# Personal Neoantigen Cancer Vaccines: A Road Not Fully Paved

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## ABSTRACT

Personal neoantigen–based cancer vaccines are designed to target antigens arising from tumor-specific mutations within individual cancers and present a tremendous opportunity to capitalize on their favorable and intrinsic properties of escape from central tolerance and exquisite tumor specificity. With the endpoint of creating an optimal T-cell army to attack a tumor, neoantigen-based vaccines have demonstrated the ability to coax naïve T-cell recruits against epitopes that do not induce spontaneous immunity to raise long-lasting T-cell responses against multiple tumor-specific epitopes and subsequently to extend the breadth of responses, as immunity begets immunity via epitope spreading. Importantly, on both preclinical and clinical fronts, the association of T-cell responses to neoantigens and favorable outcomes has been demonstrated time and time again. We recognize, however, that the path forward remains long and winding and requires the field to address several key challenges, particularly overcoming evolved tumor escape mechanisms and optimizing vaccine-induced immunity. Some challenges stem from gaps in science that enable *in silico* prediction of antigen presentation and recognition by T-cell receptors, whereas others stem from the logistical obstacles and cost of personalization. Nevertheless, with perseverance and innovative solutions, we have little doubt that the ability of neoantigen vaccination to induce potent cancer-specific T cells will fundamentally succeed in enabling greater effectiveness of a broad array of immunotherapies. We provide our perspective on the progress and the remaining challenges to realizing the opportunity of personal neoantigen cancer vaccines.

## The Concept of Immunotherapy with Personalized Neoantigen Vaccines

Although many genetic abnormalities can drive cancer and are found recurrently across cancer types and multiple patients, they are often accompanied by "passenger" changes that may not affect tumor growth and yet are considered "personal" (found exclusively within the tumors of individual patients). Exploiting this repertoire of mutations for targeting by a cancer vaccine has the earmarks of a winning strategy, as we noted in 2013 (1). Mutations in the coding regions of the genome—missense point mutations, deletions or insertions, splice-site mutations, or gene fusions—can alter the sequence of translated gene products and create novel proteins that are processed into immunogenic epitopes (i.e., mutated neoantigens, referred to as neoantigens below) that are presented on MHC molecules and recognized by T cells. Somatically mutated neoantigens, by definition, are not expressed in healthy tissues, and their cognate T cells are not shaped by central tolerance. Thus, neoantigens provide a unique opportunity for inducing potent tumor-specific T cells, which are central to the effectiveness of almost all forms of immunotherapy. Counterbalancing these apparent advantages, though, are the formidable challenges of having to identify these mutations by sequencing individual tumors, accurately predicting which mutations produce epitopes that would be presented to T cells by tumor cells and antigen-presenting cells, and preparing vaccines that are suitable for only a single patient.

## Testing the Concept

Over the past five years, three studies pioneered the clinical translation of multiepitope, personalized neoantigen–based cancer vaccination (2–4). These first-in-human trials targeted advanced melanoma with typically high tumor mutation burden and used long peptides with adjuvant, RNA-encoding long peptides, or autologous dendritic cells (DC) loaded with epitope-length HLA class I peptides to target multiple predicted patient-specific neoantigens. All three studies show the induction of potent *de novo* (i.e., not observed prior to therapy) polyfunctional T-cell responses, many comparable with responses observed for viral peptides (5). One study reports infiltration of induced neoantigen-specific T cells into the tumor (2) and observed loss of beta-2-microglobulin on one tumor. Surprisingly, in both studies using long peptides or RNA-encoded long peptides, the responses were dominated by $CD4^{++}$ neoepitope-specific T cells, despite being included in the vaccine on the basis of HLA class I binding, which remains a mystery in terms of mechanism and impact on tumor control. Curiously, the study by Ott and colleagues did not detect preexisting responses, whereas one third of the selected epitopes by Sahin and colleagues and Carreno and colleagues show weak preexisting responses, perhaps due to the prior therapies in these patients. Subsequent studies have extended the testing of these vaccines in the setting of glioblastoma, a low mutation burden tumor (6–8), and encouragingly, in the study by Keskin and colleagues, *de novo* neoantigen vaccine–induced T cells are shown to traffic to the brain. Importantly, immune responses generated by such vaccines have been shown to be long-lasting (3–4 years) and retain cytolytic potential (9). Equally important, after vaccination alone, induction of T cells recognizing mutated epitopes not part of the vaccines

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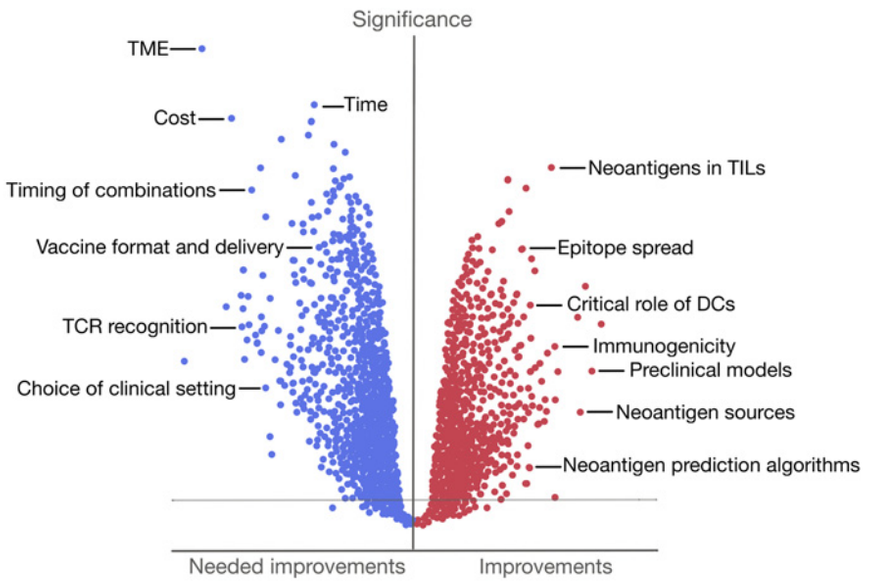


**Figure 1.**
Plotting the progress and the need. A volcano plot is used to figuratively show the areas where the development of personal neoantigen cancer vaccines has progressed ("Improvements") and areas where more research and breakthroughs are needed ("Needed improvements"). Significance increases from bottom to top of the *y*-axis. TCR, T-cell receptor; TME, tumor microenvironment.

("epitope spreading") is observed (9, 10). In aggregate, these trials suggest a reduction in disease recurrence and potentially an enhancement of clinical activity by subsequent checkpoint blockade, raising the possibility that the vaccine enhances checkpoint therapies, as we originally predicted (1). Randomized controlled trials will, of course, be required to draw more definitive conclusions.

The available data supporting the neoantigen concept provide a compelling start to the journey in 2013 and in the 7 years since our previous *Cancer Immunology at the Crossroads* review (1), in which we noted that we were entering a new era of cancer immunotherapy based on neoantigen vaccines. Although we have made substantial progress, we have not yet entered into that new era, and the path forward has been far from straight (**Fig. 1**).

## Should We Still Be Encouraged? Challenges and Opportunities

New preclinical and orthogonal clinical data sets have continued to demonstrate the power and criticality of neoantigens in the control of tumors, whether alone or in conjunction with immunotherapy or conventional therapies. First, preclinical data reveal that mouse tumors can be effectively controlled by a neoantigen vaccine, alone or in combination with other immune modulators and in multiple transplantable or autochthonous murine tumor models, including models resistant to checkpoint blockade (11–14), extending the early-demonstrated advantage of non-self versus self-antigen immunogenicity (15). Although typically focused on $CD8^+$ T-cell epitopes, the importance and critical role of $CD4^+$ T-cell epitopes has also emerged from preclinical studies (13, 16), whether directly or indirectly cytolytic (17, 18) or to provide critical help to $CD8^+$ T cells (19). It remains to be determined if the unexpected $CD4^+$ T cells induced by long-epitope vaccines, selected based on class I epitopes, are of the requisite "quality" to regularly elicit these effects. Second, promising studies dissecting human responses to existing immunotherapies highlight a central role for tumor neoantigens. Approved checkpoint therapies show induction of patient and mutated neoantigen-specific T cells (20, 21). Moreover, human tumor-infiltrating lymphocyte (TIL) therapy, for many years the shining example of the potential of immunotherapy (mostly for melanoma and a black box with respect to specificity), regained excitement and a new direction when Tran, Rosenberg, and colleagues demonstrated the curative potential of a single neoantigen-specific T-cell population in a tumor of epithelial origin (22). Third, large clinical studies show that long-term survival, particularly following checkpoint therapy, is positively associated with tumor mutational burden, a surrogate for neoantigen load (reviewed in ref. 23). Deeper analyses of neoantigen features, such as clonality, indels, lack of self-similarity, similarity to microbial antigens, and models of tumor fitness, have provided further support for this association (24–27). Finally, supporting these direct links, the broadening of the T-cell receptor (TCR) repertoire in patients responding to immunotherapy (28), and the emerging critical role of cross-presenting DCs in immunotherapy (reviewed in ref. 29) have pointed to the importance of novel T-cell specificities (30), many of which appear to be neoantigen directed (31).

These advances, however, have been tempered by a deeper appreciation of the formidable challenge of eradicating late-stage tumors, where tumors have had years to evolve and potentially evade immune responses. The seemingly dominating and multifaceted immunosuppressive tumor microenvironment, a suppression that extends beyond the boundaries of the tumor (32) and maybe even establishes the niche before its earliest metastatic seeding (33), evolves to support the tumor, and only the fittest tumors survive. Certainly, blockade of the CTLA-4:CD80/86 and the PD-1:PD-L1 axes has provided notable therapeutic successes, and these experiences have motivated the ongoing frenzied efforts to test combination immunotherapies. However, the tremendous investment of clinical resources and dollars has proven largely fruitless, a reflection of our still rudimentary understanding of the complexity and dynamism of the tumor–immune interaction. Adding A and B together is a simple concept, but, variables like sequencing and timing require consideration. In the race to test combinations, these important variables are often overlooked or ignored (34–36). Given the critical role of immune checkpoints in normal physiology, there may well be underlying unintended consequences of disturbing such well-tuned homeostatic systems.

T-cell immunity does not exist in a vacuum, and regulatory T cells, myeloid-derived suppressor cells, one or more types of tumor-associated macrophage, and cancer-associated fibroblasts have been firmly established to contribute to "cold" tumors and "immune deserts." Mitigating approaches to address the cellular components of the tumor microenvironment are under active development, with demonstrated improvement in vaccine immunogenicity (37, 38).

Finally, immune editing, the loss or reduced expression of target antigens due to immune pressure (39, 40), may eliminate the strongest target antigens. Indeed, the power of this evolutionary pressure has been thought to shape the observable landscape of the dominant mutation–based oncogenic drivers of cancer (41). Tumor/immune evolution pushes to reduce the burden of spontaneously immunogenic mutations (or enhance suppression or both) in a downward spiral as tumors progress in patients (42), leaving weakly immunogenic mutated targets. For neoantigen vaccines to tip the balance in favor of T-cell immunity, epitope selection and highly immunogenic vaccine delivery will be critical.

Selection of which neoantigens to target has seen advances, primarily through integration of data from mass spectrometric identification of the HLA class I and II peptidomes from cell lines (43, 44) or tumor samples (45) and through application of machine learning approaches (43, 45). Mass spectrometry (MS)–based data integrate multiple aspects of the antigen presentation pathway (expression, processing, editing, affinity, and stability) not reflected by affinity measurements alone, and have been used to assess the relative contribution of each of these parameters (46). With the growing identification of immunogenic epitopes, based on detection of endogenous or vaccine-induced T cells, these prediction advances can be pressure tested (47, 48). A key aspect of predictions still relatively unexplored is which features of presented peptide:HLA complexes contribute most to immunogenic T-cell recognition and differentiation from the cognate nonmutated peptide. Such parameters are useful for the majority of nonsynonymous single-nucleotide variants, most of which are not anchor-residue altering, and so may directly affect the structure of the presented complex (49). Additionally, analysis of the dynamics of peptide:HLA:TCR interaction identifies "catch bonds" (tension-induced strengthening of the force between the TCR and the peptide:HLA complex) as a feature of peptide epitopes not yet predictable but critical to effective TCR stimulation (50).

Two studies have described comparisons between nonmutated, tumor-associated self-antigens with neoantigens in the same patients. In the study by Sahin and colleagues, responses to neoantigen epitopes are significantly stronger than tumor-associated self-antigens delivered to the same patients. In the study by Hilf and colleagues, when patients were immunized with mutated and nonmutated peptides that were selected based on class I binding prediction or MS detection, respectively, of presented peptides, 5 of 13 mutated peptides, versus 1 of 6 nonmutated peptides, induced $CD8^+$ T-cell responses as a result of vaccination, supporting the viewpoint that mutated peptides are more immunogenic.

Epitopes arising from recurrent mutations, particularly in oncogenic driver mutations, in principle are desirable neoantigens to target, but the diversity of HLA restriction, and potentially evolutionary selection working against the observation of immunogenic recurrent driver mutations (41), have resulted in limited success. Mutations at the *KRAS* G12 position, arguably the most recurrently mutated position among oncogenic drivers and suppressors, have been examined extensively, but without success as a vaccine target. However, in-depth TIL and peripheral blood screening first identified T cells, and the corresponding TCRs, targeting the abundant G12D mutation against a relatively uncommon HLA-C allele (51), the less abundant KRASG12V mutation restricted by the much more common HLA-A11:01 allele (52), and HLA class II–restricted T cells against both KRASG12D and a commonly mutated position in the p53 tumor suppressor gene (53). At the other extreme, Okada and colleagues have identified T cells and TCRs targeting an HLA-A02:01–restricted mutated epitope in histone H3, a mutation common in the very rare but highly lethal childhood brain tumor, diffuse intrinsic pontine glioma (54). Whether or not shared neoantigen targets emerge as a major contributor to neoantigen vaccine therapies thus still remains to be seen.

Like cancer, the adaptive immune system also responds to evolutionary pressures. Thus, an important tactical question is whether or not to focus on preexisting responses. A study examining TCR avidity longitudinally following chronic CMV infection saw a significant drop in avidity over time, due potentially to selective survival of lower avidity cells following chronic antigen exposure (55), a condition endemic to tumors. A currently related, but important unresolved question, is whether preexisting or *de novo* responses are critical to the success of checkpoint therapy. At present, targeting a broad range of neoantigens and induction of *de novo* responses to capture more powerful T cells, if they actually still exist in the repertoire and have not already been edited by such pruning, continues to seem most prudent.

Beyond identifying the properties of more effective neoantigen peptides, multiple lines of investigations reveal a deeper well of opportunities for finding neoantigens beyond specific mutations, including splicing defects (56); epigenetic changes that contribute to induced expression (57), particularly for endogenous retroviral elements that may be tumor-specific; stochastic or deliberate modification of peptide processing and transport (58); and other "dark matter" such as aberrant translation from untranslated regions, lncRNAs, or noncoding strands (59–61). New sources of nonmutation-based neoantigens may be critically important. Using a systematic series of mixed mouse tumor cell lines bearing similar but non-identical mutations, Wolf and colleagues show that intratumoral heterogeneity drives resistance, independent of mutation burden (62). In line with the work of McGranahan and colleagues on truncal mutations (24), neoantigens created by more clonal events, such as dysregulated expression, splicing, or translation, may be more effective.

The optimal format for vaccine delivery, especially for personal epitopes and in the setting of advanced cancer with an immunosuppressive tumor microenvironment, is a critical component of success but still a work in progress. Peptide, conventional and self-replicating RNA, DNA, and viral-vectored defined epitope neoantigen vaccines are all in the clinic, as are vaccines utilizing whole cells or lysates, which in principle contain all neoantigen epitopes. The key immunologic challenge for direct *in vivo* immunization is delivering antigen and stimulatory signals (adjuvant, costimulation, etc.) to the proper cells at the same time, and no clear winner has yet emerged. Interestingly, some early DC-based vaccine studies have now begun to reveal promising clinical outcomes, and similar to other immunotherapy approaches, hints of extended survival have emerged (31, 63, 64). Whether DC generation and direct antigen delivery *ex vivo*, outside the immunosuppressive environment, is a root cause for these apparent positive clinical outcomes is still to be determined. However, these promising observations may be buttressed by observations of defects in endogenous antigen-presenting cells *in vivo* (65) and the critical role DCs appear to have for checkpoint blockade of the PD-1 axis (66),

suggesting this as a fundamental defect in the immune response to advanced cancer.

## A Major Speed Bump Ahead

Are we ready for success? Personal neoantigen vaccines have rapidly transitioned from clinical proof of concept of immunogenicity, with hints of clinical activity, to larger trials as a hoped-for step toward registration. If successful, the challenge will be bespoke manufacturing. The cost and timing for analysis and production already have affected development both logistically and biologically. Expensive therapies will not get developed for adjuvant treatment of minimal disease, where arguably vaccines have the best opportunity for success, and timing is critical for advanced disease, necessitating incorporation with available therapies, even if that poses biological challenges (35). Competition will be intense from easier- and faster-to-use combination therapies with off-the-shelf drugs, severely limiting the breadth of opportunity available to personal neoantigen–based approaches. Out-of-the-box approaches are needed to leapfrog over these barriers.

## Outlook

Thus, although mutated epitopes represent a powerful and validated opportunity to enhance tumor immunity, many practical challenges remain. Learnings from the early studies and the field of cancer immunotherapy in general have provided some clear targets to overcome. Nimbleness will be critical. As observed from the plethora of checkpoint combination trials, the system is too complex to just stumble onto the right next step. It will take testing, analysis, and retesting, and the mindset to smartly and quickly adapt to realize that opportunity.

### Disclosure of Potential Conflicts of Interest

E.F. Fritsch reports other from BioNTech (equity holder and consultant) and BioEntre (equity holder) outside the submitted work, as well as a patent for compositions and methods for personalized neoplasia vaccines pending, licensed, and with royalties paid from BioNTech, a patent for formulations for neoplasia vaccines pending, licensed, and with royalties paid from BioNTech, a patent for combination therapy for neoplasia vaccine pending, licensed, and with royalties paid from BioNTech, and a patent for methods for profiling the T-cell receptor repertoire issued, licensed, and with royalties paid from BioNTech. U.E. Burkhardt reports a patent for methods for profiling the T-cell receptor repertoire licensed and with royalties paid from BioNTech and is an equity holder in BioNTech and BioEntre. N. Hacohen reports other from BioNTech (equity) outside the submitted work, as well as a patent (Compositions and methods for personalized neoplasia vaccines) pending, licensed, and with royalties paid to BioNTech, a patent (Combination therapy for neoplasia vaccine) pending, licensed, and with royalties paid to BioNTech, and a patent (Methods for profiling the T cell receptor repertoire) issued, licensed, and with royalties paid to BioNTech. C.J. Wu reports other from BioNTech (equity) outside the submitted work, as well as a patent (Compositions and methods for personalized neoplasia vaccines) pending, licensed, and with royalties paid to BioNTech, a patent (Combination therapy for neoplasia vaccine) pending, licensed, and with royalties paid to BioNTech, and a patent (Methods for profiling the T cell receptor repertoire) issued, licensed, and with royalties paid to BioNTech.

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