

Getting Personal with Neoantigen-Based Therapeutic Cancer Vaccines

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Abstract

Despite years of preclinical efforts and hundreds of clinical studies, therapeutic cancer vaccines with the routine ability to limit or eliminate tumor growth in humans have been elusive. With advances in genome sequencing, it is now possible to identify a new class of tumor-specific antigens derived from mutated proteins that are present only in the tumor. These "neoantigens" should provide highly specific targets for antitumor immunity. Although many challenges remain in producing and testing neoantigen-based vaccines customized for each patient, a neoantigen vaccine offers a promising new approach to induce highly focused antitumor T cells aimed at eradicating cancer cells. *Cancer Immunol Res*; 1(1); 11–15. ©2013 AACR.

Introduction

The immune system exploits massive genetic variability, evolutionary selection, and population expansion to recognize and attack foreign invaders. Cancer researchers have long sought to harness this ability and persuade the immune system to similarly survey cell surfaces throughout the body for the presence of proteins or HLA-bound peptides that specifically mark a malignant change in the cell, and thereby attack tumors as well. The prospect of eliciting tumor-specific cytotoxic T cells (CTL) to eradicate malignant cells was inspired by examples of spontaneous tumor regression and has led to hundreds of animal studies and clinical trials of cancer vaccines. However, despite extensive attempts to induce an effective immune response, the clinical outcomes have been disappointing (1). This failure can be attributed to many causes, including immunosuppression by some tumors (which has led to the exciting recent work targeting checkpoint blockades) and ineffective immunologic adjuvants in the vaccine. However, one critical feature of all vaccines is the choice of antigen. Most cancer vaccines have used self antigens that are selectively expressed or overexpressed in tumors (Fig. 1A). A fundamental challenge with such approaches is that they require overcoming both central tolerance (whereby autoreactive T cells are deleted in the thymus during development) and peripheral tolerance (whereby mature T cells are suppressed by regulatory mechanisms). In contrast, vaccination against pathogens

bypasses central tolerance because it involves foreign antigens. How could a cancer vaccine mimic this approach?

The advent of massively parallel sequencing (2, 3) has now made it possible to sequence the entire genome or exome (coding regions) of tumor and matched normal cells to identify all of the mutations that have occurred. Researchers are now rapidly generating increasingly comprehensive maps of cancer genomes and identifying recurring mutations at high and moderate frequencies across tumors [i.e., "mountains and hills" (4)]. These maps bring to our attention promising new targets and theories, but also reveal the enormous diversity of mutations in each tumor, arising from an independent evolutionary process in each patient. In addition to the mountains and hills, we find the "lumps and bumps" of the flatlands, the personal mutations unique to each patient and that dramatically outnumber oncogenes. The subset of those mutations that alters protein coding sequence also creates personal, novel antigens – neoantigens – which may provide the "foreign" signal needed for cancer immunotherapy.

The Case for Neoantigens

As early as 1994, Mandelboim and colleagues (5) purified a peptide derived from a mutated transmembrane protein (Connexin 37) bound to mouse HLA molecules on the surface of Lewis lung carcinoma cells. The same team showed that immunization with synthetic peptides representing mutated Connexin 37 could induce antitumor CTLs and protect mice from spontaneous tumor metastasis and reduce metastatic load (6). Since then, a series of seminal murine and human studies have revealed that multiple other gene products with missense mutations can encode peptides recognized by cognate CTLs [refs. 7–13; see Sensi and Anichina for a comprehensive review (14)].

A particularly thorough and revealing study by Lennerz and colleagues (15) reported a potential role for CTLs against mutated antigens in controlling metastatic melanoma. These investigators had the foresight to collect tumor and blood samples from a patient who became a 5-year survivor with metastatic melanoma despite multiple disease recurrences.

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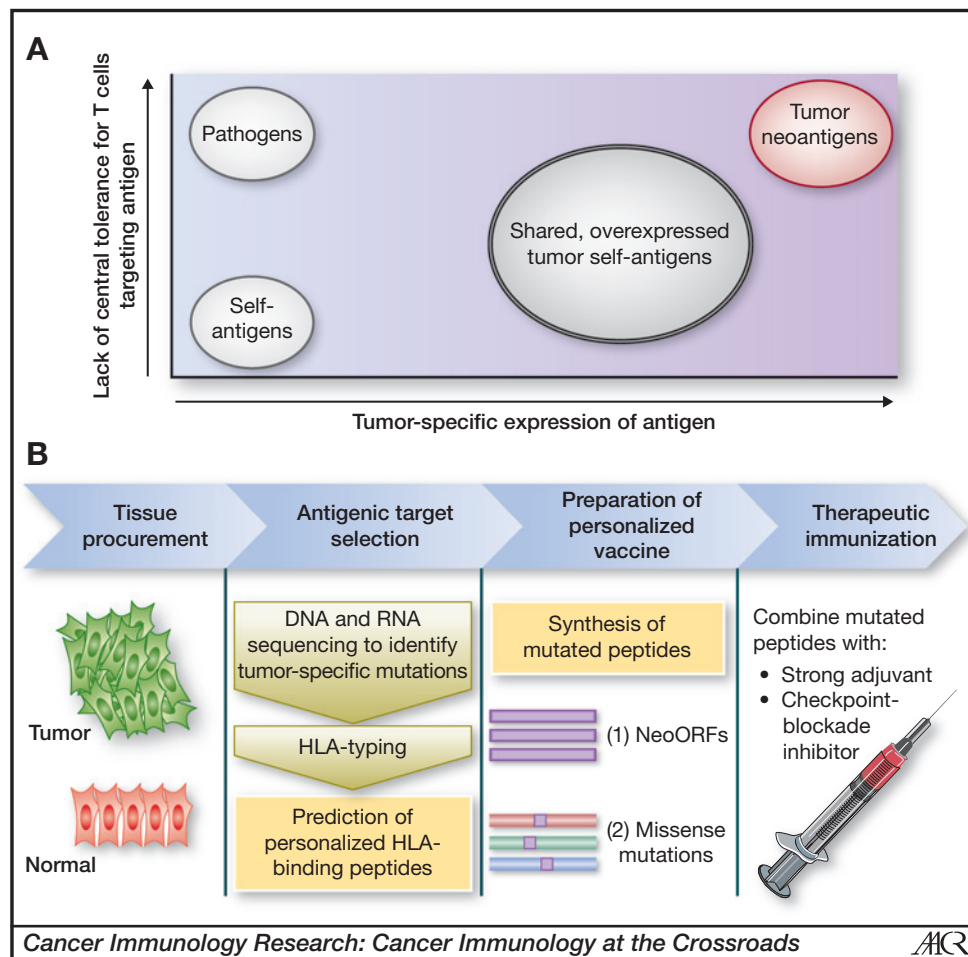


Figure 1. A, tumor neoantigens may be ideal targets for a therapeutic vaccine. Tumor neoantigens (top right) are present in tumor cells but not normal cells and do not induce deletion of their cognate antigen-specific T cells in the thymus (i.e., central tolerance). In these 2 dimensions, they appear more comparable with pathogen-derived antigens (top left) than to self-antigens (bottom left). In contrast, the most commonly identified and used tumor antigens (bottom middle) are selectively overexpressed in the tumor but can have residual expression in nontumor cells and induce central tolerance in the thymus. B, a therapeutic vaccine strategy based on tumor neoantigens. First, tumor mutations are discovered rapidly through DNA and RNA sequencing of tumor and normal tissue. Second, personalized tumor-specific mutated peptides are identified on the basis of predictive HLA-binding algorithms. Third, peptides based on neoORFs and missense neoantigens are synthesized. Finally, the peptides are delivered to patients with a powerful immune adjuvant and coupled with complementary immunotherapeutics, such as checkpoint-blockade inhibitors.

Painstaking analysis of the T-cell responses at multiple time points showed that the most dominant and enduring responses targeted proteins with missense mutations ("missense neoantigens"), and the less robust responses targeted over- and selectively-expressed self-antigens. Moreover, the cytolytic activity of these T cells was directed against mutated but not wild-type peptides derived from these genes. Other investigators found that long, completely novel stretches of amino acids (novel open reading frames or "neoORFs") can be generated in some tumors by out-of-frame insertions or deletions (due to defects in DNA mismatch repair causing microsatellite instability) and could also be recognized by the patient's T cells (16, 17). Consistent with these findings, Huang and colleagues showed that a frameshift mutation in melanoma cells was the primary target of a tumor-infiltrating T cell clone that was used for adoptive transfer, resulting in nearly complete regression of multiple metastatic melanoma lesions

in one patient (18). These studies showed that neoORFs generated by frameshift mutations (which are not subject to central tolerance) induce highly specific antitumor immunity, and are thus highly valuable as vaccine antigens.

Three exciting murine studies in early 2012 further showed the importance of strongly immunogenic mutated epitopes in the immune control of cancer. In two of these studies, the dominant epitope recognized by CD8 T cells in mice rejecting tumors was a missense neoantigen in a single highly expressed gene in a transplantable chemically induced tumor (19) and a highly immunogenic neoORF in a transgenic, inducible tumor (20). Together, these studies support the role of neoantigens as natural targets of the immune system. Within these models, tumors escaped immune control by shutting off expression of the mutated gene, and escape could be reversed through forced expression of the gene. Remarkably, transplanted tumors that contained multiple strongly immunogenic neoepitopes never

escaped, presumably due to the low likelihood of reduced expression of multiple, unrelated genes (20). In a separate series of investigations, Castle and colleagues used *de novo* sequencing to identify tumor-specific mutations in the murine melanoma tumor B16F10 and applied an algorithm to predict potential immunogenic epitopes generated by these mutations (21). This murine study showed that immunization with neoantigens—systematically identified by sequencing the genomes of tumors—can control disease both prophylactically and therapeutically.

In humans, there is also mounting evidence for the effectiveness of targeting tumor neoantigens. A recent clinically successful therapeutic vaccine (that is, a vaccine that treats rather than prevents disease) consisted of peptides derived from human papillomavirus (HPV), a known causative agent of vulvar intraepithelial neoplasia. Nearly 50% of the 19 patients receiving 3 to 4 vaccinations developed and maintained a complete response for 24 months or more (22). HPV genes encode long stretches of sequence entirely novel to the host immune system and are analogous to the novel neoORF-derived peptides generated by frameshift insertions, deletions and gene-fusions, read-through mutations in stop codons, or translation of improperly spliced RNA. The most extensively tested class of nonpathogen-associated neoantigens is the idiootype vaccine immunogens derived from malignant B cells expressing rearranged and multiply mutated surface immunoglobulins. Although complex and time-consuming to prepare, phase II studies using idiootype immunogens were encouraging, but failed to reach the clinical endpoints in 2 of 3 pivotal studies, with the single successful trial using an outdated induction therapy (23). Finally, immune targeting to a single mutated tumor antigen in humans can lead to immune escape. Sampson and colleagues immunized glioblastoma patients whose tumors carried a common in-frame deletion of the EGF receptor (EGFRviii) with a peptide corresponding to the novel sequence at the junction of the deletion, and found that more than 90% of recurrent tumors showed dramatically reduced or absent expression of EGFRviii by surface immunohistochemistry (24).

Overcoming Barriers

Neoantigens comprise a novel class of cancer immunogens with exquisite specificity for tumor cells and the advantage that one does not have to overcome central tolerance—both major improvements over using typical (unmutated) self-antigens. They also represent an opportunity to make cancer immunotherapy personalized, reflecting the nature of the disease itself and thereby "fighting fire with fire." At a practical level, however, what obstacles have to be overcome to realize this vision of a personalized neoantigen vaccine?

First, how should one identify tumor neoantigens for immunization? The initial step would be to sequence DNA and RNA from a patient's tumor and normal tissue to identify mutations that create neoantigens (both missense and neoORFs), ideally in genes expressed in the tumor cell (Fig. 1B). One would then want to identify peptides most likely to generate a robust immune response, by using algorithms (e.g., NetMHC, IEDB) that predict peptides binding to the cleft of patient-specific class I HLA molecules (25–27). For example,

2 studies in human patients with leukemia used this approach to identify CTLs targeting HLA-binding peptides derived from mutated regions of known oncogenes, nucleophosmin in acute myelogenous leukemia (28), and *BCR-ABL* in chronic myelogenous leukemia (29). In addition, to carry out an unbiased search for neoantigens, we recently used whole-exome sequencing to identify all the leukemia-specific mutations in 91 patients with chronic lymphocytic leukemia (30). Within a subset of patients with identified HLA alleles, we used NetMHC to predict which mutated peptides bind to patient-specific HLAs, validated their binding biochemically, and confirmed the presence of CTLs targeting a subset of these neoantigen-derived peptides. These studies provide proof-of-concept for the strategy. Furthermore, improved prediction rules (based on additional steps in antigen processing; refs. 31, 32) may further improve the odds. Among the neoepitopes, one would prioritize neoORFs because they provide long stretches of completely novel protein sequence (which bypass central tolerance and have no counterpart in any normal cell). In addition, one might prioritize targets harboring mutations in genes that are required for tumor cell survival (e.g., "oncogenic drivers;" ref. 33) or that diminish fitness when reduced in expression, as well as those that are present in all cancer cells (i.e., clonal) rather than only a subpopulation (i.e., subclonal).

Second, how many neoantigens should be targeted? Immunizing with multiple neoantigens has 2 advantages: (i) it increases the likelihood of generating a robust immune response against at least some of the neoantigens, and (ii) it decreases the likelihood of a tumor escaping the immune response by immunoediting, because it must downregulate multiple targets. Consistent with this notion, the results of Castle and colleagues (21) are encouraging, showing that neoantigens arising from at least 17% of murine melanoma missense mutations are "strongly immunogenic" in the syngeneic host mouse. We thus anticipate that immunization with 20 or more epitopes would provide effective presentation of a handful of epitopes by the patient's HLA molecules and limit escape. Moreover, recurrence due to immunoediting might be treated by a vaccine containing a new combination of neoantigens. Tumors also escape T-cell recognition by mutations that disrupt the basic mechanisms that present antigens on the cell surface; however, the absence of cell-surface HLA may activate the antitumor activity of natural killer (NK) cells (whose normal function is to kill virus-infected cells in which the virus has caused HLA downregulation).

Third, what is the form of the antigen and can a personalized vaccine be produced in a timely and cost-effective manner? An individual-specific multitargeted vaccine requires production of many unique immunogens per patient in a GMP environment. On the basis of a long history of successful production and safe use, synthetic peptides are an attractive choice. Especially promising are long peptides (>20 amino acids in length) that undergo efficient internalization, processing, and cross-presentation in professional antigen-presenting cells such as dendritic cells (34). Designing and implementing a streamlined process for producing a constantly varying set of heterogeneous peptides represents a paradigm shift for peptide GMP manufacture and will require close interaction between manufacturers and regulatory authorities.

Finally, which patients will benefit from this approach? A personalized neoantigen vaccine requires a period of time (that can be compressed as the process is optimized) for sequencing, analysis, and production of the vaccine. Ideally, a personalized neoantigen approach would be applied early in disease evolution, when intratumoral heterogeneity is more limited, and immunologic intervention may effectively eliminate incipient disease cells.

The clinical use of neoantigen-based vaccines will require careful optimization. As with any vaccine, it will be important to optimize the adjuvant, schedule, and mode of delivery. Moreover, evaluating the vaccine in combination with checkpoint-blockade antibodies is warranted (35–38). The combination may reduce the impact of local immune suppression while allowing a highly focused antitumor response to develop. With rapid advances across many relevant fields (sequencing, prediction algorithms, immune adjuvants, checkpoint blockade, antigen delivery, etc.), we are entering a new era of cancer

immunotherapy in which a sophisticated vaccine loaded with patient-specific neoantigens is poised to generate a powerful yet precisely targeted antitumor immune response.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Authors' Contributions

Writing, review, and/or revision of the manuscript: N. Hacohen, E.F. Fritsch, T.A. Carter, E.S. Lander, C.J. Wu

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