



The Potential of Donor T-Cell Repertoires in Neoantigen-Targeted Cancer Immunotherapy

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T cells can recognize peptides encoded by mutated genes, but analysis of tumor-infiltrating lymphocytes suggests that very few neoantigens spontaneously elicit T-cell responses. This may be an important reason why immune checkpoint inhibitors are mainly effective in tumors with a high mutational burden. Reasons for clinically insufficient responses to neoantigens might be inefficient priming, inhibition, or deletion of the cognate T cells. Responses can be dramatically improved by cancer immunotherapy such as checkpoint inhibition, but often with temporary effects. By contrast, T cells from human leukocyte antigen (HLA)-matched donors can cure diseases such as chronic myeloid leukemia. The therapeutic effect is mediated by donor T cells recognizing polymorphic peptides for which the donor and patient are disparate, presented on self-HLA. Donor T-cell repertoires are unbiased by the immunosuppressive environment of the tumor. A recent study demonstrated that T cells from healthy individuals are able to respond to neoantigens that are ignored by tumor-infiltrating T cells of melanoma patients. In this review, we discuss possible reasons why neoantigens escape host T cells and how these limitations may be overcome by utilization of donor-derived T-cell repertoires to facilitate rational design of neoantigen-targeted immunotherapy.

Keywords: neoantigen, immunotherapy, T cell, donor, allogeneic hematopoietic stem cell transplantation, donor lymphocyte infusion, minor histocompatibility antigen, graft versus tumor effect

INTRODUCTION

Neoantigens derived from somatic mutations in cancer cells and recognized as foreign by T cells are arising as the most attractive targets of cancer immunotherapy. They are expressed exclusively in malignant cells, making them truly tumor specific, and the T-cell repertoire recognizing them is not affected by central tolerance mechanisms. Recent studies have demonstrated a correlation between the clinical benefit of cancer immunotherapies such as checkpoint inhibition, with mismatch-repair deficiency, burden of somatic nonsynonymous mutations and neoantigen load (1–5), and neoantigen-reactive T cells have been detected in many tumors (Table 1).

Many tumors harbor a large number of mutations that potentially can give rise to neoepitopes (26). All mutations leading to single amino acid substitutions, reading-frame alterations, splice variants, inversions, fusions, and aberrant posttranslational modifications, have the potential to generate neoantigens. For a neoantigen to be immunogenic, it has to be expressed at sufficient levels, have the correct subcellular localization to enter proteasomes, be efficiently processed and

Abbreviations: alloHSCT, allogeneic hematopoietic stem cell transplantation; CAR, chimeric antigen receptor; DLI, donor lymphocyte infusion; GvHD, graft versus host disease; GvT, graft versus tumor; HLA, human leukocyte antigen; mHAg, minor histocompatibility antigen; TCR, T-cell receptor; TIL, tumor-infiltrating lymphocyte.

TABLE 1 | Only a small fraction of candidate neoantigens elicits spontaneous responses in the cancer patient's autologous T-cell repertoire.

Tumor type (source of neoantigen-reactive T cells)	Total number of nonsynonymous mutations/number of patients analyzed	Mutations encoding neoepitopes/mutations screened (immunogenic peptides identified/peptides screened^a)	% Mutations encoding neoepitopes (% immunogenic peptides^a)	Test method (pipeline for preselection)	Reference
Autologous, spontaneous					
Melanoma (TIL)	1,084/1	2/448 ^a	0.4 ^a	pMHC (RNAseq, NetChopCterm3.0, NetMHC3.2)	(6)
Melanoma (TIL)	1,116/3	7/191 11/227 ^a	3.7 4.8 ^a	IFNg (NetMHCpan2.4)	(7)
Melanoma (TIL)	np/2	2/288	0.7	TM and IFNg (none)	(8)
Ovarian cancer (TAL)	93/3	1/79	1.3	ELISpot (NetMHCpan2.4)	(9)
Gastrointestinal cancers (TIL)	773 ^b /10	18/1,452	1.2	TM and ELISpot/CD137 (none)	(10, 11)
Melanoma (FTD and TIL)	19,597/8	10/369 ^a	2.7 ^a	pMHC (RNAseq, IEDB)	(12)
Melanoma [TIL or PBMC-derived infusion product]	2,386/5	8/1,543	0.5	ELISA (RNAseq)	(13)
Melanoma (CD8 ⁺ PD-1 ⁺ PBMC)	1,479/4	7/691	1.0	TM and ELISpot/CD137 (RNAseq)	(14)
Melanoma (TIL)	1,100/3	2/201 2/391 ^a	1.0 0.5 ^a	pMHC (RNAseq, NetChopCterm3.0, NetMHC3.2, or NetMHCpan2.0)	(15)
Melanoma (TIL)	>4,000/1	10/720	1.4	TM and IFNg ELISA (RNAseq, IEDB)	(16)
Melanoma (TIL)	np/4 ^c	12/675	1.8	TM and CD137 (IEDB)	(17)
NSCLC (TIL and T cells from adjacent normal tissue)	np/2	3/642 ^a	0.5 ^a	pMHC (NetMHCpan2.8)	(18)
Melanoma (TIL)	1,019/1	1/2	50.0	ELISA (MS/MS)	(19)
NSCLC (TIL and PBMC)	np/2	9/705 ^a	1.3 ^a	DNA-barcoded pMHC staining (NetMHCpan2.8)	(20)
Autologous, therapeutically induced					
NSCLC (PBL after anti-PD-1)	324/1	1/99 1/148 ^a	1.0 0.7 ^a	pMHC (NetMHC3.4)	(3)
Colorectal cancer (PBMC after anti-PD-1)	1,477 ^b /1	3/15 3/15 ^a	20.0 20.0 ^a	ELISpot (ImmunoSelect-R)	(5)
Melanoma (PBMC after anti-CTLA-4)	2,329/1	2/8 2/8 ^a	25.0 25.0 ^a	ELISpot (LC-MS/MS)	(21)
Melanoma (PBMC after peptide loaded dendritic cell vaccination)	1,099 ^d /3	9/21 9/21 ^a	42.9 42.9 ^a	pMHC (MS/MS)	(22)
Lung squamous cell carcinoma (PBMC after peptide vaccination)	93/1	4/5 6/11 ^a	80.0 54.5 ^a	ELISpot (NetMHC3.4)	(23)
Melanoma (PBMC after peptide vaccination)	4,729/6	15/91 58/97	16.5 (CD8) 59.8 (CD4)	ELISpot (RNAseq, NetMHCpan2.4)	(24)
Donor derived					
CLL (PBMC after alloHSCT)	51/2	3/25 3/48 ^a	12.0 6.3 ^a	ELISpot (NetMHCpan2.4)	(25)

(Continued)

TABLE 1 | Continued

Tumor type (source of neoantigen-reactive T cells)	Total number of nonsynonymous mutations/number of patients analyzed	Mutations encoding neoepitopes/mutations screened (immunogenic peptides identified/peptides screened ^a)	% Mutations encoding neoepitopes (% immunogenic peptides ^a)	Test method (pipeline for preselection)	Reference
Melanoma (PBMC from healthy individuals)	6,413/5	4/11 4/11 ^a	36.4 36.4 ^a	ELISpot (LC-MS/MS)	(21)
Melanoma (PBMC from healthy individuals)	1,100/3	10/45 11/57 ^a	22.2 19.3 ^a	pMHC (RNAseq, NetMHC4.0)	(15)

Summary of studies in which mutations encoding candidate neoantigens were identified by whole-exome sequencing and systematically screened for recognition by T-cells. For each study, the table indicates the number of mutations encoding neoepitopes (immunogenic peptides) that were identified among the number of mutations screened, and/or, when indicated by an ^a, the number of immunogenic peptides (neoepitopes) identified among the total number of peptides screened (multiple candidate peptides can be screened for a single mutation).

^bTotal number of mutations (when number of nonsynonymous mutations was not reported).

^cOnly the four patients for which tandem minigene constructs were available are included.

^dNonsynonymous mutations in lymph node or axilla.

np, information not provided; TIL, tumor-infiltrating lymphocytes; TAL, tumor-associated lymphocytes; FTD, fresh tumor digest; PBMC, peripheral blood mononuclear cells; NSCLC, non-small-cell lung carcinoma; PBL, peripheral blood lymphocytes; CLL, chronic lymphocytic leukemia; TM, tandem minigene; MS/MS, tandem mass spectrometry; LC-MS/MS, liquid chromatography-tandem mass spectrometry; pMHC, peptide-major histocompatibility complex molecule multimers; IEDB, The Immune Epitope Database and Analysis Resource.

transported to the endoplasmic reticulum, be loaded on human leukocyte antigen (HLA)-molecules with high enough affinity, form a complex with HLA with sufficient stability, and be efficiently recognized by the patient T-cell repertoire. Current sequencing techniques and computational sequence analysis tools enable rapid calling of somatic mutations from individual tumors. However, identification of verified neoepitopes from the large pool of candidate peptides remains a major challenge on the way to efficiently exploit each patient's unique set of neoantigens for targeted immunotherapy. The vast majority of neoantigens are personal and few shared neoantigens have been found to be immunogenic, highlighting the need for efficient strategies for fast identification of personal neoepitopes. Prediction methods for HLA-binding affinity, at least for the most frequent HLA class I alleles, can narrow down the number of candidate neoepitopes, but are insufficient in predicting proteasomal processing, transport of the peptides, stability of the peptide-HLA complexes, and recognition by T cells. This makes the identification of clinically relevant neoepitopes with therapeutic potential challenging.

In this review, we discuss possible reasons for the insufficiency of the patient's T cells to respond to neoantigens and experience gained from the use of donor T cells in the treatment of hematological malignancies. We will also focus on recent insights gained from the use of T-cell repertoires from healthy individuals to identify immunogenic neoantigens and present possibilities these insights open for the efficient clinical exploitation of personal neoantigens.

THE AUTOLOGOUS T-CELL REPERTOIRE OF THE PATIENT FREQUENTLY FAILS TO CONTROL CANCER PROGRESSION

The potential of neoantigen-specific T cells to induce cancer regression has unequivocally been demonstrated. Infusion of selected patient-derived, neoantigen-reactive T cells were shown to induce objective clinical responses in a patient with epithelial cancer, treated with enriched CD4 T cells recognizing

a mutant *erbb2* interacting protein (ERBB2IP)-derived peptide (10), and in a colorectal cancer patient treated with a cytotoxic T-cell pool consisting of four different clonotypes specific for KRAS G12D-derived peptides presented by HLA-C*08:02 (27). Analysis of T cells derived from tumor-infiltrating lymphocytes (TIL) or peripheral blood has, however, shown that the frequency of neoantigens that can elicit such responses is low: Only about 1.2% of mutations with neoantigenic potential are spontaneously recognized in patients with melanoma, gastrointestinal, lung, and ovarian cancers. T cells responsive to 68 of the 5,842 candidate neoantigens screened were found in 36 patients included in 11 studies (Table 1; 7–11, 13–17, 19). Similarly, very few mutations were identified that evoke an immune response in patients treated with checkpoint inhibition (3). A multitude of mechanisms might collectively be responsible for this. Tumors can actively suppress existing T-cell responses, extensively reviewed elsewhere (28). Strategies include secretion of immunosuppressive cytokines, attraction of regulatory T cells or myeloid-derived suppressor cells, upregulation of the expression of inhibitory molecules, such as immune checkpoint receptors on T cells (29) and their ligands in tumor cells, loss or mutation of HLA-molecules (30–33), target antigens (34), or activating co-receptors resulting in induction of T-cell anergy. Peripheral tolerance mechanisms can also lead to clonal deletion and permanent loss of T cells recognizing abundantly expressed antigens in murine models (35). Parallel tracking of neoantigen-specific T cells and cognate tumor cells in an ovarian cancer patient with progressive disease showed that expansion of a tumor clone was accompanied by disappearance of the reactive T cells, suggesting deletional tolerance (9, 36). The possibility to therapeutically reverse immunosuppression has been demonstrated by checkpoint inhibition, which has led to impressive clinical responses in multiple cancer types, reviewed in Ref. (37). A large number of patients do, however, not respond, and the great majority of treated patients eventually relapse. Two recent papers have shed light on potential mechanisms for this.

Persistent stimulation by cognate antigen or exposure to inflammatory signals can lead to T cell exhaustion, which impairs

T-cell effector functions. This dysfunctional state is characterized by an altered transcriptional program, including high expression of multiple inhibitory receptors such as programmed cell death 1 (PD-1). Recent studies indicate that exhausted T cells acquire an epigenetic profile that is distinct from that of effector and memory T cells and can only minimally be remodeled by PD-1 blockade therapy (38, 39). This epigenetic programming of T cells from a functional to dysfunctional state is suggested to occur in two phases, initially to a plastic state from which T cells can be rescued, and subsequently to a fixed dysfunctional state where T cells are resistant to reprogramming (40). This could possibly explain frequent clinical relapses following treatment with immune checkpoint inhibitors and has profound implications for the development of future immunotherapies. If the tumor-responsive T-cell repertoire of the patient is incapable of exerting lasting tumor control even if inhibitory signals are discontinued, new and innovative ways to activate the immune system are required.

BROADENING THE ENDOGENOUS T-CELL RESPONSE TO NEOANTIGENS

Several studies have demonstrated that personal neoantigen vaccination protocols can elicit neoantigen-specific T-cell responses that are not detectable before vaccination (22–24), suggesting that insufficient priming partially accounts for limited neoantigen-specific T-cell responses in cancer patients. Two clinical studies pioneering mRNA-based (33) and peptide-based (24) personalized neoantigen vaccines in metastatic melanoma recently demonstrated clinical relevance of vaccination-induced responses. Thus, reduction in the frequencies of metastatic events (33) and direct recognition of tumor by some of the induced T-cell specificities (24, 33) were observed. T-cell responses to the majority of the vaccine antigens were *de novo* responses, supporting the view that the potential of the T-cell repertoire can be optimized by more effective priming. Such *de novo* responses are expected to mobilize naïve T cells that are not exhausted or dysfunctional. In the study by Ott et al., 16% of the peptides used for vaccination induced a CD8 response and 60% a CD4 response (24). Clinical response rates might, however, increase if an even higher number of verified CD8 epitopes could be included and a larger fraction of the induced T-cell specificities would translate into tumor-reactive responses (24). Thus, further studies to improve on antigen selection might be advantageous.

DONOR-DERIVED T CELLS CAN MEDIATE GRAFT-VERSUS-TUMOR EFFECTS FOLLOWING ALLOGENEIC HEMATOPOIETIC STEM CELL TRANSPLANTATION (alloHSCT)

In alloHSCT, donor-derived T cells can overcome the insufficiency of patient immunity. Alone or in combination with donor lymphocyte infusions (DLI), alloHSCT is frequently used to treat hematological cancers and still remains the only potentially curative treatment for many hematological malignancies [reviewed in Ref. (41)]. In an HLA-matched alloHSCT, the desired

graft-versus-tumor reactivity (GvT) is thought to be mainly mediated by donor T cells recognizing peptides from polymorphic proteins, so called minor histocompatibility antigens (mHAg). These are generated by genetic differences between the donor and the host and presented by matched HLA on the malignant cells [reviewed in Ref. (42)]. Immunogenic mHAg in the recipient are recognized by T cells from a donor lacking the immunogenic allele. mHAg can be encoded by the Y chromosome or be autosomal. Autosomal mHAg are most commonly derived from nonsynonymous single nucleotide polymorphisms, which result in single amino acid differences in the encoded proteins. Thus, mHAg are seen as “neoantigens” by the donor T cells. Hematopoietic cells are preferentially recognized as they are more easily accessible than cells in solid tissues and they frequently express high levels of HLA class I and II, costimulatory receptors and adhesion molecules [reviewed in Ref. (42)]. However, donor T-cell reactivity to broadly expressed immunogenic mHAg on healthy tissues bears the risk of potentially detrimental graft-versus-host disease (GvHD). DLIs can induce complete remissions in patients with relapsing leukemia after alloHSCT (43–46). The GvT effect is considered to be dependent on the presence of host antigen-presenting cells capable of efficiently displaying recipient’s hematopoietic lineage-restricted mHAg to the donor T cells (47).

The powerful immune responses of GvT and GvHD demonstrate the ability of donor T cells to attack and kill defined cell types dependent on recognition of antigens differing between host and donor by a single amino acid. In fact, it is possible that tumor-specific neoantigens also serve as clinically relevant targets mediating GvT following alloHSCT. This could be suggested by the fact that syngeneic alloHSCT from a genetically identical twin can result in similar long-term disease-free survival rates as alloHSCT from an HLA-matched donor, but in the absence of allogeneic GvT (48–50). The relevance of neoantigens as targets for GvT was shown by a study in which two chronic lymphocytic leukemia patients with durable remission after alloHSCT were monitored for cytotoxic T-cell responses against predicted tumor-specific neoantigens and found to mount long-term responses against personal neoantigens derived from three different genes (25). As donor T cells have not been exposed to the peripheral tolerance mechanisms of the tumor, they can strongly recognize defined mHAg or neoantigens presented by patient cancer cells. The possibility to specifically target donor T cells to patient neoantigens has, however, not been therapeutically explored thus far.

DONOR-DERIVED T-CELL RESPONSES REVEAL A HIGH FREQUENCY OF IMMUNOGENIC NEOANTIGENS

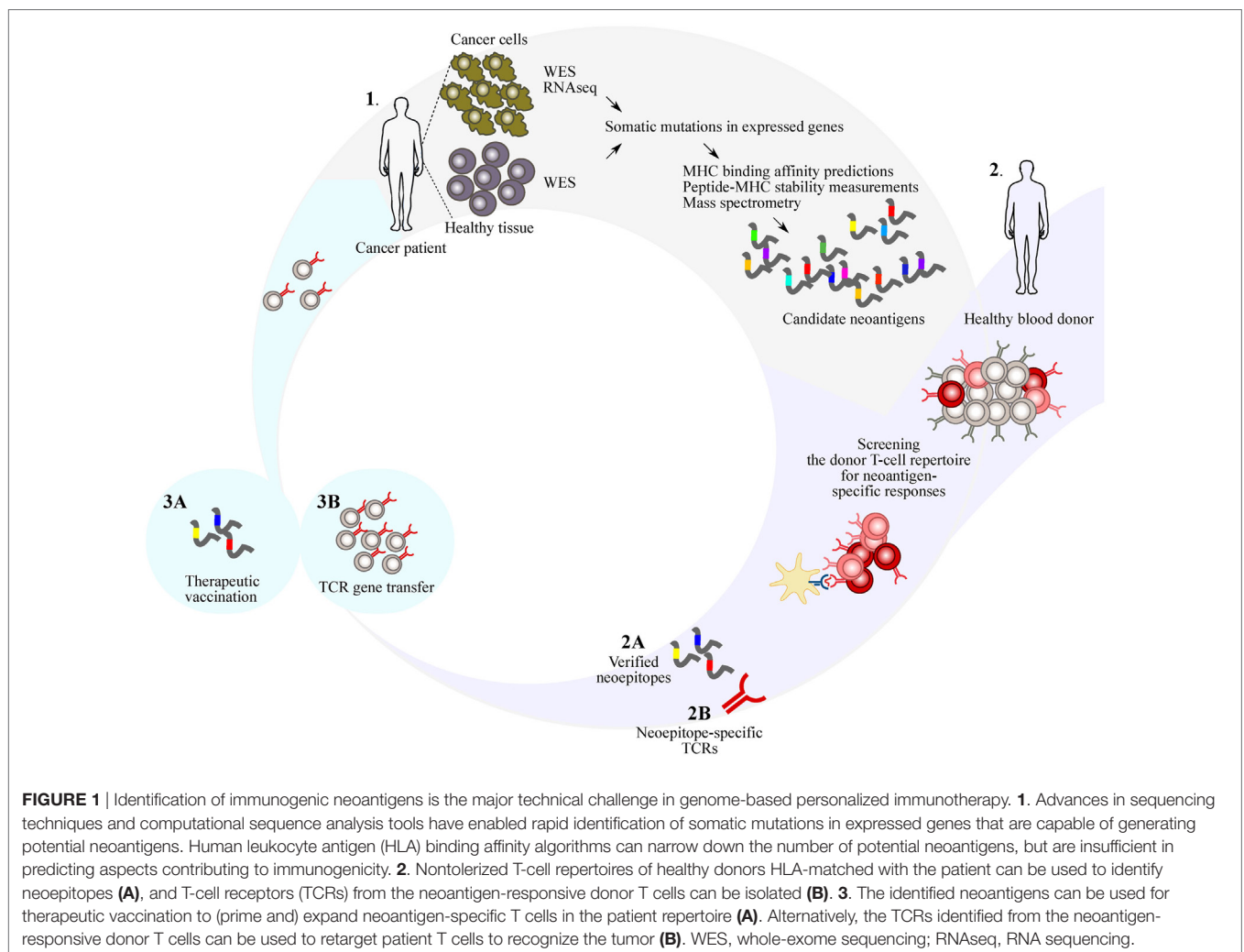
Advancements in sequencing techniques and computational sequence analysis tools have enabled fast identification of somatic mutations in expressed genes in individual tumors. The precision level of computer algorithms predicting potential neoepitopes recognized by T cells is, however, not known. A main challenge, therefore, remains to rapidly select among the large number of candidate neoantigens those that translate into clinically efficient immune responses. The uncompromised T-cell repertoires from HLA-matched donors hold an unrealized potential

when addressing these challenges as they retain their inherent capability to respond to immunogenic neoantigens. Donor T-cell repertoires could thus be applied for identification of neoepitopes, independently of blood sampling from the patient. This was recently demonstrated by Strønen et al. by coculturing the non-adherent fraction of peripheral blood mononuclear cells with autologous monocyte-derived dendritic cells electroporated with *in vitro*-translated tandem minigene library RNA encoding potential neoepitopes from three melanoma patients, and by detecting neoantigen-responsive T cells with fluorescently labeled HLA-multimers. The study revealed that 11 out of 57 predicted HLA-A*02:01-binding neoepitopes were recognized by the nontolerized CD8 T-cell repertoires of healthy blood donors, 10 of which were ignored by the autologous TILs of the patient (15). Importantly, donor T-cell populations also recognized cognate neoepitopes when endogenously presented by the patient's tumor cells, suggesting high-avidity T-cell responses. This study showed that a much higher frequency of neoantigens was immunogenic than was anticipated from analyzing the patient's autologous *in vivo* T-cell responses. T-cell receptors (TCRs) isolated from the neoantigen-reactive donor T cells efficiently retargeted third party T cells to recognize patient-derived melanoma cells

harboring the targeted mutations, suggesting that patient T cells redirected with neoantigen-targeted TCR could be effective in gene therapy (15).

DONOR-DERIVED IMMUNITY TO DESIGN PERSONALIZED IMMUNOTHERAPY

When the endogenous T-cell repertoire of the cancer patient is insufficient at controlling the disease, donor-derived immunity might provide rescue. One of the most successful examples is the adoptive transfer of T cells genetically modified by chimeric antigen receptors (CARs). T cells engineered to express CARs, harboring the antigen-recognition domain of an antibody grafted onto signaling domains that confer T-cell activation, can mediate selective killing of defined cell subsets. Treatment with CD19-targeted CAR T-cells consistently leads to complete response rates of 70–90% in acute lymphoblastic B-cell leukemia and has shown promise in non-Hodgkin B-cell lymphoma (51). The success of CAR T-cells to treat B-cell malignancies has, however, yet to be extended to other hematological cancers or solid tumors. A major obstacle is the identification of cell-type specific cell-surface



molecules that can be safely targeted. In contrast to CARs, TCRs can recognize also intracellular antigens when presented in the context of surface MHC-molecules, opening the possibility to employ neoantigen-responsive TCRs to redirect patient T cells. TCRs from neoantigen-responsive donor T cells can be isolated and introduced into patient's naïve or central memory T cells with high proliferative and functional potential. Adoptive transfer of genetically engineered T cells can create specificities not present or irreversibly inhibited in the patient, thus broadening the spectrum of the naturally occurring antitumor immunity (Figure 1). However, to make gene therapy with personalized, neoantigen-specific TCRs a feasible clinical option, the time required to identify clinically effective and safe TCRs has to be reduced. Furthermore, the cost and labor-intensive procedures associated with, and facilities required for, current retro- or lentiviral protocols for gene transfer are prohibiting widespread use of neoantigen-specific TCR gene therapy. Non-viral approaches for gene transfer might represent promising alternatives [discussed in Ref. (52)]. To this end, a recent study demonstrated efficient *in vivo* delivery of CAR genes using T-cell-targeted nanoparticles in mice, possibly representing a practical way to rapidly deliver genes (53).

A systematic screening for immunogenic neoantigens from the big pool of candidate epitopes using donor T-cell repertoires could advance our understanding of the rules determining immunogenicity. This could in turn enable development of more accurate prediction tools to identify neoepitopes. The need for sampling of blood from often heavily pretreated patients, and the use of patient T cells impaired by a variety of immunosuppressive mechanisms, would thus be circumvented. Proof of principle that such a screening with donor T-cell repertoires is possible was shown in Ref. (15), but the development of faster culture protocols and rapid identification of high-affinity TCRs would be desirable.

Screening for the ability of candidate neoantigenic peptides to induce responses in CD8 T cells from healthy, donor-derived T-cell repertoires was combined with a novel, flow cytometry-based assay to measure peptide-MHC stability (15). The results demonstrated that immunogenic neoantigens had a significantly longer half-life than the non-immunogenic ones. In fact, addition of measured peptide-HLA stability to predicted binding-affinity of the peptide to HLA significantly improved the precision levels by which the immunogenic peptides could be identified. These data corroborate well with previous studies of microbial peptides (54, 55), indicating that peptide-MHC stability is a better predictor of immunogenic peptides than peptide-MHC binding-affinity. Thus, development of assays which facilitate high-throughput stability measurements are called upon.

CONCLUDING REMARKS

Tumors implement several immunosuppressive mechanisms to evade the immune defense of the cancer patient. These peripheral tolerance mechanisms can either reversibly or irreversibly impede the effector function of the patient's tumor-targeting T-cell repertoire. Immunotherapies with expanded TILs or checkpoint blockade rely on specificities present in the patient's own T-cell

repertoire. Although clinical benefits are remarkable, they are often transient.

Transplantation of the immune system from an HLA-matched donor, which has not been compromised by tumor-induced peripheral tolerance, can induce cures in patients with hematological malignancies. The beneficial and powerful GvT effect of alloHSCT is mainly driven by donor T cells recognizing single amino acid differences in polymorphic peptides. However, since the targets are unknown and unpredictable, the desired GvT effect may be accompanied by potentially detrimental GvHD.

Personalized immunotherapies aim to explicitly target tumor-specific neoantigens, minimizing the risk of T-cell attack on healthy tissues. However, several hurdles have to be overcome to make genome-based approaches a treatment option for large groups of patients. Whole-exome sequencing can rapidly identify possible neoantigens in individual tumors, but defining those neoantigens that are immunogenic and clinically applicable remains a time-consuming, demanding task. Here, the unlimited source of donor T-cell repertoires can prove very informative. Identification of immunogenic neoantigens can guide the design of personalized vaccination and adoptive T-cell transfer therapies, and educate algorithms to become more accurate in predicting neoantigen immunogenicity. Neoantigen-reactive donor T cells can also provide TCRs, which can be used to retarget patient's naïve T cells to attack the tumor. Simultaneous targeting of multiple neoantigens expressed homogeneously in the tumor and essential for maintaining the tumorigenic phenotype, thus unlikely to be lost, might be ideal to achieve durable clinical responses. Off-the-shelf TCRs targeting neoantigens derived from driver mutations recurrent in large patient groups and in prevalent tumor types would be most practical. Such neoantigens are, however, scarce and appear poorly immunogenic when presented on patient HLA (56). Thus, there is a high demand for strategies to rapidly define clinically applicable personal neoantigens, a challenge that potentially can be answered by donor T-cell repertoires.

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Both authors made substantial intellectual contribution to the manuscript, and approved it for publication.

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Conflict of Interest Statement: JO is engaged in a research collaboration with Kite Pharma.

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