OncoImmunology 3, e28836; May 2014; © 2014 Landes Bioscience

High sensitivity of cancer exome-based CD8 T cell neo-antigen identification

Marit M van Buuren, Jorg JA Calis, and Ton NM Schumacher*
Department of Immunology; The Netherlands Cancer Institute; Amsterdam, The Netherlands

Recent data suggest that T-cell reactivity against tumor-specific neo-antigens may be central to the clinical efficacy of cancer immunotherapy. The development of personalized vaccines designed to boost T-cell reactivity against patient specific neo-antigens has been proposed largely on the basis of these findings. Work from several groups has demonstrated that novel tumor-specific antigens can be discovered through the use of cancer exome sequencing data, thereby providing a potential pipeline for the development of patient-specific vaccines. Importantly though, it has not been established which fraction of cancer neo-antigens that can be recognized by CD8$^+$ T cells is successfully uncovered with the current exome-based epitope prediction strategies. Here, we use a data set comprising human cancer neo-antigens that was previously identified through the use of unbiased, computational-independent strategies to describe the potential of cancer exome-based neo-antigen discovery. This analysis shows a high sensitivity of exome-guided neo-antigen prediction of approximately 70%. We propose that future research should focus on the analysis and optimization of the specificity of neo-antigen prediction, and should undoubtedly entail the clinical evaluation of patient-specific vaccines with the aim of inducing immunoreactivity against tumor-displayed neo-antigens in a physiologically relevant context.

Keywords: epitope prediction, immune monitoring, neo-antigens, tumor vaccine, whole exome sequencing

Abbreviations: BLAST, basic local alignment search tool; CTLA4, cytotoxic T-lymphocyte antigen 4; MUM2, multiple myeloma protein 2; NSCLC, non-small-cell lung cancer; PD1, programmed cell death protein 1; PDL1, PCD ligand 1 (PD-L1); PMBEC, peptide:MHC binding energy covariance; SCLC, small-cell lung cancer; SNV, single nucleotide variant; TCR, T-cell receptor; TIL, tumor infiltrating lymphocyte

*Correspondence to: Ton N.M. Schumacher; Email: t.schumacher@nki.nl

Submitted: 04/03/2014

Accepted: 04/09/2014

Published Online: 05/14/2014

Citation: van Buuren MM, Calis JA, Schumacher TNM. High sensitivity of cancer exome-based CD8 T cell neo-antigen identification. OncoImmunology 2014; 3:e28836; http://dx.doi.org/10.4161/onci.28836

T-cell Immunity Directed toward Human Tumors

The capacity of immune cells to recognize, and potentially eradicate, distinct types of human tumors has been proven. The most recent—and compelling—evidence comes from a number of therapeutic studies using antibodies directed against T-cell checkpoint molecules. Specifically, administration of anti-cytotoxic T-lymphocyte antigen 4 (anti-CTLA4) to advanced melanoma patients has been shown to improve overall survival,[1] with long-term clinical responses in 10–20% of treated patients.[2] Furthermore, striking results have been obtained in early phase studies assessing the effects of blocking antibodies directed against the T cell survival regulatory programmed cell death 1 (PD1)—programmed cell death 1 ligand 1 (PD-L1) axis, revealing objective clinical responses in around 30% of melanoma patients.[3,4] Finally, early evidence for synergy between PD1 and CTLA4 therapeutic targeting has also been obtained.[5] So far it has proven difficult to determine precisely which immune cell subset, or subsets, drives the apparent clinical responses to T-cell checkpoint blockade therapy. However, a study by Dudley and colleagues has shown that cytotoxic T cells are responsible for at least some of the clinical activity of melanoma immunotherapy by tumor regression in response

to adoptive transfer of autologous and ex vivo enriched CD8+ T cells.[6]

Whereas the clinical activity of anti-CTLA4 antibody treatment has, thus far, only been convincingly demonstrated for the treatment of metastatic melanoma, anti-PD1/ anti-PD-L1 treatment has also been shown to exhibit clinical activities in patients with a variety of tumor types, such as ovarian cancer, renal cell cancer and non-small-cell lung cancer (NSCLC) (ranging from 6–33% of patients).[7] Interestingly, the observation that clinical activity of PD1/ PD-L1 targeting antibodies is prominent in patients with melanoma and NSCLC, both characterized by very high mutation loads, is consistent with the hypothesis that T cell recognition of tumor-specific neo-antigens—antigens that appear on the tumor as a consequence of a tumor-specific mutation—may be of particular importance.[8] In line with this hypothesis, PD1 blockade has shown relatively higher clinical activity in smoking-associated NSCLC, a condition in which mutation loads are particularly high, vs. non-smoking associated NSCLC, with patient response rates of 26% and 10%, respectively.[7]

On the basis of these observations, as well as the fact that T-cell reactivity against neo-antigens should not be negatively affected by central tolerance,[9] and further, should not induce toxicity against healthy tissues, the development of vaccines that enhance neo-antigen specific T-cell reactivity is considered attractive. An important consideration in the development of such vaccines is that the majority of mutations in human tumors are 'passengers' that are essentially unique to individual patients. As such, molecularly defined vaccines aiming to induce or boost neo-antigen specific T-cell reactivity should be developed in a patient-specific manner.

The possibility that tumor-specific mutations could result in the presentation of peptides recognized as foreign by the autologous CD8+ T-cell repertoire has for long intrigued cancer immunologists.[10-12] Seminal work by Wölfel and colleagues, for instance, demonstrated that T-cell recognition of mutated epitopes in human melanoma could dominate the tumor-specific T-cell response.[12] However, the experimental approach used in these early experiments was substantially too involved to describe tumor-specific neo-antigens in large cohorts of patients. Furthermore, within these experiments, pre-existent reactivity of autologous T cells formed the experimental basis for neo-antigen identification. As such, antigen identification by this approach is restricted to those tumor-specific neo-antigens for which T-cell reactivity is already present, thereby ignoring the possible repertoire of neo-antigens against which T-cell reactivity could be induced by vaccination.

The development of next generation sequencing technology has permitted the analysis of potential neo-antigens expressed in human tumors by a fundamentally different approach, a method that could potentially be transformed into a high-throughput, standardized process. Sahin and colleagues were the first investigators to demonstrate how whole-exome sequencing data could be used to identify neo-antigens that could be recognized by CD8+ T cells in the B16F10 murine melanoma models. Subsequent analysis of the immunogenicity of the predicted neo-antigens by peptide vaccination showed that a marked proportion of these peptides were able to elicit an immune response, and, that a fraction of the induced T-cell populations were able to recognize B16F10 tumors.[13] In parallel work, Schreiber and colleagues utilized exome-guided analysis of a carcinogen-induced mouse tumor to identify a neo-antigen recognized by endogenous T cells during in vivo tumor outgrowth. This work additionally showed that such a neo-antigen could be lost under immune pressure,[14] providing a clear rationale for the targeting of multiple neo-antigens in parallel.[8]

Work from our group as well as from the National Institute of Health Surgery Branch has brought the concept of cancer exome-based neo-antigen discovery to the analysis of human malignancies. In both studies, cancer exome information was used to identify mutations in human melanomas, and T-cell reactivity against some of the identified potential epitopes was evaluated.[15,16] In the work of Robbins and colleagues, neo-antigen specific T-cell responses were observed in four out of five patients analyzed, with the side-note that this analysis focused on patients with a clinical response upon tumor-infiltrating lymphocyte (TIL) adoptive-transfer therapy.[15] In our study, we initially analyzed the TILs of a melanoma patient who responded to anti-CTLA4 treatment. In this patient, exome-guided analysis of tumor cells and autologous healthy tissue resulted in the identification of two neo-antigen-specific T-cell responses within the TIL product. Notably, one of these neo-antigen-specific T-cell responses was also detected in the peripheral blood of this patient, and increased markedly after the start of anti-CTLA4 treatment, thereby providing the first evidence that neo-antigens identified on the basis of cancer exome sequencing data can be used to assess the effects of immunotherapeutic interventions.[16] With an additional seven melanoma patients analyzed, we have now been able to demonstrate neo-antigen specific T-cell responses in six out of eight patients tested (range from 0.002% - 65% of CD8+ T cells at the tumor site; van Buuren et al., unpublished results). Furthermore, in recent work, Nelson and colleagues used cancer exome data to reveal neo-antigen reactivity in one out of three patients with ovarian cancer.[17] Collectively, these results indicate that neo-antigen specific T-cell reactivity is a common phenomenon in melanoma and can also be detected in other tumor types with substantially lower mutation loads, such as in ovarian cancer.

Bearing the aforementioned data in mind, the development of patient-specific vaccines designed to boost or induce T-cell responses targeting mutated epitopes appears highly attractive. Importantly though, while the above data demonstrate that T-cell responses against neo-antigens can readily be identified on the basis of cancer exome sequencing, these data reveal neither the specificity nor the sensitivity of this approach. In order to develop clinically relevant vaccines, particularly for tumors with lower mutation loads, it will be important that such vaccines do not leave out a large fraction of the true T-cell reactive neo-antigens (i.e., sensitivity must be sufficient). At the same time, it will be equally important that these vaccines are not primarily composed of epitopes that are incorrectly predicted

as possible neo-antigens, (i.e., specificity must be sufficient). Here, we address the first of these two issues, by defining the sensitivity of cancer exome-based neo-antigen identification on the basis of an independently obtained data set.

What is the Sensitivity of Cancer Exome-Based Neo-Antigen Prediction?

In order to assess the sensitivity of cancer exome-based neo-antigen prediction, we assembled a data set of human cancer neo-antigens that had been previously discovered by unbiased and non-computational strategies. To this end, we performed an extensive literature search that led us to the identification of 17 previously reported neo-antigens that met the following criteria: (1) Evidence of natural processing and presentation of the neo-antigen, as shown by recognition of tumor cells by autologous T cells; (2) The identified neo-peptides were the result of a single nucleotide variant (SNV); and (3) Unbiased epitome identification method, without the use of cancer exome data or major histocompatibility complex (MHC) pathway predictors. The majority of the epitopes that fulfilled these criteria had been previously discovered by the expression-based screening of tumor-derived cDNA libraries with tumor-reactive T cells. A large majority was derived from melanoma patients (n = 10), but neo-antigens originating from NSCLC, small-cell lung cancer (SCLC), bladder cancer and renal cell cancer were also identified (**Table 1**).

For analysis of this set of neo-antigens, the parental protein sequences were downloaded from NCBI, after a basic local alignment search (BLAST) on human coding sequences using the non-mutated peptide. The parental protein sequence was then altered to include the mutated amino acid, and subsequently, these protein sequences were used in our prediction pipeline. As based on our prior analyses, epitopes were considered as discoverable by exome-based analysis when meeting the following criteria:[16] (1) Sufficient coverage of the encoding sequence within cancer exome data to allow calling of the mutant base. For this purpose, sequence coverage for each neo-epitope was analyzed for three representative data sets of tumor tissue and matched healthy control tissue, using a cutoff of 5 reads or more. (2) NetChop cleavage probability of 0.5 or higher[18]; (3) NetMHCpan predicted binding affinity of 500 nM or less[19]; and (4). A low 'similarity-to-self', as based on a test that evaluates the likelihood that the T-cell repertoire can distinguish the mutant and wild-type epitopes, either by altered levels of epitope presentation, or by an altered structure of the MHC-presented epitope. In this analysis, similarity to self is scored in two ways. First, the core region of a potential neo-epitope is defined as the peptide sequence between the two anchor residues and is considered to be the T-cell receptor (TCR)-exposed surface.[9,20] If this region is considerably different from the core-region of the parental sequence (amino acid change has a peptide:MHC binding energy covariance (PMBEC) value of ≤ 0.05[21]), the mutant peptide is considered non-similar-to-self and is thus retained. Alternatively, if the parental sequence is not predicted to be an MHC-ligand (Netchop cleavage probability lower than 0.5 or predicted binding affinity higher than 500 nM), the peptide is also considered non-similar-to-self and retained.

Analysis of the 17 previously discovered neo-antigens by this in silico analysis pipeline reveals that the current technology for the initial step in exome-based neo-epitope prediction, the cancer exome sequencing itself, requires little if any improvement. Specifically, read coverage of the gene sequence in which the mutations were originally discovered was sufficient for all three tumors for which this was tested for 16 out of the 17 previously described neo-epitopes. Read numbers for the sequence covering the remaining previously identified mutation (multiple myeloma protein 2; MUM2[22]) was sufficient for 2 out of 3 tumors. With respect to the subsequent epitope predictions, the use of a 500 nM predicted binding affinity as a cut-off resulted in the correct identification of 14 out of 17 neo-epitopes, with two of the failures occurring for HLA alleles for which the prediction algorithm still requires further optimization (HLA-B*52:01 and HLA-C*06:02).[19] Also, 15 out of 17 epitopes correctly passed the NetChop filter, and 15 out of 17 correctly passed the self-similarity filter. The combination of these different thresholds shows that 12 out of 17 neo-antigens would have been identified in two out of three cases, and 11 out of 17 neo-antigens would have been correctly identified in all 3 cases (Table 1), resulting in an overall sensitivity of 69%. It is noted that there may be cases in which neo-antigens are derived from alternative open-reading frames that are not taken into account in epitope predictions, or from intronic sequences that are not spanned by exome sequencing strategies. The former is unlikely to be a major confounder considering that such epitopes would also have appeared within the unbiased data set assembled here had they been common. The potential relevance of the second subset, intron-derived neo-antigens, is presently more difficult to gauge, as such mutations may also not be efficiently identified in cDNA-based screening methods if these transcript are rare. On a related note, identification of indels within sequencing data has, thus far, been less reliable than identification of SNVs, and while typically occurring infrequently in certain tumor types, such as melanoma, failure to correctly identify all indels may reduce the sensitivity of neo-antigen prediction in those tumors in which these alterations are more prevalent.

Most importantly, overall the current data indicate that the sensitivity of neo-epitope prediction from analysis of exonic SNVs in cancer exome sequencing data requires little improvement. It is important to emphasize the necessity to also describe the specificity of these epitope predictions in future work. Using the above-described prediction pipeline, endogenous T-cell responses have, so far, only been observed against a relatively small fraction of the predicted neo-epitopes (van Buuren et al., unpublished). These experiments do not, however, reveal whether this low 'hit rate' is due to a large fraction of predicted neo-epitopes that are false positives, or alternatively, reflects an inability of the immune system to generate an immune response against all truly presented neo-epitopes during natural disease progression. Analysis of the potential

Table 1. Neo-antigens identified from literature

Peptide number	HLA	Parental peptide sequence	Parental binding affinity (nM)	Parental netchop score	Mutant peptide sequence	Mutant binding affinity (nM)	Mutant netchop score	PMBEC value	Coverage in cancer exome	Mutated in TCR exposed surface	Protein	Cancer type	Reference
1	A*02:01	FLDEFMEAV	2.24	0.734636	FLDEFMEGV	2.72	0.700886	0.0383	3 out of 3 exomes	YES	ME-1	NSCL cancer	Karanikas V et al.[25]
2	A*02:01	LLLDDSLVSI	6.89	0.954141	LLLDDLLVSI	5.39	0.94917	-0.0585	3 out of 3 exomes	YES	Prdx5	melanoma	Sensi M et al.[26]
3	A*02:01	SLADEAEVHL	38.56	0.969244	SLADEAEVYL	12.38	0.958266	0.0366	3 out of 3 exomes	YES	GAS7	melanoma	Zhou J et al.[27]
4	A*03:01	KIFSEVTPK	16.08	0.972275	KIFSEVTLK	14.09	0.971104	-0.051	3 out of 3 exomes	YES	SIRT2	melanoma	Lennerz V et al.[12]
5	A*68:02	ETVSEESNV	27.15	0.516073	ETVSEQSNV	19.44	0.671327	0.0281	3 out of 3 exomes	YES	Elongation Factor 2/EEF2	SCL cancer	Hogan KT et al.[28]
6	A*02:01	FIASKGVKLV	43.7	0.921914	FIASNGVKLV	29.49	0.938058	-0.034	3 out of 3 exomes	YES	a-Actinin-4/ ACTN4	NSCL cancer	Echchakir H et al.[29]
7	A*24:02	SYLDSGIHS	18745.5	0.297074	SYLDSGIHF	41.4	0.973556	-0.0868	3 out of 3 exomes	YES	B-catenin/ CTNNB1	melanoma	Robbins PF et al.[30]
8	A*03:01	KILDAVVAQE	14976.43	0.128682	KILDAVVAQK	48.23	0.969326	-0.0695	3 out of 3 exomes	YES	SNRP116/ EFTUD2	melanoma	Lennerz V et al.[12]
9	A*03:01	EINKNPKYKK	6061.38	0.850773	KINKNPKYKK	157.05	0.850773	-0.0695	3 out of 3 exomes	YES	Myosin class I	melanoma	Zorn E et al.[31]
10	A*03:01	TLGWLLQTPK	178.74	0.675804	TLDWLLQTPK	282.03	0.714946	-0.0087	3 out of 3 exomes	YES	GPNMB	melanoma	Lennerz V et al.[12]
11	B*44:03	AEPIDIQTW	258.23	0.965739	AEPINIQTW	287.87	0.968687	0.0194	3 out of 3 exomes	YES	KIAA0205	bladder cancer	Guéguen M et al.[32]
12	B*44:02	SELFRSRLDSY	182.33	0.763436	SELFRSGLDSY	184.25	0.908365	-0.0232	**2 out of 3 exomes**	YES	MUM-2	melanoma	Chiari R et al.[22]
13	B*52:01	QQITQTEV	4077.72	0.962417	QQITKTEV	**5030.87**	0.944965	-0.0332	3 out of 3 exomes	YES	NFYC	NSCL cancer	Takenoyama M et al.[33]
14	C*06:02	FRSRLDSYV	3508.71	0.73499	FRSGLDSYV	**5762.85**	0.872751	-0.0232	**2 out of 3 exomes**	YES	MUM-2	melanoma	Chiari R et al.[22]
15	A*02:01	ARDPHSGHFV	25221.62	0.668099	ACDPHSGHFV	**11191.79**	**0.475792**	-0.0483	3 out of 3 exomes	NO	CDK4	melanoma	Wölfel T et al.[11]
16	A*02:01	SLFEGIDFYT	6.78	0.0445	SLFEGIDIYT	23.2	**0.04132**	**0.0629**	3 out of 3 exomes	YES	hsp70–2	renal cell carcinoma	Gaudin C et al.[34]
17	B*07:02	GPHVPESAF	**68.08**	0.96896	RPHVPESAF	9.67	0.96843	-0.0232	3 out of 3 exomes	**NO**	RBAF600/ UBR4	melanoma	Lennerz V et al.[12]

Cancer neo-antigens (n = 17) that had been previously discovered by unbiased and non-computational strategies were analyzed to assess the sensitivity of our cancer exome-based neo-antigen prediction algorithm pipeline. Neo-antigens and their corresponding values as predicted by netMHCpan-2.4, netchop Cterm3.0 and the peptide:MHC binding energy covariance (PMBEC) matrix are shown. Presence of a non-synonymous mutation within the TCR exposed surface is indicated. Peptides 1–11 would have been identified by exome guided analysis, peptide 12 would have been identified in two out of three patients, peptides 13–17 would not have been identified.[25-34] Bold numbers indicate exlusion criteria.

occurrence of such a 'neglected' neo-epitope repertoire is an important outstanding question remaining to be addressed.

The data described here convincingly demonstrate how exome-guided analysis can be utilized to predict potential neo-antigen specific T-cell responses in patients, and, together with data from Wu and colleagues, suggest that this method will help to identify suitable candidates for the creation of patient-specific vaccines.[23] A recent analysis by Stratton and colleagues shows that while melanoma has the highest mutational load of all common human cancers, the range of mutation load overlaps significantly with that of many other common tumor types.[24] Assuming that the mutation load within tumors strongly correlates with the resultant production of neo-epitopes that can potentially be recognized by T cells, these data suggest that exome-guided analysis of neo-antigen specific T-cell responses will be relevant to a variety of human malignancies. With clear evidence for the clinical activity of a number of immunotherapies and with emerging evidence for a role of patient specific neo-antigens in these therapies, it is tempting to speculate that the combination of anti-checkpoint antibodies with patient-specific vaccines will provide further patient benefit.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Acknowledgments

We would like to thank Alexandra Piet for assembling the list of previously described neo-antigens, Sander Kelderman and Lorenzo Fanchi for critical reading of the manuscript, and members from the Schumacher lab for discussions. This work was supported by Dutch Cancer Society grant KWF 2012-5463, and by a Stand Up To Cancer - Cancer Research Institute Cancer Immunology Translational Cancer Research Grant. Stand Up To Cancer is a program of the Entertainment Industry Foundation administered by the American Association for Cancer Research.

References

1. Hodi FS, O'Day SJ, McDermott DF, Weber RW, Sosman JA, Haanen JB, Gonzalez R, Robert C, Schadendorf D, Hassel JC, et al. Improved survival with ipilimumab in patients with metastatic melanoma. N Engl J Med 2010; 363:711-23; PMID:20525992; http://dx.doi.org/10.1056/NEJMoa1003466
2. Ott PA, Hodi FS, Robert C. CTLA-4 and PD-1/PD-L1 blockade: new immunotherapeutic modalities with durable clinical benefit in melanoma patients. Clin Cancer Res 2013; 19:5300-9; PMID:24089443; http://dx.doi.org/10.1158/1078-0432.CCR-13-0143
3. Topalian SL, Hodi FS, Brahmer JR, Gettinger SN, Smith DC, McDermott DF, Powderly JD, Carvajal RD, Sosman JA, Atkins MB, et al. Safety, activity, and immune correlates of anti-PD-1 antibody in cancer. N Engl J Med 2012; 366:2443-54; PMID:22658127; http://dx.doi.org/10.1056/NEJMoa1200690
4. Hamid O, Robert C, Daud A, Hodi FS, Hwu WJ, Kefford R, Wolchok JD, Hersey P, Joseph RW, Weber JS, et al. Safety and tumor responses with lambrolizumab (anti-PD-1) in melanoma. N Engl J Med 2013; 369:134-44; PMID:23724846; http://dx.doi.org/10.1056/NEJMoa1305133
5. Wolchok JD, Kluger H, Callahan MK, Postow MA, Rizvi NA, Lesokhin AM, Segal NH, Ariyan CE, Gordon RA, Reed K, et al. Nivolumab plus ipilimumab in advanced melanoma. N Engl J Med 2013; 369:122-33; PMID:23724867; http://dx.doi.org/10.1056/NEJMoa1302369
6. Dudley ME, Gross CA, Langhan MM, Garcia MR, Sherry RM, Yang JC, Phan GQ, Kammula US, Hughes MS, Citrin DE, et al. CD8+ enriched "young" tumor infiltrating lymphocytes can mediate regression of metastatic melanoma. Clin Cancer Res 2010; 16:6122-31; PMID:20668005; http://dx.doi.org/10.1158/1078-0432.CCR-10-1297
7. Champiat S, Ferté C, Lebel-Binay S, Eggermont A, Soria JC. Exomics and immunogenics: Bridging mutational load and immune checkpoints efficacy. Oncoimmunology 2014; 3:e27817; PMID:24605269; http://dx.doi.org/10.4161/onci.27817
8. Heemskerk B, Kvistborg P, Schumacher TN. The cancer antigenome. EMBO J 2013; 32:194-203; PMID:23258224; http://dx.doi.org/10.1038/emboj.2012.333
9. Kessels HW, de Visser KE, Tirion FH, Coccoris M, Kruisbeek AM, Schumacher TN. The impact of self-tolerance on the polyclonal CD8+ T cell repertoire. J Immunol 2004; 172:2324-31; PMID:14764701; http://dx.doi.org/10.4049/jimmunol.172.4.2324
10. Baurain JF, Colau D, van Baren N, Landry C, Martelange V, Vikkula M, Boon T, Coulie PG. High frequency of autologous anti-melanoma CTL directed against an antigen generated by a point mutation in a new helicase gene. J Immunol 2000; 164:6057-66; PMID:10820291; http://dx.doi.org/10.4049/jimmunol.164.11.6057
11. Wölfel T, Hauer M, Schneider J, Serrano M, Wölfel C, Klehmann-Hieb E, De Plaen E, Hankeln T, Meyer zum Büschenfelde KH, Beach D. A p16INK4a-insensitive CDK4 mutant targeted by cytolytic T lymphocytes in a human melanoma. Science 1995; 269:1281-4; PMID:7652577; http://dx.doi.org/10.1126/science.7652577
12. Lennerz V, Fatho M, Gentilini C, Frye RA, Lifke A, Ferel D, Wölfel C, Huber C, Wölfel T. The response of autologous T cells to a human melanoma is dominated by mutated neoantigens. Proc Natl Acad Sci U S A 2005; 102:16013-8; PMID:16247014; http://dx.doi.org/10.1073/pnas.0500090102
13. Castle JC, Kreiter S, Diekmann J, Löwer M, van de Roemer N, de Graaf J, Selmi A, Diken M, Boegel S, Paret C, et al. Exploiting the mutanome for tumor vaccination. Cancer Res 2012; 72:1081-91; PMID:22237626; http://dx.doi.org/10.1158/0008-5472.CAN-11-3722
14. Matsushita H, Vesely MD, Koboldt DC, Rickert CG, Uppaluri R, Magrini VJ, Arthur CD, White JM, Chen YS, Shea LK, et al. Cancer exome analysis reveals a T-cell-dependent mechanism of cancer immunoediting. Nature 2012; 482:400-4; PMID:22318521; http://dx.doi.org/10.1038/nature10755
15. Robbins PF, Lu YC, El-Gamil M, Li YF, Gross C, Gartner J, Lin JC, Teer JK, Cliften P, Tycksen E, et al. Mining exomic sequencing data to identify mutated antigens recognized by adoptively transferred tumor-reactive T cells. Nat Med 2013; 19:747-52; PMID:23644516; http://dx.doi.org/10.1038/nm.3161
16. van Rooij N, van Buuren MM, Philips D, Velds A, Toebes M, Heemskerk B, van Dijk LJ, Behjati S, Hilkmann H, El Atmioui D, et al. Tumor exome analysis reveals neoantigen-specific T-cell reactivity in an ipilimumab-responsive melanoma. J Clin Oncol 2013; 31:e439-42; PMID:24043743; http://dx.doi.org/10.1200/JCO.2012.47.7521
17. Wick DA, Webb JR, Nielsen JS, Martin SD, Kroeger DR, Milne K, Castellarin M, Twumasi-Boateng K, Watson PH, Holt RA, et al. Surveillance of the tumor mutanome by T cells during progression from primary to recurrent ovarian cancer. Clin Cancer Res 2014; 20:1125-34; PMID:24323902; http://dx.doi.org/10.1158/1078-0432.CCR-13-2147
18. Keşmir C, Nussbaum AK, Schild H, Detours V, Brunak S. Prediction of proteasome cleavage motifs by neural networks. Protein Eng 2002; 15:287-96; PMID:11983929; http://dx.doi.org/10.1093/protein/15.4.287
19. Nielsen M, Lundegaard C, Blicher T, Lamberth K, Harndahl M, Justesen S, Røder G, Peters B, Sette A, Lund O, et al. NetMHCpan, a method for quantitative predictions of peptide binding to any HLA-A and -B locus protein of known sequence. PLoS One 2007; 2:e796; PMID:17726526; http://dx.doi.org/10.1371/journal.pone.0000796
20. Calis JJ, de Boer RJ, Keşmir C. Degenerate T-cell recognition of peptides on MHC molecules creates large holes in the T-cell repertoire. PLoS Comput Biol 2012; 8:e1002412; PMID:22396638; http://dx.doi.org/10.1371/journal.pcbi.1002412
21. Kim Y, Sidney J, Pinilla C, Sette A, Peters B. Derivation of an amino acid similarity matrix for peptide: MHC binding and its application as a Bayesian prior. BMC Bioinformatics 2009; 10:394; PMID:19948066; http://dx.doi.org/10.1186/1471-2105-10-394
22. Chiari R, Foury F, De Plaen E, Baurain JF, Thonnard J, Coulie PG. Two antigens recognized by autologous cytolytic T lymphocytes on a melanoma result from a single point mutation in an essential housekeeping gene. Cancer Res 1999; 59:5785-92; PMID:10582700
23. Fritsch EFR. M.; Ott, P.A.; Brusic, V.; Hacohen, N.; Wu, C.J. HLA-bindng properties of tumor neoepitopes in humans. Cancer Immunology Research 2014.
24. Alexandrov LB, Nik-Zainal S, Wedge DC, Aparicio SA, Behjati S, Biankin AV, Bignell GR, Bolli N, Borg A, Børresen-Dale AL, et al.; Australian Pancreatic Cancer Genome Initiative; ICGC Breast Cancer Consortium; ICGC MMML-Seq Consortium; ICGC PedBrain. Signatures of mutational processes in human cancer. Nature 2013; 500:415-21; PMID:23945592; http://dx.doi.org/10.1038/nature12477

25. Karanikas V, Colau D, Baurain JF, Chiari R, Thonnard J, Gutierrez-Roelens I, Goffinet C, Van Schaftingen EV, Weynants P, Boon T, 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 2001; 61:3718-24; PMID:11325844
26. Sensi M, Nicolini G, Zanon M, Colombo C, Molla A, Bersani I, Lupetti R, Parmiani G, Anichini A. 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 2005; 65:632-40; PMID:15695408
27. Zhou J, Dudley ME, Rosenberg SA, Robbins PF. 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 2005; 28:53-62; PMID:15614045; http://dx.doi.org/10.1097/00002371-200501000-00007
28. Hogan KT, Eisinger DP, Cupp SB 3rd, Lekstrom KJ, Deacon DD, Shabanowitz J, Hunt DF, Engelhard VH, Slingluff CL Jr., Ross MM. 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 1998; 58:5144-50; PMID:9823325
29. Echchakir H, Mami-Chouaib F, Vergnon I, Baurain JF, Karanikas V, Chouaib S, Coulie PG. 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 2001; 61:4078-83; PMID:11358829
30. Robbins PF, El-Gamil M, Li YF, Kawakami Y, Loftus D, Appella E, Rosenberg SA. A mutated beta-catenin gene encodes a melanoma-specific antigen recognized by tumor infiltrating lymphocytes. J Exp Med 1996; 183:1185-92; PMID:8642260; http://dx.doi.org/10.1084/jem.183.3.1185
31. Zorn E, Hercend T. A natural cytotoxic T cell response in a spontaneously regressing human melanoma targets a neoantigen resulting from a somatic point mutation. Eur J Immunol 1999; 29:592-601; PMID:10064075; http://dx.doi.org/10.1002/(SICI)1521-4141(199902)29:02<592::AID-IMMU592>3.0.CO;2-2
32. Guéguen M, Patard JJ, Gaugler B, Brasseur F, Renauld JC, Van Cangh PJ, Boon T, Van den Eynde BJ. An antigen recognized by autologous CTLs on a human bladder carcinoma. J Immunol 1998; 160:6188-94; PMID:9637538
33. Takenoyama M, Baurain JF, Yasuda M, So T, Sugaya M, Hanagiri T, Sugio K, Yasumoto K, Boon T, Coulie PG. 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 2006; 118:1992-7; PMID:16287085; http://dx.doi.org/10.1002/ijc.21594
34. Gaudin C, Kremer F, Angevin E, Scott V, Triebel F. A hsp70-2 mutation recognized by CTL on a human renal cell carcinoma. J Immunol 1999; 162:1730-8; PMID:9973436