

# Neoantigens in cancer immunotherapy

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The clinical relevance of T cells in the control of a diverse set of human cancers is now beyond doubt. However, the nature of the antigens that allow the immune system to distinguish cancer cells from noncancer cells has long remained obscure. Recent technological innovations have made it possible to dissect the immune response to patient-specific neoantigens that arise as a consequence of tumor-specific mutations, and emerging data suggest that recognition of such neoantigens is a major factor in the activity of clinical immunotherapies. These observations indicate that neoantigen load may form a biomarker in cancer immunotherapy and provide an incentive for the development of novel therapeutic approaches that selectively enhance T cell reactivity against this class of antigens.

Immunotherapies that boost the ability of endogenous T cells to destroy cancer cells have demonstrated therapeutic efficacy in a variety of human malignancies. Until recently, evidence that the endogenous T cell compartment could help control tumor growth was in large part restricted to preclinical mouse tumor models and to human melanoma. Specifically, mice lacking an intact immune system were shown to be more susceptible to carcinogen-induced and spontaneous cancers compared with their immunocompetent counterparts (1). With respect to human studies, the effects of the T cell cytokine interleukin-2 in a small subset of melanoma patients provided early clinical evidence of the potential of immunotherapy in this disease. In 2010, the field was revitalized by a landmark randomized clinical trial that demonstrated that treatment with ipilimumab, an antibody that targets the T cell checkpoint protein CTLA-4, improved overall survival of patients with metastatic melanoma (2). As a direct test of the tumoricidal potential of the endogenous T cell compartment, work by Rosenberg and colleagues demonstrated that infusion of autologous ex vivo expanded tumor-infiltrating lymphocytes can induce objective clinical responses in metastatic melanoma (3), and at least part of this clinical activity is due to cytotoxic T cells (4). Importantly, recent studies demonstrate that T cell-based immunotherapies are also effective in a range of other human malignancies. In particular, early-phase trials of antibodies that interfere with the T cell checkpoint molecule PD-1 have shown clinical activity in tumor types as diverse as melanoma, lung cancer, bladder cancer, stomach cancer, renal cell cancer, head and neck cancer, and Hodgkin's lymphoma (5). Based on the relationship between

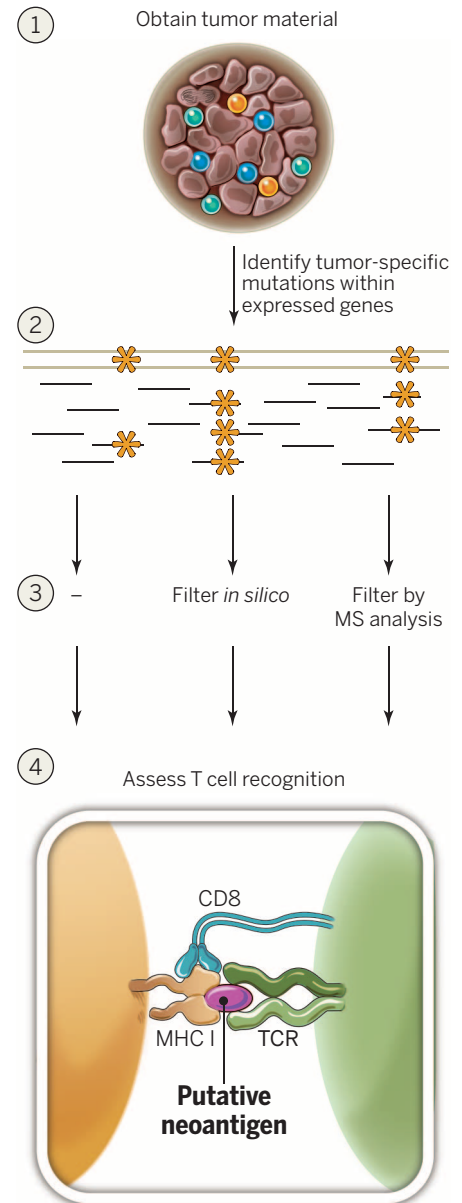
pretherapy CD8+ T cell infiltrates and response to PD-1 blockade in melanoma, cytotoxic T cell activity also appears to play a central role in this form of cancer immunotherapy (6).

An implicit conclusion from these clinical data is that in a substantial fraction of patients, the endogenous T cell compartment is able to recognize peptide epitopes that are displayed on major histocompatibility complexes (MHCs) on the surface of the malignant cells. On theoretical grounds, such cancer rejection epitopes may be derived from two classes of antigens. A first class of potential cancer rejection antigens is formed by nonmutated proteins to which T cell tolerance is incomplete—for instance, because of their restricted tissue expression pattern. A second class of potential cancer rejection antigens is formed by peptides that are entirely absent from the normal human genome, so-called neoantigens. For the large group of human tumors without a viral etiology, such neo-epitopes are solely created by tumor-specific DNA alterations that result in the formation of novel protein sequences. For virus-associated tumors, such as cervical cancer and a subset of head and neck cancers, epitopes derived from viral open reading frames also contribute to the pool of neoantigens.

As compared with nonmutated self-antigens, neoantigens have been postulated to be of particular relevance to tumor control, as the quality of the T cell pool that is available for these antigens is not affected by central T cell tolerance (7). Although a number of heroic studies provided early evidence for the immunogenicity of mutation-derived neoantigens [reviewed in (8)], technology to systemically analyze T cell reactivity against these antigens only became available recently. Here, we review our emerging understanding of the role of patient-specific neoantigens in current cancer immunotherapies and the implications of these data for the development of next-generation immunotherapies.

## Exome-guided neoantigen identification: Process considerations

A large fraction of the mutations in human tumors is not shared between patients at



**Fig. 1. Cancer exome-based identification of neoantigens.** Tumor material is analyzed for nonsynonymous somatic mutations. When available, RNA sequencing data are used to focus on mutations in expressed genes. Peptide stretches containing any of the identified nonsynonymous mutations are generated in silico and are either left unfiltered (16, 17), filtered through the use of prediction algorithms [e.g., (10–13)], or used to identify MHC-associated neoantigens in mass spectrometry data (15, 20). Modeling of the effect of mutations on the resulting peptide-MHC complex may be used as an additional filter (20). Resulting epitope sets are used to identify physiologically occurring neoantigen-specific T cell responses by MHC multimer-based screens (13, 22) or functional assays [e.g., (11, 12)], within both CD8+ [e.g., (11–13, 19, 39)] and CD4+ (16, 18) T cell populations. Alternatively, T cell induction strategies are used to validate predicted neoantigens [e.g., (10, 20)].

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meaningful frequencies and may therefore be considered patient-specific. Because of this, technologies to interrogate T cell reactivity against putative mutation-derived neoantigens need to be based on the genome of an individual tumor. With the development of deep-sequencing technologies, it has become feasible to identify the mutations present within the protein-encoding part of the genome (the exome) of an individual tumor with relative ease and thereby predict potential neoantigens (9). Two studies in mouse models provided the first direct evidence that such a cancer exome-based approach can be used to identify neoantigens that can be recognized by T cells (10, 11). In brief, for all mutations that resulted in the formation of novel protein sequence, potential MHC binding peptides were predicted, and the resulting set of potential neoantigens was used to query T cell reactivity. Subsequent studies have demonstrated that cancer exome-based analyses can also be exploited in a clinical setting, to dissect T cell reactivity in patients who are treated by either tumor-infiltrating lymphocyte (TIL) cell therapy or checkpoint blockade (12, 13). Furthermore, following this early work, the identification of neoantigens on the basis of cancer exome data has been documented in a variety of experimental model systems and human malignancies (10–22).

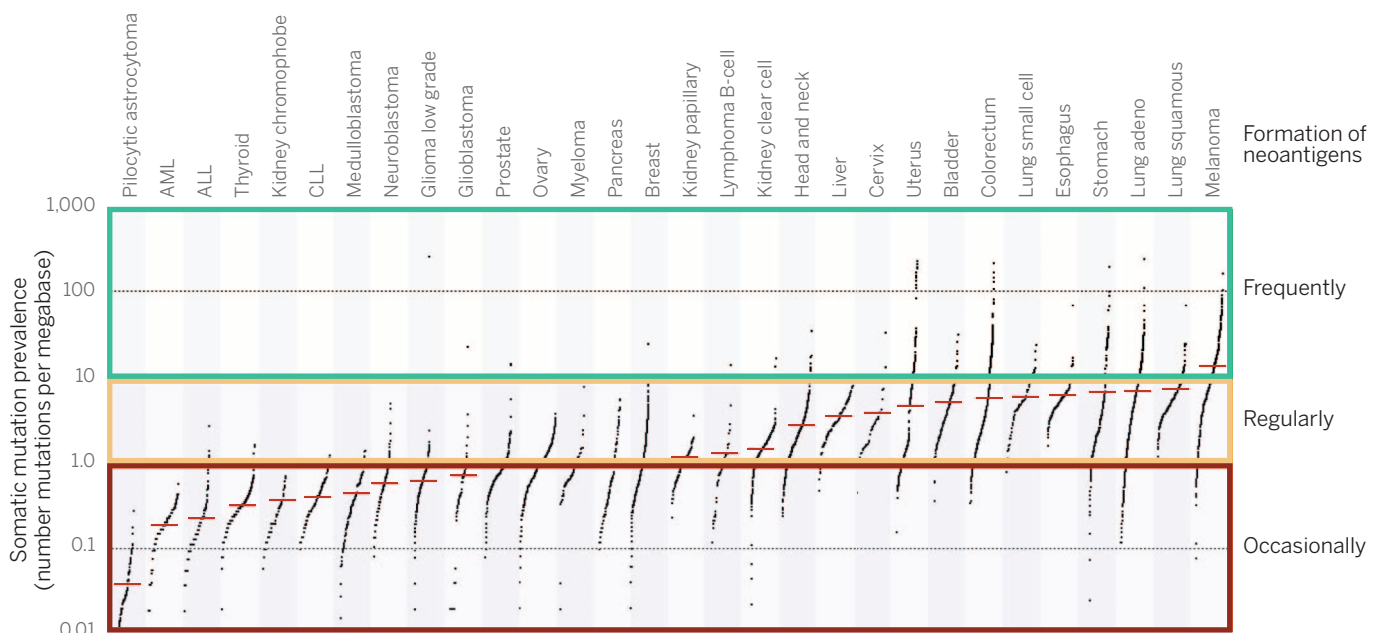
The technological pipeline used to identify neoantigens in these different studies has varied substantially, and further optimization is likely possible (Fig. 1). Accepting the limitations of probing the mutational profile of a tumor in a single biopsy (23), the genetic analysis of the tumor itself can be considered a robust process. Specifically, based on the analysis of neoantigens previously identified by other means, the false-negative rate of cancer

exome sequencing is low—i.e., the vast majority of neoantigens occur within exonic sequence for which coverage is sufficient (24). At the same time, it is apparent from unbiased screening efforts—in which the entire collection of identified mutations was used to query T cell reactivity—that the vast majority of mutations within expressed genes do not lead to the formation of neoantigens that are recognized by autologous T cells (16, 17). Because of this, a robust pipeline that can be used for the filtering of cancer exome data is essential, in particular for tumors with high mutational loads.

How can such filtering be performed? With the set of mutations within expressed genes as a starting point, two additional requirements can be formulated. First, a mutated protein needs to be processed and then presented as a mutant peptide by MHC molecules. Second, T cells need to be present that can recognize this peptide-MHC complex. In two recent preclinical studies, presentation of a handful of predicted neoantigens by MHC molecules was experimentally demonstrated by mass spectrometry (15, 20), and this approach may form a valuable strategy to further optimize MHC presentation algorithms. At the same time, the sensitivity of mass spectrometry is presently still limited, thereby likely resulting in a substantial fraction of false negatives. For this reason, but also because of logistical issues, implementation of this approach in a clinical setting is unlikely to happen soon. Lacking direct evidence for MHC presentation, as can be provided by mass spectrometry, presentation of neoantigens by MHC class I molecules may be predicted using previously established algorithms that analyze aspects such as the likelihood of proteasomal processing, transport into the endoplasmic reticulum, and affinity for the relevant MHC class I alleles. In addition,

gene expression levels (or perhaps preferably protein translation levels) may potentially also be used to help predict epitope abundance (25).

Although most neoantigen identification studies have successfully used criteria for epitope prediction that are similar to those previously established for the identification of pathogen-derived epitopes [e.g., (12, 13)], Srivastava and colleagues have argued that neoantigens in a transplantable mouse tumor model display very different properties from viral antigens and generally have a very low affinity for MHC class I (14). Although lacking a satisfactory explanation to reconcile these findings, we do note that the vast majority of human neoantigens that have been identified in unbiased screens do display a high predicted MHC binding affinity (24, 26). Likewise, minor histocompatibility antigens, an antigen class that is conceptually similar to neoantigens, are correctly identified by classical MHC binding algorithms (27). Moreover, the mutations that were identified in a recent preclinical study as forming tumor-specific mutant antigens that could induce therapeutic tumor rejection when used in tumor vaccines (15) were not predicted to be significant using the Srivastava approach. Another potential filter step that has been suggested examines whether the mutation is expected to improve MHC binding, rather than solely alter the T cell receptor (TCR)-exposed surface of the mutant peptide. However, with examples of both categories in both mouse models and human data, the added value of such a filter may be relatively modest (11, 15, 20, 26). For MHC class I restricted neoantigens, conceivably the biggest gain in prediction algorithms can be made with respect to identification of the subset of MHC binding peptides that can successfully be recognized



**Fig. 2. Estimate of the neoantigen repertoire in human cancer.** Data depict the number of somatic mutations in individual tumors. Categories on the right indicate current estimates of the likelihood of neoantigen formation in different tumor types. Adapted from (50). It is possible that the immune system in melanoma patients picks up on only a fraction of the available neoantigen repertoire, in which case the current analysis will be an underestimate. A value of 10 somatic mutations per Mb of coding DNA corresponds to ~150 nonsynonymous mutations within expressed genes.

by the TCR repertoire. With respect to this, the nature of the central TCR-exposed residues of MHC-bound peptides has been shown to be associated with peptide immunogenicity (28). By the same token, alterations at these sites may potentially be picked up by the immune system more readily (20). However, a substantial further experimental effort is required to evaluate to what extent algorithms that predict immunogenicity can facilitate the identification of MHC class I-restricted neoantigens. For MHC class II-restricted neoantigens, it will be important to obtain a better understanding not only of peptide immunogenicity but also of the basic factors that determine the efficiency of epitope presentation.

### Size and nature of the neoantigen repertoire

Large-scale analyses of neoantigen-specific T cell reactivity have now been carried out for a substantial number of patients, mostly in melanoma (12, 13, 16, 17). With the caveat of a potential selection bias toward patients with a clinical benefit upon immunotherapeutic intervention, these analyses provide a first estimate of the frequency with which the immune system recognizes the neoantigens that are formed as a consequence of mutations. The first and arguably most important conclusion that can be drawn from these analyses is that the T cell-based immune system reacts to both MHC class I-restricted (12, 13, 17) and MHC class II-restricted neoantigens (16) in a large fraction of melanoma patients. The second conclusion that can be drawn from these analyses is that only a very small fraction of the nonsynonymous mutations in expressed genes in these tumors leads to the formation of a neoantigen for which CD4+ or CD8+ T cell reactivity can be detected within tumor-infiltrating lymphocytes.

What do these observations mean for the potential formation of neoantigen repertoires in other human malignancies? Most human melanomas have a mutational load above 10 somatic mutations per megabase (Mb) of coding DNA, and this is apparently sufficient to lead to the frequent formation of neoantigens that can be seen by T cells. Based on these data, formation of neoantigens that can potentially be recognized by autologous T cells is expected to also be common for other tumors with a mutational load above 10 somatic mutations per Mb (corresponding to approximately 150 nonsynonymous mutations within expressed genes) (Fig. 2). This group contains a sizable fraction of high-prevalence tumor types such as lung cancer and colorectal cancer. If formation of neoantigens is a frequent event in tumors with mutational loads above 10 somatic mutations per Mb, many tumors with a mutational load of 1 to 10 per Mb may still be expected to carry neoantigens that

can be recognized by T cells. However, as based on the fact that even for melanomas with a mutational load around 10 mutations per Mb, T cell reactivity is not always observed (16), tumor types with a mutational load below 1 mutation per Mb appear less likely to commonly express neoantigens that can be recognized by autologous T cells.

Although this analysis provides a useful first sketch of the expected relevance of neoantigens

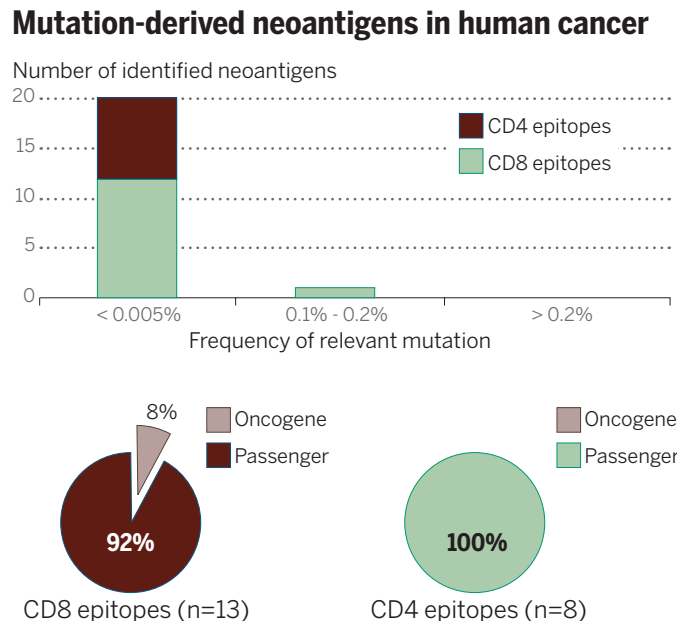
high mutational load, neoantigen-specific T cell reactivity is lacking or, vice versa, in which a tumor with only a handful of mutations will express an MHC class I- or class II-restricted neoantigen. Third, although we here make a prediction with regard to the frequency with which neoantigens that can potentially be recognized by the TCR repertoire are formed, it should be kept in mind that the presence of a neoantigen does not equal the induction of T cell reactivity. Human tumors vary substantially in the composition of their microenvironment, and this is likely to influence the ability of the T cell pool to respond to mutated antigens. Related to this, from a conceptual point of view, therapeutic manipulation of T cell reactivity would seem particularly attractive for tumor types that do express large numbers of antigens but in which the tumor microenvironment hinders the activation of the T cells that recognize them.

What are the characteristics of mutation-derived neoantigens in human cancer, both with respect to the genes from which they are derived and the frequency with which they occur within the patient population? In an ideal world, neoantigens would be derived from essential oncogenes and occur in large patient groups, to both reduce the likelihood of escape and facilitate clinical interventions that enhance T cell reactivity against them. Clearly, T cell responses do sometimes occur against MHC class I-restricted (30) and MHC class II-restricted neoantigens in validated oncogenes that are shared between

subgroups of patients (31). At the same time, it is apparent that, at least in melanoma, the bulk of the neoantigen-specific T cell response is directed toward mutated proteins that are essentially unique to that tumor and that are unlikely to play a key role in cellular transformation (Fig. 3, top and bottom) (16). A direct implication of this bias in neoantigen-specific T cell reactivity toward patient-specific passenger mutations is that the targeting of defined neoantigens will likely require the development of personalized immunotherapies.

### Extrinsic influences on the tumor antigenic landscape

The neoantigen repertoire expressed in a clinically apparent cancer may have been substantially influenced by the developing tumor's interaction with the immune system that occurs even before it becomes clinically apparent. This is the process of "cancer immunoediting" that has been well documented in preclinical cancer models (1, 32, 33). In its most complex form, cancer immunoediting may occur in three phases: elimination, in which the innate and adaptive immune systems work



**Fig. 3. Characteristics of melanoma neoantigens.** (Top) For a group of CD4+ T cell neoantigens (8 epitopes) and CD8+ T cell neoantigens (13 epitopes) identified by cancer exome-based screens, the frequency of mutation of that residue in a cohort of ~20,000 human tumor samples (51) is depicted. (Bottom) For the same group of CD4+ T cell and CD8+ T cell neoantigens, the fraction of encoding mutations that occurs within known oncogenes (52) is depicted.

in different tumor types, three important factors should be taken into account. First, by relying on the presence of preexisting T cell reactivity as a readout, the human studies carried out to date will only detect neoantigens that were immunogenic during in vivo tumor outgrowth (either spontaneously or boosted by therapy). It is conceivable that not all tumor-expressed neoantigens induce an autologous T cell response—for instance, because they are not efficiently cross presented. In addition, at least in preclinical models, there is evidence for immunodominance of tumor antigens, where the immune system becomes so fixated on particular antigens that it ignores other antigens that are both present and detectable in the tumor (29). If only a fraction of the available neoantigens would normally elicit T cell reactivity, the analyses carried out to date may underestimate the actual neoantigen repertoire. As a second consideration, it is important to realize that the formation of neoantigens is a probabilistic process in which each additional mutation increases the odds that a relevant neoantigen is created. Thus, in this "neoantigen lottery," there will be cases in which despite a



together to recognize a developing tumor and destroy it before it becomes clinically apparent; equilibrium, in which residual occult tumor cells not destroyed in the elimination phase are held in a state of tumor dormancy as a consequence of adaptive immune system activity and undergo “editing”; and escape, in which edited tumor cells are no longer recognized or controlled by immune processes, begin to grow progressively, induce an immunosuppressive tumor microenvironment, and then emerge as clinically apparent cancers. Recent work has demonstrated that T cells play a major role in shaping the immunogenicity of developing cancers—i.e., “edit” tumor immunogenicity—and exert this effect by at least two mechanisms. First, T cells can shape tumor antigenicity/immunogenicity through an immunoselection process by destroying tumor cells that express strong tumor-specific mutant antigens, leaving behind tumor cells that either express weaker antigens (some of which may still be mutant tumor antigens) or are incapable of expressing antigens (e.g., those that have developed mutations in antigen processing or presentation) (11). Second, chronic T cell attack on a tumor has been shown to silence expression of certain tumor-specific antigens through epigenetic mechanisms in a preclinical model (34). Strikingly, a recent study, based on analysis of thousands of the Cancer Genome Atlas solid tumor samples, showed that, in particular in colorectal cancer, mutated peptides predicted to bind to autologous MHC class I molecules are less frequent than expected by chance, an observation that is consistent with immune-based selection (35). By extension, the combination of cell-extrinsic forces such as cancer immunoeediting and the stochastic nature of epitopes arising from tumor-specific mutations may help drive the heterogeneous mutational—and by inference, antigenic—landscapes that have been noted in certain tumors (23). As such, the antigenic heterogeneity of tumors might explain some of the differences in response that individual patients display to checkpoint blockade therapy. Individuals who develop durable responses to checkpoint blockade may be those whose tumors retain sufficient antigenicity to render them sensitive to the heightened immune function that accompanies cancer immunotherapy, despite not being controlled by naturally occurring antitumor immune responses.

### Role of neoantigens in cancer immunotherapy

On theoretical grounds, two factors should determine the relative importance of neoantigens and nonmutated self-antigens in the effects of cancer immunotherapies such as checkpoint blockade and TIL therapy: first, the frequency with which T cell responses against the two antigen classes occur; second, the relative potency of T cell responses specific for the two antigen classes. Recent work in mouse models using transplantable carcinogen-induced cancers has demonstrated that checkpoint blockade alters both the quality of the neoantigen-specific intratumoral T cell response (as reflected by common- and

treatment-specific changes in gene expression in CD8+ TILs isolated from tumor-bearing mice treated with antibodies to CTLA-4 and/or PD-1) and the magnitude of this T cell response (seen with CTLA-4 or combined CTLA-4/PD-1 blockade but not with PD-1 blockade only) (15). Because the neoantigens identified in this model serve as cancer rejection antigens, these data provide compelling evidence that checkpoint blockade acts at least in part through neoantigen-specific T cell reactivity in this setting. However, in the case of human melanoma, where autochthonous tumors may be in contact with the immune system for years, the situation is more complicated. As discussed above, T cell reactivity against neoantigens is common in melanoma. Furthermore, a case report has shown that such reactivity can be enhanced by anti-CTLA-4 treatment (13). However, T cell reactivity against nonmutated shared antigens is also observed in the majority of melanoma patients, and broadening of this T cell response has been documented following both TIL therapy and anti-CTLA-4 treatment (36, 37). Thus, although the murine data show that neoantigen-specific T cell reactivity can be critical to the effects of checkpoint blockade, the human data are presently only consistent with this possibility.

What other data are available with respect to this issue? If recognition of neoantigens is an important component of cancer immunotherapy, one would expect tumor types with high numbers of mutations to be characterized by strong T cell

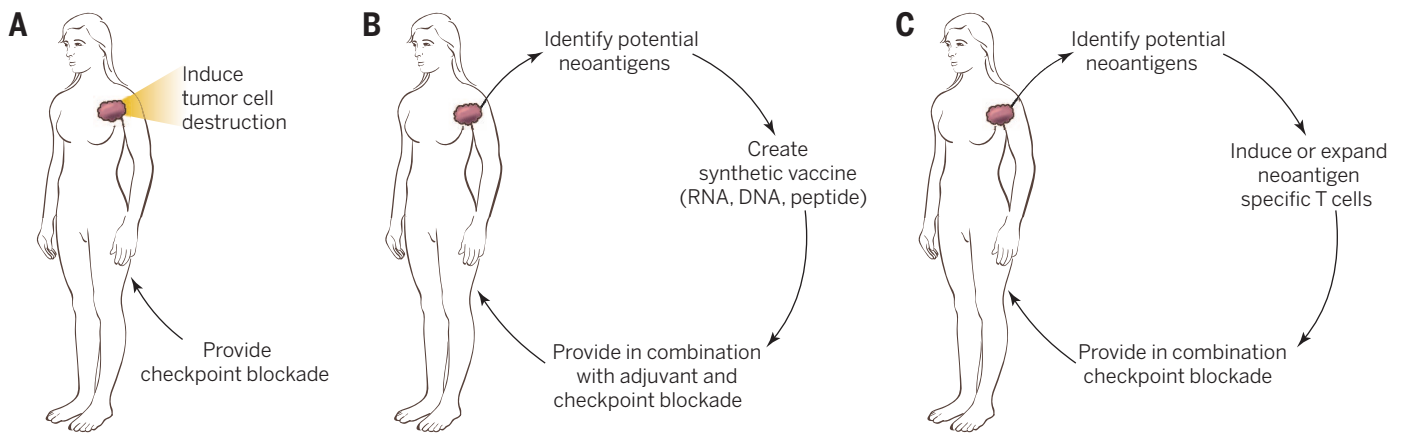
***“The genetic damage that on the one hand leads to oncogenic outgrowth can also be targeted by the immune system to control malignancies.”***

responses and to be particularly sensitive to immunotherapy. Furthermore, also within a given tumor type, response rate should correlate with mutational load. Evidence for a role of neoantigens in driving the strength of the intratumoral T cell response is provided by the observation that the presence of CD8+ T cells in cancer lesions, as read out using RNA sequencing data, is higher in tumors with a high mutational burden (38). Furthermore, an extensive analysis by Hacohen and colleagues has demonstrated that the level of transcripts associated with cytolytic activity of natural killer cells and T cells correlates with mutational load in a large series of human tumors (35). With respect to the effects of immunotherapy in tumors with different mutational loads, in non-small cell lung cancer patients treated with anti-PD-1, mutational load shows a strong correlation with clinical response (22). Likewise, in melanoma patients treated with ipilimumab, an antibody to CTLA-4, long-term benefit is also associated with a higher

mutational load, although the effect appears less profound in this setting (39). A striking observation in the latter study has been that the predicted MHC binding neoantigens in patients with a long-term clinical benefit were enriched for a large series of tetrapeptide motifs that were not found in tumors of patients with no or minimal clinical benefit. An appealing interpretation of these data is that the neoantigen-specific T cell response is preferentially directed toward a subset of mutant sequences, something that could facilitate bioinformatic identification of neoantigens for therapeutic targeting. However, analysis of the sequence properties of human neoantigens identified in other studies does not show the profound bias toward these tetrapeptide signatures that would be predicted if their role were central in the tumor-specific T cell response (40), and conceivably the identified tetrapeptide motifs play a different role.

It will be valuable to extend the analysis of genomic determinants of tumor cell sensitivity to cancer immunotherapeutics to other malignancies. However, because of the probabilistic nature of neoantigen generation, mutational load will by itself always remain an imperfect biomarker, even in a situation in which neoantigen reactivity is the sole tumor-specific T cell reactivity that is relevant to tumor control. Furthermore, the formation of tumor-specific antigens is only one of a number of essential conditions for a successful immune attack on cancer cells, a concept that is well described by the cancer-immunity cycle introduced by Chen and Mellman (41). As an example, genetic inactivation of the  $\beta_2$ -microglobulin subunit of MHC class I molecules is a relatively frequent event in some tumor types (42). In addition, a recent analysis of genetic alterations that are present in tumors with high immune activity provides evidence for a series of other escape mechanisms (35). In such cases, in which the cancer-immunity cycle is disrupted at another site, the number of neoantigens produced is unlikely to still be of much relevance. Because of this interdependence of different phases of the cancer-immunity cycle, the combined use of assay systems that report on these different phases appears warranted.

Arguably the most direct data on the relevance of neoantigen-specific T cells in human tumor control comes from a small number of clinical studies that involve infusion of defined T cell populations or infusion of TCR-transduced T cells. Encouragingly, a recent case report demonstrated regression of a metastatic cholangiocarcinoma by infusion of a CD4+ T cell product that was highly enriched for reactivity against an MHC class II-restricted neoantigen (18). Combined with the observation that, at least in melanoma, CD4+ T cell recognition of neoantigens is a frequent event (16), these data underscore the potential clinical relevance of MHC class II-restricted neoantigens. Comparison of the clinical effects of TIL therapy with that of T cells modified with TCRs recognizing different shared antigens can also be considered informative. Infusion of T cells modified with TCRs directed against the gp100 and MART-1



**Fig. 4. Strategies to target the patient-specific neoantigen repertoire.** (A) Immunotherapy is given in combination with interventions such as radiotherapy that enhance exposure to autologous neoantigens. (B) Potential neoantigens are identified as in Fig. 1 steps 1 to 3, a patient-specific vaccine is produced, and this vaccine is given together with adjuvant and T cell checkpoint-blocking antibodies. (C) Potential neoantigens are identified as in Fig. 1 steps 1 to 3, T cells that are specific for these neoantigens are induced or expanded in vitro, and the resulting T cell product is given together with T cell checkpoint-blocking antibodies.

melanocyte differentiation antigens, a prominent class of self-antigens in melanoma, shows a relatively modest clinical effect that is accompanied by substantial on-target toxicity against healthy melanocytes (43). Because this toxicity is relatively infrequent in TIL therapy, these data strongly suggest that T cell reactivity against the melanocyte differentiation antigens is not a major driver of the antitumor activity of this therapy. At the same time, there is data showing that T cell products directed against NY-eso-1, one of the nonmutant self-antigens from the family of cancer/germline antigens that show very limited expression in healthy tissue, can display substantial antitumor activity (44, 45). Thus, although the available data support the notion that T cell recognition of neoantigens contributes substantially to the effects of the currently used immunotherapies, it would not be justified to dismiss a potential contribution of T cell responses against a subset of nonmutant antigens. A direct comparison of the antitumor activity of neoantigen-specific and self-antigen-specific T cells obtained from individual patients would be useful to further address this issue.

### Therapeutic use of the patient-specific neoantigen repertoire

Based on the fact that, at least in tumors with high mutational loads, the amount of DNA damage is sufficient for the immune system to see one or multiple epitopes as foreign, it becomes of interest to stimulate neoantigen-specific T cell responses in cancer patients. Such stimulation can obviously only be of value if the strength of the neoantigen-specific T cell response is otherwise a limiting factor in tumor control. Human data on this important issue are lacking. However, in mouse models, vaccination with defined neoantigens has been shown to result in increased tumor control (10, 14, 15, 20), providing sufficient rationale for the clinical development of neoantigen-directed therapeutics. Because the majority of possible neoantigens are specific to the individual being

treated (Fig. 3), such therapeutic approaches will in most cases entail personalized immunotherapies that exploit either the antigen repertoire in the tumor cells themselves or information on that repertoire, as obtained by tumor sequencing (Fig. 4). As a first approach, a combination of checkpoint-blocking antibodies with therapeutic interventions—such as tumor radiotherapy, oncolytic viruses, or autologous tumor cell vaccines—that can increase neoantigen exposure to the T cell-based immune system may be synergistic (Fig. 4A). As a downside, as compared to molecularly defined vaccines, the neoantigens released by such strategies will be diluted by the large amount of nonmutant peptides that are also present. In addition, control over the maturation signals received by antigen-presenting cells is relatively limited. Nevertheless, because of the relative ease of clinical development of some of these combination therapies, extensive testing of such therapies is warranted.

To allow a more defined targeting of the neoantigen repertoire in human tumors, two alternative approaches should be considered, in both cases relying on sets of potential neoantigens as identified by sequencing of tumor material (Fig. 4, B and C). First, synthetic vaccines may be produced that contain or encode a set of predicted neoantigens. Although still a substantial departure from the classical pharmaceutical model, clinical development of such personalized vaccines is within reach (46–48). Mouse model data support the clinical translation of this approach, and the two most pressing questions appear to be (i) whether our ability to predict the most relevant neoantigens is already sufficiently advanced and (ii) how such vaccines may best be administered. Second, the information obtained from tumor sequencing may be used to create neoantigen-specific T cell products in vitro. This may involve either the expansion of neoantigen-specific T cell populations that can already be detected within tumor tissue or in blood or the de novo induction of such cells.

Regardless of the strategy used to enhance neoantigen-specific T cell reactivity, it will likely prove important to target multiple neoantigens simultaneously in order to prevent tumor escape by editing of the mutated epitope concerned (1). In addition, it may be prudent to avoid the targeting of mutations in gene products that are seen by the immune system in autoimmune disease to avoid induction of or exacerbation of cancer-associated autoimmune disease (49).

### Concluding remarks

Based on data obtained over the past few years, it is plausible that neoantigen-specific T cell reactivity forms a major “active ingredient” of successful cancer immunotherapies. In other words, the genetic damage that on the one hand leads to oncogenic outgrowth can also be targeted by the immune system to control malignancies. Based on this finding, it will be important to engineer therapeutic interventions by which neoantigen-specific T cell reactivity is selectively enhanced. Because of the tumor-restricted expression of the antigens that are being targeted, these personalized cancer immunotherapies offer the promise of high specificity and safety. Conceivably, the boosting of neoantigen-specific T cell reactivity that can be achieved with such personalized immunotherapies will further increase the spectrum of human malignancies that respond to cancer immunotherapy.

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#### REVIEWS

# T cell exclusion, immune privilege, and the tumor microenvironment

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**Effective immunotherapy promotes the killing of cancer cells by cytotoxic T cells. This requires not only that cancer-specific T cells be generated, but also that these T cells physically contact cancer cells. The coexistence in some patients of cancer cells and T cells that recognize them indicates that tumors may exhibit the phenomenon of immune privilege, in which immunogenic tissue is protected from immune attack. Here, we review the evidence that stromal cells of the tumor microenvironment mediate this restriction by excluding T cells from the vicinity of cancer cells. Overcoming this T cell checkpoint may thus enable optimal immunotherapy.**

The microenvironment of tumors contains numerous cell types in addition to cancer cells, which include bone marrow–derived inflammatory cells, lymphocytes, blood vessels, fibroblastic cells, and the extracellular matrix composed of collagen and proteoglycans (1, 2). The importance of a stromal microenvironment, especially one that has characteristics of a “wound” or regenerating tissue, has been recognized for at least a century (3), but its possible role in blunting an immune attack of cancer cells awaited the discovery of adaptive cellular immunity. In 1960, Klein and colleagues found that when mice developed primary methylcholanthrene-induced sarcomas, they also developed an anti-tumor immune response mediated by lymph node cells to a secondary challenge comprising cancer cells derived from the primary tumor (4). The paradoxical and critical finding of the study was that this anticancer immune response did not control the growth of the primary tumor, despite its ability to prevent the establishment of a secondary tumor comprising cancer cells derived from the primary tumor. In traditional immunological terminology, the primary tumor evaded immune control by establishing an immune-privileged microenvironment that is functionally analogous to that of certain normal tissues, such as the eye (5).

Unambiguous evidence for the inability in humans of a systemic immune response to eliminate immunogenic cancer cells was provided by Boon’s studies 30 years later of the antigens that elicit specific CD8<sup>+</sup> T cell responses in melanoma patients (6). Cloned CD8<sup>+</sup> T cells from a melanoma patient were used to identify the antigen expressed by that patient’s cancer: MAGE-A1. The explicit demonstration of the coexistence of a progressing melanoma with melanoma-specific T cells in this patient implicitly raised the question of

why the T cells did not control the growth of the cancer. Immunoediting, or the elimination of immunogenic cancer cells (7), could be excluded, which left the possibility of immune suppression by the tumor microenvironment (TME). Despite this evidence that the presence of antigen-specific CD8<sup>+</sup> T cells alone may not be sufficient for the control of cancer, a major pharmaceutical company recently conducted phase III trials in patients with non–small cell lung cancer (NSCLC) of the clinical efficacy of vaccination with the MAGE-A3 antigen (MAGRIT, NCT00480025). The study did not meet its primary end point of extending disease-free survival and was discontinued in 2014. Moreover, Rosenberg and colleagues reported evidence of disease recurrence in melanoma patients despite very high levels of vaccine-induced circulating T cells and no evidence of antigen loss by the cancer cells (8).

The discovery of melanoma-specific T cells in patients led to another strategy to increase the frequency of cancer-specific T cells in patients, that of adoptively transferring large numbers of in vitro expanded tumor-infiltrating lymphocytes (TILs). As discussed elsewhere in this issue of *Science* (9), this approach has shown some efficacy, which has been of major importance to the field by serving as proof that the immune system has the potential to control cancer (10). However, adoptive T cell therapy (ACT) with TILs has not had the dramatic success of ACT with virus-specific CD8<sup>+</sup> T cells to immunodeficient bone marrow transplant recipients with cytomegalovirus infection (11) or Epstein-Barr virus–associated lymphoproliferative disorders (12). Differences in the microenvironments of virally infected tissues and cancers may account for these distinct outcomes, with the latter being immune-suppressive. Another important point of comparison is that the TME of solid cancers is likely to be fundamentally different to that of the leukemias, in which clinical trials of ACT with T cells expressing chimeric antigen receptors, so-called CAR T cells, have demonstrable efficacy (9). These findings raise the possibility that increasing the frequency of cancer-specific T cells, by whatever means, may be more effective if combined with an approach that alters the immune-suppressive TME.

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