Honing in on perfect targeting

the pros and cons of different delivery modalities for personalised cancer vaccines

Introducing neoantigens directly into a patient in order to elicit an anti-tumour killing immune response is known as personalised cancer vaccine therapy. Currently, there is a huge debate among the neoantigen community as to what the 'best' modality is for initiating this immune response.

By Dr Samantha Zaroff

As many will unfortunately understand first hand or through a personal connection, cancer is the world's second-leading cause of death only behind cardiac arrest. In 2018 alone, 17 million people were diagnosed with cancer in the United States, with unfortunately 56% of them passing due to the disease. So with a 70% increase in cancer diagnosis over the past 20 years, and 9.5 million people dying from cancer in the US alone in 2018, researchers and clinicians have realised that standard methods of care such as chemotherapy, radiation and other one-size-fits-all treatments are not negating the disease as quickly as cancer mutates to avoid therapeutic efficacy. Therefore, we need a new standard method of care such as personalised immunotherapy.

Immunotherapies for cancer treatment have been around for almost 10 years, however, current treatments will only impact shared targets such as oncogenes, tumour-associated antigens or immune checkpoints. Checkpoints are receptors on the surface of T cells which, when bound by their ligand, are able to inhibit T cell-based immune responses against target cells. Check point inhibitors are proteins which block specific immune checkpoints, such as PD-1, and initiate a T cell mediated immune response against the tumour through binding of the T-cell receptor, or TCR, and the tumor-associated antigen. Clinical trials using check point inhibitors have shown great potential, although the effect is limited in many cases, especially in solid tumours where CPIs cannot make direct contact with the tumour microenvironment.

Another common immunotherapy against shared tumour-associated antigens are T cell therapies. There are many different forms of T cell therapy, however the goal of each form is the same, to target patient or healthy donor T cells to the tumour to bind to a tumour biomarker and initiate an anti-tumour killing response. To generate T cell therapies, patient's autologous T lymphocytes or donor-matched allogenic T cells are genetically modified to express TCRs which bind to specific tumour-associated antigens. These T cells are then expanded *in vitro* and reinfused back into the patient. These TCRs can be standard TCRs or Chimeric Antigen Receptors better known as CARs which, on top of having a TAA binding domain, contain an internal switch

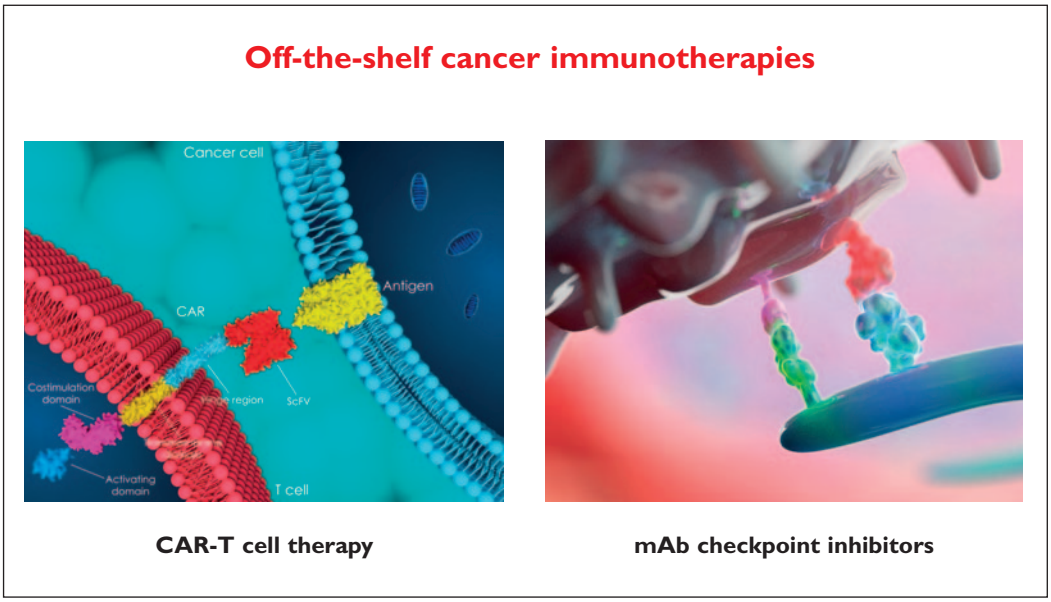

Figure 1
Different types of one-size-fits-all cancer immunotherapies. Off-the-shelf-style cancer immunotherapies target common or shared tumour-associated antigens. The two major types of these one-size-fits-all therapies are Chimeric Antigen Receptor T Cell Therapies and Monoclonal Antibody Checkpoint Inhibitors

board which, when bound to its target, activates the T cell to initiate an immune cascade (**Figure 1**). Clinical trials have shown great promise for these therapeutics as well, however they also show poor efficacy in solid tumours and tumours with high mutational burden due to immune escape.

The switch from 'one-size-fits-all' to personalised oncology

Despite showing promise, one-size-fits-all or 'off the shelf'-style immunotherapies have shown limited efficacy in about 70% of tumours, but why? Well, there are two common ways cancer evades the immune system as well as TAA targeted therapeutics, antigen escape and weak immunogenicity. Antigen escape is a natural caveat of cancer's high mutational nature, in which shared tumour antigens are either completely or partially downregulated, allowing tumours to evade single target therapies such as CPIs and CAR-T. Weak immunogenicity occurs when oncogenic tumour-associated antigens are also expressed in healthy tissue, just on a lower scale, and therefore are recognised as 'self-antigen'. Because of this, immune cells will not initiate an immune response and cancerous tissue will remain unscathed. However, both of these common problems can be avoided by using personalised immunotherapies, as opposed to one-size-fits-all treatments, but what is precision medicine?

In precision medicine, individual patient tumours are biopsied and used for NGS whole exome sequencing, RNA sequencing and proteome identification. Through bioinformatics analysis of these sequences compared to healthy tissue, clinicians can identify treatments which will work for the specific tumour biomarkers present in that patient's tumour. There are two types of precision medicine: first is to match patient tumour biomarkers to a database of clinical trials and therapeutics known to be efficacious for those specific biomarkers. Second are personalised therapeutics, which are designed from scratch to target patient tumour-specific antigens known as neoantigens. Neoantigens are 'new' or 'novel' cancer antigens which are present on the cancerous tissue but not expressed within wild type tissue. They are expressed within individual tumours or tumour cells of a specific patient, rather than being shared among patients suffering from one type of cancer. Neoantigens are expressed as peptides within cancerous tissue from single amino acid deletions and/or insertions. In rare instances, neoantigens can also be expressed from frameshift mutations. Because of how they are generated, neoantigens tend be highly hydrophobic in nature as well as having a strong tendency to aggregate. However, since they are minimally affected by antigen escape or weak immunogenicity, they make great targets for immunotherapy.

There are two main therapeutic types which target cancer neoantigens: personalised cancer vaccines or PCVs and T cell therapies. Personalised cancer vaccines (**Figure 2**) work similarly to infectious disease vaccines, in that they introduce small peptide components of the biological threat you

are trying to initiate an immune response against, in the case of PCVs this will be cancer neoantigens. Since every PCV is generated for a specific patient, the first step in production will be to take a tumour biopsy. This sample will then be sent to the lab where it will be used for next generation sequencing, RNA sequencing and immuno-peptidome identification. These sequences will be compared with sequences from healthy wild type tissue using bioinformatics tools in order to identify the mutanome, or mutations present within cancerous tissue that are not expressed in wild type cells. The mutanome will then be put through bioinformatics algorithms to identify the predictive immunogenicity and PCV efficacy of each individual neoantigen. The top neoantigen peptide candidates will be synthetically produced and used for *in vitro* functionality screening in order to confirm the predicted activity of each candidate. From these results, clinicians will first decide on the 20 or so neoantigens which they plan to use for their patient's PCV and secondly decide which modality they will use to introduce those neoantigens to the patient's lymphatic system. The most common modalities include neoantigen DNA, mRNA, peptide or pulsed antigen presenting cells mixed with varying delivery vehicles and adjuvants. Once formalised, the vaccine will be placed directly into the patients lymphatic system through injection in order to be processed and presented by patient APCs and initiate a T cell-based anti-tumour killing immune response against cancerous tissue expressing those neoantigen epitopes.

The benefits of using neoantigens as cancer vaccine epitopes compared with common tumour-associated antigens

Cancer immunotherapies targeting tumour-associated antigens have been around for quite some time, especially with the popularity of CAR-T style therapeutics. However, TAA-based vaccines have shown limited efficacy against non-viral-based cancers. The reason for this is that cancer vaccines and T cell therapies have completely different mechanisms of action.

Unlike CAR-T cells, rather than simply using the TAA as a target to bind to and induce an immune response, the antigen itself must be immunogenic enough to initiate an immune cascade on its own. Because common TAAs are also expressed in healthy tissue, they are unable to illicit any sort of immune response on their own, as they are automatically recognised as self-antigen, despite being significantly over-expressed in cancerous tissue. Unlike TAAs, neoantigens are somatic mutations which are only expressed in cancerous tissue, and therefore when surveyed by the immune system will almost never be recognised as self-antigen, making them much more likely to initiate a strong anti-tumour immune

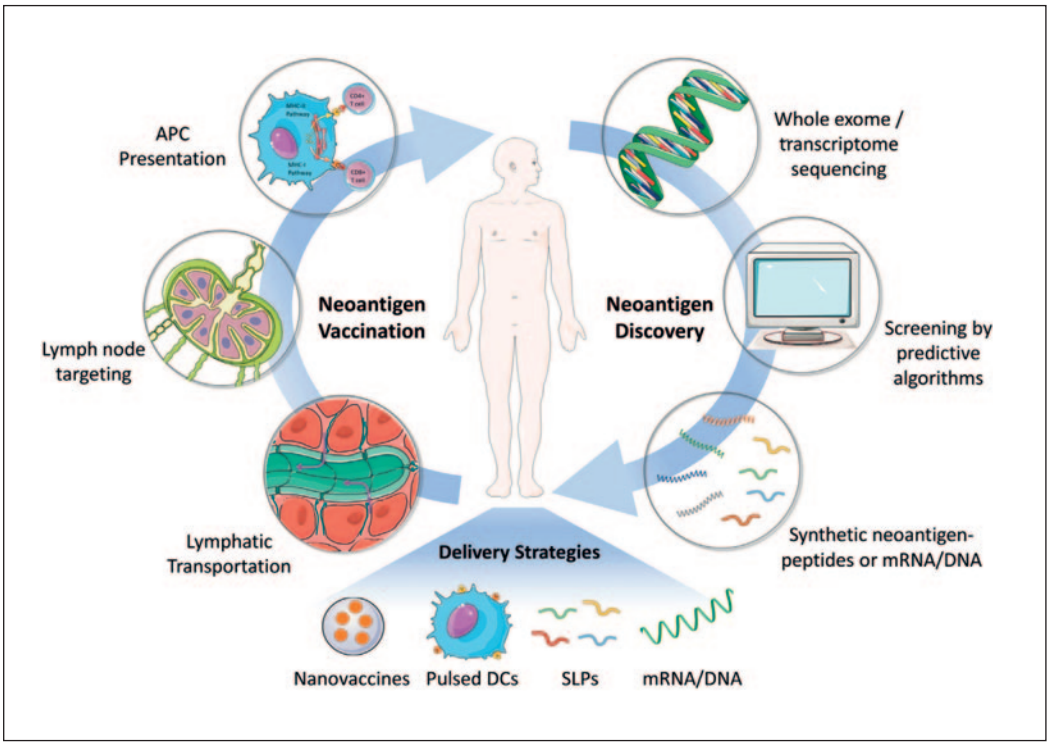

Figure 2
Development of personalised cancer vaccines. PCVs are generated by first identifying the mutanome through whole exome sequencing. Neoantigen candidates are narrowed down through bioinformatics and *in vitro* functionality screens using neoantigen peptides. Synthetic neoantigens are generated as nanovaccines, pulsed dendritic cells, synthetic long peptides or nucleotides and introduced directly into a patient's lymphatic system in order to induce anti-tumour killing immunity. *(Source: Gou Y, et al. Neoantigen Vaccine Delivery for Personalised Anticancer Immunotherapy. Frontiers in Immunology (9) 2018)*

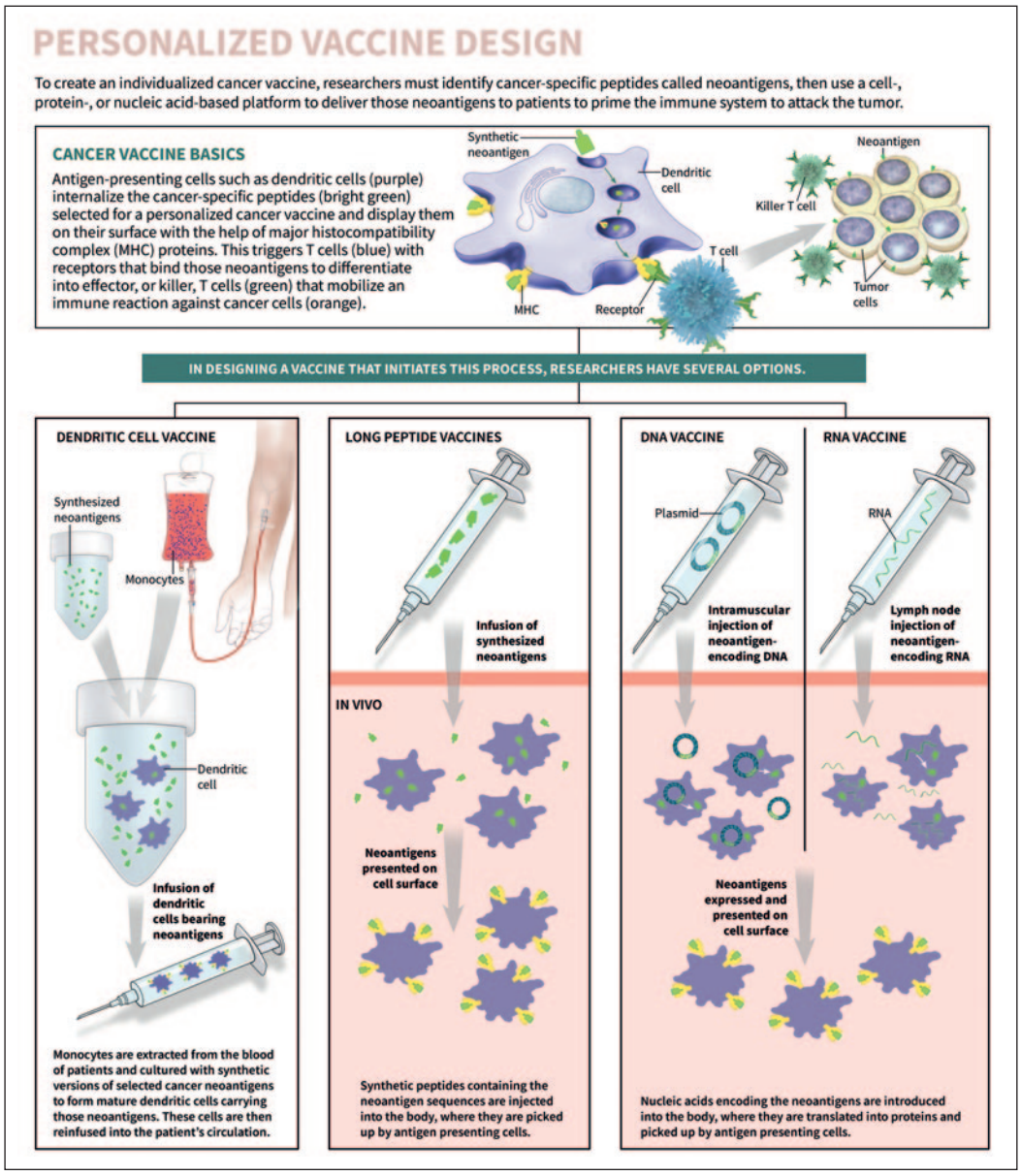

Figure 3
Design of personalised cancer vaccine in different delivery formats. Neoantigen-based personalised cancer vaccines introduce small componenets of neoantigen peptides directly into a patient's lymphatic system through various delivery formats. Some of the most common forms of delivery include nucelotides (plasmid DNA and mRNA), Synthetic Long Peptides (SLPs) and pulsed dendritic cells. *(First published in* The Scientist *© 2019 Terese Winslow LLC)*

response. This mechanism makes them a preferred target for cancer vaccines, however, since neoantigens are unique to individual patient tumour profiles, they are commonly used to generate personalised cancer vaccines as opposed to one-size-fits-all treatments.

There are two major concerns when generating personalised cancer vaccines. The first, is that identifying highly-immunogenic neoantigens can be a cumbersome task which not only involves deep whole exome sequencing of cancerous and healthy tissue, but also RNA sequencing, immunopeptidome identification, bioinformatics analysis of predictive HLA binding and immunogenicity, and even *in vitro* functionality screening of bioinformatics candidates. Secondly, once these neoantigens are identified, it is a daunting task to figure out the best delivery strategy to target neoantigens to APCs for eliciting a strong and robust immune response. Recently, there have been numerous preclinical and clinical trials investigating the potential immunogenicity of neoantigens for PCV development. Interestingly, naturally-occurring neoantigens induce cytotoxic T cell activity, however this activity is limited and rare as neoantigens are not presented well without intervention in the form of a PCV. These issues can be solved by utilising various different delivery formats, however each has their own pros and cons. The main issues which these delivery formats need to address are that,

despite neoantigens having high immunogenicity, they tend to be too small to reach their APC target, and therefore show poor accumulation which can lead to limited immune effect. Secondly, utilising immune boosting adjuvants has a tendency to lead to immune tolerance, as opposed to enhancing immunogenicity, therefore the delivery mechanisms needs to try to circumvent this issue. Lastly, it is extremely important to stimulate both $CD4^+$ and $CD8^+$ T cells in order to have a robust immune response, however it can be difficult to induce $CD8^+$ responses using non-living components, such as nucleotides or other macromolecules, therefore the neoantigen selection and delivery format must be optimised to elicit both $CD4^+$ and $CD8^+$ responses. So with all of these issues to resolve in order to generate an efficacious neoantigen personalised cancer vaccine, how do varying delivery modalities deal with these problems?

The many delivery options for neoantigen-based personalised cancer vaccines

There are a number of different approaches to developing a neoantigen-based personalised cancer vaccine, and everyone from small biotechnology companies to large biopharmaceutical companies are racing to find the most efficacious format. The most common delivery formats include nucleotides such as DNA and mRNA and synthetic long peptide (SLP) injection. However, recently there has been an increase in popularity for neoantigen pulsed dendritic cells and the highly-efficacious biomaterial-assisted neoantgien vaccines in which common formats are encompassed in a delivery vehicle such as nanoparticles and scaffolds (**Figure 3**).

The pros and cons of nucleotide-based PCVs

Nucleotide-based PCVs come in two 'flavours', either DNA-based, which can be delivered as linear DNA or as a plasmid, and *in vitro*-transcribed mRNA. By utilising small nucleotides, it is easier to enhance self-adjuvating activity, control translation into the cytoplasm of cells to induce $CD8^+$ T cell toxicity and maintain a simple and inexpensive manufacturing process. In terms of utilising DNA based PCVs, it is very easy to inject upwards of 50 neoantigens simultaneously using a very small amount of plasmids. However, there is a strong risk of insertional mutagenesis by introducing foreign DNA directly into the system. For this reason, many researchers prefer to use mRNA-based vaccines as they are very rarely integrated into the host's genome. However, mRNA vaccines come with their own difficulty of controlling translational efficiency. Overall, mRNA can be taken up by different kinds of cells, as a result only a small part of the injected mRNA could be captured by APCs and reach the cytoplasm for translation and presentation. To avoid this issue, some laboratories are carrying out direct injection of nucleotide PCVs directly into lymph nodes through ultrasound-guided percutaneous injections, also known as intra-nodel. Utilising i.n injection, researchers can enhance MHCI and MHCII presentation on dendritic cells, however, this treatment method can take up to 20 injections over time, which may not be tolerable by every patient.

The pros and cons of peptide-based PCVs

Unlike nucleotide-based PCVs, peptide-based PCVs directly introduce small peptide components of the neoantigen into a patient's lymphatic system. These peptides will be taken up by APCs and processed and presented on either MHC class I or class II depending on how they are initially designed. The presented epitopes will then be recognised by surveying T cells in order to initiate an anti-tumour killing immune response targeting those presented neoantigen epitopes. This direct interaction with T cells offers a substantial benefit in inducing anti-neoantigen immunity, especially since neoantigens offer low toxicity, as well as ease of synthesis, despite being slightly harder to receive quickly in a GMP format than its nucleotide predecessors. The main issue with peptide-based PCVs, are that neoantigen peptides tend to be too small to directly stimulate an immune response and rapidly diffuse into the peripheral blood vessels, which limits the amount of peptide actually delivered directly to the lymph node. To circumvent this, recent studies have detailed methods of pre-loading APCs with neoantigen peptides or packaging these peptides into delivery vehicles.

The pros and cons of cell-based PCVs

In order to get around the issues with peptide-based and nucleotide-based PCVs, while still maintaining the immunogenicity of these neoantigens, some researchers have begun pulsing patient-derived APCs with neoepitopes *ex vivo* in order to have pre-processed and presented dendritic cells able to be reinfused back into the patient in order to initiate anti-tumour toxicity. These pre-processed APCs are able to activate a much wider range of the TCR repertoire, as each neoantigen can be processed

multiple ways and expanded before reinfusion. Clinically, this approach has shown high efficacy, however, unlike standard PCVs, pulsed APCs are extremely costly and labour-intensive and require expert-level technicians in order to generate the raw materials for the vaccine, making large scale treatment very difficult to achieve.

The pros and cons of biomaterial-assisted PCVs

One of the main issues with using nucleotide or SLP-based PCVs are the delivery of neoantigens to APCs within patients' lymphatic tissue in order for neoeptiopes to be presented on MHC to bind to TCRs and initiate an immune cascade. In order to direct neoantigen DNA, mRNA, or SLPs to APCs and/or lymphatic tissue, researchers have been utilising biomaterial-assisted PCVs. These vehicles eliminate many of the cons of nucleotide or peptide based PCVs by using a novel biomaterial to deliver the neoantigen in tandem with adjuvants and direct targeting for enhanced cancer vaccines. There are many different kinds of biomaterial-assisted neoantigen vaccines, but they usually contain an encapsulating agent to encompass the neoantigens themselves (nucleotide or SLP), an adjuvant or a bacterial/viral particle in order to boost the immune response, and a targeting agent, such as an APC ligand or an antibody to make sure that the PCV is delivered to APCs and the lymphatic system. A main benefit of using these delivery vehicles is that once the mechanics of the delivery format are figured out, a clinician can interchange neoantigens based on the genetic make-up of individual patient's tumours, making PCV delivery much easier. However, it takes a lot of time and research in order to generate a novel PCV modality, as there are unlimited options within a design to choose from.

Conclusion

Personalised cancer vaccines are able to evade cancer's natural immune defences to trick patients' immune systems to target and kill tumours based on a subset of rare, cancer-specific, somatic mutations. In combination with other immunotherapies such as checkpoint inhibitors and standard methods of care such as chemotherapy and radiation, despite being young in the clinic, PCVs have shown great efficacy in a very short period of time. Given the large range of delivery modalities for PCVs, and the individuality of personalised immunotherapies, the combinations of treatments seem endless. However, with the direction PCV research is going today, it is only a matter of time before personalised medicine is placed at the forefront of cancer treatment, as a possible cure for the incurable. DDW

Dr Samantha Zaroff *received her PhD in 2017 from Rowan University focusing on the molecular mechanisms of neurodegenerative disease. Dr Zaroff then became the marketing specialist for GenScript's custom antibody and antibody drug services lines. Dr Zaroff currently works as the product manager of neoantigen peptide synthesis services at GenScript.*