Cancer immunotherapy targeting neoantigens

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ABSTRACT

Neoantigens are antigens encoded by tumor-specific mutated genes. Studies in the past few years have suggested a key role for neoantigens in cancer immunotherapy. Here we review the discoveries of neoantigens in the past two decades and the current advances in neoantigen identification. We also discuss the potential benefits and obstacles to the development of effective cancer immunotherapies targeting neoantigens.

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1. Introduction

The foundation of immunology is based on self-nonself discrimination [1]. Most of the pathogens contain molecular signatures that can be recognized by the host and trigger immune responses [2]. Unlike pathogens, these molecular signatures are not generally expressed by tumor cells, making them more difficult to be distinguished from normal cells. However, T cells can recognize tumor antigens expressed by tumor cells. A class of tumor antigens, named tumor-associated antigens, is expressed in some normal tissues at low levels but is over-expressed in malignant cells. Many of the tumor-associated antigens have been identified as the targets of tumor-reactive T cells, isolated from tumor infiltrating lymphocytes (TILs), from draining lymph nodes or from peripheral blood [3]. However, expression of these antigens in normal cells can trigger central and peripheral tolerance mechanisms that lead to the selection of T cells with low-affinity T cell receptors (TCR). Conversely, attempts to target tumor-associated antigens with high-affinity TCRs can lead to severe toxicities due to normal tissue destruction [4,5].

Another class of tumor antigens is tumor-specific neoantigens, which arise *via* mutations that alter amino acid coding sequences (non-synonymous somatic mutations). Some of these mutated peptides can be expressed, processed and presented on the cell surface,

and subsequently recognized by T cells. Because normal tissues do not possess these somatic mutations, neoantigen-specific T cells are not subject to central and peripheral tolerance, and also lack the ability to induce normal tissue destruction. As a result, neoantigens appear to represent ideal targets for T cell-based cancer immunotherapy.

2. Approaches to identify T cell neoantigens

2.1. Classical approaches

Some of the initial attempts were focused on common shared mutations that have been well-characterized. Short peptides were synthesized based on the sequences of mutated BRAF [6,7], KRAS [8–11] and p53 [12]. T cells from patients' or healthy donors' blood were stimulated several times by peptide-pulsed target cells, and expanded T cells were studied for their ability to kill tumors carrying these mutations. Alternatively, cells with overexpressed mutated cDNA, such as mutated NRAS cDNA, could serve as target cells to detect and isolate neoantigen-reactive T cells [13].

However, the majority of the neoantigen-reactive T cells recognized unique mutations not shared between cancer patients. Most of the unique neoantigens were identified by cDNA library screening in the past two decades. In this approach, cDNA library and MHC molecules were over-expressed in cell lines, and then co-cultured with T cells to identify antigens that could induce the T cell activation, measured by cytokine secretion or 4-1BB up-regulation. Table 1 is the list of published neoantigens identified by this approach.

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Table 1
Human neoantigens discovered by classical approaches.

Cancer type	Year	Mutated gene name	Approach	Source of T cells	Reference
Melanoma	1995	CDK4	cDNA library	PBL	[14]
Melanoma	1995	MUM1	cDNA library	PBL	[57]
Melanoma	1996	CTNNB1	cDNA library	TIL	[15]
Melanoma	1999	CDC27	cDNA library	TIL	[58]
Melanoma	1999	TRAPPC1	cDNA library	PBL	[59]
Melanoma	1999	TPI	Chromatographic purification	TIL	[60]
Melanoma	2000	ASCC3	cDNA library	PBL	[61]
Melanoma	2001	HHAT	cDNA library	TIL	[62]
Melanoma	2002	FN1	cDNA library	TIL	[63]
Melanoma	2002	OS-9	cDNA library	PBL	[64]
Melanoma	2003	PTPRK	cDNA library	TIL	[65]
Melanoma	2004	CDKN2A[**], HLA-A11	cDNA library	TIL	[18]
Melanoma	2005	GAS7, GAPDH	cDNA library	TIL	[66]
Melanoma	2005	SIRT2, GPNMB, SNRP116, RBAF600, SNRPD1	cDNA library	PBL	[67]
Melanoma	2005	Prdx5	cDNA library	PBL	[68]
Melanoma	2011	CLPP	cDNA library	TIL	[69]
Melanoma	2013	PPP1R3B	cDNA library	TIL	[46]
Lung cancer	1998	EF2	Chromatographic purification	PBL	[20]
Lung cancer	2001	ACTN4	cDNA library	TIL	[70]
Lung cancer	2001	ME1	cDNA library	PBL	[71]
Lung Cancer	2006	NF-YC	cDNA library	draining lymph node	[72]
Renal cancer	1996	HLA-A2	cDNA library	PBL	[17]
Renal cancer	1999	HSP70-2	cDNA library	TIL	[73]
Renal cancer	2005	KIAA1440	cDNA library	PBL	[74]
HNSCC	1997	CASP8	cDNA library	PBL	[16]

TIL: tumor infiltrating lymphocytes; PBL: peripheral blood lymphocytes; HNSCC: Head and neck squamous cell carcinoma.

[**] Frame-shift mutation.

Neoantigens have been identified predominantly in melanoma, likely due to the relatively high mutation rate in this tumor type. Nonetheless, neoepitopes have also been identified in multiple tumor types including lung and renal cancers. The majority of neoantigens were encoded by point-mutated gene products, although frameshift deletion and insertions have also been found to generate neoepitopes. Some mutated gene products recognized by T cells appear to be driver mutation products and play a role in tumorigenesis. These include CDK4, β-catenin (CTNNB1) and Caspase-8 (CASP8) proteins [14–16]. Interestingly, HLA-A2 and A11 containing point-mutations have also been identified as tumor-specific T cell antigens, suggesting that somatic mutations in HLA molecules can be a source of neoantigens [17–19].

In another approach, a mutated (EF2) and non-mutated (gp100:154) T cell epitopes have been identified by using mass spectrometry to sequence peptides that were eluted from HLA molecules isolated from the surface of tumor cells [20]. This approach has been limited by the sensitivity of these methods. Nevertheless, recent advances in the sensitivity and throughput of these techniques may facilitate the application of this approach to identifying tumor antigens.

2.2. Approaches utilizing next-generation sequencing techniques

Although the classical cDNA library screening approach led to the discovery of multiple neoantigens, this approach is labor-intensive and low-throughput. In addition, some large transcripts, GC-rich transcripts and low-expression transcripts cannot be cloned easily, leading to the failure of identifying some mutated antigens. Recently, several investigators have taken advantage of whole-exome sequencing technologies to identify non-synonymous mutations in tumors. These mutated genes were subjected to *in silico* analysis to predict potential high-affinity epitopes that bind to MHC molecules [21–23]. Additional filters could be applied to eliminate (1) epitopes predicted to be poorly processed by the immunoproteasome and (2) epitopes with lower binding affinity than the corresponding wild-type sequences [24]. Candidate mutated peptides were synthesized and screened to

identify T cell neoantigens. This approach could be very efficient, and could identify neoantigens where previous cDNA library screening failed to discover [25].

This peptide-based screening approach, while effective in identifying many epitopes, may nevertheless be limited by the accuracy of MHC/HLA-binding prediction algorithms, which have not been thoroughly examined for MHC class II alleles and infrequent HLA alleles. In addition, the expression of potential T cell epitopes on the cell surface is influenced by a variety of mechanisms, especially the expression of multiple forms of the proteasome, which limit the number of peptides that are truly processed and presented [26,27]. An alternative approach is pulsing antigen presenting cells with relatively long synthetic peptides that encompass minimal T cell epitopes [28]. In a recent report, nonsynonymous mutated epitopes were identified in three melanoma lesions by evaluating the response of CD4^{+} TIL to autologous B cells that were pulsed with 31 amino-acid long peptides encompassing individual mutations. Use of this approach resulted in the identification of mutated CIRH1A, GART, ASAP1, RND3, TNIK, RPS12, ZC3H18 and LEMD2 T cell epitopes. Furthermore, in a recent report, a peptide screening was carried out based on the combination of two peptide libraries: (1) 15-mer overlapping long-peptides (2) peptides based on MHC-binding prediction. This screening led to the identification of mutated HSDL1-reactive T cells isolated from an ovarian tumor [29].

To solve all the potential issues mentioned above, we have developed a tandem minigene screening approach [30]. A tandem minigene construct comprised 6 to 24 minigenes that encoded polypeptides containing a mutated amino acid residue flanked on their N- and C-termini by 12 amino acids. Tandem minigene constructs were synthesized and used to transfect autologous APCs or cell lines co-expressing autologous HLA molecules. Using this approach, mutated KIF2 C and POLA2 epitopes were identified in two melanoma patients [30]. In addition, a mutated ERBB2IP epitope was identified in a patient with cholangiocarcinoma [31]. Recent studies using this approach have led to the identification of mutated antigens express on gastrointestinal, breast and ovarian cancers [32] (and unpublished data). Notably, the neoantigen

reactivity could be identified from TILs isolated from patients with cholangiocarcinoma or gastrointestinal cancer, which has a relatively low number of mutations.

A recent approach combined whole-exome/transcriptome sequencing analysis, MHC binding prediction, as well as mass spectrometric technique to detect peptides eluted from HLA molecules [33]. Interestingly, only a small fraction of predicted high-binding peptides were confirmed by mass spectrometry. The relative small number of mutated peptides identified by mass spectrometry might be due to the sensitivity of the peptide purification and mass spectrometry, but it could also suggest that natural antigen process and presentation in cells could be very inefficient. Among 7 neoepitopes identified by this approach, mutated Adpgk, Reps1 and Dpagt1 epitopes were confirmed to be immunogenic [33].

3. The correlation between neoantigens and tumor regressions

It has been established that T cells recognizing non-mutated tumor antigens could induce tumor regression [34]. This has been demonstrated by clinical trials involving adoptive transfer of T cells targeting HLA-A*0201-restricted NY-ESO-1. Objective responses were observed in 61% of patients with synovial cell sarcoma, 55% of patients with melanoma and 80% of patients with myeloma [35–37]. However, it remains unclear whether T cells recognizing neoantigens can also induce tumor regression in human.

In the past few years, there have been significant advances in checkpoint blockade cancer immunotherapy. Tumor regressions were observed in a portion of cancer patients who underwent CTLA-4, PD-1 or PD-L1 blockade clinical trials [38]. Currently, the widely acceptable hypothesis is that tumors with more mutations likely generate more neoepitopes, which can be recognized by tumor infiltrating T cells. Checkpoint blocking antibodies reactivate these T cells *in vivo* and induce tumor regressions. As a result, cancers with high mutation rates, such as melanoma and lung cancer, are more susceptible to checkpoint blockade therapies. (One notable exception for this hypothesis is renal cell carcinoma, which is susceptible to checkpoint blockade therapies but with low mutation load). A recent study in a murine model has provided some evidence for this hypothesis. In an anti-PD-1 and/or anti-CTLA-4 immunotherapy model, two dominant neoantigens asparagine-linked glycosylation 8 (Alg8) and laminin alpha subunit 4 (Lama4) were identified from a sarcoma cell line d42m1-T3. Mutated Lama4 and Alg8 long peptide vaccines could induce tumor rejection comparable to checkpoint blockade immunotherapy. In addition, the checkpoint blockade immunotherapy increased the number and enhanced the activity of neoantigen-specific CD8+ T cells [24]. These results suggested that neoantigens and neoantigen-specific T cells were strongly associated with tumor regressions after checkpoint blockade therapy.

In human, recent studies have established the correlation between the number of mutations/neoantigens and clinical outcomes. In a PD-1 blockade clinical trial comparing colorectal cancer patients with or without mismatch-repair deficiency, prolonged progression-free survival was associated with high somatic mutations, which were found in tumors with mismatch repair-deficiency [39]. Other studies utilized *in silico* analyses to predict potential high-affinity neoepitopes for calculating the numbers of neoantigens. In two anti-CTLA-4 melanoma immunotherapy studies, the number of neoantigens was significantly associated with clinical benefits after CTLA-4 blockade therapies [40,41]. However, it's unclear whether the presence of "tetrapeptide" signatures within predicted T cell epitopes could be employed to predict the clinical outcomes for the anti-CTLA-4 immunotherapy [40,41]. Similar to CTLA-4 blockade studies, higher neoantigen burden was correlated with clinical benefit and progression-free survival in

PD-1 blockade immunotherapy for patients with non-small cell lung cancer [42]. These studies suggested that the number of neoantigens was positively associated with clinical benefit after immune checkpoint blockade therapies. Lastly, adoptive transfer of mixed TILs could induce tumor regressions in melanoma patients [43]. In a recent study from our group, objective tumor regressions in three melanoma patients were associated with adoptive transferring of TILs, which all recognized neoantigens [25]. In a subsequent study, two TIL products were associated with complete tumor regressions observed in two melanoma patients, and each TIL could recognize one unique neoantigen [30]. Taken together, these correlative studies suggested that neoantigen-reactive T cells were likely the dominant player inducing tumor regressions in patients.

4. Current evidence of cancer immunotherapy targeting neoantigens

The first approach to target neoantigens is *via* cancer vaccine. In an initial mouse study, candidate mutated epitopes were identified by whole-exome sequencing of the B16F10 murine melanoma. Fifty selected mutation-coding long peptides were injected into mice to elicit immune responses, and 11 out of 50 peptides induced immune responses preferentially recognizing the mutated epitopes. Among these 11 peptides, mutated Kif18b (K739 N) was found to be the dominant mutated antigens, and mice immunized with mutated Kif18b peptide could slow tumor growth and improve survival [22]. In another study, MC-38 tumor-bearing mice vaccinated with mutated peptides (Adpgk, Reps1 and Dpagt1) showed sustainable inhibition of tumor growth [33]. Recently, a synthetic RNA "pentatope" vaccine was developed. Each pentatope contained five 27-mer minigenes with the mutated amino acids in the center, and each fused to each other by 10-mer glycine-serine linker (pentatopes). The immunization of this RNA pentatope conferred disease control and survival benefit in a murine CT26 tumor model. Notably, mutated MHC class II epitopes were more immunogenic than class I epitopes in this study [44]. A recent study has brought a neoantigen cancer vaccine to a clinical trial in which whole-exome sequencing was carried out to identify somatic mutations in tumors from 3 patients with melanoma. Candidate HLA-A*02:01 epitopes containing residues arising from mutations were initially filtered using an HLA binding prediction algorithm, and then evaluated using competitive binding assays. Three patients were vaccinated with autologous dendritic cells that had been pulsed with the top 7 highest binding peptides identified from each tumor. The breadth and diversity of neoantigen-specific T cells were increased in all 3 patients after the vaccination. In addition, the results indicated that T cells generated by vaccination with 7 out of the 21 epitopes could recognize target cells transfected with the corresponding tandem minigene constructs, indicating that these neoantigens could be endogenously processed and presented [45]. While these results demonstrated that neoantigen cancer vaccines could elicit neoantigen-specific T cells in cancer patients, no clinical benefits to these patients were shown in this study. It remains unclear whether current cancer vaccine therapies are potent enough to provide therapeutic benefits in cancer patients with bulky disease.

The second approach to target neoantigens is by transferring neoantigen-specific T cells directly into host. Although so far no evidence in mouse models has shown that such T cells can induce tumor regressions *in vivo*, some evidence has linked neoantigen-reactive T cells to tumor regressions in human. A clue came from a report studying a unique melanoma TIL product, without any reactivity against non-mutated antigens. Instead, ~50% of this TIL product showed predominant reactivity against

Table 2
Outstanding questions for cancer immunotherapy targeting neoantigens.

1. How does this approach overcome the heterogeneity of tumor?
2. Does the number of neoantigens correlate with the mutation rate in each tumor specimen?
3. Can this approach apply to a variety types of cancers, especially cancers with low mutation rate?
4. Can neoantigens induce tolerance? Can Treg specifically recognize neoantigens and induce tolerance?
5. Are the number and fitness of neoantigen-specific T cells important for the efficacy of cancer immunotherapy?

HLA-A*01-restricted mutated PPP1R3B. Following adoptive transfer of this TIL product, the patient experienced a complete response ongoing beyond 10 years. In addition, mutated PPP1R3B-reactive T cells could be still detected in the patient's peripheral blood 5 years after the immunotherapy [46]. The most direct clinical evidence came from an adoptive cell transfer immunotherapy using nearly pure neoantigen-reactive T cells. A patient with metastatic cholangiocarcinoma was treated with one billion mutated ERBB2IP-reactive CD4$^+$ T cells, and she experienced a partial response ongoing for more than 2 years since treatment [31]. This suggested that neoantigen-reactive T cells could induce long-term tumor regressions in cancer patients, and it also brought up the possibility of applying immunotherapy to a variety of cancer types other than melanoma.

5. Conclusions and future perspectives

Based on current knowledge, neoantigens are ideal targets for cancer immunotherapy. Because neoantigens are specifically expressed in the tumor, it is less likely to induce tolerance and nearly impossible to induce normal tissue toxicity. However, it remains challenging to reduce the cost of such highly personalized therapy.

Many outstanding questions remain unanswered (Table 2). How can we apply cancer immunotherapy to a variety of cancer types by targeting neoantigens? Does the average number of neoantigens discovered per tumor correlate with the mutation rate in each cancer type? If yes, how can we apply immunotherapy to cancers with low mutation rates? Does the tumor microenvironments in different cancer types influence the efficacy of immunotherapy? Can the fitness or the number of T cells overcome the

potentially harsh tumor microenvironment in different cancer types?

One major concern is the heterogeneity of tumor. Neoantigens may be expressed in some tumor cells, but not all tumor cells in an individual patient, leading to tumor escape from immunotherapy. Conversely, we and others have observed complete tumor regressions in dozens of patients after immunotherapy. Some potential approaches may address this concern. One is to target multiple neoantigens at the same time, so all tumor cells expressing at least one neoantigen can be destroyed. Another approach is to target a single neoantigen, which is ideally expressed in all tumor cells within a patient.

Targeting driver mutations can be an effective strategy [30]. Aggressive tumor cells are actively expressing driver mutations, which are essential for carcinogenesis or metastasis. Although some tumor cells may not express driver mutations and may escape from immunotherapy, those tumor cells likely lose the metastatic potential and cannot grow aggressively. Nonetheless, defining driver mutations versus passenger mutations remains a challenging issue. The most definitive way to identify driver mutations is based on the functional studies found in the literature. From the neoantigens reviewed in this article, CDK4, β-catenin and Caspase-8 are likely driver mutations. The majority of neoantigens are likely random mutations (passenger mutations) recognized by T cells, but it is also possible that some of the mutations are simply not well-characterized. The Cancer Genome Atlas attempted to address this issue by sequencing and analyzing hundreds of tumor specimens. The recurrent mutations were identified as the potential driver mutations [47–49].

Recent checkpoint blockade immunotherapies have shown some efficacies in lung cancer, bladder cancer and renal cancer [50–53]. Based on the assumption that neoantigen-reactive T cells are responsible for tumor regressions in checkpoint blockade immunotherapies, directly transferring neoantigen-reactive T cells into cancer patients may achieve a much higher response rate. Additionally, neoantigen cancer vaccines may enhance the strength and persistence of T cells, and ultimately improve the efficacy of immunotherapy. With the recent advances in technology, we can identify neoantigens and isolate neoantigen-specific TCRs from individual patients in a timely and cost effective manner [54–56] (and unpublished data). It is possible that patients with a wide variety of cancer types, including cancers with very few mutations, can receive this proposed cancer immunotherapy targeting neoantigens (Fig. 1).
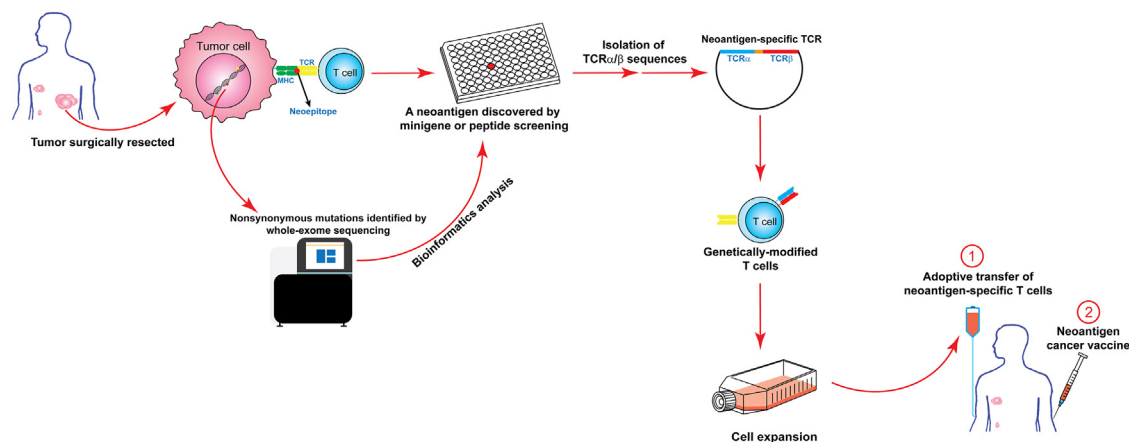

Fig. 1. Cancer immunotherapy targeting neoantigens by the adoptive transfer of genetically-modified T cells. In this proposed study, a tumor will be surgically resected from a cancer patient. Part of the tumor specimen will be subjected to whole-exome sequencing to identify nonsynonymous mutations. The rest of the tumor specimen will be used to grow TILs. Potential neoantigens will be identified by minigene or peptide screening approach described in the text. Neoantigen-specific TCRs will be isolated and introduced into patient's peripheral blood T cells. These genetically-modified T cells will be adoptively transferred back to the patient to fight cancer. A neoantigen cancer vaccine will be injected to enhance the strength and persistence of T cells.

Acknowledgements

References

[1] F.M. Burnet, The Clonal Selection Theory of Acquired Immunity, Cambridge University Press, London, 1959.

[2] C.A. Janeway Jr., R. Medzhitov, Innate immune recognition, Annu. Rev. Immunol. 20 (2002) 197–216.

[3] M.A. Cheever, J.P. Allison, A.S. Ferris, O.J. Finn, B.M. Hastings, T.T. Hecht, et al., The prioritization of cancer antigens: a national cancer institute pilot project for the acceleration of translational research, Clin. Cancer Res. 15 (2009) 5323–5337.

[4] L.A. Johnson, R.A. Morgan, M.E. Dudley, L. Cassard, J.C. Yang, M.S. Hughes, et al., Gene therapy with human and mouse T-cell receptors mediates cancer regression and targets normal tissues expressing cognate antigen, Blood 114 (2009) 535–546.

[5] M.R. Parkhurst, J.C. Yang, R.C. Langan, M.E. Dudley, D.A. Nathan, S.A. Feldman, et al., T cells targeting carcinoembryonic antigen can mediate regression of metastatic colorectal cancer but induce severe transient colitis, Mol. Ther. 19 (2011) 620–626.

[6] M.S. Sharkey, G. Lizee, M.I. Gonzales, S. Patel, S.L. Topalian, CD4(+) T-cell recognition of mutated B-RAF in melanoma patients harboring the V599E mutation, Cancer Res. 64 (2004) 1595–1599.

[7] R. Somasundaram, R. Swoboda, L. Caputo, L. Otvos, B. Weber, P. Volpe, et al., Human leukocyte antigen-A2-restricted CTL responses to mutated BRAF peptides in melanoma patients, Cancer Res. 66 (2006) 3287–3293.

[8] B. Fossum, T. Gedde-Dahl 3rd, T. Hansen, J.A. Eriksen, E. Thorsby, G. Gaudernack, Overlapping epitopes encompassing a point mutation (12 Gly→Arg) in p21 ras can be recognized by HLA-DR, -DP and -DQ restricted T cells, Eur. J. Immunol. 23 (1993) 2687–2691.

[9] E.S. Bergmann-Leitner, J.A. Kantor, W.L. Shupert, J. Schlom, S.I. Abrams, Identification of a human CD8+ T lymphocyte neo-epitope created by a ras codon 12 mutation which is restricted by the HLA-A2 allele, Cellular Immunol. 187 (1998) 103–116.

[10] B. Kubuschok, R. Schmits, F. Hartmann, C. Cochlovius, R. Breit, J. Konig, et al., Use of spontaneous Epstein-Barr virus-lymphoblastoid cell lines genetically modified to express tumor antigen as cancer vaccines: mutated p21 ras oncogene in pancreatic carcinoma as a model, Hum. Gene Ther. 13 (2002) 815–827.

[11] Y. Shono, H. Tanimura, M. Iwahashi, T. Tsunoda, M. Tani, H. Tanaka, et al., Specific T-cell immunity against Ki-ras peptides in patients with pancreatic and colorectal cancers, Br. J. Cancer 88 (2003) 530–536.

[12] Y. Ichiki, M. Takenoyama, M. Mizukami, T. So, M. Sugaya, M. Yasuda, et al., Simultaneous cellular and humoral immune response against mutated p53 in a patient with lung cancer, J. Immunol. 172 (2004) 4844–4850.

[13] B. Linard, S. Bezieau, H. Benlalam, N. Labarriere, Y. Guilloux, E. Diez, et al., A ras-mutated peptide targeted by CTL infiltrating a human melanoma lesion, J. Immunol. 168 (2002) 4802–4808.

[14] T. Wolfel, M. Hauer, J. Schneider, M. Serrano, C. Wolfel, E. Klehmann-Hieb, et al., A p16INK4a-insensitive CDK4 mutant targeted by cytolytic T lymphocytes in a human melanoma, Science 269 (1995) 1281–1284.

[15] P.F. Robbins, M. El-Gamil, Y.F. Li, Y. Kawakami, D. Loftus, E. Appella, et al., A mutated beta-catenin gene encodes a melanoma-specific antigen recognized by tumor infiltrating lymphocytes, J. Exp. Med. 183 (1996) 1185–1192.

[16] S. Mandruzzato, F. Brasseur, G. Andry, T. Boon, P. van der Bruggen, A CASP-8 mutation recognized by cytolytic T lymphocytes on a human head and neck carcinoma, J. Exp. Med. 186 (1997) 785–793.

[17] D. Brandle, F. Brasseur, P. Weynants, T. Boon, B. Van den Eynde, A mutated HLA-A2 molecule recognized by autologous cytotoxic T lymphocytes on a human renal cell carcinoma, J. Exp. Med. 183 (1996) 2501–2508.

[18] J. Huang, M. El-Gamil, M.E. Dudley, Y.F. Li, S.A. Rosenberg, P.F. Robbins, T cells associated with tumor regression recognize frameshifted products of the CDKN2A tumor suppressor gene locus and a mutated HLA class I gene product, J. Immunol. 172 (2004) 6057–6064.

[19] S.A. Shukla, M.S. Rooney, M. Rajasagi, G. Tiao, P.M. Dixon, M.S. Lawrence, et al., Comprehensive analysis of cancer-associated somatic mutations in class I HLA genes, Nat. Biotechnol. 33 (2015) 1152–1158.

[20] K.T. Hogan, D.P. Eisinger, S.B. Cupp 3rd, K.J. Lekstrom, D.D. Deacon, J. Shabanowitz, et al., The peptide recognized by HLA-A68.2-restricted, squamous cell carcinoma of the lung-specific cytotoxic T lymphocytes is derived from a mutated elongation factor 2 gene, Cancer Res. 58 (1998) 5144–5150.

[21] H. Matsushita, M.D. Vesely, D.C. Koboldt, C.G. Rickert, R. Uppaluri, V.J. Magrini, et al., Cancer exome analysis reveals a T-cell-dependent mechanism of cancer immunoediting, Nature 482 (2012) 400–404.

[22] J.C. Castle, S. Kreiter, J. Diekmann, M. Lower, N. van de Roemer, J. de Graaf, et al., Exploiting the mutanome for tumor vaccination, Cancer Res. 72 (2012) 1081–1091.

[23] A. Cai, D.B. Keskin, D.S. DeLuca, A. Alonso, W. Zhang, G.L. Zhang, et al., Mutated BCR-ABL generates immunogenic T-cell epitopes in CML patients, Clinical Cancer Res. 18 (2012) 5761–5772, an official journal of the American Association for Cancer Research.

[24] M.M. Gubin, X. Zhang, H. Schuster, E. Caron, J.P. Ward, T. Noguchi, et al., Checkpoint blockade cancer immunotherapy targets tumour-specific mutant antigens, Nature 515 (2014) 577–581.

[25] P.F. Robbins, Y.C. Lu, M. El-Gamil, Y.F. Li, C. Gross, J. Gartner, et al., Mining exomic sequencing data to identify mutated antigens recognized by adoptively transferred tumor-reactive T cells, Nat. Med. 19 (2013) 747–752.

[26] D. Valmori, D. Lienard, G. Waanders, D. Rimoldi, J.C. Cerottini, P. Romero, Analysis of MAGE-3-specific cytolytic T lymphocytes in human leukocyte antigen-A2 melanoma patients, Cancer Res. 57 (1997) 735–741.

[27] S. Morel, F. Levy, O. Burlet-Schiltz, F. Brasseur, M. Probst-Kepper, A.L. Peitrequin, et al., Processing of some antigens by the standard proteasome but not by the immunoproteasome results in poor presentation by dendritic cells, Immunity 12 (2000) 107–117.

[28] S. Zwaveling, S.C. Ferreira Mota, J. Nouta, M. Johnson, G.B. Lipford, R. Offringa, et al., Established human papillomavirus type 16-expressing tumors are effectively eradicated following vaccination with long peptides, J. Immunol. 169 (2002) 350–358.

[29] D.A. Wick, J.R. Webb, J.S. Nielsen, S.D. Martin, D.R. Kroeger, K. Milne, et al., Surveillance of the tumor mutanome by T cells during progression from primary to recurrent ovarian cancer, Clin. Cancer Res. 20 (2014) 1125–1134, an official journal of the American Association for Cancer Research.

[30] Y.C. Lu, X. Yao, J.S. Crystal, Y.F. Li, M. El-Gamil, C. Gross, et al., Efficient identification of mutated cancer antigens recognized by T cells associated with durable tumor regressions, Clin. Cancer Res. 20 (2014) 3401–3410.

[31] E. Tran, S. Turcotte, A. Gros, P.F. Robbins, Y.C. Lu, M.E. Dudley, et al., Cancer immunotherapy based on mutation-specific CD4+ T cells in a patient with epithelial cancer, Science 344 (2014) 641–645.

[32] E. Tran, M. Ahmadzadeh, Y.C. Lu, A. Gros, S. Turcotte, P.F. Robbins, et al., Immunogenicity of somatic mutations in human gastrointestinal cancers, Science Express (2015).

[33] M. Yadav, S. Jhunjhunwala, Q.T. Phung, P. Lupardus, J. Tanguay, S. Bumbaca, et al., Predicting immunogenic tumour mutations by combining mass spectrometry and exome sequencing, Nature 515 (2014) 572–576.

[34] S.A. Rosenberg, N.P. Restifo, Adoptive cell transfer as personalized immunotherapy for human cancer, Science 348 (2015) 62–68.

[35] P.F. Robbins, S.H. Kassim, T.L. Tran, J.S. Crystal, R.A. Morgan, S.A. Feldman, et al., A pilot trial using lymphocytes genetically engineered with an NY-ESO-1-reactive T-cell receptor: long-term follow-up and correlates with response, Clin. Cancer Res. 21 (2015) 1019–1027.

[36] P.F. Robbins, R.A. Morgan, S.A. Feldman, J.C. Yang, R.M. Sherry, M.E. Dudley, et al., Tumor regression in patients with metastatic synovial cell sarcoma and melanoma using genetically engineered lymphocytes reactive with NY-ESO-1, J. Clin. Oncol. 29 (2011) 917–924.

[37] A.P. Rapoport, E.A. Stadtmauer, G.K. Binder-Scholl, O. Goloubeva, D.T. Vogl, S.F. Lacey, et al., NY-ESO-1-specific TCR-engineered T cells mediate sustained antigen-specific antitumor effects in myeloma, Nat. Med. 21 (2015) 914–921.

[38] L.T. Nguyen, P.S. Ohashi, Clinical blockade of PD1 and LAG3—potential mechanisms of action, Nat. Rev. Immunol. 15 (2015) 45–56.

[39] D.T. Le, J.N. Uram, H. Wang, B.R. Bartlett, H. Kemberling, A.D. Eyring, et al., PD-1 blockade in tumors with mismatch-repair deficiency, N. Engl. J. Med. 372 (2015) 2509–2520.

[40] A. Snyder, V. Makarov, T. Merghoub, J. Yuan, J.M. Zaretsky, A. Desrichard, et al., Genetic basis for clinical response to CTLA-4 blockade in melanoma, N. Engl. J. Med. 371 (2014) 2189–2199.

[41] E.M. Van Allen, D. Miao, B. Schilling, S.A. Shukla, C. Blank, L. Zimmer, et al., Genomic correlates of response to CTLA-4 blockade in metastatic melanoma, Science 350 (2015) 207–211.

[42] N.A. Rizvi, M.D. Hellmann, A. Snyder, P. Kvistborg, V. Makarov, J.J. Havel, et al., Cancer immunology. Mutational landscape determines sensitivity to PD-1 blockade in non-small cell lung cancer, Science 348 (2015) 124–128.

[43] S.A. Rosenberg, J.C. Yang, R.M. Sherry, U.S. Kammula, M.S. Hughes, G.Q. Phan, et al., Durable complete responses in heavily pretreated patients with metastatic melanoma using T-cell transfer immunotherapy, Clin. Cancer Res. 17 (2011) 4550–4557.

[44] S. Kreiter, M. Vormehr, N. van de Roemer, M. Diken, M. Lower, J. Diekmann, et al., Mutant MHC class II epitopes drive therapeutic immune responses to cancer, Nature 520 (2015) 692–696.

[45] B.M. Carreno, V. Magrini, M. Becker-Hapak, S. Kaabinejadian, J. Hundal, A.A. Petti, et al., Cancer immunotherapy. A dendritic cell vaccine increases the breadth and diversity of melanoma neoantigen-specific T cells, Science 348 (2015) 803–808.

[46] Y.C. Lu, X. Yao, Y.F. Li, M. El-Gamil, M.E. Dudley, J.C. Yang, et al., Mutated PPP1R3B is recognized by T cells used to treat a melanoma patient who experienced a durable complete tumor regression, J. Immunol. 190 (2013) 6034–6042.

[47] M.S. Lawrence, P. Stojanov, P. Polak, G.V. Kryukov, K. Cibulskis, A. Sivachenko, et al., Mutational heterogeneity in cancer and the search for new cancer-associated genes, Nature 499 (2013) 214–218.

[48] M.S. Lawrence, P. Stojanov, C.H. Mermel, J.T. Robinson, L.A. Garraway, T.R. Golub, et al., Discovery and saturation analysis of cancer genes across 21 tumour types, Nature 505 (2014) 495–501.

[49] N. Cancer Genome Atlas, Genomic classification of cutaneous melanoma, Cell 161 (2015) 1681–1696.

[50] S.L. Topalian, F.S. Hodi, J.R. Brahmer, S.N. Gettinger, D.C. Smith, D.F. McDermott, et al., Safety, activity, and immune correlates of anti-PD-1 antibody in cancer, N. Engl. J. Med. 366 (2012) 2443–2454.

[51] J.R. Brahmer, S.S. Tykodi, L.Q. Chow, W.J. Hwu, S.L. Topalian, P. Hwu, et al., Safety and activity of anti-PD-L1 antibody in patients with advanced cancer, N. Engl. J. Med. 366 (2012) 2455–2465.

[52] R.S. Herbst, J.C. Soria, M. Kowanetz, G.D. Fine, O. Hamid, M.S. Gordon, et al., Predictive correlates of response to the anti-PD-L1 antibody MPDL3280A in cancer patients, Nature 515 (2014) 563–567.

[53] T. Powles, J.P. Eder, G.D. Fine, F.S. Braiteh, Y. Loriot, C. Cruz, et al., MPDL3280A (anti-PD-L1) treatment leads to clinical activity in metastatic bladder cancer, Nature 515 (2014) 558–562.

[54] E. Kobayashi, E. Mizukoshi, H. Kishi, T. Ozawa, H. Hamana, T. Nagai, et al., A new cloning and expression system yields and validates TCRs from blood lymphocytes of patients with cancer within 10 days, Nat. Med. 19 (2013) 1542–1546.

[55] C. Linnemann, B. Heemskerk, P. Kvistborg, R.J. Kluin, D.A. Bolotin, X. Chen, et al., High-throughput identification of antigen-specific TCRs by TCR gene capture, Nat. Med. 19 (2013) 1534–1541.

[56] C.J. Cohen, J.J. Gartner, M. Horovitz-Fried, K. Shamalov, K. Trebska-McGowan, V.V. Bliskovsky, et al., Isolation of neoantigen-specific T cells from tumor and peripheral lymphocytes, J. Clin. Invest. 125 (2015) 3981–3991.

[57] P.G. Coulie, F. Lehmann, B. Lethe, J. Herman, C. Lurquin, M. Andrawiss, et al., A mutated intron sequence codes for an antigenic peptide recognized by cytolytic T lymphocytes on a human melanoma, Proc. Natl. Acad. Sci. U.S.A. 92 (1995) 7976–7980.

[58] R.F. Wang, X. Wang, A.C. Atwood, S.L. Topalian, S.A. Rosenberg, Cloning genes encoding MHC class II-restricted antigens: mutated CDC27 as a tumor antigen, Science 284 (1999) 1351–1354.

[59] R. Chiari, F. Foury, E. De Plaen, J.F. Baurain, J. Thonnard, P.G. Coulie, Two antigens recognized by autologous cytolytic T lymphocytes on a melanoma result from a single point mutation in an essential housekeeping gene, Cancer Res. 59 (1999) 5785–5792.

[60] R. Pieper, R.E. Christian, M.I. Gonzales, M.I. Nishimura, G. Gupta, R.E. Settlage, et al., Biochemical identification of a mutated human melanoma antigen recognized by CD4(+) T cells, J. Exp. Med. 189 (1999) 757–766.

[61] J.F. Baurain, D. Colau, N. van Baren, C. Landry, V. Martelange, M. Vikkula, et al., High frequency of autologous anti-melanoma CTL directed against an antigen generated by a point mutation in a new helicase gene, J. Immunol. 164 (2000) 6057–6066.

[62] Y. Kawakami, X. Wang, T. Shofuda, H. Sumimoto, J. Tupesis, E. Fitzgerald, et al., Isolation of a new melanoma antigen, MART-2, containing a mutated epitope recognized by autologous tumor-infiltrating T lymphocytes, J. Immunol. 166 (2001) 2871–2877.

[63] H.Y. Wang, J. Zhou, K. Zhu, A.I. Riker, F.M. Marincola, R.F. Wang, Identification of a mutated fibronectin as a tumor antigen recognized by CD4+ T cells: its role in extracellular matrix formation and tumor metastasis, J. Exp. Med. 195 (2002) 1397–1406.

[64] N. Vigneron, A. Ooms, S. Morel, G. Degiovanni, B.J. Van Den Eynde, Identification of a new peptide recognized by autologous cytolytic T lymphocytes on a human melanoma, Cancer Immunity 2 (2002) 9.

[65] L. Novellino, N. Renkvist, F. Rini, A. Mazzocchi, L. Rivoltini, A. Greco, et al., Identification of a mutated receptor-like protein tyrosine phosphatase kappa as a novel, class II HLA-restricted melanoma antigen, J. Immunol. 170 (2003) 6363–6370.

[66] J. Zhou, M.E. Dudley, S.A. Rosenberg, P.F. Robbins, Persistence of multiple tumor-specific T-cell clones is associated with complete tumor regression in a melanoma patient receiving adoptive cell transfer therapy, J. Immunother. 28 (2005) 53–62.

[67] V. Lennerz, M. Fatho, C. Gentilini, R.A. Frye, A. Lifke, D. Ferel, et al., The response of autologous T cells to a human melanoma is dominated by mutated neoantigens, Proc. Natl. Acad. Sci. U.S.A. 102 (2005) 16013–16018.

[68] M. Sensi, G. Nicolini, M. Zanon, C. Colombo, A. Molla, I. Bersani, et al., Immunogenicity without immunoselection: a mutant but functional antioxidant enzyme retained in a human metastatic melanoma and targeted by CD8(+) T cells with a memory phenotype, Cancer Res. 65 (2005) 632–640.

[69] V. Corbiere, J. Chapiro, V. Stroobant, W. Ma, C. Lurquin, B. Lethe, et al., Antigen spreading contributes to MAGE vaccination-induced regression of melanoma metastases, Cancer Res. 71 (2011) 1253–1262.

[70] H. Echchakir, F. Mami-Chouaib, I. Vergnon, J.F. Baurain, V. Karanikas, S. Chouaib, et al., A point mutation in the alpha-actinin-4 gene generates an antigenic peptide recognized by autologous cytolytic T lymphocytes on a human lung carcinoma, Cancer Res. 61 (2001) 4078–4083.

[71] V. Karanikas, D. Colau, J.F. Baurain, R. Chiari, J. Thonnard, I. Gutierrez-Roelens, et al., High frequency of cytolytic T lymphocytes directed against a tumor-specific mutated antigen detectable with HLA tetramers in the blood of a lung carcinoma patient with long survival, Cancer Res. 61 (2001) 3718–3724.

[72] M. Takenoyama, J.F. Baurain, M. Yasuda, T. So, M. Sugaya, T. Hanagiri, et al., A point mutation in the NFYC gene generates an antigenic peptide recognized by autologous cytolytic T lymphocytes on a human squamous cell lung carcinoma, Int. J. Cancer 118 (2006) 1992–1997.

[73] C. Gaudin, F. Kremer, E. Angevin, V. Scott, F. Triebel, A hsp70-2 mutation recognized by CTL on a human renal cell carcinoma, J. Immunol. 162 (1999) 1730–1738.

[74] X. Zhou, D.Y. Jun, A.M. Thomas, X. Huang, L.Q. Huang, J. Mautner, et al., Diverse CD8+ T-cell responses to renal cell carcinoma antigens in patients treated with an autologous granulocyte-macrophage colony-stimulating factor gene-transduced renal tumor cell vaccine, Cancer Res. 65 (2005) 1079–1088.