Uncovering cancer:

How enlisting T cells can boost the power of immunotherapy

By Amanda Keener

Five years ago, Mikael Pittet, a cancer immunologist who studies lung cancer at Harvard University Medical School in Boston, embarked on a challenging experiment. He wanted to get immune cells to attack lung tumors in a strain of mice that is genetically engineered to develop lung tumors modeling adenocarcinoma. In humans, adenocarcinoma is to blame for more than 1 million deaths per year worldwide, making it the leading cause of cancer deaths. The problem for Pittet, however, was that this type of tumor is also known for its failure to attract the very immune cells that he wanted to activate—a type of cancer-killing immune cell called CD8 T cells.

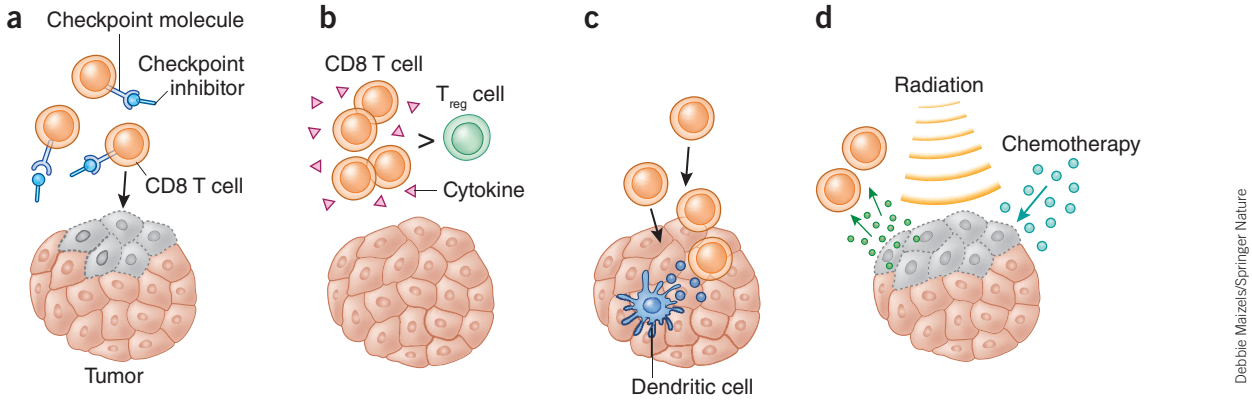
Some of Pittet's colleagues told him to drop the project, saying that he was shooting himself in the foot by choosing such a difficult-to-treat model. But, he says, "the fact that we have these tumors that are essentially unresponsive is really important to me, because that's the problem that we face in the clinic."

Immunotherapies such as checkpoint-blockade inhibitors, which release the brakes on T cells, have transformed cancer therapy by making it possible to turn a body's own immune system against the cancer. But these therapies don't work for everyone. Even Pittet's mice proved unresponsive to checkpoint inhibitors when he administered the therapy to the animals. It's not clear, however, why this is the case, either in mice or in people. For most types of cancer tested so far, only about 20% of individuals respond to checkpoint-inhibitor therapies such as ipilimumab (anti-CTLA-4) or nivolumab (anti-PD-1) when given the drug alone[1]. Cancer researchers think that a lack of immune activity in some tumor types could be part of the reason for this failure. Lack of understanding continues to be a problem, because most chemotherapies have been screened in cell-culture dishes and immunodeficient mice, which don't have a well-functioning immune system, Pittet says.

Before the development of checkpoint inhibitors, there was little incentive to select drugs that would make cancer cells interesting to the immune system—or more immunogenic—as they died. But with the advent of immunotherapy, the cancer field was forced to pay attention to how, if at all, cancer cells attracted the immune system. To this end, Pittet and his team homed in on a group of molecular triggers that cancer cells sometimes release as they die. These triggers, collectively known as damage-associated molecular-pattern molecules (DAMPs), activate several components of the antitumor immune response and draw T cells into the tumor tissue.

Pittet's doggedness paid off. When he and his team screened US Food and Drug Administration–approved chemotherapies for their ability to induce the release of DAMPs, they identified two drugs, called oxaliplatin and mafosfamide. These drugs in combination caused cancer cells in culture dishes to release DAMPs and caused CD8 T cells to flood the animals' tumors[2]. When combined with anti-PD-1 and anti-CTLA-4, the dual chemotherapy regimen prevented tumor growth for more than 200 days in all five treated mice. "If we increase survival by several weeks, we're actually pretty happy," Pittet says. By contrast, mice given just the checkpoint inhibitors or just the chemotherapy regimen all experienced tumor growth during the same time frame.

In recent years, Pittet and other researchers



Tackling tough tumors: New cancer therapies in development use a variety of approaches to make tumors more susceptible to T cell attack. (**a**) If they can reach a tumor, CD8 T cells kill cancer cells (dying cells in gray) with the help of checkpoint-inhibitor therapy. (**b**) Therapy with cytokines, such as interleukin 2, can tip the balance in favor of CD8 T cells over regulatory T cells. (**c**)The activation of dendritic cells releases cytokines (blue dots) that entice T cells into tumors. (**d**) Radiation and certain types of chemotherapy cause dying cancer cells (gray) to release molecules that attract T cells to the tumor.

have begun to coalesce around the idea that an increased presence of T cells in a tumor makes the tumor more likely to respond to immunotherapies. Building on this understanding, several companies and researchers are developing strategies to awaken a T cell response against tumors that have previously been impervious to immune attack. Whether through enhancing immunogenic tumor cell death or by tinkering with immune cell populations, these strategies aim to flood tumors with T cells and give checkpoint-inhibitor therapy a boost against otherwise unresponsive cancers. When T cells infiltrate tumors, "there is a better chance to control the tumors," says Pittet.

A good death

Many companies are now using immunogenic cell death as the basis for new approaches to increase the success of checkpoint inhibitors against tumors that tend to remain hidden from T cells. Intensity Therapeutics, a biotech company based in Westport, Connecticut, has developed a formulation called INT230-6, which pairs two common chemotherapies, vinblastine and cisplatin, with a molecule that enhances the drugs' ability to penetrate cell membranes.

Intensity has been working with vaccine immunologists at the US National Cancer Institute (NCI), and has tested the drug against mouse pancreatic, colon, and breast cancer tumors grown under the skin of mice. At the annual meeting of the American Association for Cancer Research (AACR) in April, NCI scientists reported that daily injections of INT230-6 into colon cancer tumors grown under the skin of mice for five days resulted in a surge of CD8 T cells into the tumors.

When the NCI researchers combined INT230-6 with anti-PD-1 therapy, the tumors were untraceable in five of the nine treated animals. "These mice have about two weeks left to live when we start the treatment, and 55% get cured and never get cancer again," says Lew Bender, Intensity's CEO and founder. By contrast, there were no changes to the tumors of any of the mice, which were immunocompetent, when they were treated with checkpoint inhibitors alone. The company plans to test its drugs in combination with an anti-PD-1 antibody in its ongoing phase 1/2 trial, in which 60 patients with various forms of cancer will receive injections into their tumors once a month for five months.

Anja Bloom, an immunologist at the NCI who headed up these experiments, says that she is now working to define the mechanisms that entice CD8 T cells into INT230-6-treated tumors. So far, she has found that the drug attracts to the tumors innate immune cells called dendritic cells, which digest and display pieces of dying cancer cells to train T cells to recognize cancer as something foreign. It's not clear yet, however, whether the drug induces the release of DAMPs.

Other groups in the field are also focused on understanding how cell death activates T cells and a broader immune response. In the late 2000s, a team headed by Guido Kroemer and Laurence Zitvogel at the Gustave Roussy Institute of Oncology near Paris began to define what makes a dying tumor cell immunogenic. In 2007, they reported that a chemotherapy that caused dying cancer cells in culture to move a protein called calreticulin from the cytoplasm to the cell membrane allowed those cells to trigger an antitumor immune response once injected into mice[3]. That immune response also protected the mice from developing tumors after researchers later injected live cancer cells. They went on to add high-mobility-group box 1 protein (HMGB1)—which normally acts in the nucleus to regulate gene transcription—and the energy-containing molecule adenosine triphosphate (ATP) to the list of DAMPs released by some dying cells.

Chemotherapy is not the only way to induce immunogenic cell death. For example, Pittet is now working with the Norwegian company Lytix Biopharma to develop a peptide that breaks apart cancer cells and causes them to release DAMPs that attract T cells[4]. Meanwhile, dozens of trials around the world are testing whether they can improve treatment by combining radiation with either ipilimumab or nivolumab. Laurent Levy, CEO and founder of the Paris-based company Nanobiotix, says that things really got exciting for his company about two years ago, when it started taking note of what its lead candidate, NBTXR3, does to tumor immunology. When cancer cells treated with NBTXR3 are hit with radiation, Levy says, the nanoparticles produce reactive atoms called free radicals. These molecules in turn amplify damage to the tumor cells, causing them to release more DAMPs and set off an antitumor immune response. "Instead of using biology or chemistry to kill cancer, we use physics," Levy says.

This year, at an immunotherapy workshop co-sponsored by the NCI, the Society for Immunotherapy of Cancer, and the American Society for Radiation Oncology, Levy and his colleagues reported preliminary results from a phase 2/3 trial to treat individuals with soft-tissue sarcoma. Their findings showed that

Mikael Pittet, MGH

T cell tester: Immunologist Mikael Pittet.

combining NBTXR3 treatment with radiation increased the average number of CD8 T cells in eight patient tumors. Tumors from another set of six people who received only radiation, without NBTXR3, on the other hand, displayed no increase in CD8 T cells. The trial, which is still ongoing, was not designed to test the role of the immune response in overall survival, but Levy says that the company is taking advantage of biopsy samples from the trial to explore how the nanoparticles alter immune cells and gene expression in treated tumors. Nanobiotix is now planning a trial of its product in combination with checkpoint inhibitors. "Potentially, we can apply this across oncology," Levy says.

Although researchers are relying on the presence of CD8 T cells in a tumor to determine whether the tumor may be more likely to respond to checkpoint-inhibitor therapy, Adi Diab, an oncologist at MD Anderson Cancer Center in Houston, cautions against using these T cells as tell-tale markers. "In reality, we do not have absolute predictors for immunotherapy [response]," Diab says.

Tipping the balance

Some scientists think that cancers may go unrecognized by the immune system because of immunosuppressive signals from the tumor and its surrounding cells. For example, molecular signals coming from a tumor itself can skew the ratio of CD8 T cells to regulatory T (T_{reg}) cells toward more of the latter, which blunt the antitumor response. The pharmaceutical company Nektar in San Francisco is developing a drug called NKTR-214 to skew the ratio in favor of CD8 T cells. NKTR-214 resembles a cytokine called interleukin (IL)-2, which normally promotes the production of T_{reg} cells. But NKTR-214 binds only one of the three subunits that make up the IL-2 receptor. This subunit, called CD122, is more highly expressed by CD8 T cells and another cancer-killing cell type called natural killer cells than by T_{reg} cells. This allows the drug to promote CD8 T cell, but not T_{reg} cell, production in tumors while also avoiding the major side effects of IL-2 therapy. Last year, the company reported that in mice, the ratio of CD8 cells to T_{reg} cells was 20 times higher in the tumors of NKTR-214-treated mice than in mice that received a recombinant IL-2 protein[5].

Last August, Nektar teamed up with Diab to initiate a phase 1 study to test NKTR-214 in people with any type of solid tumor. At the annual meeting for the American Society of Clinical Oncology this June, scientists from Nektar and Diab's lab reported that the drug increased natural killer cell and CD8 T cell infiltration into the tumors of 26 individuals who received the drug through intravenous injection once every two to three weeks. The numbers of T_{reg} cells in tumors were unaffected. CD8 T cells extracted from the patient's tumors or blood expressed a gene called marker of proliferation Ki-67 (*MKI67*), which indicated that the T cells were increasing in number up to eight days after treatment. The phase 1 trial was not designed to test NKTR-214 in combination with checkpoint inhibitors, but the researchers reported that after the treatment ended, three individuals also received anti-PD-1 therapy, and their tumors shrank. The company is now working on a phase 2 trial to test nivolumab in combination with NKTR-214 against multiple kinds of tumors in a 140-patient cohort.

As part of their work with NKTR-214, Diab and his team are also monitoring a T cell population called T_H17 cells, which are involved in autoimmune diseases. He says that immune-enhancing therapies—including checkpoint inhibitors administered on their own—carry the risk of causing harmful inflammation and autoimmunity. "They might increase infiltration of CD8 T cells, but they may also activate T_H17s," he says. Currently, he says, clinical researchers don't typically look for signs of autoimmunity or dangerous levels of inflammation either when testing a new drug alone or in combination with immunotherapy, but he expects that more biomarkers will be developed over the next decade. Early data suggest that NKTR-214 alone increases CD8 T cell numbers without affecting T_H17 cells, but Diab says that combining therapies calls for extra caution.

Despite these encouraging data with NKTR-214, Diab says, "I do not believe that this will be the complete answer for every patient." He says other immune cell types found in tumors, such as dendritic cells and macrophages, also require consideration.

"I think we're now [moving] from just T cell activation to, 'Let's get everybody into the tumor,'" says Edith Janssen, an immunologist at Cincinnati Children's Hospital Medical Center. In 2014, her team reported that a receptor inside dendritic cells, called stimulator of interferon genes (STING), senses DNA released by dying cells and signals for dendritic cells to produce the inflammatory molecule type 1 interferon. Type 1 interferon, in turn, activates CD8 T cells[6]. Around the same time, other groups reported similar findings in the context of cancer and infection[7]. Janssen says that the cancer field took notice because type 1 interferons were already known to cause strong antitumor immunity.

Now, Janssen is a scientific advisor for Venn Therapeutics, which is based in Mason, Ohio, and is helping the company to develop an adeno-associated virus engineered to produce strands of DNA that stimulate STING and program dendritic cells in tumors to engage T cells. The virus-based drug, currently called VTX001, is in preclinical development.

Pittet's team is now also studying the role of other non–T cell immune cells, including macrophages, in their mouse model of lung cancer. The team found that its proimmunogenic chemotherapy regimen attracted a type of macrophage that helped to bring CD8 T cells to the tumors. But whereas some subsets of macrophages may help to fight tumors, others could promote tumor progression, he says. "The response that we observed is complex." Pitett's group is still collecting clues about all the immune cell types involved in the antitumor response, and how they affect each other and influence outcomes of cancer immunotherapies. "Now we are really in detective mode," he says.

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