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

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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.

Main text (900 words max)

Immune checkpoint blockades (ICB) have revolutionized the therapeutic approach in cancer whereby, targetting 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 and renal cell carcinoma (RCC) as well as malignancy considered non-immunogenic such as non–small cell lung cancer (NSCLC) or mismatch-repair-deficient colorectal cancer. 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. In parallel, another group demonstrated the importance of Bifidobacterium in maturing intratumoral DC after anti-PDL-1 mAb.

Recently, three independent groups, Routy et al., Gopalakrishnan et al., and Matson et al. 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 uterine 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 recherche agronomique (INRA). The commensal that was most significantly associated with favorable clinical outcome or progression-free survival superior than 3 months was...
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.\textsuperscript{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.\textsuperscript{9} analyzed 43 fecal samples by 16S RNA gene sequencing, showing an enrichment of Clostridiales, Ruminococaceae 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 Faecalibacterium spp. in R patients. Matson et al.\textsuperscript{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, Cloinserella aerofaciens, Enterococcus faecium, Lactobacillus rhamnosus, Parabacteroides merdae, Roseburia intestinalis and Veillonella parvula as bacteria associated with beneficial response.

Gopalakrishnan et al.\textsuperscript{9} and Matson et al.\textsuperscript{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 Faecalibacterium 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 fectium, Faecalibacterium 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\textsuperscript{+} and CD8\textsuperscript{+} T cells memory toward A. muciniphila predicted a longer progression-free survival. Whereas, in melanoma enrichment of Faecalibacterium was associated with a higher frequency of cytotoxic CD8\textsuperscript{+} T cell infiltration in the tumor bed. Similarly, in mice intratumoral CD8\textsuperscript{+} 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.

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