OPEN ACCESS

Precision Medicine COMMENTARY

A microbial key to unlock immunotherapy resistance: Clostridium butyricum

Yiyi Chen,[1,2] Lingna Xu,[1,2] Xiya Jia,[1,2] Chuanxi Lai,[1,2] and Sheng Dai[1,2,*]

[1]Department of Colorectal Surgery, Sir Run Run Shaw Hospital, School of Medicine, Zhejiang University, Hangzhou 310016, China

[2]Provincial Key Laboratory of Precise Diagnosis and Treatment of Abdominal Infection, Sir Run Run Shaw Hospital, School of Medicine, Zhejiang University, Hangzhou 310016, China

*Correspondence: daimd@zju.edu.cn

Received: September 3, 2025; Accepted: December 10, 2025; Published Online: December 12, 2025; https://doi.org/10.59717/j.xinn-med.2026.100177

Citation: Chen Y., Xu L., Jia X., et al. (2026). A microbial key to unlock immunotherapy resistance: Clostridium butyricum. *The Innovation Medicine* **4**:100177.

Immune checkpoint blockade (ICB), particularly therapies targeting the PD-1/PD-L1 axis, has revolutionized cancer treatment. However, its efficacy remains limited in colorectal cancer (CRC), especially in microsatellite-stable (MSS) patients who often show poor response. Overcoming this primary resistance is a major therapeutic challenge, driving intense research into converting immunologically "cold" tumors into "hot" ones that are more susceptible to T-cell attack. In that setting, the emerging field of oncobiomics-that studies the microbes associated with tumors-has proven a fertile ground for new discoveries.[1] A recent ground-breaking paper by Xie et al. in Cancer Cell represents a giant step forward in this area. The study delineates that the probiotic bacterium *Clostridium butyricum (C. butyricum)* synergizes with anti-PD-1 therapy to overcome immunosuppression through a novel mechanism: direct physical interaction between the bacteria and tumor cells.[2] (Figure 1)

The research systematically moved from human epidemiology to molecular mechanism. Based on the analysis of fecal samples from CRC patients and healthy individuals, as well as other research findings, the analysis indicated that the abundance of *C. butyricum* (a type of probiotic resident in the human gut) in CRC patients was reduced. The core of their work lies in functionally characterizing a human-derived strain, Hkyucb11, and a commercial reference strain (C.b19398). The authors demonstrate convincingly across an array of in vivo models, including syngeneic orthotopic grafts, $Apc^{Min/+}$mice, and chemical (AOM/DSS) induced CRC. Through these models, they show that oral administration of *C. butyricum* not only suppresses tumor growth on its own but also significantly enhances the effect of anti-PD-1 therapy. This effect is notably consistent in both MSI-H and, importantly, MSS models, the latter being the most prevalent and therapy-resistant form of CRC.

The strength of this research is its intelligent, hierarchic experimental design, which carefully unravels the mechanism of action. In single-cell RNA sequencing and flow cytometry they deconstruct the reshaping of the immune microenvironment: *C. butyricum* increases cytotoxic CD8+ T cells (CTLs) infiltration and cytotoxic role (IFNγ, TNFα, Granzyme B) and decreases tumor-associated macrophages (TAM), particularly M2 phenotype. Specific CTL killing proved their necessity for the probiotic antitumor activity.

Arguably, the most groundbreaking discovery is direct bacterial-tumor cell communication. The authors go a step beyond the commonly held vision of probiotics working indirectly through secreted factors or alterations in gut microbiome. Xie et al. find that *C. butyricum* physically attaches CRC tumors through a highly specific protein pair: the bacterial surface protein secD binds to the host receptor GRP78 (glucose-regulated protein 78), which is selectively overexpressed on the surface of cancer cells. This interaction is not merely for adhesion, it is functionally critical. Using germ-free mice, antibiotic-treated models, and engineered E. coli expressing SecD, the authors demonstrate that the SecD–GRP78 axis is both necessary and sufficient for the observed antitumor and immunostimulatory effects. Mutational knock-out of GRP78 in tumor cells eliminates the benefit of *C. butyricum* altogether. GRP78, a stress protein, is often upregulated on cancer cell surfaces. This raises the tantalizing possibility that GRP78 may serve as a general "docking site" for other commensal bacteria, and that similar direct communication pathways might exist in other tumor types.

The downstream signaling is nicely traced. Unlike conventional cytotoxic approaches that directly kill cells, the secD-GRP78 binding acts by modulating the hyperactive PI3K-AKT-NF-κB pathway in CRC cells. That leads to reduction in the secretion of the interleukin (IL)-6, a powerful immunosuppressive cytokine hampering CTL function, and strongly associated with poor ICB outcomes. The authors then close the loop exquisitely: showing that recombinant IL-6 abolishes, and IL-6 neutralizing antibodies enhance, the *C. butyricum*-driven CTL activation. This effect, as reported by Xie et al., correlates with the reactivation of the LCK-ZAP-70 signaling axis in T cells, which is the critical first step of T-cell receptor activation. While previous studies have established that the anti-colorectal cancer mechanisms of *C. butyricum* are associated with the induction of apoptosis, cell cycle arrest, and the inhibition of pro-tumorigenic inflammation.[3]

Previous studies have revealed that microbes can modulate the tumor immune microenvironment through multiple mechanisms. By engaging Toll-like receptors via surface molecules such as LPS and flagellin, they promote the infiltration of innate immune cells into tumors. They also enhance tumor antigen presentation and activate the STING pathway. Furthermore, microbial metabolites, including short-chain fatty acids, can contribute to the remodeling of the immune microenvironment.[4] While Xie et al. uncover a fundamentally different paradigm: targeted, signal-direct modulation. This work offers a molecular connection from a bacterial colonizer to the central trigger of the adaptive immune response. Unlike indirect immune modulation, SecD-GRP78 acts directly on oncogenic PI3K-AKT signaling within tumor cells.

This work also has high clinical translatability, a feature not always seen in basic microbiome research. The authors confirm their results in a $huCD34^{+}$ humanized mouse model, which is the "gold standard" model to assess human specific immune response, and where *C. butyricum* is able to drive the infiltration and activation of human CTLs and to enhance anti-PD-1 efficacy. The validation in the autologous patient-derived organoid and tumor-infiltrating lymphocyte (TIL) co-culture system is perhaps even more impressive, as it captures the closest replica of the human tumor microenvironment,[5] where *C. butyricum* enhanced the tumor-killing by patients' own T cells, particularly when employed in conjunction with anti-PD-1.

In the future, the study by Xie et al. raises several appealing scenarios. One is to view GRP78 not just a stress indicator, but a microbial "anchor" for precisely probiotic treatment. Another is to re-engineer or administer the secD protein itself (as a biologic drug) to mimic the probiotic treatment without using a living bacteria. And of course, these findings pave the way for clinical trials combining oral *C. butyricum* supplementation with anti-PD-1 in CRC patients, particularly those with MSS disease who have few other options. Furthermore, exploring whether the SecD–GRP78 axis is specific to colorectal cancer or extends to other GRP78-high tumors (e.g., hepatocellular carcinoma, glioblastoma) could significantly broaden the translational potential of this strategy.

As for the study, the research also has several shortcomings.

First, the efficacy and safety of this therapy in human patients remain to be validated in clinical trials. Although this study has demonstrated that the bacterial protein SecD alone can inhibit tumors and promote an immunotherapeutic response, eliminating the need for live bacteria and avoiding their associated side effects. But further investigation is still required to assess potential risks such as excessive immune activation that may lead to cytokine storm. In addition, drug delivery methods and manufacturing constraints will need to be systematically addressed.

Second, the gut microbiota is a complex consortium, and the interactions between Clostridium and other commensal bacteria may be crucial for sustained effects. Engineered bacteria may compete with the natural microbial community in tumors for nutrients and space. Metabolites produced by

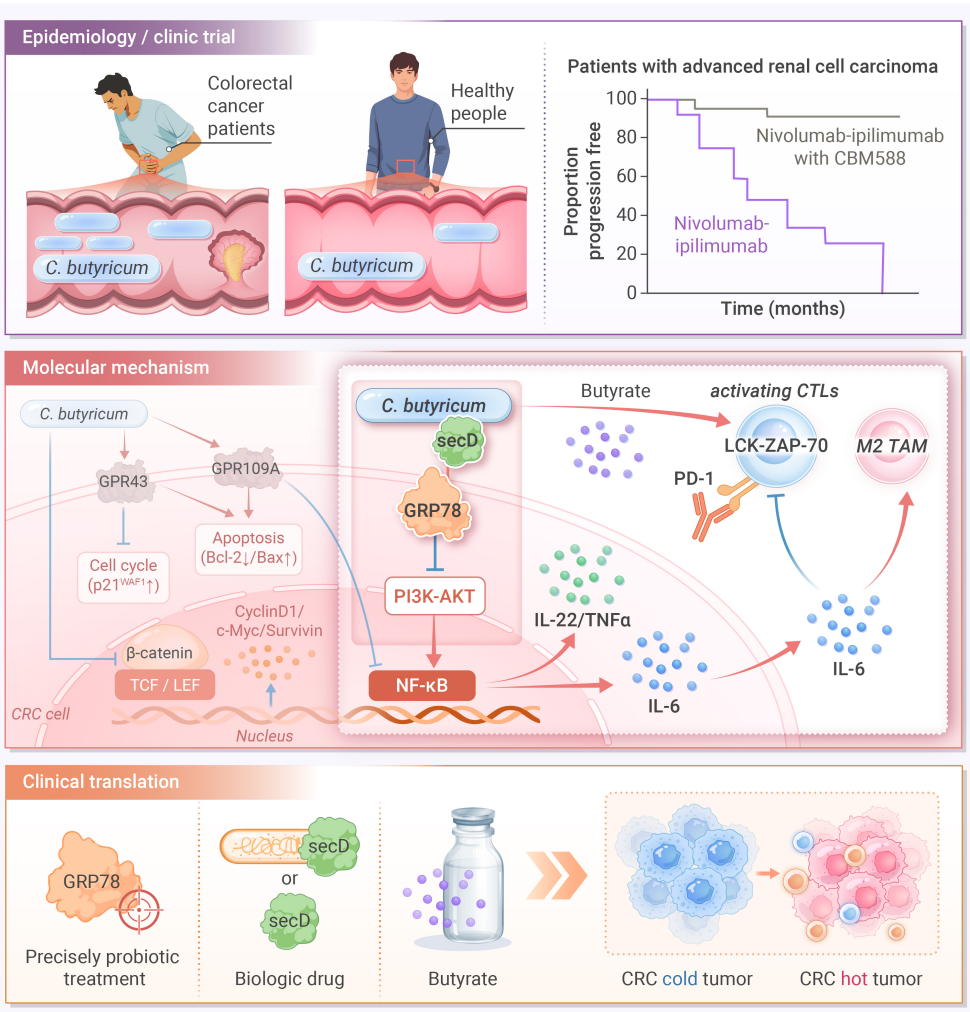

Figure 1. From bed to bench to bed: mechanism of Clostridium butyricum in suppressing colorectal cancer via direct binding and immune activation
C.butyricum is significantly reduced in CRC patients compared to healthy individuals. And a clinical study in patients with advanced renal cell carcinoma showed that combining CBM588 (which contains *C. butyricum*) with immunotherapy significantly improved progression-free survival rates. The bacterial surface protein SecD binds specifically to GRP78 overexpressed on CRC cells. This binding inhibits the PI3K-AKT-NF-κB oncogenic signaling pathway, resulting in decreased secretion of IL-6, an immunosuppressive cytokine. Reduced IL-6 levels alleviate suppression of cytotoxic T lymphocytes (CTLs), leading to reactivation of the LCK-ZAP-70 signaling axis and enhanced production of effector cytokines IFNγ and TNFα. Meanwhile, previous studies have shown that *C. butyricum* fights colorectal cancer through apoptosis, cell cycle arrest, and the suppression of protumorigenic inflammation. From a translational perspective, the findings of this study suggest that GRP78 represents a novel therapeutic target. Additionally, the bacterial surface protein SecD and SecD-expressing engineered bacteria also demonstrate therapeutic potential. Furthermore, butyrate produced by *C. butyricum* has been shown to suppress colorectal tumor. All these diverse clinical translation strategies hold the potential to convert cold tumors into hot ones.

different microbes can also interact, affecting treatment outcomes. Moreover, introducing external microbes risks disrupting the stability of the body's own microbiota.

Third, this work demonstrated that C. butyricum exhibits a higher binding affinity for CRC cells than for normal or immune cells, and confirmed its colonization within orthotopic tumors. However, two key questions remain for further exploration: the durability of this colonization in a clinical setting, and the underlying mechanism of how orally administered probiotics achieve tumor-specific homing and colonization, such as via tumor vascular leakiness or immune cell-mediated transport.

Last but not least, GRP78 plays a role in adapting to chronic stress and exerts an anti-apoptotic function in cells, thus targeting GRP78 poses a risk of off-target effects on stressed but non-malignant tissues, which could lead to adverse effects. However, this work has pointed out that GRP78 was specifically localized to the membrane of CRC cells and absent in normal NCM460 cells, indicating that secD potentially targets cancer cells specifically.

In conclusion, Xie et al. have presented a textbook example of translational cancer research by using advanced models and mechanistic dissection to rise above correlation to clearly delineate causality. Their work not only promises such an adjuvant strategy to overcome ICB-resistance in CRC, but also offers a vision for precision medicine that utilizes engineered probiotics or their biomolecules. The path forward is clear: cautiously validate these results in humans and ask if this microbial key could break doors to treatment in even more diseases.

REFERENCES

1. Zhang Z., Gao Q., Ren X., et al. (2023). Characterization of intratumor microbiome in cancer immunotherapy. *The Innovation* **4**:100482. DOI:10.1016/j.xinn.2023.100482
2. Xie M., Yuan K., Zhang Y., et al. (2025). Tumor-resident probiotic Clostridium butyricum improves aPD-1 efficacy in colorectal cancer models by inhibiting IL-6-mediated immunosuppression. *Cancer Cell* **43**:1885–1901.e10. DOI:10.1016/j.ccell.2025.07.012
3. Stoeva M. K., Garcia-So J., Justice N., et al. (2021). Butyrate-producing human gut symbiont, Clostridium butyricum, and its role in health and disease. *Gut Microbes* **13**:1–28. DOI:10.1080/19490976.2021.1907272
4. Gurbatri C. R., Arpaia N. and Danino T. (2022). Engineering bacteria as interactive cancer therapies. *Science* **378**:858–864. DOI:10.1126/science.add9667
5. Han X., Cai C., Deng W., et al. (2024). Landscape of human organoids: Ideal model in clinics and research. *The Innovation* **5**:100620. DOI:10.1016/j.xinn.2024.100620

FUNDING AND ACKNOWLEDGMENTS

Not applicable.

AUTHOR CONTRIBUTIONS

All authors contributed to the manuscript and approved the final version.

DECLARATION OF INTERESTS

The authors declare no competing interests.