International Journal of Nutrology
(Official Journal of the ABRAN - Brazilian Association of Nutrology)

REVIEW ARTICLE

DOI: 10.54448/ijn25105

Action of probiotics and nutrients in the management of tregs cells in patients with obesity and cancer: a systematic review

Rafael Tinoco Alves[1*], Deangelo Cláudio Gomes de Lima[2], Jose Manuel Torres Garcia[3], Iuri Sanzio Souto[4], Karlla Vieira Campos Ricatto[5], Luciene Pereira de Oliveira[6], Renatto Souza Nunes Cabral[7], Celso Alexandre Alves[8], Natashira Soares Torres[9], Jussara Santos Sousa[10]

[1] Aimê Institute, Praça Doutor César Leite, 228 First Floor - Downtown, Manhuaçu – Minas Gerais, Brazil.
[2] Salvus Clinic, Pamplona Street, 925, Jardim Paulista, São Paulo, Brazil.
[3] ABC Foundation, Lauro Gomes Avenue 2000, Santo André, São Paulo, Brazil.
[4] FHEMIG - Minas Gerais State Hospital Foundation - João Paulo II Children's Hospital - Pediatric Intensive Care Unit, Belo Horizonte, Minas Gerais, Brazil.
[5] Santa Monica Health Center. Mayor Manoel Gonçalves Street, 593, downtown, Barra de São Francisco, Espírito Santo, Brazil.
[6] Acre State Hospital Foundation, nephrology, Acre, Brazil.
[7] Salvalus Hospital. Bresser, 1954, Mooca, São Paulo, Brazil.
[8] Unined BH - Belo Horizonte, Minas Gerais, Brazil.
[9] Crateus Medical Center, Ceara, Brazil.
[10] Felício Rocho Hospital, Belo Horizonte, Minas Gerais, Brazil.

*Corresponding author: Dr. Rafael Tinoco Alves.
Aimê Institute, Praça Doutor César Leite, 228 First Floor - Downtown, Manhuaçu – Minas Gerais, Brazil.
Zipcode: 36900073.
E-mail: rafaeltinocoalves@hotmail.com

DOI: https://doi.org/10.54448/ijn25105
Received: 11-17-2024; Revised: 01-21-2025; Accepted: 01-25-2025; Published: 01-30-2025; IJN-id: e25105

Editor: Dr. Manuel Ignacio Moreno Gonzalez, MD, MSc.

Abstract

Introduction: In the context of immunomodulation in patients with obesity and cancer, Bifidobacterium is one of the most commonly used probiotics and activates the anti-CTLA-4 antibody, a checkpoint inhibitor that frequently causes autoimmunity in humans undergoing cancer treatment. Immunotherapy enhances the host's immune system to produce antitumor effects, primarily by stimulating Treg cells. **Objective:** It was to highlight how probiotics and nutrients can stimulate regulatory T cells in patients with obesity and cancer, to immunomodulate and treat these patients. **Methods:** The PRISMA Platform systematic review rules were followed. The search was carried out from April to June 2024 in the Scopus, PubMed, Science Direct, Scielo, and Google Scholar databases. The quality of the studies was based on the GRADE instrument and the risk of bias was analyzed according to the Cochrane instrument. **Results and Conclusion:** A total of 137 articles were found, and 39 articles were evaluated in full, and 31 were included and developed in the present systematic review study. Considering the Cochrane tool for risk of bias, the overall assessment resulted in 27 studies with a high risk of bias and 25 studies that did not meet GRADE and AMSTAR-2. Most studies showed homogeneity in their results, with X^2=68.9%>50%. It was concluded that oral administration of butyrate, propionate, and acetate, individually or in combination, led to an increase in the number of Treg cells in the colon. Also, all-trans retinoic acid (atRA), a bioactive form of vitamin A stimulates Treg cells in the human intestine. Probiotics, together with gut microbiota, have been increasingly proposed to improve immune checkpoint blockade treatments against cancer by activating Treg cells.

Keywords: Immunomodulation. Regulatory T cells. Nutrology. Probiotics. Cancer.

Introduction

In the context of immunomodulation in patients with obesity and cancer, the introduction of Bifidobacterium,

one of the most commonly used probiotics, not only colonizes the intestine but also alters the entire microbiota landscape. This treatment has been found to activate the anti-CTLA-4 antibody, a checkpoint inhibitor that often causes autoimmunity in humans undergoing cancer treatment. This effect is due to the effect of this probiotic treatment on CD4+ regulatory cells, whose metabolic and immunosuppressive functions are altered. These CD4+ regulatory T cells are known to be a key mechanism in controlling autoreactivity in the immune system in humans **[1]**.

A revolutionary approach in the field of obesity and cancer treatment, immunotherapy enhances the host immune system to produce antitumor effects, in which immune checkpoint blockade has produced remarkable advances in the treatment of a wide range of malignancies **[2,3]**. This treatment can block negative immune regulatory factors in the tumor microenvironment by monoclonal antibodies to improve tumor immunosurveillance and enhance host immune activity against tumors **[4,5]**.

In this field, the most attractive inhibitors in both scientific research and clinical applications include cytotoxic T lymphocyte-associated molecule-4 (CTLA-4), programmed cell death receptor-1 (PD-1), and programmed cell death ligand-1 (PD-L1) **[6–8]**. However, only a small percentage of patients have significant responses to immune checkpoint blockade therapy **[9]**. Therefore, new strategies, such as the action of probiotics on the activation and regulation of regulatory T cells (Tregs), are urgently needed to improve host responses and the outcomes of immune checkpoint inhibition therapy.

Although many new immune checkpoint molecules and pathways have been explored to enhance the efficacy of existing immune checkpoint blockade therapy, they may increase the incidence and severity of immune-related adverse events **[10]**. However, emerging evidence supports that the efficacy of immune checkpoint blockade therapy strongly correlates with the baseline gut microbiota of recipients **[11–13]**. Interestingly, dysbiotic gut microbiota have been implicated in ineffective cancer therapies **[14,15]** while "healthy" gut microbiota often lead to beneficial immunotherapy outcomes **[16,17]**.

As a corollary, modulation of the gut microbiota combined with inhibition of immune checkpoints may be a promising and powerful strategy for the development of next-generation antitumor treatments **[18,19]**. Probiotics have attracted much attention due to their strong ability to modulate gut microbiota to promote gut or host health. Exogenous probiotics can directly or indirectly produce beneficial metabolites *in vivo* to activate and promote the antitumor immune response **[20]**. For example, enrichment or supplementation of Bifidobacterium species (such as B*. longum, B. breve*, and *B. bifidum*) enhanced dendritic cell (DC) antigen presentation, further promoting cytotoxic T lymphocyte (CTL) infiltration and activation in tumors, which increased the efficacy of PD-1/PD-L1 blockade-based immunotherapies **[21,22]**. Although these studies have demonstrated a role for the microbiota in antitumor immunity, the underlying events related to checkpoint antibody-induced autoimmunity remain elusive. Optimization of the gut microbiota depends on the presence of Tregs **[1]**.

Given this, a systematic review was conducted to demonstrate how probiotics can stimulate regulatory T cells in patients with obesity and cancer to immunomodulate and treat these patients.

Methods

Study Design

This study followed the international systematic review model, following the PRISMA (preferred reporting items for systematic reviews and meta-analysis) rules. Available at: http://www.prisma-statement.org/?AspxAutoDetectCookieSupport=1. Accessed on: 10/04/2024. The AMSTAR-2 (Assessing the methodological quality of systematic reviews) methodological quality standards were also followed. Available at: https://amstar.ca/. Accessed on: 10/04/2024.

Data Sources and Search Strategy

The literature search process was carried out from April to June 2024 and developed based on Scopus, PubMed, Lilacs, Ebsco, Scielo, and Google Scholar, covering scientific articles from various periods to the present day. The following descriptors (DeCS /MeSH Terms) were used: "Immunomodulation. Regulatory T cells. Nutrology. Probiotics. Cancer", and using the Boolean "and" between MeSH terms and "or" between historical findings.

Study Quality and Risk of Bias

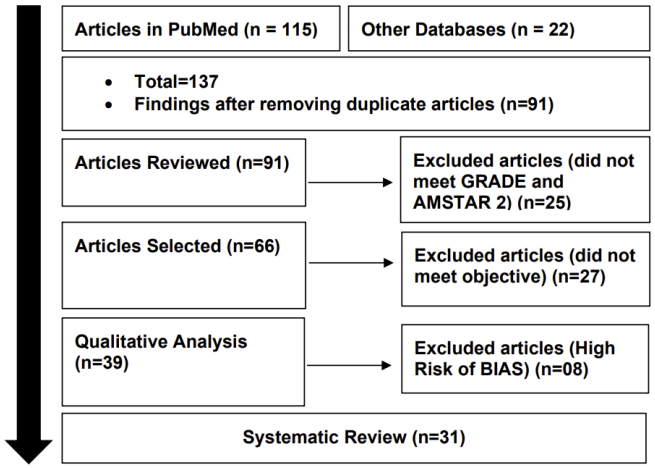
Quality was classified as high, moderate, low, or very low regarding the risk of bias, clarity of comparisons, precision, and consistency of analyses. The most evident emphasis was on systematic review articles or meta-analyses of randomized clinical trials, followed by randomized clinical trials. Low quality of evidence was attributed to case reports, editorials, and brief communications, according to the GRADE instrument. The risk of bias was analyzed according to the Cochrane instrument by analyzing the Funnel Plot graph (Sample size versus Effect size), using Cohen's d test.

Results and Discussion

Summary of Findings

A total of 137 articles were found that were submitted to eligibility analysis, and 31 final studies were selected to compose the results of this systematic review. The studies listed were of medium to high quality (Figure 1), considering the level of scientific evidence of studies such as meta-analysis, consensus, randomized clinical, prospective, and observational. Biases did not compromise the scientific basis of the studies. According to the GRADE instrument, most studies presented homogeneity in their results, with X^2=68.9%>50%. Considering the Cochrane tool for risk of bias, the overall assessment resulted in 27 studies with a high risk of bias and 25 studies that did not meet GRADE and AMSTAR-2.

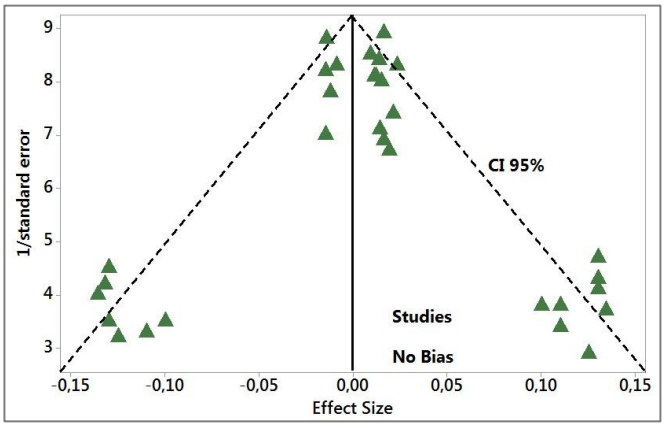
Figure 1. Flowchart showing the article selection process.



Source: Own authorship.

Figure 2 presents the results of the risk of bias of the studies using the Funnel Plot, showing the calculation of the Effect Size (Magnitude of the difference) using Cohen's Test (d). Precision (sample size) was determined indirectly by the inverse of the standard error (1/Standard Error). This graph had a symmetrical behavior, not suggesting a significant risk of bias, both among studies with small sample sizes (lower precision) that are shown at the bottom of the graph and in studies with large sample sizes that are shown at the top.

Figure 2. The symmetrical funnel plot does not suggest a risk of bias among the studies with small sample sizes that are shown at the bottom of the graph. Studies with high confidence and high recommendation are shown above the graph (n=31 studies).



Source: Own authorship.

Main Outcomes – Probiotics and Immunomodulation by Tregs

According to the literature findings, the specific mechanism by which symbionts stimulate Treg accumulation is not fully understood. It is believed that Clostridia spp. may function synergistically to stimulate Treg induction **[23]**. One proposed mechanism of action is the cooperative production of short-chain fatty acids (SCFAs), such as butyrate, propionate, and acetate, through fermentation of dietary fiber by commensals, including Clostridium and Bacteroides spp. in the colon. SCFAs are taken up by passive diffusion and H+-coupled active transport via MCT1 (SLC16A1) and MCT4 (SLC16A3), two electroneutral monocarboxylate transporters, or by electrogenic Na+-coupled monocarboxylate transporters SMCT1 (SLC5A8) and SMCT2 (SLC5A12) **[23]**.

Oral administration of butyrate, propionate, and acetate, individually or in combination, led to an increase in the number of colonic Treg cells **[24]**. Butyrate is known to participate in Treg differentiation by facilitating the acetylation of histone H3 in the promoter region and CNS1 and 3 of the Foxp3 gene **[25]**. Recognition of butyrate by G-protein-coupled receptors, such as GPR43 and GPR15 expressed by colonic Treg cells and by GPR109A expressed by dendritic cells (DCs) and macrophages **[23-25]** also promotes Treg differentiation. Interestingly, treatment with SCFA increased the number of Helios+ Tregs, indicating that SCFA also promotes the expansion of Tregs **[24]**. Furthermore, the aryl hydrocarbon receptor (AhR) is a nuclear sensor expressed by Tregs that helps them detect and react to compounds that act as AhR ligands. Intestinal pTregs have higher AhR expression than Tregs in any other tissue. Dietary tryptophan, an essential amino acid, is metabolized by IDO (indoleamine 2,3-dioxygenase) and TDO (tryptophan 2,3-dioxygenase). Kynurenine increases Foxp3+ Treg generation *in vitro* in the presence of TGFβ1. Lactobacillus spp. can also metabolize tryptophan to many AhR ligands **[26]**.

Another dietary metabolite that drives Treg cell development in the intestine is all-trans retinoic acid (atRA), a bioactive form of vitamin A. Dietary vitamin A, specifically retinol, is absorbed by intestinal epithelial cells via passive diffusion. Although epithelial cells are capable of synthesizing atRA from dietary vitamin A, a process regulated by commensal bacteria, the vast majority of studies to date have focused on dendritic cells, macrophages, and eosinophils as sources of atRA for iTreg induction **[27]**.

In addition, atRA has also been reported to suppress IL6-induced conversion of Foxp3+ Tregs into inflammatory Th17 cells, a mechanism likely mediated by the retinoic acid receptor α. Although vitamin A plays the most prominent role in Treg development, other vitamins affect this lymphocyte population. Dietary vitamin D3 is metabolized to 1,25-hydroxyvitamin D3, which binds to the vitamin D nuclear receptor element (VDRE) in the CNS noncoding region from +1714 to +2554 nt of the human Foxp gene. FR4, the vitamin B9 (folic acid) receptor, is highly expressed by Tregs and is known to promote intestinal Treg survival through upregulation of the anti-apoptotic factor BCL2 **[27]**.

Also in this context, mucosal surfaces are distinct sites exposed to environmental, dietary, and microbial antigens. Particularly in the intestine, the host continuously and actively adapts through complex interactions between the microbiota and dietary compounds immune cells, and other tissues. Treg cells are essential for fine-tuning the intestinal immune response to self and non-self antigens in the intestine. Their importance in intestinal homeostasis is illustrated by the onset of overt inflammation caused by impaired generation, function, or stability of Tregs in the intestine. A substantial imbalance in Tregs has been observed in intestinal tissue during pathogenic conditions when a tightly regulated and balanced system becomes dysregulated and leads to chronic and unimpeded immune responses **[28]**.

Human CD4+ Treg cells are characterized by the expression of the transcription factor forkhead box protein P3 (FOXP3, scurfin), high surface expression of CD25, and low or no expression of CD127. Tregs express high levels of CD25 (the α chain of the IL-2 receptor) due to their high dependence on interleukin 2 (IL-2) for their development and maintenance of peripheral homeostasis **[1]**.

In addition, the systematic interrogation of tumor-infiltrating lymphocytes is critical for the development of immunotherapies and the prediction of their clinical responses in cancers. The authors Zheng et al. (2017) **[29]** performed single-cell RNA sequencing on 5,063 single T cells isolated from peripheral blood, tumor, and adjacent normal tissues of six patients with hepatocellular carcinoma. Transcriptional profiles of these individual cells, together with assembled T cell receptor (TCR) sequences, allow us to identify 11 T cell subsets based on their molecular and functional properties and delineate their developmental trajectory. Specific subsets, such as exhausted CD8+ T cells and Tregs, are preferentially enriched and potentially clonally expanded in hepatocellular carcinoma (HCC), and signature genes for each subset were identified. One of the genes, leilin, is upregulated in activated CD8+ T cells and Tregs and represses CD8+ T cell functions *in vitro*. This compendium of transcriptome data has provided valuable insights and a rich resource for understanding the immunological landscape in cancer.

In this scenario, probiotics have been increasingly proposed to improve immune checkpoint blockade treatments against cancer. However, their causal relationship with immunotherapeutic efficacy remains unclear, leading authors Gao et al. (2023) **[30]** to explore whether and how the probiotic *Lacticaseibacillus rhamnosus* Probio-M9 manipulates the gut microbiota to achieve the expected results. It was evaluated the effects of Probio-M9 on anti-PD-1 treatment against colorectal cancer in mice through a multiomics approach. The results indicated that Probio-M9 intervention strengthened anti-PD-1-based tumor inhibition. Both prophylactic and therapeutic administration of Probio-M9 showed remarkable performance in controlling tumor growth with ICB treatment. Probio-M9 supplementation modulated the enhanced immunotherapy response by promoting beneficial microbes (e.g., Lactobacillus and *Bifidobacterium animalis*), producing beneficial metabolites including butyric acids in the gut, and accumulating blood-derived α-ketoglutaric acid, N-acetyl-l-glutamic acid, and pyridoxine in particular, which promoted the infiltration and activation of cytotoxic T lymphocytes (CTLs) and suppressed the function of Treg cells in the tumor microenvironment.

Finally, Tregs have important functions in the tumor microenvironment, mainly in inducing immune evasion. To find the underlying mechanism of Treg dysregulation in breast cancer tissues, the authors Moallemi-Rad et al. (2023) **[31]** evaluated the expression of five Treg-related long non-coding RNAs (lncRNAs) namely FLICR (FOXP3 Regulating Long Intergenic Non-Coding RNA), NEST (IFNG-AS1), RMRP (Mitochondrial RNA processing endoribonuclease RNA component), MAFTRR (MAF transcriptional regulatory RNA) and TH2-LCR (Th2 cytokine locus control region) in paired breast cancer and nearby non-cancerous tissues. The expression levels of RMRP, TH2-LCR, MAFTRR, and GATA3-AS1 were significantly higher in

breast cancer samples compared to non-tumor tissues. There were significant positive associations between RMRP gene expression level in tumor tissues and nuclear grade, tubule formation, and tumor size. Furthermore, there was a significant positive association between MAFTRR gene expression levels in tumor tissues and nuclear grade. Furthermore, FLICR expression levels were different among tumors with different HER2/neu receptor levels. Therefore, Treg-associated long noncoding RNAs (lncRNAs) may contribute to the pathogenesis of breast cancer.

Conclusion

It was concluded that oral administration of butyrate, propionate, and acetate, individually or in combination, led to an increase in the number of Treg cells in the colon. In addition, all-trans retinoic acid (atRA), a bioactive form of vitamin A, also stimulates Treg cells in the human intestine. Probiotics, together with gut microbiota, have been increasingly proposed to improve immune checkpoint blockade treatments against cancer in patients with obesity by activating Treg cells.

CRediT

Author contributions: **Conceptualization -** Rafael Tinoco Alves, Deangelo Cláudio Gomes de Lima, Jose Manuel Torres Garcia, Iuri Sanzio Souto, Karlla Vieira Campos Ricatto, Luciene Pereira de Oliveira, Renatto Souza Nunes Cabral; **Data curation-** Rafael Tinoco Alves, Deangelo Cláudio Gomes de Lima, Jose Manuel Torres Garcia, Iuri Sanzio Souto, Karlla Vieira Campos Ricatto, Luciene Pereira de Oliveira, Renatto Souza Nunes Cabral, Celso Alexandre Alves; **Formal Analysis -** Rafael Tinoco Alves, Deangelo Cláudio Gomes de Lima, Jose Manuel Torres Garcia, Iuri Sanzio Souto, Karlla Vieira Campos Ricatto, Luciene Pereira de Oliveira, Renatto Souza Nunes Cabral, Celso Alexandre Alves, Natashira Soares Torres, Jussara Santos Sousa; **Investigation -** Rafael Tinoco Alves, Natashira Soares Torres, Jussara Santos Sousa; **Methodology-** Rafael Tinoco Alves; **Project administration-** Rafael Tinoco Alves; **Supervision-** Rafael Tinoco Alves, Deangelo Cláudio Gomes de Lima; **Writing - original draft-** Rafael Tinoco Alves, Deangelo Cláudio Gomes de Lima, Jose Manuel Torres Garcia, Iuri Sanzio Souto, Karlla Vieira Campos Ricatto, Luciene Pereira de Oliveira, Renatto Souza Nunes Cabral, Celso Alexandre Alves, Natashira Soares Torres, Jussara Santos Sousa ; **Writin- greview & editing-** Rafael Tinoco Alves, Deangelo Cláudio Gomes de Lima, Jose Manuel Torres Garcia, Iuri Sanzio Souto, Karlla Vieira Campos Ricatto, Luciene Pereira de Oliveira, Renatto Souza Nunes Cabral, Celso Alexandre Alves, Natashira Soares Torres, Jussara Santos Sousa.

Acknowledgment

Not applicable.

Ethical Approval

Not applicable.

Informed Consent

It was applicable.

Funding

Not applicable.

Data Sharing Statement

No additional data are available.

Conflict of Interest

The authors declare no conflict of interest.

Similarity Check

It was applied by Ithenticate@.

Peer Review Process

It was performed.

References

1. Sun S, Luo L, Liang W, Yin Q, Guo J, Rush AM, Lv Z, Liang Q, Fischbach MA, Sonnenburg JL, Dodd D, Davis MM, Wang F. Bifidobacterium alters the gut microbiota and modulates the functional metabolism of T regulatory cells in the context of immune checkpoint blockade. Proc Natl Acad Sci U S A. 2020 Nov 3;117(44):27509-27515. doi: 10.1073/pnas.1921223117
2. Pardoll D.M. The blockade of immune checkpoints in cancer immunotherapy. Nat Rev Cancer. 2012;12(4):252–264.
3. Havel J.J., Chowell D., Chan T.A. The evolving landscape of biomarkers for checkpoint inhibitor immunotherapy. Nat Rev Cancer. 2019;19(3):133–150.
4. Chen D.S., Mellman I. Oncology meets immunology: the cancer-immunity cycle. Immunity. 2013;39(1):1–10. [PubMed] [Google Scholar]
5. Postow M.A., Callahan M.K., Wolchok J.D. Immune checkpoint blockade in cancer therapy. J

Clin Oncol. 2015;33(17):1974–1982. [PMC free article] [PubMed] [Google Scholar]

6. Wei S.C., Duffy C.R., Allison J.P. Fundamental mechanisms of immune checkpoint blockade therapy. Cancer Discov. 2018;8(9):1069–1086.
7. Seidel J.A., Otsuka A., Kabashima K. Anti-PD-1 and anti-CTLA-4 therapies in cancer: mechanisms of action, efficacy, and limitations. Front Oncol. 2018;8:86.
8. Doroshow D.B., Bhalla S., Beasley M.B., et al. PD-L1 as a biomarker of response to immune-checkpoint inhibitors. Nat Rev Clin Oncol. 2021;18(6):345–362.
9. Ribas A., Wolchok J.D. Cancer immunotherapy using checkpoint blockade. Science. 2018;359(6382):1350–1355.
10. Marin-Acevedo JA, Kimbrough EO, Lou Y. Next generation of immune checkpoint inhibitors and beyond. J Hematol Oncol. 2021;14(1):45.
11. Sepich-Poore GD, Zitvogel L., Straussman R., Hasty J., Wargo J.A., Knight R. The microbiome and human cancer. Science. 2021;371(6536).
12. Lam KC, Araya RE, Huang A, et al. Microbiota triggers STING-type I IFNdependent monocyte reprogramming of the tumor microenvironment. Cell. 2021;184(21):5338–5356.e21.
13. Sivan A, Corrales L, Hubert N, et al. Commensal Bifidobacterium promotes antitumor immunity and facilitates anti-PD-L1 efficacy. Science. 2015;350(6264):1084–1089.
14. Helmink BA, Khan MAW, Hermann A, Gopalakrishnan V, Wargo JA. The microbiome, cancer, and cancer therapy. Nat Med. 2019;25(3):377–388.
15. Mohiuddin JJ, Chu B, Facciabene A, et al. Association of antibiotic exposure with survival and toxicity in patients with melanoma receiving immunotherapy. J Natl Cancer Inst. 2021;113(2):162–170.
16. Gopalakrishnan V., Helmink B.A., Spencer C.N., Reuben A., Wargo J.A. The influence of the gut microbiome on cancer, immunity, and cancer immunotherapy. Cancer Cell. 2018;33(4):570–580.
17. Almonte A.A., Rangarajan H., Yip D., Fahrer A.M. How does the gut microbiome influence immune checkpoint blockade therapy? Immunol Cell Biol. 2021;99(4):361–372.
18. Wang Y., Ma R., Liu F., Lee S.A., Zhang L. Modulation of gut microbiota: a novel paradigm of enhancing the efficacy of programmed death-1 and programmed death ligand-1 blockade therapy. Front Immunol. 2018;9:374.
19. Zitvogel L., Ma Y., Raoult D., Kroemer G., Gajewski T.F. The microbiome in cancer immunotherapy: diagnostic tools and therapeutic strategies. Science. 2018;359(6382):1366–1370.
20. Panebianco C., Latiano T., Pazienza V. Microbiota manipulation by probiotics administration as emerging tool in cancer prevention and therapy. Front Oncol. 2020;10:679.
21. Matson V., Fessler J., Bao R., et al. The commensal microbiome is associated with anti-PD-1 efficacy in metastatic melanoma patients. Science. 2018;359(6371):104–108.
22. Lee S.H., Cho S.Y., Yoon Y., et al. Bifidobacterium bifidum strains synergize with immune checkpoint inhibitors to reduce tumour burden in mice. Nat Microbiol. 2021;6(3):277–288.
23. Atarashi K, Tanoue T, Oshima K, Suda W, Nagano Y, Nishikawa H, Fukuda S, Saito T, Narushima S, Hase K, Kim S, Fritz JV, Wilmes P, Ueha S, Matsushima K, Ohno H, Olle B, Sakaguchi S, Taniguchi T, Morita H, Hattori M, Honda K (2013) Treg induction by a rationally selected mixture of Clostridia strains from the human microbiota. Nature 500(7461):232–236. 10.1038/nature12331.
24. Smith PM, Howitt MR, Panikov N, Michaud M, Gallini CA, Bohlooly YM, Glickman JN, Garrett WS (2013) The microbial metabolites, short-chain fatty acids, regulate colonic Treg cell homeostasis. Science 341(6145):569–573. 10.1126/science.1241165.
25. Furusawa Y, Obata Y, Fukuda S, Endo TA, Nakato G, Takahashi D, Nakanishi Y, Uetake C, Kato K, Kato T, Takahashi M, Fukuda NN, Murakami S, Miyauchi E, Hino S, Atarashi K, Onawa S, Fujimura Y, Lockett T, Clarke JM, Topping DL, Tomita M, Hori S, Ohara O, Morita T, Koseki H, Kikuchi J, Honda K, Hase K, Ohno H (2013) Commensal microbe-derived butyrate induces the differentiation of colonic regulatory T cells. Nature 504(7480):446–450. 10.1038/nature12721
26. Ye J, Qiu J, Bostick JW, Ueda A, Schjerven H, Li S, Jobin C, Chen ZE, Zhou L (2017) The aryl hydrocarbon receptor preferentially marks and promotes gut regulatory T cells. Cell Rep 21(8):2277–2290. 10.1016/j.celrep.2017.10.114.
27. Grizotte-Lake M, Zhong G, Duncan K, Kirkwood J, Iyer N, Smolenski I, Isoherranen N, Vaishnava S (2018) Commensals suppress intestinal epithelial cell retinoic acid synthesis to regulate interleukin-22 activity and prevent microbial dysbiosis. Immunity 49(6):1103–1115. e1106. 10.1016/j.immuni.2018.11.018.
28. Figliuolo da Paz VR, Jamwal DR, Kiela PR.

Intestinal Regulatory T Cells. Adv Exp Med Biol. 2021;1278:141-190. doi: 10.1007/978-981-15-6407-9_9.

29. Zheng C, Zheng L, Yoo JK, Guo H, Zhang Y, Guo X, Kang B, Hu R, Huang JY, Zhang Q, Liu Z, Dong M, Hu X, Ouyang W, Peng J, Zhang Z. Landscape of Infiltrating T Cells in Liver Cancer Revealed by Single-Cell Sequencing. Cell. 2017 Jun 15;169(7):1342-1356.e16. doi: 10.1016/j.cell.2017.05.035.
30. Gao G, Shen S, Zhang T, Zhang J, Huang S, Sun Z, Zhang H. Lacticaseibacillus rhamnosus Probio-M9 enhanced the antitumor response to anti-PD-1 therapy by modulating intestinal metabolites. EBioMedicine. 2023 May;91:104533. doi: 10.1016/j.ebiom.2023.104533.
31. Moallemi-Rad L, Ghorbani A, Dadyar M, Hussen BM, Rasul MF, Eslami S, Taheri M, Jamali E, Ghafouri-Fard S. Expression of Treg-associated lncRNAs in breast cancer. Pathol Res Pract. 2023 Jan;241:154270. doi: 10.1016/j.prp.2022.154270.