

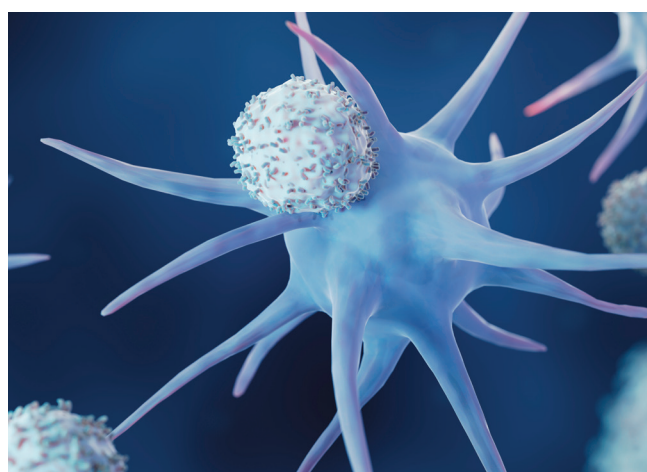
Connecting the Gut Microbiome to Cancer Treatment Efficacy

Immune checkpoint blockade therapy (ICT) is a type of immunotherapy used to treat previously incurable cancers. Various host factors influence ICT efficacy, but it is unknown how gut microbiota, particularly bacteria, affect anticancer immune responses in extraintestinal tumors. In a recently published paper in *Science Immunology*, Yongbin Choi and his colleagues from the University of Texas Southwestern Medical Center described how they used PhenomeX's IsoCode® Single-Cell Secretome platform and IsoSpark™ system to investigate alterations to ICT efficacy in a preclinical melanoma model following antibiotic treatment.¹

Investigating gut bacterial translocation during ICT treatment

ICT employs immune checkpoint inhibitors to block proteins, such as cytotoxic T lymphocyte-associated antigen 4 (CTLA-4) or programmed cell death protein 1 (PD-1), on the lymphocyte's surface. This enables the immune system to mediate tumor cell killing. Yet, up to 50 percent of patients do not respond to ICT.² In prior studies, researchers revealed that tumor-containing mice depleted of gut bacteria, either through antibiotic treatment or living in a germ-free environment, do not respond well to ICT treatment.^{3,4} These results indicate that the gut microbiome affects ICT efficacy, where ICT treatment could exploit the bacteria-activated immune system for cancer cell killing.

For patients with tumors located in the gastrointestinal tract, it is easy to understand how gut bacteria could promote an anticancer immune response, given the relative proximity of the bacteria to the tumor site. However, researchers do not know how gut bacteria



alter the immune response to cancers located in distant sites, including melanoma and lung cancer. To investigate if gut bacteria could translocate into melanoma tumors, Choi and his research team treated mice containing B16-F10 melanoma tumors every four days with the immune checkpoint inhibitors anti-PD-1 and anti-CTLA-4. Twelve days after the initiation of ICT treatment, they harvested the tumors and cultured the bacteria within the tissue homogenates. The researchers detected bacterial components in the melanoma tumors after the first dose of ICT, suggesting that ICT induces gut bacteria translocation into tumors. Because secondary lymphoid organs are critical for a positive response to ICT, they also investigated if bacteria could translocate into the spleen, mesenteric lymph nodes (MLNs), or tumor-draining lymph nodes (TDLNs).⁵ Cultured bacterial levels in each organ increased upon ICT treatment, which indicated that ICT augments gut bacterial translocation into secondary lymphoid organs.

Assessing the role of MLNs in ICT treatment

Choi and his colleagues examined which secondary lymphoid organ was required for a high ICT efficacy by surgically resecting spleens, MLNs, and TDLNs from tumor-containing mice before applying ICT treatment. They determined that MLN removal significantly increased tumor size and decreased survival time, which indicated that MLNs are critical for ICT's anticancer response. Moreover, MLN resection reduced the bacterial load within the TDLNs and the tumor site, suggesting that MLNs enable gut bacteria to move to distant sites within the body. Lastly, mice lacking MLNs had a lower abundance of CD11c⁺ dendritic cells (DCs) and CD8⁺ T cells in their TDLNs. Both immune cell types are vital for antitumor immunity, where DCs become activated through interactions with bacteria and go on to prime CD8⁺ T cells to produce the immune response.

Characterizing cytokine secretion from immune cells

Patients with cancer have a reduced response to ICT when treated with antibiotics, potentially due to reduced gut bacterial loads and, thus, diminished immune system activation. To investigate if antibiotics affect the T cell-mediated antitumor response, Choi and his research team treated mice with the antibiotics streptomycin and penicillin G before tumor inoculation and ICT addition. They then isolated CD8⁺ T cells from the MLNs and TDLNs of these mice and examined their cytokine profiles using PhenomeX's IsoCode® Single-Cell Secretome platform and the IsoSpark™ system. The researchers analyzed their data and generated visuals using the PhenomeX IsoSpeak™ software suite. They observed a reduction in the abundance of CD8⁺ T cells that secrete effector cytokines, such as interferon-γ or granzyme B, in the MLNs and TDLNs of antibiotic-treated mice as compared to mice not treated with the antibiotics. Additionally, the antibiotics decreased the ability of CD8⁺ T cells to secrete two or more cytokines, known as T cell polyfunctionality. Because scientists have linked high T cell polyfunctionality to enhanced patient responses to ICT treatment, these

results indicated that antibiotics reduce ICT efficacy by altering cytokine secretion from CD8⁺ T cells.^{6,7} Moreover, the researchers also assessed the cytokine profiles of DCs isolated from the TDLNs of mice treated with or without antibiotics using PhenomeX's CodePlex® platform to measure bulk cytokine levels. They observed diminished cytokine levels from antibiotic-treated mice compared to untreated mice, indicating that antibiotics also decrease ICT efficacy by reducing DC activation.

Cytokine profiling of immunotherapies

Using the IsoCode® Single-Cell Secretome platform and CodePlex® platform with the IsoSpark™ instrument, Choi and colleagues determined that gut microbiome health has a direct effect on T cell polyfunctionality, DC activation, and, consequently, ICT efficacy in mice with melanoma tumors. This study could provide the framework for future investigations into how gut bacteria affect ICT efficacy in patients with other extraintestinal cancers, as well as how the gut microbiome influences the effectiveness of additional immunotherapies.

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