathogenesis of CRC



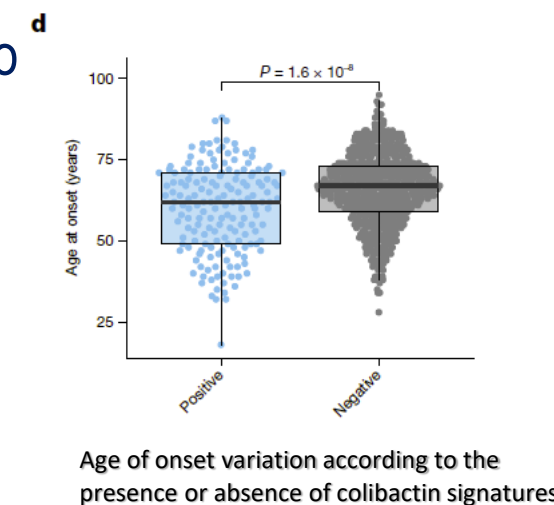
**Escherichia coli** produces colibactin, causing DNA interstrand crosslinks and double-strand breaks with a unique mutational signature.

**Fusobacterium nucleatum** Fap2 binds Gal-GalNAc, while FadA engages E-cadherin to activate Wnt-β-catenin and MYC, and its LPS triggers NF-κB-driven proliferation.

**Bacteroides fragilis** secretes BFT to cleave E-cadherin and disrupt tight junctions, inducing MYC and proliferation; its LPS via TLR4 upregulates JMJD2B, SOX2, and NANOG to promote stemness.

- **Colibactin-Related Mutational Signatures SBS88 and ID18**, associated with colibactin (a genotoxin from *E. coli*), were 3.3 more prevalent in EO CRC compared to AOCRC

Díaz-Gay M et al., Nature. 2025



Cullin et al. Cancer Cell 2021

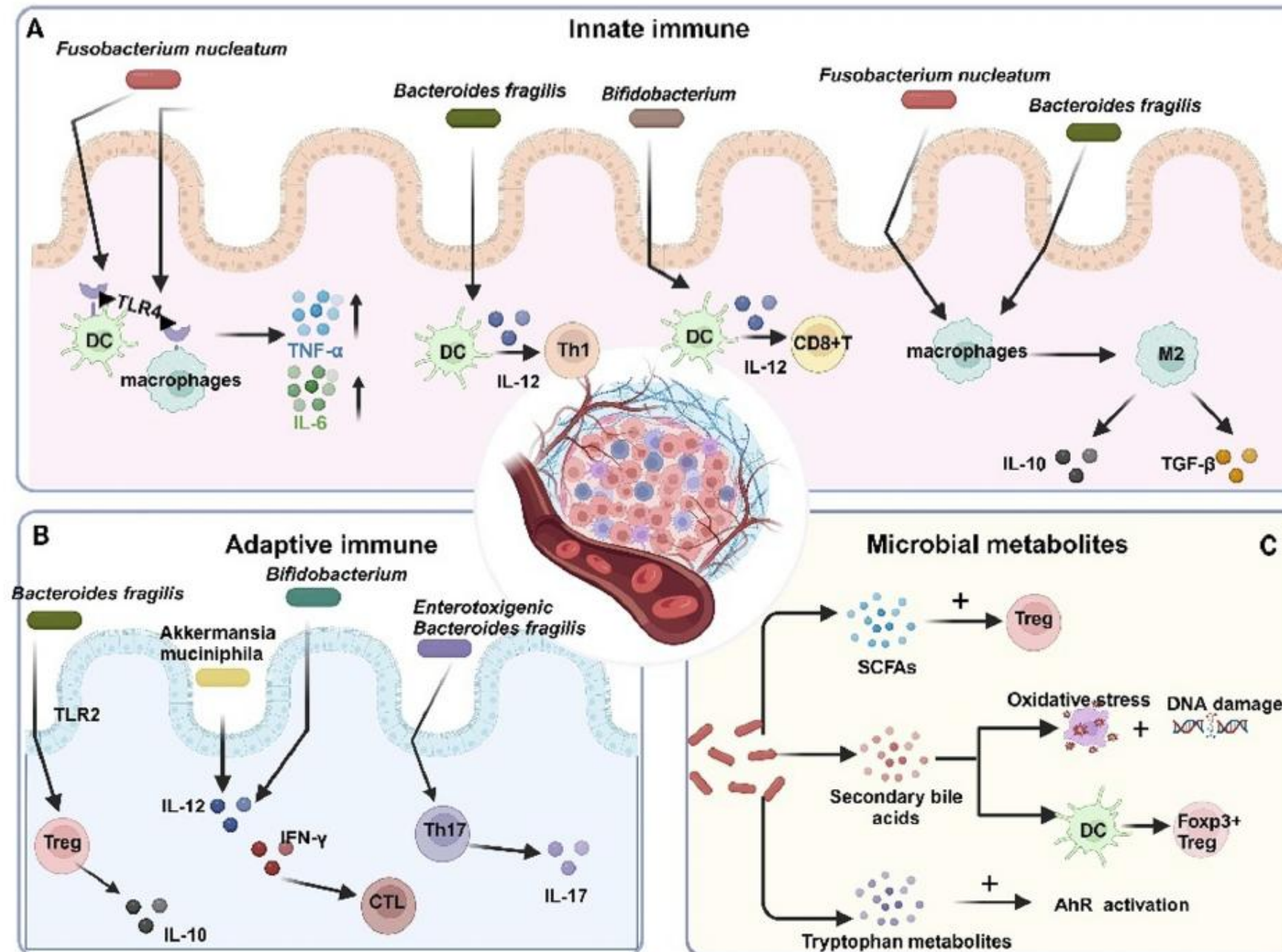
# Gut microbiota and their metabolites – interplay with immune cells and signaling pathways

## Innate immune:

- *Fusobacterium nucleatum* interacts with TLR4 on DC and macrophages, triggering the production of pro-inflammatory cytokines
- *Bifidobacterium* enhanced DCs activation, stimulating IL-12 secretion, improving CD8+T cell responses,
- *Bacteroides fragilis* induced DCs maturation and stimulating IL-12-dependent Th1 immune responses.
- *F. nucleatum* and *B. fragilis* promote M1 macrophage polarization, which release immunosuppressive cytokines

## Adaptive immune:

*Bacteroides fragilis* stimulate Treg differentiation via TLR2 signaling, leading to an increase in IL-10 production.



## Microbial metabolites:

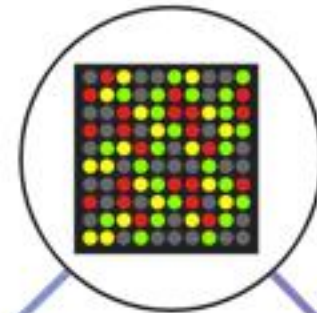
- Short- chain fatty acids SCFAs can promote Treg differentiation.
- Secondary bile acids support tumorigenesis by inducing oxidative stress and DNA damage, reduce immunostimulatory effects on dendritic cells, leading to increased Foxp3 expression, upregulation of Treg numbers
- Tryptophan metabolites: can activate the aryl hydrocarbon receptor (AhR).

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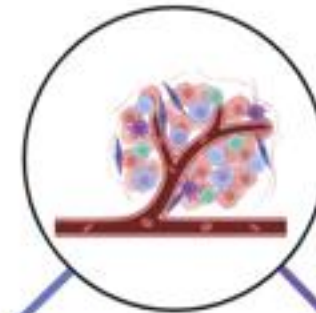
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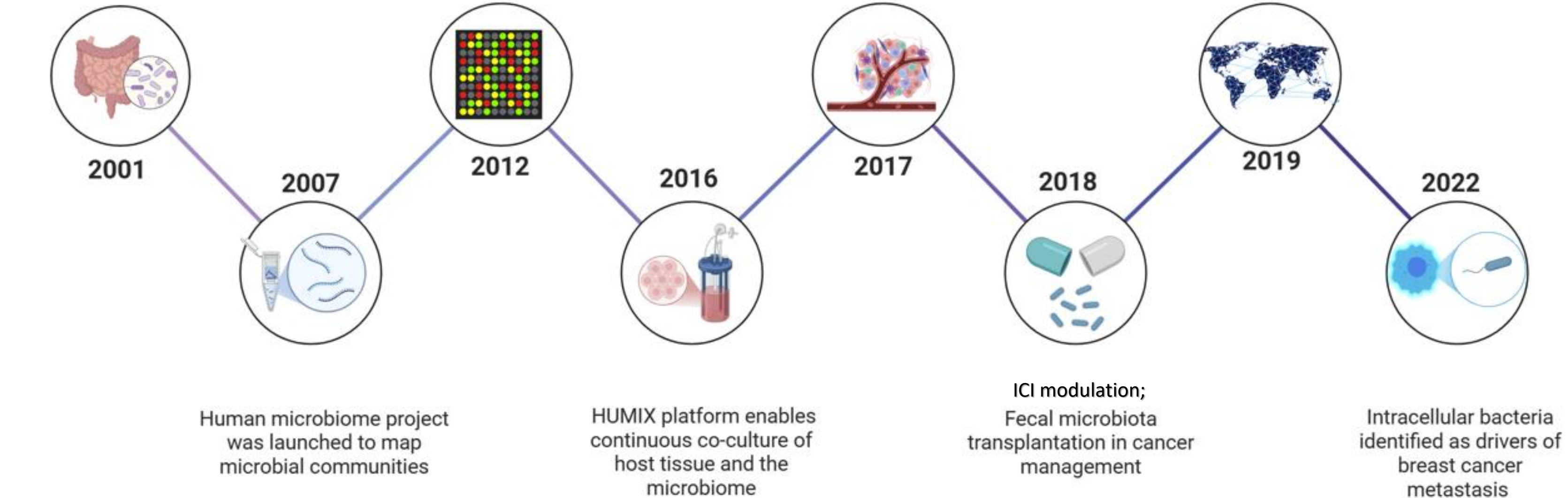


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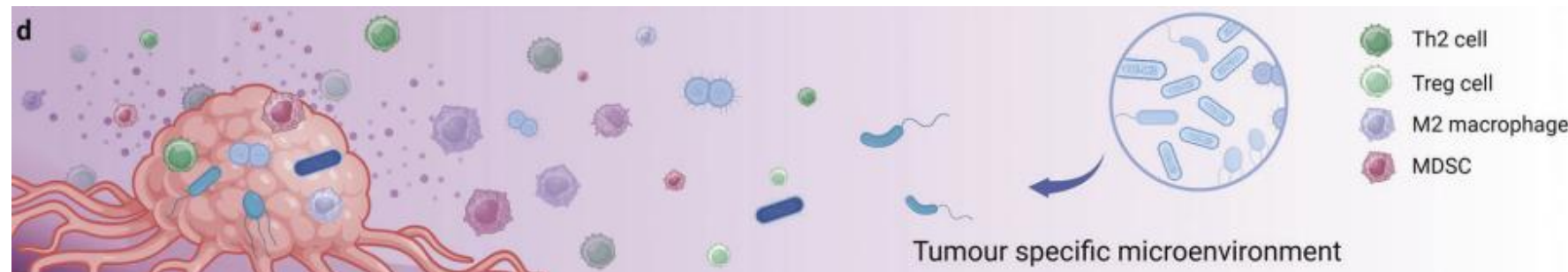
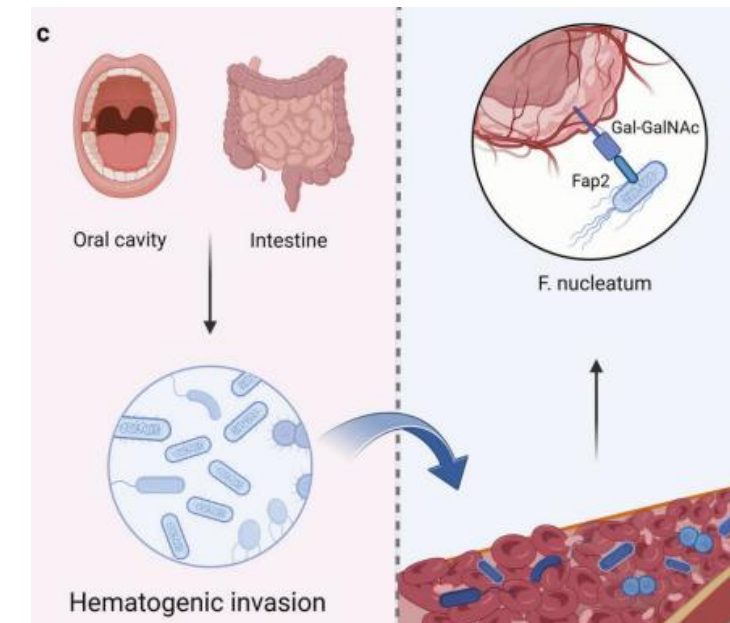
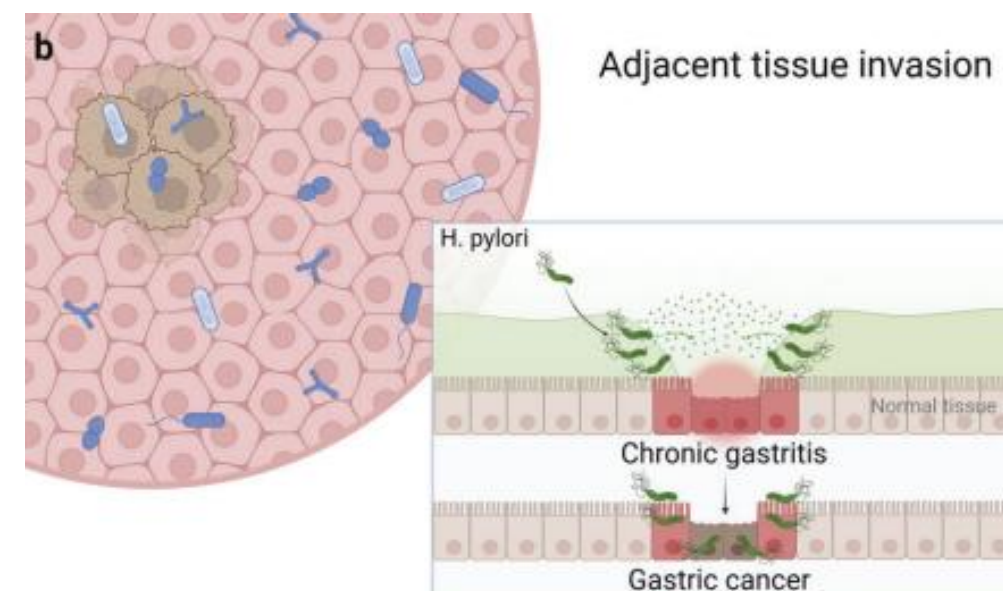
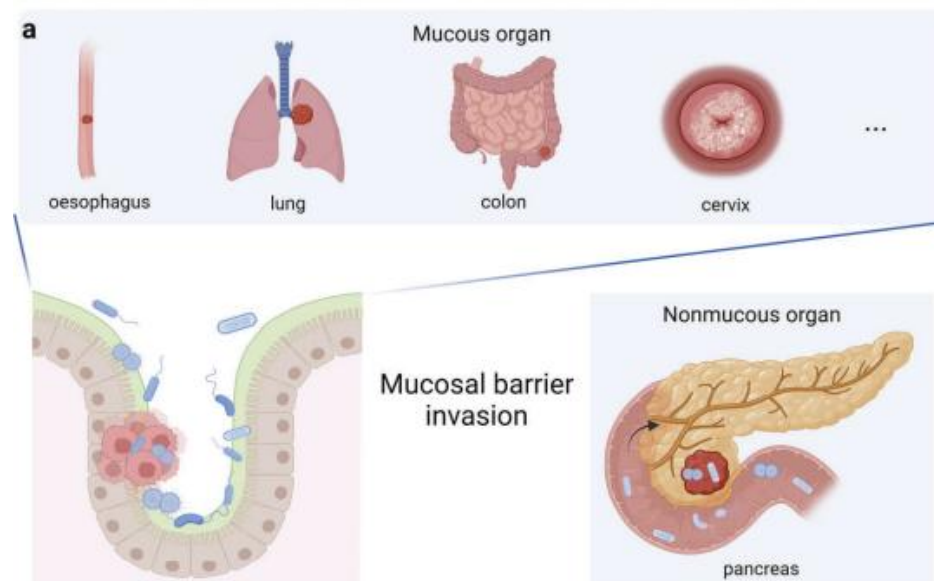
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# Tumor microbiome

- The concept of intratumoral microbes as distinct low-biomass ecosystems, which may impact the TME, including inflammatory mediators such as tissue resident and peripherally recruited immune cells, fibroblasts, endothelial cells, adipocytes, and pericytes has been evolved in recent years and shed better light on the microbiome role.
- It is still uncertain whether these microbes constitute a predetermined niche or rather represent a transient stochastic colonization, nevertheless evidence indicate that intratumoral microbiota enter the tumor tissue mainly in four ways:

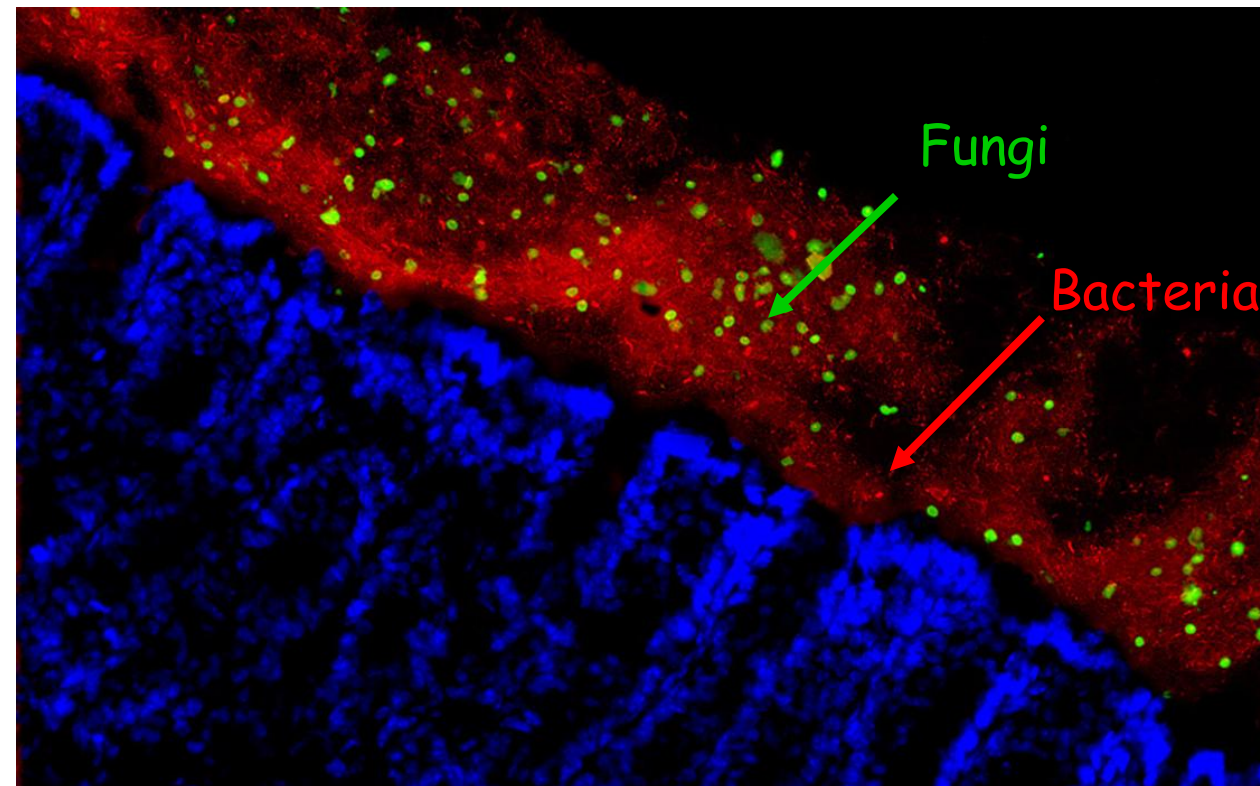


Yang et al., 2022

Cia et al, Signal Transduction and Targeted Therapy (2024)

# Intratatumoral microbiome

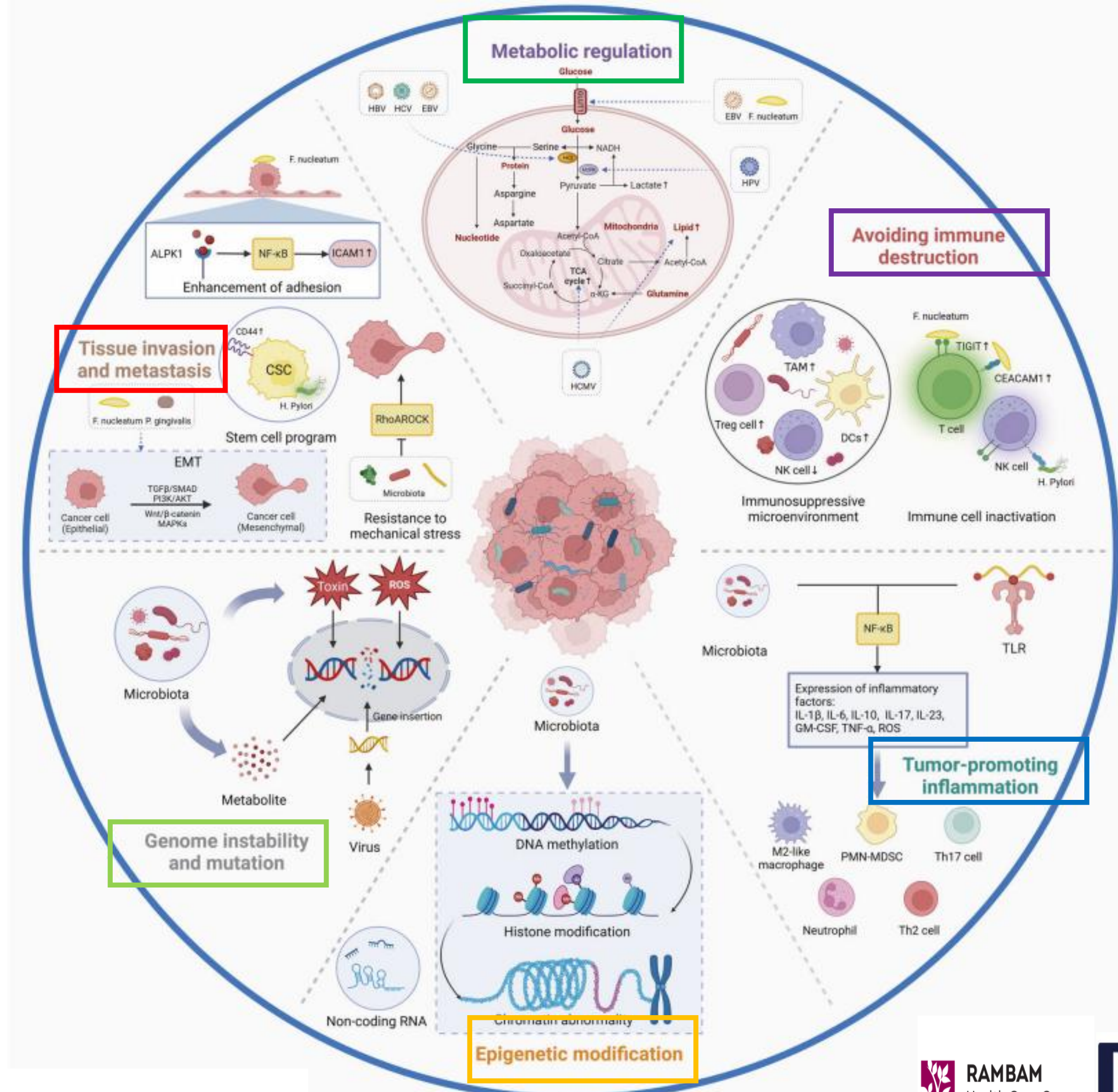
- Had been characterized in various tumors
- Not only bacteria but also fungi and viruses
- In CRC – 14 fungal biomarkers were associated with a bacterial dysbiosis



Nejman et al, *Science* 2020

Narunsky-Haziza et al. *Cell* 2022

- Mechanistic studies have demonstrated that intratumoral microbiota potentially promote the initiation and progression of tumors by six major mechanisms:

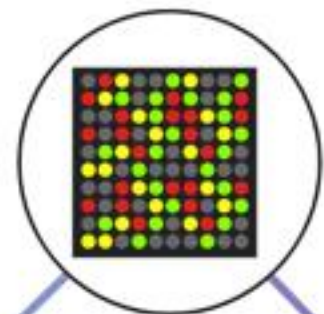


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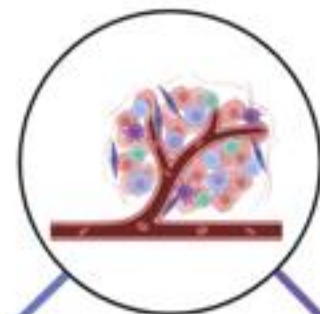
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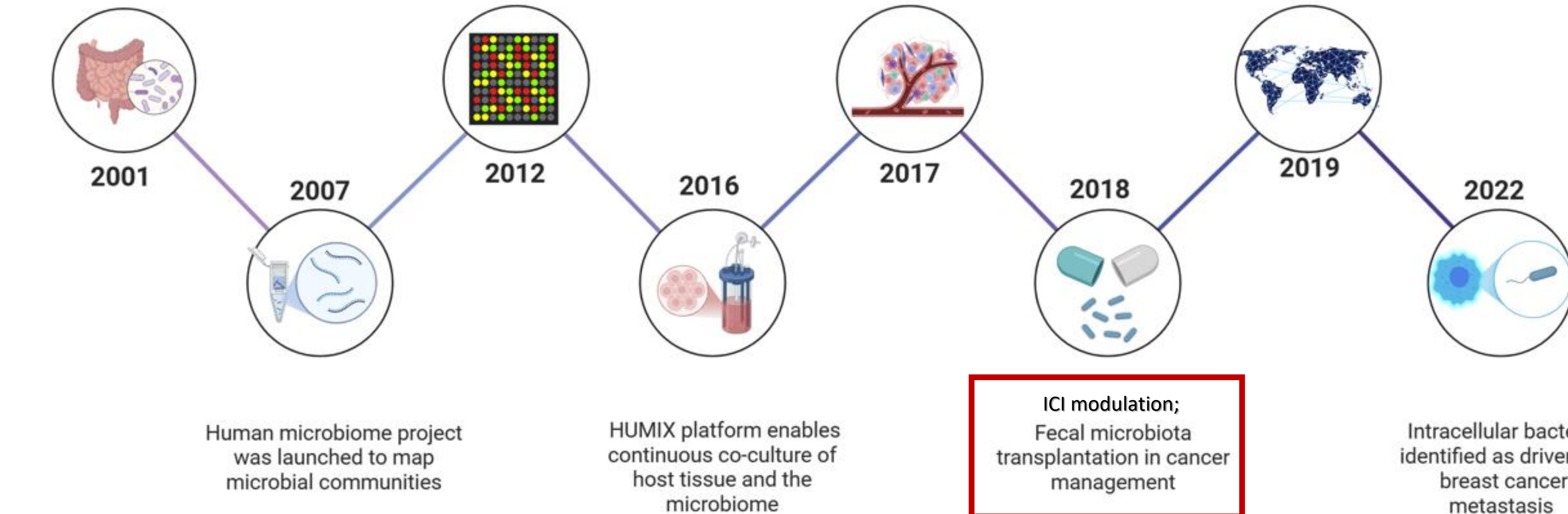


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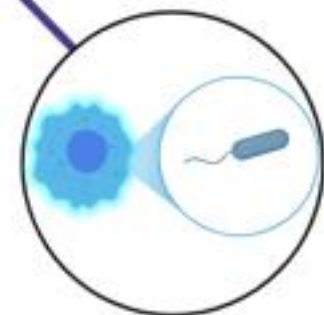
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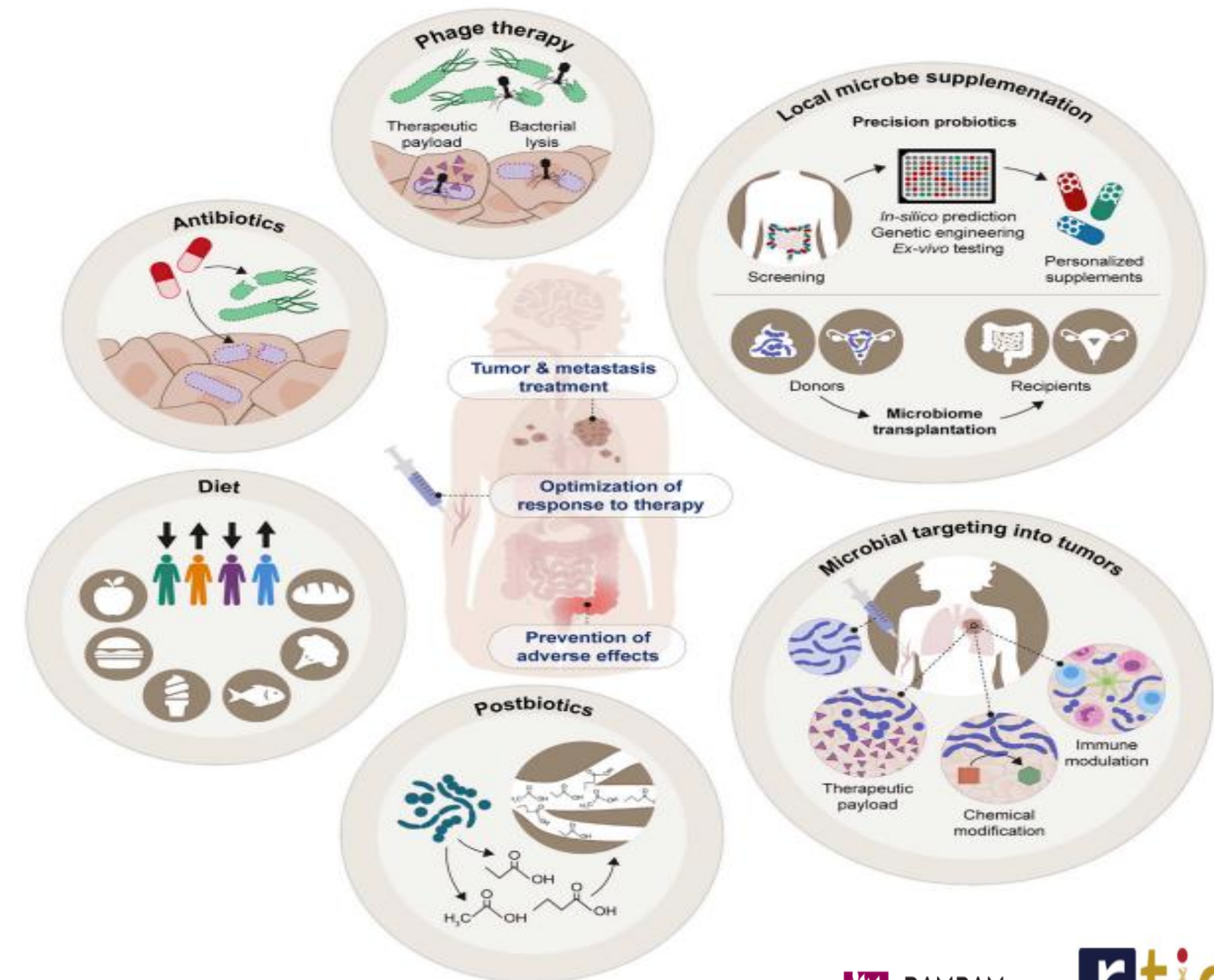


2022

# Microbiome Modulation Toward Improved Cancer Therapy

A unique feature of the microbiome, regarded by many as a “second genome,” is that in contrast to the human genome, it is amenable to modulation.

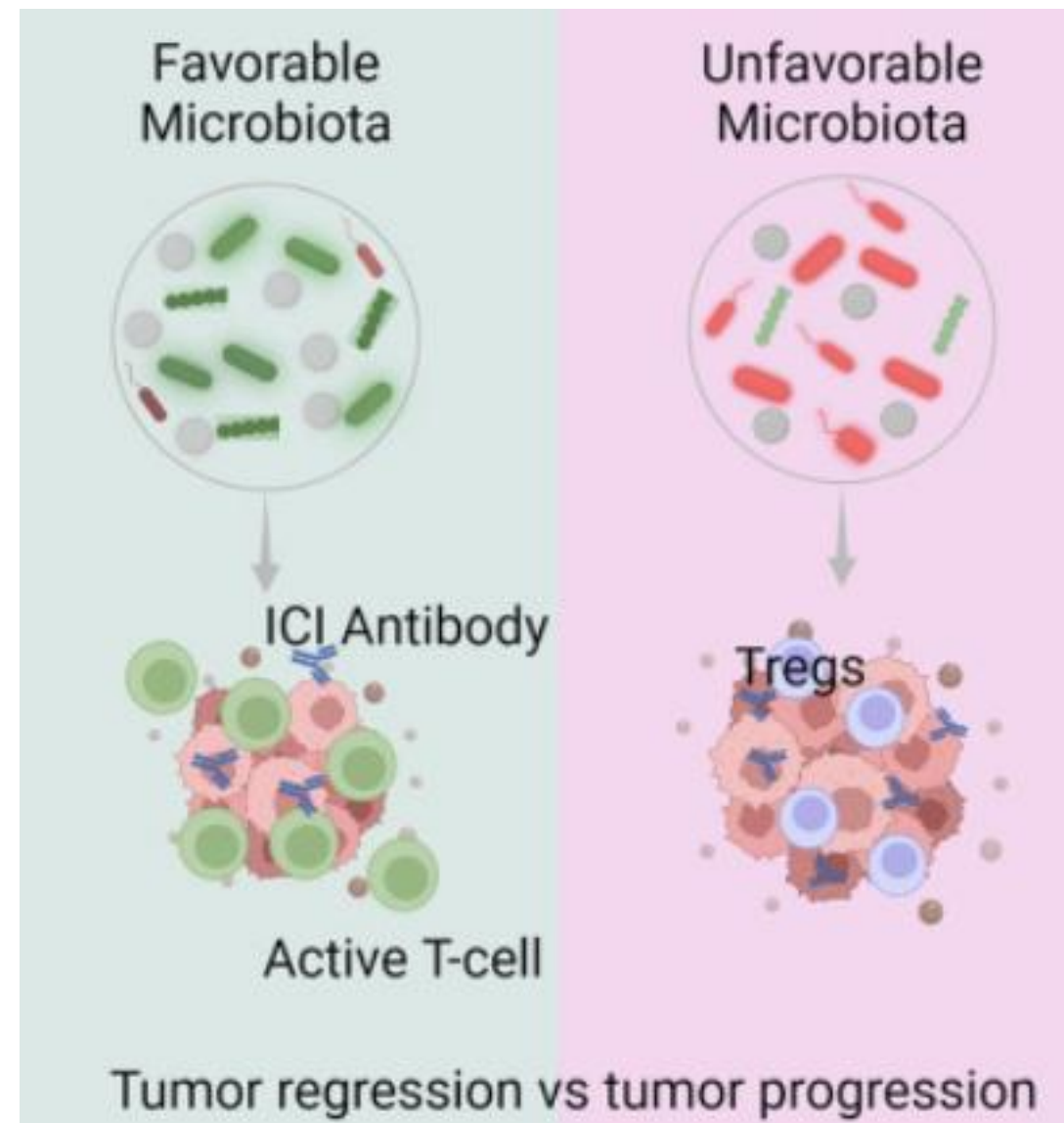
**MSS-CRC are a main target to modulation in order to increase sensitivity to ICI**



- The impact of gut microbiota on response to ICIs was initially demonstrated in a mouse model, with landmark studies published in Science in 2015 highlighting the influence of gut microbiota composition on response to CTLA-4 and PD-L1 inhibitors.

**Points for discussion:**

- MSI status
- Implications for research and practice



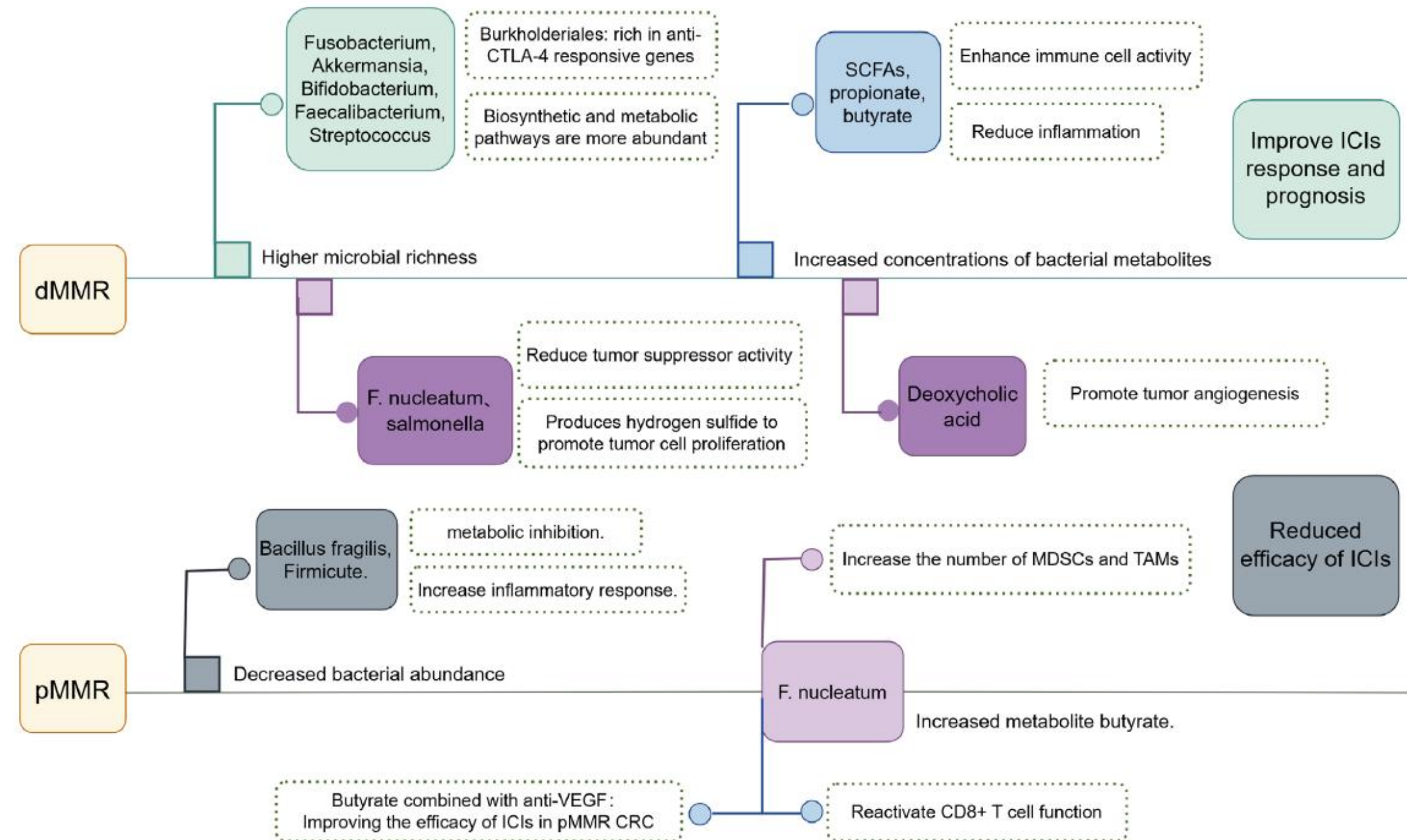
**Anticancer immunotherapy by CTLA-4 blockade relies on the gut microbiota**

Marie Vétizou,<sup>1,2,3</sup> Jonathan M. Pitt,<sup>1,2,3</sup> Romain Daillère,<sup>1,2,3</sup> Patricia Lepage,<sup>4</sup> Nadine Waldschmitt,<sup>5</sup> Caroline Flament,<sup>1,2,6</sup> Sylvie Rusakiewicz,<sup>1,2,6</sup> Bertrand Routy,<sup>1,2,6,8</sup> Maria P. Roberti,<sup>1,2,6</sup> Connie P. M. Duong,<sup>1,2,6</sup> Viehnon Poirier-Colame,<sup>1,2,6</sup> Antoine Roux,<sup>1,2,7</sup> Sonia Becharef,<sup>1,2,6</sup> Silvia Formenti,<sup>9</sup> Encouse Golden,<sup>9</sup> Sascha Cording,<sup>9</sup> Gerard Eberl,<sup>9</sup> Andreas Schlitzer,<sup>10</sup> Florent Ginhoux,<sup>10</sup> Sridhar Mani,<sup>11</sup> Takahiro Yamazaki,<sup>1,2,6</sup> Nicolas Jacquelot,<sup>1,2,3</sup> David P. Enot,<sup>1,7,12</sup> Marion Bérard,<sup>13</sup> Jérôme Nigou,<sup>14,15</sup> Paule Opolon,<sup>1</sup> Alexander Eggermont,<sup>1,2,16</sup> Paul-Louis Woerther,<sup>17</sup> Elisabeth Chaachaty,<sup>17</sup> Nathalie Chaput,<sup>1,18</sup> Caroline Robert,<sup>1,18,19</sup> Christina Mateus,<sup>1,19</sup> Guido Kroemer,<sup>7,13,20,21,22</sup> Didier Raoult,<sup>23</sup> Ivo Gomperts Boneca,<sup>24,25\*</sup> Franck Carbonnel,<sup>2,26\*</sup> Mathias Chamaillard,<sup>2\*</sup> Laurence Zitvogel<sup>1,2</sup>



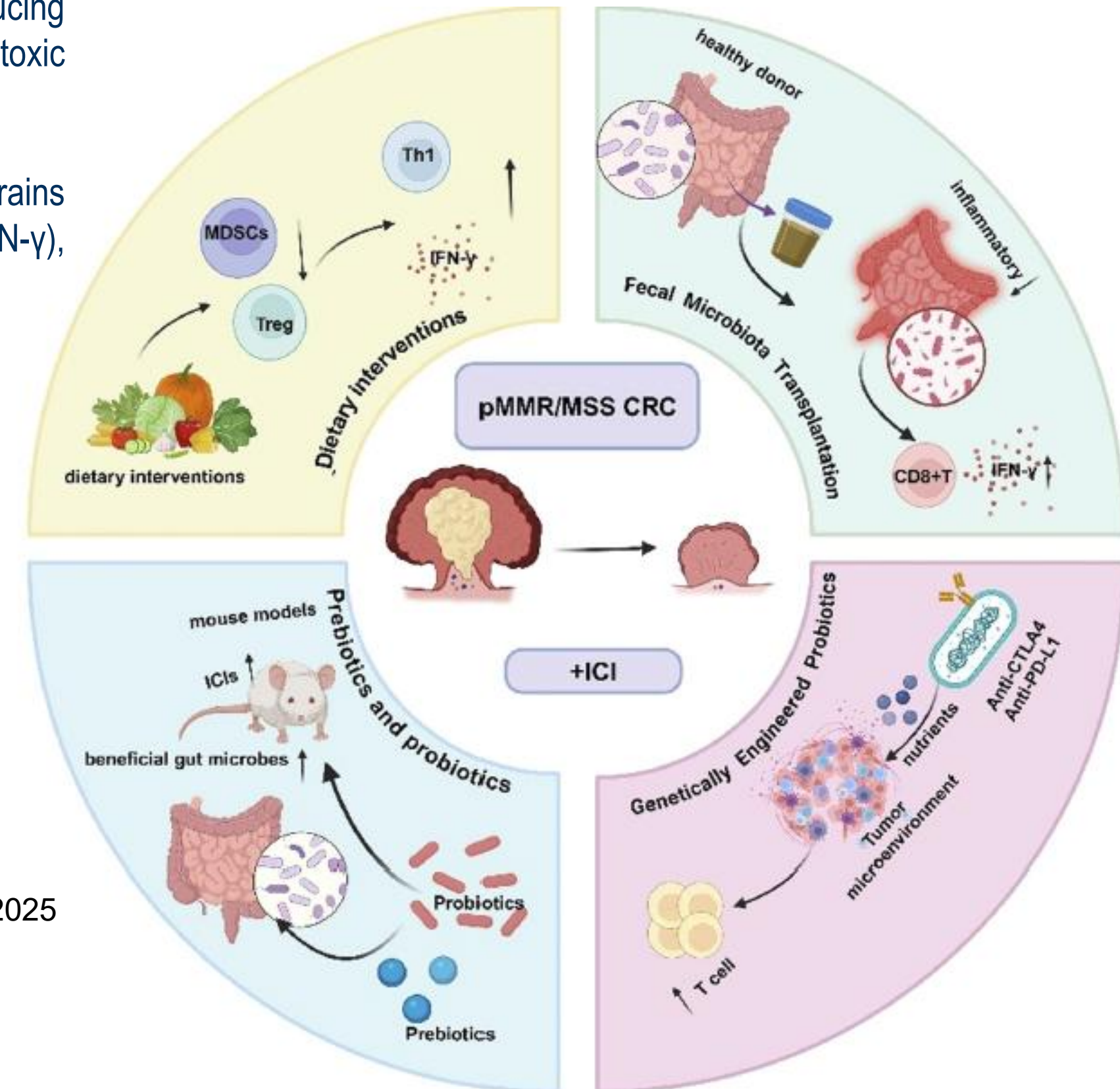
# Bacterial-derived immune modulation and MSI status:

- pMMR patients demonstrate lower bacterial abundance, which diminishes the positive regulatory effects on immune cells and the TME.
- However, certain controversial 'harmful bacteria' (e.g., *Fusobacterium nucleatum*) may still enhance the immunotherapy response in pMMR patients to some extent.



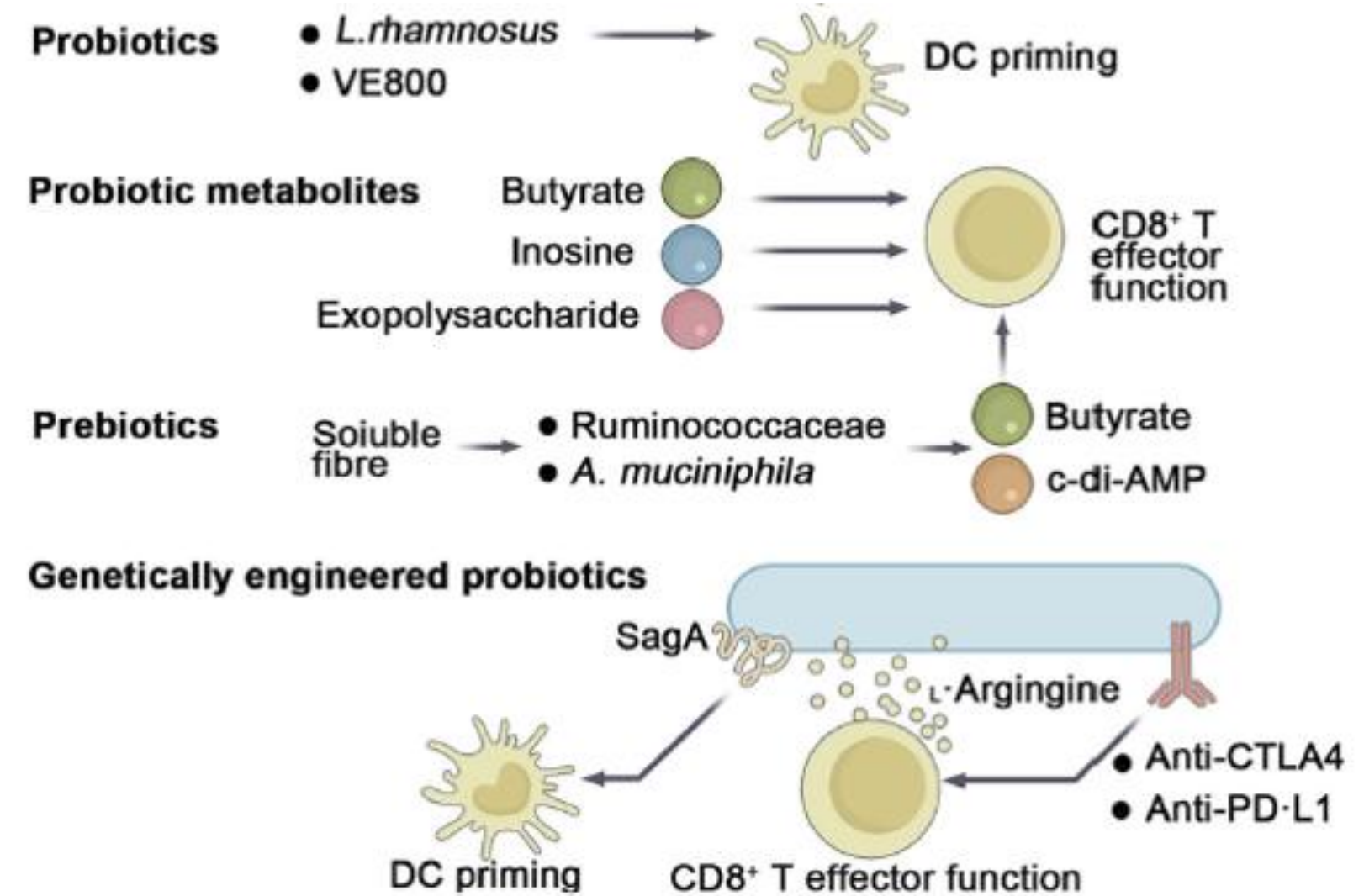
# Modulation of gut microbiota, TME and immune checkpoint inhibitors.

- **Dietary interventions** significantly boosts anticancer immunity by reducing immunosuppressive myeloid cells and regulatory T cells, enhancing Th1-type and cytotoxic responses in the TME and upregulating immune-related gene expressions
- **FMT** can mitigate inflammatory responses potentially reducing tumor formation, strains isolated from healthy donor feces could induce CD8+T cells to produce interferon- $\gamma$  (IFN- $\gamma$ ), thereby enhancing T cell function and improving the antitumor effects of ICIs.



# Modulation of the gut microbiota, TME and immune checkpoint inhibitors.

- **Probiotics** enhance gut barrier function, can bolster the activity of ICIs in mice.
- **Prebiotics**, such as soluble fibers like inulin and pectin, selectively stimulate the growth of beneficial gut microbes, can bolster the activity of ICIs in mouse models.
- **Genetically engineered probiotics** can express antibody fragments targeting PD-L1 and CTLA-4, these probiotics may enhance T cell function by providing essential nutrients, such as arginine, within tumor microenvironments.



# Modulation of the gut microbiota, TME and immune checkpoint inhibitors.

- Nevertheless, most of the current ongoing studies are in mice and only the minority are at the clinical setting
- Future studies should focus on gene-edited probiotic strains with targeted modifications and the synergistic effects of multiple probiotics and on conducting high-quality, multicenter clinical trials.

**Table 1** Characteristics of the Included animal studies

Author	Country	Year	Animal	n	Groups	MSI Model	Tumor Model	Intervention Strategy	Duration(day)	Outcome Measures
Lu et al. [24]	China	2023	mice	20	4	MSI-H	Subcutaneous	LGG-EV	28	Tumor Weight, Tumor Volume
Wang et al. [25]	China	2024	mice	NA	4	MSI-L+MSS	Subcutaneous+Orthotopic	Fusobacterium nucleatum	182	Tumor Weight, Tumor Volume, Survival Time
Zhang et al. [26]	China	2022	mice	NA	2	MSI-H	Subcutaneous	FMT+Lactobacillus paracasei sh2020	30	Tumor Weight, Tumor Volume, Survival Rate
Gao et al. [27]	China	2023	mice	48	2	MSI-L	Subcutaneous	Lactobacillus rhamnosus Probio-M9	33	Tumor Weight, Tumor Volume
Fong et al. [28]	China	2023	mice	NA	4	MSI-L+MSI-H	Subcutaneous+Orthotopic	Lactobacillus gallinarum	22	Tumor Weight, Tumor Volume, Tumor Number
Wei et al. [29]	USA	2022	mice	20	3	MSI-H	Subcutaneous	Lactobacillus rhamnosus GG	28	Tumor Weight, Tumor Volume, Tumor Incidence
Huang et al. [30]	China	2022	mice	40	2	MSI-L	Subcutaneous	Fecal Microbiota Transplantation(FMT)	24	Tumor Weight, Tumor Volume, Survival Rate
Kang et al. [31]	China	2023	mice	38	4	MSI-H+MSI-L	Subcutaneous+Orthotopic	Roseburia intestinalis	30	Tumor Incidence, Tumor Weight, Tumor Volume, Tumor Number

**Table 2** Characteristics of the Included clinical studies

Study	Country	Year	Enrollment	MSI / MMR	Intervention Strategy	Primary outcome measures	Research status	Results
ChiCTR2100046768	China	2021	20	MSS/pMMR	FMTplus tislelizumab and fruquintinib	ORR DCR	Recruiting	YES[32]
NCT05279677	China	2022	30	MSS/pMMR	FMT+PD-1 ICI+Sintilimab Injection+ Fruquintinib	ORR	Recruiting	NO
NCT04729322	China	2021	15	dMMR	Pembrolizumab/Nivolumab+FMT	ORR	Recruiting	NO
ChiCTR2100052431	China	2021	—	MSI-H/dMMR	FMT+PD-1 ICI	ORR PFS OS	Active, not recruiting	NO
NCT04130763	China	2019	10	—	FMT+PD-1 ICI	ORR	Recruiting	NO

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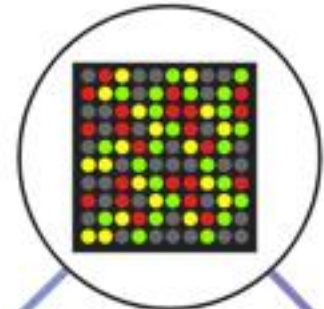
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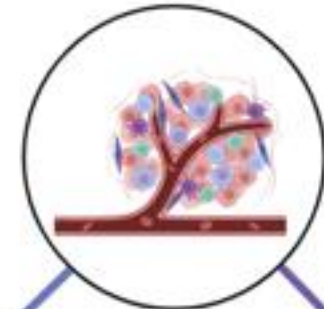
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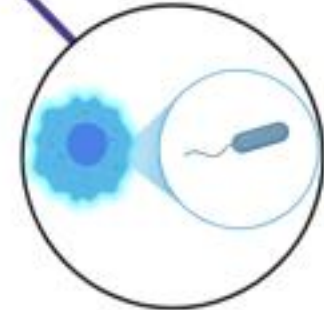
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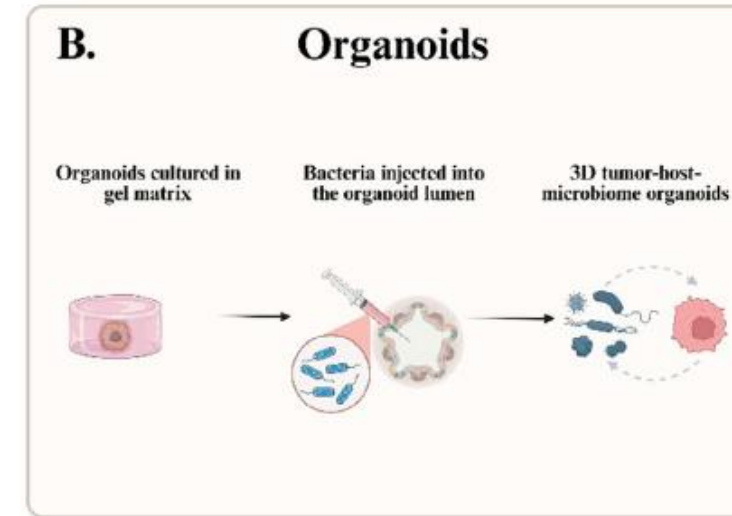
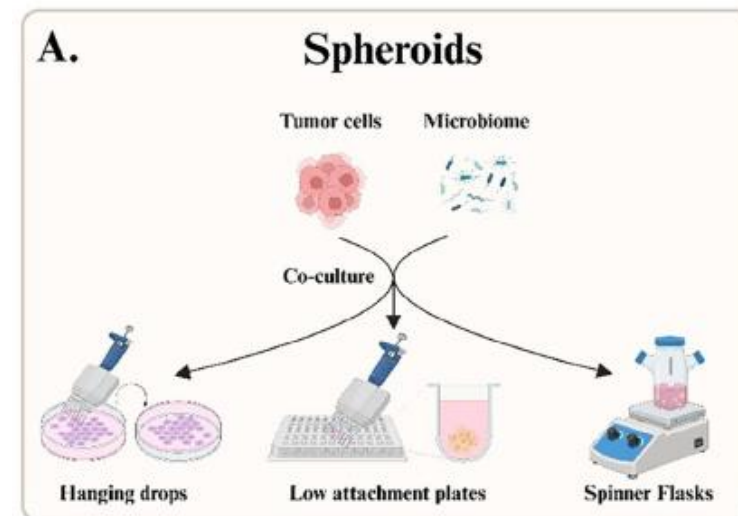
2022

# Landmark developments in tumor modeling technologies and microbiome–cancer insights

Several breakthroughs in tumor modeling systems have been developed and integrated into current research and practice, and enabled dynamic, physiologically relevant *in vitro* studies of tumor biology:

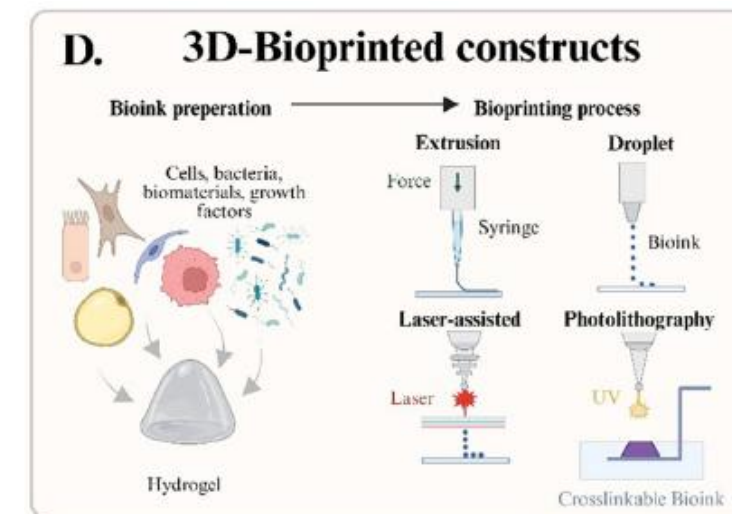
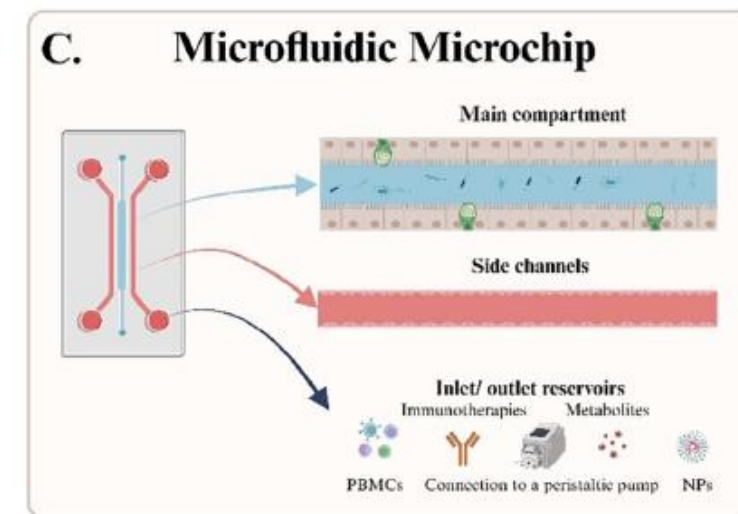
## Experimental platforms for modeling tumor–microbiome interactions in 3D models

**Spheroids:** Tumor cells and microbiome components can be co-cultured using different techniques such as hanging drops, low-attachment plates, or spinner flasks to generate tumor spheroids.



**Organoids:** Tumor-derived organoids cultured in gel matrices can be microinjected with bacteria into their lumen, enabling the establishment of complex 3D tumor– host-microbiome organoids

**Microfluidic systems:** Tumor-microbiome co-cultures integrated into organ-on-chip devices allow perfusion through the side channels, with inlet/outlet reservoirs enabling the delivery of immunotherapies, nanoparticles, peripheral blood mononuclear cells (PBMCs), or metabolites under dynamic flow conditions.



**Bioprinted constructs:** Tumor cells, stromal components, immune cells, and microbiome elements incorporated in bioinks can be processed using extrusion, droplet, laser-assisted, or photolithography bioprinting. Crosslinking can yield spatially defined 3D tumor-microbiome constructs.

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Green Buzhor et al., *Adv. Healthcare Mater.* 2025

# Future implications



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# Bacterial engineering – Lessons from melanoma

- Using engineered *Salmonella* to target tumor methionine dependency and to enhance anti-tumor immunity
- The attenuated *Salmonella* strain was shown to be safe in phase 1 studies.
- It accumulates in tumors and have affinity to APC.
- A recent study in melanoma showed that methionine restriction with this bacterial strain can also activate anti-tumor immunity and have synergistic effect with ICI.

Open access Original research

Journal for ImmunoTherapy of Cancer

## *Salmonella*-mediated methionine deprivation drives immune activation and enhances immune checkpoint blockade therapy in melanoma

Sujin Zhou<sup>1</sup>, Shiwei Zhang<sup>1</sup>, Kexin Zheng<sup>1</sup>, Zixuan Li<sup>1</sup>, Enyu Hu<sup>1</sup>, Yunping Mu<sup>1</sup>, Jialuo Mai<sup>2</sup>, Allan Zhao<sup>1</sup>, Zhenggang Zhao<sup>1</sup>, Fanghong Li<sup>1</sup>

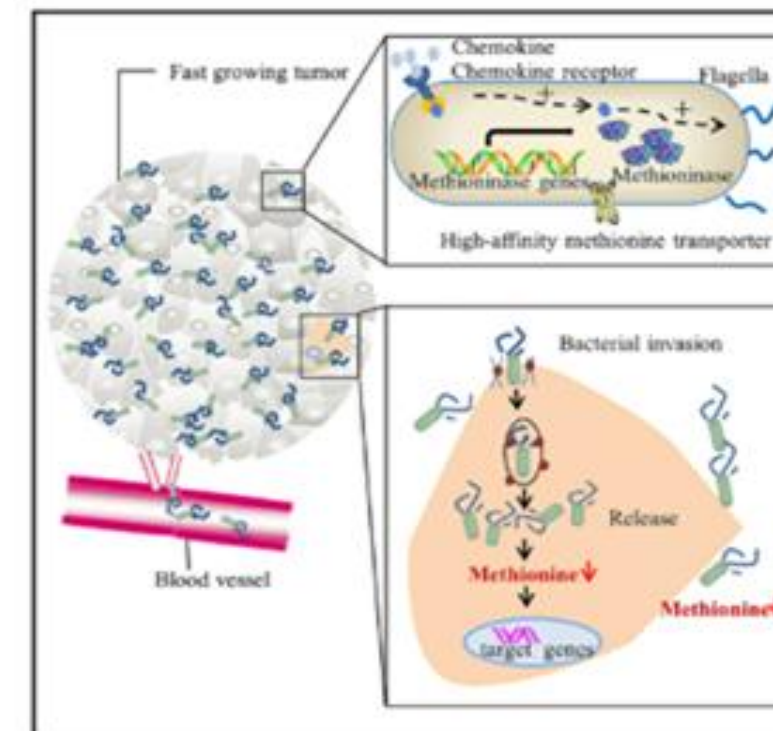
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Cell Reports  
Medicine

Article

Targeted deprivation of methionine with engineered *Salmonella* leads to oncolysis and suppression of metastasis in broad types of animal tumor models



(Zhou et al, 2023)

RECRUITING

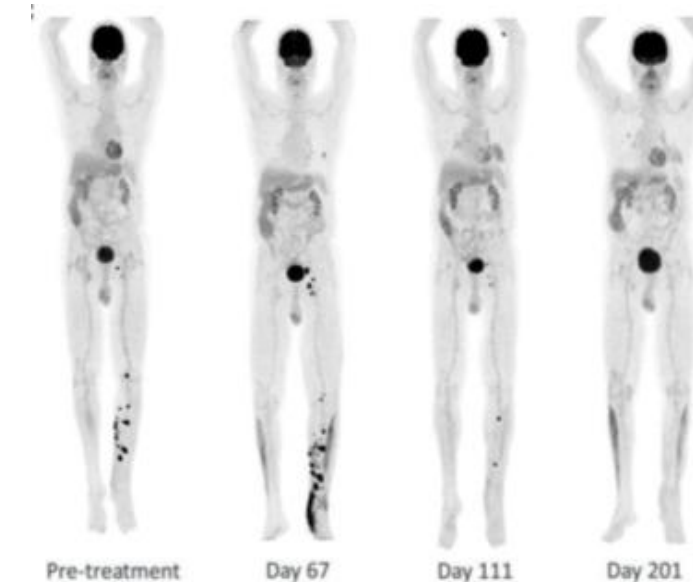
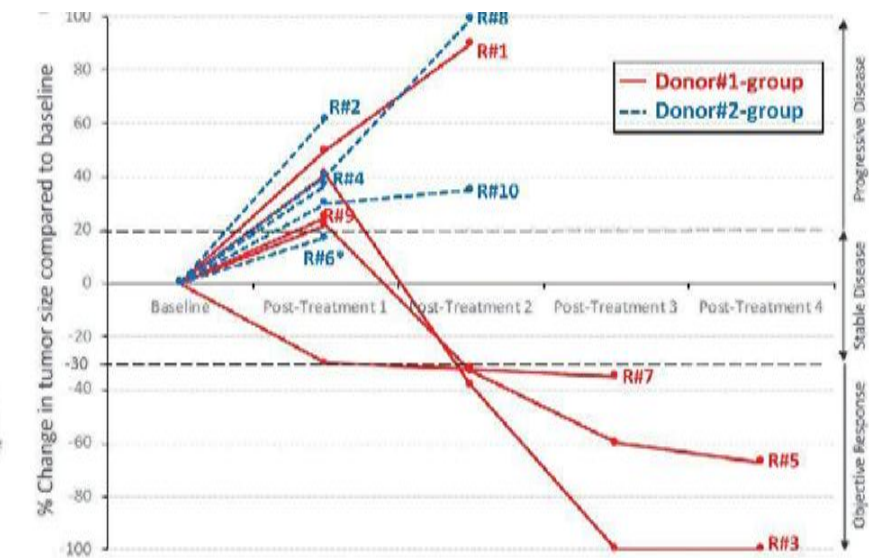
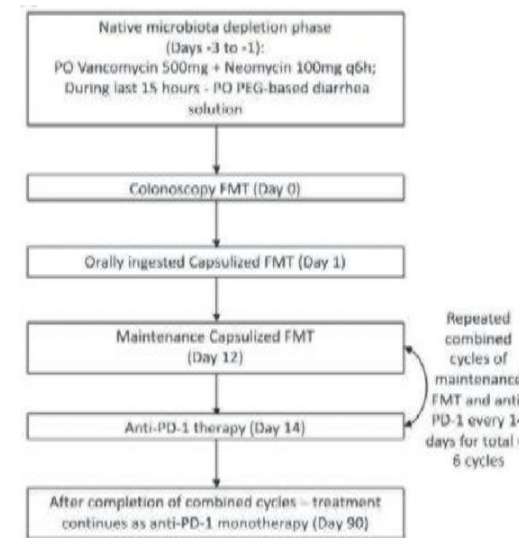
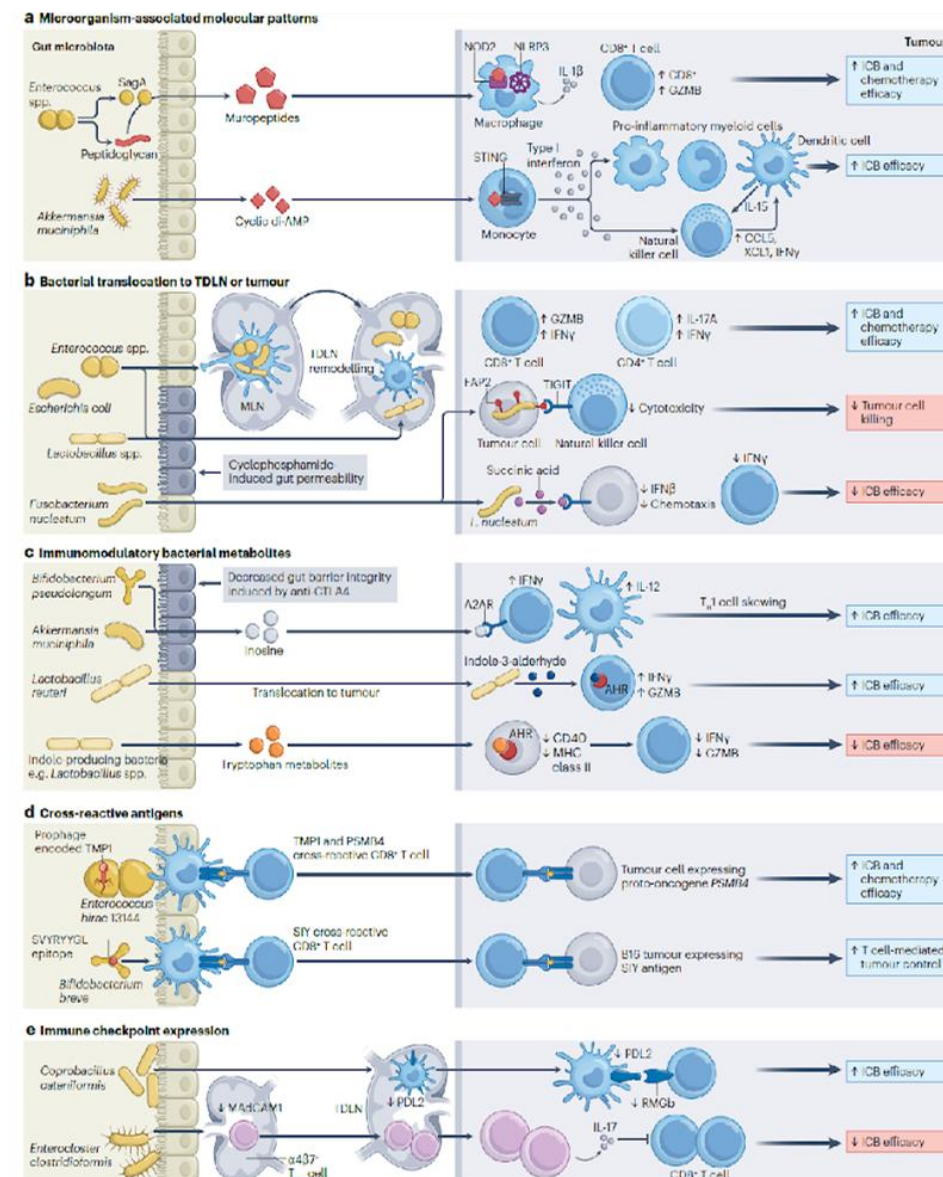
Study of SGN1 in Patients With Advanced Solid Tumor

ClinicalTrials.gov ID NCT05038150

Sponsor Guangzhou Sinogen Pharmaceutical Co., Ltd

# Identify dysbiotic non-immunogenic microbiome and design logical microbial modulation

- In immunogenic tumor (such as melanoma and RCC) microbial modulation can overcome resistance and improve response to immunotherapy.
- Mechanisms of action of microbial modulation (whether through molecular mimicry, immunogenic metabolites, activation of TME or activation of anti-cancer drugs) are all relevant to non-immunogenic tumors as well.



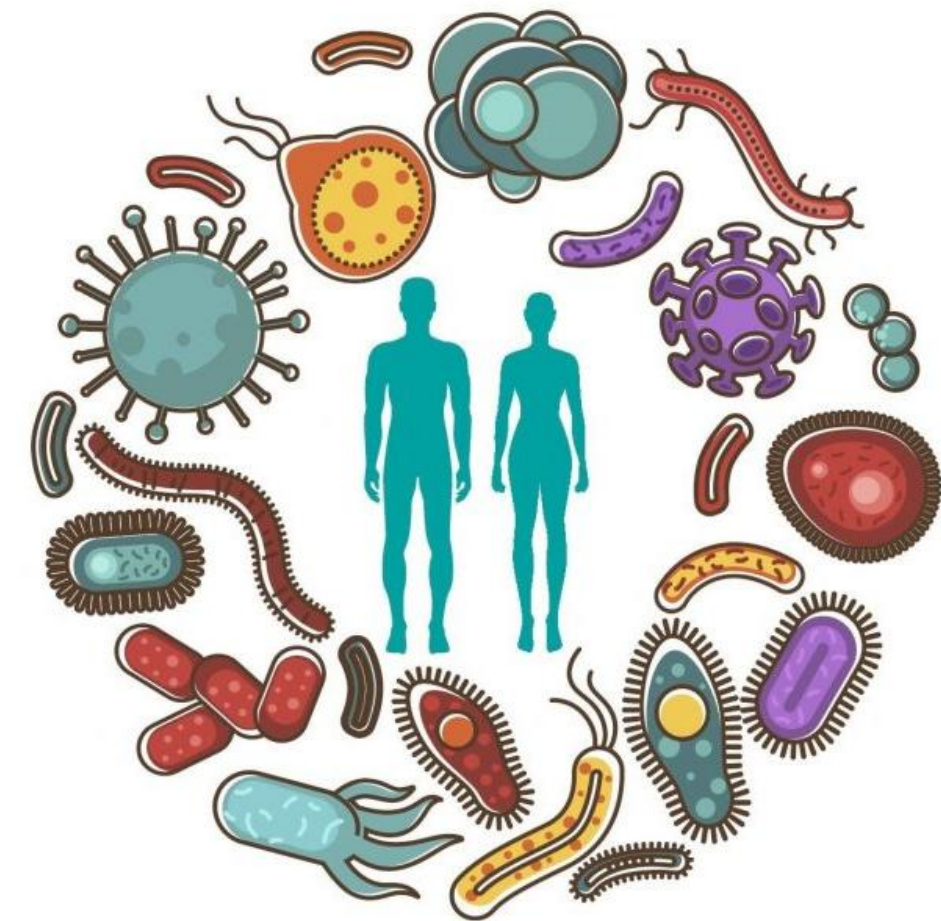
Baruch et. al Science 2021  
Blake et al Nat Rev Immunol 2023

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## Future directions:

- With the deepening realization that the microbiome may impact cancer pathogenesis and treatment, utilization of microbiome-derived personalized data finds an exciting avenue of research aimed at integrating microbiome readouts into the precision oncology setting.
- Recent studies have provided evidence that unique microbial DNA and RNA signatures can be detected in blood samples.
- Future studies will shed light on:
  - Effect of bacteria on other cancer hallmarks
  - Bacteria-cancer crosstalk
  - Homing of bacteria to tumors
  - Manipulation of TME for optimizing response to therapy



**THANK YOU!**

[i\\_benaharon@rambam.health.gov.il](mailto:i_benaharon@rambam.health.gov.il)

