CANCER NEOANTIGENS PROVIDE NEW RESEARCH LEADS FOR PERSONALIZED VACCINE DEVELOPMENT

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ANDREW BROOKES / GETTY

Information derived from DNA and RNA sequencing could soon lead to development of personalized cancer vaccines.

Since the first full cancer genome was revealed in 2008, researchers have explored how to use genomic information to selectively target the disease. By comparing the DNA from various cancer cells with that in healthy cells from the same individual, researchers have found that tumours contain unique mutations.

Cancer vaccines that make use of these mutations represent a promising therapeutic strategy. "By stimulating immune responses against tumours through vaccines, we have an opportunity to generate long-term anti-tumour immunity," says Benjamin Vincent, an immune-oncologist in the Division of Hematology/Oncology at the University of North Carolina at Chapel Hill. Not only do vaccines limit the damage to healthy cells, they can also prevent cancer recurrence by training the immune system to respond to those cells in the future.

Vincent is using immunogenomic approaches in his research to understand tumour biology and develop clinically relevant biomarkers and new cancer therapies. Cancer-associated mutations frequently lead to the production of neoantigens: unique protein fragments that the immune system recognizes as foreign. Consequently, the total number of mutations in cancer cells (the tumour mutational burden, TMB) is emerging as a biomarker for predicting response to immunotherapy across all cancer types. "TMB is now used as a surrogate for the number of predicted neoantigens," he says.

Tumours with a high TMB are more likely to respond to immune checkpoint inhibitor drugs, although this has not been found in all cancer types[1]. "Even if neoantigens are present in the tumour, there can be multiple reasons for immunotherapy failure," says Vincent. These reasons include the patient's T cells not recognizing the neoantigens, or their neoantigen-recognizing T cells are somehow suppressed or unable to reach the tumour site.

Checkpoint inhibitors, which interfere with cancer cells' mechanism for evading the immune response, are likened to 'releasing the brakes' of the immune system. But these drugs fail to eradicate

the tumour in the majority of cases. In these instances, it could be more effective to 'hit the accelerator' by priming the immune system to kill cancer cells directly, using a vaccination strategy that targets their mutant proteins. "The hope is that, by both expanding the repertoire of neoantigen-recognizing T cells with a vaccine and removing the brakes that tumour cells put on the immune system with checkpoint inhibitors, we can obtain a synergistic effect," says Vincent.

Finding the relevant cancer antigen

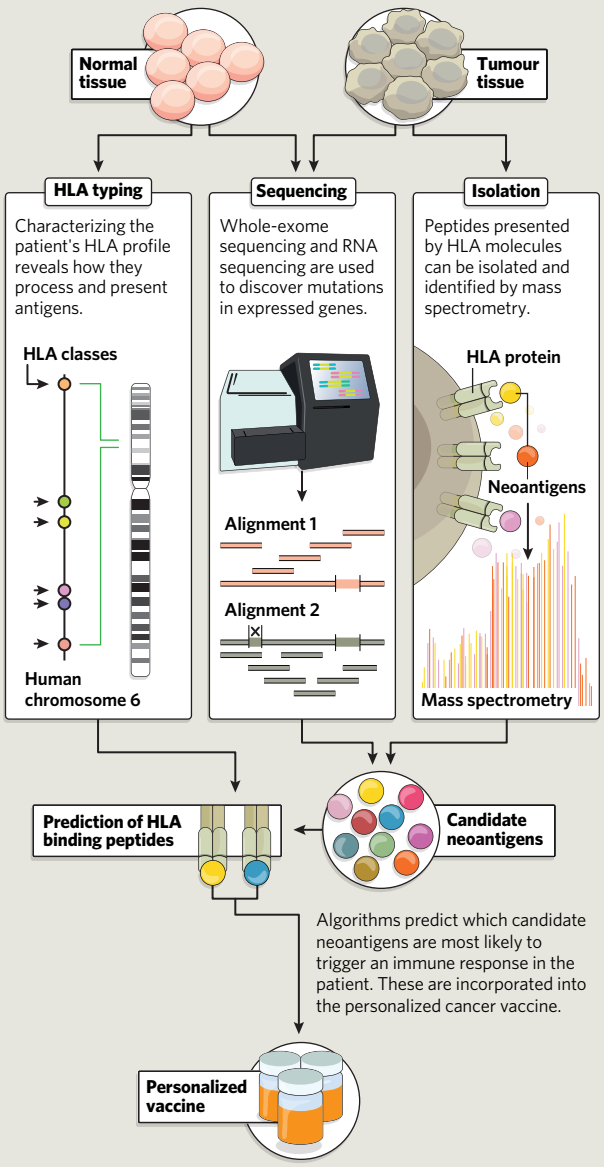
Early attempts to prime the immune system used tumour-associated antigens (TAAs) — completely normal peptides that are expressed in healthy tissue but are overexpressed in cancer cells. However, these vaccines ran into efficacy and safety issues owing to the difficulty of driving an immune response against protein fragments that the immune system recognizes as 'self'. Targeting the 'non-self' peptides that arise from cancer neoantigens should be a much better approach.

In 2017, two proof-of-principle studies by teams in the United States and Europe demonstrated that neoantigen vaccines can direct immune responses against cancer cells in patients with recurrent melanoma. In the US study[2], researchers created cancer vaccines containing up to 20 different neoantigen peptides for each patient, which led to cancer-free survival in 4 out of 6 patients. The other two also became cancer-free after further therapy with an immune checkpoint inhibitor. In the European study[3], researchers in Germany and Austria created a vaccine containing a mix of 10 RNA molecules encoding neoantigen peptides, which boosted immune activity against tumours in 9 out of 13 patients.

These positive results suggested that personalized immunotherapy might have clinical benefit, and led to a resurgence in cancer vaccine research. A recent review[4] of the global immuno-oncology pipeline showed that, between September 2017 and September 2018, there was a 133% increase in the number of agents targeting patients' neoantigens. By contrast, there was a decrease in the number of agents targeting TAAs, suggesting that the immunotherapy field is moving towards personalized approaches.

NEOANTIGEN-BASED CANCER VACCINES

Using normal and tumour tissue samples, researchers can determine what cancer antigens might be best suited to use therapeutically.



The identification of neoantigens is possible thanks to advances in next-generation sequencing (NGS) of both DNA and RNA, which can rapidly scan and compare millions of base pairs in tumour and healthy cells. These technologies, together with advances in manufacturing, make it feasible to quickly produce the diverse molecules required for individualized vaccines.

Predicting immunogenicity

There are several challenges to address to develop personalized cancer vaccines. For a start, once the tumour-specific mutations have been identified, researchers must determine how they will be expressed and recognized by the patient's immune system. Mutations such as single nucleotide variants (SNVs) may change the amino acid sequence of the protein, whereas other kinds of mutations can lead to the insertion or deletion of a whole sequence of nucleotides (indels) or gene fusions that produce abnormal (and potentially immunogenic) proteins. Indeed, a recent study identified a fusion-associated neoantigen that was able to trigger a T cell-mediated cancer killing response in an advanced-cancer patient with low TMB and minimal immune infiltration[5].

Vincent's group is using exome sequencing to identify the DNA differences, followed by RNA sequencing to confirm which DNA variants are being transcribed, and thus likely to produce neoantigens. This process is yielding interesting findings. "Although we are able to reduce the number of potential vaccine targets derived from DNA sequencing by more than 50% using this approach, RNA sequencing is also revealing a whole new set of 'alternative' neoantigens that

are not derived from genetic mutations but from differences in RNA expression," he says.

To be effective, neoantigens must be recognized as foreign by cancer-killing T cells. In this process, immune cells turn neoantigens into smaller peptides, load them onto major histocompatibility complex (MHC) proteins (also known as the human leukocyte antigen (HLA) system) and display this MHC/peptide complex to T cells. Because of genetic variation in the HLA molecules, some people's immune systems are better at processing and recognizing tumour neoantigens than others. NGS can be used to determine a person's HLA type and hence their susceptibility to disease and their response to immunotherapies. Computer algorithms use the patient's HLA type to prioritize which neoantigens to include in their vaccine, based on the likelihood that their immune system will process the neoantigens appropriately (see 'Neoantigen-based cancer vaccines).

TO BOOST THE CHANCES OF SUCCESS, MOST TRIALS USE VACCINE COCKTAILS CONTAINING 10–20 NEOANTIGENS

Computationally predicted neoantigens can be validated using mass spectrometry and T cell screening assays. Michal Bassani-Sternberg, a biochemist in the Center of Experimental Therapeutics at the University of Lausanne and the Ludwig Institute for Cancer Research, Switzerland, is using mass spectrometry in her research to characterize MHC-binding peptides from cancerous tissue and matching the results to personalized reference databases containing neoantigen peptides predicted from the genetic sequence of that individual's cancer cells[5]. "This methodology allows us to identify mutated MHC-binding peptides that are actually presented to T cells in vivo," Bassani-Sternberg explains.

Immune system recognition is not the only feature that is required from a 'good' neoantigen. "Neoantigen targets should not only be tumour specific and highly immunogenic," says Bassani-Sternberg, "ideally, they should be stably and widely expressed as well." Identifying and targeting neoantigens that are present in every cancer cell, rather than those present in only a subset of cells, will increase the likelihood of eliminating the whole cancer. Not many neoantigens meet all the desired criteria. Therefore, to boost the chances of success, most trials use vaccine cocktails containing 10–20 neoantigens.

Optimizing production and efficiency

There are still significant hurdles to overcome before research into personalized cancer vaccines can yield clinical therapies. The speed and cost of production are often cited as the main challenges; it is crucial that scientists reduce the time it takes to perform a tumour biopsy, sequence its DNA and RNA, analyse the data and create a personalized vaccine.

The production process involves identification of neoantigens from sequencing data obtained from a very small tumour tissue samples, underscoring the need for accuracy. "Illumina's NGS technologies combine the faithful detection of mutations with high-throughput sequencing to provide a cost effective and flexible read-out," says Illumina's VP of Product Management, Kevin Meldrum. By optimizing sample preparation methods and working with software companies to improve the interpretation of sequencing data, Meldrum says that the aim is to speed up the entire process from weeks to days.

SEQUENCING CANCER-DERIVED DNA WILL ALSO IDENTIFY NEW NEOANTIGENS

When it comes to designing an effective vaccine, there are many factors to consider, including the number of neoantigens to include and the delivery method. Sequencing at the single-cell level will help to incorporate as much of the cancer's variability into the vaccine as possible. This will reduce the chance of cells escaping the directed immune response. However, the optimum number of neoantigens to include in a cancer vaccine is still unclear. A study in mice[6] suggested that too many neoantigens could lead to competition for MHC binding and potentially dilute the immune response.

Different vaccine delivery approaches could also help improve efficacy. For example, delivering RNA molecules that encode neoantigen peptides might be better at stimulating an immune response because the immune system is primed to detect certain forms of RNA as foreign[7]. However, Vincent and colleagues are pursuing a different approach to improve the efficacy of free peptide vaccines. Working in collaboration with material scientists, they are using nanoparticles that deliver neoantigens in a way that is more stimulatory to antigen presenting cells. Early work in mice seems to show that this technique activates more cancer-killing T cells and leads to better tumour clearance.

Further research into the timing and dosage of vaccination, and combination therapies with immune checkpoint inhibitors or radiotherapy, will help to optimize response and outcomes. "We are aware of the need for a multipronged approach," says Vincent, "but we still don't know how many prongs it will involve."

Further targets are emerging from NGS analyses of tumour stromal cells and from research into how the tumour microenvironment contributes to regulating neoantigen expression and immune-cell infiltration[8]. In addition, sequencing cancer-derived DNA in blood samples (liquid biopsies) will not only help monitor response but also identify new neoantigens selected during tumour evolution that can be used for sequential vaccine development. ■

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