DECODING THE SIGNS OF RESPONSE TO CANCER IMMUNOTHERAPY

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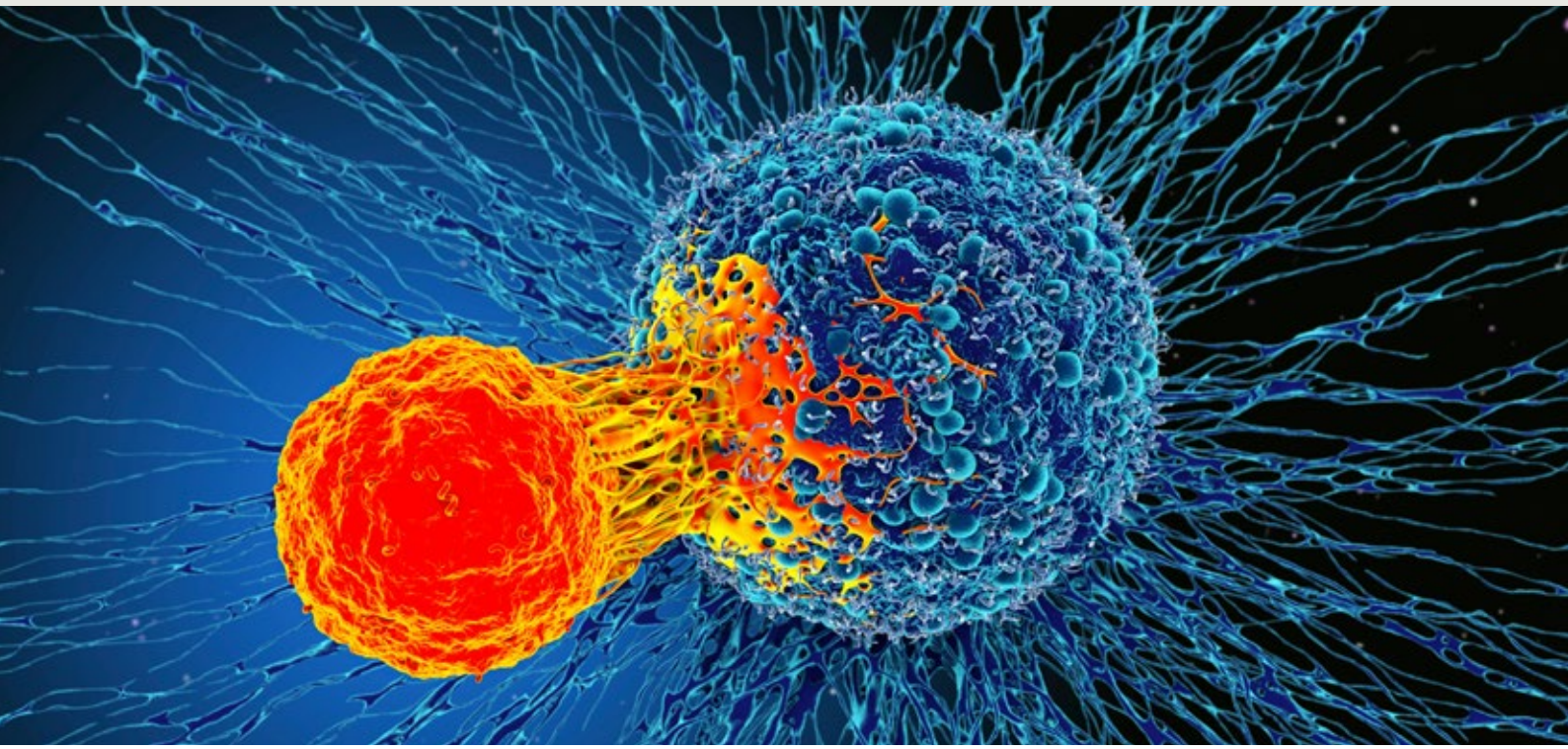

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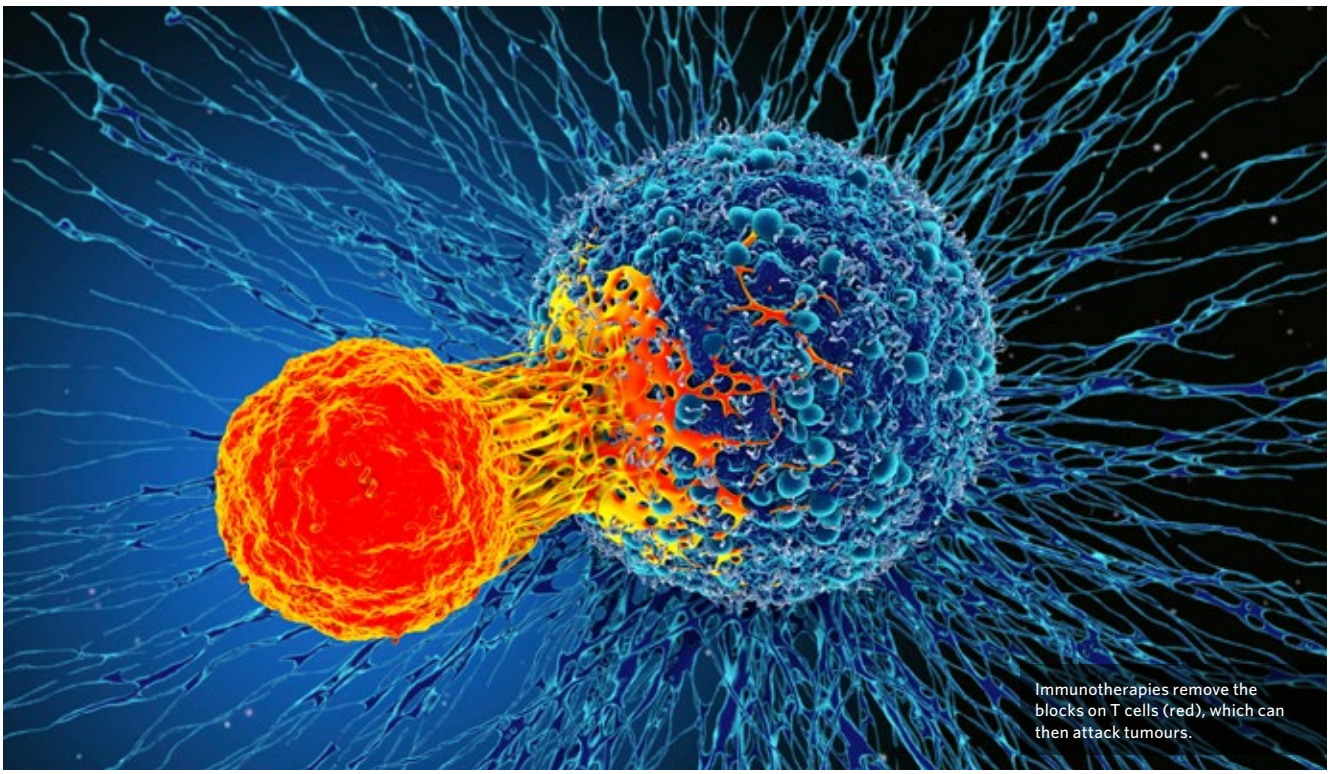

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Immunotherapies remove the blocks on T cells (red), which can then attack tumours.

Cancer therapies that activate the immune system have shown remarkable results for many patients. Immune checkpoint inhibitors (ICIs) work by interfering with the mechanism that cancer cells use to evade the host's immune response. Drugs that block cytotoxic T lymphocyte associated protein 4 (CTLA-4), programmed cell death protein 1 (PD-1) and programmed death ligand 1 (PD-L1) activate the cancer-killing T cells that had been suppressed. ICIs can shrink tumours and improve survival rates, even for patients for whom other cancer therapies have failed.

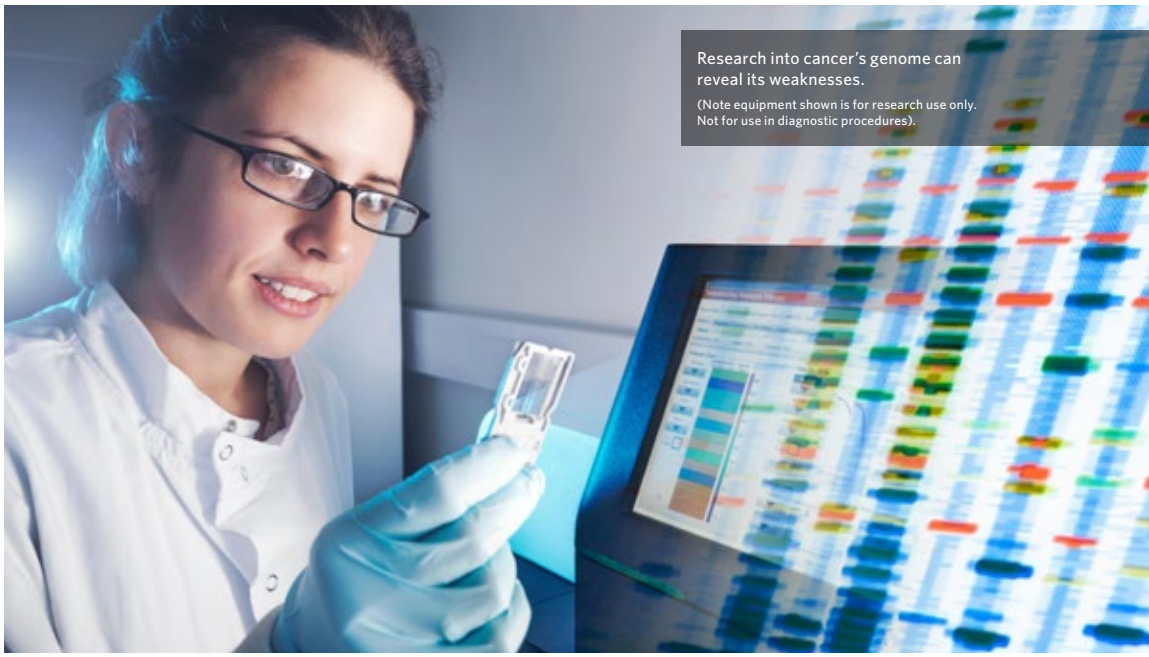
Only 20-40% of patients respond to immunotherapy[1] and, because these drugs can activate a broad range of immune cells, they can sometimes trigger severe auto-immune reactions. If clinicians can predict who will be a non-responder, they will save treatment costs and spare patients from side-effects.

Next-generation sequencing (NGS) technologies are starting to reveal the hallmarks of treatment response. Assays that measure the genetic profile of tumours and the host's immune activity are helping to guide treatment decisions in clinical trials with new immuno-oncology drugs or combination therapies, and they are making their way into routine clinical practice.

Since 2017 the FDA has approved two comprehensive diagnostic tests that rely on NGS technology to characterize the genetic profile of any type of solid tumour, without being tied to any particular drug. Victor Weigman, director of translational genomics at Q^2 Solutions, a laboratory services provider for global clinical trials, based in North Carolina, sees these tests as the start of a new era for NGS in the clinic. "The flexibility of NGS to provide multi-analyte reporting creates a strong pull for its use in immune-oncology."

There are huge advantages in terms of cost and clinical benefit of moving away from using one test for one drug for one indication, and these are accelerating efforts to develop new tests. "The field is advancing at dramatic speed; only a few years ago, it didn't seem possible that NGS would be able to tell us much about patients' response to immunotherapy," says Timothy Chan, director

of the Immunogenomics and Precision Oncology Platform at the Memorial Sloan Kettering Cancer Centre in New York City. "Today, these genomic assays are producing actionable results and evidence of their clinical utility is growing."



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Research into cancer's genome can reveal its weaknesses.

(Note equipment shown is for research use only. Not for use in diagnostic procedures).

Quantifying multiple cancer biomarkers

Many hospitals and clinics carry out single biomarker testing for cancer diagnostics. Genetic screens for mutations within the *BRCA1, EGFR* or *KRAS* genes help oncologists determine a patient's risk of developing certain cancers or drug resistance. Tests that assess the expression levels of PD-L1 also help to determine the likelihood of clinical benefit from anti-PD-1/PD-L1 drugs and to inform treatment options.

However, single-biomarker assays are unable to capture the full genetic complexity of a tumour. Genetic profiling of cancer tissue by targeted NGS is a cost-efficient and rapid way of analysing multiple targets simultaneously, and is allowing researchers to identify new biomarkers. Recently, research efforts have focused on using NGS to quantify the number of mutations in cancer cells (the tumour mutational burden — TMB) and to identify genetic patterns that impair DNA repair mechanisms and cause mutations to accumulate (microsatellite instability — MSI). TMB and MSI are important because cancer cells carrying a high number of mutations are more likely to produce proteins that the immune system will recognize as foreign — and thus are more likely to be susceptible to an immune response. High TMB[2] or a high level of MSI[3] have been shown to correlate with better prognosis for patients with various cancer types undergoing ICI treatments.

A combinatorial approach

While MSI is currently approved as a pan cancer biomarker for access to the ICI pembrolizumab, there are still some unresolved issues regarding the use of TMB and MSI as routine biomarkers. There are many ways to measure TMB, and its value can vary across tumour types, highlighting the need for consistent standards for measurement and the establishment of disease-specific TMB thresholds

Originally, TMB was determined by sequencing all protein-coding regions in the cancer genome. To reduce costs and turnaround time, most assays target a panel of several hundred genes to measure TMB. The lack of standardization for TMB calculation and reporting has led to initiatives such as the Friends of Cancer Research's TMB Harmonization Project that aims to create a universal reference standard using NGS technologies and identify sources of variability among TMB scores obtained from different targeted panels.

The correlation between high TMB and favourable response to ICI is not observed in all immunotherapy-treated patients, suggesting that it may not be possible to rely on one universal definition of high TMB[2]. In addition, a study of different microsatellite-stable tumours that took into consideration genomic features beyond mutational burden highlighted the role of specific genes or signalling pathways in responsiveness to immunotherapy[4].

"THE FIELD IS ADVANCING AT DRAMATIC SPEED."

In fact, results from a phase II trial with atezolizumab, a therapeutic anti-PD-L1 antibody, in patients with metastatic urothelial cancer, indicated that biomarkers such as PD-L1 expression and TMB provide independent and complementary information about the tumours' response to the treatment[5]. The emerging picture is that a combination of markers is going to be required to predict a patient's response.

Garret Hampton, senior vice president of clinical genomics at Illumina, Inc, a leading sequencing technology provider, based in San Diego, says that "the use of combinatorial biomarker approaches is likely to provide more precise measures of the benefits relating to immunotherapies".

Dissecting the cancer-immune interplay

It's not just the tumour's genetic profile that can affect immunotherapy response. Several studies have highlighted the role of the host's baseline immune response in determining treatment effectiveness[6] — and the need to better understand the interaction between cells in the tumour microenvironment.

In particular, increased infiltration of T cells into tumours seems to be associated with patient survival and immunotherapy response. But the factors that determine if a tumour has high ('hot') or low ('cold') level of T-cell infiltration are only starting to be understood.

Chan has conducted a large

Next-generation sequencing can be used to analyse cancer-derived DNA in the blood.

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genetic analysis of melanoma and lung cancer patients treated with checkpoint inhibitors[7]. His team found a relationship between treatment response and human leukocyte antigen (HLA) genes. Similar to previous findings in patients infected with HIV, hepatitis B or malaria, certain HLA profiles are associated with better outcomes in cancer. "People who have more variation in their HLA genes have a greater ability to recognize what is 'self' and what is not," he says.

To further understand the mechanisms driving or preventing the infiltration of T cells into tumours, researchers are examining gene expression changes in T cells and cancer cells. Cancer transcriptome sequencing in single cells (scRNA-seq) provides valuable information about cell-type-specific changes in gene expression that cannot be ascertained from bulk tissue analysis.

"Unlike bulk approaches, scRNA-seq is enabling us go beyond simply identifying the cellular composition of a tumour and to characterize the dynamics, functional states and cross-talk between cell populations of the tumour microenvironment," explains Jacqui Shields, group leader at the MRC Cancer Unit, University of Cambridge.

With the help of computational models, scRNA-Seq studies have revealed malignant cell programmes of gene expression that are associated with T cell exclusion and T cell dysfunction and that can be used to predict resistance to immunotherapy. In her latest study[8], Shields and colleagues identified three distinct types of cancer-associated fibroblasts in mouse melanoma that can modulate the immune response as the tumour progresses.

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"What is really exciting about scRNA-seq is that it allows us to identify novel or rare populations that would frequently be 'overpowered' in bulk approaches and to examine their contribution to disease," she says. "ScRNA has provided a platform to dramatically increase our understanding of the interaction between the tumour and host cells at an unparalleled resolution."

From research to clinical settings

Drug developers and clinical trial laboratories routinely use NGS-based assays to both prospectively identify patients that are most likely to benefit from ongoing clinical trials, and to retrospectively assess genetic signatures that predict a favourable response to the treatment.

"Rather than relying on clinical parameters, we are able to filter for patients that are more likely to respond based on a panel of specific biomarkers," says Weigman. Drug developers have rapidly adopted this approach to advance drug candidates and combination treatments in an increasingly crowded field. It is also helping to prevent clinical trial failure because of lack of patient response.

There are still some technical challenges to overcome before NGS can be widely adopted in clinical practice. Advances in sequencing methods are addressing issues related to the size and quality of the tumour tissue samples that are obtained in the clinic, as well as reducing turnaround time, which is critical for patients with aggressive tumours. An exciting development is the use of NGS for liquid biopsies to analyse cancer-derived DNA that might be present in blood, circulating cancer cells, or T cells. Both activated circulating T cells[9] and TMB measured using DNA shed from tumour cells[10] correlate with patients' response to cancer immunotherapy. The possibility of obtaining useful information on treatment response from a simple blood draw provides new options for monitoring and for making treatment decisions.

Weigman is optimistic about the future of NGS-based diagnostics in clinical practice. "Within the next four to five years, therapeutic decisions in the clinic will be made based on genomic biomarker tests," he says.

Given that a growing number of drugs are approved with an associated biomarker (nearly 1 in every 4 in the US) and that accompanying diagnostics have a proven impact on sales and prescriptions[11], the demand for such tests has never been greater. "Clearly defined clinical utility and short turnaround time will be key for any NGS technology to be adopted for routine testing," says Weigman.

To help usher in this new era, Illumina is partnering with pharmaceutical and diagnostic companies to develop companion-diagnostic versions of NGS-based tests. "We are excited that a number of Illumina products are being leveraged by this research," says Hampton. Such tests will, ultimately, allow us to understand more about cancer — and for more patients to benefit from immunotherapies. ■

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