# Neo-antigen peptide vaccines as emerging cancer therapeutics

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## The problem in cancer therapeutics

Aging-related diseases, including cancer, will form the major socio-economic health burdens of Western societies this coming century. Enormous expenditure on innovative technologies including combinatorial chemistry, whole genome sequencing, crystallography, high-throughput drug screening and computational science have generated significant advances in understanding the molecular basis of cancer. These advances have rapidly generated hundreds of promising drug leads to key oncogenic targets. However, despite this increased expenditure and research and development (R&D), the number of effective drugs reaching the clinic is in steady decline. There are many possible explanations for this, including political and infrastructure drag. A technical problem is the lack of robust age-dependent, sporadic immune-competent models of human cancer that predict toxicity and response in patients. An ideological hurdle is that, until recently, we have had to use 'models' of cancer, such as yeast, worms and flies to identify druggable targets. These models reflect features of a cancer cell but do not mimic tumour tissue *in vivo*. It is difficult to experimentally model an actual tumour. A tumour could be considered a tissue that includes cancer cells themselves, normal supporting tissue including nutrient conduits, a complex local environment that could be very low in oxygen and many types of immune cells that carry out diverse functions.

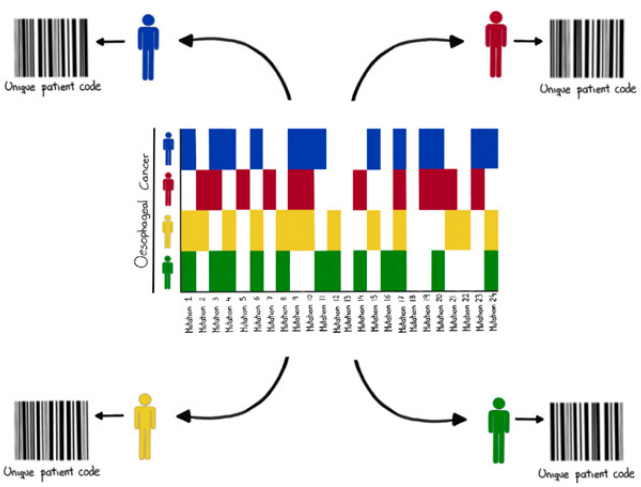


**Figure 1.** The cancer barcode. Cancer from individual patients can share mutations in several genes, but the combined total cancer genome mutations between patients are not identical. This resembles a barcode and offers the opportunity of developing personalized or precision therapeutics based on each unique mutation pattern.

One of the key features emerging from cancer genome sequencing is the striking patient-specific nature of cancer mutations that create a 'cancer barcode' (Figure 1). This highlights the fact that, although cancers from different patients can share common mutations, no two cancers are identical at the genetic level. In addition, recent genomics analysis in one cancer type has identified a large number of cancer-driver mutations in apparently normal tissue that accrue as we age. This suggests that there are powerful tumour suppressor pathways within the mutated landscape that prevent catastrophic cancer development. The unique, 'person-specific' molecular mutation pattern of any one cancer makes it difficult to find common drug targets, so-called 'magic bullets' that could benefit large numbers of cancer patients. However, some cancer patients can be stratified, i.e., subdivided into appropriate treatment groups, using drugs targeted at their specific mutations. For example, the pharmaceutical industry can use epidermal growth factor receptor (EGFR) kinase inhibitors in lung cancer patients who are stratified based

 

on EGFR mutated cancers. The number of such cancer patients are relatively rare, but the numbers add up when collected globally.

An emerging therapeutic application of 'cancer genomics' is to exploit the many so-called 'passenger mutations' in a given human cancer by developing personalized vaccines that enable the immune system to detect cancer-specific, mutated protein fragments, called neo-antigens. Cancer cells producing neo-antigens are seen as foreign or 'non-self' by the immune system and can be eradicated. Recent developments in immunotherapies that target the immune system, rather than the tumour itself, have had striking impacts in the treatment of some cancers. The 2018 Nobel Prize in Physiology or Medicine was awarded to Allison, Honjo and colleagues, for their pioneering work on cancer therapies that relieve negative immune regulation (as discussed earlier in this issue by Sam Hill, Tim Elliott and Peter Johnson). This review will provide a brief history of what we know about immunity based on pathogen vaccines and how this knowledge of infectious agents has informed our view of cancer immunity. We will then address a key question for biochemists, one which will drive our future development of therapeutic cancer vaccines, namely: where do mutated cancer neo-antigens come from in a cell?

## How does the immune system eradicate pathogens?

*'The remarkable fact that one attack of many of the infectious diseases confers immunity from a second attack has led to many explanations… [a new explanation is that] the germs themselves leave behind some material which acts as a poison to succeeding germs of the same disease'*
Dr Carrington Purvis (1890)

Our understanding of cancer immunity in the 21st century is built upon the strategies for treating infectious diseases. At the end of the 19th century preventative vaccines to infectious agents were developed, reducing the incidence of smallpox, anthrax and cholera. In the 20th century, biological materials derived from attenuated or inactivated infectious agents were used as vaccines to reduce the worldwide spread of poliovirus, measles and many other pathogens. How do the antiviral or antibacterial vaccines work? One of two fundamental mechanisms exist whereby the immune system can create immunity to a virus, for example, measles (Figure 2a). In one arm of the immune system, a protein produced by a pathogen can activate a B cell that can, in turn, create antibodies specific to the foreign protein. A long-lasting, so-called memory B cell response can then produce high-affinity antibodies that act to neutralize any subsequent infection or propagation by the same pathogen.

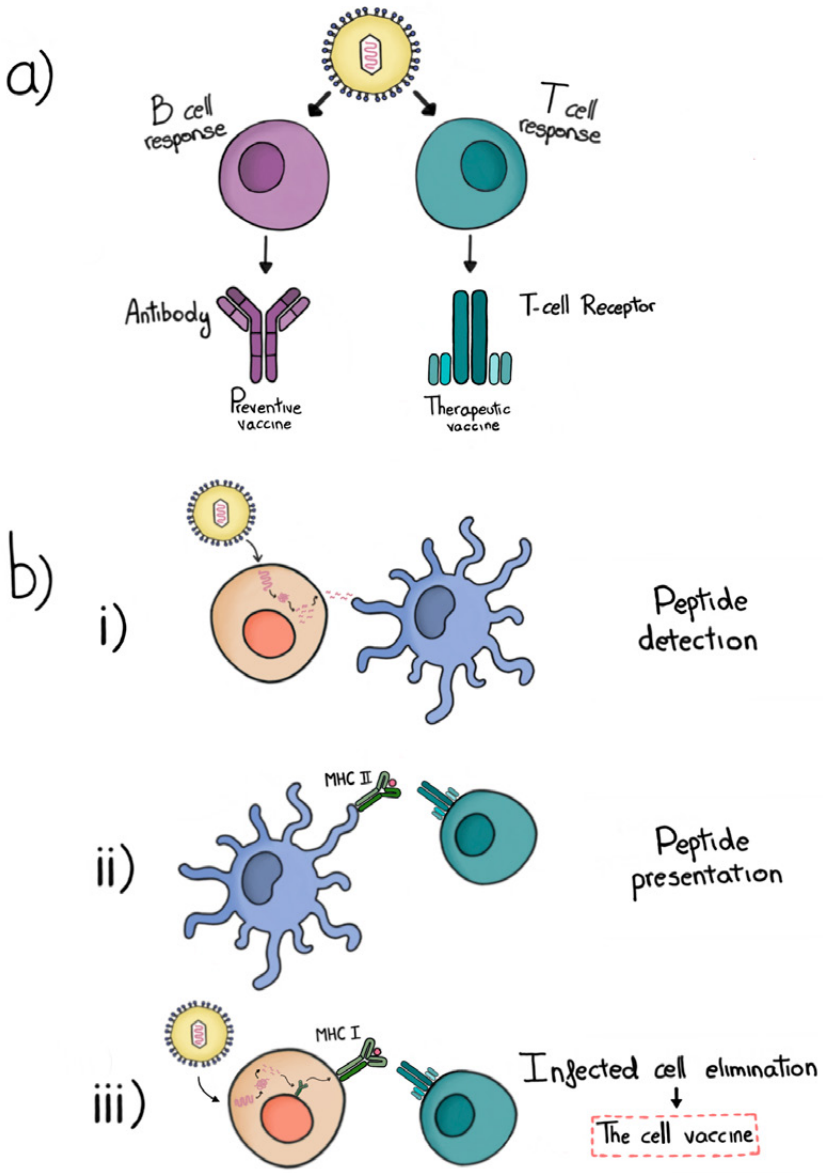


**Figure 2.** Two different immune responses to pathogens. **a)** A viral infection (in yellow) can result in both B cell and T cell responses which lead to either (left) the production of antibodies from a B cell that can detect a foreign protein or (right) a T cell receptor expressing immune cell that can detect a pathogenic protein on an infected cell surface. Both of these branches of the immune system can lead to preventative or therapeutic vaccines. **b)** The classic multi-cell step eradication of an infected cell by the immune system. Peptides (in red) from a virus can be detected by dendritic cells. The dendritic cell can capture the peptides, and presents them on their cell surface through MHC class II molecules to T cells. This activates a T cell response that amplifies cells that produce T cell receptors which, in turn, can detect MHC class I–viral peptide complexes on other virus-infected cells. The production of viral peptides or cancer neo-antigens can be channelled into similar pathways to produce T cells that can detected MHC class I peptide molecules.

Into the 21st century, preventative vaccines exist even for a cancer-causing virus, the cervical cancer associated human papillomavirus (HPV). The preventative HPV vaccine contains recombinant biological material that mimics the HPV coat proteins and produces a memory B cell response that reduces the HPV incidence rate of infection. In the decade since HPV vaccines were introduced in the West, we have already observed a reduction in the incidence of HPV-dependent cervical cancers. However, the HPV vaccine is not a therapeutic vaccine; it does not eliminate HPV-infected cells or reduce disease incidence in an affected individual.

In 1890, *The Lancet* published a letter by Dr Carrington Purvis that discussed his previously unpublished ideas for developing a therapeutic vaccine to treat infectious disease. He proposed an 'experiment' to transfer blood from an individual who survived scarlet fever to a patient who had just caught scarlet fever. Purvis suggested that 'white blood corpuscles' in our bodies were the likely 'germ destroyers'. Would this 'therapeutic' blood-based vaccine reduce the life-threatening symptoms of the newly infected patient? This focused on what we now know to be the second branch of the immune system, whereby a T cell can detect a pathogenic protein fragment on the surface of an infected cell (Figure 2a). Recognition results in the death of the infected cell and elimination of the pathogen from the body. The proposal to carry out a therapeutic blood transfusion might now be considered the forerunner of current immune cell vaccines designed to target and kill, for example, HIV-infected cells.

In his letter, Purvis highlighted the mechanism by which immunity to a viral pathogen might be imprinted as a 'memory'. One idea required the germ to leave behind a piece of itself (i.e., a 'poison') in the host. The germ-specific poison could then be propagated in the body conferring permanent immunity. We might consider this poison to be a 'neo-antigen'; a new, foreign, 'non-self' peptide fragment. In one sense, the neo-antigen becomes genetically encoded in the form of neo-antigen-detecting T cells that kill any future infected cell appearing on the scene.

The neo-antigen paradigm invokes the concept that a protein produced by a pathogen inside an infected cell can be detected as a foreign agent by the immune system (Figure 2b). Following virus infection into the host cell, a race starts which balances the evolutionary adaptation of the virus to replicate itself against the orchestrated defence systems of the infected cell that attempts to neutralize the virus. If the host defence system wins this race, it will minimize tissue damage. In the example highlighted (Figure 2b), a virus begins to produce viral proteins inside a cell. In response, the host cell engages its first defence system. A dendritic cell captures viral protein fragments using its major histocompatibility complex (MHC) molecule (Figure 2b-i). The MHC molecule in the dendritic cell can travel to the cell surface and expose the viral peptide to a T cell receptor (TCR) molecule on a naïve T cell (Figure 2b-ii). This activates a T cell population growth spurt that has the ability to detect the same viral peptide sequence on the surface of other infected host cells. Upon continued host cell infection, the affected cell itself raises the alarm and exports the viral neo-antigen to its surface, thus enabling the pre-activated T cell to detect and kill the virus-infected cell (Figure 2b-iii). This is the mechanism whereby T cells can eliminate pathogen-infected cells from the body. The virus, in essence, produces the seeds of its own destruction; it makes its own poison. A therapeutic anti-viral vaccine can function in a similar manner to create or amplify T cells that can detect MHC class I peptide molecules on the cell surface and kill pathogen-infected cells. Strategies to develop therapeutic vaccines based on viral peptides that can bind to MHC class I molecules are now being developed for two cancer-causing viruses, the Epstein-Barr virus and HPV.

## How do cancers evade the immune system?

Infectious disease was not the only field of medicine in the late 19th century that was interested in the concept of vaccines. In the 1890s, the oncologist Dr William Coley observed that many sarcoma patients who experienced a severe bacterial infection called erysipelas, caused by streptococcal bacteria, underwent spontaneous tumour remission. *The New York Times* recently reported that Coley was not alone in thinking that therapeutic bacterial vaccines could be used to treat cancer; 'In a letter to a colleague in 1890, the Russian physician and playwright Anton Chekhov wrote of erysipelas: "It has long been noted that the growth of malignant tumors halts for a time when this disease is present". Coley went on to develop inactivated bacterial vaccines that were claimed to have a relatively high degree of success in treating cancer patients. However, Coley's anti-cancer vaccine did not take off and X-ray therapy, which emerged at the same time, was the preferred therapeutic route for physicians of the day. Nevertheless, such early evidence suggests that the immune system can be activated by a vaccine to suppress cancer growth and that cancer can find a way to evade immune eradication.

By 1957, a new generation of medical scientists, including Burnet and Thomas, put forward the theory that genetic mutations that increase as a function of age could kick-start cancer development. They suggested that certain immune cells would be able to detect and eradicate emerging tumour cells, giving rise to the immunosurveillance hypothesis. Since then, genes such as STAT1, RAG-2, IFNGR1 or IFNG, which produce defects in T cell, B cell, γδ-T cell and natural killer (NK) immune cell populations, have been shown to alter tumour growth rates.



 

There are several stages at which immune cells can reject emerging tumours. According to the immunosurveillance hypothesis, emerging cancer cells could produce mutated proteins that can be captured by dendritic immune cells, producing tumour-detecting T cells. In turn, such T cells can recognize MHC class I mutant peptides on the cancer cell surface resulting in cancer cell eradication (Figure 3a). Although cancer cell killing at early stages, by the classic dendritic cell–T cell cascade (Figure 3a), is perhaps best understood, how cancers actually evade the immune system is more complex. Growing tumours that escape the immune cell surveillance system through evolution (by natural selection) can acquire additional mutations that ensure survival even in the presence of immune cells. Cancer cells exist in equilibrium with immune cells and some immune cells such a regulatory T-cell (Treg) that can play a positive role in tumour stasis (Figure 3b). This provides a therapeutic strategy for developing drugs that stimulate tumour rejection.

Finally, in more advanced cancers, further mutations accumulate that result in a highly immune-suppressive environment in which immune cells are attenuated (Figure 3c). In this immunosuppressed state, there are receptors on the immune cells that are inhibited by molecules in the cancer cell thus dampening the immune cell response. One of the most well-studied immune cell receptors, named PD1, can be targeted with monoclonal antibodies that results in the stimulation of immune cells and significant reduction in cancer growth in patients. In addition to the targeting of immune cells by monoclonal antibodies, there are now several clinical trials in progress using dendritic cell vaccines, RNA vaccines, synthetic peptide vaccines and DNA vaccines. These vaccine approaches aim to deliver mutated peptides to the immune system to stimulate the production of T cells that can detect and eradicate neo-antigen-expressing tumour cells. Accordingly, discovering the most potent MHC class I binding neopeptides is becoming the new 'drug-discovery frontier' in anti-cancer vaccine research.

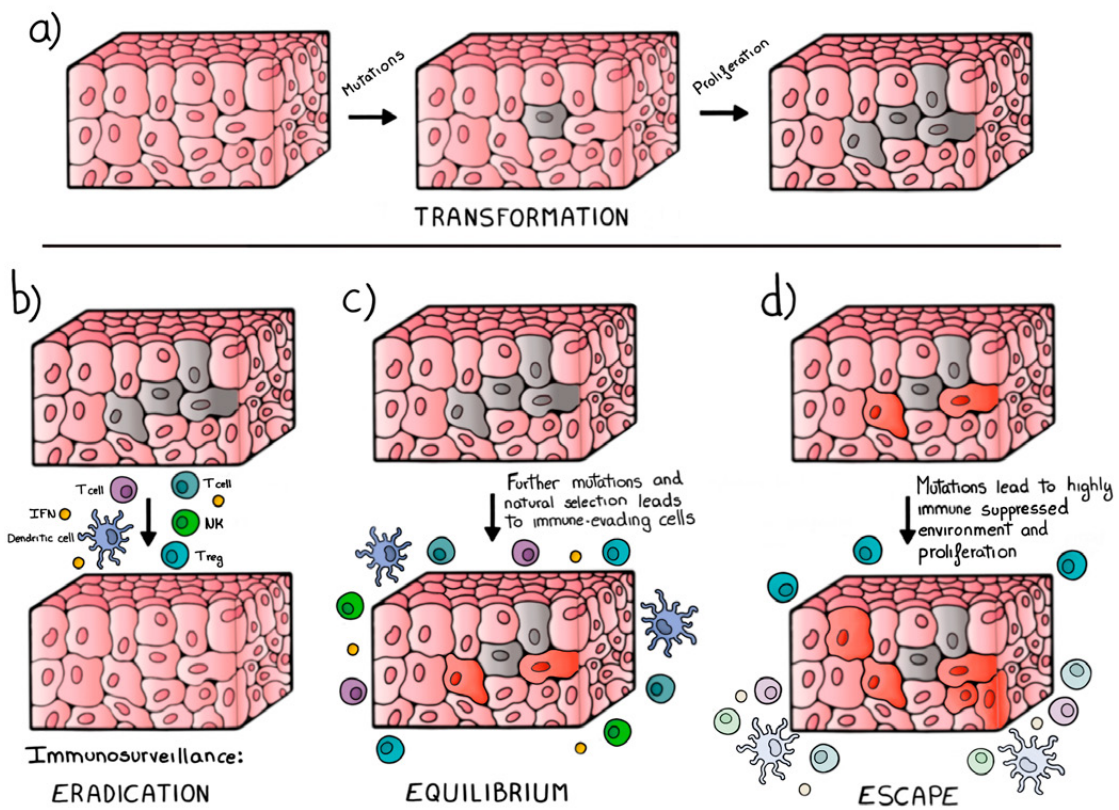


**Figure 3.** The immunosurveillance hypothesis that invokes three stages in the evolution of cancer. The eradication stage of cancer (left panel) is when emerging tumour cells (in grey) are detected as foreign bodies through the production of mutated neo-antigens and these cells are destroyed by the dendritic-T-immune cell cascade (Figure 2b). The equilibrium stage is when additional mutations (in red) result in cancer cells that become invisible to the immune system (perhaps suppressing MHC class I molecules so that reduced neo-antigens are produced on the cell surface). The escape stage is when the tumour has accumulated further adaptive changes that result in immune cell suppression. It is in the immune cell-suppressed stage that the new immunotherapies target reactivate immune cell functions.

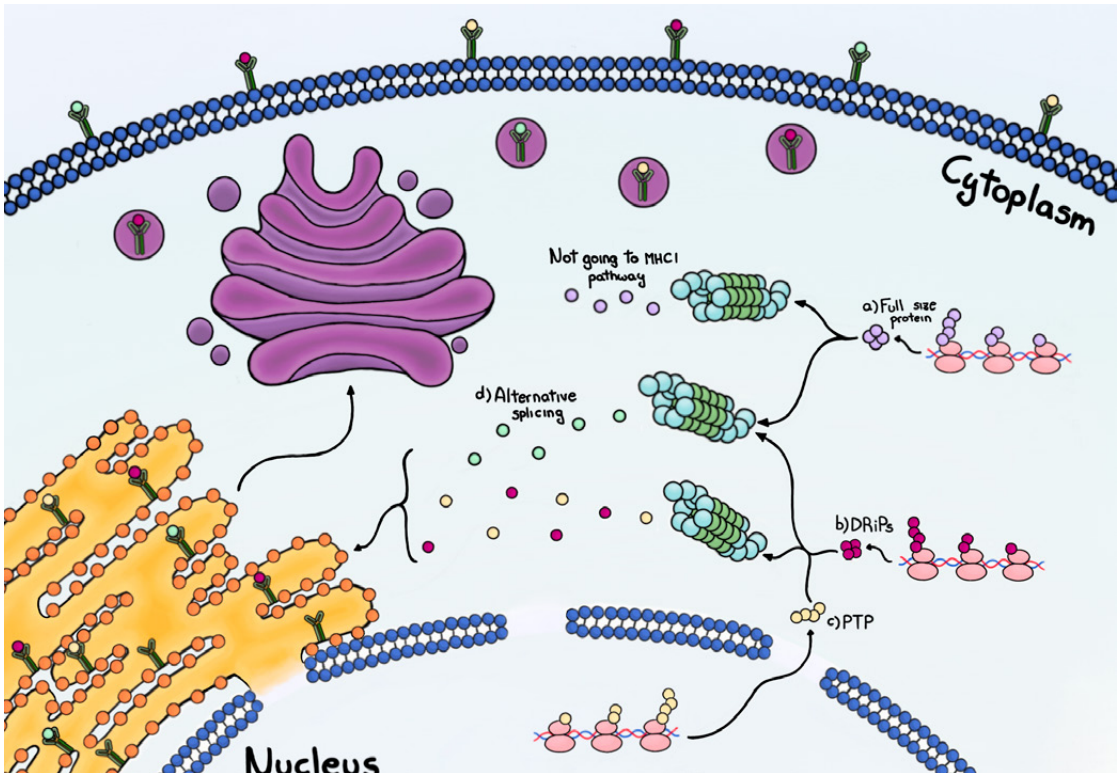


**Figure 4.** Origins of neo-antigens in tumour cells. Textbooks highlight the general view that full-length proteins can produce peptides through degradation that can be channelled into the MHC class I antigen presentation pathway. However, a new paradigm (DRIPs) argues that defective protein folding of a protein during translation can result in its degradation and then processing through the MHC class I pathway. Another source of MHC class I peptides is derived from pre-spliced RNA in the nucleus thus highlighting a relatively untapped source of neo-antigens for potential vaccine developments. Another source of MHC class I peptides stems from peptide splicing at the proteasome. Dissecting the biochemical mechanisms of how these various pathways can produce MHC class I peptides, and whether these pathways are suppressed in advanced cancers, will facilitate the development of therapeutic anticancer vaccines in the future.

## Where do neo-antigens come from in cancer cells?

The presentation of antigenic peptides on MHC class I molecules allows the immune system to detect and destroy cells expressing non-self antigens and forms the basis for generating vaccines (Figure 2b). Deeper understanding of the origin of neopeptides is becoming an important part of anti-cancer vaccine development since neopeptides act like drugs that stimulate cancer rejection. Most current peptide neo-antigen discovery platforms focus on the use of mutated exomic regions in cancer genes to identify potential neo-antigens. The current state of the art in tumour-specific antigen discovery includes the use of next-generation exome sequencing that facilitates the discovery of tumour-specific antigens using (i) mass spectrometry, (ii) molecular docking onto available MHC class I structures and/or (iii) functional mutant antigen-specific T cells.

It has long been thought that the degradation of full-length proteins is the major source of peptides destined for the MHC class I pathway (Figure 4). However, there has been a shift away from this theory to alternative peptide (AltPep) sources. The DRIPs model of antigen presentation (defective ribosomal in-frame translation products) gives rise to polypeptides from in-frame translation of mRNA that are not assembled into their native structures (Figure 4). This model holds that defects in translation, or protein folding, that is sensed as inaccurate can lead to processing of peptides through the MHC class I pathway. In addition, there is emerging evidence for a source of peptide translation products that come from introns (Figure 4). This so-called 'pioneer' round of translation can take place on pre-spliced mRNAs and is consistent with earlier discoveries showing the existence of intron-derived MHC class I peptides in cancer cells. The pioneer translation products (PTPs) can be a source for direct presentation and are cross-presented from tumour cells to dendritic cells, perhaps through exosomes, and are highly efficient in promoting cancer rejection in animal models. PTPs can be made from newly synthesized mRNA during an RNA quality control step. This might help the immune system to fight viral infections by guaranteeing that the peptides encoded by a viral mRNA are presented to the immune system, ensuring early pathogen detection.

Most recently, it was suggested that MHC class I peptides can also be generated via peptide splicing within the proteasome (Figure 4). A spliced peptide repertoire from a cell would greatly increase the combinatorial diversity of MHC class I peptides, although it is difficult to understand how this increase in non-genome encoded peptides can be recorded as 'self' by the immune memory. Evidence for spliced peptides in cancers being detected by tumour-reactive T cells is in its infancy. However, there is evidence in mouse models that T cells can be detected binding to MHC class I peptides that come from spliced peptides. Together, these different observations (Figure 4) have now opened the door to a biochemical understanding of where neo-antigens come from in tumour cells, which gene products regulate neo-antigen flux, what their role



 

is in presenting tumour neo-antigens and how we can exploit this knowledge to produce more accurate neo-antigen vaccines to stimulate cancer rejection by the immune system.

## Conclusions

The Nobel Prize in Physiology or Medicine was awarded in 2018 for the groundbreaking discovery of monoclonal antibodies that can stimulate immune cells and promote cancer rejection. This provided a solid proof-of-concept that cancer therapeutic strategies can be modified to include drugs that target the immune system. These discoveries built on over 100 years of knowledge gained from producing preventative vaccines to infectious diseases. We now know that the mechanisms whereby the immune system can eradicate pathogen-infected cells is very similar to that used to promote cancer rejection; the immune system can recognize both viral antigens and mutated cancer associated antigens as 'foreign'. This knowledge is driving R&D in the areas of preventative and therapeutic vaccine developments that aim to target mutated cancer antigens, or viral-causing cancers, to eliminate tumours from the body. Fundamental to the R&D pipeline is understanding where neo-antigens come from in cancer cells so that we are better able to produce personalized vaccines. The origin of neo-antigens in cancer cells is far more complex than current textbooks propose (Figure 4). This opens the doors for a new generation of biochemists to make further discoveries in the fundamental pathways that lead to the production of mutant peptides in cancers that can be exploited as anticancer vaccines. ■

*Professor Ted Hupp was educated in microbiology and chemistry (USA). Following a PhD in Biochemistry at Michigan State University, he applied enzymology principles to the cancer research field to study the structure and function of the tumour suppressor p53. His current position is Chair of Experimental Cancer Research, University of Edinburgh (UK) and Co-Director of the International Centre for Cancer Vaccine Science (ICCVS) (Gdańsk, Poland).*

*Professor Robin Fahraeus trained as a clinician in Sweden and carried out postdoctoral work in the UK. He currently directs the therapeutics in the cancer group with INSERM (Paris). His research focuses on the physiological implications of mRNA translation control in cancer. Together with Ted Hupp he co-directs the ICCVS (Poland).*

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*Professor Kathryn Ball trained as a biochemist specializing in enzymology (UK). She then carried out postdoctoral research on protein structure function relationships in the UK and USA before moving to Dundee where she set-up a group studying the biochemistry of interferon signalling in health- and age-related disease. She is currently the Professor of Biochemistry and Cell Signalling at the IGMM (Edinburgh). Email: Kathryn.Ball@ed.ac.uk*

### Further reading


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