Miriam Merad/Icahn School of Medicine at Mount Sinai

Mutations as munitions:

Neoantigen vaccines get a closer look as cancer treatment

By Alla Katsnelson

In 2010, the Dendreon company received the news it had been hoping for: the US Food and Drug Administration had approved its therapeutic cancer vaccine Provenge for prostate cancer. At the time of Provenge's approval, the headlines hailed it as groundbreaking, and they noted a surge in the price of Dendreon's stock as the company announced its $93,000 price tag for the therapy. But enthusiasm fizzled when the company later revealed that fewer people used the therapy than expected, and in November 2014 the company filed for bankruptcy.

Now, however, there is a new infusion of interest in cancer vaccines. The main difference between current and earlier vaccines is the use of a different type of antigen. "Up until recently, the major effort has been on tumor-associated antigens—shared antigens that are expressed in both tumors and normal cells," says Robert Schreiber, a cancer immunologist at Washington University in St. Louis. "But the fact is, those antigens have been present in the host since birth." This means that the immune system has developed a tolerance to them, which makes it "an uphill battle" to use them to elicit a therapeutic response.

Instead, Schreiber and others are focusing on 'neoantigens'—peptides that include amino acids encoded by somatic gene mutations in cancerous cells. Such mutations are tumor-specific, so the peptides that they produce are not present in the body before the cancer develops. Researchers are banking on that specificity to draw the cancer out of its cloak of immune tolerance and to provoke a T cell response that can control or kill it. "These mutations are not really subject to self-tolerance and could potentially be a very nice target for vaccines," says Timothy Chan, an oncologist and geneticist at Memorial Sloan Kettering Cancer Center in New York.

Because every cancer's set of tumor-specific mutations is unique, neoantigen-based vaccines will probably need to be fully personalized for each individual, at least initially. Judging by recent investment into the approach, however, not everyone sees this as a barrier to commercialization. In October, two biotechnology companies developing neoantigen vaccines—Neon Therapeutics and Gritstone Oncology—announced venture capital–backed launches, and a handful of other companies (see table, page 124) are also pursuing the approach. (Schreiber is a co-founder of Neon, and Chan is a co-founder of Gritstone.)

A slew of studies has reported that neoantigens are correlated with a T cell response. Despite the excitement brewing around the possibility of an effective neoantigen vaccine, however, the therapy's prospects are still far from certain. "I think it is still in a sense an unproven hypothesis," says Howard Kaufman, a cancer immunologist at Rutgers University in New Brunswick, New Jersey. Moreover, no clinical trial has yet demonstrated the efficacy of such a vaccine. Although multiple clinical trials are now under way, the first report of a neoantigen-based vaccine being administered to humans was published only in April. In that study[1], three people with advanced melanoma received a personalized dendritic cell vaccine containing neoantigens identified through cancer genome sequencing; all three subsequently demonstrated a boost in neoantigen-specific $CD8^+$ T cell responses. Because the recipients had received prior treatment, however, the vaccine's clinical efficacy could not be assessed.

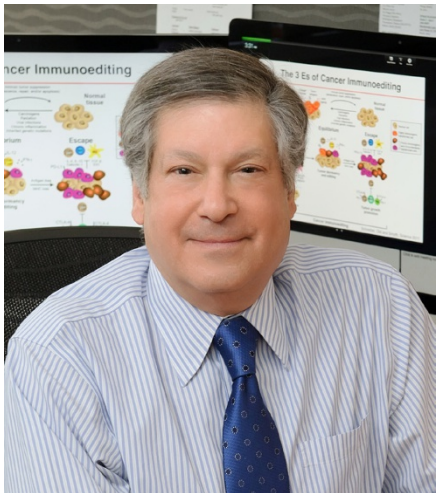
First clues

Despite the recent momentum in neoantigen-

vaccine development, neoantigens are not a new discovery. Studies of carcinogen- or ultraviolet-induced cancers in mice as far back as the 1950s have suggested that each individual tumor can express unique antigens, says Cornelis Melief, an immuno-oncologist at the Leiden University Medical Center in the Netherlands and chief scientific officer of ISA Pharmaceuticals[2]. In the mid-1990s, Mandelboim and colleagues[3] purified neoantigens from murine lung carcinoma and showed that immunizing mice with a synthetic versions of the antigens protected the mice from metastases. "But [neoantigens] were so hard to pull out and identify that, commercially, it just wasn't possible to think about utilizing them as a therapy," says Schreiber.

Over the past decade, gene-sequencing technologies improved, and it became conceivable to scan a tumor's DNA and RNA quickly and cheaply enough to use the results in the clinic. Schreiber's group and Ugur Sahin's team at Johannes Gutenberg-University of Mainz in Germany independently published mouse studies in 2012 that represented early efforts to show that neoantigens can be pinpointed and used in individualized cancer vaccines. Their work used tumor exome sequencing and computer algorithms that predict how strongly epitopes bind to major histocompatibility complex (MHC) molecules[4,5], which present antigenic peptides on the cell surface for recognition by T cells.

Additionally, the FDA's approval in 2011 of another immunotherapy—the checkpoint-blockade antibody therapy Yervoy (ipilimumab)—for melanoma helped to set the stage for neoantigen-based vaccines. "This is the proof we had been waiting for that T cell responses work" to shrink tumors, says Miriam Merad, an oncologist and immunologist at the Icahn School of Medicine at Mt. Sinai in New York. "Checkpoint blockade has really defined a new era."



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Perusing peptides: Schreiber sees promise in neoantigens. Opposite page: Merad emphasizes the importance of understanding the tumor micro-environment.

Checkpoint inhibitors such as ipilimumab, and subsequently nivolumab and pembrolizumab (which are both approved for advanced melanoma and for non-small-cell lung cancer) , work by releasing a molecular brake used by tumors to dampen immune responses. The discovery of this class of drugs was the first home run for cancer immunotherapy, because it demonstrated that the treatment could induce durable remissions. But checkpoint therapies may directly potentiate neoantigen responses: in one person with melanoma who responded to ipilimumab, researchers could detect T cells that recognized a specific neoantigen. This suggested that the drug had a role in eliciting the T cell response and thus helped to drive the therapy's efficacy[6]. When another person with melanoma, this time in Steve Rosenberg's group at the National Cancer Institute, showed a similar result after treatment with a T cell immunotherapy called adoptive cell transfer, the field took off[7]. "In the last year and a half, there's been an explosion of literature supporting that neoantigens are an antigen class of interest," says Catherine Wu, a medical oncologist at the Dana-Farber Cancer Institute in Boston and a co-founder of Neon.

But researchers have not yet fully worked out how to select the neoantigens with the best chance of eliciting a strong T cell response. Scientists who are studying the association between antigenic peptides and MHC molecules have developed predictive algorithms to determine which peptides are likely to bind to different MHC molecules. If a cancer has 100 or so tumor-specific mutations, each peptide sequence must be run through an MHC-binding algorithm to determine which peptides will bind most strongly. Computer prediction tools for MHC class I molecules, which bind CD8 T cells, are relatively robust, although some researchers think that antigens thus identified still need to be validated *in vivo*. The tools for predicting epitopes that bind to MHC class II molecules, and that thereby engage receptors on CD4 T cells, are substantially less accurate "We need some faster way to assess functional immunogenicity," Wu says. The vaccine that Neon Therapeutics is testing takes about three months to produce, although the time necessary for MHC prediction is just one factor. Wu thinks that this duration can ultimately be reduced to a period of less than a month.

Probably the biggest unresolved issue, however, is whether neoantigen vaccines will expand the pool of people—and the range of cancer types—that checkpoint-blockade

Timothy Chan

Mulling mutations: Chan and his colleagues have explored mutational load in cancers.

therapies and immunotherapies in general can successfully treat. Although response to checkpoint blockade is relatively high for a few types of cancer—around 40% of people with metastatic melanoma and 20% of those with advanced lung cancer show improvement with checkpoint inhibitor drugs —responses in other cancers are paltry or nonexistent. A pair of papers by Chan and colleagues proposed that the difference is explained by the number of mutations that each cancer type tends to accrue: the higher the load, the stronger the response[8,9]. In the context of neoantigens, this makes sense, because high mutation burden is essentially a surrogate for neoantigen frequency. Subsequently, a study of more than 100 people with metastsatic melanoma confirmed that those with the highest number of tumor neoantigens were most likely to respond to ipilimumab[10].

Some researchers argue that this by definition limits the use of neoantigen-based vaccines. Different types of tumors might carry anywhere from dozens to hundreds of expressed mutations. Fewer than 1% of mutations are thought to be spontaneously immunogenic. Sahin's 2012 paper reported that when neoantigens are presented as vaccine targets, their immunogenicity is closer to 20% or 30%. But because good MHC class I targets have been hard to pinpoint—particularly in cancers with low mutational burdens—identifying those with an MHC-binding ability strong enough to rev up the immune system "is like looking for a needle in a haystack," says Mary 'Nora' Disis, an immuno-oncologist at the University of Washington in Seattle. And indeed, many clinical trials start with the usual high mutation burden suspects, such as melanoma, lung cancer and bladder cancer.

But recent work suggests that strong vaccine targets may not actually be so rare. Rosenberg and colleagues showed that a patient with cholangiocarcinoma—an epithelial cancer with a low mutation rate—still expressed neoantiogens that generated a T cell response[11]. More recently, Sahin, who also serves as CEO of BioNTech, and

Selected companies developing neoantigen-based anti-cancer vaccines

Company	Selected product	Vaccine platform	Stage	Indication
BioNTech	IVAC Mutanome	Synthetic RNA vaccine	Phase 1	Melanoma and triple-negative breast cancer
Neon Therapeutics	NEO-PV-01	Synthetic peptide vaccine	Expected clinical trial launch in 2016	Melanoma, non-small-cell lung cancer and bladder cancer
ISA Pharmaceuticals	N/A	Synthetic peptide vaccine	Preclinical	Lung cancer, bladder cancer and melanoma
Gritstone Oncology	N/A	Undisclosed technology	Preclinical	Non-small-cell lung cancer
Agenus	AutoSynVax	Synthetic peptide vaccine	Preclinical	Undisclosed
Caperna	N/A	Synthetic RNA vaccine	Preclinical	Undisclosed

his colleagues conducted an experiment that found 80–90% of the immunogenic neo-epitopes that they tested were recognized by CD4 T cells—not by CD8 T cells[12]—demonstrating that MHC class II molecules are also viable candidates. They also developed a bioinformatic algorithm that can accurately predict the most potent MHC class II antigens by using sequencing data alone, without the need to test immunogenicity *in vivo*. The neoantigens identified in the study elicited antitumor immunity in three different mouse models. The team applied the same algorithm to more than 1,000 human cancer genomes and found that human cancers—including those with fewer mutations—are also rich in class II neoantigens. "My prediction is that class II neoantigens will become prime targets for tumors with lower number of mutations," he says.

Bringing in the blockade

Combination therapies are the norm in cancer therapy, if for no other reason than their tendency to limit resistance to treatment. It is perhaps unsurprising then that researchers involved in the initial testing of neoantigen vaccines plan to combine them with checkpoint-inhibitor therapies. Neon's vaccine, for example, will be administered concomitantly with nivolumab. If checkpoint blockade boosts T cell recognition of neoantigens, the two approaches should theoretically potentiate each other, and the approach might even increase the percentage of people who benefit from checkpoint inhibitors.

Doubling up with checkpoint inhibitors also serves another role: their modulation of the immune system could help the T cells that are activated by the vaccine to overcome the notoriously immunosuppressive tumor microenvironment. Merad doubts, however, that checkpoint inhibitors will be enough to solve the microenvironment problem. "It's not clear whether checkpoint inhibitors can modify or modulate the T cell's ability to infiltrate the tumor," she says. Early data on checkpoint blockers suggested that even when T cells were activated, the therapy could fail because the T cells were stuck outside the tumor. Disis agrees. "I don't think we've fully integrated the tumor microenvironment into our therapeutic thinking," she says, noting that the microenvironment probably changes according to the stage of disease, other treatments that the person has received and multiple other factors.

Several more immune modulators that could act on the microenvironment are being examined, including inhibitors of indoleamine 2,3-dioxygenase, an enzyme that can shut down T cells, of granulocyte-macrophage colony-stimulating factor, a protein that promotes antigen presentation by dendritic cells, and of colony stimulating factor 1, a cytokine that depletes macrophages. But to really make sense of how the body responds to immunotherapies, the field will have to expand immune-monitoring capabilities so that researchers can follow the effects of a treatment throughout the course of the disease. "If we don't study what's going on at the tissue site as deeply as possible," Merad says, "then we are going to continue to waste years."

Other factors besides tumor microenvironment will undoubtedly also affect neoantigen vaccines' efficacy. For example, two mouse studies recently showed that intestinal microbiota could affect the outcome of anti-tumor immunotherapy[13,14]. Ultimately, all of these parameters are empirical matters that ongoing clinical trials will address. "I suspect in the course of next year, we will know once and for all if it will work," Schreiber says, "and how to make it work even better."

Thomas Hartmann /Johannes Gutenberg University of Mainz-Germany

Exploring epitopes: Sahin and his colleague Özlem Türeci have published studies on how epitopes drive therapeutic immune responses to cancer.

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