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The role of the commensal microbiota in carcinogenesis and cancer therapy
Disclosure

This presenter has no financial interest or other relationships with manufacturers of commercial products, suppliers of commercial services, or commercial supporters.
On September 17, 1683 Antonie van Leeuwenhoek wrote to the Royal Society in London about observations on the plaque between his own teeth with his homemade microscope:

"I then most always saw, with great wonder, that in the said matter there were many very little living animalcules, very prettily a-moving".
We can culture only a fraction of the bacterial species that live in our body.
Humans have co-evolved with microbial partners

- We are a composite of species: bacteria, archea, protozoa, fungi, viruses, bacteriophages

- Commensal microorganisms
  - inhabit all barrier surfaces of our organism
  - are at least as numerous as human cells
  - their DNA (the microbiome) contains 100 times more genes than our ‘own’ human genome
Humans are **metaorganisms** composed of the large host (human) cells and commensal microbial cells.

The commensal microbiota is indispensable for the survival of the metaorganism and the cross-talk between the host and its microbiota regulates many physiological functions.
Changes in our microbiota...

**Dysbiosis**: a microbiota imbalance that may be associated with illnesses

- **Geographical location**
- **Lifestyle**
- **Nutrition**
- **Hygiene**
- **Probiotics**
- **Antibiotics**
- **Infections**

- **Age**
- **Mode of delivery**
- **Host Genetics**
- **Diet & Lifestyle**
  - Window of opportunity for microbial modulation
  - >65 years
  - Diet & Lifestyle
  - Living structure & Medications

(genetics accounts for between 1 to 8% of microbiota diversity)
Variable regions of the 16 rRNA gene

Amplicons

Shotgun Metagenomics & Metatranscriptomic

MICROBIOTA

HOST

Modified from: Ji B, Nielsen J. From next-generation sequencing to systematic modeling of the gut microbiome. Front Genet. 2015

Transkingdom Networks: A Systems Biology Approach to Identify Causal Members of Host-Microbiota Interactions
Nutrient absorption, Synthesis of Vitamins, Metabolism of bile and hormones, Fermentation of undigestable carbohydrates, Barrier fortification, Mucosal Immunity

Inflammatory bowel diseases

Immune response to respiratory viruses, Allergy

Behavior and cognitive functions

Development of the immune system

Metabolic (Obesity, metabolic syndrome, insulin resistance)

Autoimmunity

Innate and adaptive skin immunity

Skin Microbiota

Cancer as a disease of the human metaorganism

Seed and soil

[Oncogene (Ras, Ret, Src)-induced] intrinsic inflammation

Activation of signaling pathways (e.g. β-catenin) regulates the immune infiltrate in the TME

Passenger/driver mutations as tumor neoantigens

TUMOR CELLS
(cancer genetics)

Genetic and epigenetic control of oncogenes and tumor suppressor gene expression

Tumor microenvironment
Inflammation

Innate and adaptive immune response

Host genetics

Environmental and lifestyle factors

Commensal Microbiota
Cancer, Inflammation & the Microbiota

Commensal Microbiota

Chronic inflammation (infections, aseptic)
Genomic mutations
Intrinsic / oncogene induced inflammation
Tumor promotion
Predisposing conditions (obesity, metabolic syndrome)

Cancer associated (extrinsic) inflammation
Tumor growth
Angiogenesis
Tissue remodeling
Infiltration and Metastasis
Immunoevasion
Anti-cancer immune response
Cancer Co-morbidities
Response to therapy
Primary tumor
Mets

Cancer, Inflammation & the Microbiota
Rous Sarcoma Virus induces tumors in adult birds at the site of injection or injury but not in sterile embryos even if the cells in the embryo express the Src viral oncogene and show a transformed phenotype when cultured in vitro.


Local and distant effects of the microbiota on cancer

- Stomach cancer (*Helicobacter pylori*)
- Colon rectal carcinoma (*Escherichia coli*, *Fusobacterium* spp., enterotoxigenic *Bacteroides fragilis*, *Streptococcus gallolyticus*)
- Gallbladder carcinoma (*Salmonella enterica* Thyphi)
- Pancreatic cancer
- Lung cancer
- CRC metastases
  - Inactivation of chemotherapeutic drugs (*gemcitabine*)
  - Either immunosuppression or immune activation

Cancer at epithelial barrier surfaces

- Cancer at sterile sites
  - Systemic effects

Intratumoral bacteria

Commensal Microbiota

Effectiveness and Toxicity of Chemotherapy and Immunotherapy against sterile tumors

- Malt lymphoma
- Hepatocellular carcinoma
- Mammary carcinoma
- Thymic lymphoma
- Sarcoma
- Ovarian Cancer
The microbiota affects cancer cell genetic stability and proliferative pathways as well as inflammation and immunity in the tumor microenvironment.

**Fusobacterium nucleatum** is present at higher frequency in the colon of CRC patients and particularly in the tumor area where it may form microfilms.

- Activation of E-cadherin–β-catenin signalling via the adhesion protein FadA
- Establishment of a pro-tumorigenic inflammatory microenvironment
- Inhibition of antitumour immunity via the Fap2–TIGIT (T-cell immunoreceptor with Ig and ITIM domains)
- Upregulation of microRNA-21 to enhance cancer cell proliferation and invasion
- Stimulation of cancer cell autophagy to promote resistance to chemotherapy

Colorectal carcinoma are enriched for biofilms harboring symbionts with capacity for tumorigenesis.

Colorectal carcinoma are enriched for biofilms harboring symbionts with capacity for tumorigenesis.

Biofilm-positive samples were stained with DAPI (blue) and probes against four bacterial membership groups: **Fusobacterium (yellow)**, **Bacteroidetes (green)**, **Lachnospiraceae (red)**, and **Proteobacteria (magenta)**.

Intratumoral bacteria: Pancreatic Ductal Adenocarcinoma (PDA)

Intratumor bacteria, primarily Gammaproteobacteria, inactivate the chemotherapeutic drug gemcitabine by expressing the enzyme cytidine deaminase.

• PDAC long-term survivors display high tumor microbial diversity and immunoactivation
• A PDAC tumoral microbiome signature predicts PDAC long-term survival
• The gut microbiome modulates the PDAC tumor microbiome landscape
• Fecal microbial transplants can modulate tumors immunosuppression and growth


Intratumoral bacteria: Lung Microbiota and Cancer

- Higher microbiome $\alpha$-diversity in normal lung tissue (but not tumor tissue) of NSCLC patients was associated with reduce disease-free survival
- The presence of Koribacteraceae was associated with increased survival, whereas greater abundance of families Bacteroidaceae, Lachnospiraceae, and Ruminococcaceae were associated with reduced survival


- Depletion of commensal microbiota suppresses lung adenocarcinoma development induced in mice by Kras mutation and p53 loss
- Lung cancer development is associated with local dysbiosis and inflammation
- Microbiota drive proliferation and activation of $\gamma\delta$+T cells in lung cancer
- $\gamma\delta$ T cells promote neutrophil infiltration and tumor cell proliferation


- This study of 142 lung cancer patients and 33 healthy control showed both microbiome-gene and microbiome-exposure interactions in lung cancer tissue.
- Specifically, squamous cell carcinoma harboring TP53 mutations, which can impair epithelial function, have a unique bacterial consortium that in smoking-associated tumors shows higher relative abundance of certain species such as Acidovorax.

Bacterial digestion products (SCFA) ↓ Inflammasome ↓ IL-18 ↓ IL-18R (MyD88) ↓ IFN-γ IL-22

Lack of IL-18R signaling in CD11b+ myeloid cells induces a transmissible dysbiosis that affects both tumor initiation and promotion.

Bacterial β-glucuronidase is responsible for reactivation of other carcinogens such as the environmental pollutant benzopyrene and the food-borne carcinogen 2-amino-3-methylimidazo[4,5-f]quinoline, is a biomarker for colon cancer and is responsible for the intestinal toxicity of the drug Irinotecan.

Bacterial antibiotics (Colicin from O7H7 E. coli and lantibiotic from B. coccoides) may play a role in the dominance of the transmissible dysbiosis into wild type mice.
Host genetics may affect the mouse phenotype by modifying the composition of the microbiota.

Phenotype
(e.g. spontaneous colitis, susceptibility to inducer of colitis and carcinogenesis, tumor growth, response to anti-cancer therapy, response to vaccination)

Genetically altered mouse
(e.g. MyD88-/-, TLR5-/-, IL-18-/-)

Bacterial Dysbiosis
Virus infection
(e.g. norovirus)

Reactivation of endogenous retroviruses (ERVs) (lymphomas, mouse) or Pro-inflammatory ERV transcript (humans)

Mycosis → Oral and esophageal carcinogenesis (humans and mouse)

Protozoan parasites
(e.g. Trichomonas muris)
Protozoan viruses

Wild type mice
(co-housed, fecal transplant, fostering)

No phenotype
Partial phenotype
Full phenotype
Is the response to cancer therapy regulated by the commensal bacteria?

Cancer
Immunotherapy
&
Chemotherapy

Gut microbiota

Tumor
Is the response to cancer therapy regulated by commensal bacteria?

Systemic anti-IL-10R + Intratumor CpG-OGN immunotherapy
Platinum compound (oxaliplatin, cisplatin) chemotherapy

ANTIBIOTICS
Neomycin
Vancomycin
Imipenem

or Germ-free mice

Sterile subcutaneous transplanted tumor

Intestinal microbiota

Noriho Iida, Amiran Dzutsev, C. Andrew Stewart, ……… Giorgio Trinchieri, Romina S. Goldszmid
Commensal bacteria control cancer response to therapy by modulating the tumor microenvironment
Science, 2013; 342:967-70
Antibiotics (ABX) suppress the anti-tumor effect of immune and chemo therapy.

Antibiotics (ABX) suppress TNF-mediated early necrosis of the tumor and decrease inflammatory cytokine production following anti-IL-10R/CpG.

1 cm

MC38 tumor, 72 h after CpG treatment
ABX impair oxaliplatin therapy by preventing production of ROS from NOX2 + myeloid cells that is required for DNA damage after formation of platinum DNA adducts.

Oxaliplatin induces ROS production in tumors of control but not ABX-treated mice.

- EL4 tumors-bearing B6 mice were treated with 10mg/kg oxaliplatin.
- ROS-induced bioluminescence using the L-012 probe was analyzed 24 hours after oxaliplatin injection.
The composition of the (gut) microbiome determines the efficacy of cancer therapy by training infiltrating myeloid cells in distant tumors.
Recent papers have established in clinical studies that the composition of the gut microbiome modulates the effectiveness of anti-PD1 cancer therapy. 

Anti-PD1 treated melanoma patients

Hassane Zarour, Diwakar Davar and John Kirkwood

Melanoma and Skin Cancer program, U. Pittsburgh

- **Ruminococcaceae**
- **Clostridiales**
- **Faecalibacterium prausnitzii**
- **Akkermansia muciniphila**
- **Alistipes spp**
- **Bifidobacterium longum**
- **Collinsella aerofaciens**
- **Enterococcus faecium**
Gut microbiome modulates response to anti-PD-1 immunotherapy in melanoma patients

Melanoma

Gut microbiome influences efficacy of PD-1-based immunotherapy against epithelial cancer

Lung and renal cancer

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

Melanoma

Environment dominates over host genetics in shaping human gut microbiota

Daphna Rothschild1,2, Omer Weissbrod1,2, Elad Barkan1,2, Alexander Kurilshikov1, Tal Korem1,2, David Zeevi1,2, Paul I. Costa1,2, Anastasia Godneva1,2, Iris N. Kalka1,2, Noam Bar1,2, Smadar Shilo1,2, Dar Lador1,2, Arnau Vich Vila1,4, Niv Zmora5,6,7, Meirav Pevsner-Fischer3, David Israeli8, Noa Kosower1,2, Gal Malka1,2, Bat Chen Wolf1,2, Tali Avnit-Sagi1,2, Maya Lotan-Pompan1,2, Adina Weinberger1,2, Zamir Halpern7,9, Shai Carmi10, Jingyuan Fu3,11, Cisca Wijmenga3,12, Alexandra Zhernakova3, Eran Elinav5,8 & Eran Segal1,2
Microbiota taxonomic identification in cancer patients may be affected by geography, disease and sequencing technology.

**Study**
- Chicago (Gajewski)
- Pittsburgh (Zarour)
- Houston (Wargo)
- Paris (Zitvogel)

**Tumor type**
- Melanoma
- Not_Melanoma

**Sequencing method**
- IDENTICAL BIOINFORMATIC ANALYSIS (JAMS)
  - Genes are found *ab initio*, from contigs.
  - Standard annotation using Prokka - hierarchical pipeline which takes into consideration quality of annotated database: UniProt + GenBank RefSeq.
Melanoma patients have different microbiota composition than healthy donors and both alpha and beta diversity change with disease progression.

Mouse and, in part, clinical studies have established an important modulating role of the gut microbiome composition on the immune-checkpoint inhibition cancer therapy.
Can we target the microbiome to improve therapy response?

Targeting the microbiota

- Diet
- Antibiotics
- Probiotics
- Prebiotics
- Fecal transplant
- Oral formulation of bacteria or their spores
**Lung and renal cancer**

**Melanoma**

FMT

Bacteria consortium in a pill

Selected Bifidobacterium spp probiotics

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**Ruminococcaceae**

Clostridiales

Faecalibacterium prausnitzii

Akkermansia muciniphila

Alistipes spp

Bifidobacterium longum

Collinsella aerofaciens

Enterococcus faecium

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**NCT03817125: Melanoma Checkpoint and Gut Microbiome Intervention (MCGRAW).** Parker Institute for Cancer Immunotherapy and Seres Therapeutics, Inc.

Administration (SER-401® consortium of live bacteria (spores?) with or without preconditioning with vancomycin) in Combination With Anti-PD1 Therapy in Adult Melanoma Patients

Bristol-Myers Squibb and Vedanta Biosciences to treat advanced or metastatic cancer patients with anti-PD1 and VE800

VE800 is a lyophilized preparation (pills) of 8 bacterial strains in experimental animals based on Kenya Honda work

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**NCT03775850 A Study of EDP1503 in Patients With Colorectal Cancer, Breast Cancer, and Checkpoint Inhibitor Relapsed Tumors.** Evelo Biosciences, Inc.

Monoclonal microbial EDP1503 is an orally available preparation derived from a single clone of Bifidobacterium spp. with potential immunomodulatory and antineoplastic activities based on Tom Gajewski work
Associations of OTC probiotic use with features of the gut microbiome and response to melanoma therapy

Spencer, C., McQuade, J.L., Vetizou, M., McCulloch, J., Trinchieri, G., Daniel-McDouglas C., Wargo, J.

Submitted
Ruminococcaceae
Clostridiales
Faecalibacterium prausnitzii

Akkermansia muciniphila
Alistipes spp

Bifidobacterium longum
Collinsella aerofaciens
Enterococcus faecium

Fecal microbiome transplant (FMT)
12 anti-PD1 refractory patients have been transplanted with the fecal microbiome from responsive patients and treated again with pembrolizumab®:

Enrolled: 12; Evaluable: 8
Best Response: 1 PR, 1 CR, 5 SD, 1 PD
Current Response: 1 PR, 1 CR, 2 SD, 4 PD

We are working with the University of Pittsburgh by characterizing the changes in microbiota composition in the transplanted patients by metagenomic analysis and testing the transplanted microbiomes in gnotobiotic mice in conventional conditions or following diet alterations.

NCT03353402: Fecal Microbiota Transplantation (FMT) in Metastatic Melanoma Patients Who Failed Immunotherapy. Sheba Medical Center Tel HaShomer, Israel

NCT03341143: Fecal Microbiota Transplant (FMT) in Melanoma Patients. University of Pittsburgh

NCT03772899: Fecal Microbial Transplantation in Combination With Immunotherapy in Melanoma Patients (MIMic). Lawson Health Research Institute, London, CA

AACR April 2019 General Meeting
Patient 1: PR (7 months)
Patient 2: PD
Patient 3: PR (2 months), PD

Gal Markel and Ben Boursi
Sheba Medical Center in Ramat Gan, Israel
NCT03341143: Fecal Microbiota Transplant (FMT) in Melanoma Patients.
University of Pittsburgh

Phase II Feasibility Study of FMT in PD-1 Resistant Melanoma

Stage 1 (N=13)
- No active CNS disease
- Accessible tumor for biopsy
- Amenable to FMT therapy

Stage 2 (N=7)
- Simon Two Stage
  Stage 1 → 2 if ≥1 response
  FMT worthy of further study if >4 responses in total (2/cohort)

PD-1 non-responders at 12 weeks

- Biopsy (tumor & gut via colonoscopy)
- Labs
- FMT
- Scans
- Pembrolizumab
- Stool sampling

Response Evaluation
NCT03341143: Fecal Microbiota Transplant (FMT) in anti-PD1 refractory Melanoma Patients. University of Pittsburgh

Enrolled: 12; Evaluable: 8
Best Response: 1 PR, 1 CR, 5 SD, 1 PD
Current Response: 1 PR, 1 CR, 2 SD, 4 PD
NCT03341143: Fecal Microbiota Transplant (FMT) in Melanoma Patients. Metagenomic analysis of microbiome in donors and recipients

FMT: PT180005 to PT180007
Response: Partial Response

FMT: PT180002 to PT180018
Response: Progressive Disease
Germ-free mice

Humanized gnotobiotic mice

Human fecal microbiota

Donor 1
PT-18-0002

2 recipients:
-PD (PT-18-0017)
-SD to PD (PT-18-0018)

Donor 3
PT-18-0005

2 recipients:
-SD to PR (PT-18-0007)
-PD (PT-18-0001)

Donor 6
PT-18-0008

4 recipients:
-SD to PD (PT-18-0033)
-SD to SD (PT-18-0034)
-pending (PT-18-0008)
-pending (PT-18-0010)

Donor 5
TPF-18-195

4000-

NCT03341143: Fecal Microbiota Transplant (FMT) in Melanoma Patients.
Testing FMT donors in gnotobiotic mice

Responder patients as FMT donors

Non-responder patient as FMT donors

Marie Vetizou
Effect of dietary fiber intake on anti-PD1 Response

Effect of high/low fiber diet on anti-PD1 response in melanoma patients

- Responders: 69%
- Non-responders: 36%

p = 0.05 OR: 1.02-26.25
(McQuade, SMR 2018)

Spencer, C.
McQuade, J.L.
Vetizou, M.

McCulloch, J.
Trinchieri, G.
Daniel-McDouglas C.
Wargo, J.

Submitted
Effect of dietary fiber intake on anti-PD1 Response

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McCulloch, J.
Trinchieri, G.
Daniel-McDouglas C.
Wargo, J.
Submitted

Marie Vetizou

PCA (taxonomic) of shotgun metagenomic sequencing of fecal microbiota

Isotype control treated groups

Anti-PD1 treated groups
Mouse and, in part, clinical studies have established an important modulating role of the gut microbiome composition on the immune-checkpoint inhibition cancer therapy.
Discovery of reliable microbiome-related biomarkers for prediction of response and stratification of patients

Identification of favorable microbiomes for fecal transfer from responder patients or healthy donors

Identification of consortia of commensal bacteria that favor a clinical response

Identification of perturbations (diet, prebiotics, etc.) able to induce or maintain a favorable microbiome composition

Identification and therapeutic use of bacterial metabolites enhancing anti-tumor immunity

Targeting molecular pathways by which the host-microbiome cross-talk enhance the anti-cancer response

Mouse and, in part, clinical studies have established an important modulating role of the gut microbiome composition on the immune-checkpoint inhibition cancer therapy

Large individual and geographical diversity
Noriho Iida
Amiran Dzutsev
Andy Charles Stewart
Romina Goldszmid

Hassane Zarour
Diwakar Davar
John Kirkwood

Christine Spencer
Jennifer McQuade
Carrie Daniel-MacDougall
Jennifer Wargo
MD Anderson CC

Marie Vetizou (CRI Fellow)
John McCulloch
Colm O’hUigin
Jane Yuan
Vishal Thovarai
Richard Rodrigues

Antonio Difilippantonio
Misty Hawes

Cancer and Inflammation Program, CCR, NCI, Bethesda and Frederick, MD

Dysbiosis & mouse genetics
Prepartum ABX
Chemotherapy toxicity & cachexia
Colon carcinogenesis
Anti-PD1 therapy

Cancer therapy

Cancer therapy

Bowel carcinogenesis
Anti-PD1 therapy

Chemotherapy toxicity & cachexia

Prepartum ABX

Dysbiosis & mouse genetics

Colon carcinogenesis
Anti-PD1 therapy

Final scene from Federico Fellini’s "E la nave va" (1983)