Doggybones, DNA vaccines and skin-penetrating fluids: whatever it takes to win the fight against cancer

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Nucleic acid-based vaccines have risen in popularity following the success of mRNA vaccines during the COVID-19 pandemic. Growing research in response to the pandemic has now seen DNA vaccines enter the stage, with phase III trials currently underway in India. As with mRNA vaccines, DNA vaccines can be made to encode protein or peptides that are thought to be expressed on the surface of pathogens and infected and cancerous cells. With over 17 million people reported to be diagnosed with a cancer worldwide in 2018, cancer continues to impose a very real threat. Although conventional treatments such as chemotherapy have reduced mortality, cancerous cells often evolve, adapting mechanisms that help them survive and evade immune recognition. DNA vaccines present an immunotherapeutic tool that addresses this issue by improving the visibility of cancer cells. Here we discuss the role of DNA vaccines in a dynamic arms race against cancer.

He is on the run. Turning into a busy London street, he tactically moves between the crowds, knowing he fares a better chance at losing them here than in the empty park. They should have known! They should have called for reinforcements! He knew that their hopes of closing in on him were diminishing by the minute with every step he took…

While this might sound like an excerpt from a crime novel, it is in fact a window into the plight of the immune system in its fight against cancer.

As you read this article, the trillions of cells in your body are undergoing a tightly regulated, multi-step process to help maintain the balance between cell death and cell birth. However, this process is not perfect, and faulty DNA replication machinery often introduces mutations in the cell. When these mutations affect control mechanisms, they can lead to uncontrolled cell division, resulting in cancer. With over a 1000 new cases being reported every day in the UK, cancer is a life-changing disease that remains at the forefront of biomedical research. With numerous cells in the human body, and a process that is not fool proof, stray cancer cells can be a common occurrence. Nevertheless, our bodies are armed with a remarkable defence mechanism that keeps these cells in check.

Enter: the immune system!

The immune system consists of a diverse group of cells that keep pathogens out, help maintain internal processes and destroy malignant cells. The immune system is equipped to detect antigens presented on the surface of cells as small nine amino acid peptides. These peptides help the immune system differentiate between the good, the bad and the ugly! Yet, the battle against the immune system and cancer is not straightforward. It is an arms race. When under attack, cancer cells evolve mechanisms that help them evade immune recognition and inhibit immune cells. Immunotherapy offers the much-needed reinforcements that facilitate the immune response against cancer.

Cancer cells are almost identical to healthy cells but with a few unique mutations in their DNA. These mutations may result in mutated peptides on the surface of these cells which can be identified by the immune system. However, these mutated antigens are often very similar to those on healthy cells, so while we might expect cancer cells to stand out like a white poppy in a field of red, the reality is far from it. By blending in with their surroundings, the cancer cells go unnoticed, just like a felon on the run in a busy London street. Vaccines help the immune system

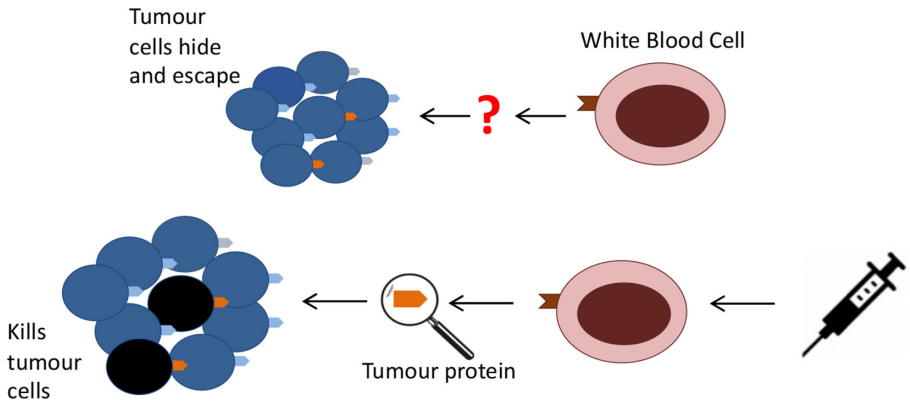

Figure 1. Making cancer cells visible – DNA vaccines encode peptides that are presented on the surface of cancer cells. They help the immune system differentiate between healthy and transformed, cancer cells.

better identify and eradicate these well-disguised cancer cells (Figure 1).

A game-changing tip-off

Cancer vaccines can be prophylactic or therapeutic. Cervarix, Gardasil and Gardasil 9 are some of the most prominent prophylactic cancer vaccines to enter clinical use against the human papilloma virus (HPV) and have successfully reduced the incidence of HPV-driven cervical cancer in various studies. In spite of this success, prophylactic vaccines are largely limited to cancers of viral origin. Therapeutic cancer vaccines allow for curative immunotherapy by targeting antigenic peptides specifically expressed on cancer cells. They can be used to target differentiation antigens, antigens overexpressed in transformed cells, antigens normally expressed in the testis, viral antigens and neoantigens generated from mutated proteins specific to cancer cells. Therapeutic cancer vaccines can vary greatly from whole tumour cells to nucleic acids encoding these tumour antigens.

Nucleic acid-based vaccines such as mRNA vaccines have risen in popularity following their phenomenal success in the fight against COVID-19. They have shown favourable safety profiles and have effectively mounted antigen-specific immune responses against COVID-19 and in small cohorts of patients with melanoma. Despite being effective in the clinic, RNA is prone to degradation and must be stored at extremely low temperatures to maintain their structural integrity. In contrast, DNA-based vaccines offer a more stable and economical alternative, and can be stored and transported at room temperature, making them more accessible than RNA vaccines. DNA vaccines consist of a plasmid backbone encoding antigens of interest and can encode sequences that promote nuclear localization, gene expression and immune activation. For example, researchers at the University of Southampton have shown that vaccine efficacy can be boosted by incorporating an immunogenic fragment from the tetanus toxin as it promotes the recruitment of multiple immune mechanisms. Furthermore, DNA vaccines are easy to manipulate, result in prolonged expression of target antigens and allow post-translational modifications and antigen processing before presentation on the cell surface. Studies in murine models have also suggested that the size of the plasmid and the arrangement of the epitopes are unlikely to affect the efficacy of the vaccine *in vivo*; however, it is currently unclear if plasmid size limits nuclear transfer.

Candidate antigens for vaccination are selected in a multi-step process (Figure 2), where thousands of mutations are screened to identify those that are aberrantly or exclusively expressed in cancer cells and predicted to mount an immune response. Selected antigens are compiled into a plasmid DNA vaccine, which, upon injection, is taken up by a specific subset of immune cells known as dendritic cells that go on to train the key fighters, T cells, to recognize and kill cancer cells.

Hot off the press!

DNA-based cancer vaccines have shown favourable safety profiles and efficacy in preclinical and clinical settings, with those encoding tumour antigens, including neoantigens potently delaying tumour growth and improving survival. In addition, tumour regression and tumour-specific immune responses have also been observed in phase I and II studies for breast, prostate, colorectal and HPV-driven cancers. Moreover, as with mRNA vaccines, the COVID-19 pandemic has accelerated research on DNA vaccines, with at least three DNA vaccines currently in phase I,

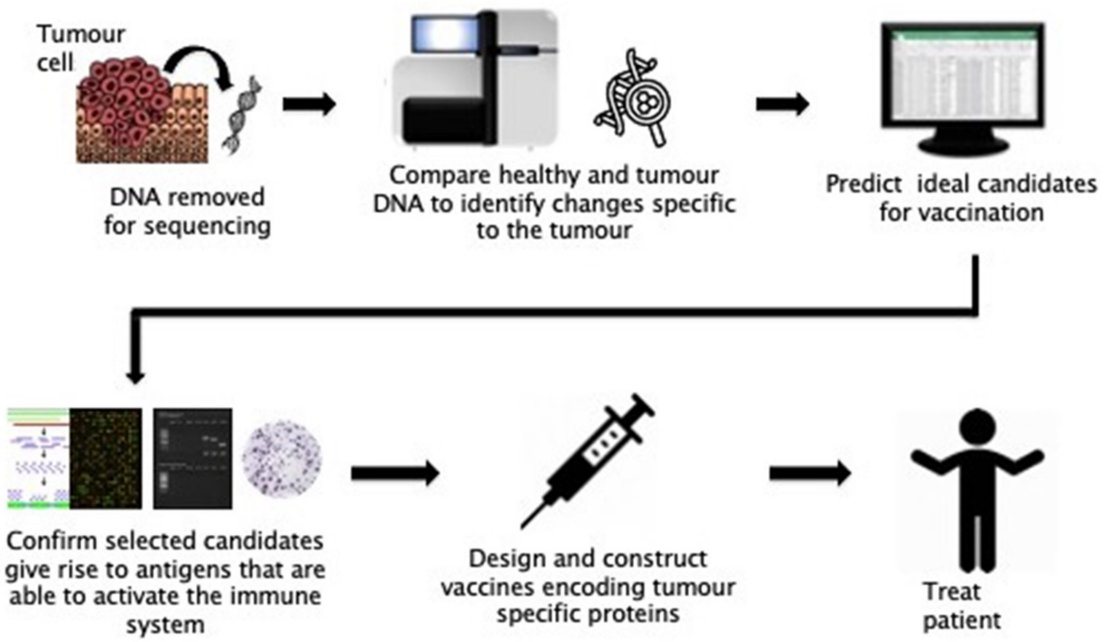

Figure 2. Steps involved in designing DNA vaccines against antigenic peptides expressed on cancer cells

II and III trials around the world including Covigenix VAX-001 in phase I and II trials in Canada, ZyCoV-D DNA vaccine currently in phase III trials in India and the INO-4800 DNA vaccine in phase II and III trials.

It's all in the delivery

Plasmid DNA vaccines are often delivered intramuscularly, where they are picked up by myocytes and the target antigen presented on dendritic cells to activate T cells. In early days the application of DNA vaccines was limited by inefficient uptake into cells. Various studies in preclinical models and in patients have indicated an increase in antigen-specific immune responses when delivered with electroporation, where an electrical field is applied to increase the permeability of the cell membrane to promote cellular uptake, allowing the plasmid to enter the cytosol, and thereby enhance antigen expression. Upcoming results from clinical trials testing DNA vaccines against COVID-19 and cancer of head and neck have indicated that vaccine uptake can also be improved by the use of a novel, needle-free injector that delivers the plasmid using a narrow, high-velocity fluid stream to penetrate the layers of the skin or muscle. This system is increasingly favoured by patients and doctors alike as it mitigates risk of needlestick injuries and cross-contamination.

Are Doggybones the future?

If the developments with electroporation and high-velocity fluids weren't exciting enough, there's more! Plasmid DNA vaccines are generated in bacteria and therefore require further purification to remove toxins before use in patients. These can be bypassed in an enzymatic reaction that produces closed, linear Doggybone DNA that encodes the target antigen. The name 'Doggybone' arises from the unique shape of the molecule which consists of DNA concatemers joined into a linear cassette that is closed at either end with hairpin loops. Preclinical models have demonstrated that Doggybone DNA is as effective as plasmid DNA in mounting an antigen-specific immune response. These results hold great promise for the future of DNA vaccines. With next-generation Doggybone DNA vaccines in the horizon, and improvements in vaccine delivery, we ought to watch this space, as this could be DNA vaccine's big break in the fight against cancer! ■

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