ONCOIMMUNOLOGY
2018, VOL. 7, NO. 6, e1434468 (3 pages)
https://doi.org/10.1080/2162402X.2018.1434468

Taylor & Francis
Taylor & Francis Group

AUTHOR'S VIEW



# The intestinal microbiota determines the clinical efficacy of immune checkpoint blockers targeting PD-1/PD-L1

Lisa Derosa[a,b,c,d], Bertrand Routy[a,b,c,d,e,f], Guido Kroemer [g,h,i,j,k,l,m,n], and Laurence Zitvogel[a,b,c,d,e,f,g,h,i,j,k,l,m,n,o]

[a]Gustave Roussy Cancer Campus (GRCC), Villejuif, France; [b]Institut National de la Santé Et de la Recherche Medicale (INSERM), Villejuif, France; [c]Equipe Labellisée—Ligue Nationale contre le Cancer, Villejuif, France; [d]Univ. Paris-Sud, Université Paris-Saclay, Gustave Roussy, Villejuif, France; [e]Hematology-Oncology Division, Department of Medicine, Centre Hospitalier de l'Université de Montréal (CHUM), Montréal, Qc, Canada; [f]Centre de recherche du Centre hospitalier de l'Université de Montréal (CRCHUM), Montréal, Qc, Canada; [g]Metabolomics and Cell Biology Platforms, GRCC, Villejuif, France; [h]Paris Descartes University, Sorbonne Paris Cité, Paris, France; [i]Equipe 11 Labellisée—Ligue Nationale contre le Cancer, Centre de Recherche des Cordeliers, INSERM, Paris, France; [j]Pôle de Biologie, Hôpital Européen Georges Pompidou, Assistance Publique—Hôpitaux de Paris, Paris, France; [k]Institut National de la Santé et de la Recherche Médicale, Paris, France; [l]Pierre et Marie Curie University, Paris, France; [m]Pôle de Biologie, Hôpital Européen Georges Pompidou, Assistance Publique—Hôpitaux de Paris, Paris, France; [n]Department of Women's and Children's Health, Karolinska University Hospital, Stockholm, Sweden; [o]Center of Clinical Investigations in Biotherapies of Cancer (CICBT), Villejuif, France

**ABSTRACT**

Cancer immunotherapists have been searching for biomarkers predicting patient responses to PD-1/PD-L1 blockade in neoplastic cells as well as in the immune system. Now, accumulating evidence indicates that the composition of the intestinal microflora has a major impact on patient prognosis. Here, we enumerate the bacterial species that are associated with favorable outcome of immunotherapy.

**ARTICLE HISTORY**
Received 25 January 2018
Accepted 25 January 2018

**KEYWORDS**
Microbiota; immune checkpoint blockers; PD-1/PD-L1; NSCLC; RCC; Biomarkers; Inflammation and cancer; New target

## Main text (900 words max)

Immune checkpoint blockades (ICB) have revolutionized the therapeutic approach in cancer whereby, targeting the tumor microenvironment (TME) and reinvigorating the immune systems open a new era for immuno-oncologists. This innovative strategy has provided benefit in immunogenic cancers including melanoma[1] and renal cell carcinoma (RCC)[2] as well as malignancy considered non-immunogenic such as non–small cell lung cancer (NSCLC)[3,4] or mismatch-repair–deficient colorectal cancer.[5] However, ICB responses are heterogeneous, transient and a significant proportion of patients among all cancer types exhibit resistance. Consequently, large efforts are being dedicated to identify biomarkers that reliably predict clinical outcomes and avoid life-threatening immune related toxicity more specifically colitis and pneumonitis. Tumor and immune related biomarkers have been developed but lack robust sensitivity and specificity. Without taking into account the tumor heterogeneity, PD-L1-negative patients consistently experience strong response and not all PD-L1-positive tumors benefit from anti–PD-(L)1 therapy.

The unexpected link between the gut microbiota and ICB anti-tumor response has recently been unraveled. The role of antibiotics induced gut dysbiosis in mice highlighted the contribution of commensals *Bacteroides fragilis* on the anti-CTLA-4 mAb response.[6] In parallel, another group demonstrated the importance of *Bifidobacterium* in maturing intratumoral DC after anti-PDL-1 mAb.[7]

Recently, three independent groups, Routy et al., Gopalakrishnan et al., and Matson et al.[8-10] clearly demonstrated that the diversity and composition of the intestinal microbiota play a key role on response to cancer immunotherapy and might have a prognostic value in patients. Indeed, based on the composition of their intestinal microbiome, patients can be stratified in responders (R) versus non-responders (NR) to PD-1 blockade using RECIST1.1 criteria. Bacteria genera or species associated with favorable responses to anti-PD-1 are listed in Table 1.

Furthermore, our group showed that antibiotics (ATB) related dysbiosis impaired ICB efficacy in patients with various cancers. This negative impact was observed in patients with NSCLC, RCC or urothelial carcinoma who received ATB within 2 months before or 1 month after the first administration of anti-PD-(L)1 mAb. To corroborate the link between microbiota and response to ICB, we prospectively explored the gut microbiota composition of 100 NSCLC and RCC patients using whole genome shotgun sequencing (WGS) in collaboration with Institut national de la recherché agronomique (INRA). The commensal that was most significantly associated with favorable clinical outcome or progression-free survival superior than 3 months was

**CONTACT** Guido Kroemer Kroemer@orange.fr Centre de Recherche des Cordeliers, 15 rue de l'Ecole de Médecine, Paris 75006, France; Laurence Zitvogel Laurence.zitvogel@orange.fr

**Table 1.** Bacteria genera or species associated with favorable responses to immune checkpoint blockade targeting the PD-1 interaction (listed by alphabetic order).

| | Association with responder status in | | |
|---|---|---|---|
| Bacteria | Routy et al.[8] | Gopalakrishnan et al. [9] | Matson et al.[10] |
| *Akkermansia muciniphila* | Yes | No | Yes |
| *Alistipes indisctintus* | Yes | No | No |
| *Bifidobacterium spp.* | No | No | Yes |
| *Colinsiella aerofaciens* | No | No | Yes |
| *Clostridiales* | Yes | Yes | No |
| *Enterococcus fecium* | Yes | No | Yes |
| *Eubacterium spp.* | Yes | Yes | No |
| *Fecalibacterium* | No | Yes | No |
| *Firmicutes* | Yes | Yes | No |
| *Lactobacillus animalis* | No | No | Yes |
| *Parabacteroides merdae* | No | No | Yes |
| *Roseburia intestinalis* | No | No | Yes |
| *Ruminococcus spp.* | Yes | Yes | Yes |
| *Veillonella parvula* | No | No | Yes |

*Akkermansia muciniphila* in the whole cohort ($p = 0.028$) and excluding patients on ATB ($p = 0.007$). *A. muciniphila* was present in 69% of responders compared to 34% of non-responders ($p = 0.007$). This observation was confirmed in a validation cohort of 53 patients.[8]

To demonstrate the relevance of this clinical finding, we performed fecal microbiota transplantation (FMT) from feces of R patients or feces of NR patients in germ free or ATB-treated mice. Feces from patients with clinical response conveyed a stronger immune response against the tumor compared to feces from NR. Subsequently, we demonstrated that mice transplanted with NR feces patients and supplementation with *A. muciniphila* restored the anti-cancer activity of anti-PD-1 treatment.

Similar approaches were used to demonstrate that the anti-tumor effects of PD-1 in melanoma patients depend on distinct bacteria species.

Gopalakrishnan et al.[9] analyzed 43 fecal samples by 16SRNA gene sequencing, showing an enrichment of *Clostridiales, Ruminococcaceae* and *Faecalibacterium* in R to anti-PD-1 treatment and *Bacteroidales* in NR patients. Twenty-five samples from the same cohort were analyzed by WGS, confirming the enrichment of *Feacalibacterium spp.* in R patients. Matson et al.[10] collected 38 stool samples from melanoma patients on anti-PD-1 treatment and after 16S RNA sequencing and quantitative PCR analysis he identified *Bifidobacterium spp, Colinsella aerofaciens, Enterococcus faecium*, *Lactobacillus animalis, Parabacteroides merdae, Roseburia intestinalis and Veillonella parvula* as bacteria associated with beneficial response.

*Gopalakrishnan et al.*[9] *and Matson et al.*[10] also showed that FMT from R patients into germ-free mice improved tumor control and response to anti-PD-L1 treatment.

Unequivocally, these three studies highlighted the significance of distinct commensals in the favorable response of PD-1 against cancer types: *A. muciniphila* on NSCLC or RCC patients and *Fecalibacterium spp.* or *Bifidobacterium spp.* on melanoma patients. Of course, this variation may be linked to the cancer histology but others factors must be considered such as obesity, diet and methods used for bacterial identification. WGS improved the accuracy of species detection and has multiple advantages compared with the 16S RNA, including enhanced detection of diversity and bacterial species and increased prediction of genes. Although, updated databases are need to be developed considering new strains of same species or new species isolated by culturomics. Nevertheless, when analyzing in more details the identified bacteria, we observe commonality in the three R profiles namely: *Akkermansia muciniphila*, *Clostridiales, Enterococcus fecium, Eubacterium spp., Firmicutes* and *Ruminococcus spp.*

Converging data from these three studies also revealed a strong interaction between specific immunogenic bacteria and the systemic immune response. In NSCLC patients specific $CD4^+$ and $CD8^+$ T cells memory toward *A. muciniphila* predicted a longer progression-free survival. Whereas, in melanoma enrichement of *Faecalibacterium* was associated with a higher frequency of cytotoxic $CD8^+$ T cell infiltration in the tumor bed. Similarly, in mice intratumoral $CD8^+$ T cell infiltration post anti-PD-L1 treatment correlated with a favorable microbiota composition.

Therefore, it remains open corundum if a universal or an individual gut microbiota fingerprint is associated with immunotherapy response profile across various histological types. Irrespective of these remaining questions, targeting the microbiota as a novel ICB biomarker will most likely change the concept of immuno-oncology and push even further the frontiers of personalized medicine to improve patient care.

## Disclosure of potential conflicts of interest

No potential conflicts of interest were disclosed.

## Acknowledgments

LD and BR were supported by Gustave Roussy Course of Excellence in Oncology- Fondation Philanthropia. LZ and GK were supported by the Ligue contre le Cancer (équipe labelisée); Agence Nationale de la Recherche (ANR) – Projets blancs; ANR under the frame of E-Rare-2, the ERA-Net for Research on Rare Diseases; Association pour la recherche sur le cancer (ARC); Cancéropôle Ile-de-France; Institut National du Cancer (INCa); Institut Universitaire de France; Fondation pour la Recherche Médicale (FRM); a donation by Elior; the European Commission (ArtForce); the European Research Council (ERC); Fondation Carrefour; Institut National du Cancer (INCa); Inserm (HTE); Institut Universitaire de France; the LeDucq Foundation; the LabEx Immuno-Oncology; the RHU Torino Lumière; the Searave Foundation; the SIRIC Stratified Oncology Cell DNA Repair and Tumor Immune Elimination (SOCRATE); the SIRIC Cancer Research and Personalized Medicine (CARPEM); the Paris Alliance of Cancer Research Institutes (PACRI) and by philanthropia (Mrs E. Badinter and Mrs N. Meyer).

## ORCID

Guido Kroemer http://orcid.org/0000-0002-9334-4405

## References

1. 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;350:1079–84. doi:10.1126/science.aad1329. PMID:26541610.

2. 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. N Engl J Med. 2015;373:1803–13. doi:10.1056/NEJMoa1510665. PMID:26406148.
3. Carbone DP, Reck M, Paz-Ares L, Creelan B, Horn L, Steins M, Felip E, van den Heuvel MM, Ciuleanu T-E, Badin F, et al. First-line nivolumab in stage IV or recurrent non–small-cell lung cancer. N Engl J Med. 2017;376:2415–26. doi:10.1056/NEJMoa1613493. PMID:28636851.
4. 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. N Engl J Med. 2015;373:1627–39. doi:10.1056/NEJMoa1507643. PMID:26412456.
5. Le DT, Uram JN, Wang H, Bartlett BR, Kemberling H, Eyring AD, Skora AD, Luber BS, Azad NS, Laheru D, et al. PD-1 blockade in tumors with mismatch-repair deficiency. N Engl J Med. 2015;372:2509–20. doi:10.1056/NEJMoa1500596. PMID:26028255.
6. 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;350:1079–84. doi:10.1126/science.aad1329. PMID:26541610.
7. 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;350:1084–9. doi:10.1126/science.aac4255. PMID:26541606.
8. 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;359:91–7. doi:10.1126/science.aan3706. PMID:29097494.
9. 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;359:97–103. doi:10.1126/science.aan4236. PMID:29097493.
10. 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;359:104–8. doi:10.1126/science.aao3290. PMID:29302014.