CASE STUDY

Cancer is a personal issue – a worldwide scientific effort focuses on making the treatment match the individual

Neoantigen vaccines promise to bring personalized cancer therapy to a new level

The dream of designing therapies that effectively destroy tumors but spare healthy tissue is the Holy Grail in clinical oncology. Efforts to improve on traditional chemotherapy have led to, for example, immunotherapy, and antibody-based therapies. A new approach in immunotherapy that involves vaccines based on peptide neoantigens promises to bring therapeutic precision to the level of individual tumors in individual patients.

The immune system and cancer

The importance of immunotherapy was highlighted this year when the 2018 Nobel Prize in Physiology or Medicine was awarded to James P. Allison and Tasuku Honjo for "their discovery of cancer therapy by inhibition of negative immune regulation". Their pioneering work revealed the importance of immune checkpoints that act as 'brakes' on the immune system, and their inhibition enables T cells to more effectively eradicate cancer cells (see, for example, https://www.nature.com/collections/gqznlfngkz).

Immunotherapy has been suggested as a promising alternative to conventional cancer therapy for more than a century, mainly driven by case reports of immune-mediated tumor control. In the late nineteenth century, William Coley noted occasional examples of tumor regression in patients with erysipelas, bacterial skin infection, and high fever. This prompted him to inoculate sarcomas with bacteria to engage the patient's immune system in the fight against cancer. The results could be spectacular for individual patients and Coley has been referred to as the "father of cancer immunotherapy" (1).

More recently, studies on mice have highlighted the role the immune system plays in recognizing and attacking tumor cells at all stages in carcinogenesis in a process called immune surveillance. Added to that, T cells have been shown to recognize tumor cells. As a result, the fundamental influence of the immune system on cancer progression has been designated a hallmark of cancer, which stimulated the development of immunotherapy as a more targeted method of cancer treatment.

The promise and limitations of conventional immunotherapy

Immunotherapy treatment activates T- and B cells that target specific tumor antigens expressed by each patient's unique and frequently changing population of cancer cells. This supports the immune system in its adaption to mutations in the patient's tumor cells that can lead to resistance to traditional anticancer therapies. Initial efforts in immunotherapy focused on tumor-associated antigens, but these are seldom tumor-specific and are also found in normal cells.

Immunotherapy involving checkpoint inhibitors has been very effective. These block the inhibitory checkpoints that tumors use to protect themselves from immune system attacks, thus restoring immune system function. For example, the first checkpoint inhibitor approved by the FDA, ipilimumab targets proteins that act as molecular brakes for T cells, enabling them to kill cancer-causing cells. These agents are now commonly referred to as checkpoint modulators, or CPMs, since many in development are agonists for the immune system. Unfortunately, not all patients respond to checkpoint inhibitors and of those that do, many relapse. Neoantigens, on the other hand, exploit one of the most important characteristics of T cells – their high specificity, making them specific to tumor cells and, more importantly, the individual tumor cells of a particular cancer patient. This makes neoantigens unique targets for personalized medicine.

What are neoantigens?

Cancer is characterized by a high frequency of genetic mutations that result in mutated proteins, uncontrolled division, and proliferation of abnormally functioning cells. These mutated proteins can be processed into peptides and presented as immune signals on the surface of cancer cells. It is these peptides that are called neoantigens (Figure 1). The T cells can recognize and target these antigens as foreign, leading to the death of the cancer cell. Neoantigens are specific to the tumor in the individual patient making them a highly promising target for personalized, or rather individualized[1] immunotherapy using cancer vaccines. Certain cancers, such as melanoma, result in more mutations than others, making the production of neoantigens more likely.

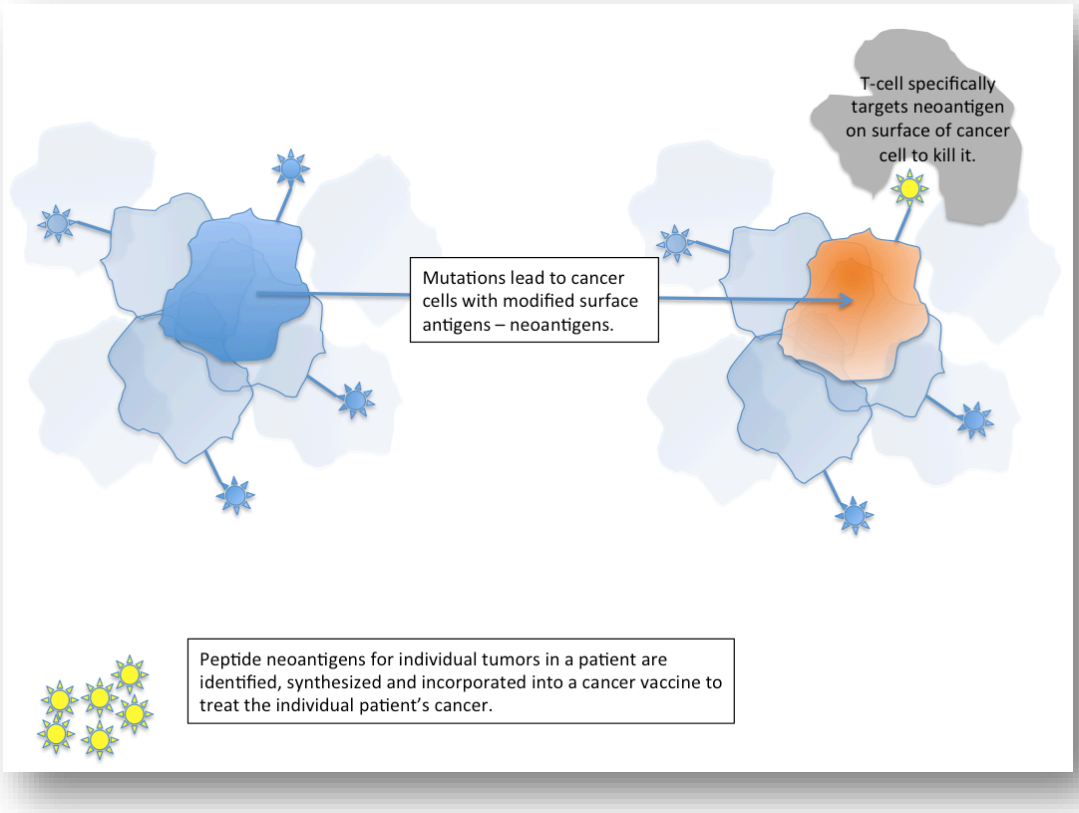

Figure 1: *The principle of immunotherapy based on neoantigens.*

[1] Since 'personalized' can be limited to picking the right drug for a specific patient, many in the field prefer the term 'individualized' i.e. designing therapy that is specific to the individual patient and even tumor.

Cancer vaccines

Cancer vaccines are designed to train the immune system to attack cancer cells. These vaccines are created using antigens specific to cancer cells combined with a delivery vehicle such as a virus that alerts the immune system to the antigen that should be eliminated from the body. Cancer vaccines are generally well tolerated and can be combined with traditional anti-cancer therapies and also other immunotherapies, including checkpoint modulators. In the case of neoantigens, the vaccine strengthens the body's response to the neoantigen that it already recognizes and helps the immune system to target neoantigens that it did not respond to before.

How are neoantigens used in therapy?

Cancer therapy based on neoantigens involves generating a vaccine designed to target the individual cancer cells in a particular patient. The first step in this process is to pinpoint missense mutations in tumor-expressed proteins by sequencing exons of cancer biopsies and normal tissue from the patient. Transcriptome data is also included to indicate antigen abundance. The most critical step is the use of algorithms to identify those mutated proteins that are processed into 8- to 11-residue peptides by the proteasome for later recognition by CD8+ T cells. Once the neoantigens have been identified, they are synthesized using conventional peptide synthesis. Cocktails of the neoantigens are incorporated into vaccines designed to stimulate the immune system to attack the specific cancer cells in the individual patient that express just these neoantigens, although there are also other approaches, as mentioned below. The result is truly individualized immunotherapy, which can be combined with other therapies such as checkpoint modulators and monoclonal antibodies.

Speed is key in this process, since the dynamics of the mutation spectrum of individual tumors means that neoantigen-based therapy can be compared to firing a rifle bullet ('the vaccine') at a predator ('the tumor') that can move in any direction at any moment – maximizing your chances of success is based on hitting the target before it has moved too far away from its original position. This means that the genomic snapshot of the tumor must be quickly converted into a functioning vaccine, with vaccine manufacturers aiming at a turnaround time of just a few weeks.

Neoantigen vaccines have been made possible by major technological advances, including next generation sequencing that enables the timely and cost-effective sequencing of individual genomes. Powerful computer algorithms help identify the best neoantigens to include in a vaccine, although further development is needed to reduce the number of candidates to those that specifically trigger antitumor responses. In addition, advances in manufacturing enable rapid production of small quantities of diverse molecules for individual vaccines.

What is needed from peptide synthesis?

Aside from the usual requirements for high purity and yield, parallel peptide synthesis is also important to generate the pool of neoantigens needed. Speed is a critical factor since timing the delivery of a vaccine can make all the difference to the outcome.

Neoantigens may be further altered through posttranslational modifications (PTMs) that occur in malignant but not healthy cells, and are therefore an additional source of unique antigens that are specific to the individual patient. Such modified neoantigens have been isolated from a number of blood cancers and research clearly shows that PTM-neoantigens make promising targets for immunotherapy (2). Mass spectrometry (MS) analysis has helped in the discovery of a large range of attractive target antigen candidates, such as phosphopeptides (3), that may be used for immunotherapy.

The synthesis of neoantigens requiring modifications may present challenges in synthesis and handling depending on the modification itself and the peptide sequence being synthesized. Generating phosphorylated peptides can be quite straightforward but requires specialized amino acids such as pTyr, pSer, or pThr. However, synthesis with modified amino acids still needs much work. Currently, there are only mimics of pHist and introducing $Lys(Me)_2$ or $Lys(Me)_3$ can be difficult and may reduce the overall quality of peptide synthesis (4). Heating during a deprotection step can result in dephosphorylating the amino acids during synthesis and pSer and pThr can require extra base and longer coupling times (see, for example, reference 4).

Success with neoantigens

The challenge of predicting which neoantigens will generate a response has limited the testing of neoantigen vaccines to cancer forms that have a high mutation rate, such as melanoma. New advances in MS technology, however, now enable us to rapidly detect neoantigens from MHC peptide elutions, even in cancers with low mutational load.

Recent successes in human melanoma cancer vaccine trials have reinvigorated interest in an extremely exciting time in cancer immunotherapy. Early work from Carreno (5) showed that patients given neoantigen vaccines developed antigen specific responses to multiple antigens delivered by dendritic cells. Following up on this, Hacohen and Wu (6) administered peptide vaccines containing up to 20 neoantigens with promising results. Of the six patients treated, four were cancer free two and a half years later, and tumors could no longer be detected in the other two patients once checkpoint inhibitors had been added. In a similar study of neoantigen vaccines, all of the 13 patients developed vaccine specific immune responses and the rate of metastatic disease was drastically decreased post cytoreductive surgery (7).

Trials in the pipeline will investigate the efficacy of neoantigen vaccines for treating a number of other cancer forms, including pancreatic cancer, follicular lymphoma, glioblastoma, non small-cell lung cancer, and bladder cancer. Treatment efficacy may also be enhanced by combining vaccination with other forms of immunotherapy and several clinical trials are underway to assess the safety and feasibility of neoantigen vaccines in combination with radiotherapy or chemotherapy in patients with glioblastoma or breast cancer (8).

Companies active in this field

The promise of neoantigen vaccines has mobilized a lot of commercial activity, including Agenus <http://agenusbio.com>, BioNTech<https://biontech.de >, JPT< https://www.jpt.com/products/pepmix-peptide-pools/>, and Neon Therapeutics <https://neontherapeutics.com>. It was the founders of Neon Therapeutics, for example, who published the initial feasibility, safety, immunogenicity and clinical outcomes for a personal neoantigen vaccine in patients with adjuvant melanoma (6). Agenus is very active in providing vaccines based on individualized vaccines, while BioNTech, founded by Sahin (7), is also aiming to commercialize neoantigen vaccines.

The future challenges and promise of neoantigen vaccines

Neoantigen vaccines are clearly a major step forward in refining immunotherapy, but there remains much to be done before they become a clinical reality. Continued improvement in next generation sequencing will power the analysis of individual genomes, but much better algorithms are needed to predict neoepitopes, and being able to apply this therapeutic approach to cancers with low mutational load would be a great step forward. Future challenges also include the development of vaccine strategies to overcome evolving tumor escape and the spread of tumor antigens after successive treatment.

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References and further reading

1. Coley's toxins in perspective. Starnes CO. Nature (1992) 357:11–2. doi:10.1038/357011a0
2. Identification of glycopeptides as posttranslationally modified neoantigens in leukemia. Malaker SA et al, Cancer Immunol Res. 2017 May;5(5):376-384. doi: 10.1158/2326-6066.CIR-16-0280. Epub 2017 Mar 17.
3. Direct identification of clinically relevant neoepitopes presented on native human melanoma tissue by mass spectrometry. Bassani-Sternberg M et al, Nat Commun. 2016 Nov 21;7:13404. doi: 10.1038/ncomms13404
4. Peptide microarrays to interrogate the "Histone Code". Rothbart, SB. Methods Enzymol. 2012 ; 512: 107–135. doi:10.1016/B978-0-12-391940-3.00006-8.
5. A dendritic cell vaccine increases the breadth and diversity of melanoma neoantigen-specific T cells. Carreno BM et al, Science. 2015 May 15; 348(6236): 803–808. doi:10.1126/science.aaa3828.
6. An immunogenic personal neoantigen vaccine for patients with melanoma. Ott PA, et al. Nature (2017) 547:217–21. doi:10.1038/nature22991
7. Personalized RNA mutanome vaccines mobilize poly-specific therapeutic immunity against cancer. Sahin U, et al. Nature (2017) 547:222–6. doi:10.1038/nature23003
8. Combination immunotherapy: Taking cancer vaccines to the next level. Grenier JM, et al., Front Immunol. 2018 Mar 22;9:610. doi: 10.3389/fimmu.2018.00610.
9. Immunotherapy: past, present and future. Waldmann TA. Nat Med. 2003 Mar;9(3):269-77. DOI: 10.1038/nm0303-269