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Commentary

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Cancer neoantigens targeted by adoptive T cell transfer: private no more

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Effector T cell responses directed toward cancer neoantigens mediate tumor regression following checkpoint blockade or adoptive T cell immunotherapy, but are generally “private”, thus requiring considerable effort for their identification. In this issue of the *JCI*, Malekzadeh et al. show that a fraction of patients with epithelial cancers mount antigen-specific T cell responses to “hot spot” p53 mutations that in some cases are shared among patients. This work suggests that other genes frequently mutated in human cancer can be immunogenic, thus offering a rapid way to screen for cancer neoantigens that can be targeted by subsequent T cell-based therapies.

Genetic mutations of oncogenes lead to altered protein functions, resulting in increased cellular proliferation, differentiation blockade, and, ultimately, neoplastic transformation. With progression, tumors accumulate mutations and mutational load is generally considered deleterious because they generate subclones of tumor cells with different biological properties, thus making cancer more difficult to treat with standard therapies (1). Patients with specific mutations, such as the melanoma V600E BRAF mutation, can be treated with targeted therapies inhibiting the pathway, although clinical responses do not last long (2). Recent evidence indicates that a higher tumor mutational burden, as found in melanoma, non-small cell lung cancer, and colorectal cancer with microsatellite instability (3, 4), is beneficial for clinical responsiveness to immunotherapies such as antiprogrammed death 1 (PD1) immune checkpoint blockade (ICB) (5). In fact, peptides resulting from degradation of altered protein sequences caused by missense mutations (neoantigens) can be recognized by the adaptive immune system when presented by human leukocyte

antigens (HLA). Such antigens have the potential to be truly foreign to the immune system (no expression during thymic selection) and can prime an effector T cell response, whose reinvigoration is thought to be a key component in successful ICB.

A plethora of genes are generally mutated in human cancer, with each patient bearing his or her own private array of mutations and, as a consequence, neoantigen-specific T cell responses. This information is important to develop personalized therapies that are tailored to the genetic architecture of the tumor, including the adoptive cell transfer (ACT) of neoantigen-specific T cells (either tumor-infiltrating lymphocyte-engineered [TIL-engineered] or T cell receptor-engineered [TCR-engineered] T cells) or anticancer vaccines containing private neoepitopes, both of which were recently demonstrated to be feasible and effective against metastatic tumors in patients in clinical trials (6, 7). However, the process for neoantigen identification is time consuming and requires considerable patient-specific effort at the technical and bioinformatic level because it implies

whole-exome and transcriptome sequencing, prediction of personal HLA-binding peptides, and extensive testing in vitro to assess T cell reactivity (8). Importantly, we now know that only a miniscule number of potential neoantigens are presented by tumor cells (9), and we as a field do not yet know how to identify these targetable neoantigens using computational tools. These aspects pose a challenge for the utilization of such treatment strategies on a large scale.

Some genes are more frequently mutated than others, called genetic hot spots, thus raising the possibility that cancer patients do not only harbor their own private neoantigen repertoire and corresponding T cell responses, but also mount responses to “public” neoantigens. This possibility is elegantly suggested by the report published in this issue of the *JCI* by Malekzadeh et al. (10). *TP53*, encoding the p53 tumor suppressor, is the most frequently mutated gene in human cancer, thus leading the authors to perform a systematic evaluation of intratumoral T cell responses directed toward p53 hot spot mutations for therapeutic purposes. Of the 140 patients with epithelial tumors tested, 91 carried *TP53* mutations, 36% of which mapped to previously identified *TP53* hot spots. Of TILs isolated from 28 patients, 39% were found to display a T cell response directed to p53 hot spot mutations. The application of a recently described high-throughput method generating a tandem minigene (TMG) library (11) containing *TP53* mutations allowed the identification of TILs that are reactive to the hot spots. The authors electroporated immature dendritic cells with TMGs and cocultured them with TIL fragment cultures. IFN- γ secretion and CD137 (also known as 4-1BB) expression two times higher than the background identified positive responses, thereby revealing that patients harbor complex features of neoantigen-specific T cell responses, featur-

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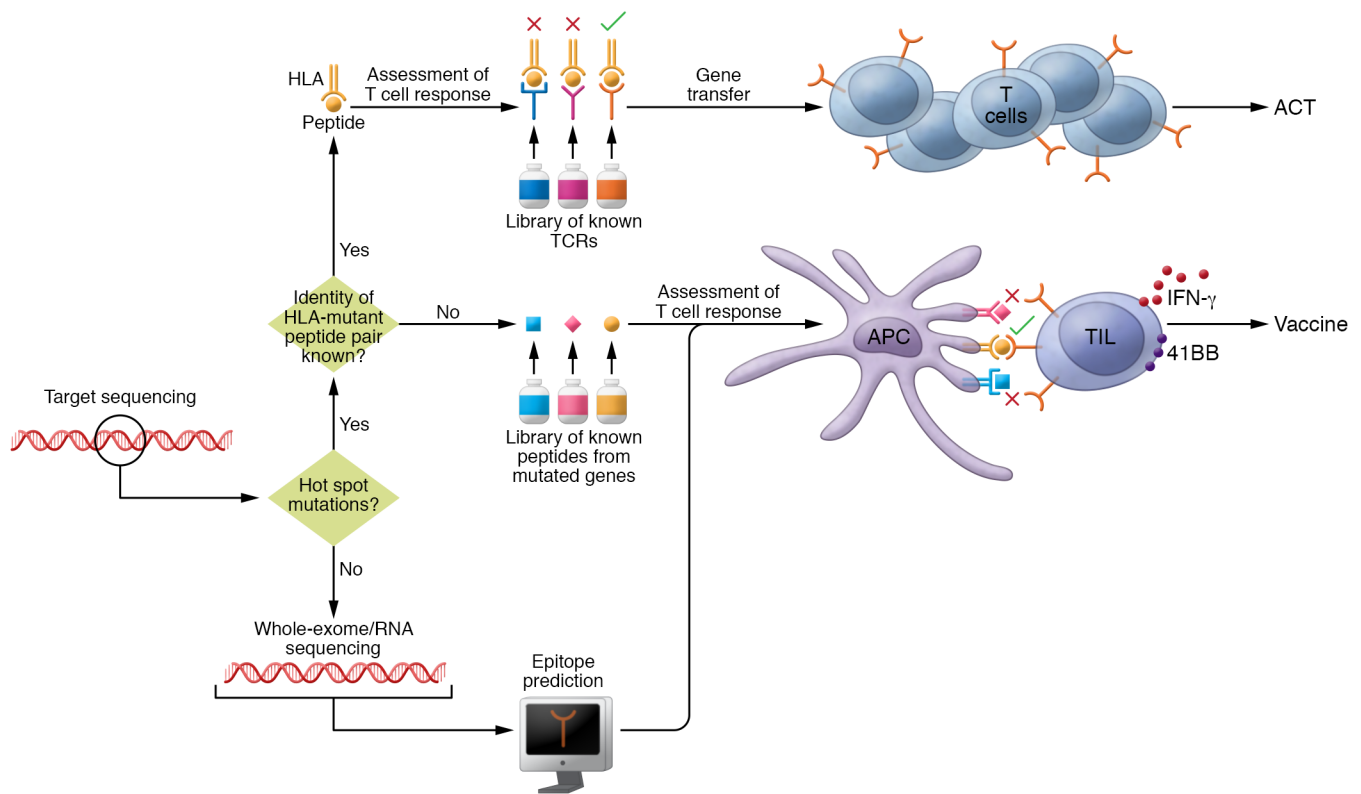


Figure 1. Accelerated neoantigen identification pipeline. A panel of hot spot mutations is tested by target sequencing. Should a known HLA/mutant peptide pair be identified, the patient’s own T cells can be engineered by TCR gene therapy as an off-the-shelf strategy from a previously created library of TCRs. Alternatively, the patient’s TILs can be assessed for neoantigen reactivity by using a collection of mutant peptides from genes frequently mutated in human cancer and by using IFN- γ secretion or 41BB upregulation as a readout. These approaches will decrease the time requested to produce the engineered cell products or vaccine formulations for personalized therapy. Should the patient not harbor hot spot mutations in the tumor, the standard pipeline for neoantigen identification using whole-exome/RNA sequencing and epitope prediction is applied. Assessment of candidate peptides by T cell assays in vitro may be performed as an optional validation step.

ing either CD8⁺ or CD4⁺ or both, that are capable of producing IFN- γ . This is in line with additional, recent evidence showing neoantigen-specific CD4⁺ T cell responses in cancer (6, 12). Selected cultures were rescreened to confirm 10-fold higher avidity to the mutated epitope compared with the wild type. Overall, the procedure for neoantigen-specific T cell identification from a patient lung lesion took 34 days. Of note, as peptide-pulsed antigen-presenting cells (APCs) produced results generally comparable to TMG in the identification of reactive TILs, the whole process might be accelerated even further by stimulating T cells directly with peptides from a previously created library (Figure 1).

Next, the authors set the basis of genetic modification of T cells for cell-based therapy by generating a library of 9 TCRs capable of recognizing 7 different p53 neoantigens. They identified a mutated p53 neoepitope shared by two patients presented by HLA-A*0201 as well as two

different neoantigens from two additional patients presented by HLA-DRB3*02:02. By defining the HLA alleles presenting the p53 neoantigens, they found that these alleles are present in approximately 50% of the population, indicating that an increased number of individuals (~12% of the total population with p53 mutations) could benefit from ACT directed to mutated p53. Patients might, in any case, respond to p53 mutations that are not located in hot spots. Engineering T cells with p53-specific TCRs demonstrated their ability to recognize naturally processed p53 neoepitopes in vitro, including the ability of CD4⁺ T cells to produce proinflammatory cytokines as well as to express CD107a (i.e., to degranulate), thus implying that both CD4⁺ and CD8⁺ are potentially involved in the killing activity. The same authors previously demonstrated the ability of infused CD4⁺ T cells recognizing a neoantigen from the mutated *erbb2* interacting protein (ERBB2IP) to mediate tumor regression

in a patient with metastatic cholangiocarcinoma (6). In healthy individuals, CD4⁺ T cells with cytotoxic traits occur more infrequently than CD8⁺ T cells. The role of CD4⁺ T cells in the immune response is generally neglected, although multiple lines of evidence indicate they might play an important role in the immune response against chronic viruses, including CMV (13) and HIV (14, 15). On the basis of these results, we anticipate that this topic will be extensively explored in the future.

A limitation of the study is the lack of formal proof that p53 neoantigen-specific T cells can mediate tumor regression in vivo, an aspect the authors want to tackle directly in humans by transferring the T cells isolated from TILs or by redirecting their specificity by TCR gene transfer. This is important, because ACT directed to a single mutated neoepitope rather than to multiple ones may result in reduced efficacy due to the restricted TCR repertoire of the transferred cells. Nevertheless, the study

provides a framework for potentially accelerating the discovery of neoantigen-specific T cell responses in patients with cancer. The presence of *TP53* hot spots in a wide variety of cancer types (16), as well as for other genes that are frequently mutated in cancer, including, but not limited to, *KRAS*, *EGFR*, and *PI3KCA*, marks the strategy defined within this report as highly relevant to impact a larger population of patients. Should the known hot spot mutations and HLA pairs be identified, cloned TCRs could be used as off-the-shelf ACT therapy. Alternatively, one could envisage a strategy based on target-sequencing of frequently mutated genes instead of interrogating the whole genome, followed by testing for neoantigen-specific T cell responses by using TMG or peptides that are readily available from previously created libraries.

Even though vaccines represent the holy grail of cancer treatment by maximizing benefits while minimizing toxicities, the definition of TCRs and HLA restrictions, as proposed in the paper by Malekzadeh et al., may offer a rapid approach to generating T cell products for ACT (10). Alternatively, hot spot neoantigen-specific T cells may be grown from selected subsets of TILs with increased functionality (17) or directly from the naive T cell repertoire (18), so to direct the differentiation of precursors to stem-like T cells (19) that have proven enhanced antitumor immunity owing to their increased persistence in vivo (20). Further studies will define the cohort of patients that might benefit from using T cell responses against neoantigens frequently mutated in human cancers.

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