Enhancing the clinical coverage and anticancer efficacy of immune checkpoint blockade through manipulation of the gut microbiota

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ABSTRACT
Although anticancer therapy with immune checkpoint blockers has seen unprecedented success, it fails to control neoplasia in most patients and often causes immune-related adverse events (irAEs). Our recent research shows the immunostimulatory and antitumor effects of CTLA-4 blockade depend on distinct Bacteroides species of the gut microbiota, signifying novel approaches to improve such immunotherapies.

The equilibrium linking the intestinal microbiota, the intestinal epithelium and the host immune system determines host health and homeostasis. Perturbation of the mutualistic symbiosis between the intestinal microbiota and the host can result in chronic inflammatory and autoimmune immunopathologies. Previous studies from our group and others have suggested the constitution of the gut microbiota can also influence the immunostimulatory capacity of cancer therapies, particularly those that harness the anti-tumor immune response to mediate their efficacy.

In our latest research published in Science, we questioned whether the therapeutic efficacy of CTLA-4 blockade, as mediated by the FDA/EMEA-approved clinical immunotherapy ipilimumab, might also be determined by components of the intestinal microbiota. By comparing the efficacy of anti-CTLA-4 Ab against various established tumors in mice maintained under specific pathogen-free (SPF) and germ-free (GF) conditions, we identified that tumor progression was controlled in SPF but not in GF animals. The antitumor effects of CTLA-4-specific Ab were similarly compromised in mice treated with a broad-spectrum antibiotic cocktail to eliminate gut microbiota. In addition, anti-CTLA-4-mediated activation of tumor-infiltrating lymphocytes (TILs) and splenic lymphocytes was significantly reduced in GF and antibiotics-treated mice.

We next investigated anti-CTLA-4-mediated effects at the gut–microbiota interface, given that this is where microbiota alteration is initially sensed by the immune system and equally where blockade of CTLA-4 by ipilimumab often results in irAEs. We identified alteration of the mucosal barrier following administration of anti-CTLA-4 Ab in mice, consistent with a “subclinical colitis”. This was more prominent in SPF than in GF animals, suggesting a role for distinct commensals in this anti-CTLA-4 Ab-promoted pathology. Antibody treatment additionally increased the proliferation and the T cell-mediated apoptosis of intestinal epithelial cells (IECs). Using intestinal crypt-derived enteroid cultures, this apoptosis was found to be induced by intraepithelial lymphocytes (IELs) from anti-CTLA-4-treated (but not isotype-treated) mice, and to be dependent on the presence of microbial products. Coinciding with these mucosal alterations, FISH analysis revealed an accumulation of distinct Bacteroides spp. in the inner part of the mucus layer, potentially within reach of mucosal dendritic cells (DCs). Taken together, these findings signified that CTLA-4 Ab dysregulates the equilibrium between IECs, IELs and the microbiota at intestinal barriers (Fig. 1).

To identify potential changes in bacterial species caused by this dysregulation, we performed high-throughput pyrosequencing of 16S ribosomal RNA gene amplicons from murine feces. CTLA-4 blockade induced underrepresentation of Bacteroidales and Burkholderiales, and a relative increase in Clostridiales. We recolonized antibiotics-treated or GF mice with bacterial species associated with these perturbations, and identified that Bacteroides fragilis, Bacteroides thetaiotaomicron, Burkholderia cepacia, or the combination of B. fragilis and B. cepacia could each restore the anti-CTLA-4 Ab-mediated anticancer responses. Notably, oral feeding with B. fragilis induced Th1 immune responses in tumor-draining lymph nodes and promoted DC maturation in tumor beds. The microbiota-dependent immunostimulatory effects produced by CTLA-4 blockade required IL-12, this cytokine likely produced by mobilized B. fragilis-stimulated CD11b+ DC from the lamina propria.
To test whether these findings translated to the clinical setting, we analyzed the gut microbiome composition in metastatic melanoma patients before and after ipilimumab treatment. Three distinct microbiome clusters (enterotypes) were revealed, for which segregation was determined by the *Bacteroides* and *Prevotella* genera (*Alloprevotella/Prevotella* driving cluster A, and distinct *Bacteroides* spp. driving clusters B and C). Fecal microbial transplantation of feces highlighted that the microbial composition of cluster C, rich in immunogenic *Bacteroides* species (e.g., *B. fragilis*), could restore anti-CTLA4 Ab efficacy, while cluster B enriched in tolerogenic *Bacteroides* species mediated complete resistance to the Ab. Taken together, this suggests ipilimumab adjusts the level of immunostimulatory *Bacteroides* spp. in the gut, to facilitate its antitumor efficacy.

Given the exceptional clinical outcomes and lengthened overall survival that can be induced by ipilimumab, it is unfortunate that many patients receiving this immunotherapy develop irAEs. Interestingly, we identified that administration of the combination of *B. fragilis* and *Burkholderia cepacia*, mandatory to restore the efficacy of CTLA4 blockade in antibiotics-treated animals, failed to induce signs of subclinical colitis, rather inducing a protection against anti-CTLA4-induced intestinal lesions. This protection was associated with the capacity of *B. fragilis* to promote the proliferation of plasmacytoid DC seen to accumulate and mature in mesenteric lymph nodes after *B. fragilis* colonization of GF mice treated with anti-CTLA4 Ab. In support of this, blockade of ICOS or IL-10 plus anti-CTLA4 Ab treatment resulted in an overt and deadly colitis in tumor bearers reared in SPF conditions. Therefore, efficacy and toxicity following CTLA-4 blockade could be uncoupled in this model of *B. fragilis* and *B. cepacia* bicolonization.

We therefore show that the efficacy of CTLA-4 blockade is facilitated by constituents of the microbiota, especially certain *Bacteroides* spp. and *Burkholderiales*, which enhance tumor control via stimulation of Th1 immune responses during anti-CTLA-4 therapy. Our findings put forward the possibility to (re)establish a favorable enteric microbiota in patients with an ineffective pre-existing enteric microbial microflora that may be associated with a poor prognosis to ipilimumab therapy. Taking our findings, and recent evidence that the anticancer actions of PDL-1 blockade are enhanced in the presence of *Bifidobacterium* spp., the search is on for components of the microbiota that enhance the action of other immunotherapies.

**Disclosure of potential conflicts of interest**

The authors have declared no conflicts of interest. Gustave Roussy has signed a collaborative contract agreement with Enterome.
References

1. Eberl G. A new vision of immunity: homeostasis of the superorganism. Mucosal Immunol. 2010; 3:450-60; PMID:20445502; http://dx.doi.org/10.1038/mi.2010.20

2. Zitvogel L, Galluzzi L, Viaud S, Vetizou M, Daillere R, Merad M, Kroemer G. Cancer and the gut microbiota: an unexpected link. Sci. Transl. Med. 2015; 7:271ps1; PMID:25609166; http://dx.doi.org/10.1126/scitranslmed.3010473

3. Viaud S, Saccheri F, Mignot G, Yamazaki T, Daillere R, Hannersi D, Enot DP, Pflerschke C, Engblom C, Pittet MJ et al. The intestinal microbiota modulates the antitumor immune effects of cyclophosphamide. Science 2013; 342:971-6; PMID:24264990; http://dx.doi.org/10.1126/science.1240537

4. 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; 342:967-70; PMID:24264989; http://dx.doi.org/10.1126/science.1240527

5. Vetizou M, Pitt JM, Daillere R, Lepage P, Waldschmitt N, Flament C, Rusakiewicz S, Routy B, Roberti MP, Duong CP et al. Antitumor immunotherapy by CTLA-4 blockade relies on the gut microbiota. Science 2015; 350:1079-84; PMID:26541610; http://dx.doi.org/10.1126/science.aad1329

6. 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. N. Engl. J. Med. 2010; 363:711-23; PMID:20525992; http://dx.doi.org/10.1056/NEJMoa1003466

7. Beck KE, Blansfield JA, Tran KQ, Feldman AL, Hughes MS, Royal RE, Kammula US, Topalian SL, Sherry RM, Kleiner D et al. Enterocolitis in patients with cancer after antibody blockade of cytotoxic T-lymphocyte-associated antigen 4. J. Clin. Oncol. 2006; 24:2283-9; PMID:16710025; http://dx.doi.org/10.1200/JCO.2005.04.5716

8. Dasgupta S, Erturk-Hasdemir D, Ochoa-Reparaz J, Reinecker HC, Kasper DL. Plasmacytoid dendritic cells mediate anti-inflammatory responses to a gut commensal molecule via both innate and adaptive mechanisms. Cell Host Microbe 2014; 15:413-23; PMID:24721570; http://dx.doi.org/10.1016/j.chom.2014.03.006

9. Sivan A, Corrales L, Hubert N, Williams JB, Aquino-Michaels K, Earley ZM, Benaymin FW, Lei YM, Jabri B, Alegre ML et al. Commensal Bifidobacterium promotes antitumor immunity and facilitates anti-PD-L1 efficacy. Science 2015; 350:1084-9; PMID:26541606; http://dx.doi.org/10.1126/science.aac4255