Personalized vaccines for cancer immunotherapy

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Cancer is characterized by an accumulation of genetic alterations. Somatic mutations can generate cancer-specific neoepitopes that are recognized by autologous T cells as foreign and constitute ideal cancer vaccine targets. Every tumor has its own unique composition of mutations, with only a small fraction shared between patients. Technological advances in genomics, data science, and cancer immunotherapy now enable the rapid mapping of the mutations within a genome, rational selection of vaccine targets, and on-demand production of a therapy customized to a patient’s individual tumor. First-in-human clinical trials of personalized cancer vaccines have shown the feasibility, safety, and immunotherapeutic activity of targeting individual tumor mutation signatures. With vaccination development being promoted by emerging innovations of the digital age, vaccinating a patient with individual tumor mutations may become the first truly personalized treatment for cancer.

river mutations promote the oncogenic process, whereas passenger mutations have long been considered as functionally irrelevant (1). Both types of mutations, however, can alter the sequence of proteins and create new epitopes, which are processed and presented on major histocompatibility complex (MHC) molecules. The sequence-altered proteins are termed “neoantigens,” and their mutated epitopes that are recognized by T cells are called “neoepitopes” (2). Neoepitopes are absent from normal tissues and new to a given individual’s immune system. In 1916, Ernest Tyzzer, who introduced the term “somatic mutation,” recognized their role in the “acquisition of new immunogenic characteristics” by cancer cells (3). The idea of taking advantage of their “foreignness” and using mutations as targets against cancer has attracted generations of scientists. How to realize a specific targeting of cancer, however, has remained obscure since its proposal by Paul Ehrlich in 1909 (4).

In the 1980s, studies to understand why mice with syngeneic carcinogen-induced tumors are protected from rechallenge with the same cancer cells led to the concept of adaptive tumor immunity (5). In the 1970s, tumor-derived T cell clones were shown to recognize human tumor cell lines and were identified as cellular correlates of adaptive immunity. The molecular nature of tumor antigens remained unknown until cloning techniques were introduced in the late 1980s (6). Screening of patient tumor-derived expression libraries with autologous tumor-reactive CD4+ or CD8+ T cells revealed two categories of spontaneously recognized T cell antigens: (i) nonmutated proteins with tumor-associated expression and (ii) mutated gene products (7). An oncogenic loss-of-function mutation of cyclin-dependent kinase 4 (CDK4) was the first example of the latter category in humans (8).

However, the vast majority of discovered mutated antigens were unique to individual patients, and a viable concept for exploiting “personal” targets for therapy could not be envisaged. Therefore, in the 1990s and 2000s, nonmutated tumor antigens shared by patients were favored for cancer vaccine development, yet outcomes were disappointing. Technological and scientific breakthroughs brought somatic mutations back into focus. Next-generation sequencing (NGS) allows rapid sequencing of genomes at low cost. Together with dedicated bioinformatics tools, NGS enables comprehensive mapping of all mutations in a cancer (collectively called the “muta-nome”) and prediction of MHC molecule-binding neoepitopes. Neoepitope-specific T cells have been shown to be associated with durable clinical responses mediated by immune checkpoint blockade and adoptive transfer of autologous tumor-infiltrating lymphocytes (TILs) (9, 10). Mutational burden, tumor immune cell infiltration, and survival correlate across various cancer types (11, 12). Collectively, these findings strongly indicate that immune recognition of neoepitopes is clinically meaningful. However, profiling of individual cancer patients revealed spontaneous immune responses against only a small fraction of their mutations (<1%) (13), casting doubt on the immunogenicity of mutations per se. This led to the presumption that only tumors with a high mutational load, and accordingly a higher diversity of spontaneously occurring cancer-reactive T cell specificities, may qualify for neoantigen-based immunotherapy.

Preclinical studies and clinical translation of personalized mutanome vaccines

Whether the examples of mutated tumor rejection antigens were rare cases of incidental immunogenicity, or were the tip of the iceberg, remained unclear until systematic studies in syngeneic mouse models provided deeper insights. To address which portion of a mutation induced a neoepitope-specific immune response, our lab-oratory vaccinated mice with long peptides or antigen-encoding RNA, representing 50 mutations identified by NGS in their syngeneic tumor; a considerable fraction of the mutations were immunogenic and mediated tumor rejection (14, 15). Notably, the vast majority of neoepitopes, accounting for 20 to 25% of the randomly selected mutations, were recognized by CD4+ T helper cells. Vaccination with these neoepitopes resulted in growth control of advanced mouse tumors. Most cancers are constitutively MHC class II-negative, and recognition by CD4+ T cells requires uptake and presentation of released tumor antigens by dendritic cells (DCs) in the tumor microenvironment or draining lymph node. As this mechanism should work most efficiently for highly expressed antigens, MHC class II binding prediction and expression thresholds of the mutated allele were combined to enrich for immune-dominant MHC class II neoepitopes. Mice were vaccinated with a computationally designed synthetic mRNA incorporating multiple predicted MHC class II neoepitopes. The mice experienced complete re-rejection of established tumors associated with strong CD4+ T cell responses and a CD8+ T cell response against an epitope not represented in the vaccine, indicating antigen spread (16).

In a concurrent study, the feasibility of using NGS and MHC class I prediction to identify MHC class I-restricted tumor rejection antigens was shown in a highly immunogenic mouse sarcoma model (17). The antitumor effect of checkpoint blockade in this model was mediated by neoepitope-specific CD8+ T cells and was fully reproducible by vaccination with long synthetic peptides representing an identified neoepitope (17). Another study systematically tested protective antitumor activity of mutated 9-amino acid oligomer (9-mer) peptides in mice. The difference in predicted affinity for a given wild-type/mutant peptide pair, and the predicted conformational stability of MHC class I peptide interaction, were both found to positively correlate with the likelihood of the mutated peptide to be recognized by CD8+ cytotoxic T lymphocytes (CTLs) (18). A combination of mass spectrometry and exome sequencing was shown to enrich for immune-dominant MHC class I neoantigens in mouse models (19).

Clinical translation from syngeneic mice to humans who have “one-of-a-kind” cancers is more complex because it requires personalization of the process, including identification of mutations, prediction of potential neoantigens, and design and manufacture of the vaccine (Fig. 1). This was recently accomplished by three first-in-human studies in malignant melanoma patients (20–22). In one trial, three melanoma patients received autologous DCs loaded ex vivo with seven synthetic 9-mer peptides representing individual mutations of each patient predicted to bind to the frequent class I haplotype HLA-A2 (20). Vaccine-induced CD8+ T cell immune responses with confirmed specificity for the respective immunogen were detected against 9 of the 21 peptides. However, recognition of autologous melanoma cells was not assessed, and the relevance of the vaccine responses remained unclear. Two subsequently
Fig. 1. Customizing a patient-specific cancer vaccine. Patient tumor biopsies and healthy tissue (e.g., peripheral blood white blood cells) are subjected to next-generation sequencing. By comparing the sequences obtained from tumor and normal DNA, tumor-specific nonsynonymous single-nucleotide variations or short indels in protein-coding genes are identified. A computational pipeline is used to examine the mutant peptide regions for binding to the patient’s HLA alleles (based on predicted affinity) and other features of the mutated protein deemed relevant for prioritization of potential vaccine targets. These data can facilitate selection of multiple mutations to design unique neoepitope vaccines that are manufactured under GMP conditions.

reported clinical trials in resected stage III-IV melanoma patients exploited the broader potential of the concept irrespective of the HLA haplotype. In one trial, six patients were vaccinated subcutaneously with long peptides representing up to 20 mutations per patient coadministered with adjuvants (22). In the second trial, 13 patients were injected with RNA encoding 10 of their individual mutations as 27-mers (22). Thus, induction of T cell responses in both studies required processing and presentation of neoepitopes by the patient’s antigen-presenting cells. Both studies showed a high overall immunogenicity rate of 60%, as demonstrated by analyzing T cell responses against the individual mutations in interferon-γ (IFN-γ) secretion assays. Each patient developed strong T cell reactivity against several of their tumor mutations. Preexisting T cells were expanded; moreover, the majority of vaccine-induced T cell responses in both studies were newly primed and not detectable before vaccination.

In accordance with the preclinical findings, the majority of neoepitopes induced functional CD8+ T helper 1 (T2H1) cells, both in the RNA trial (which combined MHC class I and class II prediction for neoepitope selection) and in the peptide trial (which relied on MHC class I binding prediction only). Neoepitope-specific CD8+ T cell responses were detected against 25% versus 16% of the mutations in the RNA or the peptide vaccine, respectively. In most cases, the identified minimal epitopes recognized by these CD8+ T cells showed a strong predicted binding affinity, supporting the usefulness of this criterion for neoepitope prioritization. In the RNA trial, multiple CD8+ responses were of high magnitude, allowing detectability without prior expansion. A number of neoepitopes were also concurrently recognized by both CD4+ and CD8+ T cells, the meaning of which is currently unclear. Both trials showed recognition of autologous tumor cell lines for selected vaccine-induced immune responses. In two RNA-vaccinated patients, there was evidence of CTL infiltration and tumor cell killing with neoepitope-specific T cells.

Despite the small cohort sizes, these studies provide intriguing evidence for clinical activity of the vaccine alone and in combination with subsequently administered checkpoint inhibitors. In the RNA trial, vaccination significantly reduced the cumulative number of disease recurrences in the 13 high-risk melanoma patients, translating to a long progression-free survival. Eight of the patients did not have radiographically detectable lesions at study entry and remained recurrence-free for the entire follow-up period. In five patients with progressing disease at entry, there were two objective responses attributable to the vaccine-mediated durable clinical responses have indicated that mobilization of the host’s immune system represents a powerful therapeutic modality. In cancer patients, the complex interactions between immune system and tumor are considered dysfunctional. A key principle of rational immunotherapy is to restore this process, known as the cancer immunity cycle (23, 24), and to expand and broaden the CTL response against cancer cells (Fig. 2). For many years, tumor rejection has mainly been attributed to cytotoxic CD8+ T cells, and vaccine approaches have relied on potential MHC class I epitopes. There is a growing body of data, however, indicating that neoantigen-specific CD4+ T cells contribute critically to the effectiveness of cancer immunotherapy (25). As the cancer mutanome turned out to provide an exceptionally rich source for potent MHC class II neoepitopes, mutanome vaccines have potential for mobilizing the broad repertoire of T1H1 CD4+ T cells (15).

Whereas the primary mode of action of CD8+ effectors is cytotoxic killing of cells presenting their cognate antigen (26), CD4+ effectors have a wider range of functions, including orchestration of various cell types of the adaptive and innate immune system (27). CD4+ T cells are gatekeepers for the induction of effective CD8+ T cells. T1H1 CD4+ T cells in different compartments activate DCs presenting antigens released from tumor cells through cognate CD40 ligand/CD40 receptor interaction. DCs then undergo maturation, produce interleukin-12 and chemoattractants, and up-regulate costimulatory molecules. These events are characteristically linked to the generation of sustained CTL responses to tumor antigens, which are released by tumor cell death and cross-presented. Multi-neoepitope vaccines have been shown to concurrently mobilize neoantigen-specific CD8+ as well as CD4+ T cells, and by furnishing collaborative synergy they may actuate a non-performing cancer immunity cycle at several key points (Fig. 2): In the priming phase of the vaccine...

“...a personalized mutanome vaccine has the potential to become a universally applicable therapy irrespective of cancer type.”

alone (one complete, one partial response), one mixed response, and one stable disease. One patient discontinued vaccination because of fast progression and had a rapid complete remission after subsequent checkpoint blockade therapy. In the peptide trial, four of six vaccinated patients remained recurrence-free for the full follow-up period, while two developed progressive disease and achieved complete tumor regression after subsequent anti–PD-1 treatment.

Using neoantigens to stimulate tumor immunity

Whereas clinical oncology has relied on targeting oncogenic pathways for decades, lessons learned in recent years by studying immunotherapy-mediated durable clinical responses have indicated that mobilization of the host’s immune system represents a powerful therapeutic modality. In cancer patients, the complex interactions between immune system and tumor are considered dysfunctional. A key principle of rational immunotherapy is to restore this process, known as the cancer immunity cycle (23, 24), and to expand and broaden the CTL response against cancer cells (Fig. 2). For many years, tumor rejection has mainly been attributed to cytotoxic CD8+ T cells, and vaccine approaches have relied on potential MHC class I epitopes. There is a growing body of data, however, indicating that neoantigen-specific CD4+ T cells contribute critically to the effectiveness of cancer immuno...
response in the lymphatic compartment, effective licensing of DCs by T<sub>H1</sub> cells can robustly induce potent neoeptope-specific CTLs with improved ability to infiltrate into the tumor and can generate long-lived memory CD8<sup>+</sup> T cells (28). In the tumor, vaccine-induced T<sub>H1</sub> CD4<sup>+</sup> T cells may promote an inflammatory microenvironment by acting on various immune cell types (15, 29). IFN-γ, the key cytokine of T<sub>H1</sub> cells, up-regulates MHC class I on tumor cells to improve killing by CD8<sup>+</sup> effectors. Concurrently, by inducing MHC class II expression, IFN-γ sensitizes tumor cells for recognition and direct killing by cytotoxic T<sub>H1</sub> CD4<sup>+</sup> effectors. Overall, by promoting tumor cell death and neoantigen release for uptake by DCs in combination with an immunogenic microenvironment, CD4<sup>+</sup> T cells may crank up consecutive cycles of T cell priming, expansion, and antigen spread, thus broadening the antitumor T cell repertoire.

**Mutation discovery, neoepitope prediction, and selection of target antigens for vaccine design**

One of the critical challenges for personalized cancer vaccines is to accurately map the cancer mutanome, so as to select the most suitable mutations for optimal immune responses. Mutations are detected by comparing exome sequencing data generated by NGS from tumor tissue and a matched healthy tissue sample (e.g., the patient’s blood cells), thereby preventing the incorrect classification of germline variants as neoepitopes. NGS analysis is being continuously improved, and clinical application requires standard operating procedures that ensure data reproducibility, quality control, and privacy. Current protocols allow efficient nucleic acid extraction in NGS-grade quality from fresh, frozen, and formalin-fixed paraffin-embedded tissues. Typically, these analyses rely on a small biopsy from a single tumor lesion collected for routine diagnostics, and thus sequence data may not be representative of the tumor’s full clonal spectrum. Another concern to be addressed is erroneous mutation determination. Standardized algorithms to sensitively determine true mutations in individual NGS data sets work well for single-nucleotide variations (SNVs). SNVs are the most abundant type of tumor mutations, and if they occur in an expressed protein and are nonsynonymous, they can give rise to T cell–recognized neoepitopes. Other mutation types of potential relevance are gene fusions or small insertions and deletions (indels) that can lead to highly immunogenic frameshifts (30). Besides these well-defined mutation classes, less characterized cancer-associated epigenetic, transcriptional, translational, or posttranslational aberrations may generate neoepitopes and expand the discovery space for vaccine targets considerably (31). Their cancer specificity and usability as neoantigens are difficult to assess with existing technologies and are currently under investigation.

The processing and presentation of antigens is a complex, multistep process following stochastic principles in which protease cleavage products of thousands of proteins compete for binding into pockets of MHC molecules. Only a portion of mutated sequences are presented on MHC at levels sufficient to trigger an effective T cell response. Technical feasibility and costs limit the number of mutations that can be incorporated into a drug product. Selecting the mutations with the highest likelihood of immunogenicity and therapeutic relevance is critical for designing personalized vaccines. So far, there is no consensus on how to prioritize mutations in this regard. The minimum requirements are (i) expression of the mutated gene in the tumor, and (ii) its capability of producing a sequence-altered epitope presented on one of the patient’s MHCs. NetMHC and IEDB consensus methods are most frequently used to estimate MHC binding and enrich for neoepitopes recognized by CD8<sup>+</sup> T cells [reviewed in (32)]. The stability of the MHC-peptide complex is considered a better predictor for immunogenicity than MHC binding affinity alone. However, the currently available prediction algorithms for MHC-peptide stability cover only a few MHC alleles and rely on small data sets. Only a fraction of mutant peptides predicted as high-affinity binders to human MHC class I alleles are naturally presented MHC class I ligands. Presentation on MHC class I is affected by the transcription level of the mutated gene. Generally, in mammalian cells, the gene expression level, the amount of translated protein, the cell surface density of MHC ligands derived from it, immune recognition, and lysis of the respective cell are all positively...

Fig. 2. Neoeptope vaccines promote a functional cancer immunity cycle. The goal of cancer immunotherapy is to ensure self-propagating revolution of the dysregulated cancer immunity cycle through its various steps (24). Vaccine-induced neoeptope-specific CD4<sup>+</sup> T<sub>H1</sub> cells may intersect at discrete rate-limiting steps considered as potentially difficult to overcome. These include the promotion of T cell priming and expansion, proinflammatory reshaping of the tumor microenvironment, and recruitment of CD4<sup>+</sup> T cells for direct killing of tumor cells. By concomitantly inducing CD8<sup>+</sup> and CD4<sup>+</sup> T cell responses, multi-neoeptope vaccines may contribute to tipping the balance from tolerance toward productive immunity against tumor cells, rendering the cancer immunity cycle functional.
correlated (33). Consequently, high expression can to some extent compensate for weak MHC class I binding and vice versa (34), which suggests that neoantigen prioritization can be achieved by combining predicted MHC class I binding and the expression level of the mutation. Expression levels can be mined from NGS data via the number of mRNA sequencing reads containing the mutation. However, not every mutant MHC class I ligand (that is presented) is immunogenic. Even so, the current class I prediction algorithms seem to enrich sufficiently for processed, strongly immunogenic CTL neoepitopes (2, 22).

Accurate prediction of ligands binding to MHC class II is challenging. MHC II binds denatured proteins or large peptides that are subsequently trimmed, whereas MHC I relies on pre-generated, sized peptides. Because the binding groove of MHC II molecules is open at both ends, the length and binding register of peptides are less defined and thus less predictable. On the other hand, this may be one of the reasons for higher abundance of MHC class II binding epitopes relative to MHC class I binding epitopes, and may explain why MHC class II binding prediction efficiently enriches for immunogenic neoantigens. Depending on the affinity prediction cutoff, 70%, 45%, or 34% of mutations selected for a HLA class II binding score of <1, 1 to 10, or >10, respectively, induced neoepitope-specific CD4+ T cell responses (22).

One concept currently being explored to enrich for tumor rejection–mediating neoantigens is to target mutations that are expressed across all clones within a heterogeneous tumor. This is expected to reduce the likelihood of outgrowth of antigen-negative clones and is supported by the observation that advanced non–small cell lung cancer and melanoma patients with tumors enriched for clonal neoantigens respond better to checkpoint blockade (35). An alternative explanation may be that subclonal antigens have occurred later in a tumor’s life cycle, or under conditions of potent immunosuppression, which are less likely to generate immunity. This again would be better addressed by a vaccine combining mutations of various subclones and capable of de novo priming of immune responses. Inclusion of mutated driver genes critical for proliferation, survival, or metastasis of tumor cells into a multi-neoantigen vaccine may further mitigate immune editing of tumors. A caveat is that functional validation and authentication as a driver gene is not trivial.

In patients treated with immune checkpoint inhibitors, the composition of the gut microbiome affects the efficacy of cancer immunotherapy (36–38). A detailed discussion of the gut microbiome and immunotherapy is provided in a recent review (39). One explanation may be a direct effect of the microbiome on antitumor immunity—for example, T cell modulation by tertiary bile acids produced by specific microbial communities, or induction of inflammation by pattern recognition receptor signaling. Another explanation is molecular mimicry between microbial and cancer neoantigens. An interesting idea in this regard is that neoantigens sharing structural features with microbial antigens are more likely immune-dominant and recognized by the T cell receptor (TCR) repertoire evolutionarily optimized for the detection of pathogen-derived epitopes. Shared epitope-string patterns in neoantigens of patients responding to anti-CTLA-4 checkpoint blockade were hypothesized (40) but not confirmed in two meta-analyses in larger patient cohorts (40). Recent studies introduced a composite neoantigen quality model, which confers a clinically relevant stronger immunogenicity to neoantigens with (i) sequence homology with pathogen-derived peptides and (ii) stronger predicted HLA binding affinity of the neoepitope relative to its wild type (differential neoepitope presentation). When this “neoantigen quality” model was applied to cancer mutanome data from pancreatic cancer, lung cancer, and melanoma patient cohorts, it was capable of discriminating long- and short-term survivors (41, 42).

### Table 1. Current vaccine formats explored for delivery of neoepitopes.

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<tr>
<th>Vaccine format</th>
<th>Advantages</th>
<th>Challenges</th>
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<tbody>
<tr>
<td>Synthetic peptides (45)</td>
<td>Cell-free manufacturing</td>
<td>Lack of clinical-grade manufacturability of a substantial portion of sequences</td>
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<td></td>
<td>Automated synthesis established</td>
<td>High variability in the physicochemical properties of individual peptides, complicating manufacturing</td>
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<td>Proven clinical activity of long peptides</td>
<td>Irrelevant immune responses against artificial epitopes</td>
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<td></td>
<td>Compatible with a wide range of formulations to improve delivery</td>
<td>Created by peptide degradation in the extracellular space</td>
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<td>Transient activity and complete degradation</td>
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<tr>
<td>Messenger RNA (46)</td>
<td>Cell-free manufacturing</td>
<td>Fast extracellular degradation of mRNA if not protected by appropriate formulation</td>
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<td></td>
<td>Inherent adjuvant function via TLR7, TLR8, and TLR3 signaling</td>
<td>Interpatient variability of TLR7-driven adjuvant activity</td>
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<td>Proven clinical activity</td>
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<td></td>
<td>Highly efficient systemic delivery into DCs established</td>
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<td>Transient activity and complete degradation</td>
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<td></td>
<td>All types of epitopes can be encoded</td>
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<tr>
<td>DNA plasmids (47)</td>
<td>Cell-free manufacturing</td>
<td>Potential safety risks by insertional mutagenesis</td>
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<td></td>
<td>Inherent adjuvant activity driven by TLR9</td>
<td>Successful safety testing requires entry into nucleus, thereby limiting effective delivery of vaccines into DCs</td>
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<td></td>
<td>Cost-effective and straightforward manufacturing</td>
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<td>All types of epitopes can be encoded</td>
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<tr>
<td>Viral vectors (48)</td>
<td>Strong immunostimulatory activity Extensive clinical experience with vector formats in the infectious disease field All types of epitopes can be encoded</td>
<td>Complex manufacturing</td>
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<td>(adenoviral and vaccinia)</td>
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<td>Immune responses against components of the viral vector backbone, limiting successful in vivo vaccine delivery and efficacy</td>
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<tr>
<td>Engineered attenuated bacterial vectors (49)</td>
<td>Strong immunostimulatory activity</td>
<td>Complex manufacturing and “sterility” testing</td>
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<tr>
<td>(Salmonella, Listeria)</td>
<td>Could be combined with plasmid DNA</td>
<td>Immune responses against bacterial components, limiting</td>
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<td>All types of epitopes can be encoded</td>
<td>Vaccine delivery and vaccine immunogenicity</td>
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<td>Potential safety risks due to delivery of live, replication-competent bacteria</td>
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<tr>
<td>Ex vivo antigen-loaded DCs (50)</td>
<td>Strong immunostimulatory activity</td>
<td>Higher costs and resources required for adoptive cell therapy approaches</td>
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<tr>
<td></td>
<td>Proven clinical efficacy of DC vaccines</td>
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<td></td>
<td>Can be loaded with various antigen formats</td>
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and timely delivery of the individually tailored vaccine. The turnaround time for vaccine production depends on the vaccine format, which is also critical for the magnitude and quality of the induced immune response. Formats under consideration for personalized vaccines are long peptides, RNA, DNA plasmids, viral vectors, engineered bacteria, and antigen-loaded DCs (Table 1). The recently reported fully personalized clinical trials used either a mixture of peptides (15 to 30 amino acids in length) corresponding to the mutated sequences with poly-ICLC (carboxymethylcellulose, polyinosinic-polyribonucleic acid, and polylysine double-stranded RNA) as adjuvant or mRNA with intrinsic adjuvant activity and encoding a strand of multiple predicted neoepitopes. The overall timeline for good manufacturing practice (GMP)–compliant on-demand production, from the start of processing of the patient’s sample for mutation discovery to vaccine release for administration, was about 3 to 4 months. Patients were treated with other standard or experimental compounds until their personal vaccine had been produced. For both peptide and mRNA vaccine platforms, reduction of lead times to less than 4 weeks is expected.

Another challenge is to define the most suitable clinical setting for mutanome vaccination. A therapeutic vaccine most likely works particularly well in the adjuvant or minimal residual disease settings, where tumor load is low and immune-suppressive mechanisms are not firmly established. Efficient control of a larger tumor load may require combination immunotherapies. Neoepitope vaccination can turn “cold” tumors into “hot” ones and mediate up-regulation of PD-L1 in the tumor microenvironment. Thus, it may extend application of anti–PD-1/PD-L1 therapies to patients without preexisting T cell response. This is particularly attractive for patients with a lower tumor mutational burden, who are less likely to have endogenous immunity to be unleashed by anti–PD-1/PD-L1 treatment. In addition, neoepitope vaccine–primed Tₘ⁺ and CD8⁺ memory T cells may enhance durability of anti–PD-1/PD-L1–mediated effects by promoting robust memory responses. Clinical trials NCT02897765 and NCT03289962, evaluating PD-1/PD-L1 blockade in combination with neoepitope vaccination, are currently recruiting patients. Similarly, inhibition of factors such as CTLA4, LAG-3, TIM-3, IDO, or TGF-β, as well as stimulation of costimulatory molecules (e.g., OX40, 4-1BB, CD137) and addition of T cell–agonistic cytokines, were preclinically shown to synergize with cancer vaccines. With regard to escape mechanisms, the risk of outgrowth of neoantigen loss variants can be mitigated by the multi-neoepitope–targeting nature of personalized mutanome vaccines. In contrast, selection of clones with defects in the antigen processing and presentation machinery (e.g., HLA or β₂-microglobulin loss) is likely to occur (22, 43, 44). This risk again can be addressed by combining mutanome vaccines with compounds that do not depend on intact MHC class I presentation. These could be, for example, bispecific T cell engagers or antibodies against cancer cell surface targets, which are capable of Fc-mediated activation of natural killer cells or of complement.

The broader impact of realizing personalized mutanome vaccines

Mutanome vaccines may become the first therapeutic modality to truly realize personalized treatment of cancer. In the era dominated by the “one-size-fits-all” paradigm, stratified treatment has been considered a synonym for personalized medicine. However, this is not strictly the case. Stratified therapies identify patients carrying a defined biomarker (generally a shared cancer-driving genetic aberration) and subject them to a treatment targeting this biomarker. Unfortunately, such therapeutically targetable biomarkers are not available for most cancer types and are restricted to patient subsets. Thus, each stratified drug excludes the majority of patients who do not harbor the respective aberration (Fig. 3). In contrast, true patient-specific therapy should be achievable by neontigen vaccination. The large repertoire of individual driver and passenger mutations (irrespective of their functional relevance) can be leveraged, and all patients with a sufficient frequency of cancer mutations could be offered their tailored and targeted treatment. The number of somatic mutations in tumors ranges from less than 10 to several thousand, and they are largely unique for every patient. This is not the only dimension of heterogeneity (Fig. 4).

A delicate interplay between the tumor and a set of host and environmental factors (e.g., HLA haplotype and other genetic polymorphisms, the microbiome, age, comorbidity, the immune cell repertoire, the composition of the tumor microenvironment), which define the immunological status (“cancer-immune set point”), shapes each individual cancer (23) (Fig. 4). Personalized mutanome vaccines hold promise to address tumor heterogeneity, which accounts for the failure of conventional anticancer treatments. A patient’s vaccine composition can be directed against various clones because of its ability to target multiple epitopes within a tumor and can be adjusted upon tumor changes.

Concluding thoughts

Personalized cancer vaccines have moved beyond the first critical hurdle of clinical translation. Challenges remaining on the path forward include identifying the most suitable clinical settings, reducing production turnaround time, upscaling manufacturing, and ensuring affordability. New trends and technologies of the digital age, such as big data science, cloud and high-performance computing, and digitalized manufacturing solutions, are expected to add momentum. Predictive neoepitope algorithms will continue to improve by applying machine learning tools to big data sets.
Higher-resolution analysis of tumors, the micro-environment, and immunity is becoming feasible by TCR repertoire analysis, high-throughput single-cell sequencing, and circulating tumor DNA detection. Computational inference of the phenotypic and functional status of infiltrating cells from transcriptome data may support the selection of combination treatments. Lastly, insights into immunotherapy success or failure are expected to increase the spectrum of biomarkers for vaccine design and combination treatment. It is well worth the effort, as mutations constitute critical promoters of the oncogenic process and treatment failure and are the common denominator across all cancers. Therefore, a personalized mutanome vaccine has the potential to become a universally applicable therapy irrespective of cancer type.

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14. J. C. Castle et al., Cancer Res. 72, 1081–1091 (2012).

Fig. 4. The interconnected dimensions of cancer heterogeneity. The interaction between cancer and immune system is shaped by various host, tumor and environmental factors. The complex interplay of these sources of interpatient heterogeneity affects both the course of disease and the efficacy of immunotherapy, and calls for personalized approaches.
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