Modulation of Gut Microbiota: A Novel Paradigm of Enhancing the Efficacy of Programmed Death-1 and Programmed Death Ligand-1 Blockade Therapy

Yiming Wang, Rena Ma, Fang Liu, Seul A. Lee and Li Zhang*

School of Biotechnology and Biomolecular Sciences, University of New South Wales, Sydney, NSW, Australia

Blockade of programmed death 1 (PD-1) protein and its ligand programmed death ligand 1 (PD-L1) has been used as cancer immunotherapy in recent years, with the blockade of PD-1 being more widely used than blockade of PD-L1. PD-1 and PD-L1 blockade therapy showed benefits in patients with various types of cancer; however, such beneficial effects were seen only in a subgroup of patients. Improving the efficacy of PD-1 and PD-L1 blockade therapy is clearly needed. In this review, we summarize the recent studies on the effects of gut microbiota on PD-1 and PD-L1 blockade and discuss the new perspectives on improving efficacy of PD-1 and PD-L1 blockade therapy in cancer treatment through modulating gut microbiota. We also discuss the possibility that chronic infections or inflammation may impact on PD-1 and PD-L1 blockade therapy.

Keywords: gut microbiota, programmed death 1, programmed death ligand 1, cancer immunotherapy, efficacy

INTRODUCTION

The immune system uses various effector cells and molecules to control and eradicate infectious agents and cancer cells. Cytotoxic T cells (CTL) are the major effector cells in anti-tumor immune responses (1, 2). However, the functions of these effector cells are inhibited in the tumor microenvironment, which contributes to cancer cell immune evasion (3). In recent years, the blockade of immune checkpoint proteins and molecules that deliver inhibitory signals to activated T cells, have shown great promise in cancer treatment. However, the beneficial effects of these treatment strategies were seen only in a subgroup of patients (4). In this review, we summarize the emerging evidence of improving immune checkpoint protein blockade therapy efficacy by modulating gut microbiota and discuss the possibility that chronic infections or inflammation may impact on programmed death 1 (PD-1) and programmed death ligand 1 (PD-L1) blockade therapy.

PD-1 AND ITS LIGANDS

Programmed death 1, also known as cluster of differentiation 279 (CD279), is a cell surface receptor that was discovered in 1992 (5). PD-L1 and PD-L2, the two molecules that interact with PD-1, were identified in the following years (6, 7). PD-L1 is also known as CD274 or B7 homolog 1 (B7-H1) and PD-L2 known as CD273 or B7-DC.

Programmed death 1 is expressed on T, B cells, and myeloid cells (8). PD-L1 is expressed by a variety of cells in the immune system and non-immune cells. However, the expression level of PD-L1 in normal human tissues is low; despite the presence of PD-L1 mRNA, PD-L1 protein is rarely detected on the cell surfaces in most of normal human tissues except for a subset of human tissue macrophages.
Programmed death 1 and its ligands are members of the immune checkpoint proteins delivering inhibitory signals to activated T cells. The interaction of PD-1 with PD-L1 or PD-L2 leads to suppression of T cells, a regulatory mechanism to prevent overstimulation of immune responses and autoimmunity (6, 7, 9, 16–21). However, such a mechanism is hijacked in the tumor microenvironment, providing opportunities for tumor cells to evade the attack from the immune system.

**PD-1 AND PD-L1 BLOCKADE IN CANCER IMMUNOTHERAPY**

In anti-tumor immune responses, the tumor antigens generated by gene mutations, are recognized by the immune system and specific CD8+ CTLs targeting tumor antigens are generated (22). These specific effector CTLs recognize the target tumor cells and induce tumor cell apoptosis. However, tumor cells employ various strategies to escape the attack from the immune system, one of which is to resist the killing effects from the anti-tumor CTLs by increasing PD-L1 expression in tumor tissues (9, 23, 24). Most normal human tissues do not express detectable PD-L1 on their cell surface, in contrast PD-L1 is abundantly expressed by tumor cells, the immune and non-immune cells in various tumor tissues (6, 9, 25–30). IFN-γ released by the anti-tumor CTLs infiltrating into tumor tissues plays a major role in inducing the expression of PD-L1 (9–11, 14–16). Other cytokines, such as tumor necrosis factor (TNF)-α, interleukin (IL)-4, and IL-10 can also increase PD-L1 expression (31, 32).

The interaction of PD-L1 with PD-1 in the tumor microenvironment enables the tumor cells to resist the endogenous anti-tumor activities from the immune system. PD-L1 expressed in tumor tissues interacting with PD-1 expressed on the activated T cells leads to the dysfunction of the effector T cells, via multiple mechanisms, such as promoting T cell apoptosis, anergy, and exhaustion (6, 7, 9, 16–21). More recently, it was found that interaction of PD-L1 with PD-1 expressed on tumor-associated macrophages inhibits the phagocytic potency of macrophages against tumor cells (33). The importance of PD-L1 and PD-1 interaction in tumor cell evasion has led scientists to explore the use of these molecules as therapeutic targets in cancer immunotherapy (Figure 1).

**FIGURE 1** The role of programmed death 1 (PD-1) and programmed death ligand 1 (PD-L1) in tumor evasion and cancer immunotherapy. In the tumor microenvironment, T cells were activated after antigen-presenting cells recognized tumor neoantigens. The IFN-γ produced by activated T cells induced the expression of PD-1 ligands on cancer cells and immune cells. Afterward, the engagement of PD-1 by PD-L1 between T cells and antigen-presenting cells will lead to T cell dysfunction. PD-1/PD-L1 blockade using relevant antibodies can inhibit this process, therefore, offering a chance for T cells to continue being effectors. Abbreviations: TCR, T-cell receptor; MHC, major histocompatibility complex; IFN-γ, interferon gamma; IL-10, interleukin 10.
Dong et al. showed that PD-L1 positive human tumor cells induced apoptosis of co-cultured activated effector T cells and this effect was blocked by an anti-human PD-L1 monoclonal antibody (mAb). They also showed that the growth of PD-L1 positive murine tumors in syngeneic mice was suppressed by an anti-murine PD-L1 mAb (9). Other researchers later reported similar findings in examination of different types of cancer cells using mice models (24, 34–36). These important laboratory observations led to numerous clinical trials of using monoclonal antibodies targeting PD-1 or PD-L1 in cancer immunotherapy for a variety of cancers. In addition to affecting the immunological pathways, PD-L1 and PD-1 blockade may also work in part by disrupting autologous PD-1 and PD-L1 signaling within tumors (37, 38).

To date, the U.S. Food and Drug Administration (FDA) has approved the use of five monoclonal antibodies targeting PD-L1 or PD-1 in cancer treatment. The details of the clinical trials of these five monoclonal antibodies are summarized in Table 1. Despite the clear benefits of PD-L1 and PD-1 blockade in treating some cancer patients, not all cases responded to treatment (Table 1). Given this, strategies to improve the efficacy of cancer immunotherapy are needed. Emerging evidence suggests that modulation of the gut microbiota is a promising approach.

**MODULATION OF GUT MICROBIOTA ENHANCES THE ANTI-TUMOR EFFICACY OF PD-1 AND PD-L1 BLOCKADE THERAPY**

A very interesting study by Sivan et al. provided strong evidence that the efficacy of PD-L1 blockade therapy can be improved by the modulation of gut microbiota (70). In this study, Sivan et al. examined the subcutaneous growth of B16.SIY melanoma in genetically similar C57BL/6 mice raised in the Jackson Laboratory (JAX) and Taconic Farms (TAC), and found that the tumor growth was more aggressive in TAC mice as compared to that in JAX mice and that TAC mice had a significantly lower intratumoral CD8+ T cell accumulation. They then conducted various experiments, which demonstrated that gut microbiota contributed to this difference.

They first showed that prophylactic transfer of fecal material from JAX mice to TAC mice was sufficient to delay tumor growth. To examine whether microbial community alone was effective as a therapy, they administered fecal material from JAX mice alone or in combination with anti-PD-L1 mAbs to TAC mice. These experiments showed that fecal material alone was sufficient to significantly inhibit tumor growth and that the combination treatment further improved tumor control. To identify the responsible bacterial species, they used 16S ribosomal RNA (16S rRNA) sequencing and identified *Bifidobacterium* species, particularly *Bifidobacterium breve*, *Bifidobacterium longum*, and *Bifidobacterium adolescentis* as the candidate species. The role of these *Bifidobacterium* species in enhancing protective immunity against tumors were further investigated by administering TAC mice bearing established tumors with a cocktail of *Bifidobacterium* species containing *B. breve* and *B. longum* by oral gavage. This experiment resulted in *Bifidobacterium*-treated mice having significantly improved tumor control as compared to mice that did not receive *Bifidobacterium*. Sivan et al. also showed that the possible mechanisms by which *Bifidobacterium* species inhibited tumor growth were through activating DCs, which in turn, improves the effector function of tumor-specific CD8+ T cells. Given that the enhanced anti-melanoma effect from *Bifidobacterium* species had occurred at the innate immunity level, the authors anticipated that *Bifidobacterium* species also provide anti-tumor beneficial effects to other types of tumors. However, the mechanisms by which *Bifidobacterium* species activated DCs improved the effects of anti-tumor CD8+ cells still need to be clarified.

The findings by Sivan et al. using mice models suggest that it is possible to enhance the anti-tumor efficacy of PD-L1 blockade therapy in treating cancer patients by modulating their gut microbiota and their findings are summarized in Figure 2. Interestingly, a very recent study by Matson et al. examining the stool samples collected from patients with metastatic melanoma before anti-PD-1 immunotherapy found that *B. longum*, *Collinsella aerofaciens*, and *Enterococcus faecium* were more abundant in the anti-PD-1 immunotherapy responders, supporting the anti-tumor effects of *Bifidobacterium* species (71).

Several additional studies also compared the gut microbiota in patients with metastatic melanoma receiving anti-PD-1 therapy. A recent study by Frankel et al. using metagenomic shotgun sequencing method showed that melanoma patients who responded to immune checkpoint inhibitors were enriched with *Bacteroides caccae* (72). Furthermore, they showed that the bacteria that are enriched within responders are most likely to be antibody dependent. Patients who responded to nivolumab (PD-1 antibody) were enriched with *Fecalibacterium prausnitzii*, *Bacteroides thetaiotaomicron*, and *Holdemania filiformis*, whereas patients who responded to pembrolizumab (another PD-1 antibody), their gut microbiota enriched with *Dorea formicigenerans*. However, the mechanisms responsible for these changes are not clear. Studies comparing the gut microbiota changes prior to and following anti-PD-1 therapy of individual patients are required, which will provide information regarding whether anti-PD-1 antibodies directly affect gut bacterial species.

A study by Wargo et al. examined the human gut microbiota and metabolites of metastatic melanoma patients who received anti-PD-1 therapy using 16S rRNA and whole genome shotgun sequencing (73). They found that bacterial diversity and composition in patients that responded to the therapy were significantly different from that in patients who did not respond to the therapy. The responding patients had a higher diversity of bacteria and a higher abundance of *Clostridiales*, and the non-responders had a higher abundance of *Bacteroidales*. In a very recent study with multiple first authors and J. A. Wargo being the responding author, they further compared the gut microbiota of patients with metastatic melanoma receiving anti-PD-1 therapy (74). They found that patients who responded to anti-PD-1 therapy were associated with a significantly higher bacterial diversity and abundance of bacteria from the *Ruminococcaceae* family, which belongs to the *Clostridiales* order, as compared to patients who did not respond to the therapy. Furthermore, they performed fecal microbiota transplantation experiments in germ-free mice,
TABLE 1  | Five monoclonal antibodies targeting programmed death ligand-1 (PD-L1) or programmed death 1 (PD-1) were approved by the U.S. Food and Drug Association to treat cancer.

| Commercial name (active ingredient) | Target Treatment of disease | Targeting patients | Clinical cases | Clinical phase | Overall response rate (95% CI) | Objective response rate (95% CI) | Clinical study (clinical trial ID) | Reference |
|-------------------------------------|-----------------------------|--------------------|---------------|---------------|-------------------------------|---------------------------------|----------------------------------|-----------|
| Bavencio (Avelumab) PD-L1 Metastatic MCC | Metastatic MCC patients whose disease had progressed on or after chemotherapy administered | 88 | Phase 2 | 33% (23.3%, 43.8%) | Not applicable | JAVELIN Merkel 200 Trial (NCT02155647) |  | (39, 40) |
| Tecentriq (Atezolizumab) PD-L1 Advanced or metastatic urothelial carcinoma | Cisplatin-ineligible patients with locally advanced or metastatic urothelial carcinoma | 119 | Phase 2 | 23.5% (16.2%, 32.2%) | Not applicable | IMvigor210 (NCT02951767) |  | (41) |
|  | Previously treated patients with locally advanced or metastatic urothelial carcinoma | 310 | Phase 2 | 14.8% (11.1%, 19.3%) | Not applicable | IMvigor210 (NCT02951767) |  | (41) |
|  | Metastatic NSCLC | Previously treated patients with metastatic non-small cell lung cancer | 22 | Phase 2 | Not applicable | 15% (10%, 22%) | POPLAR (NCT01903993) | (42) |
| Imfinzi (Durvalumab) PD-L1 Locally advanced or metastatic urothelial carcinoma | Patients with locally advanced or metastatic urothelial carcinoma in total | 182 | Phase 1 and 2 | Not applicable | 17.0% (11.9%, 23.3%) | Study 1108 (NCT01693562) |  | (43–45) |
|  | Patients with locally advanced or metastatic urothelial carcinoma who showed high PD-L1 expression on tumor cells | 95 | Phase 1 and 2 | Not applicable | 26.3% (17.8%, 36.4%) | Study 1108 (NCT01693562) |  | (43–45) |
|  | Patients with locally advanced or metastatic urothelial carcinoma who showed low or non-PD-L1 expression on tumor cells | 73 | Phase 1 and 2 | Not applicable | 4.1% (0.9%, 11.5%) | Study 1108 (NCT01693562) |  | (43–45) |
| Keytruda (Pembrozumab) PD-1 Melanoma | Patients with Ipilimumab-Naive melanoma (receive KEYTRUDA at a dose of 10 mg/Kg every 3 weeks) | 277 | Phase 3 | 33% (27%, 39%) | Not applicable | KEYNOTE-006 (NCT01866319) |  | (46, 47) |
|  | Patients with Ipilimumab-Naive melanoma (receive KEYTRUDA at a dose of 10 mg/Kg every 2 weeks) | 279 | Phase 3 | 34% (28%, 40%) | Not applicable | KEYNOTE-006 (NCT01866319) |  | (46, 47) |
|  | Patients with Ipilimumab-refractory melanoma (receive KEYTRUDA at a dose of 2 mg/Kg every 3 weeks) | 180 | Phase 2 | Not applicable | 21% (15%, 28%) | KEYNOTE-002 (NCT01704287) |  | (48) |
|  | Patients with Ipilimumab-refractory melanoma (receive KEYTRUDA at a dose of 10 mg/Kg every 3 weeks) | 181 | Phase 2 | Not applicable | 25% (19%, 32%) | KEYNOTE-002 (NCT01704287) |  | (48) |

(Continued)
| Commercial name (active ingredient) | Target Treatment of disease | Targeting patients | Clinical cases | Clinical phase | Overall response rate (95% CI) | Objective response rate (95% CI) | Clinical study (clinical trial ID) | Reference |
|----------------------------------|-----------------------------|--------------------|----------------|----------------|-----------------------------|-------------------------------|-------------------------------|------------|
| **NSCLC**                        | Metastatic NSCLC patients with first-line treatment with a single agent | 154                | Phase 3        | Not applicable | 45% (37%, 53%)             | Not applicable                 | KEYNOTE-024 (NCT02142738) | (49)       |
|                                  | Metastatic NSCLC patients with first-line treatment in combination with pemetrexed and carboplatin | 60                 | Phase 1 and 2  | 55% (42%, 68%) | Not applicable             | KEYNOTE-021 (NCT02039674)   |                               | (50)       |
|                                  | Previously treated NSCLC patients (all randomized patients who receive KEYTRUDA at a dose of 2 mg/Kg every 3 weeks) | 344                | Phase 2 and 3  | Not applicable | 18% (14%, 23%)             | KEYNOTE-010 (NCT01905667)   |                               | (51)       |
|                                  | Previously treated NSCLC patients (all randomized patients who receive KEYTRUDA at a dose of 10 mg/Kg every 3 weeks) | 346                | Phase 2 and 3  | Not applicable | 19% (15%, 23%)             | KEYNOTE-010 (NCT01905667)   |                               | (51)       |
| **HNSCC**                        | HNSCC patients whose disease had progressed on or after chemotherapy administered | 174                | Phase 1        | 16% (11%, 22%) | Not applicable             | KEYNOTE-012 (NCT01848834)   |                               | (52)       |
| **Urothelial Carcinoma**         | Cisplatin-ineligible patients with urothelial carcinoma | 370                | Phase 2        | Not applicable | 29% (24%, 34%)             | KEYNOTE-052 (NCT02335424)   |                               | (53)       |
|                                  | Previously treated urothelial carcinoma patients | 270                | Phase 3        | Not applicable | 21% (16%, 27%)             | KEYNOTE-045 (NCT02256436)   |                               | (54)       |
| **cHL**                          | Patients with cHL           | 210                | Phase 2        | 69% (62%, 75%) | Not applicable             | KEYNOTE-087 (NCT02453594)   |                               | (55, 56)   |
| **MSI-H**                        | Patients with MSI-H or mismatch repair deficient (dMMR) | 149                | Phase 1        | Not applicable | 39.6% (31.7%, 47.9%)       | KEYNOTE-012 (NCT01848834)   |                               | (52, 57–59)|
|                                  |                             |                    | Phase 2        | Not applicable |                         | KEYNOTE-016 (NCT01878511)   |                               |            |
|                                  |                             |                    | Phase 1        | Not applicable |                         | KEYNOTE-028 (NCT02054806)   |                               |            |
|                                  |                             |                    | Phase 2        | Not applicable |                         | KEYNOTE-158 (NCT02628067)   |                               |            |
|                                  |                             |                    | Phase 2        | Not applicable |                         | KEYNOTE-164 (NCT02460198)   |                               |            |

(Continued)
| Commercial name (active ingredient) | Target of disease | Clinical cases | Clinical phase | Overall response rate (95% CI) | Objective response rate (95% CI) | Clinical study (clinical trial ID) | Reference |
|------------------------------------|------------------|----------------|----------------|-------------------------------|---------------------------------|---------------------------------|----------|
| Opdivo (Nivolumab) PD-1            | Unresectable or metastatic melanoma | 316            | Phase 3        | Not applicable                | 40% (34%, 46%)                  | CheckMate-067 (NCT01844505)     | (60, 61) |
|                                    | Previously treated patients with unresectable or metastatic melanoma in the treatment of OPDIVO | 314            | Phase 3        | Not applicable                | 50% (44%, 55%)                  | CheckMate-067 (NCT01844505)     | (60, 61) |
| Metastatic NSCLC                    | NSCLC patients who had experienced disease progressed during or after one prior platinum doublet-based chemotherapy regimen | 272            | Phase 3        | Not applicable                | 20% (14%, 28%)                  | CheckMate-017 (NCT01642004)     | (62)     |
|                                    | Patients with metastatic non-squamous NSCLC who had experienced disease progressed during or after one prior platinum doublet-based chemotherapy regimen | 292            | Phase 3        | Not applicable                | 19% (15%, 24%)                  | CheckMate-057 (NCT01673867)     | (63)     |
| Renal cell carcinoma               | Patients with advanced RCC who had experienced disease progressed during or after one or two prior anti-angiogenic therapy regimes | 410            | Phase 3        | Not applicable                | 21.5% (17.6%, 25.8%)            | CheckMate-025 (NCT01668784)     | (64, 65) |
| cHL                                | Adult patients with cHL | 258            | Phase 2        | Not applicable                | 69% (63%, 75%)                  | CheckMate-205 (NCT02181738)     | (66, 67) |
|                                    | Recurrent or metastatic SCCHN | 240            | Phase 3        | Not applicable                | 13.3% (9.3%, 18.3%)             | CheckMate-141 (NCT02105636)     | (68, 69) |

Five monoclonal PD-L1 or PD-1 antibodies granted after May 2017 by FDA for cancer treatments were not included in the table. MCC, metastatic Merkel cell carcinoma; NSCLC, non-small cell lung cancer; HNSCC, head and neck squamous cell cancer; cHL, classical Hodgkin lymphoma; MSI-H, microsatellite instability-high cancer; dMMR, mismatch repair deficient; SCCHN, recurrent or metastatic squamous cell carcinoma of the head and neck.
in which they showed that germ-free mice transplanted with stool samples from patients who responded to anti-PD-1 and anti-PD-L1 therapy had a significantly reduced tumor growth and improved responses to anti-PD-1 and anti-PD-L1 therapy, coupled with a higher density of CD8+ T cells. However, it is not clear which bacterial species in the Ruminococcaceae family has played the role in enhancing the PD-1 blockade therapy.

Another recent study by Routy et al. investigated the effects of gut microbiota in PD-1 blockade therapy (75). In their study, data from 140 patients with advanced non-small-cell-lung cancer, 67 patients with renal cell carcinoma, and 42 patients with urothelial carcinoma were collected, and they found that 69 patients who took antibiotics before or soon after starting the PD-1 blockade therapy had shorter progression-free survival and overall survival. They then explored the composition of the gut microbiota using shotgun sequencing, which showed that Akkermansia muciniphila was enriched in patients who responded to anti-PD-1 therapy. This suggests that A. muciniphila may enhance patient response to PD-1 blockade therapy. They verified this observation by transplanting the patients stool samples in specific pathogen-free mice or germ-free mice and observed tumor growth in these mice. They also found that A. muciniphila alone was able to restore the anti-tumor effects of PD-1 blockade that was inhibited by antibiotics. However, the mechanism by which A. muciniphila enhancing PD-1 blockade therapy is not known.

Bacterial species that are positively associated with PD-1 and PD-L1 blockade therapy are summarized in Table 2. Some bacterial species have also been demonstrated to affect CTLA-4 blockade immunotherapy, which were not reviewed here (76, 77).

**POTENTIAL MECHANISMS OF GUT MICROБES ON IMPROVING THE EFFICACY OF PD-1 AND PD-L1 BLOCKADE THERAPY**

Despite the exciting findings in this research field, the underlying molecular mechanisms by which the identified gut bacterial species in the above studies enhance PD-1 and PD-L1 blockade therapy remain largely unknown.

Recently, unmethylated CpG oligodeoxynucleotides, which are abundant in bacterial DNA, were found to enhance CD8+ T cell anti-tumor immunity by downregulating PD-1 expression via the IL-12 pathway, suggesting that gut bacterial species that
| Bacteria Model Methods | Main findings | Reference |
|------------------------|--------------|-----------|
| **Bifidobacterium breve, Bifidobacterium longum, Bifidobacterium adolescentis** Mouse Fecal transplantation | Some Bifidobacterium species enhanced the efficacy of anti-PD-L1 therapy in vivo | (70) |
| **Fecalibacterium prausnitzii, Bacteroides thetaiotamicron, Holdemania filiformis, Dorea formicogenerans** Human Metagenomic shotgun sequencing | Melanoma patients who responded to nivolumab (PD-1 antibody) were enriched with F. prausnitzii, B. thetaiotamicron, and H. filiformis Melanoma patients who responded to pembrolizumab (another PD-1 antibody), their gut microbiota enriched with D. formicogenerans | (72) |
| **Clostridiales** Human 16S rRNA gene sequencing Whole genome shotgun sequencing Immunohistochemistry Flow cytometry Cytokines assay Gene expression profiling | Melanoma patients who responded to anti-PD-1 therapy had a higher diversity of bacteria and a higher abundance of Clostridiales | (73) |
| **Ruminococcaceae** Mouse Human 16S rRNA gene sequencing Whole genome shotgun sequencing Immunohistochemistry Flow cytometry Cytokines assay Gene expression profiling Fecal microbiota transplantation | Melanoma patients who responded to anti-PD-1 therapy had a higher diversity of bacteria and a higher abundance of Ruminococcaceae Germ-free mice transplanted with stool samples from patients responded to anti-PD-1 and anti-PD-L1 therapy had a significantly reduced tumor growth and improved responses to anti-PD-1 and anti-PD-L1 therapy coupled with higher density of CD8+ T cells in tumor | (74) |
| **Akkermansia muciniphila** Mouse Human Metagenomic shotgun sequencing Fecal microbiota transplantation Immunohistochemistry Flow cytometry Cytokines assay Immunohistochemistry | 27% cancer patients who took antibiotics before or soon after starting the PD-1 blockade therapy had shorter progression-free survival and overall survival A. muciniphila was found enriched in those patients who respond to anti-PD-1 therapy A. muciniphila alone was able to restore the anti-tumor effects of PD-1 blockade that was inhibited by antibiotics. | (75) |
| **B. longum, Collinsella aerofaciens, Enterococcus faecium** Mouse Human 16S rRNA gene sequencing Metagenomic shotgun sequencing Species-specific quantitative PCR Immunohistochemistry Fecal transplantation | Melanoma patients who responded to anti-PD-1 therapy had a higher abundance of B. longum, C. aerofaciens, and E. faecium Germ-free mice transplanted with fecal material from responding patients could lead to improved tumor control, augmented T cell responses, and greater efficacy of anti-PD-L1 therapy | (71) |

*aBacteria of Ruminococcaceae family belongs to the Clostridiales order.

*bPatients here include patients with advanced non-small-cell-lung cancer, renal cell carcinoma, and urothelial carcinoma.
are positively associated with PD-1 and PD-L1 blockade therapy may release components that directly downregulate PD-1 or PD-L1 expression (78, 79).

It is also possible that the gut bacterial species indirectly affect PD-1 and PD-L1 expression through locally or systematically regulating immune responses, thereby affecting the efficacy of PD-1 and PD-L1 blockade therapy. Gut microbiota has been shown to impact on both innate and adaptive immune cells. Germ-free animals had a reduced number of intestinal DCs and administration of Escherichia coli in these animals was able to recruit sufficient DCs to the intestines (80, 81). In Germ-free pigs, systemic circulating macrophages were also reduced and their functions were compromised (82). Germ-free mice had markedly decreased presence of lamina propria CD4+ T cells and absence of lymphocyte zones in spleens and mesenteric lymph nodes (83, 84). Polysaccharide A from Bacteroides fragilis was found to induce the Th1 response (83). Reduction of commensal microbiota in mice by using broad-spectrum antibiotics resulted in defective T and B cell responses against influenza infection (85). The findings that gut microbes can affect the immune functions, both locally and systematically suggest that bacterial species positively associated with PD-1 and PD-L1 blockade therapy may enhance PD-1 and PD-L1 immunotherapy through regulation of the immune response. The previous study by Sivan et al. showed that Bifidobacterium species that inhibited tumor growth activated DCs, further supporting this view (70).

**THE POSSIBLE IMPACT OF CHRONIC INFECTIONS AND INFLAMMATION ON PD-1 AND PD-L1 BLOCKADE THERAPY**

Several microbes cause chronic infections in humans, some of which are known to increase host PD-1 and PD-L1 expression (86–94). However, studies have not examined whether existing chronic infections in patients with cancer affect the efficacy of PD-1 and PD-L1 blockade therapy.

An example of a chronic infection is *Helicobacter pylori* infection. *H. pylori* are a Gram-negative bacterium that colonizes the stomach of more than 50% of the world population. While most of the individuals colonized with *H. pylori* are asymptomatic, some may develop chronic gastritis and peptic ulcers, and *H. pylori* colonization is also a risk factor for gastric cancer (95). Previous studies have shown that patients with *H. pylori* infection have a significantly higher level of pro-inflammatory cytokines, such as TNF-α (96–98). Das et al. showed that *H. pylori* increased the gastric epithelial expression of PD-L1 using a gastric epithelial cell line model (86). Furthermore, they showed that gastric epithelial cells exposed to *H. pylori* inhibited the proliferation of CD4+ T cells isolated from blood and the inhibitory effect can be blocked using antibodies PD-L1. Similarly, Wu et al. observed increased PD-L1 expression in gastric biopsies of individuals infected with *H. pylori*, and co-culture of *H. pylori* infected primary gastric epithelial cells with T cells isolated from blood induced T cell apoptosis (87). These results suggest that *H. pylori* infection may cause the non-specific inhibition of circulating T cells, including tumor-specific T cells. In addition to *H. pylori*, several viruses, such as the hepatitis B virus, hepatitis C virus, human papillomavirus, and Epstein–Barr virus are also able to establish chronic infections in humans and increase host PD-1 or PD-L1 expression (88–94). Future studies are needed to examine whether chronic infections or inflammation impact on the efficacy of PD-1 and PD-L1 blockade. A recent study by Kottke et al. using a mouse model showed that pro-inflammatory cytokine TNF-α promoted tumor recurrence, while TNF-α blockade prevented tumor recurrence (99–102). Some bacterial species that are known to reduce chronic inflammation after administration orally may be examined to see whether they can improve cancer treatment (103–108). If chronic infections or inflammation reduce the efficacy of PD-1 and PD-L1 blockade, it would be through mechanisms other than the induction of the PD-1 and PD-L1 expression in the tumor tissues, as previous studies observed better responses to PD-1 blockade in patients with higher expression of PD-L1 in tumor tissues (51).

---

**TABLE 3 | Suggested future directions.**

| Modulation of gut microbiota |
|-----------------------------|
| Identify the gut bacteria that are positively associated with PD-1 and PD-L1 blockade therapy in humans at species and strain level and understand their anti-tumor mechanisms |
| Explore the mechanisms of the anti-tumor effects of *Bifidobacterium* species |
| Examine the impact of chronic inflammation on PD-1 and PD-L1 blockade therapy and develop treatment strategies accordingly |
| Enhance the efficacy of PD-1 and PD-L1 blockade therapy |
FUTURE DIRECTIONS

As discussed, despite the clear benefits of PD-1 and PD-L1 blockade in treating some cancer patients, the efficacy and the recurrence of tumor are issues that remain to be tackled. Emerging evidence suggests that modulation of the gut microbiota is a promising approach for improving PD-1 and PD-1 blockade therapy. However, future studies are needed to further develop this research area.

The *Bifidobacterium* species, particularly *B. longum*, increased anti-PD-1 efficacy in mice models and was positively associated with anti-PD-1 efficacy in metastatic melanoma patients. Future studies are needed to understand the molecular mechanisms of these *Bifidobacterium* species in enhancing PD-1 and PD-L1 blockade therapy. In addition to the *Bifidobacterium* species, various studies reported positive associations of gut microbes with PD-1 and PD-L1 blockade therapy at genus level. These microbes need to be identified at species and strain level and their potential anti-tumor mechanisms require further investigation.

Several bacterial and viral pathogens are known to cause chronic human infections and the pro-inflammatory cytokines are known to induce host PD-1 and PD-L1 expression. In addition, some of these pathogens are known to directly attack immune cells. Whether chronic infections caused by different pathogens impact on PD-1 and PD-L1 blockade therapy should be investigated, and appropriate strategies to enhance PD-1 and PD-L1 blockade therapy in these patients can then be developed accordingly. A suggested course of action is outlined in Table 3.

AUTHOR CONTRIBUTIONS

Wrote the paper: YW, LZ. Figures and tables: YW. Revised the paper: YW, LZ, RM, FL, SL. All authors have approved the final version of the manuscript.

ACKNOWLEDGMENTS

The authors would like to thank Dr. Jiezhong Chen (School of Biomedical Sciences, University of Queensland) for reading the manuscript and providing feedback on this article.

FUNDING

This work is supported by a Faculty Research Grant awarded to LZ from the University of New South Wales (Grant No.: PS46772).

REFERENCES

1. Ercolini AM, Ladle BH, Manning EA, Pfannenstiel LW, Armstrong TD, Machiels J-PH, et al. Recruitment of latent pools of high-avidity CD8+ T cells to the antitumor immune response. *J Exp Med* (2005) 201(10):1591–602. doi:10.1084/jem.20042167
2. Melief CJ. Tumor eradication by adoptive transfer of cytotoxic T lymphocytes. *Adv Cancer Res* (1992) 58:143–75. doi:10.1016/S0065-230X(08)60294-8
3. Zou W. Immunosuppressive networks in the tumour environment and their therapeutic relevance. *Nat Rev Cancer* (2005) 5(4):263–74. doi:10.1038/nrc1586
4. Postow MA, Callahan MK, Wolchok JD. Immune checkpoint blockade in cancer therapy. *J Clin Oncol* (2015) 33(17):1974–82. doi:12.1001/jco.2014.459.4358
5. Ishida Y, Agata Y, Shibahara K, Honjo T. Induced expression of PD-1, a novel member of the immunoglobulin gene superfamily, upon programmed cell death. *EMBO J* (1992) 11(11):3887.
6. Dong H, Zhu G, Tamada K, Chen L. PD-1, a novel marker of the immunoglobulin gene superfamily, upon programmed cell death. *EMBO J* (1999) 18(12):3165–9. doi:10.1093/emboj/18.12.3165
7. Freeman GJ, Long AJ, Iwai Y, Bourque K, Chernova T, Nishimura H, et al. Engagement of the PD-1 immunoinhibitory receptor by a novel B7 family member leads to negative regulation of lymphocyte activation. *J Exp Med* (2000) 192(7):1027–34. doi:10.1084/jem.192.7.1027
8. Keit ME, Butte MJ, Freeman GJ, Sharpe AH. PD-1 and its ligands in tolerance and immunity. *Annu Rev Immunol* (2008) 26:677–704. doi:10.1146/annurev.immunol.26.021607.090331
9. Dong H, Strome SE, Salomao DR, Tamura H, Hirano F, Flies DB, et al. Co-inhibitory molecules of the B7–CD28 family in the control of T-cell immunity. *Nat Rev Immunol* (2008) 4(5):336–47. doi:10.1038/nri1349
10. Zou W, Chen L. Immunoregulatory function of PD-1 and its ligand, B7-H1. *J Exp Med* (2003) 198(1):31–8. doi:10.1084/jem.20030242
11. Tamura H, Dong H, Zhu G, Sica GL, Flies DB, Tamada K, et al. B7-H1 costimulation preferentially enhances CD28-independent T-helper cell function. *Blood* (2001) 97(6):1809–16. doi:10.1182/blood.V97.6.1809
12. Tseng S-Y, Otsuji M, Gorski K, Huang X, Slansky JE, Pai SI, et al. Recruitment of latent pools of high-avidity CD8+ T cells to the antitumor immune response. *J Exp Med* (2005) 201(10):1591–602. doi:10.1084/jem.20042167
13. Shin T, Kennedy G, Gorski K, Tsuchiya H, Koseki H, Azuma M, et al. Cooperative B7–1/2 (CD80/CD86) and B7–DC costimulation of CD4+ T cells independent of the PD-1 receptor. *J Exp Med* (2003) 198(1):31–8. doi:10.1084/jem.20030242
14. Tamura H, Dong H, Zhu G, Sica GA, Flies DB, Tamada K, et al. B7-H1 costimulation preferentially enhances CD28-independent T-helper cell function. *Blood* (2001) 97(6):1809–16. doi:10.1182/blood.V97.6.1809
15. Mazanet MM, Hughes CC. B7-H1 is expressed by human endothelial cells and suppresses T cell cytokine synthesis. *J Immunol* (2002) 169(7):3581–8. doi:10.4049/jimmunol.169.7.3581
16. Selenko-Geibauer N, Majdic O, Szekeres A, Höfler G, Guthann E, Korthauer U, et al. B7-H1 (programmed death-1 ligand) on dendritic cells is involved in the induction and maintenance of T cell anergy. *J Immunol* (2003) 170(7):3637–44. doi:10.4049/jimmunol.170.7.3637
17. Ghiotto M, Gauthier L, Serriari N, Pastor S, Truneh A, Nünès JA, et al. PD-L1 and PD-L2 differ in their molecular mechanisms of interaction with PD-1. *Int Immunol* (2010) 22(8):651–60. doi:10.1093/intimm/dxq049
18. Chen L. Co-inhibitory molecules of the B7–CD28 family in the control of T-cell immunity. *Nat Rev Immunol* (2004) 4(5):336–47. doi:10.1038/nri1349
19. Zou W, Chen L. Inhibitory B7-family molecules in the tumor microenvironment. *Nat Rev Immunol* (2008) 8(6):467–77. doi:10.1038/nri2352
20. Tsushima F, Yao S, Shin T, Flies A, Flies S, Xu H, et al. Interaction between B7-H1 and PD-1 determines initiation and reversal of T-cell anergy. *Blood* (2007) 110(1):180–5. doi:10.1182/blood-2006-11-060087
21. Goldberg MV, Maris CH, Hipkiss EL, Flies AS, Zhen L, Tuder RM, et al. Role of PD-1 and its ligand, B7-H1, in early fate decisions of CD8 T cells. *Blood* (2007) 110(1):186–92. doi:10.1182/blood-2006-12-062422
22. Engels B, Engelhard VH, Sidney J, Sette A, Binder DC, Liu RB, et al. Relapse or eradication of cancer is predicted by peptide-major histocompatibility complex affinity. *Cancer Cell* (2013) 23(4):516–26. doi:10.1016/j.ccr.2013.03.018
23. Mühlbauer M, Fleck M, Schütz C, Weiss T, Froh M, Blank C, et al. PD-L1 is induced in hepatocytes by viral infection and by interferon-α and γ and mediates T cell apoptosis. *J Hepatol* (2006) 44(4):520–8. doi:10.1016/j.jhep.2006.05.007
24. Iwai Y, Ishida M, Akazaki T, Honjo T, Minato N. Involvement of PD-L1 on tumor cells in the escape from host immune system and tumor immunotherapy by PD-L1 blockade. *Proc Natl Acad Sci U S A* (2002) 99(19):12293–7. doi:10.1073/pnas.192461099
25. Konishi I, Yamazaki K, Azuma M, Kinoshita I, Dosaka-Akita H, Nishimura M. B7-H1 expression on non-small cell lung cancer cells and its relationship with tumor-infiltrating lymphocytes and their PD-1 expression. *Clin Cancer Res* (2004) 10(15):5094–100. doi:10.1158/1078-0432.CCR-04-0428

26. Brown JA, Dorfman MA, Ma F-R, Sullivan EL, Munoz O, Wood CR, et al. Blockade of programmed death-1 ligands on dendritic cells enhances T cell activation and cytokine production. *J Immunol* (2003) 170(3):1257–66. doi:10.4049/jimmunol.170.3.1257

27. Curiel TJ, Wei S, Dong H, Alvarez X, Cheng P, Mottram P, et al. Blockade of B7-H1 improves myeloid dendritic cell-mediated antitumor immunity. *Nat Med* (2003) 9(5):562–7. doi:10.1038/nm863

28. Kuang D-M, Zhao Q, Peng C, Xu J, Zhang J-R, Wu C, et al. Activated monocytes in peritumoral stroma of hepatocellular carcinoma foster immune privilege and disease progression through PD-L1. *J Exp Med* (2009) 206(6):1327–37. doi:10.1084/jem.20082173

29. Liu Y, Zeng B, Zhang Z, Zhang Y, Rang R-B. B7-H1 on myeloid-derived suppressor cells in immune suppression by a mouse model of ovarian cancer. *Clin Immunol* (2008) 129(3):471–81. doi:10.1016/j.clim.2008.07.030

30. Pardoll DM. The blockade of immune checkpoints in cancer immunotherapy. *Nat Rev Cancer* (2012) 12(4):252–64. doi:10.1038/nrc3429

31. Sznol M, Chen L. Antagonist antibodies to PD-1 and B7-H1 (PD-L1) in the treatment of advanced melanoma and renal cell carcinoma. *Clin Cancer Res* (2004) 10(15):5094–100. doi:10.1158/1078-0432.CCR-04-0428

32. Konishi I, Yamazaki K, Tamura H, Zhao W, Touji T, Shimizu M, et al. Interferon-γ and tumor necrosis factor-α induce an immunoinhibitory molecule, B7-H1, via nuclear factor-κB and tumor necrosis factor-γ in peritoneal macrophages. *Blood* (2010) 116(7):1124–31. doi:10.1182/blood-2009-12-255125

33. Gordon SR, Maute RL, Dulken BW, Hutter G, George BM, McCracken MN, et al. PD-1 expression by tumour-associated macrophages inhibits phagocytosis and tumour immunity. *Nature* (2017) 545(7655):495–9. doi:10.1038/nature22396

34. Blank C, Brown I, Peterson AC, Spriott M, Iwai Y, Honjo T, et al. PD-L1 inhibits the effector phase of tumor rejection by T cell receptor (TCR) transgenic CD8+ T cells. *Cancer Res* (2004) 64(3):1140–5. doi:10.1158/0008-5472.CAN-03-3259

35. Petroff M, Chen L, Phillips T, Hunt J. B7 family molecules: novel immunomodulators at the maternal-fetal interface. *Placenta* (2002) 23:595–101. doi:10.1053/plac.2002.0813

36. Hirano F, Kaneko K, Tamura H, Dong H, Wang S, Ichikawa M, et al. Atezolizumab (MEDI4736): an experimental, phase 2 cohort of the open-label KEYNOTE-021 study. *Lancet Oncol* (2016) 17(1):1497–508. doi:10.1016/S1470-2045(16)33049-3

37. Le DT, Yoshino T, Jäger D, Andre T, Bendell JC, Wang R, et al. KEYNOTE-164: Pembrolizumab in untreated, microsatellite instability-high advanced colorectal carcinoma. *J Clin Oncol* (2017) 35(15):2568. doi:10.1200/JCO.2017.35.15_suppl.2568

38. Ibrahim R, Stewart R, Shalabi A. PD-L1 blockade for cancer treatment: MDPI. *Semin Oncol* (2015) 42:474–83. doi:10.1053/j.seminoncol.2015.02.007

39. Syed Y. Durvalumab: first global approval. *Drugs* (2017) 77(12):1369–76. doi:10.1007/s40265-017-0782-5

40. Ribas A, Hamid O, Daud A, Hodi FS, Wolchok JD, Kefford R, et al. Association of pembrolizumab with tumor response and survival among patients with advanced melanoma. *JAMA* (2016) 315(15):1600–9. doi:10.1001/jama.2016.4059

41. Reck M, Rodríguez-Abreu D, Robinson AG, Hui R, Csőszi T, Fülop A, et al. Pembrolizumab versus chemotherapy for PD-L1–positive non–small-cell lung cancer. *N Engl J Med* (2016) 375(18):1823–33. doi:10.1056/NEJMoa1606774

42. Schachter J, Raubitschek A, Long GV, Arance A, Grob JJ, Mortier L, et al. Pembrolizumab versus ipilimumab for advanced melanoma: final overall survival analysis of KEYNOTE-006. *J Clin Oncol* (2016) 34(15):9504. doi:10.1200/JCO.2016.34.15_suppl.9504

43. Ribas A, Puzanov I, Dummer R, Schadendorf D, Hamid O, Robert C, et al. Pembrolizumab versus investigator-choice chemotherapy for ipilimumab-refractory melanoma (KEYNOTE-002): a randomised, controlled phase 2 trial. *Lancet Oncol* (2015) 16(8):908–18. doi:10.1016/S1470-2045(15)00893-2

44. Balar AV, Abreu D, Robinson AG, Hui R, Csőszi T, Fülop A, et al. Pembrolizumab versus docetaxel for previously treated, PD-L1–positive, advanced non–small-cell lung cancer (KEYNOTE-010): a randomised controlled trial. *Lancet* (2016) 387(10027):1540–50. doi:10.1016/S0140-6736(16)30381-7

45. Seiwert TY, Burtis R, Mehra R, Weiss J, Berger R, Eder JP, et al. Safety and clinical activity of pembrolizumab for treatment of recurrent or metastatic squamous cell carcinoma of the head and neck (KEYNOTE-012): an open-label, multicentre, phase 1b trial. *Lancet Oncol* (2016) 17(7):956–65. doi:10.1016/S1470-2045(16)30066-3

46. Balar AV, Castellano DE, O’Donnell GH, Grivas P, Vuky J, Powles T, et al. Pembrolizumab as first-line therapy in cisplatin-ineligible advanced urothelial cancer: results from the phase 2 KEYNOTE-028 study. *J Immunother Cancer* (2017) 5(6):284. doi:10.1200/JCO.2017.35.6_suppl.284

47. Bellmunt J, de Wit R, Vaughn DJ, Fradet Y, Lee J-L, Fong L, et al. Pembrolizumab as second-line therapy for advanced urothelial carcinoma. *N Engl J Med* (2017) 376(11):1015–26. doi:10.1056/NEJMoa1613683

48. Moskowitz CH, Zinzani PL, Fanale MA, Armand P, Johnson NA, Radford JA, et al. Pembrolizumab in relapsed/refractory classical Hodgkin lymphoma: primary end point analysis of the phase 2 KEYNOTE-087 study. *Blood* (2016) 128(22):1107.

49. Chen R, Zinzani PL, Fanale MA, Armand P, Johnson NA, Brice P, et al. Pembrolizumab in advanced urothelial carcinoma (UC) and other solid tumors. *J Clin Oncol* (2017) 35(15):2568. doi:10.1200/JCO.2017.35.15_suppl.2568

50. Ibrahim R, Stewart R, Shalabi A. PD-L1 blockade for cancer treatment: MDPI. *Semin Oncol* (2015) 42:474–83. doi:10.1053/j.seminoncol.2015.02.007

51. Wang et al. Gut Microbiota and PD-1/PD-L1 Blockade
Gut Microbiota and PD-1/PD-L1 Blockade

76. Vézizou M, Pitt JM, Dailère L, Lepage P, Waldschmitt N, Flamant C, et al. Anticancer immunotherapy by CTLA-4 blockade relies on the gut microbiota. Science (2015) 350(6264):709–14. doi:10.1126/science.aaa3129

77. Pitti M, Vézizou M, Gomperts Boneca L, Lepage P, Chamaillard M, Zitvogel L. Enhancing the clinical coverage and anticancer efficacy of immune check point blockade through manipulation of the gut microbiota. Oncoimmunology (2017) 6(1):e1121377. doi:10.2147/oncotarget.113217

78. Yin P, Liu X, Mansfield AS, Harrington SM, Li Y, Yan Y, et al. Cpg-induced antitumor immunity requires IL-12 in expansion of effector cells and down-regulation of PD-1. Oncotarget (2016) 7(43):70223. doi:10.18632/oncotarget.11883

79. Wang S, Campos J, Gallotta M, Gong M, Crain C, Naik E, et al. Intratumoral injection of a Cpg oligonucleotide reverses resistance to PD-1 blockade by expanding multifunctional CD8+ T cells. Proc Natl Acad Sci U S A (2016) 113(46):E7240–9. doi:10.1073/pnas.1608551133

80. Haverson K, Rehakova Z, Sinkora J, Sver L, Bailey M. Immune development in jejunal mucosa after colonization with selected commensal gut bacteria: a study in germ-free pigs. Vet Immunol Immunopathol (2009) 119(3):243–53. doi:10.1016/j.vetimm.2007.05.022

81. Williams AM, Probert CS, Stepankova R, Tskalovka-Hogenova H, Phillips A, Bland PW. Effects of microflora on the neonatal development of gut mucosal T cells and myeloid cells in the mouse. Immunology (2006) 119(4):470–8. doi:10.1111/j.1365-2567.2006.02438.x

82. Zhang W, Wen K, Azevedo M, Naranjo A, Salif LI, Li G, et al. Lactic acid bacterial colonization and human rotavirus infection influence distribution and frequencies of monocytes/macrophages and dendritic cells in neonatal gnotobiotic pigs. Vet Immunol Immunopathol (2008) 121(3):222–31. doi:10.1016/j.vetimm.2007.10.001

83. Mazmanian SK, Liu CH, Tzianabos AO, Kasper DL. An immunomodulatory molecule of symbiotic bacteria directs maturation of the host immune system. Cell (2005) 121(1):107–18. doi:10.1016/j.cell.2005.05.007

84. Macpherson A, Martinic M, Harris N. The functions of mucosal T cells in containing the indigenous commensal flora of the intestine. Cell Mol Life Sci (2002) 59(12):2088–96. doi:10.1007/s000180200009

85. Ichinohe T, Pang IK, Kumamoto Y, Peaper DR, Ho JH, Murray TS, et al. Microbiota regulates immune defense against respiratory tract influenza A virus infection. Proc Natl Acad Sci U S A (2011) 108(13):5354–9. doi:10.1073/pnas.101937108

86. Das S, Suarez G, Beswick EJ, Sierra JC, Graham DY, Reyes VE. Expression of B7-H1 on gastric epithelial cells: its potential role in regulating T cells during Helicobacter pylori infection. J Immunol (2006) 176(5):3000–9. doi:10.4049/jimmunol.176.5.3000

87. Wu Y, Lin CW, Cheng KS, Lin C, Wang YM, Lin IT, et al. Increased programmed death-ligand-1 expression in human gastric epithelial cells in Helicobacter pylori pylori infection. Clin Exp Immunol (2010) 161(3):551–9. doi:10.1111/j.1365-2249.2010.04217.x

88. Xie Z, Chen Y, Zhao S, Yang Z, Yao X, Guo S, et al. Intraepithelial PD-1/PD-L1 up-regulation closely correlates with inflammation and virus replication in patients with chronic HBV infection. Immunol Invest (2009) 38(7):624–38. doi:10.1080/0882013090362210

89. Peng G, Li S, Wu W, Tan X, Chen Y, Chen Z. PD-1 upregulation is associated with HBV-specific T cell dysfunction in chronic hepatitis B patients. Mol Immunol (2008) 45(4):963–70. doi:10.1016/j.molimm.2007.07.038

90. Golden-Mason L, Palmer B, Klarquist J, Mengshol JA, Castelblanco N, et al. Intrahepatic PD-1/PD-L1 with HBV-specific T cell dysfunction in chronic hepatitis B patients. Hepatology (2007) 46(5):1468–75. doi:10.1002/hep.21425

91. Matson V, Fessler J, Bao R, Chongwuat T, Zha Y, Alegre M-L, et al. The commensal microbiome is associated with anti-PD-1 efficacy in metastatic melanoma patients. Science (2018) 359(6371):104–8. doi:10.1126/science.aao3290

92. Franklin AE, Coughlin LA, Kim J, Froehlich TW, Xie Y, Frenkel EP, et al. Metagenomic shotgun sequencing and unbiased metabolomic profiling identify specific human gut microbiota and metabolites associated with immune checkpoint therapy efficacy in melanoma patients. Neoplasia (2017) 19(10):848–55. doi:10.1016/j.neo.2017.08.004

93. Wargo JA, Gopalakrishnan V, Spencer C, Karpinets T, Reuben A, Andrews MC, et al. Association of the diversity and composition of the gut microbiome with responses and survival (PFS) in metastatic melanoma (MM) patients (pts) on anti-PD-1 therapy. J Clin Oncol (2017) 35(15):3008. doi:10.1200/JCO.2017.35.15_suppl.3008

94. Gopalakrishnan V, Spencer C, Nezi I, Reuben A, Andrews M, Karpinets T, 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

95. ROUTY B, Le Chatelier E, Derosa L, Duong CP, Alou MT, Daillère R, et al. Gut microbiome influences efficacy of PD-1–based immunotherapy against epithelial tumors. Science (2018) 359(6371):91–7. doi:10.1126/science.aan3706
102. Wang X, Yang L, Huang F, Zhang Q, Liu S, Ma L, et al. Inflammatory cytokines
101. Gowrishankar K, Gunatilake D, Gallagher SJ, Tiffen J, Rizos H, Hersey P.
100. Chen J, Jiang C, Jin L, Zhang X. Regulation of PD-L1: a novel role of pro-
97. Bodger K, Wyatt J, Heatley R. Gastric mucosal secretion of interleukin-10:
96. Crabtree J, Shallcross T, Heatley R, Wyatt J. Mucosal tumour necrosis factor
93. Fang W, Zhang J, Hong S, Zhan J, Chen N, Qin T, et al. EBV-driven LMP1
92. Parsonnet J, Friedman GD, Vandersteen DP, Chang Y, Vogelman JH, Orentreich N, et al.
91. Derks S, Liao X, Chiaravalli AM, Xu X, Camargo MC, Solcia E, et al.
90. Perri F, Clemente R, Festa V, De Ambrosio C, Quitadamo M, Fusillo M, et al.
89. Kottke T, Evgin L, Shim KG, Rommelfanger D, Boisgerault N, Zaidi S, et al.
88. Zhang L, Su P, Henriksson A, O'Rourke J, Mitchell H. Investigation of the
87. Vieira AT, Galvao I, Amaral FA, Teixeira MM, Nicoli JR, Martins FS. Oral
86. Tien MT, Girardin SE, Regnault R, Le Bourhis L, Dillies MA, Coppee JY, et al.
85. Liu Y, Fatheree NY, Mangalat N, Rhoads JM. Human-derived probiotic
84. Fang W, Zhang J, Hong S, Zhan J, Chen N, Qin T, et al. EBV-driven LMP1
83. Fang W, Zhang J, Hong S, Zhan J, Chen N, Qin T, et al. EBV-driven LMP1
82. Matsumoto S, Hara T, Hori T, Mitsuya K, Nagaoka M, Tomiyasu N, et al. Probiotic Lactobacillus-induced improvement in murine chronic
81. Liu Y, Fatheree NY, Mangalat N, Rhoads JM. Human-derived probiotic
80. Parsonnet J, Friedman GD, Vandersteen DP, Chang Y, Vogelman JH, Orentreich N, et al.
79. Bodger K, Wyatt J, Heatley R. Gastric mucosal secretion of interleukin-10:
78. Perri F, Clemente R, Festa V, De Ambrosio C, Quitadamo M, Fusillo M, et al.
77. Kottke T, Evgin L, Shim KG, Rommelfanger D, Boisgerault N, Zaidi S, et al.
76. Derks S, Liao X, Chiaravalli AM, Xu X, Camargo MC, Solcia E, et al.
75. Parsonnet J, Friedman GD, Vandersteen DP, Chang Y, Vogelman JH, Orentreich N, et al.
74. Fang W, Zhang J, Hong S, Zhan J, Chen N, Qin T, et al. EBV-driven LMP1
73. Fang W, Zhang J, Hong S, Zhan J, Chen N, Qin T, et al. EBV-driven LMP1
72. Wang X, Yang L, Huang F, Zhang Q, Liu S, Ma L, et al. Inflammatory cytokines
71. Gowrishankar K, Gunatilake D, Gallagher SJ, Tiffen J, Rizos H, Hersey P.
70. Parsonnet J, Friedman GD, Vandersteen DP, Chang Y, Vogelman JH, Orentreich N, et al.
69. Crabtree J, Shallcross T, Heatley R, Wyatt J. Mucosal tumour necrosis factor
68. Bodger K, Wyatt J, Heatley R. Gastric mucosal secretion of interleukin-10:
67. Matsumoto S, Hara T, Hori T, Mitsuya K, Nagaoka M, Tomiyasu N, et al. Probiotic Lactobacillus-induced improvement in murine chronic
66. Parsonnet J, Friedman GD, Vandersteen DP, Chang Y, Vogelman JH, Orentreich N, et al.
65. Derks S, Liao X, Chiaravalli AM, Xu X, Camargo MC, Solcia E, et al.
64. Parsonnet J, Friedman GD, Vandersteen DP, Chang Y, Vogelman JH, Orentreich N, et al.
63. Fang W, Zhang J, Hong S, Zhan J, Chen N, Qin T, et al. EBV-driven LMP1
62. Parsonnet J, Friedman GD, Vandersteen DP, Chang Y, Vogelman JH, Orentreich N, et al.
61. Derks S, Liao X, Chiaravalli AM, Xu X, Camargo MC, Solcia E, et al.
60. Parsonnet J, Friedman GD, Vandersteen DP, Chang Y, Vogelman JH, Orentreich N, et al.
59. Fang W, Zhang J, Hong S, Zhan J, Chen N, Qin T, et al. EBV-driven LMP1
58. Parsonnet J, Friedman GD, Vandersteen DP, Chang Y, Vogelman JH, Orentreich N, et al.
57. Derks S, Liao X, Chiaravalli AM, Xu X, Camargo MC, Solcia E, et al.
56. Parsonnet J, Friedman GD, Vandersteen DP, Chang Y, Vogelman JH, Orentreich N, et al.
55. Fang W, Zhang J, Hong S, Zhan J, Chen N, Qin T, et al. EBV-driven LMP1
54. Parsonnet J, Friedman GD, Vandersteen DP, Chang Y, Vogelman JH, Orentreich N, et al.
53. Derks S, Liao X, Chiaravalli AM, Xu X, Camargo MC, Solcia E, et al.
52. Parsonnet J, Friedman GD, Vandersteen DP, Chang Y, Vogelman JH, Orentreich N, et al.
51. Fang W, Zhang J, Hong S, Zhan J, Chen N, Qin T, et al. EBV-driven LMP1
50. Parsonnet J, Friedman GD, Vandersteen DP, Chang Y, Vogelman JH, Orentreich N, et al.
49. Derks S, Liao X, Chiaravalli AM, Xu X, Camargo MC, Solcia E, et al.
48. Parsonnet J, Friedman GD, Vandersteen DP, Chang Y, Vogelman JH, Orentreich N, et al.
47. Fang W, Zhang J, Hong S, Zhan J, Chen N, Qin T, et al. EBV-driven LMP1
46. Parsonnet J, Friedman GD, Vandersteen DP, Chang Y, Vogelman JH, Orentreich N, et al.
45. Fang W, Zhang J, Hong S, Zhan J, Chen N, Qin T, et al. EBV-driven LMP1
44. Parsonnet J, Friedman GD, Vandersteen DP, Chang Y, Vogelman JH, Orentreich N, et al.
43. Fang W, Zhang J, Hong S, Zhan J, Chen N, Qin T, et al. EBV-driven LMP1
42. Parsonnet J, Friedman GD, Vandersteen DP, Chang Y, Vogelman JH, Orentreich N, et al.
41. Fang W, Zhang J, Hong S, Zhan J, Chen N, Qin T, et al. EBV-driven LMP1
40. Parsonnet J, Friedman GD, Vandersteen DP, Chang Y, Vogelman JH, Orentreich N, et al.
39. Fang W, Zhang J, Hong S, Zhan J, Chen N, Qin T, et al. EBV-driven LMP1
38. Parsonnet J, Friedman GD, Vandersteen DP, Chang Y, Vogelman JH, Orentreich N, et al.
37. Fang W, Zhang J, Hong S, Zhan J, Chen N, Qin T, et al. EBV-driven LMP1
36. Parsonnet J, Friedman GD, Vandersteen DP, Chang Y, Vogelman JH, Orentreich N, et al.
35. Fang W, Zhang J, Hong S, Zhan J, Chen N, Qin T, et al. EBV-driven LMP1
34. Parsonnet J, Friedman GD, Vandersteen DP, Chang Y, Vogelman JH, Orentreich N, et al.
33. Fang W, Zhang J, Hong S, Zhan J, Chen N, Qin T, et al. EBV-driven LMP1
32. Parsonnet J, Friedman GD, Vandersteen DP, Chang Y, Vogelman JH, Orentreich N, et al.
31. Fang W, Zhang J, Hong S, Zhan J, Chen N, Qin T, et al. EBV-driven LMP1
30. Parsonnet J, Friedman GD, Vandersteen DP, Chang Y, Vogelman JH, Orentreich N, et al.
29. Fang W, Zhang J, Hong S, Zhan J, Chen N, Qin T, et al. EBV-driven LMP1
28. Parsonnet J, Friedman GD, Vandersteen DP, Chang Y, Vogelman JH, Orentreich N, et al.
27. Fang W, Zhang J, Hong S, Zhan J, Chen N, Qin T, et al. EBV-driven LMP1
26. Parsonnet J, Friedman GD, Vandersteen DP, Chang Y, Vogelman JH, Orentreich N, et al.
25. Fang W, Zhang J, Hong S, Zhan J, Chen N, Qin T, et al. EBV-driven LMP1
24. Parsonnet J, Friedman GD, Vandersteen DP, Chang Y, Vogelman JH, Orentreich N, et al.
23. Fang W, Zhang J, Hong S, Zhan J, Chen N, Qin T, et al. EBV-driven LMP1
22. Parsonnet J, Friedman GD, Vandersteen DP, Chang Y, Vogelman JH, Orentreich N, et al.
21. Fang W, Zhang J, Hong S, Zhan J, Chen N, Qin T, et al. EBV-driven LMP1
20. Parsonnet J, Friedman GD, Vandersteen DP, Chang Y, Vogelman JH, Orentreich N, et al.
19. Fang W, Zhang J, Hong S, Zhan J, Chen N, Qin T, et al. EBV-driven LMP1
18. Parsonnet J, Friedman GD, Vandersteen DP, Chang Y, Vogelman JH, Orentreich N, et al.
17. Fang W, Zhang J, Hong S, Zhan J, Chen N, Qin T, et al. EBV-driven LMP1
16. Parsonnet J, Friedman GD, Vandersteen DP, Chang Y, Vogelman JH, Orentreich N, et al.
15. Fang W, Zhang J, Hong S, Zhan J, Chen N, Qin T, et al. EBV-driven LMP1
14. Parsonnet J, Friedman GD, Vandersteen DP, Chang Y, Vogelman JH, Orentreich N, et al.
13. Fang W, Zhang J, Hong S, Zhan J, Chen N, Qin T, et al. EBV-driven LMP1
12. Parsonnet J, Friedman GD, Vandersteen DP, Chang Y, Vogelman JH, Orentreich N, et al.
11. Fang W, Zhang J, Hong S, Zhan J, Chen N, Qin T, et al. EBV-driven LMP1
10. Parsonnet J, Friedman GD, Vandersteen DP, Chang Y, Vogelman JH, Orentreich N, et al.
9. Fang W, Zhang J, Hong S, Zhan J, Chen N, Qin T, et al. EBV-driven LMP1
8. Parsonnet J, Friedman GD, Vandersteen DP, Chang Y, Vogelman JH, Orentreich N, et al.