Credit: James Moon, John Gideon Searle

Using the body's own immune defenses to shut down cancer is an idea that has long tantalized researchers and clinicians. But tumors arise from the body's own cells, which means ramping up an immune response risks attacking normal cells as well. Not to mention that tumors co-opt some of the body's built-in strategies to shut down immune defenses and protect cells.

This is the delicate balance that makes immunotherapy tricky. "The immune system is always integrating all these signals. It's never on or off. It's just always a balance," says Christopher Jewell, a chemical engineer at the University of Maryland in College Park.

Many existing targeted tumor therapies are antibody-based, such as Herceptin for Her2 positive breast tumors. Within the last several years, a new class of immune therapies, checkpoint inhibitors, have also exploded into clinical use. These therapies target pathways that tumor cells exploit to shut down T-cell responses.

Although they were a landmark discovery, checkpoint inhibitors only work with a small subset of patients. Instead of activating and targeting T cells toward a specific response, these drugs remove the brakes that block T cells from attacking the tumor; therefore, to benefit from these drugs, patients need to have T cells that are already activated against the tumor. This means that to extend the benefits of these drugs to more patients, researchers might need to combine checkpoint inhibitors with other immunotherapies that trigger T-cell responses to tumors.

Nanotechnology offers one such approach to activate T cells. Alongside developments in cancer immunotherapy, researchers have also been using nanoscience to probe some of cancer's unanswered questions. Nanoparticles can serve as a tool for en-

hancing tumor imaging and targeting chemotherapies to cells, or for strategies that combine those functions. Increasingly, researchers in oncology, immunology, engineering, chemistry, and biology are seeing nanoparticles as a way to ramp up the immune response to cancer.

The abscopal effect

Wenbin Lin is a chemist at the University of Chicago. Lin has been working on nanoparticles for more than 2 decades, originally focusing on imaging applications, before realizing the difficulty in translating those tools into clinical use. More recently, his team moved into targeted therapies for cancer—with the idea of using nanoparticles to deliver toxic chemotherapy directly to tumor cells. Once the toxic cargo reaches the target cells, the researchers would activate the killing power of those agents with light, minimizing the damage to healthy cells, a strategy known as photodynamic therapy.

However, the focus has now shifted toward the immune system and activating T cells. The idea is "to turn so-called cold tumors into hot tumors," Lin says. If a strategy like this proves successful in the clinic and gains FDA approval, combining it with checkpoint inhibitors could greatly improve cancer therapy, he adds.

The nanoparticle core Lin and his team use is made of zinc and pyrophosphate, which form an ionic-crystalline core, surrounded by a photosensitizing lipid, which is non-toxic until illuminated. In an initial study, they infused mice possessing breast cancer tumors with these nanoparticles and irradiated the tumors to trigger cell death. They used that process to generate an immune response, which, when combined with checkpoint

inhibitors, prevented metastases and largely eliminated unirradiated distant tumors (1). When the nanoparticles were loaded with oxaliplatin, an approved chemotherapeutic, and administered in a mouse model of colon cancer, both primary and distant tumors largely disappeared (2). Lin has founded a biotech company, RiMO Therapeutics, with hopes to move these nanoparticles into clinical testing.

The systemic immune response Lin observed is known as the abscopal effect. Physicians first noted it more than 50 years ago in patients with metastatic cancer after radiation therapy. As the radiation kills tumor cells, the dying cells release proteins, some of which are tumor-specific and immunogenic. If the immune system's dendritic cells recognize them as antigens and activate T cells, they can trigger an active immune response.

Radiation oncologist Andrew Wang of the University of North Carolina Lineberger Comprehensive Cancer Center has also been thinking about the abscopal effect in connection with nanoparticles. The weak point in trying to trigger this phenomenon, he says, is in getting the immunogenic and tumor-specific peptides from an irradiated tumor site to the dendritic cells that can then present them to T cells. The immune system doesn't respond well to free proteins, he notes.

To get around this, his team developed sticky nanoparticles made of poly(lactic-co-glycolic acid) that sponge up free proteins from dying tumor cells based on their hydrophobicity, ionic interactions, or covalent linkages, via surface nucleophiles. "Then, when the immune system sees it, it doesn't see them as free proteins," he says. The nanoparticles resemble a virus in size and are likely to be much more immunogenic.

Wang's team has tested these sponge-like nanoparticles in combination with checkpoint inhibitors in a mouse model of melanoma. In a recent *Nature Nanotechnology* paper, they observed a 20% cure rate in mice compared with no response for treatment with checkpoint inhibitors alone (3).

Reprogramming T cells

Another route to activating T cells is through direct reprogramming and reengineering. For some blood-based cancers, reprogrammed T cells are available in clinical trials to patients who have exhausted other treatment options. In adoptive cell therapy, physicians collect T cells from a patient's blood and then genet-

cally engineer them to produce chimeric antigen receptors (CAR) against a target. These cells are subsequently expanded in the lab and infused back into patients.

While this could be a revolutionary technique for diseases such as acute lymphoblastic leukemia (ALL), the approach has limitations. Personalized cell therapy is expensive—roughly \$400,000 per treatment, approximately double the cost of a conventional bone marrow transplant—and potentially deadly side effects are possible. Still, the approach can have amazing success for some patients—a 90% rate of complete response in ALL, particularly in young patients. With older patients and in the heterogeneous environment of a tumor, however, the response rates might be much lower, says physician-scientist Matthias Stephan of the Fred Hutchinson Cancer Research Center.

Those challenges have led Stephan to consider nanotechnology as a less costly method of reprogramming T cells within the body. Recently, his group designed nanoparticles encapsulating DNA from critical leukemia antigens and piggyBac transposon elements within a liposome shell. When they infused these agents into mice, the nanoparticles moved into lymph nodes, where the cargo was released, allowing the new genes to be incorporated in the T cells. Seven out of 10 mice showed complete tumor eradication, results that were nearly comparable to treatment with externally reprogrammed T cells (4).

Still, this strategy is unlikely to have the same potency as full-scale CAR T-cell therapy, he says. But the primary goal was to develop a treatment option other than chemotherapy that doctors could prescribe immediately. In CAR T-cell therapy, the process of reprogramming and then expanding T cells can take weeks. Stephan's nanoparticles could be administered immediately, on the day of diagnosis. T-cell reprogramming in the body starts within 24 hours, he notes. "So, within 1 day, you all of a sudden have a small army of T cells swimming in your blood that wasn't there before, and the side effects are basically none compared to what's going on with chemotherapy." In addition, clinicians could schedule regular nanoparticle infusions to boost immunity and provide a mixture of nanoparticles containing different tumor antigens.

Like Stephan, Jewell is also interested in reprogramming T cells in vivo, but through a vaccine strategy. Jewell and his colleagues are carefully designing nanoparticles with combinations of antigens and adjuvants that are as simple as possible, with the goal of only turning on desired immune responses. The

When studying theranostic nanoparticles containing iron, Stanford's Heike Daldrop-Link and her colleagues observed an unexpected immune response. Magnetic resonance imaging shows how nanoparticles accumulate in mouse mammary tumors (red areas in second panel from left) after injection. Immunofluorescence (third panel) and staining (fourth panel) confirm uptake of iron nanoparticles in tumor-associated macrophages. Credit: Heike Daldrop-Link.

James J. Moon and Anna Schwendeman of the University of Michigan have developed nanodiscs that target cancer antigens to the lymph nodes. Credit: Courtesy of Anna Schwendeman.

primary target is triggering toll-like receptors, critical proteins on the surface of dendritic cells and other antigen-presenting cells that help to lock in T-cell programming (5).

But in addition to fine-tuning T-cell targeting, they would also like to pause the differentiation of these cells so that they become tumor-specific memory T cells. Such cells exist in small populations within the body, but their power comes from being able to proliferate at high rates when challenged. They're also testing a strategy that includes cues that control T-cell plasticity and manage the expansion of tumor-specific T cells in mice (6).

Searching for a vaccine

Jewell is not the only researcher working on cancer vaccine development. Several decades ago, many researchers invested a lot of thought and effort into the idea. But the work fizzled out, mainly because they didn't understand how immune checkpoints worked, according to Jewell. "Even if you make many, many, many tumor-specific T cells, when they get to the tumor, the tumor's shutting them down."

As with traditional vaccines against infectious disease, cancer vaccines aim to deliver antigens and adjuvants directly to the lymph nodes that nurture and activate T cells. Within those tissues, T cells become sensitized to components of tumor cells or molecules expressed in high levels on the surfaces of those cells. The goal is to produce a population of T cells that will attack and eliminate the tumor selectively.

Even a decade or so ago, the primary goal in vaccine research was a large immune response, Jewell says. But researchers have learned that when targeting cancer, the best strategy is more tailored. "We're really trying to use materials to better control the types and characteristics of immune responses that are going to be generated," Jewell says. With the availability of checkpoint inhibitors to curb the brakes that cancer has evolved to counteract the immune system, the two strategies pair well.

Anna Schwendeman and James J. Moon of the University of Michigan are also interested in applying nanoscience for delivery of tumor-based antigens to the lymph nodes. Schwendeman's expertise is in constructing small nanodiscs, ~10 nm in diameter, assembled from phospholipids and lipoproteins. Known as sHDL nanodiscs, these nanomaterials have been well-characterized in patients and have been used in an experimental treatment for cardiovascular disease. Moon has now focused on cancer immunotherapy, testing much larger liposomes to deliver vaccine antigens.

But size makes a difference in antigen delivery, and the two researchers hypothesized that attaching peptide antigens to nanodiscs could allow the structures to migrate into draining lymph nodes and interact with T cells more effectively. Publishing their results in *Nature Materials*, the team demonstrated that antigen-decorated nanodiscs did improve lymph node targeting and boosted the T-cell response. In animal studies, treated mice

Precision is a perfect fit—times 3

Lower consumables cost and reduce waste with the only 96-well plate that divides into 32-well plates.

The new Applied Biosystems™ MicroAmp™ TriFlex 3 x 32-Well PCR Reaction Plate offers one plastic solution that's verified for all of our standard 0.2 mL thermal cyclers, regardless of run size.

Reduce cost and waste at **thermofisher.com/3x32plate**

ThermoFisher
SCIENTIFIC

For Research Use Only. Not for use in diagnostic procedures. © 2017 Thermo Fisher Scientific Inc. All rights reserved. All trademarks are the property of Thermo Fisher Scientific and its subsidiaries unless otherwise specified.
COL21827 0517

Unexpected Immunity

At Stanford University, Heike Daldrop-Link's team was primarily studying nanoparticles for imaging applications. That work drew her into collaboration with a group of oncologists at the university who were developing nanoparticles to shuttle therapeutics to cancer cells to create so-called "theranostic" nanoparticles. "If we combine nanoparticles that we can see with imaging applications and therapeutic drugs, then we could actually visualize if a nanoparticle arrives in the tumor tissue and where it ar-

rives in the tumor tissue," explains Daldrop-Link.

The nanoparticles in question, ferumoxytol, consist of an iron core coated with complex carbohydrates and average 30 nm in diameter. When the researchers started testing these iron nanoparticles in mice with implanted tumors, they noticed something strange. The nanoparticles without drugs—the controls—were also inhibiting tumor growth. The question became why.

Studying the treated mice, Daldrop-Link and her colleagues

noticed that the tumor tissue of nanoparticle-treated mice contained more tumor-associated macrophages. These immune cells engulf debris and metabolize it. Activated by the iron nanoparticles, the macrophages were attacking and engulfing the dying tumor cells. The activated macrophages produced hydrogen peroxide, which reacts with iron to produce hydroxyl radicals that destroy organic material and induce cancer cell apoptosis (9).

"The process [itself] produces a killing cycle of sorts," Daldrop-Link says. —S.W.

were able to fight off a subsequent attack from tumors, and tumor growth slowed. On its own, however, the vaccine could not stop tumor growth. But when combined with anti-PD1 checkpoint inhibitors, 90% of the mice harboring melanoma tumors cleared those cancer cells, compared with only 38% of mice that received free antigens along with checkpoint inhibitors (7).

New targets

In the quest for new cancer vaccines, researchers are examining a range of immune pathways. The stimulator of interferon genes (STING) pathway, which also influences antigen-presenting cells, is one promising target, and nanoparticles might provide a good way to exploit it, according to research from University of Texas Southwestern Medical Center.

Jinming Gao's team has developed pH-sensitive nanoparticles to take advantage of the more acidic environment surrounding tumors, which result from the well-documented Warburg effect. They've used that targeting ability for more traditional therapies, but they're also turning that work toward cancer vaccines.

Collaborating with Zhijian Chen's group, they have identified one set of polymers that helps deliver tumor antigens to the lymph nodes, allowing it to be engulfed by dendritic and other antigen-presenting cells. The low pH of the endosomal compartment allows the polymers to disrupt the membrane and release antigens into the cytosol.

"What we didn't expect is that the polymer itself, after it's coming out of the endosome, is also able to bind to the STING protein, which is actually a very important adapter alerting the immune system that we're under attack," Gao says. STING activation fuels the release of cytokines, leading naive T cells to mature into antigen-specific ones. The researchers are still working out the biochemical details of this mechanism in molecular studies.

In initial tests in several mouse models of cancer, the vaccine strategy proved effective, with 50% of animals surviving for more than 1 month. When they combined the nanovaccine with checkpoint inhibitors, the results were even more dramatic: 100% of animals survived for more than 60 days, and 90% had no tumors (8).

As researchers pursue cancer immunotherapy using nanotechnology, one question that looms large is finding and selecting appropriate tumor antigens. "It's hard to find a universal antigen signal across all different tumor types," Gao says. Bioinformatics and genetics are helping researchers look for novel antigens, but right now there doesn't seem to be a magic bullet to avoid personalized therapy.

Historically, there have been concerns about the immunogenicity of nanoparticles and the adverse effects that could result from such medicines. Though any marriage of materials science and medicine has to consider such issues, more bridges are being built between these two fields. "I think there's also more acceptance that both of those fields have strengths that can be synergistic," Jewell says. Increasingly, interdisciplinary teams are saying, "Let's not avoid the immune system. Let's control it".

References

1. **Duan, X. et al.** 2016. Photodynamic therapy mediated by nontoxic core-shell nanoparticles synergizes with immune checkpoint blockade to elicit antitumor immunity and antimetastatic effect on breast cancer. *J Am Chem Soc.* 138:16686-16695.
2. **He, C. et al.** 2016. Core-shell nanoscale coordination polymers combine chemotherapy and photodynamic therapy to potentiate checkpoint blockade cancer immunotherapy. *Nat Comm.* 7:12499.
3. **Min, Y. et al.** 2017. Antigen-capturing nanoparticles improve the abscopal effect and cancer immunotherapy. *Nat Nano.* doi: 10.1038/nnano.2017.113
4. **Smith, T. T. et al.** 2017. In situ programming of leukaemia-specific T cells using synthetic DNA nanocarriers. *Nat Nano.* doi: 10.1038/nnano.2017.57
5. **Chiu, Y-C. et al.** 2016. Assembly and immunological processing of polyelectrolyte multilayers composed of antigens and adjuvants. *ACS Appl Mater Interfaces.* 8:18722-18731.
6. **Andorko, J. I. et al.** 2016. Targeted programming of the lymph node environment causes evolution of local and systemic immunity. *Cell Mol Bioeng.* 9:418-432.
7. **Kuai, R. et al.** 2016. Designer vaccine nanodiscs for personalized cancer immunotherapy. *Nat Mater.* 16:489-496.
8. **Luo, M. et al.** 2017. A STING-activating nanovaccine for cancer immunotherapy. *Nat Nanotech.* doi: 10.1038/nnano.2017.52
9. **Zanganeh, S. et al.** 2016. Iron oxide nanoparticles inhibit tumour growth by inducing pro-inflammatory macrophage polarization in tumour tissues. *Nat. Nano.* doi: 10.1038/nnano.2016.168

Written by Sarah Webb, Ph.D.

BioTechniques 63:7-13 July 2017 doi: 10.2144/000114564