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Antibiotic nanoparticles boost antitumor immunity

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Liposomes loaded with antibiotics eliminate intracellular bacteria in a colorectal cancer model, unleashing antitumor T cell immunity.

Cancer immunotherapy, which requires T cell recognition of tumor neoepitopes, can fail in the face of tumors that have few mutations. In colorectal cancer (CRC), for example, so-called microsatellite-stable tumors — those with a low mutational burden — account for around 85% of cases. Writing in *Nature Biotechnology*, Wang et al.[1] propose a solution for such tumors if they are colonized by bacteria. They show that pH-sensitive liposomes loaded with antibiotics can selectively kill anaerobic bacteria in tumors in mice, expose novel microbial epitopes and activate cross-reactive $CD8^+$ T cell immunity that eliminates both bacteria-infected and uninfected tumor cells (Fig. 1). The work introduces an approach for counteracting the poor immunogenicity of tumors with low mutational burden and offers insights into how bacterial death within tumors influences $CD8^+$ T cell immunity through microbial neoepitopes.

In cancer immunotherapy, stimulation of antitumor T cell responses requires recognition between T cell receptors and tumor-derived epitopes presented by the major histocompatibility complex class I (MHC-I)[2]. However, tumors with a low mutational burden have few neoantigens, which limits the repertoire of antitumor T cells[3]. Bacteria may enter both tumor cells and antigen-presenting cells in tumor tissues[4], suggesting that microbial neoepitopes might engage antitumor T cell immunity, and several studies have reported that microbial peptide antigens can be presented by human tumor cells and can activate T cells ex vivo[5,6]. However, it has been difficult to exploit this phenomenon for cancer therapy.

Wang et al. analyzed a French nationwide database of CRCs and breast cancers and determined that antibiotic treatment targeting anaerobic bacteria improved the survival of patients with CRC but not of those with breast cancer. This finding led them to examine whether anaerobic bacteria, in particular the oncogenic *Fusobacterium nucleatum* prevalent in human CRCs, correlate with tumor growth. Indeed, the authors observed hypoxia-induced invasion of *F. nucleatum* into CRC tumor cells in vitro. Interestingly, in mice bearing orthotopic CRC tumors, the presence of *F. nucleatum* infection increased tumor growth >30-fold, with more extensive metastasis and stronger immunosuppressive profiles.

To evaluate intratumoral anaerobic bacteria as a new therapeutic target, the authors engineered a pH-responsive liposomal formulation that encapsulates an antimicrobial silver ion-tinidazole complex (LipoAgTNZ). After intravenous administration in an orthotopic mouse model of CRC, LipoAgTNZ accumulated in tumor and liver tissues and killed intratumoral bacteria, without disrupting the gut microbiota. The therapy reversed the immunosuppressive phenotype in CRC tumors and had a stronger antitumor effect than tinidazole or LipoAg treatment alone, with >80% of LipoAgTNZ-treated mice being tumor-free and surviving, as compared to none in the controls. Furthermore, adoptive transfer of T cells from survivors previously treated with LipoAgTNZ into recipient mice bearing orthotopic CRC tumors led to substantial tumor control in *F. nucleatum*-infected as

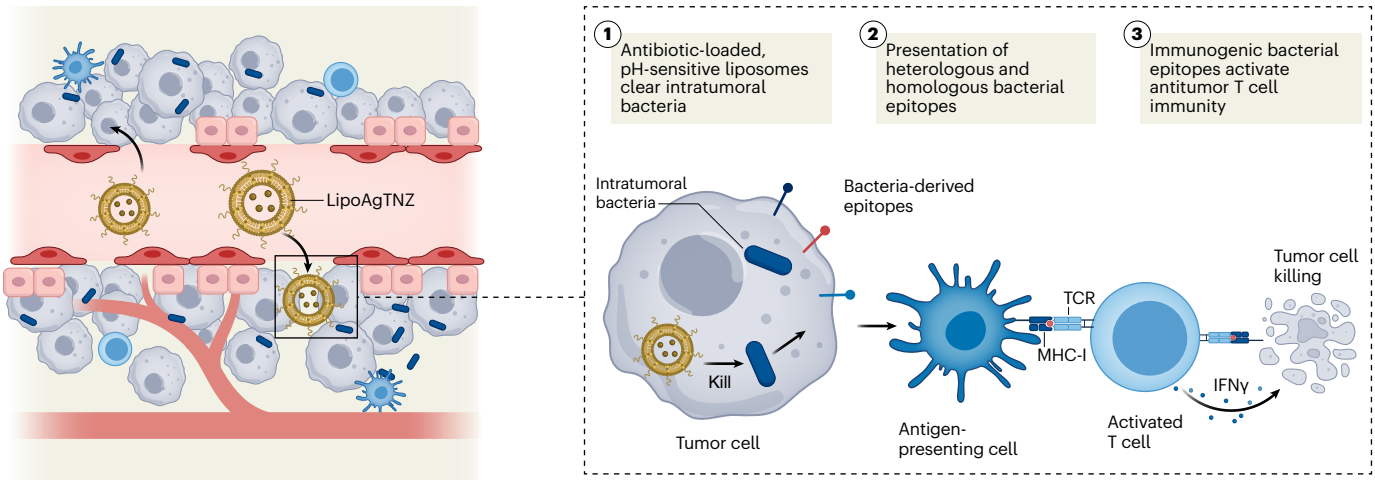

Fig. 1 | Antibiotics-loaded liposomes kill intratumoral bacteria and elicit antitumor T cell immunity. Intravenous administration of LipoAgTNZ nanoparticles carrying silver ion and tinidazole leads to their accumulation in the tumor and elimination of intratumoral bacteria (for example, *F. nucleatum*). Bacterial death releases heterologous and homologous microbial epitopes, which are presented by antigen-presenting cells to activate the antitumor T cell immune response. IFNγ, interferon-γ.

well as uninfected tumors, demonstrating that T cells were the active agent of tumor control.

To define the relationship between intratumoral bacterial death and favorable T cell immune surveillance, Wang et al. conducted a topologic analysis of the proteome and proteomics of *F. nucleatum*. They predicted MHC-I binding peptides from top-ranked cytoplasmic proteins in the quantitative proteomics of *F. nucleatum* and selected five top-ranked microbial neoantigens with a high total score and MHC-I binding affinity. They also predicted another three homologous antigens shared by bacteria and mice using the Immune Epitope Database. These bacterial and homologous epitopes were able to induce interferon-γ-secreting splenic T cells from mice treated with LipoAgTNZ. Through a coculture assay with MHC-I tetramer–peptide complex and T cells, the authors also identified the bacteria–host shared epitopes LADDNFSTI and RGVPQIEVTF as the key targets recognized by tumor-infiltrating T cells.

This work points to several directions for future studies. Although activation of host T cells by targeted bacterial epitopes after LipoAgTNZ treatment was verified ex vivo, it remains to be demonstrated in vivo. The authors' finding that exposure of patients with breast cancer to bactericidal nitroimidazole or lincomycin provided no protective effect appears at odds with a study[7] claiming that intratumoral bacterial biomass in patients is low and that breast cancer harbors a richer and more diverse microbiome than other tumor types. Furthermore, although intravenous LipoAgTNZ can kill bacteria in tumors while maintaining homeostasis of the gut microbiome, this strategy relies on the enhanced permeability and retention effect, which remains to be validated in humans with cancer[8].

The translational potential of the authors' approach depends on a host of unanswered questions. Do most tumors harbor enough intratumoral bacteria to release a sufficient quantity of microbial neoantigens? What properties of microbial neoantigens (for example, affinity or immunogenicity) are necessary to trigger cross-reactive host $CD8^+$ T cell immunity? Will the liposomal formulation be effective in spontaneous human colorectal tumors? In at least one study[9], an intratumoral microbiome signature was associated with enhanced long-term survival in patients with pancreatic adenocarcinoma. Thus, the impact of the intratumoral microbiome and antibiotics on patient outcomes should be studied further across various types of cancer.

In summary, the study of Wang et al. provides an intriguing demonstration that liposomal formulations can clear intratumoral bacteria, yield microbial sources of neoantigens and induce cytotoxic immune responses against tumors. The specific homologous epitopes identified in this work may be useful in cancer vaccines to activate antitumor cytotoxic T cell response against colorectal tumors. In addition, the authors' engineering strategy may be combined with other immunotherapeutic agents, such as immune checkpoint blockers, cancer vaccines and chimeric antigen receptor T cells, to overcome the challenge of tumors with low mutational burden.

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Competing interests

J.J.M. declares financial interests for board membership, as a paid consultant, for research funding, and/or as an equity holder in EVOQ Therapeutics and Saros Therapeutics.