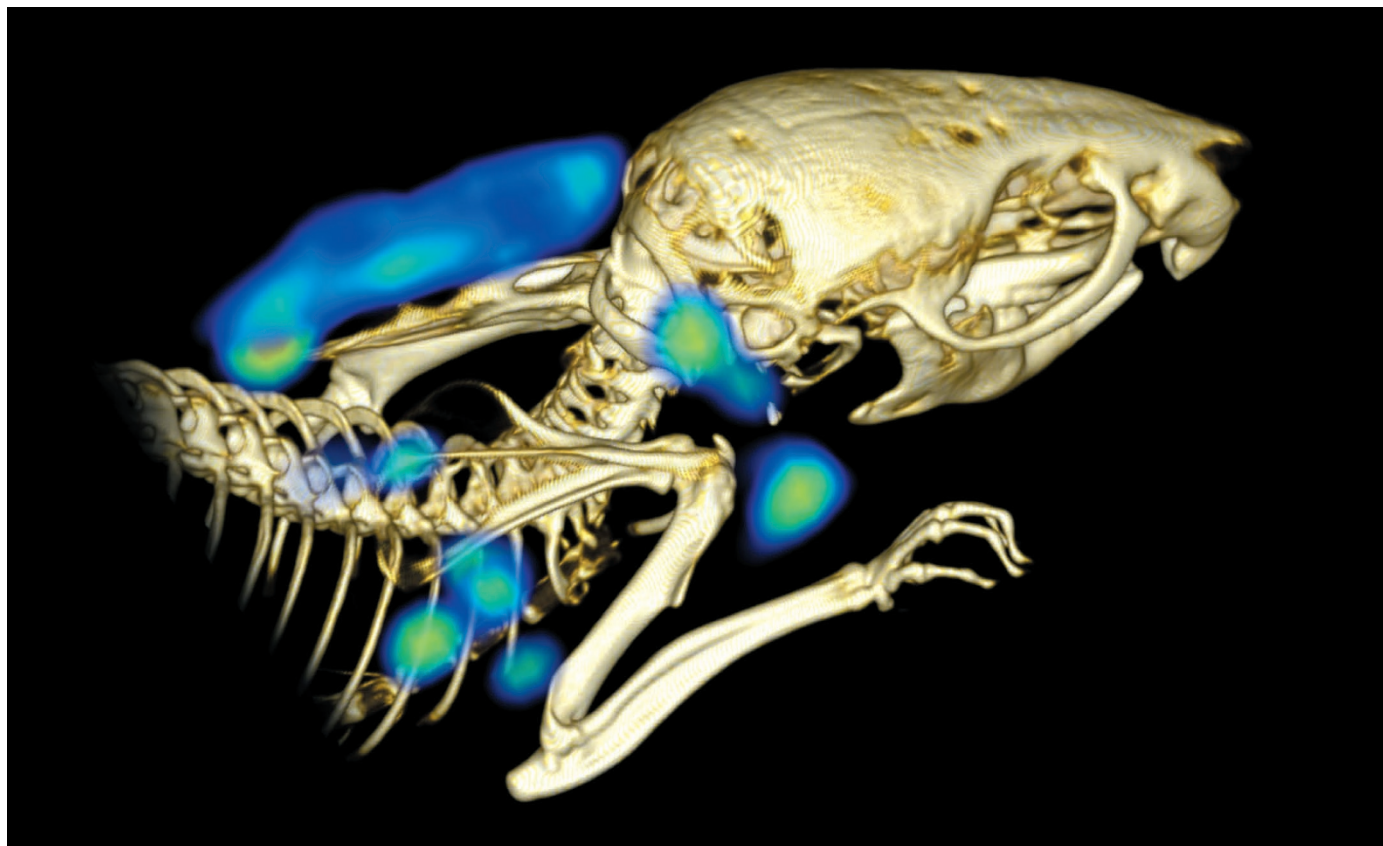# IMAGING WITH ANTIBODIES

Innovative techniques are giving researchers unprecedented access to the inner workings of the immune system.

HIDDEPLOEGH/*PNAS*

Positron-emission tomography using antibodies can help researchers to visualize the location of potential cancers in mice and other animals.

BY ROSIE MESTEL

The experimental cancer drug looked promising at first. But then the monkey results started coming in. The creatures were dying, poisoned by a treatment that was intended to target and kill pancreatic cancer cells.

Nothing in the tissue samples collected by the scientists at Genentech in South San Francisco, California, had suggested this would happen, says team member Simon Williams. But when the researchers imaged live creatures, and tracked how the drug moved through the animals' bodies, they finally found the problem: the animals' bone marrow was

greedily sucking up the antibody-based drug, killing the developing white blood cells inside the bones. The drug was abandoned.

When biological drugs go into live bodies, researchers often have little idea of what is going to happen. Surprises greet them at a drug's earliest stages, and all the way through to the clinic. Sometimes patients will respond; sometimes they won't. Either way, researchers want to know why. But often, they lack the tools to find out.

Imaging scientists and cancer researchers are now trying to change that, using antibodies and similar molecules in a technology known as immunoPET. As cancer therapies grow more precise and sophisticated, the researchers say, so too should the tools used to assess the

treatments. Modern biological therapies work only for certain patients, and physicians can't yet reliably predict who those people are. And whereas biopsies can tell you what's happening in one part of one tumour, immunoPET can take snapshots of the entire body and every tumour in it.

ImmunoPET marries positron emission tomography (PET), a technique that uses radioactive tracers to visualize the functions of human tissues, to an antibody's propensity to home in on the cells it's made to recognize. Interest in such imaging has heightened with the mushrooming of cancer immunotherapies, strategies aimed at revving up the body's immune system to fight tumours. But ▶

▶ designing an immunoPET imaging probe isn't easy. The choice of radioactive tracer, antibody design and imaging kinetics all require thought. Still, scientists are making progress. They can now identify an increasing variety of immune-cell and cancer tissues *in vivo*, and are tweaking antibody structures to improve their properties. New therapeutic and imaging strategies could be on the horizon.

This 'immuno-toolbox' is sorely needed, says Sam Gambhir, chair of radiology at Stanford University in California, who works on molecular imaging for the early detection and management of cancer. "Most therapeutic interventions we do are pretty much shooting blindly. We have no idea whether a therapy is working or not, especially in the early phases," he says. "All you can see is, does the tumour actually shrink? But if it doesn't shrink, you have no idea what went wrong." And that means you may not know what to do next.

GOOD PET
When defined narrowly, immunoPET is a tool that uses antibodies or related molecules as imaging agents. Researchers select one such molecule to recognize a specific protein on the cell of interest — PD-L1, for instance, which helps cancers to protect themselves from the patient's immune system, or CD8, which marks killer T cells. When injected into an animal, the antibody will travel through the body until it reaches its target cells and binds to them.

So that they can see those cells, researchers label the antibodies with short-lived radioactive isotopes, generally zirconium-89 or iodine-124, which have half-lives of 3.27 and 4.18 days, respectively. PET labels emit positrons, which are the antimatter version of electrons. When positrons collide with electrons in the body, they produce a pair of γ-ray particle, which rocket away from each other at 180° angles. Simultaneous detection of the paired particles reveals the location and abundance of the target in the body. Researchers can then overlay those data onto computed-tomography or magnetic resonance imaging scans, to determine the label's position relative to anatomical landmarks.

"Rarely does a patient come in with one metastasis. They can have multiple. And you can't biopsy them all."

Biologists and clinicians are using immunoPET to unravel why some patients — and not others — respond to cancer therapies.

A few years ago, for instance, Elisabeth de Vries, a medical oncologist at the University Medical Center Groningen in the Netherlands, and her colleagues conducted immunoPET imaging of 56 people with advanced breast cancer who received trastuzumab emtansine (Kadcyla)[1], a conjugate in which the anti-cancer antibody trastuzumab, which binds to the tumour protein HER2, is attached to a chemotherapy drug that poisons the target cell. Using radioactive labelling, the team found that in 29% of cases, patients' tumours didn't vigorously take up the antibody. That implies that the patients would be less likely to benefit from the therapy; indeed, the treatment in this group failed after a median of 2.8 months, compared with 15 months for those who did show antibody uptake. De Vries and her colleagues at Groningen and three other centres in the Netherlands are now conducting a trial to test whether up-front imaging such as this can improve treatment decisions for 200 women with newly diagnosed metastatic breast cancer; enrolment is slated to be completed later this year.

In a separate study[2], de Vries and others demonstrated that antibodies can reach a type of brain tumour known as a glioma when cancer has damaged the blood–brain barrier, suggesting that, contrary to conventional wisdom, antibody-based treatments may be effective against this disease. "The idea was that antibodies were too big and could not get into the brain, and that's not the case," de Vries says.

ImmunoPET's ability to scan the whole body can also help to address the fact that many

4 h		24 h		72 h		168 h	
Blk	NBlk	Blk	NBlk	Blk	NBlk	Blk	NBlk

PET images of mice at various times after injection with a PET tracer, overlaid on CT images.

cancers evolve quickly, and when they spread to other parts of the body, or metastasize, they may differ from the original tumour, and from each other. "Rarely does a patient come in with one metastasis. They can have multiple. And you can't biopsy them all," says Jason Lewis, director of the Center for Molecular Imaging and Nanotechnology at Memorial Sloan Kettering Cancer Center in New York City. But you can image them. In a 2016 immunoPET study[3] of nine patients, Lewis and his colleagues identified two women who had HER2-positive metastases whose primary tumour was HER2-negative; the women went on to benefit from trastuzumab (Herceptin) treatment.

AIDING IMMUNOTHERAPY

The many immunotherapies under development might also benefit from accompanying immunoPET analyses, says Antoni Ribas, a medical oncologist at the University of California, Los Angeles (UCLA), who is researching treatments for malignant skin cancers known as melanomas. Take, for instance, an approach that tackles cancer cells' defence against T-cell attack. This brake, called an immune checkpoint, occurs when a receptor on a tumour cell interacts with a receptor on the surface of a T cell. By administering antibodies that bind to, and block, either of those receptors, researchers should be able to free up the T cells clustered around the tumour to move in and kill.

But that, says Ribas, assumes that those killer T cells, which are identifiable through the cell-surface protein CD8, are positioned near the tumour. This isn't always the case. With immunoPET, "you could see if the immune system is ready to be turned on or not", he says. "If there's no CD8 cells pre-existing where the tumours are, there may be nothing to be activated." Such patients would warrant a different approach.

Treatments that release immune checkpoints can also induce nasty side effects when immune cells go into overdrive and wreak havoc in other tissues, Ribas notes. Gut inflammation, for example, is a common side effect of the melanoma checkpoint-inhibitor drug ipilimumab (Yervoy). Perhaps, he speculates, immunoPET could allow clinicians to detect this early.

Clinical questions such as these led Ribas to collaborate with UCLA imaging scientist Anna Wu, co-founder of the imaging company ImaginAb in Inglewood, California, who is tinkering with antibodies to optimize them for immunoPET. In 2015, Wu's team, with Ribas and others, showed that they could use immunoPET to track killer T cells in mouse studies of three types of immunotherapy[4]. The radiolabelled probe, a shortened fragment of antibody targeting the CD8 receptor, revealed T cells amassing in tumours and altering their distribution in other parts of the body.

ImmunoPET can even reveal whether a treatment is starting to work at an early stage, before the tumours start to shrink, says Wu.
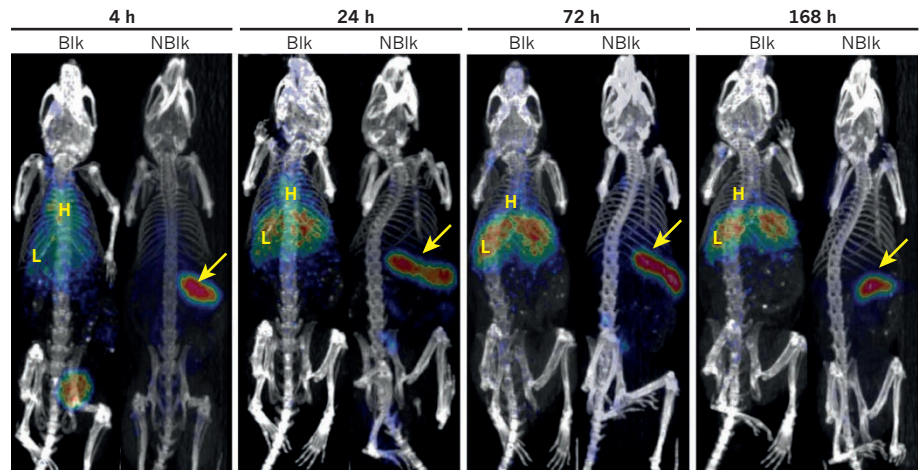
Indeed, researchers and clinicians have sometimes been led astray when tumours seemed to enlarge, rather than shrink, with initial treatment, when immune cells flooded the site and swelled the tissue. ImmunoPET can expose the difference between the cell types, and show that tumour cells are starting to respond to treatment before they visibly begin to die, says Wu.

In drug-development labs, immunoPET can help to guide decisions on whether to keep pursuing a therapeutic antibody, says Williams. In preclinical work, immunoPET confirmed the likely promise of an antibody called STEAP1, which targets metastatic prostate cancer cells and poisons them with an attached drug. In early (unpublished) clinical trials, imaging showed the antibody component could even home in on metastases in tissues once thought to be inaccessible, such as bone. "That used to be a discussion point in the field: can you deliver an antibody into bony metastases?" Williams says. "The imaging immediately showed us, yes, you can."

And then there was the case of the antibody–drug conjugate that unexpectedly harmed non-human primates. Williams's immunoPET study was instrumental in working out why that drug was so lethal.

EXPRESS DELIVERY

Monoclonal antibodies produce gorgeous immunoPET images, researchers say. But they can take up to a week to make their way to the tissue of interest and clear out of general circulation in the body, to produce images with good contrast and specificity.

Sometimes, that time scale is acceptable. In drug development, for example, researchers need to make decisions about whether to pursue drug candidates, and generally don't have time to wait for the creation of faster imaging agents of similar quality. "Management has to

> *"We want to bring targeted radiotherapy into this in a big way, and we feel it's very practical to do that."*

decide: are we going to pay for a phase III trial or not? If the imaging isn't done in time for that decision, it's pointless," Williams says.

But in the clinic, time is an overarching concern for imaging tests. "They are used in advanced-cancer patients, often with metastasis," says Sridhar Nimmagadda, a molecular-imaging scientist at Johns Hopkins University in Baltimore, Maryland. "For a patient in such a dire state to be able to come back for one more scan would be very difficult."

So researchers are pursuing molecules that retain the precision of antibodies but are smaller, and have more desirable pharmacokinetics. Wu's lab, for example, has engineered slimline antibody variants called 'minibodies' and 'diabodies'. These retain the parts of the antibody that interact with antigens (known as variable domains), but lose those that engage other parts of the immune system, such as the cells that clear away bacteria and debris. So other than the ability to bind to their targets, they are inert. And with them, patients could go from injection to imaging in under a day, Wu says.

These protein variants have other useful properties, too, Wu says. For instance, whether they exit the body through the liver or the kidney depends on their size. To improve the contrast and get a clear image of a pelvic tumour, clinicians would select a design that clears through the liver; for pancreatic cancer, a renal route would be desirable. Wu can also alter these engineered antibody fragments to render them as 'human' in sequence as possible to minimize immune reactions against the imaging agents (because they are not native human proteins), or so that the radioisotope label attaches to the protein in a uniform way, which can help to ensure consistent and reliable activity. "These are the things we can do as protein engineers," Wu says. "As long as you're going to produce a recombinant protein, you might as well optimize everything you can."

Other labs, such of that of molecular biologist Hidde Ploegh at Boston Children's Hospital in Massachusetts, exploit the antibodies of llamas, alpacas and camels to obtain faster-acting

immunoPET agents. These camelid species produce antibodies that consist of just one type of chain instead of the usual two, and weigh just one-tenth as much as conventional antibodies. But they also clear faster than conventional antibodies and penetrate more deeply into tissues.

Anything that allows a patient to get scanned and out of the office in a few hours would work, says radiologist and molecular-imaging scientist Martin Pomper, who directs Johns Hopkins' division of nuclear medicine and molecular imaging, where Nimmagadda works.

But Pomper and Nimmagadda have come to believe that the best approach for the clinic lies not with antibodies or stripped-down versions thereof, but with even smaller peptides and other low-molecular-weight molecules. The team has developed a radiolabelled peptide that latches onto PD-L1 and is ready for imaging in two hours, he says. The group is seeking even smaller molecules.

LABELLING TWO-STEP
Another trick to retain the precision of antibodies involves taking a two-step approach. Researchers infuse an antibody, then wait the needed week for any antibody that doesn't bind to a target to clear the body. Then they inject a second, smaller labelled probe, which rapidly binds to the previously administered antibody. That would allow them to use isotopes with

even faster radioactive decay, such as fluorine-18 or copper-64, which have half lives of less than 2 hours and 12.7 hours, respectively.

The method depends on the creation of 'bispecific' antibodies, which have two binding sites — one for the protein target (such as PD-L1) and one for the radiolabelled probe, called a hapten.

Like immunoPET itself, the two-step strategy is not a new idea, says Steven Larson, head of the molecular pharmacology programme at Memorial Sloan Kettering. But today's advances in imaging and antibody engineering make it an exciting one, he says. Larson is confident that the approach can breathe new life into an old therapeutic strategy: radioimmunotherapy, which uses antibody specificity to deliver radioactive poison to tumours. Modern imaging enables physicians to calibrate doses exquisitely to spare healthy tissues, Larson says.

Very few people are cured of solid tumours if they're not caught early, he adds. "We want to bring targeted radiotherapy into this in a big
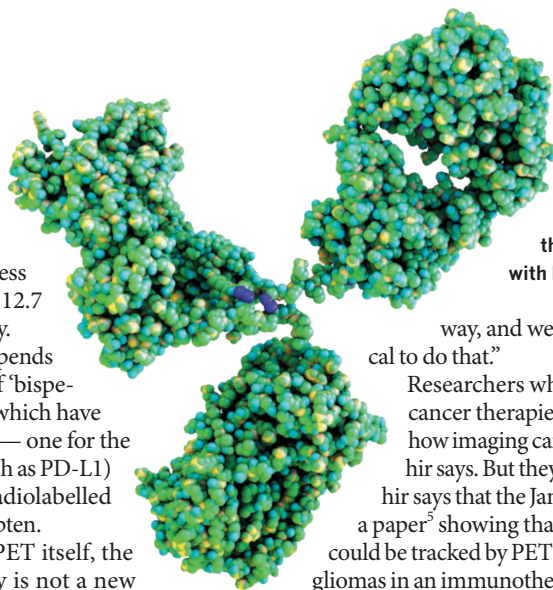
Antibodies can bind specifically to cellular targets and highlight them for visualization with PET.

way, and we feel it's very practical to do that."

Researchers who develop and test cancer therapies often don't realize how imaging can help them, Gambhir says. But they're learning. Gambhir says that the January publication of a paper[5] showing that engineered T cells could be tracked by PET as they homed in to gliomas in an immunotherapy trial generated a flurry of inquiries from industry.

"I've been inundated by drug companies," he says. "As soon as they see the human results, they get very excited." ∎

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