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The cancer vaccine resurgence

A wave of companies are developing personalized vaccines, built from tumour-specific neo-antigens, providing a timely boost for a failure-ridden area of cancer immunotherapy.

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The cancer vaccine landscape is littered with two decades of clinical failures. Since the mid-1990s, biotechs and big pharmaceutical companies alike have been unable to generate compelling data for a class of therapeutics that promises to train the immune system to recognize and destroy cancerous cells. Although the FDA did approve Dendreon's sipuleucel-T as a therapeutic vaccine for prostate cancer in 2010, the company filed for bankruptcy protection in 2014 because of a lack of demand for its complex cell-based vaccine.

But so-called tumour neo-antigens that are unique to each patient's tumour are now renewing interest in the class. Several groups have shown that neo-antigens are critical for the success of immunotherapeutic checkpoint inhibitors, raising the possibility that these antigens might also make for efficacious and truly personalized vaccines.

At least two biotechs, BioNTech and Neon Pharmaceuticals, are already in the clinic with neo-antigen vaccines (TABLE 1). Others — including Advaxis, Gritstone Oncology, ISA Pharmaceuticals, Moderna and Agenus — are set to enter the clinic soon. Large pharmaceutical companies too, are getting in on the game. In September, Genentech committed US$310 million in upfront and near-term milestones to collaborate on neo-antigen cancer vaccine development with BioNTech. In August, Amgen partnered with Advaxis in a deal that could be worth nearly $500 million for access to Advaxis's technology. In June, Merck & Co. partnered with Moderna in a $200 million deal that could provide another way in.

"There has been a renaissance of interest in this field," says Andrew Allen, CEO of Gritstone Oncology, which launched last year with a massive $102 million in funding.

Everything old is new again

Many of the first cancer vaccines were built to prime the immune system against 'tumour-associated antigens' (TAAs), antigens that are expressed in healthy tissue but overexpressed in cancer cells, providing an opportunity for increased cancer cell selectivity.

Table 1 | **Selected neo-antigen cancer vaccine companies**

Company (vaccine name)	Properties of lead neo-antigen vaccine	Indication	Status
BioNTech (IVAC mutanome)	mRNA-based vaccine; 10 neo-antigens per vaccine	Melanoma	In phase I
Neon (NEO-PV-01)	Peptide-based vaccine; 20 peptides per vaccine	Melanoma, NSCLC and bladder cancer	In phase I, in combination with nivolumab
Advaxis (ADXS-NEO)	Bacteria-based vaccine; 20–50 neo-antigens per bacterial construct, but multiple constructs can be delivered together	Undisclosed	Phase I to start in 2017
Gritstone Oncology	Virus-based vaccine; 20–50 neo-antigens per vaccine	NSCLC	Phase I to start in 2017

NSCLC, non-small-cell lung cancer.

Merck KGaA's tecemotide, for example, targets the mucin 1 (MUC1) antigen, a protein that is expressed on the surface of epithelial cells and overexpressed in breast, ovarian, lung, pancreatic and other cancers. Merck KGaA advanced tecemotide into phase III trials in 2007, but discontinued in breast cancer in 2010 after hitting a safety setback, and stopped work in non-small-cell lung cancer in 2014 because of a lack of efficacy (phase II development in prostate and colorectal cancer is ongoing).

Research groups have also previously focused on cancer germline antigens, which are expressed only in germline cells and in cancer tissue. Because germline tissues such as the testis are immune-privileged, researchers theorized that these antigens would offer better immunogenicity without the risk of autoimmunity. GlaxoSmithKline (GSK) made it as far as phase III with its melanoma-associated antigen 3 (MAGEA3) vaccine, for example. But here too the clinical data were disappointing, and GSK discontinued development of its vaccine in non-small-cell lung cancer and in melanoma in 2014 and 2015, respectively (a phase II trial in bladder cancer is ongoing).

In the past few years, however, ideas about what makes a good antigen have started to change. Improvements in DNA and RNA sequencing technology, in particular, mean that researchers can now analyse each patient's tumour exome quickly and cheaply, drilling down into the unique aspects of their disease. "This technology has given cancer vaccines new life," says Allen.

At the same time, success with checkpoint inhibitors has suggested that neo-antigens may make for better vaccines than TAAs and cancer germline antigens. In 2013, analysis of a patient who had responded to Bristol-Myers Squibb's anti-CTLA4 (cytotoxic T lymphocyte-associated antigen 4) ipilimumab found that his T cells reacted to two neo-antigens (*J. Clin. Oncol.* **31**, 439–442;s 2013). A year later, another group reported that melanoma patients fared better on ipilimumab when their tumours carried high loads of neo-antigens (*N. Engl. J. Med.* **371**; 2189–2199; 2014). When checkpoint inhibitors work, that is, accumulating evidence suggests that they do so by unleashing T cells to target neo-antigens rather than TAAs or cancer germline antigens.

Complementary data from other groups support this theory. Adoptive cell transfer of tumour-infiltrating lymphocytes — a cell-therapy approach that can induce regression in melanoma patients — also acts through T cells that are active against neo-antigens (*Immunol. Rev.* **257**, 56–71; 2014).

The same findings are also encouraging researchers to engineer specificity against neo-antigens into chimeric antigen receptor (CAR) T cell therapies.

From an immunological perspective, the importance of neo-antigens makes sense, says Robert Petit, chief scientific officer at Advaxis. "If the target that you are trying to drive a response against is a completely normal peptide that is expressed in healthy tissue, then you are always going to have an uphill battle because you need to overcome central tolerance," he says. Mutations that push 'self' proteins into the 'non-self' camp — be it through missense or frameshift mechanisms — offer a more immunogenic starting point.

Neo-antigens also offer another benefit: the ability to train the immune system to see multiple red flags. Whereas many of the first cancer vaccines trained the immune system to search out single TAAs, researchers can now prime the immune system against dozens of neo-antigens in one go. Although it remains to be seen whether the use of multiple neo-antigens boosts clinical efficacy, it stands to reason that this could help to tackle heterogeneous cancers while also raising the bar against immune escape.

Neo-antigen nuance

Several scientific unknowns, however, are tying up the field. And key among these is the decision over which neo-antigens to incorporate into any one personalized cancer vaccine. The more mutagenic the cancer, the more amenable it is likely to be to cancer vaccine treatment. But the most mutagenic cancers — melanoma and lung cancer, for example — can harbour hundreds of neo-antigens. Some neo-antigens may be more widespread throughout the tumours than others, just as some may be more provocative to the immune system than the rest. So, which do you include in a cancer vaccine?

Researchers have developed different public and in-house computational tools to guide their decisions. The general strategy, however, is to compare how strongly putative neo-antigens bind to the major histocompatibility complex (MHC), the proteins that interface between antigen-presenting cells and T cells to trigger sensitivity.

"As a field we haven't quite got antigen selection figured out yet," warns Petit. Allen agrees, estimating that as yet the community can only predict perhaps 2% of 'true' neo-antigens from sequence data alone. Even once true neo-antigens are identified, researchers struggle to predict which are likely to be the most immunogenic. The predictive shortfalls are huge.

Some vaccine delivery platforms offer the opportunity for a workaround. Advaxis, for example, uses engineered *Listeria* bacteria to introduce neo-antigens into cancer patients. Each bacterium can carry up to 50 neo-antigens, and Advaxis's production method allows it to generate multiple bacterial constructs per patient. "We can make a mixture of constructs that represent literally every neo-antigen for a patient. Until we know how to pick neo-antigens better as a field, this makes our life a little simpler," says Petit. Preliminary data suggest that the use of more neo-antigens is more effective than the use of fewer, he adds.

"There are definite advantages to this approach," says William Gillanders, a breast cancer surgeon at Washington University

School of Medicine in St Louis. Gillanders is one of several physicians who are developing cancer vaccines that have come out of academic research projects.

But he adds that other delivery vectors can be used to similar broad effect, especially if the focus is on less mutagenic cancers. He is currently trialling a cancer vaccine for triple-negative breast cancer, an intermediately mutagenic cancer. His DNA-based vaccine expresses up to 15 neo-antigens *in vivo*, providing the bandwidth to "integrate all of a patient's neo-antigens if we need to".

Gritstone is working with a viral vector for its first clinical candidate, in which the antigens are encoded into the RNA of a hybrid virus. This platform can deliver up to 50 neo-antigens, says Allen. (The company is also considering using other vectors for future projects.)

Gillanders also cautions that overuse of the shotgun approach might overload the immune system, with too much competition between multiple neo-antigens potentially diluting the immune response. "We don't know if this is a legitimate concern, but it might be," he says.

The different antigen-delivery platforms also have other strengths and weaknesses that need to be taken into consideration. BioNTech, for example, recently showed that a nanoparticle liposomal formulation of its mRNA-based vaccines can be used for systemic delivery, solving a long-standing tissue-targeting hurdle for oligonucleotide-based drugs (*Nature* **534**, 396–401; 2016). The ability of Gritstone's lentiviral approach and of Advaxis's bacterial platform to co-opt the immune system's response to pathogens, meanwhile, may promote vaccine uptake and efficacy.

"The bottom line is that nobody knows which platform is going to work best," says Cary Pfeffer, interim chief executive officer at Neon and partner at Third Rock Ventures, which backed the foundation of the company.

Different neo-antigens might ultimately be suited to different delivery platforms," adds Petit. "This is where practical vaccinology will come into play."

Mass personalization

Although companies currently need 6–12 weeks to generate a personalized cancer vaccine, they are aiming at bringing the production timelines down to as low as 1 month. "It really is just an issue of logistics," says Pfeffer. Gillanders argues that even 6-week timelines are "optimistic".

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These lag times aren't ideal for patients with advanced cancers, but the field believes it can produce vaccines quickly enough to generate proof-of-concept efficacy in the clinic. If and when cancer vaccines move into earlier stages of disease, when the immune system might be in better shape to take on cancerous tissue, the delay may become less consequential.

The personalized nature of each vaccine offers plenty of production pitfalls, however, including the need for reliable collection of high-quality tumour samples, robust sequencing set-ups, and manufacture and shipping of single-batch (in some cases, live) products across the country. These vaccines are a far cry from the mass-produced drugs that pharmaceutical companies are used to selling.

A small subset of 'shared' neo-antigens may yet offer the potential for off-the-shelf neo-antigen vaccines in the future. "We've identified at least a couple of hundred of neo-epitopes, on a fewer number of total genes, that we believe are shared between patients," says Pfeffer.

One example of such an antigen is epidermal growth factor receptor variant III (EGFRvIII), a gain-of-function mutation that leads to an in-frame deletion and a novel epitope in the receptor. Because EGFRvIII is present in 34–63% of glioblastomas, an EGFRvIII vaccine need not necessarily be manufactured each time anew.

But Sebastian Kreiter, head of Immunotherapies and Preclinical Research at BioNTech, says not to over-embrace this approach. "I don't think there is clear opinion yet over whether shared neo-antigens will prove to be a druggable concept," he says. In part, it taps into old concerns about whether a single-antigen vaccine — be it a neo-antigen or a TAA — can induce a strong enough response. So, can enough shared neo-antigens be identified for a big enough patient population to make a multi-antigen off-the-shelf product worthwhile? If not, clinical data to date do not bode well. Earlier this year, Pfizer and Celldex discontinued CDX-110, a peptide-based vaccine against EGFRvIII, after an interim analysis of a phase III glioblastoma trial suggested that the vaccine wouldn't hit its efficacy end points.

Conversely, Kreiter still sees potential for TAA-based vaccines. Despite the lack of success with previous generations of these vaccines, past failures may have been due to a number of causes. Researchers may have chosen the wrong cancer types, for example, opting for development strategies in late-stage fast-progressing cancers that were beyond the capacity of the immune system (metastatic melanoma and pancreatic cancer, for example). Failed cancer vaccines may also just have lacked power, says Kreiter. "It's not just that you have to qualitatively induce a few T cells," he says. "Quantity matters."

He and others remain committed to TAA-based approaches, trialling new antigens, delivery platforms and drug development strategies to improve outcomes. BioNTech's phase I Lipo-MERIT, for example, combines four TAAs into a single mRNA-based vaccine, in the hope that a multi-antigen strategy will yield better results. Advaxis, meanwhile, thinks that its bacterial platform can induce better responses to TAAs, and has single-TAA products in phase I/II trials for both prostate-specific antigen (PSA)- and HER2-positive cancers.

Vaccinologists think that they might also now see success thanks to new combination strategies that can help them get the most out of their products. Checkpoint inhibitors in particular may work well with both TAA and neo-antigen vaccines, unleashing the immune system even as a vaccine primes T cells to track down cancerous tissue. Bavarian Nordic's Prostvac, a TAA-based vaccine that is now in phase III trials as a monotherapy, is already being combined with ipilimumab in a phase II prostate cancer trial, and will soon be combined in phase II with both ipilimumab and Bristol-Myers Squibb's anti-PD1 (programmed cell death protein 1) inhibitor nivolumab. Neon, too, is testing its neo-antigen vaccine in combination with nivolumab. BioNTech is also now set to test its cancer vaccines in combination with Genentech's portfolio of cancer drugs, which includes PD1 ligand 1 (PDL1)-targeting atezolizumab.

"Right now, there is a lot of research interest in neo-antigens, but I don't think that interest in TAAs will go away," says Gillanders. "I think there is room for both."