Targeting the Heterogeneity of Cancer with Individualized Neoepitope Vaccines

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Abstract

Somatic mutations binding to the patient’s MHC and recognized by autologous T cells (neoepitopes) are ideal cancer vaccine targets. They combine a favorable safety profile due to a lack of expression in healthy tissues with a high likelihood of immunogenicity, as T cells recognizing neoepitopes are not shaped by central immune tolerance. Proteins mutated in cancer (neoantigens) shared by patients have been explored as vaccine targets for many years. Shared ("public") mutations, however, are rare, as the vast majority of cancer mutations in a given tumor are unique for the individual patient. Recently, the novel concept of truly individualized cancer vaccination emerged, which exploits the vast source of patient-specific "private" mutations. Concurrence of scientific advances and technological breakthroughs enables the rapid, cost-efficient, and comprehensive mapping of the "mutanome," which is the entirety of somatic mutations in an individual tumor, and the rational selection of neoepitopes. How to transform tumor mutanome data to actionable knowledge for tailoring individualized vaccines "on demand" has become a novel research field with paradigm-shifting potential. This review gives an overview with particular focus on the clinical development of such vaccines. Clin Cancer Res; 22(8); 1885–96. ©2016 AACR.

See all articles in this CCR Focus section, "Opportunities and Challenges in Cancer Immunotherapy."

Introduction

Genetic abnormalities are key drivers of cancer (1–4). A hundred years ago, Ernest Tyzzer introduced the term "somatic mutation" to describe these abnormalities. In a compellingly visionary review (5), he also predicted immune recognition of somatic mutations and their interindividual variability within tumors of a species, and postulated the existence of tumor-related immunosuppressive mechanisms (see Box 1).

Various types of genomic mutations exist: point mutations (exchange of one nucleotide by another), insertions/deletions (one or more nucleotides added or removed from the original DNA), amplifications/deletions (multiplication or loss of copies of a chromosomal region), and translocations and inversions (interchange of pieces of nonhomologous chromosomes or reversion of the orientation of a chromosomal segment; ref. 6). By altering the sequence of translated gene products, mutations may create novel immunogenic epitopes presented on MHC molecules and recognized by T cells (Fig. 1). Neoepitopes may also be created by cancer-associated alterations in mRNA translation, antigen processing, and presentation, for example, translation of untranslated regions (UTR) of mRNAs, altered proteasomal cleavage sites, or protein-splicing events (7, 8).

Neoepitopes are exempt from central tolerance, and thus, the presence of high-affinity T cells against them in the patient’s repertoire is very likely. A considerable number of the neoepitopes accumulating in the course of tumor evolution are recognized by autologous spontaneously occurring T cells in mice and in cancer patients and may constitute the Achilles’ heel of tumor cells. In mice, molecular characterization of tumor rejection antigens unraveled mutated epitopes (9–14). Various unique immunodominant mutations were identified in human cancer patients using tumor-reactive T-cell lines derived from tumor-infiltrating lymphocytes (TIL) or obtained by repeated stimulation of peripheral blood T cells with autologous tumor cell lines (refs. 15–17; reviewed in ref. 8).

Whereas these data clearly indicate sporadic immune recognition of mutant neoepitopes, the overall relevance of mutations for cancer immunotherapy remained controversial and ambiguous for a long time. Only recently, a series of independent reports revealed that neoepitope-specific T cells are crucial for clinical responses mediated by adoptive transfer of autologous TILs or by immune checkpoint inhibitors ([refs. 18–23; summarized in refs. 24–27); also see reviews by Hegde and colleagues (28) and Maus and June (29) in this CCR Focus]. In patients treated with anti–CTLA-4 or anti–PD-1, the mutational load in the tumor appeared to be an important predictor of clinical benefit establishing the relevance of neoepitopes for rejection of at least melanoma, non–small cell lung cancer (NSCLC), and mismatch repair–deficient colorectal tumors, which are the "signature" cancer types for checkpoint blockade. Concordantly, temporary expansion of mutation-specific T cells upon checkpoint blockade was reported in some patients and in tumor-bearing mice (20, 30).
Box 1. Relationships between somatic mutations and tumor immunity as predicted by Tyzzer

There are marked differences in the behavior of various tumors on transplantation in given classes of mice. Even tumors arising in homogenous races show such differences, and this may be attributed to acquisition of new characteristics, […] it appears logical to regard a tumor as a manifestation of somatic mutation[…] The tissue of the new growth has thus in certain respects become foreign to the other tissues […] Malignant tumors must have feeble antigenic power as well as sufficient resistance to the normal inhibiting influences to provide continued growth in the animal in which they originate, otherwise reactions sufficient to destroy them would occur more frequently.

Ernest Edward Tyzzer, "Tumor Immunity," The Journal of Cancer Research, 1916 (ref. 5)

Moreover, recent studies from Laurence Zitvogel’s (INSERM, U805, Institut Gustave Roussy, 39 Rue Camille Desmoulins, F-94805 Villejuif, Paris, France) as well as from Thomas Gajewski’s (Department of Pathology, University of Chicago, Chicago, IL) laboratories convincingly demonstrated that commensal gut microbes, such as Bacteroides, Burkholderia, or Bifidobacterium species, may positively affect the success of checkpoint blockade treatment in tumor-bearing mice (31, 32). Vézizou and colleagues demonstrated that adoptive transfer of bacteria-restimulated T cells from anti–CTLA-4 antibody–exposed mice is able to induce tumor growth retardation in tumor-bearing recipient mice (31). This finding indicates that microbial antigen-specific T cells might be cross-reactive with neoantigens, thereby supporting antitumoral responses after checkpoint blockade. Snyder and colleagues proposed that anti–CTLA-4 checkpoint blockade favors T-cell responses against neoantigens sharing common peptide sub-string patterns (22); however, this finding could not be confirmed in a study by Van Allen and colleagues investigating responses in a larger cohort of cancer patients (18). Further studies with isolated T-cell clones or T-cell receptor (TCR)–engineered T cells are needed to get a better understanding of potential common epitope patterns recognized by neoantigen cross-reactive microbial antigen-specific T cells.

Vaccines Targeting Individual Mutations

Mutation-based vaccination attempts represent “off-the-shelf” approaches, as they target single or multiple frequently shared neoantigens, such as mutRas, mutP53, mutVHL, mutEGFR, or mutIDH1 (Table 1), which were used to stratify patients according to the presence or absence of the respective mutations in their tumor specimen (Fig. 2).

With the introduction of next-generation sequencing technologies (NGS), it became apparent that human cancers are much more complex and bear dozens to thousands of mutations, of which, according to the analysis with algorithms for MHC-binding prediction, a considerable number in every human tumor specimen was a potential MHC class I neoepitope (33, 34).

Various attempts have been made to apply neoantigen-based vaccine strategies in mouse models. Matsushita and colleagues and Cubin and colleagues showed the feasibility of using genomic and bioinformatic approaches to identify MHC class I neoepitopes as tumor rejection antigens in a highly immunogenic methylcholanthrene (MCA)-induced murine sarcoma model (14, 30). In continuation of this work, his laboratory demonstrated in immune-edited variants of the sarcoma model that the antitumor effect of checkpoint blockade is mediated by MHC class I neoepitope-specific T cells and that vaccination with long synthetic peptides encoding these neoepitopes induces the same therapeutic activity as checkpoint inhibition.

Our group pioneered the concept of individualized neoepitope vaccines and proposed a tailored approach to exploit the full spectrum of the individual mutations of a patient (14, 30, 34, 35). Vaccination studies with synthetic peptides and mRNA-encoding mutations identified by NGS in three different mouse tumor models revealed that a significant portion of nonsynonymous point mutations (21%–45%) is immunogenic. Unexpectedly, the vast majority of these neoepitopes were not recognized by CD8⁺ but were recognized by CD4⁺ T cells. mRNA vaccines encoding single MHC class II neoepitopes were capable of controlling the growth of established mouse B16-F10 melanoma and CT26 colon cancer tumors. On the basis of these observations, we developed a process combining computational prediction of relevant MHC class II neoepitopes from NGS exome data with rapid production of synthetic poly-neoepitope mRNA vaccines. Vaccination with such mRNA vaccines induced an inflammatory tumor microenvironment, mediated induction of a CD8⁺ T-cell response by antigen spread, and resulted in complete rejection of established aggressively growing tumors. Interestingly, employing the same predictive algorithm on corresponding human cancer types demonstrated an abundance of MHC class II epitopes indicating the applicability of this approach to human cancers.

Duan and colleagues showed that prophylactic vaccination against neoepitopes identified by a prediction approach selecting neoepitopes with improved MHC class I binding inhibits growth of CMS5 or Meth A tumors in mice (36). Yadav and colleagues combined mass spectrometry (MS) and exome sequencing and demonstrated prophylactic and therapeutic activity of vaccination against identified neoepitopes in the MC38 tumor model (37).

Clinical Translation of Individualized Neoepitope Vaccines

Despite excitement that in conjunction with individualized immunotherapy, the spectrum of potential targets is now extended by unique mutations, there is no consensus so far on the path to their successful clinical application. That each and every patient’s cancer would be subjected to deep sequencing to manufacture a vaccine of unique composition “on demand” differs in key aspects from conventional “off-the-shelf” drug approaches. Putting a mutanome-based individualized treatment concept into practice requires both highly
Figure 1. Types of genomic mutations and T-cell neoepitopes resulting from them. A, nonsynonymous point mutations in the coding sequence of a gene alter a single amino acid. By creating an anchor residue or changing the TCR-binding properties (nonanchor residue), a neoepitope may be formed. Insertion of three or a multiple of 3 nucleotides in frame introduces novel amino acids into the protein sequence, potentially generating a T-cell response. Insertion or deletions of exonic nucleotides, mutations in intronic regions that affect RNA splicing, or gene fusions (altered genes after fusion marked with an apostrophe) can cause inclusion of introns into mRNA and a shift of the open reading frame. Resulting T-cell epitopes may be in part (type 1) or fully (type 2) comprised of an altered amino acid sequence. In addition, T cells may target neoepitopes formed by translated introns or fusion of distant exons and genes as a result of splice site mutations and gene fusion. B, human cells express up to 6 different MHC class I (2 alleles of HLA-A, -B, and -C) and class II molecules (2 alleles of HLA-DP, -DQ, and -DR), thereby presenting neoepitopes to T cells.
<table>
<thead>
<tr>
<th>Date</th>
<th>Reference/Sponsor</th>
<th>Cancer entity</th>
<th>Patients planned or treated</th>
<th>Target</th>
<th>Vaccine format</th>
<th>Outcome</th>
<th>Clinicaltrials.gov identifier</th>
</tr>
</thead>
<tbody>
<tr>
<td>2015</td>
<td>National Center for Tumor Diseases (Heidelberg, Germany)</td>
<td>Grade III–IV gliomas</td>
<td>39</td>
<td>IDH1R132H</td>
<td>Peptide + Montanide and topical imiquimod</td>
<td>Open study</td>
<td>NCT02454634</td>
</tr>
<tr>
<td>2015</td>
<td>(75)</td>
<td>Glioblastoma</td>
<td>65</td>
<td>EGFRvIII mutation</td>
<td>Peptide + KLH</td>
<td>Improved OS 24 months; 85% of patients analyzed have specific humoral response</td>
<td>—</td>
</tr>
<tr>
<td>2015</td>
<td>Duke University (Durham, NC)</td>
<td>Recurrent grade II glioma</td>
<td>24</td>
<td>IDH1R132H</td>
<td>Peptide + GM-CSF + Montanide</td>
<td>Ongoing study</td>
<td>NCT02193347</td>
</tr>
<tr>
<td>2014</td>
<td>(76)</td>
<td>Colorectal (38), pancreatic (11), common bile duct (1), lung (3)</td>
<td>53</td>
<td>Patients' individual RAS mutations</td>
<td>Peptide + IL2</td>
<td>20/37 patients developed a specific immune response measured in ELISPOT and/or proliferation assay</td>
<td>—</td>
</tr>
<tr>
<td>2014</td>
<td>(77)</td>
<td>KRAS-mutant lung cancer</td>
<td>24</td>
<td>7 common RAS mutations</td>
<td>4 heat-inactivated Saccharomyces cerevisiae yeast products, each expressing a unique combination of 3 RAS mutations, collectively targeting 7 RAS mutations</td>
<td>50% of patients developed a T-cell response; proof of safety</td>
<td>—</td>
</tr>
<tr>
<td>2013</td>
<td>Radboud University (Nijmegen, the Netherlands)</td>
<td>CRC, hypermutated (Lynch, MSI)</td>
<td>25</td>
<td>Frameshift-derived neoantigens; CEA</td>
<td>Synthetic peptide + KLH</td>
<td>Ongoing study</td>
<td>NCT01885702</td>
</tr>
<tr>
<td>2013</td>
<td>Dana-Farber Cancer Institute (Boston, MA)</td>
<td>Melanoma</td>
<td>20</td>
<td>Personal neoantigens</td>
<td>Peptide + Poly ICLC</td>
<td>Ongoing study</td>
<td>NCT01970358</td>
</tr>
<tr>
<td>2011</td>
<td>Celldex Therapeutics Oryx GmbH</td>
<td>Gliaoblastoma</td>
<td>700</td>
<td>EGFRvIII mutation</td>
<td>Peptide + KLH</td>
<td>Ongoing study</td>
<td>NCT0480479</td>
</tr>
<tr>
<td>2010</td>
<td>(78)</td>
<td>Glioblastoma</td>
<td>18 (group); 17 (control group)</td>
<td>EGFRvIII mutation</td>
<td>Peptide + KLH</td>
<td>Greater OS in treatment group compared with control group; 6/14 patients analyzed showed stable disease</td>
<td>—</td>
</tr>
<tr>
<td>2009</td>
<td>(79)</td>
<td>Glioblastoma</td>
<td>15</td>
<td>EGFRvIII mutation</td>
<td>Peptide-loaded DC</td>
<td>10/12 vaccinated patients showed a specific proliferative T-cell response in vitro</td>
<td>—</td>
</tr>
<tr>
<td>2008</td>
<td>(80)</td>
<td>Pancreatic and CRC</td>
<td>12</td>
<td>Patients' individual RAS mutations</td>
<td>13 mer peptide</td>
<td>Specific T-cell response in 5 patients; proof of safety</td>
<td>—</td>
</tr>
<tr>
<td>2007</td>
<td>NCI (Bethesda, MD)</td>
<td>Colorectal, pancreatic, lung cancer RAS-positive NSCLC</td>
<td>Data not available</td>
<td>RAS mutations</td>
<td>Peptide + Detox-B</td>
<td>Study completed; no outcome data available</td>
<td>NCT00019006</td>
</tr>
<tr>
<td>2007</td>
<td>(81)</td>
<td>RAS-positive NSCLC</td>
<td>4</td>
<td>7 common RAS mutations</td>
<td>Peptide mix of 7 peptides + GM-CSF</td>
<td>1 patient developed a positive DTH reaction; proof of safety</td>
<td>—</td>
</tr>
<tr>
<td>2007</td>
<td>(74)</td>
<td>Chronic myeloid leukemia</td>
<td>21</td>
<td>BCR-ABL fusion protein</td>
<td>Peptides + IFNα</td>
<td>Improved cytogenetic response in 13/21 patients, 6 complete molecular remissions</td>
<td>NCT00466726</td>
</tr>
<tr>
<td>2006</td>
<td>GlobalImmune</td>
<td>Pancreatic cancer</td>
<td>176</td>
<td>Mutated Ras</td>
<td>Yeast-derived recombinant-mutated Ras protein</td>
<td>Study completed</td>
<td>NCT00300950</td>
</tr>
<tr>
<td>2005</td>
<td>(82)</td>
<td>Various cancer entities</td>
<td>39</td>
<td>Individual mutant p53, individual mutant RAS</td>
<td>Peptide-loaded PBMC</td>
<td>16 patients developed a specific immune response; proof of safety</td>
<td>—</td>
</tr>
</tbody>
</table>

(Continued on the following page)
interdisciplinary research and an innovative drug development process. Clinical translation of this concept has just begun, and a small number of early clinical trials with exciting exploratory concepts are under way, data from which are expected to increase the level of understanding (Table 2). After several decades of preclinical and clinical cancer vaccine research, the field has matured, and many lessons have been learned as to which aspects to consider for the next generation of vaccines [see reviews by Whiteside and colleagues (38) and Bol and colleagues (39) in this CCR Focus], for example, by understanding the pros and cons of potential vaccine format platforms, the use of suitable adjuvants, and synergistic combination treatment protocols [also see review by Zarour (40) in this CCR Focus].

### Identification, Prioritization, and Selection of Mutations for Design of Cancer Vaccines for Clinical Use

The cancer mutanome is defined by comparing exome sequencing data obtained by NGS of individual healthy tissue with sequences from tumor-derived nucleic acids. Usually, the blood cells of a patient serve as a source for healthy tissue DNA. Methods to obtain DNA from tumor tissue should preferably be compatible with what is available in the context of routine diagnostics. In our experience, efficient and reproducible isolation of nucleic acids in NGS-grade quality is feasible from fresh as well as frozen or formaldehyde-fixed, paraffin-embedded biopsies. A caveat is that, in general, a limited number of tumor lesions per patient are

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### Table 1. Cancer vaccine trials using single or multiple mutations as cancer vaccine targets (Cont’d)

<table>
<thead>
<tr>
<th>Date</th>
<th>Reference/ Sponsor</th>
<th>Cancer entity</th>
<th>Patients planned or treated</th>
<th>Target</th>
<th>Vaccine format</th>
<th>Outcome</th>
<th>Clinicaltrials.gov identifier</th>
</tr>
</thead>
<tbody>
<tr>
<td>2002</td>
<td>(83) Alveolar Kettering Cancer Center (New York, NY)</td>
<td>Lung adenocarcinoma</td>
<td>Data not available</td>
<td>RAS mutations</td>
<td>Peptide + GM-CSF</td>
<td>Study completed; no outcome data available</td>
<td>NCT00005630</td>
</tr>
<tr>
<td>1999</td>
<td>NCI</td>
<td>Sporadic renal cell cancer carrying VHL mutations</td>
<td>6</td>
<td>VHL mutations</td>
<td>Peptide + IFA</td>
<td>Terminated due to poor accrual and peptide supply problems</td>
<td>NCT00001703</td>
</tr>
<tr>
<td>1999</td>
<td>(86)</td>
<td>Advanced solid cancer</td>
<td>10 (evaluable)</td>
<td>Patients’ individual RAS mutations</td>
<td>Peptide + Detox-B</td>
<td>Specific T-cell response in 4/5 patients</td>
<td>—</td>
</tr>
<tr>
<td>1999</td>
<td>Memorial Sloan Kettering Cancer Center</td>
<td>Myelodysplastic syndrome</td>
<td>Data not available</td>
<td>RAS mutations</td>
<td>Peptide + GM-CSF</td>
<td>Study completed; no outcome data available</td>
<td>NCT00003959</td>
</tr>
<tr>
<td>1999</td>
<td>NCI</td>
<td>Ovarian cancer</td>
<td>21</td>
<td>p53 mutations</td>
<td>Peptide + GM-CSF</td>
<td>Study completed; no outcome data available</td>
<td>NCT00001827</td>
</tr>
<tr>
<td>1996</td>
<td>(87)</td>
<td>Advanced pancreatic cancer</td>
<td>5</td>
<td>Patients’ individual RAS mutations</td>
<td>Peptide-loaded APC</td>
<td>2 patients generated a specific T-cell response; proof of safety</td>
<td>—</td>
</tr>
</tbody>
</table>

Abbreviations: APC, antigen-presenting cell; CEA, carcinoembryonic antigen; CRC, colorectal cancer; DC, dendritic cell; DTH, delayed-type hypersensitivity; GM-CSF, granulocyte macrophage colony-stimulating factor; MSI, microsatellite instability; OS, overall survival; PBMC, peripheral blood mononuclear cell; VHL, von Hippel-Lindau.
accessible and available, and thus, the mutanome determined by these analyses may not represent the entire clonality of an individual’s disease. On another note, no consensus has been reached so far about how to distinguish authentic cancer-associated mutations from erroneous calls. Besides artifacts introduced by tissue fixation, sequencing, and mutation calling procedures, contamination with healthy tissue or necrotic cells as well as tumor heterogeneity may compromise the mutanome data. Different statistical approaches and algorithms were developed by us and others to determine true mutations in individual NGS data (41).

T cells targeting one single mutated MHC class I or class II epitope have been shown to confer potent therapeutic antitumor activity in mice (30, 34–37, 42) and men (43). Even so, a poly-neoepitope vaccine has the added value of covering various subclones, thereby mitigating the risk of clonal selection of antigen loss variants (44–47). Moreover, as there is no way to predict relevant epitopes with absolute certainty, the polyepitope approach increases the likelihood of inducing antitumor activity. Until now, no consensus has been reached about how many mutations should be represented in a polyepitope vaccine and which criteria should be applied for their selection. The minimal requirements for selection of a mutation as vaccine target are that the neoantigen is expressed in the tumor and that it gives rise to an epitope with an altered amino acid
sequence presented on one of the patient’s MHC molecules. Quantification of the mutated allele frequencies by exome sequencing provides the clonal abundance of tumor cells carrying the respective mutation (48).

The number of RNA sequencing reads of the mutated sequence provides the expression level of the mutated allele, and also, the MHC haplotypes can be determined with high accuracy by mining the patient’s NGS data (49). Epitopes that bind to the different MHC alleles of an individual patient can be predicted by a variety of Web-based tools (55). MHC I prediction softwares (e.g., NetMHC or IEDB consensus) are good in enriching MHC class I binding epitopes with high sensitivity for a broad range of MHC class I alleles. Seq2MHIC, an MHC haplotype prediction approach our laboratory developed, provides information about expression levels of the respective MHC alleles in the tumor sample, which may further facilitate the selection of mutations (52). Accurate prediction of MHC class II epitopes (e.g., TEPITOPE, NetMHCIpan) remains a challenge (56, 57).

Many factors other than binding score determine whether a neoepitope is eventually displayed on the tumor cells, and only a minority of predicted epitopes, in fact, are processed and presented. There is no reliable method that can be implemented into the clinical setting to unequivocally verify that in silico–identified neoepitopes tested for binding on target cells expressing the patient’s respective MHC allele are in fact presented by the tumor. MS-assisted MHC ligandome analyses may be used for this purpose to validate NGS-identified mutations (37). However, due to its relatively low sensitivity as compared with T-cell recognition, failure to detect a neoepitope by MHC ligandome technology does not prove its lack of relevance. It is more important for the field that validated neoepitopes with proven immunogenicity and antitumor activity are collected in public databases and enable development of improved tools for prediction of relevant neoepitopes.

Preexisting T-cell responses for computationally predicted neoepitopes can be assessed by testing patients’ PBMCs on autologous antigen-presenting cells pulsed with neoepitope peptides or antigen-encoding RNA (58–60). Whether mutations against which the patient has mounted an apparently inefficient response are in fact good vaccine targets needs validation by in vivo studies.

Mutant class I and class II epitopes may be combined in a poly-neoepitope vaccine for synergistic effects. Whereas cytotoxic CD8+ T cells kill tumor cells directly, CD4+ T cells have a "catalytic" function orchestrating the activity of other cell types, such as macrophages, natural killer (NK) cells, B cells, dendritic cells (DC), and CD8+ T cells. Moreover, they can modify stroma functions by intratumoral secretion of inflammatory cytokines, such as IFNγ (61–63).
Once neoepitopes have been selected the patient's individualized vaccine can be manufactured. The production needs to be "on demand," cost-effective, rapid, and compliant with Good Manufacturing Practice (GMP). Currently, ongoing mutanome vaccine trials (Tables 1 and 2) use synthetic peptides and antigen-encoding DNA or RNA (Fig. 3) as formats. These vaccines types can be rapidly produced in a GMP-compliant manner and are able to deliver effective mutant MHC class I and class II neoepitopes. On the basis of the experience from ongoing early clinical studies, lead times from the start of processing of the patient's sample for determination of mutations to the release of the investigational drug product are currently about 3 to 4 months. Patients can be treated with other standard or experimental compounds until their personal vaccine has been produced. For both peptide and mRNA vaccine platforms, reduction of lead times to less than 4 weeks is expected, which is compatible with use in the vast majority of cancer indications.

The route of administration, bioavailability and pharmacokinetics, and level of uptake by DCs, as well as simultaneously coapplied adjuvants and immune modulators, all crucially determine the overall potency of a given vaccine. How to achieve strong and durable immunity against cancers by vaccination is still an ongoing quest. Our group focuses on mutanome-engineered RNA immunotherapy (MERIT) vaccines, which are designed to display multiple neoepitopes separated by nonimmunogenic linkers in a single molecule (34). These mRNAs are taken up by DCs, are endogenously translated and, due to their intrinsic and strong adjuvant activity, elicit most potent CD8+ as well as CD4+ T-cell responses (64, 65).

Clinical Translation of the Concept

Clinical translation of individualized mutanome vaccines has some unique aspects. Regulatory challenges are associated with the fact that each patient will receive a tailored vaccine that is unique. This is fundamentally different from classical molecularly defined vaccines that can be premanufactured, released, and stored until use for all patients in a defined trial. The entire process from sample acquisition, mutation discovery, vaccine design and production, drug administration, and clinical monitoring must be run in the framework of a regulatory approved clinical trial with standardized processes (66).
As the sequence composition of the individualized drug product is patient specific, formal preclinical toxicity studies, which usually inform a risk-adapted safety strategy for clinical translation, are not feasible. Instead, preclinical studies are to be designed in accordance with regulatory guidelines to test the safety of a substitute drug product generated by a representative process. On the basis of the mode of action of mutation-based vaccines, the induction of T cells cross-reactive with the nonmutated wild-type counterparts may be considered a potential risk. In preclinical mouse models, we have occasionally detected induction of such cross-reactivity (35), however, without any evidence for autoimmunity-associated toxicities. In the vast majority of cancer trials using defined mutated epitopes, no safety issues based on such cross-reactivity were reported. However, this risk cannot be fully excluded with vaccine platforms of higher potency emerging. Potential on-target toxicity organs cannot be announced a priori on the trial protocol level, as each patient is immunized with a different set of mutated epitopes, and wild-type counterparts of these are expressed in different sets of normal tissue. This problem can be addressed by providing investigators with patient-specific profiles of organs with high expression levels of wild-type counterparts of chosen mutations as the basis for personalization of safety monitoring. Generally speaking, diligent safety assessment of patients to capture potential auto-immune-driven toxicity is advisable. Strategies for safety signal detection across trials within a development program must take into account that treatment-related adverse event profiles may vary between patients, and smart solutions for this challenge need to be worked out.

The activity of individualized mutanome vaccines may be assessed throughout the clinical development by clinical, molecular, and immunologic endpoints. Imaging of tumor lesions according to the standards developed specifically for immunotherapies [e.g., immune-related response criteria (67)] implemented early on in clinical development assists in verifying activity in patients with measurable disease. Potential surrogates for tumor cell burden, such as classical tumor serum markers [e.g., PSA, CA125], circulating tumor cells, and circulating nucleic acids, may have a supportive value. The most immediate measurements for the intended pharmacodynamic effect of the vaccine are immunologic parameters, for example, the frequency, function, and phenotype of neoepitope-specific immune cell subsets in the peripheral blood, T-cell infiltrates at the vaccination site, induction of delayed-type hypersensitivity (68) reactions, and infiltration of vaccine-induced, neoepitope-specific T cells into tumor lesions. The induction of functional mutation-specific T-cell responses, which are able to recognize neoepitopes in the natural context, i.e., either on autologous tumor cells or on tumor-derived DCs, their trafficking to the tumor as determined by functional immunologic readouts and TCR repertoire profiling is a straightforward strategy to establish the clinical mode-of-action in first-in-human trials. Longitudinal analysis of antigen-specific T cells in blood samples and in tumor biopsies before, during, and after vaccination will aid in understanding of the immunogenicity of each individual mutation. Immunogenicity data generated from such trials will help to build knowledge around the key requirement for successful individualized vaccination. As the assessment of treatment-emergent T-cell responses is endpoint relevant, scientifically sound and qualified bioassays (e.g., ELISpot, flow cytometric cytokine release, MHC multimer technology) must be used according to GCLP (Good Clinical Laboratory Practice) standards and documented according to MIATA (Minimal Information about T cell Assays; ref. 69).

In the later stages of clinical development, the classical clinical endpoints, such as survival, will play a major role to provide the clinical proof of concept and to establish the risk/benefit profile as compared with the standard of care. Assessment of single-agent activity and effect size of individualized vaccines has just begun and will show in which settings (e.g., minimal residual disease, adjuvant setting) such vaccines can be used in their own right. Combining the individualized mutanome vaccine approach with potent immunomodulatory therapies, such as checkpoint blockade, immunogenic cell death–inducing chemotherapeutics, and mutation-inducing interventions, such as radiotherapy, will open additional avenues. On the other hand, the antitumor effect of checkpoint inhibitors mediated by unleashing immune effectors may be further boosted by individualized vaccines, which steer the immune system with a tailored target map of the patient’s tumor.

**Outlook**

Ongoing clinical trials furnish clear evidence for the feasibility of individualized vaccination of cancer patients with unique mutations. The concept holds several promises.

One such promise is the full exploitation of the mutational neoepitope repertoire of tumors, which is the richest so far identified source of otherwise narrow vaccine target discovery space. Mutations are the very root of cancer as supported by the unequivocal clinical benefit of drugs against shared mutations (e.g., imatinib, crizotinib). The relevance of mutated epitopes as tumor rejection antigens is validated, for example, as clinical responses to checkpoint blockade and adoptive T-cell transfer have been linked to immune responses against neoepitopes. Prevalent T-cell responses against mutations are rare in cancer patients and need to be boosted by vaccines. Mutanome vaccines may be particularly useful to induce CD4+ T-cell responses. Given the role of CD4+ T cells as orchestrators of immunity, such vaccines may result in immunogenicity of tumors that lack spontaneous immunity, thereby rendering tumors responsive to checkpoint blockade treatment (70, 71).

Second, this concept takes personalization from stratified approaches to the next level of truly individualized medicine. Not any more limited by the small common denominator of neontigen targets shared by many patients, the mutanome vaccine approach taps the large unique antigenic target repertoire of each individual patient and provides a universally applicable regimen from which each and every patient can profit. The availability of multiple vaccine targets for each patient allows us to address interindividual variability and intratumor clonal heterogeneity, which are key to the largely disappointing effects of currently used targeted therapeutics (72).

Third, generally speaking, cancer therapy is moving from a drug-centered to a patient-centered approach with different levels of personalization (73). This requires paradigm shifts along the entire drug development process, including diagnostics, drug production, logistics, regulatory aspects, and patient management. Fully individualized mutanome vaccination may become a prototype for how to open up this territory by developing best-practice blueprints for the implementation of personalized approaches.
On a related note, with health care systems becoming increasingly cost-conscious, one of the challenges is the pharmacoeconomics of individualized medicine. In the clinical development stage, individualized vaccines will be expensive, mainly due to costs for genome sequencing and manufacturing of small, patient-specific GMP product batches, and costs need to be reduced until commercialization. There is good reason to believe that the concurrence of future trends, for example, dramatically decreasing genome sequencing costs, full automation, miniaturization, and optimization of manufacturing processes, will make individualized mutanome approaches affordable.

Disclosure of Potential Conflicts of Interest
O. Türeci is CEO of Ganymed Pharmaceuticals. M. Vormehr is an employee of and has ownership interest (including patents) in BioNTech RNA Pharmaceuticals. M. Diken is a consultant/advisory board member for BioNTech. S. Kreiter is a consultant/advisory board member for BioNTech. C. Huber is a non-operative board member for, has ownership interest (including patents) in, and is a consultant/advisory board member for BioNTech. U. Sahin is CEO of and has ownership interest (including patents) in BioNTech. No other potential conflicts of interest were disclosed.

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