ONCOIMMUNOLOGY
2020, VOL. 9, NO. 1, 1–8
https://doi.org/10.1080/2162402X.2020.1774298

Taylor & Francis
Taylor & Francis Group

REVIEW

Trial watch : the gut microbiota as a tool to boost the clinical efficacy of anticancer immunotherapy

Romain Daillère[a], Lisa Derosa[b,c], Mélodie Bonvalet[b,c], Nicola Segata[d], Bertrand Routy[e,f], Manuela Gariboldi[g], Eva Budinská[h], I. Jolanda M. De Vries[i], Alessio Gordon Naccarati[j,k], Valérie Zitvogel[a], Carlos Caldas[l], Lars Engstrand[m], Sibylle Loilbl[n], Jacques Fieschi[o], Lucie Heinzerling[p], Guido Kroemer[q,r,s,t,u], and Laurence Zitvogel[b,c,t,v,w]

[a]EverImmune, Villejuif, France; [b]Gustave Roussy Comprehensive Cancer Institute, Villejuif, France; [c]INSERM U1015, Villejuif, France; [d]Department CIBIO, University of Trento, Trento, Italy; [e]Centre Hospitalier De l'Université De Montréal (CHUM), Montréal, Canada; [f]Centre De Recherche Du Centre Hospitalier De l'Université De Montréal (CRCHUM), Montréal, Canada; [g]Department of Research, Fondazione IRCCS Istituto Nazionale dei Tumori, via G. Amadeo 42, 20133, Milan, Italy; [h]RECETOX, Faculty of Science, Masaryk University, Brno, Czech Republic; [i]Department of Tumor Immunology, Radboud Institute for Molecular Life Sciences, Radboud University Medical Center, Nijmegen, Netherlands; [j]IIGM Italian Institute for Genomic Medicine, c/o IRCCS Candiolo, 10060 Candiolo, Turin, Italy; [k]Candiolo Cancer Institute, FPO-IRCCS, 100 60, Turin, Italy; [l]CRUK Cambridge Institute, University of Cambridge, Cambridge, UK; [m]Department of Microbiology, Tumor and Cell Biology and Science for Life Laboratory, Karolinska Institutet, Sweden; [n]Medicine and Research, GBG Forschungs GmbH, , Neu-Isenburg, Germany; [o]HalioDx is a biotech company; [p]Department of Dermatology, Universitätsklinikum Erlangen, Friedrich Alexander University, 91054 Erlangen, Germany; [q]Centre de Recherche des Cordeliers, Equipe labellisée par la Ligue contre le cancer, Université de Paris, Sorbonne Université, Inserm U1138, Paris, France; [r]Metabolomics and Cell Biology Platforms, Institut Gustave Roussy, Villejuif, France; [s]Pôle De Biologie, Hôpital Européen Georges Pompidou, AP-HP, Paris, France; [t]Suzhou Institute for Systems Medicine, Chinese Academy of Medical Sciences, Suzhou, China; [u]Karolinska Institute, Department of Women's and Children's Health, Karolinska University Hospital, Sweden; [v]Faculty of Medicine, Université Paris Saclay, Le Kremlin-Bicêtre, France; [w]Center of Clinical Investigations in Biotherapies of Cancer (CICBT) 1428, Villejuif, France

ABSTRACT

Accumulating evidence demonstrates the decisive role of the gut microbiota in determining the effectiveness of anticancer therapeutics such as immunogenic chemotherapy or immune checkpoint blockade in preclinical tumor models, as well as in cancer patients. In synthesis, it appears that a normal intestinal microbiota supports therapeutic anticancer responses, while a dysbiotic microbiota that lacks immunostimulatory bacteria or contains overabundant immunosuppressive species causes treatment failure. These findings have led to the design of clinical trials that evaluate the capacity of modulation of the gut microbiota to synergize with treatment and hence limit tumor progression. Along the lines of this Trial Watch, we discuss the rationale for harnessing the gut microbiome in support of cancer therapy and the progress of recent clinical trials testing this new therapeutic paradigm in cancer patients.

ARTICLE HISTORY
Received 18 May 2020
Revised Vxx xxx xxxx
Accepted 19 May 2020

KEYWORDS
Gut microbiota; anticancer therapeutics; clinical trials

The rebirth of cancer immunotherapy

The rise and success of cancer immunotherapy over the past decade has revolutionized the clinical management of a wide array of malignancies that were previously associated with poor prognosis.[1] At the forefront of immunotherapy development are immune-checkpoint blockers (ICBs), which have seen enormous and unparalleled success in cancer therapy as a result of their broad bioactivity across many histological tumor types, the durability of their responses, and therapeutic success stories that sometimes involve even metastatic and chemo-resistant diseases.[2–8]

Antibodies targeting the cytotoxic T-lymphocyte-associated protein 4 (CTLA-4) or the interaction between programmed cell death protein 1 and programmed cell death ligand 1 (PD-1/PD-L1) disrupt negative immune regulatory checkpoints, thus unleashing antitumour immune responses. Such ICBs have been approved by the regulatory agencies and are now being considered as standard of care in a wide range of solid and hematologic neoplastic diseases including advanced-stage melanoma, non-small-cell lung cancer (NSCLC), head and neck cancer, bladder or renal cell cancer (RCC).[9]

In spite of the exceptional improvement in objective response rates and overall survival in a minority (~30%) of patients, ICBs responses are heterogeneous (with occasional acceleration of the disease called "hyperprogression" and a majority of patients exhibiting primary resistance) and sometimes transient (meaning that therapeutic "success" is followed by secondary resistance). Large efforts are being dedicated to identify the parameters that govern the strength, timing and the threshold of the anticancer immunity needed to trigger the effectiveness of anticancer therapeutics, a notion defined as the "cancer immune set-point". This set-point would dictate the capacity of a particular cancer patient to mount an effective antitumor immunity counteracting tumor progression.[10,11]

Among the factors deciphering the mechanisms of primary resistance to ICBs, evidence accumulating over the past decade has highlighted the role of the gut microbiota. Indeed, the reciprocal relationship between cancer progression, immune control, primary resistance/sensitivity to ICBs and the microbiota is becoming increasingly apparent.[12–15]

CONTACT Romain Daillère romaindaillere@gmail.com Centre De Recherche Des Cordeliers, Equipe Labellisée Par La Ligue Contre Le Cancer, Université De Paris, Sorbonne Université, Paris, France

Supplemental data for this article can be accessed on the publisher's website.

The role of the gut microbiome in modulating the cancer-immune set-point

The human gut microbiome modulates many host processes, including metabolism, inflammation, immune and intestinal epithelial cell responses.[16–18] In the last decade, major progress has been made in the comprehension of cancer development in interaction with the microbiota.[19] Indeed, a 'deviated' repertoire of the gut microbiome, that has been referred to as 'intestinal dysbiosis' has been epidemiologically – and sometimes causally – associated with a variety of chronic inflammatory disorders. In parallel, discoveries made in preclinical tumor models and in cancer patients have demonstrated that the composition of the intestinal microbiota influences the effectiveness of anticancer drugs (such as immunogenic chemotherapies and ICBs) and regulates tumor immunosurveillance.[20–27] Several lines of evidence have unraveled the link between the gut microbiota and ICBs-mediated anti-tumor immune responses. Hence, studies performed in axenic (gnotobiotic) or broad-spectrum antibiotic-treated mice have supported a cause–effect relationship between dysbiosis and the failure of anticancer therapeutics.[20–22,25,27] Similar retrospective and prospective studies in advanced cancer patients across a diverse range of malignancies and geographic locations revealed that antibiotic treatment before anticancer therapeutics dampens the clinical efficacy of ICBs and immunogenic chemotherapy, highlighting that the disruption of a homeostatic microbiome (i.e. a switch from eubiosis to dysbiosis) and the loss of specific bacterial species may be detrimental for the success of anticancer therapies.[27–34] In line with this notion, Derosa et al. European Urology (In press, DOI : 10.1016/j.eururo.2020.04.044) showed that antibiotics prior to immune checkpoint inhibitors had a deleterious clinical impact, reduce the micobiome diversity and increase *Clostridium hathewayi* bacteria associated with resistance.

In addition, the functional properties of the intestinal microbiota can be studied by transferring the entire fecal microbiota from patients to axenic or antibiotic-pretreated recipient mice, a process that is called fecal microbial transplantation (FMT). Experiments in mice have established robust cause–effect relationships between the composition of the gut microbiota and the therapeutic outcome of immune-based anticancer therapeutics. Indeed, the phenotype of patients (that are either clinical responders or non-responders to ICBs) can be recapitulated in mice through FMT, demonstrating that the fecal material derived from cancer patient drives the capacity to respond to ICBs. In line with these observations, such mice can be considered to serve as 'avatars' of the patient-derived microbiota.[22,24,26,27]

Recent advances in sequencing methods studying the composition of the intestinal microbiota have ameliorated our capacity to unravel correlations between specific gut microbiota fingerprints and the onset or course of pathological processes.[35] Accordingly, exploration of the composition of the gut microbiota in cancer patients through 16 S rRNA sequencing or quantitative metagenomics has demonstrated a major impact of the gut microbiota on the clinical activity of immune checkpoint inhibitors. Indeed, a triad of papers published in 2018 in *Science* support the notion that the composition of the gut microbiota modulates the response to immunotherapy with PD-1 or PD-L1 blocking antibodies, including non-small cell lung cancer, renal cell carcinoma and melanoma.[24,26,27] In all three studies, independent patient populations were subjected to fecal microbial analyses, leading to the identification of bacterial entities that positively correlate with the clinical outcome according to the response evaluation criteria in solid tumors criteria (RECIST). This methodology has led to the identification of several bacterial species that favor anticancer immunosurveillance. Accordingly, transfer of defined bacterial species is capable of restoring responses in a variety of preclinical tumor models. For example, supplementation with *Akkermansia muciniphila* or *Enterococcus hirae*, two species that are associated with a favorable clinical outcome of PD1 blockade in NSCLC patients, reestablish the capacity of the murine immune system to mediate ICBs-stimulated anticancer responses in tumor-bearing avatar mice.[27] Additional studies have demonstrated the capacity of several other bacterial species such as *Bacteroides fragilis, Bifidobacterium longum, Barnesiella intestinihomins* or *Alistipes shahii* to support anticancer immunity by activating dendritic cells (DCs), by stimulating the production of interleukin-12 (IL-12) by DCs, by enhancing recruitment of tumor-specific cytotoxic T lymphocytes (CTLs), by triggering the production of interferon-γ (IFNγ) by tumor-infiltrating γδ T cells or by elevating the production of TNFα by intratumoural myeloid cells.[21–23,25]

Importantly, memory T cell responses associated with IFNγ production by peripheral blood $CD4^+$ and $CD8^+$ T cells upon co-culture with *A. muciniphila or E. hirae*-pulsed peripheral blood autologous monocytes were stronger in patients with NSCLC or RCC who responded to ICBs than in non-responders and predicted longer progression-free survival (PFS) in these cohorts of patients.[21,27] Interestingly, such an immune reactivity against *E. hirae* or *B. longum* also correlates with robust $CD8^+$ T cell responses and better prognosis in HBV-related hepatocellular carcinoma,[36] potentially highlighting the clinical relevance of these particular strains across different malignancies.

Therefore, accumulating evidence brings to light that modulating the composition of the gut microbiota and harnessing the immunogenicity of the intestinal microbiome may become promising strategies to circumvent primary resistance to anticancer therapeutics (Figure 1).

The gut microbiome as a tool to modulate the clinical outcome of anticancer therapeutics

William Coley has been the first to develop a combination of microbial products including *Streptococcus pyogenes* and *Serratia marcescens* which has proven antitumoral efficacy.[37] Following this pioneering discovery, multiple attempts have been launched to use microbial agents for anticancer therapy. Since 1990, *Mycobacterium bovis* Bacille Calmette Guérin (BCG) has been approved by both FDA- and EMA for the treatment of noninvasive bladder cancer.[38] BCG induces a local immune response against residual cancer cells that largely reduces the probability of relapse.[39,40] No other bacteria have reached clinical approval by the regulatory agencies so far.

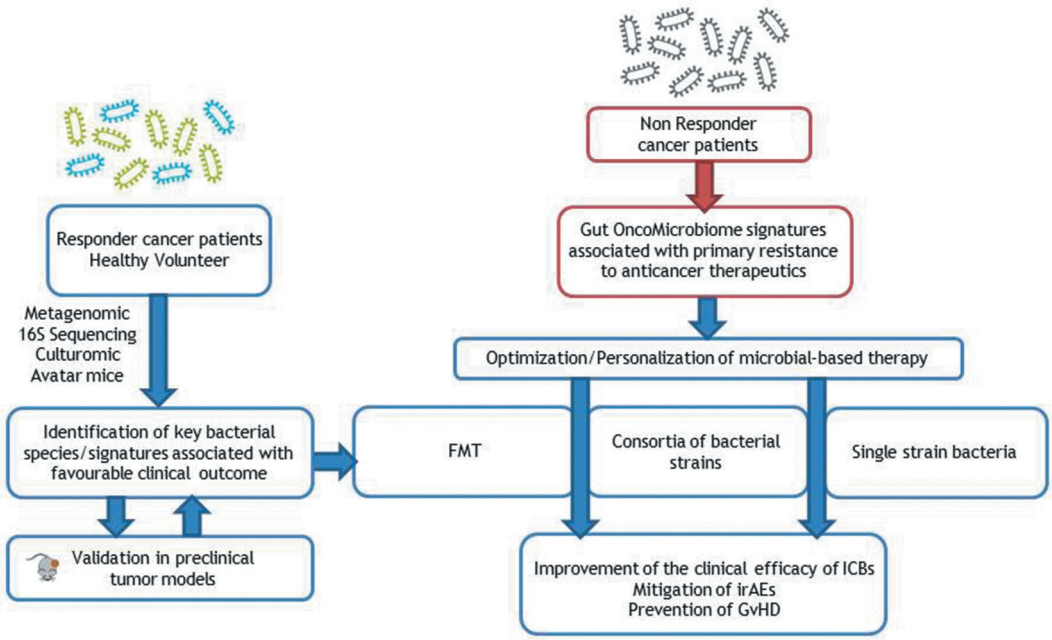

Figure 1. Therapeutic strategy involving microbial products to circumvent primary resistance to anticancer treatments. Preliminary data suggest the capacity of gut onco-microbiome signatures to predict the clinical outcome of anticancer treatments. Data from these analyses can inform on the microbiota in cancer patients and indicate which bacterial genera or species could be beneficial to patients. The identification, cultivation and functional characterization of cancer-relevant microbial species through an extensive preclinical validation process will be crucial for optimizing the process. The development of diagnostic tools predicting the response of a particular cancer patient to a particular microbial product will allow to personalize therapeutic interventions, that will rely on fecal microbial transplantation (FMT), consortia of bacterial strains or single-strain bacteria. The outcome of microbial interventions can be evaluated by monitoring the clinical efficacy of anticancer treatments as well as the incidence of immune-related adverse effects (irAEs) or signs of graft-versus-host disease (GvHD).

Although the development of anticancer agents based on live microbial agents has traditionally focused on parenteral (local or systemic) administration, investigators have recently been considering to orally administer live bacteria to safely boost the clinical efficacy of anticancer treatments or diminish toxicities associated to these therapeutics. As such, interventional approaches aiming at modulating the composition of the gut microbiota are under development and include fecal microbiota transplantation (FMT), antibiotic regimens, prebiotic and/or probiotic formulations, or other types of drug (such as the diabetes drug metformin) and dietary-based interventions, such as caloric restriction.[41]

To date, multiple clinical trials (www.ClinicalTrials.gov) are investigating the antitumoral potential of live biotherapeutic products (LBPs), ie FMT, single strain bacteria or bacterial consortia (Table 1). The majority of these clinical trials focus on cancer patients that previously failed immunotherapy. As such, the capacity of microbial products to convert non-responders cancer patients into responders is under evaluation (NCT03341143/NCT03353402/NCT04116775/NCT04130763/NCT04264975/NCT03637803/NCT03595683/NCT03775850). In parallel, strategies aiming at boosting the objective response rate to ICBs in ICBs-naïve patients are investigated (NCT04056026/NCT03686202/NCT03595683/NCT03817125/NCT04208958). Several clinical trials are testing, in the neoadjuvant setting, the capacity of LBPs to modulate the tumor microenvironment before tumor resection (NCT04139993/NCT03934827/NCT04193904). Other clinical trials are exploring the capacity of FMT to mitigate ICBs induced-diarrhea or colitis in cancer patients, therefore allowing to uncouple efficacy from toxicity (NCT04038619/NCT04040712/NCT04163289/NCT03819296). Last but not least, the capacity of the gut microbiota to circumvent corticosteroid-resistant acute graft-versus-host disease (GvHD) in hematologic malignancies is under investigation (NCT03812705/NCT02928523/NCT03678493/NCT03359980). Safety, engraftment of the microbial product, monitoring of the immune compartments as well improved objective response rates and survival are generally the main endpoints of such trials. The website ClinicalTrials.gov informs on multiple clinical trials that are either ongoing or completed, yet generally lack published information on the outcome (Table 1)

First lines of evidence

FMT has been the first strategy to enter clinical evaluation. FMT involves the transfer of lyophilized and encapsulated feces from donor, orally or rectally. This intervention has been demonstrated to be effective in other therapeutic areas such as the management of refractory *Clostridium difficile* infection.[42] Two clinical trials (NCT03341143 and NCT03353402) investigate the capacity of the gut microbiota to rescue the clinical efficacy of ICBs in metastatic melanoma patients who failed previously immunotherapy. Patients with metastatic melanoma who achieved a durable complete response to treatment are serving as FMT donors. The first Phase II clinical trial (NCT03341143) has enrolled 12 patients in which 8 patients were evaluable. Among them, one patient exhibited a complete response (CR), another one a partial response (PR), two patients stable disease (SD) while the therapeutic intervention was ineffective in the remaining four cancer patients

Table 1. Clinical trials employing microbial products for cancer therapy. AFMT: autologous fecal microbial transplantation; GvHD: graft-versus-host disease; ICBs: immune checkpoint blockers; irAE: immune-related adverse event; MDRB: multi-drug resistant bacteria; ORR: objective response rate; OS: overall survival; PFS: progression-free Survival; PSA: prostate-specific antigen; RRR: radiographic response rate; TKI: tyrosine kinase inhibitor.

Microbial intervention	Sponsor	NCT	Therapeutic intervention	Cancer type	Phase	Primary Endpoint	Secondary Endpoint
Clinical Trials investigating the capacity of the gut microbiota to boost ICBs efficacy							
FMT	Zarour, Hassane	NCT03341143	Pembrolizumab	Melanoma	II	ORR	Immune biomarkers
FMT	Sheba Medical Center	NCT03353402	PD-1 inhibitor	Melanoma	I	Safety, engraftment	ORR, immune biomarkers
FMT	Lawson Health Research Institute	NCT03772899	Pembrolizumab/ Nivolumab	Melanoma	I	Safety	ORR, microbiome, metabolome, blood biomarkers
FMT	Julie Graff	NCT04116775	Pembrolizumab	Prostate	II	PSA	RRR, PFS, OS
FMT	Peking University	NCT04130763	PD-1 inhibitor	Gastrointestinal System Cancer	I	ORR, Safety	Immune biomarkers
FMT	Asan Medical Center	NCT04264975	Immunotherapy	Solid carcinoma	Not Applicable	ORR	
FMT	ProgenaBiome	NCT04056026	Keytruda	Mesothelioma	I	PFS	
Consortia	University Health Network, Toronto	NCT03686202	PD-1/PD-L1 inhibitor	Solid tumors	I	Safety, engraftment	ORR, PFS, microbiome, immune biomarkers
Single strain	4D pharma plc	NCT03637803	Pembrolizumab	Solid tumors	I/II	Safety, Tolerability, Clinical befinit	Immune biomarkers
Single strain	University of Chicago	NCT03595683	Pembrolizumab	Melanoma	II	Response rate, adverse events	PFS
Consortia	Parker Institute for Cancer Immunotherapy	NCT03817125	Nivolumab	Melanoma	I	Adverse events	Engraftment, ORR, PFS, OS, immune biomarkers
Consortia	Vedanta Biosciences, Inc.	NCT04208958	Nivolumab	Selected types of advanced or metastatic cancer	I/II	Safety, Tolerability, Clinical befinit	Engraftment, PFS, OS, duration of response
Single strain	Evelo Biosciences, Inc	NCT03775850	Pembrolizumab	Selected types of advanced or metastatic cancer	I/II	Safety, Tolerability, ORR	PFS, OS
Clinical Trials investigating the capacity of the gut microbiota to modulate the tumor microenvironment before tumor resection							
FMT	Mayo Clinic	NCT04139993	X	Operable Stage I–III Breast Cancer	I	Safety	Engraftment, immune biomarkers
Single strain	Imperial College London	NCT03934827	X	Operable solid tumors	I	Safety, tolerability	OS, immune biomarkers
Single strain	4D pharma plc	NCT04193904	Radiation	Pancreatic	I	Safety	Immune biomarkers, OS, PFS
Clinical Trials investigating the capacity of the gut microbiota to mitigate anticancer treatments-related colitis							
FMT	M.D. Anderson Cancer Center	NCT04038619	ICBs	Genitourinary Cancer Patients	I	Safety, tolerability, efficacy	Recurrence rate
FMT	Catholic University of the Sacred Heart	NCT04040712	TKI	Renal Cell carcinoma	Not Applicable	Rate of patients with diarrhea	Rate of patients who need to stop/ reduce TKI
FMT	Lawson Health Research Institute	NCT04163289	ICBs	Renal Cell carcinoma	I	Occurence of immune-related colitis	irAE, ORR
FMT	M.D. Anderson Cancer Center	NCT03819296	ICBs	Melanoma	I	Incidence of adverse events, Toxicity	
Clinical Trials investigating the capacity of the gut microbiota to circumvent corticosteroid-resistant acute GvHD in hematologic malignancies							
FMT	Shanghai General Hospital, Shanghai Jiao Tong University School of Medicine	NCT03812705	X	Hematopoietic and Lymphoid Cell Neoplasm	II	Response Rate	Time to response, duration of response
FMT	Maat Pharma	NCT02928523	X	Acute Myeloid Leukemia	I/II	AFMT efficacy, eradication of MDRB	
FMT	Masonic Cancer Center, University of Minnesota	NCT03678493	X	Acute Myeloid Leukemia	II	Incidence of infections	Engraftment, incidence of GvHD
FMT	MaaT Pharma	NCT03359980	X	Steroid Refractory, Gastrointestinal, Acute GvHD	II	Efficacy	Safety, MDRB, incidence of GvHD
FMT	Massachusetts General Hospital	NCT02733744	X	Bone Marrow Transplantation	I	Feasability	OS, PFS, incidence of GvHD

(https://www.umms.org/sjmc/-/media/files/um-sjmc/health-services/cancer-institute/symposium-powerpoints/role-of-commensal-microdata-in-carcinogenesis-and-cancer-therapy.pdf?upd=20191120214445&la=en&hash=EA5670957E87F12C6434A047801E4E0C632910B6). In the second Phase I clinical trial (NCT03353402), three out of five patients had a partial or complete response to treatment post-FMT. Interestingly, immunohistochemical staining of biopsies demonstrated an infiltration of the melanoma by antigen presenting cells ($CD68^{+}$) as well as intra-tumoral CD8 + T-cell post-FMT.[43] Although too preliminary to draw any definitive conclusions, these two trials represent the first clinical evidence that the gut microbiota may have an impact on antitumor immunity and potentially even responses to ICBs.

Another strategy consists in providing lyophilized and encapsulated single strain bacteria for oral delivery to cancer patients. A Phase I/II clinical trial (NCT03637803) investigates the safety and efficacy of MRx0518, a bacterial strain of *Enterococcus gallinarum*, in combination with KEYTRUDA® in cancer patients with solid tumors and advanced malignancies who have progressed on PD-1/PD-L1 inhibitors. Two out of six cancer patients displayed a partial response with evidence of increased tumor infiltrating lymphocytes (https://www.londonstockexchange.com/exchange/news/market-news/market-news-detail/DDDD/14295955.html). In contrast, a clinical trial (NCT03775850) investigating the capacity of *Bifidobacterium longum* (EDP1503) to boost the efficacy of pembrolizumab in microsatellite stable colorectal cancer who had previously failed all therapies did not observe any formal clinical responses, atlhough some patients manifested extended stable disease (https://evelobio.com/portfolio/).

It is also well documented that GvHD following hematopoietic stem cell transplantation has a high mortality rate. Therapeutic options such as steroids are not always efficient, indicating that GvHD remains an unmet medical need. The role of the gut microbiota in the development of GvHD has been extensively elucidated.[44–46] The first pilot clinical study (NCT02733744) exploring the administration of oral FMT capsules to patients early after allo-HCT has unraveled the feasibility, safety and efficiency of this procedure.[47] FMT was associated with an increase in recipient microbiome diversity, while the Kaplan-Meier estimates for 12-month OS post- FMT increased to 85%. [47] An additional study (NCT03359980) has released preliminary data demonstrating that 3 out 8 steroid-refractory intestinal acute GvHD attained a complete response post-FMT (external presentation, ASH2019).

ICBs can induce immune-related adverse effects (irAEs) in some cancer patients such as colitis.[48,49] Two patients experiencing refractory ICI-associated colitis were treated with FMT from healthy donors. They exhibited a complete resolution of colitis-associated clinical symptoms, demonstrating that the gut microbiome is a potent tool for improving immune checkpoint inhibitors-associated irAEs.[50]

Outlook

Preliminary data suggest that the gut microbiota can safely boost the clinical efficacy of anticancer therapeutics and favor the success of hematopoietic stem cell transplantation (HSCT) in cancer patients (Figure 1). Interestingly, FMT-based clinical trials revealed that the efficacy of transplantion is donor-dependent, demonstrating the clinical relevance of specific gut microbiota signatures and raising the question as to how identify optimal donors. Several investigators are recruiting FMT donors among cancer patients who previously responded to their treatments while others are preferring healthy volunteers. The identification and functional characterization of the key bacterial species driving the favorable clinical outcome of ICBs seem crucial. In addition, FDA has issued safety alerts after the death of patients receiving FMT for *Clostridium difficile* infection that developed infections caused by enteropathogenic bacteria contained in the FMT. Besides, the current SARS-CoV-2 pandemics instructs us that harmful viruses for humans that have not only a tropism for the lung tissues but also for the intestinal epithelium might jeopardize the long term future of these "allogeneic FMT" based-approaches. Screening for covid in FMT trial will become mandatory. Furthermore, in one case, the obese phenotype has been transferred from a donor to a recipient,[51] calling for guidelines to exclude donors with any kind of pathology (including obesity) from the clinical protocols. Beyond obvious hygiene-related practicalities, challenges to FMT include the selection of optimal donors and the provision of sufficient material to enable long-term, repeated treatment of multiple patients.[52] Beyond these headlines, whether the donor FMT should exhibit short (within the first 3 months of ICBs) or long term (>18 months) persistance and "colonize" the recipient intestines remains an open conundrum in oncology. Finally, the necessity of a concomitant nutritional intervention or a prebiotic usage in conjunction with FMT will have to be evaluated in second generation trials.

Clinical trials will presumably establish the therapeutic impact of the gut microbiota in this scenario. However, it appears that only a subset of patients benefits from these innovative anticancer therapies, either because the recipient exhibits a primary resistance to ICBs independent from intestinal dysbiosis or because the donor FMT has not provided the appropriate set of microbes to this recipient host. Hence, there is an urgent need for suitable, robust and reliable diagnostics tools to fully identify and functionally characterize the minimalist commensal ecosystems relevant to cancer, in order to prospectively validate cancer-associated gut microbiome fingerprints of high clinical relevance. Several european (such as ONCOBIOME: https://www.oncobiome.eu/) and international consortia are currently developing "Gut Oncomicrobiome Signatures" (GOMS) across various malignancies and geographical locations (and other confounding factors), that will eventually become part of the oncological arsenal for the optimization and personalization of therapy in the future. Another issue is the capacity to manufacture microbial products at an industrial scale and with consistent levels of quality. Last but not least, it is unlikely that "one size will fit all cancer types and anticancer therapeutics". Preclinical studies that precisely define the mechanisms of action of microbial products are therefore crucial to adapt the use of microbial products to cancer patients.

Concluding remarks

As such, the gut microbiota appears to dictate the clinical efficacy of anticancer therapeutics. Without doubt, the intestinal microbiota is one of the parameters modulating the cancer immune set-point and whose therapeutic manipulation has to be incorported into the oncological arsenal. Multiple clinical trials are on the verge to be launched in this moving area. We expect the confirmation that FMT constitutes a viable and safe procedure on cancer during this calendar year. Moreover, it can be anticipated that technologies to expand the donor microbiota in bioreactors will be developed to standardize the process and provide unlimited amounts of material for FMT. As an alternative, microbially defined bacterial communities (oligoclonal consortia) or single bacterial strains (monoclonal therapies) will be developed for prolonged therapeutic interventions in cancer patients (Figure 1).

Acknowledgments

GK and LZ are supported by the Ligue contre le Cancer (équipe labellisée); Agence National de la Recherche (ANR) – Projets blancs; ANR under the frame of E-Rare-2, the ERA-Net for Research on Rare Diseases; AMMICa US23/CNRS UMS3655; Association pour la recherche sur le cancer (ARC); Association "Le Cancer du Sein, Parlons-en!"; Cancéropôle Ile-de-France; Chancelerie des universités de Paris (Legs Poix), Fondation pour la Recherche Médicale (FRM); a donation by Elior; European Research Area Network on Cardiovascular Diseases (ERA-CVD, MINOTAUR); Gustave Roussy Odyssea, the European Union Horizon 2020 Project Oncobiome; Fondation Carrefour; High-end Foreign Expert Program in China (GDW20171100085), Institut National du Cancer (INCa); Inserm (HTE); Institut Universitaire de France; LeDucq Foundation; the LabEx Immuno-Oncology (ANR-18-IDEX-0001); the RHU Torino Lumière; the Seerave Foundation; the SIRIC Stratified Oncology Cell DNA Repair and Tumor Immune Elimination (SOCRATE); and the SIRIC Cancer Research and Personalized Medicine (CARPEM). We thank Laura Lauden for coordinating the Oncobiome consortium.

ORCID

Guido Kroemer http://orcid.org/0000-0002-9334-4405
Laurence Zitvogel http://orcid.org/0000-0003-1596-0998

Conflicts of interest

RD is a full-time employee of everImmune, a biotech company dedicated to immunostimulatory bacteria. RD, GK and LZ are the scientific cofounders of everImmune. BR is on the scientific board of Vedanta.

References

1. Pardoll D. Cancer and the immune system: basic concepts and targets for intervention. Semin Oncol. 2015;42(4):523–538. doi:10.1053/j.seminoncol.2015.05.003.
2. Robert C, Thomas L, Bondarenko I, O'Day S, Weber J, Garbe C, Lebbe C, Baurain J-F, Testori A, Grob -J-J, et al. Ipilimumab plus dacarbazine for previously untreated metastatic melanoma. New England Journal of Medicine. 2011;364(26):2517–2526. doi:10.1056/NEJMoa1104621.
3. Borghaei H, Paz-Ares L, Horn L, Spigel DR, Steins M, Ready NE, Chow LQ, Vokes EE, Felip E, Holgado E, et al. Nivolumab versus docetaxel in advanced nonsquamous non–small-cell lung cancer. New England Journal of Medicine. 2015;373(17):1627–1639. doi:10.1056/NEJMoa1507643.
4. Ansell SM, Lesokhin AM, Borrello I, Halwani A, Scott EC, Gutierrez M, Schuster SJ, Millenson MM, Cattry D, Freeman GJ, et al. PD-1 blockade with nivolumab in relapsed or refractory hodgkin's lymphoma. New England Journal of Medicine. 2015;372(4):311–319. doi:10.1056/NEJMoa1411087.
5. Topalian SL, Hodi FS, Brahmer JR, Gettinger SN, Smith DC, McDermott DF, Powderly JD, Carvajal RD, Sosman JA, Atkins MB, et al. Safety, activity, and immune correlates of anti–PD-1 antibody in cancer. New England Journal of Medicine. 2012;366(26):2443–2454. doi:10.1056/NEJMoa1200690.
6. Ribas A, Hamid O, Daud A, Hodi FS, Wolchok JD, Kefford R, Joshua AM, Patnaik A, Hwu W-J, Weber JS, et al. Association of pembrolizumab with tumor response and survival among patients with advanced melanoma. JAMA. 2016;315(15):1600–1609. doi:10.1001/jama.2016.4059.
7. Motzer RJ, Escudier B, McDermott DF, George S, Hammers HJ, Srinivas S, Tykodi SS, Sosman JA, Procopio G, Plimack ER, et al. Nivolumab versus everolimus in advanced renal-cell carcinoma. New England Journal of Medicine. 2015;373(19):1803–1813. doi:10.1056/NEJMoa1510665.
8. Hodi FS, O'Day SJ, McDermott DF, Weber RW, Sosman JA, Haanen JB, Gonzalez R, Robert C, Schadendorf D, Hassel JC, et al. Improved survival with ipilimumab in patients with metastatic melanoma. New England Journal of Medicine. 2010;363(8):711–723. doi:10.1056/NEJMoa1003466.
9. Yu JX, Hubbard-Lucey VM, Tang J. Immuno-oncology drug development goes global. Nat Rev Drug Discov. 2019;18(12):899–900. doi:10.1038/d41573-019-00167-9.
10. Chen DS, Mellman I. Elements of cancer immunity and the cancer-immune set point. Nature. 2017;541(7637):321–330. doi:10.1038/nature21349.
11. Sharma P, Hu-Lieskovan S, Wargo JA, Primary RA. Adaptive, and acquired resistance to cancer immunotherapy. Cell. 2017;168(4):707–723. doi:10.1016/j.cell.2017.01.017.
12. Zitvogel L, Ma Y, Raoult D, Kroemer G, Gajewski TF. The microbiome in cancer immunotherapy: diagnostic tools and therapeutic strategies. Science. 2018;359(6382):1366–1370. doi:10.1126/science.aar6918.
13. Routy B, Gopalakrishnan V, Daillère R, Zitvogel L, Wargo JA, Kroemer G. The gut microbiota influences anticancer immunosurveillance and general health. Nat Rev Clin Oncol. 2018;15(6):382–396. doi:10.1038/s41571-018-0006-2.
14. Kroemer G, Zitvogel L. Cancer immunotherapy in 2017: the breakthrough of the microbiota. Nature Reviews Immunology. 2018;18(2):87–88. doi:10.1038/nri.2018.4.
15. Zitvogel L, Daillère R, Roberti MP, Routy B, Kroemer G. Anticancer effects of the microbiome and its products. Nature Reviews Microbiology. 2017;15(8):465–478. doi:10.1038/nrmicro.2017.44.
16. Maynard CL, Elson CO, Hatton RD, Weaver CT. Reciprocal interactions of the intestinal microbiota and immune system. Nature. 2012;489(7415):231–241. doi:10.1038/nature11551.
17. Bäckhed F. Host-bacterial mutualism in the human intestine. Science. 2005;307(5717):1915–1920. doi:10.1126/science.1104816.
18. Kamada N, Chen GY, Inohara N, Núñez G. Control of pathogens and pathobionts by the gut microbiota. Nature Immunology. 2013;14(7):685–690. doi:10.1038/ni.2608.
19. de Vos WM, de Vos EAJ. Role of the intestinal microbiome in health and disease: from correlation to causation. Nutrition Reviews. 2012;70(Suppl 1):S45–56. doi:10.1111/j.1753-4887.2012.00505.x.
20. Viaud S, Saccheri F, Mignot G, Yamazaki T, Daillère R, Hannani D, Enot DP, Pfirschke C, Engblom C, Pittet MJ, et al. The intestinal microbiota modulates the anticancer immune effects of cyclophosphamide. Science. 2013;342(6161):971–976. doi:10.1126/science.1240537.
21. Daillère R, Vétizou M, Waldschmitt N, Yamazaki T, Isnard C, Poirier-Colame V, Duong CM, Flament C, Lepage P,

Roberti MP, et al. Enterococcus hirae and barnesiella intestinihominis facilitate cyclophosphamide-induced therapeutic immunomodulatory effects. Immunity. 2016;45(4):931–943. doi:10.1016/j.immuni.2016.09.009.
22. Vétizou M, Pitt JM, Daillère R, Lepage P, Waldschmitt N, Flament C, Rusakiewicz S, Routy B, Roberti MP, Duong CPM, et al. Anticancer immunotherapy by CTLA-4 blockade relies on the gut microbiota. Science. 2015;350(6264):1079–1084. doi:10.1126/science.aad1329.
23. Sivan A, Corrales L, Hubert N, Williams JB, Aquino-Michaels K, Earley ZM, Benyamin FW, Lei YM, Jabri B, Alegre M-L, et al. Commensal Bifidobacterium promotes antitumor immunity and facilitates anti-PD-L1 efficacy. Science. 2015;350(6264):1084–1089. doi:10.1126/science.aac4255.
24. Matson V, Fessler J, Bao R, Chongsuwat T, Zha Y, Alegre M-L, Luke JJ, Gajewski TF. The commensal microbiome is associated with anti–PD-1 efficacy in metastatic melanoma patients. Science. 2018;359(6371):104–108. doi:10.1126/science.aao3290.
25. Iida N, Dzutsev A, Stewart CA, Smith L, Bouladoux N, Weingarten RA, Molina DA, Salcedo R, Back T, Cramer S, et al. Commensal bacteria control cancer response to therapy by modulating the tumor microenvironment. Science. 2013;342(6161):967–970. doi:10.1126/science.1240527.
26. Gopalakrishnan V, Spencer CN, Nezi L, Reuben A, Andrews MC, Karpinets TV, Prieto PA, Vicente D, Hoffman K, Wei SC, et al. Gut microbiome modulates response to anti–PD-1 immunotherapy in melanoma patients. Science. 2018;359(6371):97–103. doi:10.1126/science.aan4236.
27. Routy B, Le Chatelier E, Derosa L, Duong CPM, Alou MT, Daillère R, Fluckiger A, Messaoudene M, Rauber C, Roberti MP, et al. Gut microbiome influences efficacy of PD-1–based immunotherapy against epithelial tumors. Science. 2018;359(6371):91–97. doi:10.1126/science.aan3706.
28. Derosa L, Hellmann MD, Spaziano M, Halpenny D, Fidelle M, Rizvi H, Long N, Plodkowski AJ, Arbour KC, Chaft JE, et al. Negative association of antibiotics on clinical activity of immune checkpoint inhibitors in patients with advanced renal cell and non-small-cell lung cancer. Annals of Oncology. 2018;29(6):1437–1444. doi:10.1093/annonc/mdy103.
29. Pinato DJ, Howlett S, Ottaviani D, Urus H, Patel A, Mineo T, Brock C, Power D, Hatcher O, Falconer A et al. Association of prior antibiotic treatment with survival and response to immune checkpoint inhibitor therapy in patients with cancer. JAMA Oncology. 2019 Sep 12;5(12):1774. doi:10.1001/jamaoncol.2019.2785.
30. Elkrief A, El Raichani L, Richard C, Messaoudene M, Belkaid W, Malo J, Belanger K, Miller W, Jamal R, Letarte N, et al. Antibiotics are associated with decreased progression-free survival of advanced melanoma patients treated with immune checkpoint inhibitors. Oncoimmunology. 2019;8(4):e1568812. doi:10.1080/2162402X.2019.1568812.
31. Zhao S, Gao G, Li W, Li X, Zhao C, Jiang T, Jia Y, He Y, Li A, Su C, et al. Antibiotics are associated with attenuated efficacy of anti-PD-1/PD-L1 therapies in Chinese patients with advanced non-small cell lung cancer. Lung Cancer. 2019;130:10–17. doi:10.1016/j.lungcan.2019.01.017.
32. Lalani AKA, Xie W, Braun DA, Kaymakcalan M, Bossé D, Steinharter JA, Martini DJ, Simantov R, Lin X, Wei XX, et al. Effect of antibiotic use on outcomes with systemic therapies in metastatic renal cell carcinoma. Eur Urol Oncol. 2019 Sep 24. doi:10.1016/j.euo.2019.09.001.
33. Pflug N, Kluth S, Vehreschild JJ, Bahlo J, Tacke D, Biehl L, Eichhorst B, Fischer K, Cramer P, Fink A-M, et al. Efficacy of antineoplastic treatment is associated with the use of antibiotics that modulate intestinal microbiota. Oncoimmunology. 2016;5(6):6. doi:10.1080/2162402X.2016.1150399.
34. Nenclares P, Bhide SA, Sandoval-Insausti H, Pialat P, Gunn L, Melcher A, Newbold K, Nutting CM, Harrington KJ; Nenclares P, Bhide SA, Sandoval-Insausti H, Pialat P, Gunn L, Melcher A, Newbold K, Nutting CM, Harrington KJ. Impact of antibiotic use during curative treatment of locally advanced head and neck cancers with chemotherapy and radiotherapy. European Journal of Cancer. 2020;131:9–15. doi:10.1016/j.ejca.2020.02.047.
35. Claesson MJ, Clooney AG, O'Toole PW. A clinician's guide to microbiome analysis. Nature Reviews Gastroenterology & Hepatology. 2017;14(10):585–595. doi:10.1038/nrgastro.2017.97.
36. Rong Y, Dong Z, Hong Z, Jin Y, Zhang W, Zhang B, Mao W, Kong H, Wang C, Yang B, et al. Reactivity toward Bifidobacterium longum and Enterococcus hirae demonstrate robust CD8+ T cell response and better prognosis in HBV-related hepatocellular carcinoma. Experimental Cell Research. 2017;358(2):352–359. doi:10.1016/j.yexcr.2017.07.009.
37. Nauts HC, Swift WE, Coley BL. The treatment of malignant tumors by bacterial toxins as developed by the late William B. Coley, M.D., reviewed in the light of modern research. Cancer Res. 1946;6:205–216.
38. Zbar B, Tanaka T. Immunotherapy of cancer: regression of tumors after intralesional injection of living Mycobacterium bovis. Science. 1971;172(3980):271–273. doi:10.1126/science.172.3980.271.
39. Böhle A, Brandau S. Immune mechanisms in bacillus calmette-Guerin immunotherapy for superficial bladder cancer. Journal of Urology. 2003;170(3):964–969. doi:10.1097/01.ju.0000073852.24341.4a.
40. Zbar B, Bernstein I, Tanaka T, Rapp HJ. Tumor immunity produced by the intradermal inoculation of living tumor cells and living Mycobacterium bovis (strain BCG). Science. 1970;170(3963):1217–1218. doi:10.1126/science.170.3963.1217.
41. Pietrocola F, Pol J, Vacchelli E, Rao S, Enot DP, Baracco EE, Levesque S, Castoldi F, Jacquelot N, Yamazaki T, et al. Caloric restriction mimetics enhance anticancer immunosurveillance. Cancer Cell. 2016;30(1):147–160. doi:10.1016/j.ccell.2016.05.016.
42. van Nood E, Vrieze A, Nieuwdorp M, Fuentes S, Zoetendal EG, de Vos WM, Visser CE, Kuijper EJ, Bartelsman JFWM, Tijssen JGP, et al. Duodenal infusion of donor feces for recurrent clostridium difficile. New England Journal of Medicine. 2013;368(5):407–415. doi:10.1056/NEJMoa1205037.
43. Youngster I, Baruch E, Katz L, Lahat A, Brosh-Nissimov T, Schachter J, Koren O, Markel G, Boursi B. 90. fecal microbiota transplantation in metastatic melanoma patients resistant to anti-PD-1 treatment. Open Forum Infectious Diseases. 2019;6(Suppl Supplement_2):S7. doi:10.1093/ofid/ofz359.014.
44. Taur Y, Coyte K, Schluter J, Robilotti E, Figueroa C, Gjonbalaj M, Littmann ER, Ling L, Miller L, Gyaltshen Y, et al. Reconstitution of the gut microbiota of antibiotic-treated patients by autologous fecal microbiota transplant. Sci Transl Med. 2018;10(460):460. doi:10.1126/scitranslmed.aap9489.
45. Peled JU, Devlin SM, Staffas A, Lumish M, Khanin R, Littmann ER, Ling L, Kosuri S, Maloy M, Slingerland JB; Peled JU, Devlin SM, Staffas A, Lumish M, Khanin R, Littmann ER, Ling L, Kosuri S, Maloy M, Slingerland JB, et al. Intestinal microbiota and relapse after hematopoietic-cell transplantation. J Clin Oncol. 2017;35(15):1650–1659. doi:10.1200/JCO.2016.70.3348.
46. Taur Y, Jenq RR, Perales M-A, Littmann ER, Morjaria S, Ling L, No D, Gobourne A, Viale A, Dahi PB; Taur Y, Jenq RR, Perales M-A, Littmann ER, Morjaria S, Ling L, No D, Gobourne A, Viale A, Dahi PB, et al. The effects of intestinal tract bacterial diversity on mortality following allogeneic hematopoietic stem cell transplantation. Blood. 2014;124(7):1174–1182. doi:10.1182/blood-2014-02-554725.
47. DeFilipp Z, Peled JU, Li S, Mahabamunuge J, Dagher Z, Slingerland AE, Del Rio C, Valles B, Kempner ME, Smith M, et al. Third-party fecal microbiota transplantation following allo-HCT reconstitutes microbiome diversity. Blood Advances. 2018;2(7):745–753. doi:10.1182/bloodadvances.2018017731.
48. Cramer P, Bresalier RS. Gastrointestinal and hepatic complications of immune checkpoint inhibitors. Curr Gastroenterol Rep. 2017;19(1):3. doi:10.1007/s11894-017-0540-6.
49. Michot JM, Bigenwald C, Champiat S, Collins M, Carbonnel F, Postel-Vinay S, Berdelou A, Varga A, Bahleda R, Hollebecque A, et al. Immune-related adverse events with immune checkpoint blockade: a comprehensive review.

European Journal of Cancer. 2016;54:139–148. doi:10.1016/j.ejca.2015.11.016.

50. Wang Y, Wiesnoski DH, Helmink BA, Gopalakrishnan V, Choi K, DuPont HL, Jiang Z-D, Abu-Sbeih H, Sanchez CA, Chang -C-C, et al. Fecal microbiota transplantation for refractory immune checkpoint inhibitor-associated colitis. Nat Med. 2018;24(12):1804–1808. doi:10.1038/s41591-018-0238-9.
51. Alang N, Kelly CR. Weight gain after fecal microbiota transplantation. Open Forum Infect Dis. 2015;2(1):1. doi:10.1093/ofid/ofv004.
52. Laffin M, Madsen KL. Fecal microbial transplantation in inflammatory bowel disease: a movement too big to be ignored. Clinical Pharmacology & Therapeutics. 2017;102(4):588–590. doi:10.1002/cpt.747.