Suzanne Topalian (centre) and her team work on the body's immune responses against tumours.

JOHNS HOPKINS MEDICINE

BIOMARKERS

A surer bet

Researchers are seeking biological cues that can more accurately predict immunotherapy outcomes.

BY MICHAEL EISENSTEIN

Embarking on checkpoint-inhibitor immunotherapy for cancer is a bit like taking a single pull on the lever of a slot machine. For a relatively small risk — such drugs are generally safer than other types used to treat cancer — recipients can win a massive reward: years of disease-free survival. "My longest responder is from 2001, and she continues to do well long term," says Antoni Ribas, an oncologist at the University of California, Los Angeles. However, only a small proportion of people who are eligible for treatment with the drugs reap that reward — less than 40% for melanoma, for example, the type of cancer for which treatment has been most successful.

Researchers are still trying to understand the biological mechanisms that determine whether a gamble on immunotherapy will pay off. "We cannot fully explain why many patients don't respond and many types of cancer see no activity at all," says Ribas.

Equally confounding is that those who benefit can do so over wildly differing timescales, which range from several weeks to almost a year after beginning treatment. And conventional imaging can offer insights into tumour responses that are ambiguous or outright misleading. "There are plenty of patients where we've gone back and resected what looks like stable disease, and we find that there's no viable tumour left there," says Jennifer Wargo, an oncologist at the University of Texas MD Anderson Cancer Center in Houston.

The hunt is on for biological markers (biomarkers) that could effectively flag the people who are most likely to benefit from cancer immunotherapy — and also indicate the extent of the recipient's clinical response as it happens. "Hopefully, we'll get to a point where we can use what we've learned to understand what is protecting the tumour from the body's immune system," says Michael Atkins, deputy director of the Georgetown Lombardi Comprehensive Cancer Center in Washington DC. "Then we can choose combination treatments to paralyse those things that are suppressing immune function."

PUZZLING PREDICTORS

Many tumours evade destruction by immune cells by producing signalling proteins that trigger fail-safe mechanisms normally able to prevent uncontrolled inflammation or autoimmune responses. Known as immune checkpoint proteins, these molecules essentially switch off the T cells that would normally attack cancer cells. Checkpoint-inhibitor drugs interfere with the signals, reactivating T cells to put tumours back into the cross hair. The drugs most commonly used target either the checkpoint protein PD-L1 on the tumour, or its receptor, PD-1, on the T cell (see 'Checkpoint proteins in action'). Such agents are approved for use in a variety of cancers.

However, although several promising leads have been uncovered, the cellular and molecular features of tumours that predict their response are still being defined.

Initial studies homed in on the production of PD-L1 by tumours as a potential indicator of susceptibility. By probing sections of biopsied tumour with antibodies that recognize this checkpoint protein, clinicians can potentially identify cancers that are likely to respond to checkpoint-inhibition treatments.

Several clinical trials have shown that such testing can improve the odds of a response. For example, in the KEYNOTE-001 trial[1], almost half of people with lung cancer whose tumours broadly expressed PD-L1 responded to the PD-1 inhibitor pembrolizumab, compared with less than one-tenth of those with very low levels of PD-L1 expression. The US Food and Drug Administration (FDA) then set a high level of PD-L1 expression as a criterion for pembrolizumab treatment in this cancer.

However, the relationship of the level of the response with PD-L1 expression is neither straightforward nor universal. Trials have used various tests and cutoffs to determine levels of PD-L1 expression, thwarting their direct comparison. "It has generated some confusion in oncology, because the tests are all interpreted in

MICHAEL T. TETZLAFF/ALEX REUBEN/KIM ANH VU

slightly different ways," says Suzanne Topalian, director of the melanoma programme at Johns Hopkins Sidney Kimmel Comprehensive Cancer Center in Baltimore, Maryland. In cancers other than lung cancer that respond to checkpoint inhibitors, PD-L1 expression shows only modest utility in predicting such benefit.

In Topalian's view, the test serves best as a tool for helping physicians to decide whether to start a patient's treatment plan with checkpoint inhibitors when other treatment options are available. But when there are no other options, she says, the absence of PD-L1 shouldn't deter them from trying checkpoint inhibitors.

Many clinical studies suggest that only a few types of cancer respond to checkpoint inhibition. People with prostate cancer — one of the most common malignancies — tend to receive negligible benefit. The same is true for those with pancreatic cancer, which is one of the most deadly types. The extent of genomic damage also seems to be an important factor in determining the tumour response to checkpoint inhibition, which is why carcinogen-induced cancers are especially vulnerable. "A higher load of mutations may be more immunogenic, and that's why lung cancers from cigarette smoking or melanoma from ultraviolet light respond the best," says Ribas. Having a greater number of mutations probably increases the likelihood of the tumour producing abnormal proteins, known as neoantigens, that can trigger a T-cell attack.

Researchers led by Luis Diaz Jr at the Memorial Sloan Kettering Cancer Center in New York City have uncovered compelling evidence to support a correlation between mutational load and checkpoint-inhibitor response. In 2017, Diaz and his team showed that tumours with defects in certain proteins that repair gene mutations are more likely to respond to treatment with pembrolizumab, even in tumour types with an otherwise poor record of responding to checkpoint inhibition[2]. More than half of people with such tumours, which included a range of cancer types, responded to treatment, and more than 90% of people who had the strongest responses were alive three years later. "These are patients who were looking at death," Diaz says.

On the strength of these and other data, in 2017, the FDA made a landmark decision to approve pembrolizumab for any cancer that shows such repair defects. "When you have patients who would normally slip through your fingers continue to survive, that takes things to a whole different level," says Diaz.

Yet even tumours that seem to be ideal targets for immunotherapy can have features that render them less vulnerable to checkpoint inhibitors. Ribas and colleagues assembled detailed profiles of gene expression from samples of melanoma, and identified a signature that, on the basis of the activity of 26 genes, has the potential to predict resistance to PD-1 inhibition with accuracy[3]. "In 80–90% of cases, patients without a response had a gene-expression profile that was different from the responders," says Ribas.

IMMUNITY ON ALERT

The focus on cancer cells provides only a small part of the whole picture, however. The presence of T cells in the region that surrounds a tumour is an essential element of successful treatment. "The drug is not the antibody," Ribas says. "The drug is the T cell." When T cells are absent from the tumour site, there is little hope of eliciting a meaningful antitumour immune response.

A seminal study by Ribas and colleagues in 2014 highlighted the importance of assessing immunity as a predictor of response to checkpoint inhibitors[4]. Ribas says that T cells must be present in an area called the invasive margin — essentially, the front line in the war between tumour and host. If the defending army of T cells does not show up, the battle is already lost.

Any troops that are present must also be armed appropriately. This means that the T cells that infiltrate the tumour can kill cancer cells, and that they react specifically to the presence of the tumour. Ribas and his team are developing tools to measure gene expression that can reveal whether such T cells are in a state of readiness that could be fully unleashed through PD-1 inhibition.

Ideally, the potential response of a person to checkpoint inhibitors could be assessed before treatment commences. But a study by Wargo's team at the MD Anderson Cancer Center showed that the clearest indicators of an immune response might only become apparent after treatment is under way[5]. Because recipients generally experience minimal — or no — side effects from drugs that inhibit PD-1, a try-it-and-see approach could be justifiable. "If you give maybe two doses of anti-PD-1 and do an early-in-treatment biopsy, you can actually tell who's going to be a responder," Wargo says. A clear signal that the immunotherapy is getting traction might be observed within several weeks of treatment, long before the tumour reacts visibly.

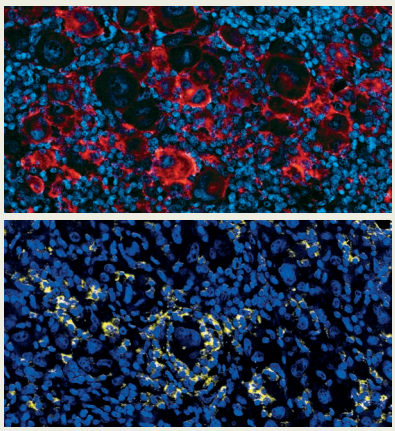
By digging deeper into the T-cell response to cancer, researchers also hope to develop less-invasive strategies than biopsy for monitoring disease. Work from researchers such as immunologist John Wherry at the University of Pennsylvania in Philadelphia offers evidence that T cells circulating in the blood could provide a useful barometer of response to treatment[6].

Such findings are starting to persuade those who had been sceptical, including Ribas. "My bias was that we wouldn't learn anything from blood," he says. "But there are high-profile papers showing that there is a subset of PD-1-positive T cells that circulate in the blood and are enriched for tumour specificity."

The presence of such cells could enable clinicians to perform 'liquid' biopsies to predict and assess a tumour's response to checkpoint inhibitors simply by analysing a sample of blood. But considerable work remains to refine liquid biopsy into a reliable diagnostic tool.

CHECKPOINT PROTEINS IN ACTION

Most current immune-checkpoint-inhibitor drugs target either the checkpoint protein PD-L1 on tumour cells (top, in red for melanoma) or its receptor, PD-1, on T cells infiltrating the tumour (bottom, yellow).



BODY OF EVIDENCE

Getting a handle on the complex interactions between tumours and the immune system is hard enough. However, the immune system also interacts extensively with the body's organ systems and external environment, creating relationships that may shape response to treatment with checkpoint inhibitors.

For instance, Wargo points to mounting evidence that the populations of bacteria in the body can influence the progression of cancer. That's just one example of why she thinks the future of immunotherapy will be built on the extensive collection and analysis of data from people undergoing treatment, including the work of multidisciplinary initiatives such as the Parker Institute for Cancer Immunotherapy in San Francisco, California.

Ribas, who is part of the Parker Institute's research programme on tumour responses to checkpoint inhibitors, concurs that monitoring recipients for several years might be the best hope for finding ways to optimize the timing, dosage and combination of immunotherapeutic agents.

"The preclinical models both in mice and *in vitro* can tell us what to take into the clinic, but they won't tell us what will work best," says Ribas. "The experimentation now is in humans." ■

Michael Eisenstein *is a freelance science writer based in Philadelphia, Pennsylvania.*

1. Garon, E. B. *et al. N. Engl. J. Med.* **372,** 2018–2028 (2015).
2. Le, D. T. *et al. Science* **357,** 409–413 (2017).
3. Hugo, W. *et al. Cell* **165,** 35–44 (2016).
4. Tumeh, P. C. *et al. Nature* **515,** 568–571 (2014).
5. Chen, P. L. *et al. Cancer Discov.* **6,** 827–837 (2016).
6. Huang, A. C. *et al. Nature* **545,** 60–65 (2017).