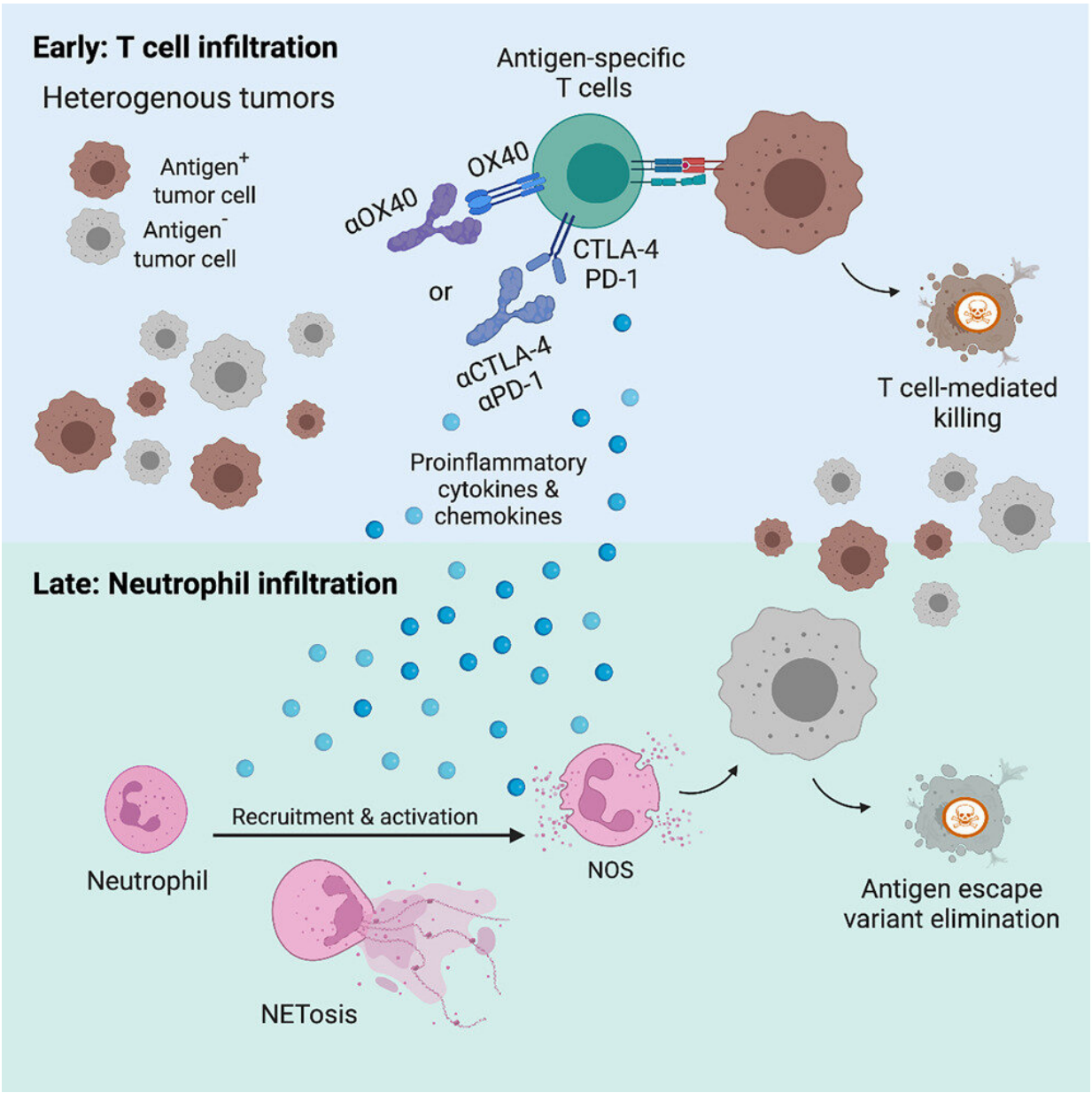
Two cancer research studies reveal essential role of neutrophils in immunotherapy

April 1 2023



Graphical abstract. Credit: Daniel Hirschhorn et al, T cell immunotherapies engage neutrophils to eliminate tumor antigen escape variants, *Cell* (2023). DOI: 10.1016/j.cell.2023.03.007

Two independent Ludwig Cancer Research studies published in the current issue of the journal *Cell* show that immune cells known as neutrophils, whose abundance in the microenvironment of tumors has traditionally been associated with poor patient prognosis, can play an important role in the success of cancer immunotherapies.

One study, co-led by Ludwig Lausanne Member Mikaël Pittet and Allon Klein of Harvard Medical School, identifies a functional state assumed by neutrophils following immunotherapy—termed the $Sell^{hi}$ state—in which they become formidable agents of antitumor immunity in mouse models of lung and colon cancer.

"Our results demonstrate that neutrophils, which play an important role in battling microbial pathogens, can also be mobilized to fight cancer if they are appropriately engaged," said Pittet.

The other Ludwig study, led by Taha Merghoub and Jedd Wolchok, co-directors of the Ludwig Collaborative Laboratory at Weill Cornell Medicine, simultaneously discovered in a mouse model of melanoma that neutrophils are essential to the complete destruction of tumors during immunotherapies such as immune checkpoint blockade (ICB). Reflecting these findings, tumor samples from patients successfully treated with ICB were found to be teeming with neutrophils.

"Our study identified a unique subpopulation of neutrophils with anti-tumor activity," said Merghoub. "When activated by an experimental immunotherapy, this subpopulation kills cancer cells that have evolved to

escape a key mechanism of immune recognition and so helps eliminate advanced melanoma tumors in mice."

Most immunotherapies in use today, including ICB, primarily activate CD8+ T cells, which recognize and destroy cancer cells. Multiple immune cell types display a degree of plasticity, which can shift them from states that support tumor growth to those that eliminate tumors. A growing body of evidence from mouse studies suggests that neutrophils too display such functional dichotomy. Yet nuanced investigation of their behavior in tumors and during immunotherapy has long been neglected, in part due to the limitations of techniques researchers use to isolate and assay individual cells in such studies.

Pittet, Klein and their colleagues, who have been exploring the subtle functional differences within neutrophil populations, found that in both mice and in human lung cancers, neutrophil numbers surge in tumors that respond to immunotherapy. They show in models of lung and colon cancer that, in responsive tumors, it is neutrophils in the $Sell^{hi}$ state that explode in number and that their expansion is essential to the success of immunotherapy.

"We found that if we block this therapy-elicited neutrophil response in mice, the benefits of immunotherapy are lost," said Pittet.

The researchers show in this study that neutrophils pushed into a $Sell^{hi}$ state during effective immunotherapy bear this gene expression pattern. Their dissection of neutrophil activation in a mouse model of colon cancer reveals that it rests on three key pillars of anti-tumor immunity. It depends, specifically, on the production by related immune cells of a factor (IL12) that activates killer T cells, which in turn produce a signaling protein named interferon-ɣ, further stimulating immune responses and—crucially—enabling the $Sell^{hi}$ neutrophil response.

Merghoub, Wolchok and their team were studying a different aspect of tumor immunology when they made their neutrophil discovery.

As tumors advance, their constituent cancer cells often evolve to stop producing antigens—protein fragments that betray disease to T cells and other immune cells—to escape immune targeting. The researchers had previously shown that a combination immunotherapy they've been developing could eliminate advanced melanoma tumors in a mouse model, apparently overcoming such immune evasion.

That combination involves a chemotherapy and a pair of experimental immunotherapies: an infusion of CD4+ T cells engineered to target a melanoma antigen, and treatment with antibodies that activate a molecule on T cells known as OX40. The latter therapy switches the CD4+ T cells, better known for orchestrating immune responses, into a cancer cell-killing mode while inactivating regulatory T cells that suppress immune responses.

In the current study, Merghoub, Wolchok and their colleagues explored the mechanisms by which their experimental therapy clears cancer cells that have evolved to escape T cell recognition using a mouse model devised for the study of antigenic heterogeneity.

Their analysis revealed that tumors in the mice were consistently infiltrated with activated neutrophils following immunotherapy, something they also saw in tumor samples from melanoma patients who had responded well to ICB therapy. Depleting neutrophils in the mice compromised the curative efficacy of the experimental therapy. They also show that these neutrophils contribute to tumor elimination in a model of colorectal cancer.

"Our findings reveal a previously unrecognized and essential role played by neutrophils in mopping up cancer cells that evade the T cell attack

stimulated by immunotherapies," said Wolchok. "They reiterate the importance of other types of immune cells in the success of ICB and other immunotherapies that activate T cell responses to treat cancer."

Echoing the findings of the Ludwig Lausanne study, the Ludwig Weill Cornell Medicine study identified unique gene expression signatures and cell surface molecular markers in tumor-targeting neutrophils. Further, activated neutrophils that helped clear cancer cell variants that had eluded T cell recognition expressed high levels of an enzyme that drives production of nitric oxide and is associated with enhanced cell killing capability in neutrophils.

"Our studies have independently reached the same conclusion about the importance of neutrophil activity to the success of distinct immunotherapies in models of three different types of tumors," noted Pittet. "This suggests we have discovered an important dimension of tumor immunology—one that, we hope, will pave the way for new treatment strategies and the improvement of existing immunotherapies against cancer."

More information: Jeremy Gungabeesoon et al, A neutrophil response linked to tumor control in immunotherapy, *Cell* (2023). DOI: 10.1016/j.cell.2023.02.032

Daniel Hirschhorn et al, T cell immunotherapies engage neutrophils to eliminate tumor antigen escape variants, *Cell* (2023). DOI: 10.1016/j.cell.2023.03.007

Provided by Ludwig Institute for Cancer Research