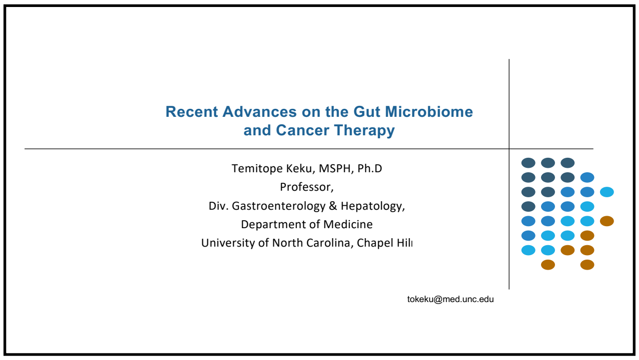
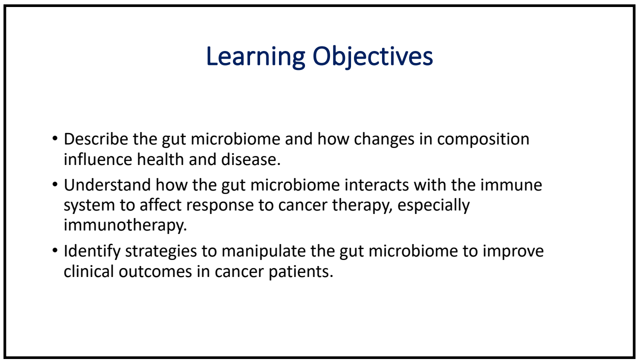


Recent Advances on the Gut Microbiome and Cancer Therapy

Temitope Keku, MSPH, Ph.D
Professor,
Div. Gastroenterology & Hepatology,
Department of Medicine
University of North Carolina, Chapel Hil

tokeku@med.unc.edu

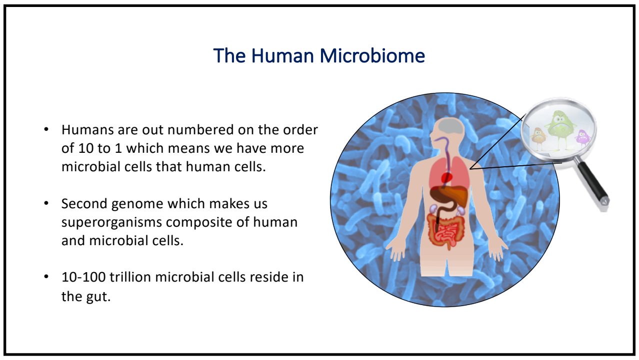


Learning Objectives

- Describe the gut microbiome and how changes in composition influence health and disease.
- Understand how the gut microbiome interacts with the immune system to affect response to cancer therapy, especially immunotherapy.
- Identify strategies to manipulate the gut microbiome to improve clinical outcomes in cancer patients.

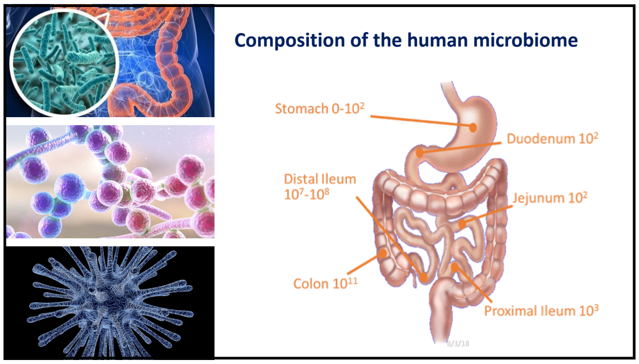
Terminology

- **Microbiota / microflora**: the microorganisms of a particular site or habitat such as the colon.
- **Microbiome**: the collective genomes of the microorganisms within a given host or environment.
- **Dysbiosis**: pathological imbalance in microbial community structure.
- **Diversity**: takes into account species richness and evenness.
- **Richness**: count of number of species/phylotypes.
- **Evenness**: distribution of the different types of bacteria within the community.
- **Germ-free**: free from living organisms other than the host species.
- **Bacterial co-metabolites:** Bioactive substances produced by bacterial metabolism within the gut.

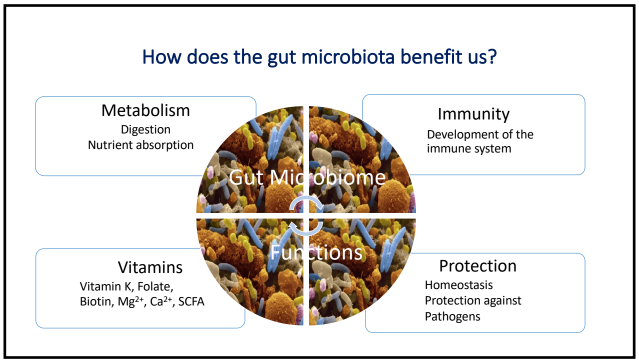


The Human Microbiome

- Humans are out numbered on the order of 10 to 1 which means we have more microbial cells that human cells.
- Second genome which makes us superorganisms composite of human and microbial cells.
- 10-100 trillion microbial cells reside in the gut.



Composition of the human microbiome



How does the gut microbiota benefit us?

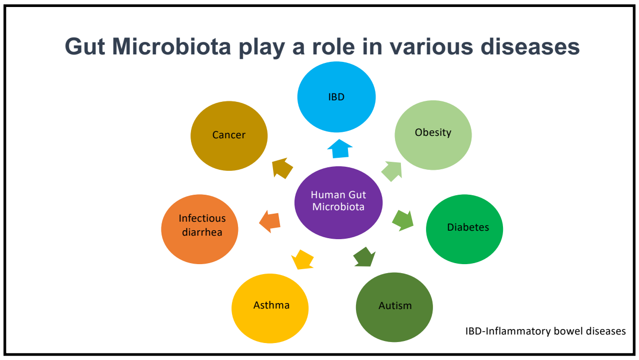
Metabolism
Digestion
Nutrient absorption

Immunity
Development of the immune system

Gut Microbiome Functions

Vitamins
Vitamin K, Folate, Biotin, Mg^{2+}, Ca^{2+}, SCFA

Protection
Homeostasis
Protection against Pathogens

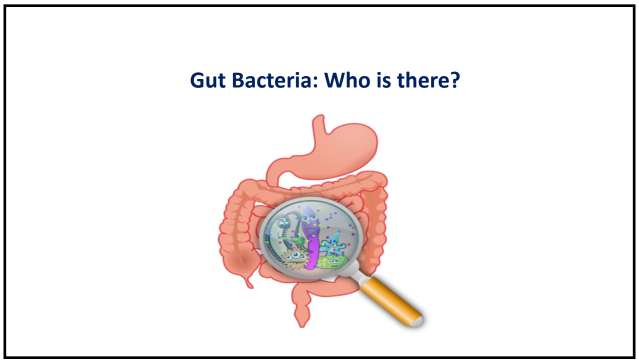


Gut Microbiota play a role in various diseases

Human Gut Microbiota

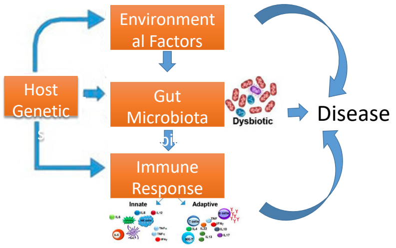
- IBD
- Obesity
- Diabetes
- Autism
- Asthma
- Infectious diarrhea
- Cancer

IBD-Inflammatory bowel diseases



Gut Bacteria: Who is there?

The Gut microbiota is influenced by a range of factors

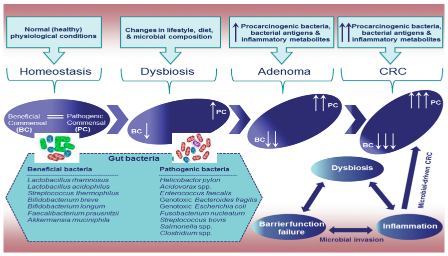


Adapted from Ahmed et al.
Microorganisms **2016**, *4*:20

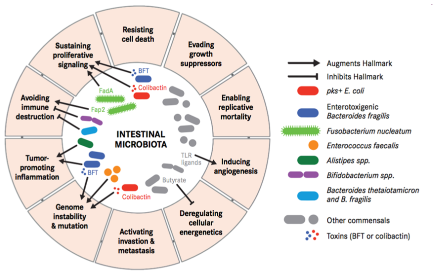
Cancers associated with gut microbiota

GI Cancers/Bacteria Implicated	Other Cancers
Stomach Cancer /*Helicobacter pylori*	Head and Neck Cancer
Gallbladder / *Salmonella enterica typhi*	Melanoma
Colorectal Cancer /*Escherichia coli*	Lung cancer
Fusobacterium spp	Breast cancer
Enterotoxigenic *Bacteroides fragilis*	Prostate
Pancreatic cancer	Sarcoma
Liver cancer	Ovarian
	Lymphoma

Microbiota Dysbiosis and Cancer



Dulal & Keku, The Cancer J. 2014;20:225

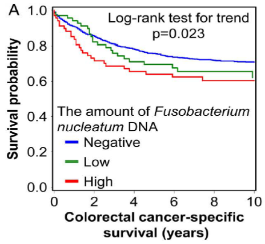


BFT indicates *Bacteroides fragilis* toxin; pks+, colibactin-producing; TLR, toll-like receptor.

Links between the gut microbiota and hallmarks of cancer

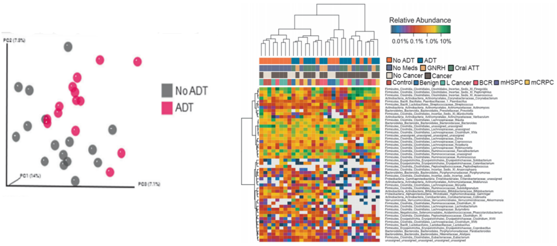
Fulbright et al. PLoS Pathog. 2017;13:1371

High *Fusobacterium nucleatum* abundance is associated with shorter colorectal cancer survival.



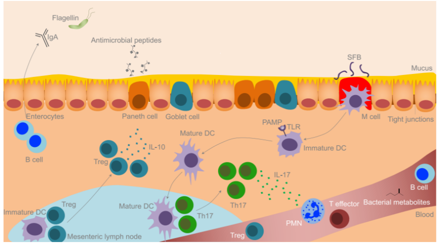
Mima et al. Gut 2015

Gut microbial composition differs in prostate cancer patients treated with androgen axis-targeted therapies (ATT)



Stanos et al. Prostate Can. Prost. Dis. 2018

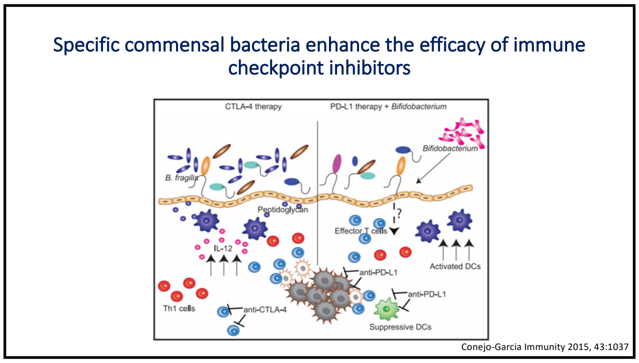
The gut microbiome interacts with the mucosal immune system



Gopalakrishnan et al. Cancer Cell Imm. 2018; 33:570

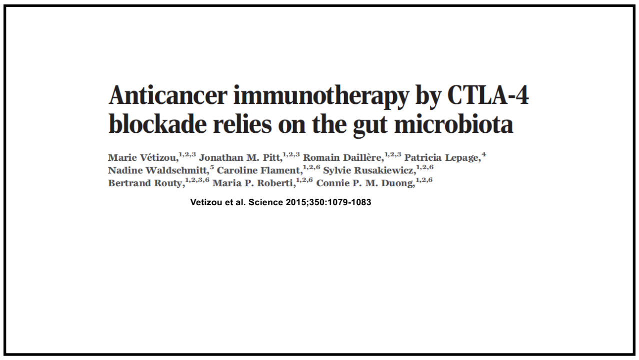
Immunotherapies

- Cancer cells express certain proteins that help them evade recognition by immune cells.
  - Cytotoxic T-lymphocyte antigen 4 (CTLA-4)
  - Programmed cell death protein (PD-1, PD-L1)
- Check point inhibitors block these proteins and enhance the immune system to fight cancer.
  - Ipilimumab, Pembrolizumab, Nivolumab



Specific commensal bacteria enhance the efficacy of immune checkpoint inhibitors

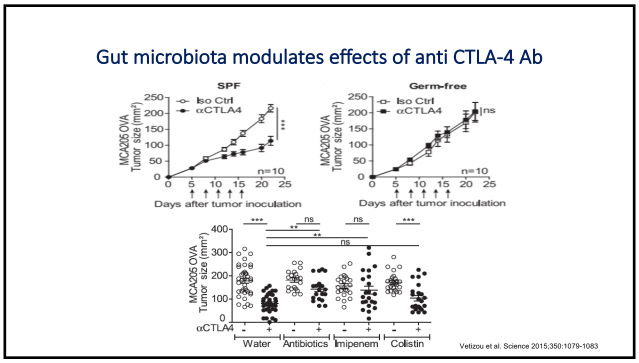
Conejo-Garcia Immunity 2015, 43:1037



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

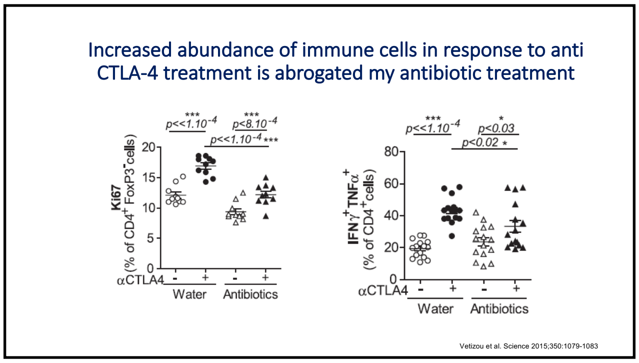
Marie Vétizou,[1,2,3] Jonathan M. Pitt,[1,2,3] Romain Daillère,[1,2,3] Patricia Lepage,[4]
Nadine Waldschmitt,[5] Caroline Flament,[1,2,6] Sylvie Rusakiewicz,[1,2,6]
Bertrand Routy,[1,2,3,6] Maria P. Roberti,[1,2,6] Connie P. M. Duong,[1,2,6]

Vetizou et al. Science 2015;350:1079-1083



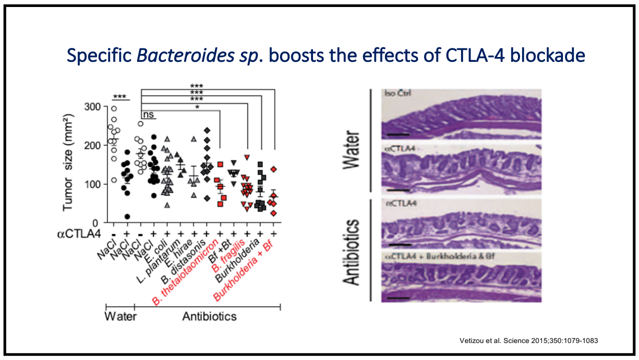
Gut microbiota modulates effects of anti CTLA-4 Ab

Vetizou et al. Science 2015;350:1079-1083



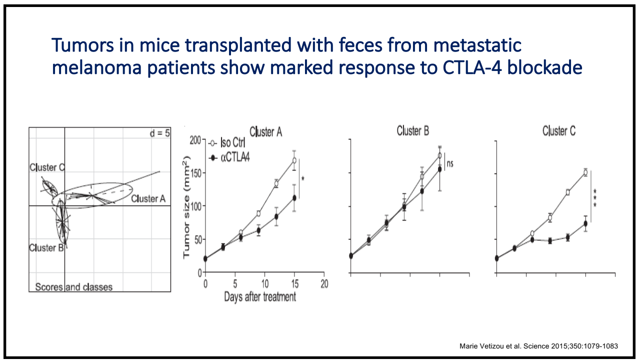
Increased abundance of immune cells in response to anti CTLA-4 treatment is abrogated my antibiotic treatment

Vetizou et al. Science 2015;350:1079-1083



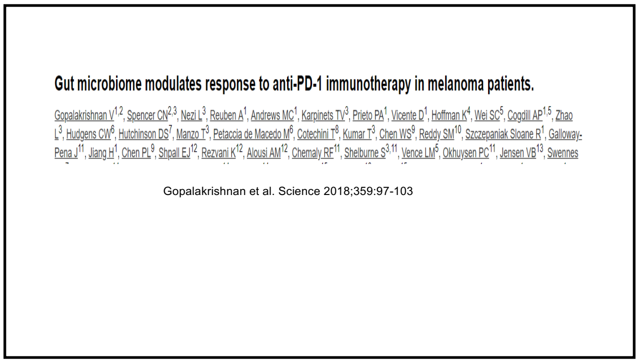
Specific *Bacteroides sp*. boosts the effects of CTLA-4 blockade

Vetizou et al. Science 2015;350:1079-1083



Tumors in mice transplanted with feces from metastatic melanoma patients show marked response to CTLA-4 blockade

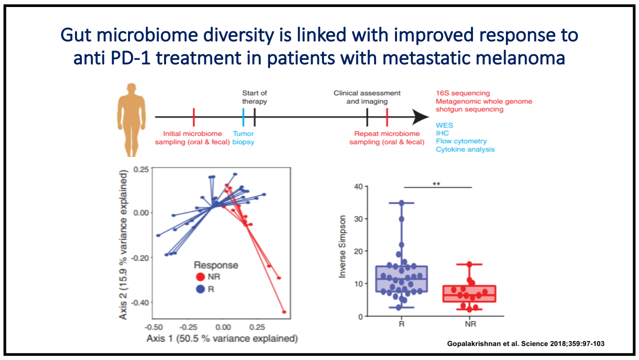
Marie Vetizou et al. Science 2015;350:1079-1083



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

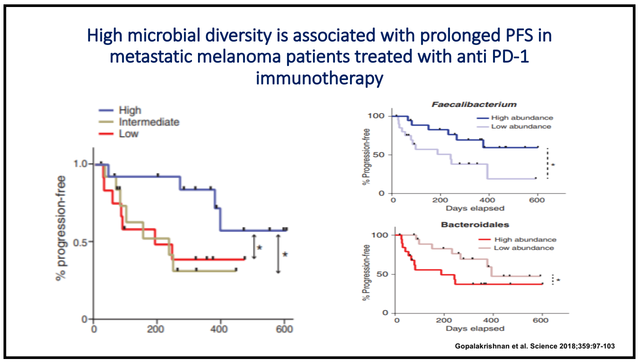
Gopalakrishnan V[1,2], Spencer CN[2,3], Nezi L[3], Reuben A[1], Andrews MC[1], Karpinets TV[3], Prieto PA[1], Vicente D[1], Hoffman K[4], Wei SC[5], Cogdill AP[1,5], Zhao L[3], Hudgens CW[6], Hutchinson DS[7], Manzo T[3], Petaccia de Macedo M[6], Cotechini T[8], Kumar T[3], Chen WS[9], Reddy SM[10], Szczepaniak Sloane R[1], Galloway-Pena J[11], Jiang H[1], Chen PL[9], Shpall EJ[12], Rezvani K[12], Alousi AM[12], Chemaly RF[11], Shelburne S[3,11], Vence LM[5], Okhuysen PC[11], Jensen VB[13], Swennes

Gopalakrishnan et al. Science 2018;359:97-103



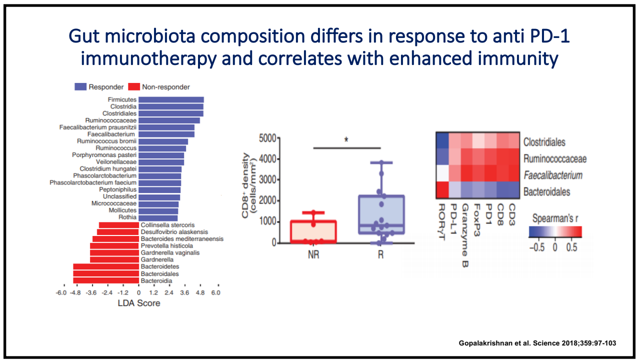
Gut microbiome diversity is linked with improved response to anti PD-1 treatment in patients with metastatic melanoma

Gopalakrishnan et al. Science 2018;359:97-103



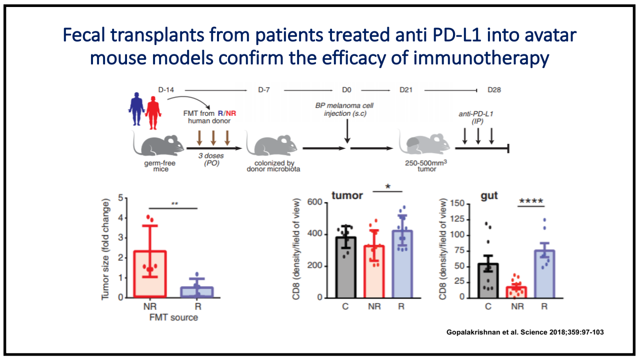
High microbial diversity is associated with prolonged PFS in metastatic melanoma patients treated with anti PD-1 immunotherapy

Gopalakrishnan et al. Science 2018;359:97-103



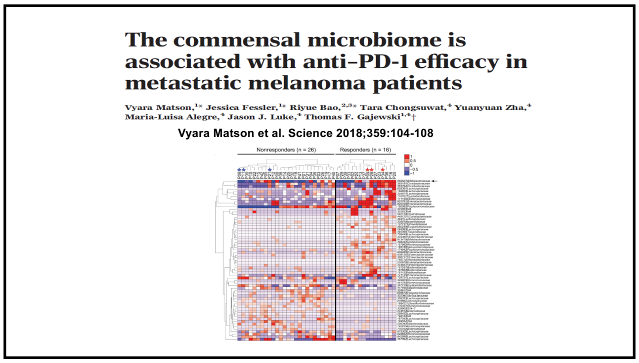
Gut microbiota composition differs in response to anti PD-1 immunotherapy and correlates with enhanced immunity

Gopalakrishnan et al. Science 2018;359:97-103



Fecal transplants from patients treated anti PD-L1 into avatar mouse models confirm the efficacy of immunotherapy

Gopalakrishnan et al. Science 2018;359:97-103



The commensal microbiome is associated with anti–PD-1 efficacy in metastatic melanoma patients

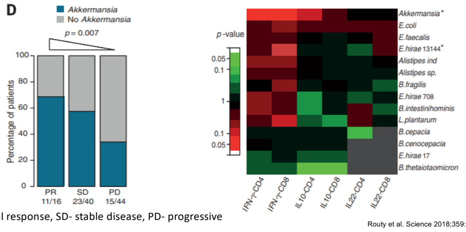
Vyara Matson,[1*] Jessica Fessler,[1*] Riyue Bao,[2,3*] Tara Chongsuwat,[4] Yuanyuan Zha,[4] Maria-Luisa Alegre,[4] Jason J. Luke,[4] Thomas F. Gajewski[1,4†]

Vyara Matson et al. Science 2018;359:104-108

Specific bacteria shown to enhance efficacy of immune check point inhibitors

- *Akkermansia muciniphila*
- *Bifidobacterium spp.*
- *Fecalibacterium praustnitzii*
- *Bacteroides fragilis*

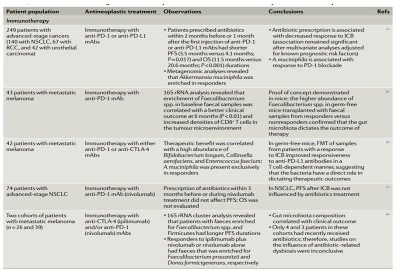
Akkermansia muciniphila correlates with response to anti PD-1 immunotherapy and enhanced immunity



(PR- partial response, SD- stable disease, PD- progressive disease)

Routy et al. Science 2018;359:91-97

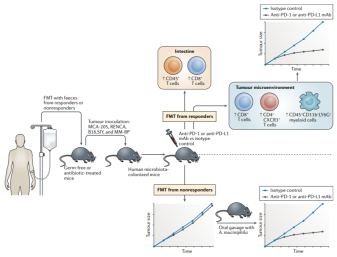
Clinical studies provide evidence that the gut microbiota modulates the efficacy of immunotherapy



Patient population	Antineoplastic treatment	Observations	Conclusions	Refs
Immunotherapy				
249 patients with advanced-stage cancers (140 with NSCLC, 67 with RCC, and 42 with urothelial carcinoma)	Immunotherapy with anti-PD-1 or anti-PD-L1 mAbs	• Patients prescribed antibiotics within 2 months before or 1 month after the first injection of anti-PD-1 or anti-PD-L1 mAbs had shorter PFS (3.5 months versus 4.1 months; $P=0.017$) and OS (11.5 months versus 20.6 months; $P<0.001$) durations • Metagenomic analyses revealed that *Akkermansia muciniphila* was enriched in responders	• Antibiotic prescription is associated with decreased response to ICB (association remained significant after multivariate analyses adjusted for known prognostic risk factors) • *A muciniphila* is associated with response to PD-1 blockade	22
43 patients with metastatic melanoma	Immunotherapy with anti-PD-1 mAb	16S rRNA analysis revealed that enrichment of *Faecalibacterium* spp. in baseline faecal samples was correlated with a better clinical outcome at 6 months ($P<0.01$) and increased densities of CD8⁺ T cells in the tumour microenvironment	Proof of concept demonstrated in mice: the higher abundance of *Faecalibacterium* spp. in germ-free mice transplanted with faecal samples from responders versus nonresponders confirmed that the gut microbiota dictates the outcome of therapy	82
42 patients with metastatic melanoma	Immunotherapy with either anti-PD-1 or anti-CTLA-4 mAbs	Therapeutic benefit was correlated with a high abundance of *Bifidobacterium longum*, *Collinsella aerofaciens*, and *Enterococcus faecium*; *A muciniphila* was present exclusively in responders	In germ-free mice, FMT of samples from patients with a response to ICB improved responsiveness to anti-PD-L1 antibodies in a T cell-dependent manner, suggesting that the bacteria have a direct role in dictating therapeutic outcomes	42
74 patients with advanced-stage NSCLC	Immunotherapy with anti-PD-1 mAb (nivolumab)	Prescription of antibiotics within 3 months before or during nivolumab treatment did not affect PFS; OS was not evaluated	In NSCLC, PFS after ICB was not influenced by antibiotics treatment	23
Two cohorts of patients with metastatic melanoma (*n* = 26 and 39)	Immunotherapy with anti-CTLA-4 (ipilimumab) and/or anti-PD-1 (nivolumab) mAbs	• 16S rRNA cluster analysis revealed that patients with faeces enriched for *Faecalibacterium* spp. and Firmicutes had longer PFS durations • Responders to ipilimumab plus nivolumab or nivolumab alone had faeces that was enriched for *Faecalibacterium prausnitzii* and *Dorea formicigenerans*, respectively	• Gut microbiota composition correlated with clinical outcome • Only 4 and 3 patients in these cohorts had recently received antibiotics; therefore, studies on the influence of antibiotic-related dysbiosis were inconclusive	84

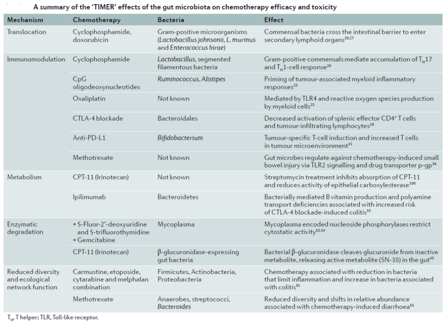
Routy et al. 2018
Nat Rev Clin Oncol.

Summary: Efficacy of immune checkpoint inhibitors



Routy et al. Nat. Rev. Clin. Onc. 2018:15: 382

Gut Microbiome and other chemotherapy

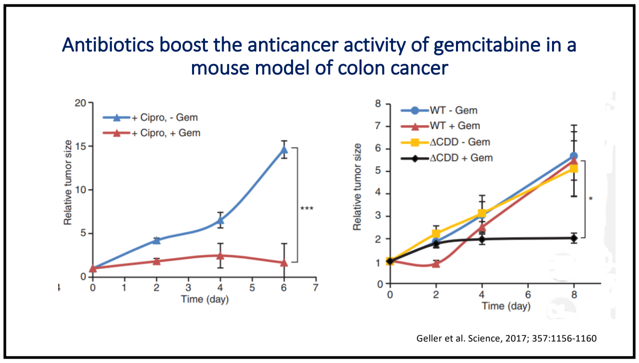


A summary of the 'TIMER' effects of the gut microbiota on chemotherapy efficacy and toxicity

Mechanism	Chemotherapy	Bacteria	Effect
Translocation	Cyclophosphamide, doxorubicin	Gram-positive microorganisms (*Lactobacillus johnsonii*, *L. murinus* and *Enterococcus hirae*)	Commensal bacteria cross the intestinal barrier to enter secondary lymphoid organs[26,27]
Immunomodulation	Cyclophosphamide	*Lactobacillus*, segmented filamentous bacteria	Gram-positive commensals mediate accumulation of T_H17 and T_H1-cell response[26]
	CpG oligodeoxynucleotides	*Ruminococcus*, *Alistipes*	Priming of tumour-associated myeloid inflammatory responses[28]
	Oxaliplatin	Not known	Mediated by TLR4 and reactive oxygen species production by myeloid cells[28]
	CTLA-4 blockade	Bacteroidales	Decreased activation of splenic effector CD4$^+$ T cells and tumour-infiltrating lymphocytes[29]
	Anti-PD-L1	*Bifidobacterium*	Tumour-specific T-cell induction and increased T cells in tumour microenvironment[31]
	Methotrexate	Not known	Gut microbes regulate against chemotherapy-induced small bowel injury via TLR2 signalling and drug transporter p-gp[36]
Metabolism	CPT-11 (Irinotecan)	Not known	Streptomycin treatment inhibits absorption of CPT-11 and reduces activity of epithelial carboxylesterase[106]
	Ipilimumab	Bacteroidetes	Bacterially mediated B vitamin production and polyamine transport deficiencies associated with increased risk of CTLA-4 blockade-induced colitis[33]
Enzymatic degradation	• 5-Fluor-2′-deoxyuridine and 5-trifluorothymidine • Gemcitabine	Mycoplasma	Mycoplasma encoded nucleoside phosphorylases restrict cytostatic activity[53,54]
	CPT-11 (Irinotecan)	β-glucuronidase-expressing gut bacteria	Bacterial β-glucuronidase cleaves glucuronide from inactive metabolite, releasing active metabolite (SN-38) in the gut[45]
Reduced diversity and ecological network function	Carmustine, etoposide, cytarabine and melphalan combination	Firmicutes, Actinobacteria, Proteobacteria	Chemotherapy associated with reduction in bacteria that limit inflammation and increase in bacteria associated with colitis[50]
	Methotrexate	Anaerobes, streptococci, *Bacteroides*	Reduced diversity and shifts in relative abundance associated with chemotherapy-induced diarrhoea[55]

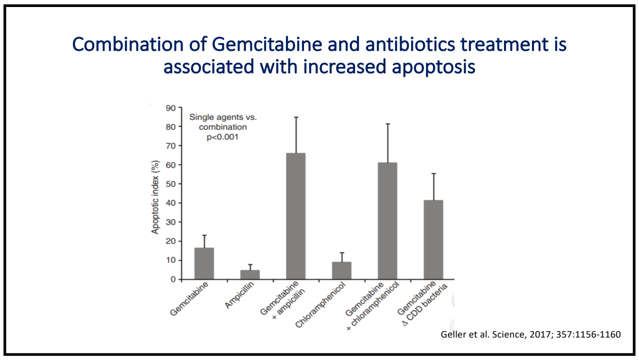
T_H, T helper; TLR, Toll-like receptor.

Alexander et al. 2017



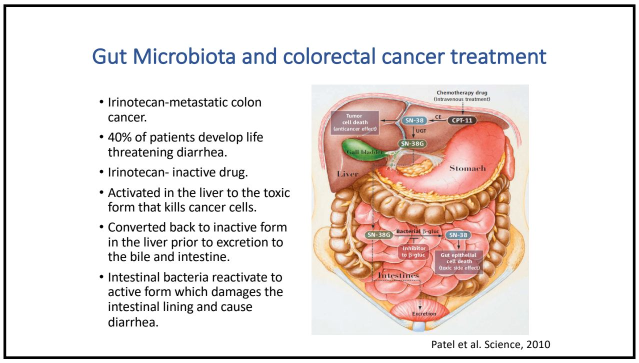
Antibiotics boost the anticancer activity of gemcitabine in a mouse model of colon cancer

Geller et al. Science, 2017; 357:1156-1160



Combination of Gemcitabine and antibiotics treatment is associated with increased apoptosis

Geller et al. Science, 2017; 357:1156-1160

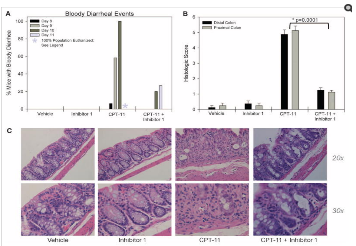


Gut Microbiota and colorectal cancer treatment

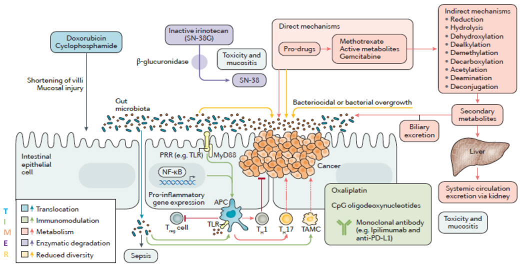
- Irinotecan-metastatic colon cancer.
- 40% of patients develop life threatening diarrhea.
- Irinotecan- inactive drug.
- Activated in the liver to the toxic form that kills cancer cells.
- Converted back to inactive form in the liver prior to excretion to the bile and intestine.
- Intestinal bacteria reactivate to active form which damages the intestinal lining and cause diarrhea.

Patel et al. Science, 2010

Irinotecan toxicity is reduced by inhibition of bacterial β-glucuronidase



Wallace et al. Science, 2010

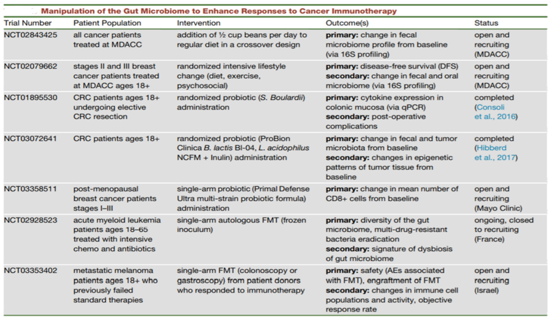


Gut microbiota mediates response to chemotherapy

Alexander et al. Nature Rev Gastro. 2017

Future directions

- Manipulation of the gut microbiota to identify beneficial strains that could improve anticancer immunosurveillance and therapeutic outcomes.
- Enrich for favorable gut bacteria
  - Prebiotics
  - Probiotics
  - Diet (Shift from Western to Mediteranian, low carbohydrate diet, ketogenic, caloric restriction)
- Fecal microbial Transplantation (FMT)
  - Lyophilized and encapsulated feces from healthy donors without cancer
  - Administered orally or rectally.
    - Challenges: selection of optimal donors
    - Sufficient material for long-term use and repeated treatment
- Identification of a defined probiotic mixture of several bacterial species could be better than use of any one species and may lead to improved therapeutic effects.
- Effective strategies to target microbial enzymes involved in drug metabolism to reduce toxic side effects from chemotherapy.



Manipulation of the Gut Microbiome to Enhance Responses to Cancer Immunotherapy

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

Goplakrushnan Cancer Cell 33, April 9, 2018