Cancer vaccine: how far are we from curing cancer?

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Although cancer treatment has developed considerably in the 21st century, there are still 8–9 million people dying of this disease each year. One strategy, using a patient's own immune system to combat their cancer, has recently led to a significant improvement in survival rates. The concept of immunotherapy is not new; as far back as 1893, William Coley reported tumour regression following repeated injections of bacterial toxin from *Streptococcus pyogenes* in a patient with advanced sarcoma. Over 100 years later, the use of cancer vaccine has come a long way, with a better understanding of the immune system's interaction with the tumour microenvironment.

Checkpoint inhibitors

To evade the immune system, tumour cells can express PD-L1 which is a ligand for the checkpoint protein PD-1, found on T cells. PD-L1 expression is important in limiting collateral damage to normal tissues. Generally, binding to PD-L1 reduces the effectiveness of T cells. If this interaction is blocked by a monoclonal antibody (mab), such as Nivolumab or Pembrolizumab, then the T cells can realize their full potential. Other checkpoint mabs include Ipilimumab, which blocks CTLA-4 interaction with its receptor CD80/86, preventing activation-induced cell death. The use of checkpoint inhibitors has been successful in many cancer types in recent years.

Cancer vaccines

Cancer vaccination involves stimulation of the immune system, specifically CD4 and CD8 T cells, in a way that promotes recognition and elimination of tumour cells. Proteins expressed on a target cell serve as antigens, which bind to receptors on T cells, leading to activation of the latter. Anticancer immunotherapy relies on effective targeting of antigens that can be recognized by high avidity T cells. Many antigens expressed on tumours are also expressed on normal tissues. Furthermore, in order to avoid T cell attack on healthy tissue, T cells are deleted if the antigen they recognize is also expressed in the thymus. The goal is to find antigens that are not thymically expressed and are found in high abundance on tumour cells but not healthy tissues.

Personalized vaccines

Each cancer has a unique signature arising from specific mutations. These mutations are represented in epitopes which act as 'neo-antigens' if they escape thymic selection. Using lymphocytes and tumour cells from a melanoma patient, a 'personalized vaccine study' demonstrated neo-antigens are associated with the long-term survival of melanoma patients. Indeed, patients whose tumours have a higher mutation rate often respond better to immunotherapy.

Different mutations can give rise to the same cancer phenotype, necessitating the use of individual vaccines for each patient. This involves sequencing the DNA and RNA of cancer cells of individual patients and then predicting the epitopes that will induce responses in CD4 and CD8 T cells, using specific algorithms and software. This method has already been used in six patients with melanoma. Following vaccination, four of the six patients were recurrence-free for 2 to 3 years. The other two patients experienced disease recurrence, however, they responded successfully to the receipt of checkpoint inhibitors, suggesting the vaccine resulted in expansion of neo-antigen-specific T cells. In another study, patients showed a decrease in cancer metastatic events and remained progression-free after treatments targeting multiple neo-antigens with the intranodal delivery of an RNA polyepitope vaccine.

The major disadvantage of designing vaccines targeting neo-antigens is that patient-specific vaccines are expensive. Furthermore, there is a high risk of tumour heterogenicity, with regions of outgrowth that do not express the target neo-antigen.

Phosphorylated peptide vaccines

Many proteins undergo post-translational modification following their biosynthesis. Phosphorylation is the most common enzymatic post-translational modification and key to cell signalling. Due to high metabolic stress and

uncontrolled proliferation, phosphorylation is increased in cancer cells. As a consequence, quantities of phosphorylated peptides are often higher in cancer cells compared with normal cells, making them an attractive target for cancer vaccines. Antigens on the surface of the cells are presented by major histocompatibility complex (MHC) class I and class II molecules to CD4 and CD8 T cells, respectively. Recent studies have shown phosphorylation of peptides is preserved during antigen presentation and phosphopeptides can be presented to both MHC class I and class II molecules, thereby activating both CD4 and CD8 T cells. Potential vaccines include pIRS-$2_{1097\text{-}1105}$ and pCDC25$b_{38\text{-}46}$. An *in vitro* study with human peripheral blood mononuclear cells (PBMCs) showed donors are able to produce a CD8 T cell-specific response to these peptides, suggesting individuals have a T cell receptor repertoire. Furthermore, both peptides were found to be presented by the MHC class I molecule in multiple carcinomas. A group in the University of Virginia have discovered more than 600 phosphopeptides that are presented by MHC class I and II on tumour cells. These peptides are attractive candidates for potential vaccines as only a few of them are displayed on healthy cells. Some of those peptides have been shown to induce memory responses when PBMCs from cancer patients were stimulated *in vitro*. This suggests the cancer patients already had an expanded pool of specific T cells that recognize phosphopeptide neo-antigens and therefore are likely to make a response upon immunization with these antigens. The development of phosphopeptide cancer vaccines is a new area, but it will not be long before they are used for treatment.

Citrullinated peptide vaccines

A further post-translational modification is citrullination, which is an enzyme-based conversion of arginine to citrulline. This process normally occurs when cells are under stress during autophagy, a process that allows cells to generate energy from the digestion of intracellular proteins/organelles. Autophagy is triggered in response to external or internal stresses such as nutrient deprivation, hypoxia, DNA damage, the presence of reactive oxygen species and protein aggregation. There is an inherent nutrient deficiency and hypoxia in tumours due to the unregulated proliferation of cancer cells. Indeed, evidence from the literature shows autophagy is highly localized in the centre of a growing tumour, prior to the occurrence of angiogenesis, while the tumour core is hypoxic. Citrullinated peptides generated during autophagy can be presented to T cells as neo-antigens, since wild-type peptides containing arginine are normally degraded by proteolytic enzymes.

How effective are citrullinated peptide vaccines?

Earlier cancer vaccines predominantly targeted CD8 cells for their cytotoxic effects against tumour cells; however, the focus has recently shifted to CD4 cells. We have developed the MODI-1 vaccine that consists of two vimentin and one α-enolase citrullinated peptides. The vaccines target and harness the power of CD4 T cells to kill the tumour. The mechanism of action of citrullinated peptides is shown in Figure 1. After vaccination, the citrullinated peptides are taken up by antigen presenting cells (APCs), such as dendritic cells, which process the peptides and present them to CD4 T cells through MHC II molecules. As these CD4 T cells infiltrate into the tumour microenvironment, they encounter citrullinated peptides expressed on the surface of APCs. As a result, the CD4 T cells become activated and secrete interferon γ (IFNγ). Tumour cells often evade the immune system by creating an anti-inflammatory microenvironment where MHC II expression is not upregulated. However, IFNγ secreted from activated CD4 T cells can shift that balance and induce upregulation of MHC II expression by tumour cells.

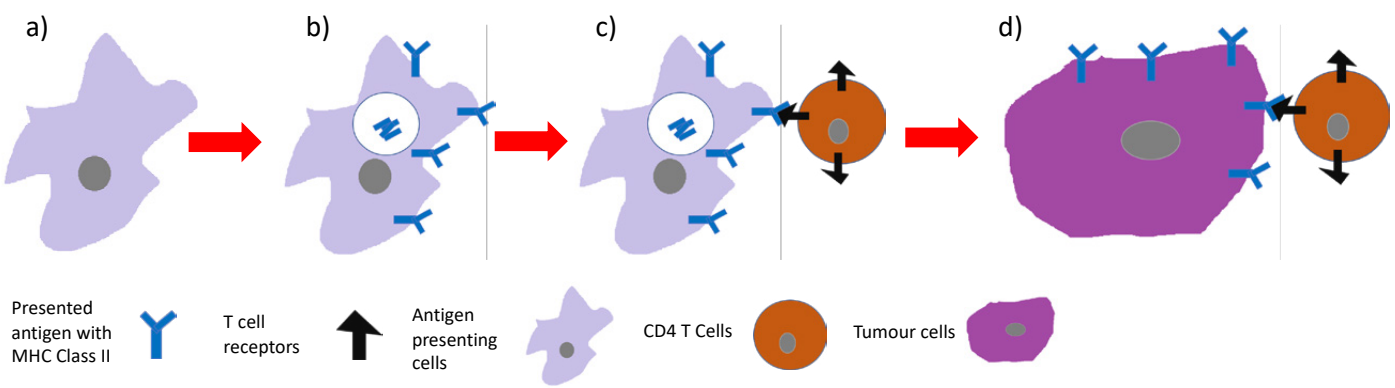

Figure 1. Schematic showing the mechanism of action of MODI-1: **a)** antigen presenting cells (APCs), **b)** after vaccination, MODI-1 citrullinated peptides are taken up by APCs; they are processed and presented on MHC class II molecules on the APCs, **c)** CD4 T cell receptors bind to the MHC class II molecules, **d)** CD4 T cells infiltrate the tumour, encounter and recognize citrullinated peptides expressed on the APCs. CD4 T cells become activated and release IFNγ which induces upregulation of MHC II expression by tumour cells. CD4 cells become further activated and kill tumour cells.

CD4 T cells become further activated by recognition of the tumour cells and directly kill the cells via the secretion of cytotoxic molecules.

MODI-1 vaccines have been extremely successful in animal studies with transgenic mice expressing human MHC II. Both vimentin and α-enolase peptides individually have been shown to induce immune responses against tumours in HLA-DR4 transgenic mice. Since these mice are expressing HLA-DR4 (a human MHC II molecule) we can effectively establish a 'human' tumour model and investigate the interaction between human CD4 T cells and human tumour *in vivo*. Vimentin peptides induced a strong CD4 anti-tumour response leading to the survival of 80% of transgenic mice. Vaccination with just α-enolase also showed significant survival against a number of tumour types including melanoma, and those in the lung and pancreas. Furthermore, CD4-specific responses were also observed when these peptides were tested *in vitro* using PBMCs from healthy individuals with several HLA types. The results from PMBCs proliferation assays suggest the citrullination-specific response is not restricted to just one HLA type, hence the vaccine can be used in a wider population. Clinical trials with MODI-1 are now being designed.

Combination therapy

Vaccines are often very effective at inducing a strong T cell response but fail to achieve regression of an established tumour, due to the immunosuppressive tumour microenvironment. Combining vaccines with checkpoint inhibitors may improve the success rate (Figure 2).

Combination therapy involving vaccines and checkpoint inhibitors is already in clinical trials, such as Neovax with Ipilimumab for treating renal cancer and NEO-PV-01 with Nivolumab for the treatment of melanoma, lung and bladder cancer.

Conclusion

Currently, if cancer is diagnosed early, physicians have several options at their disposal for treating patients and immunotherapy is slowly becoming a more popular choice. New potential ways of harnessing the power of the immune system to defeat cancer are being discovered due to advances in medicine. We are certainly closer to curing cancer than we have ever been before. It is not a matter of if, but when, we overcome the challenges of cancer and treatment with vaccines may be one way. ■

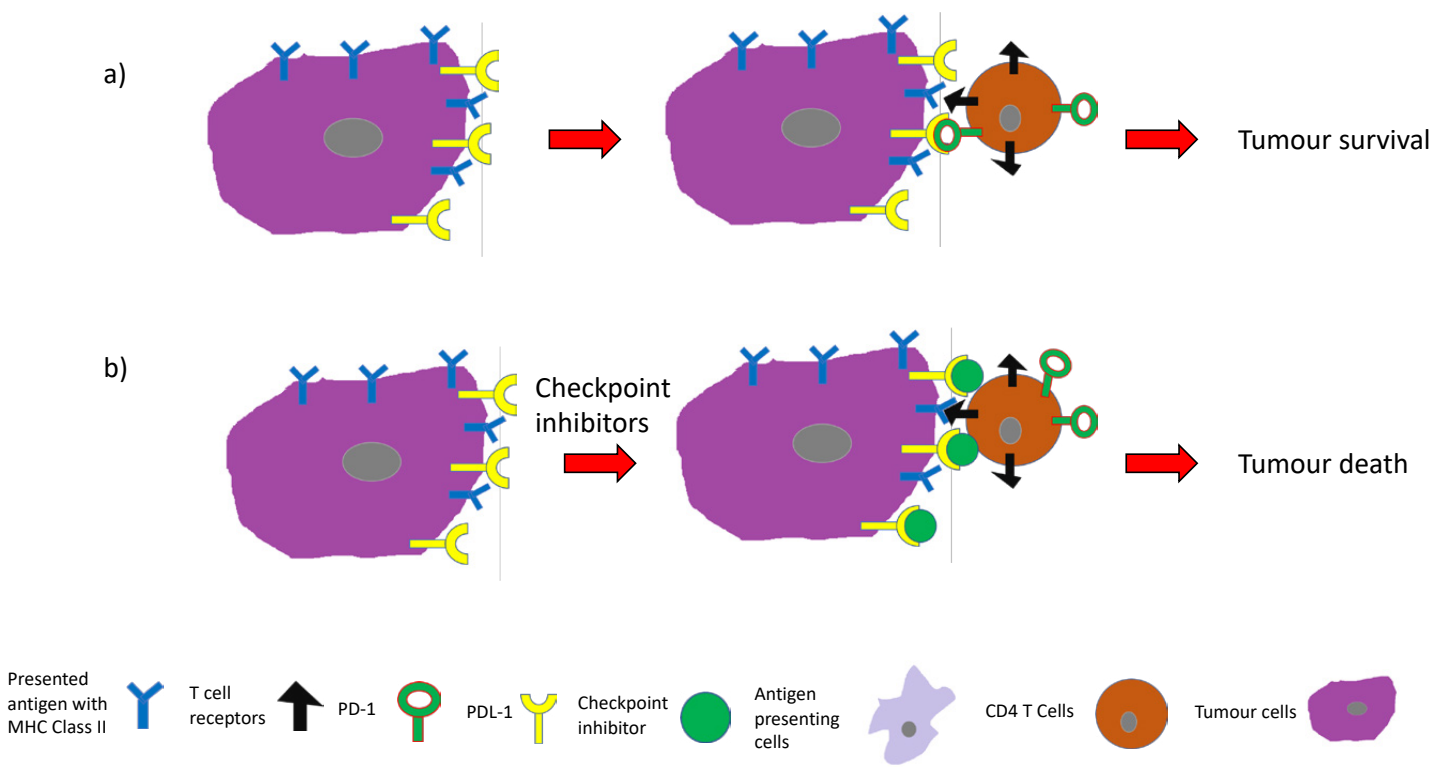

Figure 2. Schematic showing pathways with and without checkpoint inhibitors. **a)** Tumour cells express PDL-1 which interacts with PD-1 on T cells and leads to T cell apoptosis. The tumour survives despite expressing T cell receptors to the antigen expressed on tumour cells. **b)** The addition of checkpoint inhibitors blocks PD-1 and PDL-1 interaction. T cells are then able to recognize and kill the tumour cells.

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Lindy Durrant is Professor of Cancer Immunotherapy at the University of Nottingham and the co-founder of an onco-immunology company, Scancell. Lindy has worked for over 20 years in translational research. Her previous work involves developing monoclonal antibodies for diagnostic imaging and now most of her work is focused on discovering ways to use the immune system to beat cancer. Lindy's group have already developed a DNA vaccine named SCIB1 for the treatment of melanoma which is currently in clinical trials. Her group is currently working on a peptide vaccine, MODI-1, that showed promising results in mice models. A clinical trial is now being designed to test the safety and efficacy of MODI-1. Email: lindy.durrant@nottingham.ac.uk

Further reading

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