

Advances in personalized cancer immunotherapy

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Abstract There are currently three major approaches to T cell-based cancer immunotherapy, namely, active vaccination, adoptive cell transfer therapy and immune checkpoint blockade. Recently, this latter approach has demonstrated remarkable clinical benefits, putting cancer immunotherapy under the spotlight. Better understanding of the dynamics of anti-tumor immune responses (the “Cancer-Immunity Cycle”) is crucial for the further development of this form of treatment. Tumors employ multiple strategies to escape from anti-tumor immunity, some of which result from the selection of cancer cells with immunosuppressive activity by the process of cancer immunoediting. Apart from this selective process, anti-tumor immune responses can also be inhibited in multiple different ways which vary from patient to patient. This implies that cancer immunotherapy must be personalized to (1) identify the rate-limiting steps in any given patient, (2) identify and combine strategies to overcome these hurdles, and (3) proceed with the next round of the “Cancer-Immunity Cycle”. Cancer cells have genetic alterations which can provide the immune system with targets by which to recognize and eradicate the tumor. Mutated proteins expressed exclusively in cancer cells and recognizable by the immune system are known as neoantigens. The development of next-generation sequencing technology has made it possible to determine the genetic landscape of human cancer and facilitated the utilization of genomic

information to identify such candidate neoantigens in individual cancers. Future immunotherapies will need to be personalized in terms of the identification of both patient-specific immunosuppressive mechanisms and target neoantigens.

Keywords Cancer immunotherapy · Next-generation-sequencing · Neoantigen

Tumor immunology and cancer immunotherapy

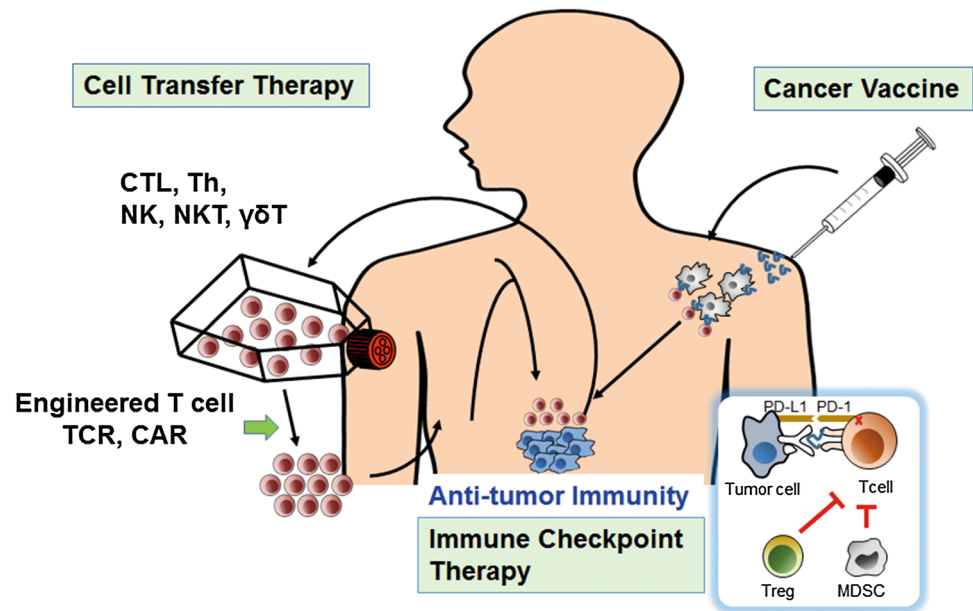
It was a controversial issue for over a century as to whether an effective immune response can be elicited against tumors and whether cancer immunotherapy is feasible [1]. It has now been accepted, however, that both the innate and adaptive arms of immunity can target tumor cells and that these immune responses can be harnessed to control cancer. Cancer immunotherapy used to be categorized into two types, cancer vaccination and cell transfer therapy (Fig. 1). Cancer vaccines rely on active immunotherapy to induce anti-tumor immunity in vivo by immunization with tumor antigens, while cell transfer therapies rely on passive adoptive treatments depending on harvesting, expanding and re-infusing autologous lymphocytes that are reactive to tumor cells [2, 3]. Cell transfer therapies may be improved by incorporating genetic engineering technologies to design T cells with specificity for tumor antigens [4, 5]. More recently, a new form of immunotherapy known as immune checkpoint blockade has been introduced in the clinic [6]. Antibodies targeting so-called immune checkpoint molecules, such as CTLA-4, PD-1 and PD-L1, re-invigorate pre-existing compromised anti-tumor T cells by releasing them from immunosuppression. All of these cancer immunotherapies depend on the augmentation of T

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Fig. 1 Cancer immunotherapy. Cancer immunotherapy is categorized into three approaches: cancer vaccination, cell transfer therapy and immune checkpoint therapy. All modalities depend on the cellular immune response to control tumor cells



cell-mediated cellular immunity with tumor-specific cytotoxic T lymphocytes (CTLs) playing a pivotal role. Advances in fundamental basic science regarding anti-tumor immunity including knowledge of tumor antigens, immune-suppressive cells, factors and signaling pathways observed in the tumor-bearing host in addition to better understanding of the effector arm of anti-tumor immunity has finally resulted in the establishment of successful cancer immunotherapies [7].

The Cancer-Immunity Cycle

The understanding of the dynamics of anti-tumor immune responses is crucial for the development of cancer immunotherapy. Recently, Chen and Mellman illustrated these points in what they called the “Cancer-Immunity Cycle” (Fig. 2) [8]. First, tumor antigens are released from dying tumor cells (Fig. 2①) and are captured by immature dendritic cells (DC). Changes at the surface of dying tumor cells, such as the expression of calreticulin (CRT) and heat shock proteins (HSPs), in addition to the release of immunostimulatory “danger” signals such as high mobility group box 1 (HMGB1) and ATP, define “immunogenic cell death” (ICD). CRT, HMGB1 and ATP bind to CD92, TLR4 and P2RX7, respectively. This can result in signals causing the maturation of DCs and can facilitate their migration to draining lymph nodes (Fig. 2②) [9]. Pro-inflammatory cytokines and even the gut microbiota can affect these events [10]. Optimally, DCs will process the captured tumor antigens and present them via their MHC

class I and II molecules to stimulate T cells, which, together with DC costimulatory signals leads to priming and activation of effector T cells reactive to tumor cells (Fig. 2③). However, the responding T cells are exposed to co-inhibitory as well as co-stimulatory signals during this process; a balance between them is thought to determine the nature of the immune response and the final outcome [6]. Suppressive cells such as regulatory T cells (Tregs) also affect T cell priming, probably via their interaction with the DCs [11]. The activated effector T cells that may finally be generated in this manner must then traffic to and infiltrate into the tumor bed (Fig. 2④⑤) so that they can recognize and kill their target cells (Fig. 2⑥⑦). Tumor-specific cytolytic effector T cells recognize MHC class I/peptide complexes on the cell surface; tumor cells with defective expression of MHC molecules can evade this process. In addition, many inhibitory mechanisms are active within the tumor microenvironment, such as Tregs and myeloid-derived suppressor cells (MDSC), which hamper the anti-tumor effector response. However, if and when tumor cells are destroyed, additional tumor antigens can be released to initiate further cycles of anti-tumor immune responses. This secondary immunity potentially targeting distinct wider ranges of tumor antigens is recognized as “antigen spreading” and contributes to increasing the breadth and depth of anti-tumor immunity in subsequent cycles [12].

At early stages of carcinogenesis, the transformed cells can be attacked by the immune system and probably eliminated. However, if and when some tumor cells eventually evade the immune system, possibly by acquiring

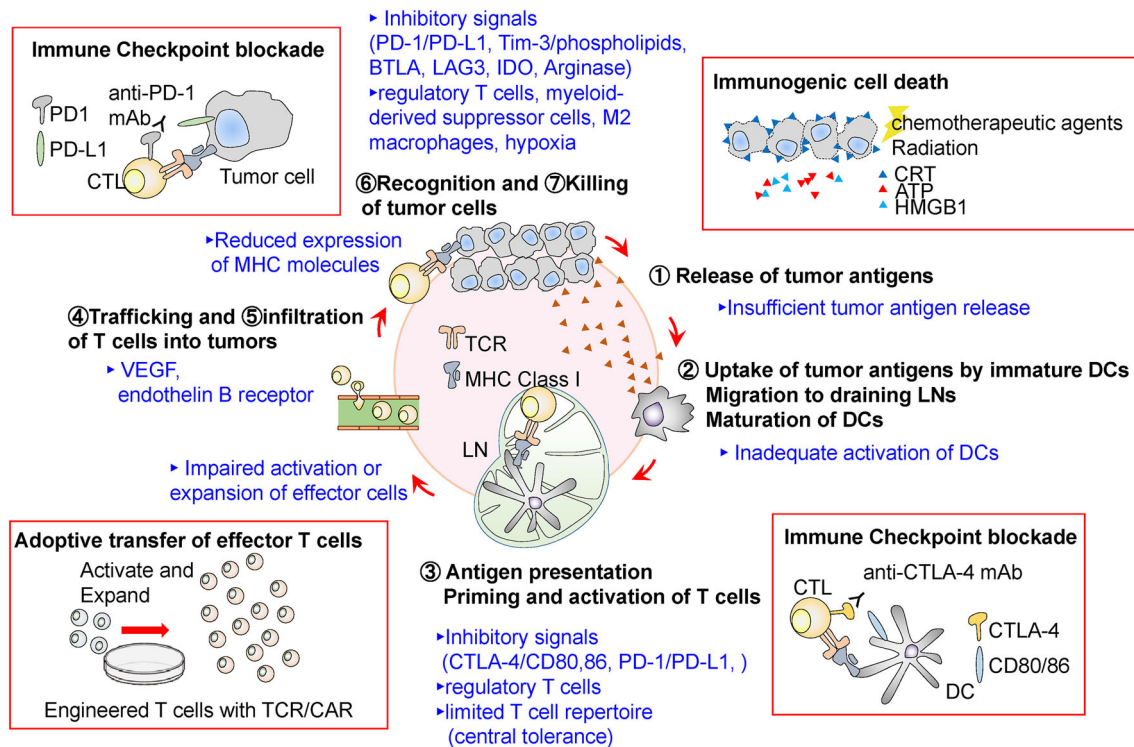


Fig. 2 Cancer-Immunity Cycle. The generation of immunity to cancer is dynamic and described as a cyclic process with 7 major steps. Each step is confronted with inhibitory factors (in blue) that can halt the cycle. Abbreviations are as follows: *CRT* calreticulin, *ATP*

adenosine triphosphate, *HMGB1* High Mobility Group Box 1, *LN* lymph node, *DCs* dendritic cells, *CTLs* cytotoxic T lymphocytes, *TCR* T cell receptor, *CAR* chimeric antigen receptor, *IDO* indoleamine 2,3-dioxygenase, *mAb* monoclonal antibody

an immunosuppressive phenotype, this results in the formation of overt tumor [13]. Tumors employ multiple strategies to attenuate the effector activities of anti-tumor immunity, often as a result of selection pressure exerted by the immune system in a process designated “cancer immunoediting” [14, 15]. As a result, the Cancer-Immunity Cycle is interrupted at one or more steps in cancer patients (Fig. 2). For this reason, cancer immunotherapy must be personalized (1) to identify the rate-limiting steps in any given patient, because these may be different in different patients; (2) to combine strategies to overcome these hurdles; (3) to trigger the Cancer-Immunity Cycle to proceed. Cancer immunotherapies are designed to bypass several crucial steps of this cycle and to amplify and propagate the anti-tumor immune response. For example, cancer vaccines can bypass both steps ① and ② to initiate an immune response. Cell transfer therapy starts the cycle from step ④. Oncologists will need to select and combine appropriate immunotherapeutic strategies to generate and maintain anti-tumor immunity by sustaining Cancer-Immunity Cycles in each patient.

For a long time, investigators focused primarily on induction and expansion of CTLs that can selectively recognize cancer cells, but now we understand that the paradigm of immunotherapy has shifted more towards

overcoming immunosuppression, rather than actively inducing anti-tumor immunity. We will discuss these points in greater detail in the next section.

Immune checkpoint therapy

Because of the remarkable clinical benefits observed in some patients due to the development of immune checkpoint therapy, cancer immunotherapy in general has once more come under the spotlight. The immune system is equipped with multiple negative feedback mechanisms to prevent excessive immunopathology and autoimmunity. Immune checkpoint pathways are the locus of action for several tumor escape mechanisms because cancer cells hijack this system to evade the immune response. For example, interactions between CTLA-4 on activated T cells and CD80/86 on DCs results in the transmission of inhibitory signals to T cells (inhibition at step ③). Another pathway is represented by the binding of PD-1 on activated T cells to PD-L1 on tumor cells, which also attenuates T cell signaling and inhibits T cell proliferation and function (inhibition at step ⑦). Immune checkpoint inhibitors, such as anti-CTLA-4, anti-PD-1, and anti-PD-L1 antibodies can re-activate impaired anti-tumor immune responses by

blocking this inhibition and re-initiating the procession of the Cancer-Immunity Cycle [7, 16, 17]. It has passed a decade until immune check point blockade has become a paradigm-shifting in the treatment for solid tumors since the groups of Dr. J. Allison and Dr T. Honjo reported that blocking CTLA-4 and PD-1 activated T cell responses and potentiated anti-cancer immunity, respectively [18, 19]. In 2011, ipilimumab (an anti-CTLA-4 mAb) was approved by the FDA for the treatment of melanoma [16]; shortly thereafter, a high response rate was also reported for the treatment of certain solid cancers by nivolumab (an anti-PD-1 mAb) [17]. Based on these and other reports of successful novel immunotherapies and T cell transfer therapies, *Science* magazine designated cancer immunotherapy “Breakthrough of the year 2013” [7]. In Japan, nivolumab was approved for the treatment of malignant melanoma and lung cancer in 2014 and 2015, respectively. It is anticipated that approval will soon be extended to renal cell carcinoma (RCC), bladder cancer, lymphoma, triple negative breast cancer and many other types of cancer over the next few years. The success of immune checkpoint blockade convinced many oncologists who had remained skeptical about cancer immunotherapy of the existence of anti-tumor immunity and of the potential benefit of its therapeutic application. Additionally, another paradigm shift is occurring whereby the targets of tumor treatment are no longer the cancer cells themselves but the cells of the patient’s immune system. In addition to the molecules expressed on T cells, other molecules and cells that are associated with an immunosuppressive microenvironment induced by the cancer cells are also promising targets for immunotherapy. Therefore, the overall potential of cancer immunotherapy will be further extended by the integration of several therapeutic drugs according to the immunosuppressive mechanisms involved at different “checkpoints” in each patient.

All cancer therapy is immunotherapy

Additional to surgery, chemotherapy and radiotherapy, immunotherapy can be considered as a fourth pillar of cancer treatment. Recently, the concept has emerged that in a way all cancer therapy is immunotherapy. For example, tumor cell killing by some chemotherapeutics such as anthracyclines and oxaliplatin (but not all agents), or by radiotherapy, results in antigen release as well as DC maturation, leading to the induction of anti-tumor immunity. As mentioned above, this type of death has been designated ICD. ICD facilitates DC migration into the tumor, enhances tumor antigen uptake and presentation, together with appropriate costimulatory signals, to T cells (intervention at steps ①–③ in Fig. 2). In addition,

chemotherapeutic agents such as cyclophosphamide and gemcitabine, and molecular targeted drugs like sunitinib, decrease the number of Tregs and/or MDSCs (intervention at steps ③ and ⑦ in Fig. 2) [20]. Surgical removal of the tumor can also facilitate anti-tumor immunity in that it not only reduces tumor burden but also normalizes the immunosuppressive state induced by the tumor. Thus, even standard care of cancer can strongly affect the immune response.

Neoantigens

In their recent review, Drs. Schumacher and Schreiber described “The genetic damage that on the one hand leads to oncogenic outgrowth can also be targeted by the immune system to control malignancies.” [21]. Somatic cells accumulate spontaneously occurring mutations, the majority of which do not have significant effects, but some of which affect a gene or regulatory element that leads to a changed phenotype that may as a consequence contribute to carcinogenesis. However, independent of their functional relevance for carcinogenesis, these genetic and cellular alterations including both driver and passenger mutations provide the immune system with potential target antigens by which they may recognize and eradicate cancer cells. There are differences in mutation load between malignant tumors and somatic mutations are found in 10–100/Mb for melanoma and non-small cell lung cancer. However, breast cancer yields rather low mutation rate of 1/Mb which might be relatively low immunogenicity [22]. The mutated proteins expressed exclusively in cancer cells and recognized by the immune system are known as neoantigens (Fig. 3) [23]. Some but not all somatic missense mutations can yield mutational epitopes (neoepitope) presented by each patient’s autologous HLA molecules. Neoantigens are not expressed in the thymus and are thus not affected by central T cell tolerance are expected to be more immunogenic than non-mutated self-antigens (Fig. 4) [24]. There is ample evidence for a role of neoantigens in determining the magnitude of intratumoral T cell responses. Analysis of RNA-seq data of 515 patients from The Cancer Genome Atlas has demonstrated a positive association between the numbers of predicted MHC Class I-associated neoepitopes present and increased patient survival paralleled by higher CD8A gene expression suggesting greater intratumoral CTL content [25]. In addition, the level of intratumoral transcripts associated with cytolytic activity correlates with a higher mutational burden in 18 human tumor types [26].

Recent studies of immune checkpoint blockade and tumor-infiltrating lymphocyte (TIL) therapy demonstrate the relative importance of neoantigens in the effects of

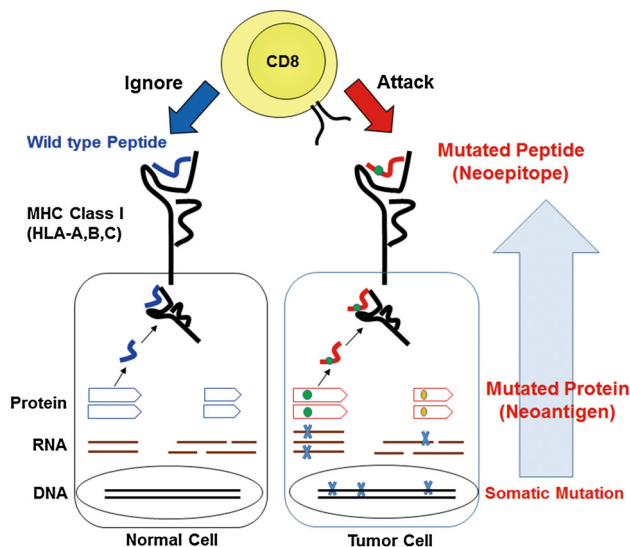


Fig. 3 Neoantigens. Mutated proteins resulting from somatic tumor-specific mutations can be targets (neoantigens) on the cancer cell and induce an immune response in the host. Only tumor cells display the mutated peptide (neoepitope) together with MHC class I molecules

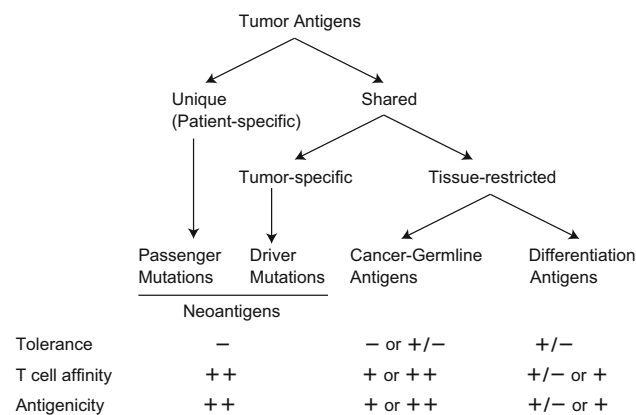


Fig. 4 Classification of human tumor antigens and their antigenicity. Neoantigens result from somatic mutations and have strong antigenicity

cancer immunotherapies. In a murine carcinogen-induced transplantable tumor model, it has been demonstrated that neoantigens can serve as cancer rejection antigens [15, 27] and that checkpoint blockade alters both the quality and the magnitude of the neoantigen-specific intratumoral T cell response [28]. In a human study, T cell reactivity against neoantigens was found to be enhanced by anti-CTLA-4 treatment [29], and it was shown that cytotoxic T cell activity in the tumor also appears to play a central role in anti-PD-1 mAb therapy for the metastatic melanoma [30]. Long-term clinical benefit in melanoma patients treated with anti-CTLA-4 mAb [31] and clinical responses in non-small cell lung cancer patients treated with anti-PD-1 mAb [32] also correlate with mutational load. In addition, mismatch-repair deficient colorectal tumors with a large

number of somatic mutations are more susceptible to anti-PD-1 mAb [33]. For breast cancer, BRCA1 associated triple negative breast cancer shows higher genomic instability [34], which might be a good candidate for immune check point blockade.

The most direct evidence for the role of neoantigen-specific T cells in anti-tumor immunity comes from TIL therapy. TILs from patients who showed clinical benefit contain neoantigen-specific populations that induce tumor regression and durable responses when adoptively transferred [35]. In addition to melanoma, neoantigen-specific CD4⁺ or/and CD8⁺ T cells were detected in gastrointestinal cancer [36, 37]. Large-scale HLA-A2 tetramer-based analysis using a panel of all the known shared non-mutated antigens has demonstrated that 99 % of human TILs in melanoma are not reactive with any of the shared antigens tested and that their reactivity is likely to be against neoantigens [38]. These data support the notion that there is a substantial contribution of neoantigen-specific T cell responses to anti-tumor immunity and immunotherapies.

Personalized cancer vaccines targeting unique antigens in each individual

The concept of mutated neoantigen-based cancer immunotherapy is not novel. Gjertsen et al. conducted the clinical study of mutant ras peptides vaccination and reported the induction of peptide-specific T cell responses (Table 1) [39]. We also conducted vaccine study indirectly targeting tumor specific mutant neoantigens [40]. We chose autologous tumor lysates as a source of tumor antigens for DC vaccination. This was despite their preparation by repetitive freezing and thawing cycles being quite old-fashioned and laborious for personalized processing and the fact that the precise contents are unidentified and differ from patient to patient. However, tumor lysate potentially contains a constellation of mutated proteins including neoantigens derived from somatic mutations in each patient's tumor that are now acknowledged as dominant tumor antigens. Therefore, our DC vaccine could be a cutting edge cancer vaccine targeting a set of neoantigens, the majority of which are unique to each individual patient. When the vaccine is administered to patients, their DCs should process and present these neoantigens, leading to the activation of a diverse repertoire of neoantigen-specific T cells. These actions amplify the Cancer-Immunity Cycles when combined with the immunoregulatory activity of sunitinib.

Autologous heat shock protein vaccines are therapeutic cancer vaccines based on similar concepts. HSPs are chaperones that bind peptides and transport them

Table 1 Neoantigen-based cancer immunotherapy

| | |
|------|---|
| 1988 | Identification of tumor antigen by expression cloning: CTLs recognized an amino acid change produced by one nucleotide mutation in P1A gene [54] |
| 1991 | Identification of human tumor antigen, MAGE-1, recognized by CTLs [44] |
| 1995 | Clinical trial of peptide vaccination with mutant ras peptides [39] |
| 2003 | Finishing the euchromatic sequence of the human genome [55] |
| 2005 | Identification of neoantigens generated by somatic point mutations in the patient's melanoma [45] Autologous TILs in melanoma patient, who experienced a complete regression recognized novel mutated transcripts (neoantigens) [56] |
| 2008 | Successful application of in silico-based epitope prediction algorithm for the identification of candidate tumor antigens (neoantigens) [49] |
| 2011 | Approval of anti-CTLA-4 Ab (ipilimumab) for the treatment of melanoma by US FDA |
| 2012 | Tumor mutations and the epitope landscape predicted by MHC-binding algorithm demonstrates that many nonsynonymous somatic mutations in tumors are immunogenic and confer anti-tumoral vaccine activity [27] |
| 2013 | Detection of neoantigen-specific T-cell reactivity in an Ipilimumab-responsive melanoma [29] <i>Science</i> selected cancer immunotherapy as breakthrough of the year [7] |
| 2014 | Approval of anti-PD-1 Ab (nivolumab) for the treatment of melanoma in Japan Approval of anti-PD-1 Ab (pembrolizumab) for the treatment of melanoma by US FDA Adoptive transfer of mutated neoantigen-reactive CD4 ⁺ T helper 1-type TIL achieved a decrease in target lesions with prolonged stabilization of disease [36] |
| 2015 | DC vaccination directed at tumor-encoded amino acid substitutions [52] Mutational landscape determines sensitivity to PD-1 blockade in non-small cell lung cancer and anti-PD-1 therapy enhances neoantigen-specific T cell reactivity [32] |

throughout the cell. HSP-peptide complexes isolated from tumor cells represent an individually unique antigenic repertoire of each cancer, including their neoantigens. In this approach, patients undergo surgical resection, and HSP-peptide complexes are isolated from resected tumor for vaccine production. Srivastava et al. demonstrated that HSP peptide complex-96 (HSPPC-96) conferred protective immunity against cancer [41]. While an HSPPC-96 vaccine (OncophageTM) was approved in Russia in 2008 for patients who have earlier-stage kidney cancer [42], it is not yet approved in the USA. However, based on the promising results in patients with recurrent glioblastoma [43], a randomized Phase II study for the treatment of glioblastoma sponsored by the NCI is currently underway.

Developing highly personalized immunotherapies based on mutational analysis of tumors

Since the MAGE-1 antigen was identified in 1991 as an immunogenic molecular entity on tumor cells that can be recognized by human T cells [44], a large number of antigens, most of which are non-mutated self-antigens aberrantly expressed in human tumors, has been identified via cDNA expression cloning, using recognition by tumor-reactive CTLs from the corresponding patients as the detection system (Table 1). As you can imagine, identification of individually distinct mutated antigens was very laborious and painstaking, until recently. Under

these circumstances, it is of note that Lennerz et al. identified five neoantigens generated by somatic point mutations in one patient's melanoma and demonstrated a dominant role of neoantigen-specific CTLs in controlling disease [45]. In the early 2000s, reverse immunology, that is, prediction and identification of immunogenic peptides from the sequence of a gene product of interest, has been combined with various mRNA/cDNA subtraction methods to identify their differential presence in normal tissues versus cancer cells. Methods have included classical cDNA subtraction techniques, representational differential analysis, differential PCR display, and comparison of cDNA profiles obtained by genome-wide cDNA microarrays to isolate tumor-associated antigens [46, 47]. Candidate genes with cancer-specific expression are analyzed using computer algorithms predicting possible peptide binding to any given patient HLA molecule. This approach resulted in the identification of a large number of tumor-associated antigens, mainly non-mutated self-antigens and cancer-germline antigens. At that time, efforts were focused on the identification of shared antigens that are expressed on the tumors of as many patients as possible; unique antigens expressed by individual tumors were excluded. T cells reactive to shared antigens, mostly non-mutated self-antigens, are likely subject to central tolerance and their receptor affinity is often low (Fig. 4). This may be one reason why it has often been observed that cancer vaccines targeting these shared antigens induce antigen-reactive T cells detectable in the

peripheral blood, but these rarely correlate with tumor regression in clinical trials.

In the late 2000s, information from large-scale sequencing studies of individual tumors, such as the Human Cancer Genome Project, began to become increasingly available [48]. Segal et al. showed that the *in silico* approach to identify potential cancer antigens using epitope prediction algorithms is feasible by demonstrating that breast and colorectal cancers possibly contain on average ~10 and ~7 HLA-A2 neopeptides, respectively. To do this, they inspected 1152 peptides containing missense mutations previously identified in 11 breast and 11 colorectal cancers [49]. The emergence of next-generation sequencing (NGS) technology has made it possible to illuminate the full genetic landscape of human cancer [50], facilitating the utilization of genomic information to identify neoantigens in individual cancers. To identify neoantigens, NGS data of a tumor are first compared with the non-transformed tissue from the same patient to exclude single nucleotide polymorphisms (SNPs) and to reveal the full range of genomic alterations within the tumor, including single nucleotide substitutions, insertions–deletions, structural rearrangements and copy number alterations (Fig. 5). Of all these, only those mutations that encode a non-germline protein sequence are screened out by algorithms such as NetMHC [51] due to their estimated strength of binding to MHC molecules. RNA-seq data obtained from tumor material can be used in parallel to determine the expression of mutated antigens in the tumor. Recently, Sahin and colleagues demonstrated that this strategy can be effectively applied to the development of personalized immunotherapy in a murine model [27]. They performed whole exome sequencing of the B16 melanoma and identified 962 nonsynonymous somatic point mutations, 563 of which were in expressed genes. Importantly, 16 of 50 validated mutations were shown to be immunogenic, 3 of which were confirmed as being endogenously processed and presented to T cells. Finally, two of these were shown to inhibit tumor growth *in vivo* when applied as tumor vaccines. These results suggest that whole genome/exome-sequencing of human tumors will be equally informative. Two European Commission-funded consortia, the Glioma Actively Personalized Vaccines Consortium (GAPVAC at <http://www.gapvac.eu>) and the Mutanome Engineered RNA Immuno-Therapy project (MERIT at <http://www.merit-consortium.eu>) are currently underway in Europe. GAPVAC is a peptide vaccine for the treatment of glioblastoma, while MERIT is an RNA-based vaccine for triple-negative breast cancer. In addition, several clinical studies have just been initiated in the US. Over the next few years, these studies will provide us with more insight as to whether neoantigens are crucial targets of antitumor immunity and whether targeting neoantigens is

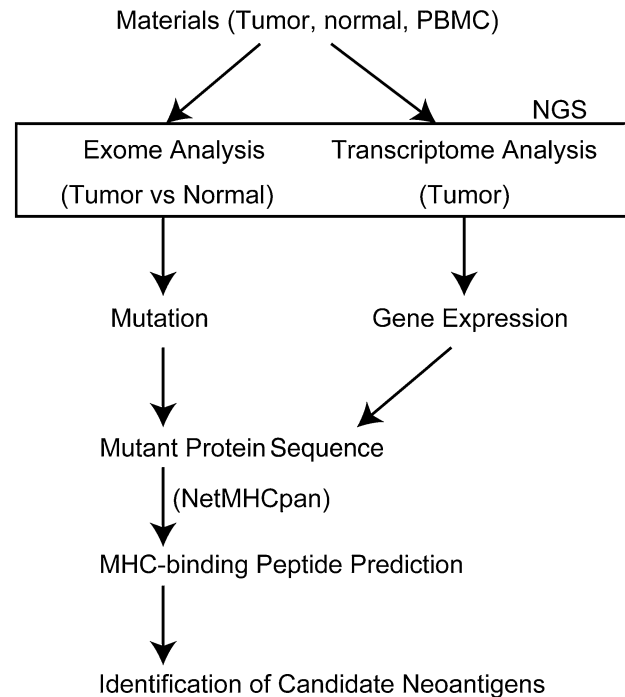


Fig. 5 Neoantigen prediction. NGS data from the tumor are first compared with those of appropriate normal tissue from the same patient to detect the full range of genomic (exomic) alterations within a tumor. Of these, only those mutations that encode mutant protein screened by algorithms such as NetMHC for their estimated binding capacity to MHC molecules are selected. Transcriptome analysis of tumor material can be used in parallel to determine the expression of mutated antigens in the tumor. The recognition of neoantigen is determined by standard immunological assays and the results are used as feedback to optimize *in silico* neoantigen identification

paramount for disease control. Of note, Carreno et al. published a first proof-of-concept study that neoantigen-specific CD8⁺ T cell responses can be enhanced through vaccination with DCs pulsed with fully defined neopeptide peptides in 3 melanoma patients [52].

Regulatory challenges to personalized immunotherapy

Several strategies to target patient-specific neoantigens are now available [21]. Once potential neoantigens have been identified from the individual patient tumor material, a synthetic vaccine can be designed in either RNA, DNA or peptide format, and this vaccine can then be given to the patient together with adjuvant and immune checkpoint inhibitors. Alternatively, neoantigens can be utilized to induce or expand neoantigen-specific T cells that are infused back to the patient. Neoantigen-based immunotherapy, especially using cancer vaccines, represents the ultimate personalized medicine in that target

antigens need to be determined in each individual patient. Neoantigen-based cancer vaccines exceed the current concepts of personalized medicine, which is characterized by biomarker-based stratification of patients and the application of treatment evaluated in each stratified patient group beforehand. This stage of personalized medicine is termed precision medicine, which in general applies to the selection of molecular targeted drugs. Neoantigen-based cancer vaccines also go beyond the current concepts of personalized medicine in that an individual cancer vaccine is manufactured for an individual patient on-demand according to the results of genomic analysis and mutation selection. While conventional personalized medicine seeks patient subgroups fitting to the drug, neoantigen-based cancer vaccines are tailored to the patient. In such a case, extensive safety and efficacy testing of each individual vaccine is not feasible, in contrast to the requirements for the development of chemical and biological drug products. Therefore, the development of personalized immunotherapies might require a paradigm shift from the currently available regulatory framework that was intended for conventional drug development. Neoantigen-based cancer vaccines will be highly personalized but their components are molecularly well-defined and can be produced by chemical synthesis. In any case, the components of the vaccine are peptides, DNA, or RNA for which safety has already been established for vaccine use. As such, safety and efficacy of neoantigen-based cancer vaccines will need to be judged on the basis of their generic components in preclinical and early clinical proof-of-principle studies. Recently, the Regulatory Research Group of the Association of Cancer Immunotherapy (CIMT) and the Innovation Task Force of the European Medicines Agency (EMA) have suggested that the existing regulatory framework for autologous cell therapies can be applied with modification to the development of neoantigen-based cancer vaccines [53].

Future directions

Until recently, a major aim of cancer immunotherapy was to identify shared tumor antigens. However, identification of neoantigens and preparation of personalized cancer vaccines in individual patients will become the mainstream of cancer immunotherapy in future because anti-tumor immunity that can control tumor growth focuses on neoantigens. In addition, to make immunotherapy effective, personalized strategies to regulate the immune suppressive microenvironment will be required. The immune checkpoints that halt the Cancer-Immunity Cycle might vary in each individual patient. Therefore, future immunotherapy needs to be personalized also in terms of the identification of

immunosuppressive mechanisms as well as target antigens and integrated with immune regulatory strategies. For that purpose, intense collaboration between academia, business and regulatory authorities will be crucial.

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Compliance with ethical standards

Conflict of interest Kazuhiro Kakimi received a research grant from Medinet Co. Ltd. Tomoharu Sugie received lecture fees from Astra Zeneca and Novartis Pharma. Other authors have no conflict of interest.

References

1. Parish CR. Cancer immunotherapy: the past, the present and the future. *Immunol Cell Biol.* 2003;81:106–13.
2. Melief CJ, van Hall T, Arens R, Ossendorp F, van der Burg SH. Therapeutic cancer vaccines. *J Clin Invest.* 2015;125:3401–12.
3. Rosenberg SA, Restifo NP. Adoptive cell transfer as personalized immunotherapy for human cancer. *Science.* 2015;348:62–8.
4. Morgan RA, Dudley ME, Wunderlich JR, Hughes MS, Yang JC, Sherry RM, et al. Cancer regression in patients after transfer of genetically engineered lymphocytes. *Science.* 2006;314:126–9.
5. Porter DL, Levine BL, Kalos M, Bagg A, June CH. Chimeric antigen receptor-modified T cells in chronic lymphoid leukemia. *N Engl J Med.* 2011;365:725–33.
6. Sharma P, Allison JP. The future of immune checkpoint therapy. *Science.* 2015;348:56–61.
7. Couzin-Frankel J. Breakthrough of the year 2013. *Cancer immunotherapy.* *Science.* 2013;342:1432–3.
8. Chen DS, Mellman I. Oncology meets immunology: the cancer-immunity cycle. *Immunity.* 2013;39:1–10.
9. Kroemer G, Galluzzi L, Kepp O, Zitvogel L. Immunogenic cell death in cancer therapy. *Annu Rev Immunol.* 2013;31:51–72.
10. Viaud S, Daille R, Boneca IG, Lepage P, Langella P, Chamaillard M, et al. Gut microbiome and anticancer immune response: really hot Sh*t! *Cell Death Differ.* 2015;22:199–214.
11. Nishikawa H, Sakaguchi S. Regulatory T cells in cancer immunotherapy. *Curr Opin Immunol.* 2014;27:1–7.
12. Corbiere V, Chapiro J, Stroobant V, Ma W, Lurquin C, Lethe B, et al. Antigen spreading contributes to MAGE vaccination-induced regression of melanoma metastases. *Cancer Res.* 2011;71:1253–62.
13. Motz GT, Coukos G. Deciphering and reversing tumor immune suppression. *Immunity.* 2013;39:61–73.
14. Schreiber RD, Old LJ, Smyth MJ. Cancer immunoediting: integrating immunity's roles in cancer suppression and promotion. *Science.* 2011;331:1565–70.
15. Matsushita H, Vesely MD, Koboldt DC, Rickert CG, Uppaluri R, Magrini VJ, et al. Cancer exome analysis reveals a T-cell-dependent mechanism of cancer immunoediting. *Nature.* 2012;482:400–4.
16. Hodi FS, O'Day SJ, McDermott DF, Weber RW, Sosman JA, Haanen JB, et al. Improved survival with ipilimumab in patients with metastatic melanoma. *N Engl J Med.* 2010;363:711–23.
17. Topalian SL, Hodi FS, Brahmer JR, Gettinger SN, Smith DC, McDermott DF, et al. Safety, activity, and immune correlates of anti-PD-1 antibody in cancer. *N Engl J Med.* 2012;366:2443–54.

18. Leach DR, Krummel MF, Allison JP. Enhancement of antitumor immunity by CTLA-4 blockade. *Science*. 1996;271:1734–6.
19. Iwai Y, Ishida M, Tanaka Y, Okazaki T, Honjo T, Minato N. Involvement of PD-L1 on tumor cells in the escape from host immune system and tumor immunotherapy by PD-L1 blockade. *Proc Natl Acad Sci USA*. 2002;99:12293–7.
20. Galluzzi L, Senovilla L, Zitvogel L, Kroemer G. The secret ally: immunostimulation by anticancer drugs. *Nat Rev Drug Discov*. 2012;11:215–33.
21. Schumacher TN, Schreiber RD. Neoantigens in cancer immunotherapy. *Science*. 2015;348:69–74.
22. Alexandrov LB, Nik-Zainal S, Wedge DC, Aparicio SA, Behjati S, Biankin AV, et al. Signatures of mutational processes in human cancer. *Nature*. 2013;500:415–21.
23. Heemskerk B, Kvistborg P, Schumacher TN. The cancer anti-genome. *EMBO J*. 2013;32:194–203.
24. Gilboa E. The makings of a tumor rejection antigen. *Immunity*. 1999;11:263–70.
25. Brown SD, Warren RL, Gibb EA, Martin SD, Spinelli JJ, Nelson BH, et al. Neo-antigens predicted by tumor genome meta-analysis correlate with increased patient survival. *Genome Res*. 2014;24:743–50.
26. Rooney MS, Shukla SA, Wu CJ, Getz G, Hacohen N. Molecular and genetic properties of tumors associated with local immune cytolytic activity. *Cell*. 2015;160:48–61.
27. Castle JC, Kreiter S, Diekmann J, Lower M, van de Roemer N, de Graaf J, et al. Exploiting the mutanome for tumor vaccination. *Cancer Res*. 2012;72:1081–91.
28. Gubin MM, Zhang X, Schuster H, Caron E, Ward JP, Noguchi T, et al. Checkpoint blockade cancer immunotherapy targets tumour-specific mutant antigens. *Nature*. 2014;515:577–81.
29. van Rooij N, van Buuren MM, Philips D, Velds A, Toebes M, Heemskerk B, et al. Tumor exome analysis reveals neoantigen-specific T-cell reactivity in an ipilimumab-responsive melanoma. *J Clin Oncol*. 2013;31:e439–42.
30. Tumeh PC, Harview CL, Yearley JH, Shintaku IP, Taylor EJ, Robert L, et al. PD-1 blockade induces responses by inhibiting adaptive immune resistance. *Nature*. 2014;515:568–71.
31. Snyder A, Makarov V, Merghoub T, Yuan J, Zaretsky JM, Desrichard A, et al. Genetic basis for clinical response to CTLA-4 blockade in melanoma. *N Engl J Med*. 2014;371:2189–99.
32. Rizvi NA, Hellmann MD, Snyder A, Kvistborg P, Makarov V, Havel JJ, et al. Cancer immunology. Mutational landscape determines sensitivity to PD-1 blockade in non-small cell lung cancer. *Science*. 2015;348:124–8.
33. Le DT, Uram JN, Wang H, Bartlett BR, Kemberling H, Eyring AD, et al. PD-1 blockade in tumors with mismatch-repair deficiency. *N Engl J Med*. 2015;372:2509–20.
34. Stefansson OA, Jonasson JG, Johannsson OT, Olafsdottir K, Steinarsdottir M, Valgeirsdottir S, et al. Genomic profiling of breast tumours in relation to BRCA abnormalities and phenotypes. *Breast Cancer Res*. 2009;11:R47.
35. Robbins PF, Lu YC, El-Gamil M, Li YF, Gross C, Gartner J, et al. Mining exomic sequencing data to identify mutated antigens recognized by adoptively transferred tumor-reactive T cells. *Nat Med*. 2013;19:747–52.
36. Tran E, Turcotte S, Gros A, Robbins PF, Lu YC, Dudley ME, et al. Cancer immunotherapy based on mutation-specific CD4⁺ T cells in a patient with epithelial cancer. *Science*. 2014;344:641–5.
37. Tran E, Ahmadzadeh M, Lu YC, Gros A, Turcotte S, Robbins PF, et al. Immunogenicity of somatic mutations in human gastrointestinal cancers. *Science*. 2015;350:1387–90.
38. Kvistborg P, Shu CJ, Heemskerk B, Fankhauser M, Thru CA, Toebes M, et al. TIL therapy broadens the tumor-reactive CD8(+) T cell compartment in melanoma patients. *Oncoimmunology*. 2012;1:409–18.
39. Gjertsen MK, Bakka A, Breivik J, Saeterdal I, Solheim BG, Sor-eide O, et al. Vaccination with mutant ras peptides and induction of T-cell responsiveness in pancreatic carcinoma patients carrying the corresponding RAS mutation. *Lancet*. 1995;346:1399–400.
40. Matsushita H, Enomoto Y, Kume H, Nakagawa T, Fukuhara H, Suzuki M, et al. A pilot study of autologous tumor lysate-loaded dendritic cell vaccination combined with sunitinib for metastatic renal cell carcinoma. *J Immunother Cancer*. 2014;2:30.
41. Srivastava PK. Immunotherapy of human cancer: lessons from mice. *Nat Immunol*. 2000;1:363–6.
42. Wood C, Srivastava P, Bukowski R, Lacombe L, Gorelov AI, Gorelov S, et al. An adjuvant autologous therapeutic vaccine (HSPPC-96; vitespen) versus observation alone for patients at high risk of recurrence after nephrectomy for renal cell carcinoma: a multicentre, open-label, randomised phase III trial. *Lancet*. 2008;372:145–54.
43. Bloch O, Crane CA, Fuks Y, Kaur R, Aghi MK, Berger MS, et al. Heat-shock protein peptide complex-96 vaccination for recurrent glioblastoma: a phase II, single-arm trial. *Neuro Oncol*. 2014;16:274–9.
44. van der Bruggen P, Traversari C, Chomez P, Lurquin C, De Plaen E, Van den Eynde B, et al. A gene encoding an antigen recognized by cytolytic T lymphocytes on a human melanoma. *Science*. 1991;254:1643–7.
45. Lennerz V, Fatho M, Gentilini C, Frye RA, Lifke A, Ferel D, et al. The response of autologous T cells to a human melanoma is dominated by mutated neoantigens. *Proc Natl Acad Sci USA*. 2005;102:16013–8.
46. Kawakami Y, Fujita T, Matsuzaki Y, Sakurai T, Tsukamoto M, Toda M, et al. Identification of human tumor antigens and its implications for diagnosis and treatment of cancer. *Cancer Sci*. 2004;95:784–91.
47. Nishimura Y, Tomita Y, Yuno A, Yoshitake Y, Shinohara M. Cancer immunotherapy using novel tumor-associated antigenic peptides identified by genome-wide cDNA microarray analyses. *Cancer Sci*. 2015;106:505–11.
48. Sjoblom T, Jones S, Wood LD, Parsons DW, Lin J, Barber TD, et al. The consensus coding sequences of human breast and colorectal cancers. *Science*. 2006;314:268–74.
49. Segal NH, Parsons DW, Peggs KS, Velculescu V, Kinzler KW, Vogelstein B, et al. Epitope landscape in breast and colorectal cancer. *Cancer Res*. 2008;68:889–92.
50. Meyerson M, Gabriel S, Getz G. Advances in understanding cancer genomes through second-generation sequencing. *Nat Rev Genet*. 2010;11:685–96.
51. Lundegaard C, Lamberth K, Harndahl M, Buus S, Lund O, Nielsen M. NetMHC-3.0: accurate web accessible predictions of human, mouse and monkey MHC class I affinities for peptides of length 8–11. *Nucleic Acids Res*. 2008;36:W509–12.
52. Carreno BM, Magrini V, Becker-Hapak M, Kaabinejadian S, Hundal J, Petti AA, et al. Cancer immunotherapy. A dendritic cell vaccine increases the breadth and diversity of melanoma neoantigen-specific T cells. *Science*. 2015;348:803–8.
53. Britten CM, Singh-Jasuja H, Flamion B, Hoos A, Huber C, Kallen KJ, et al. The regulatory landscape for actively personalized cancer immunotherapies. *Nat Biotechnol*. 2013;31:880–2.
54. De Plaen E, Lurquin C, Van Pel A, Mariame B, Szikora JP, Wolfel T, et al. Immunogenic (tum-) variants of mouse tumor P815: cloning of the gene of tum- antigen P91A and identification of the tum- mutation. *Proc Natl Acad Sci USA*. 1988;85:2274–8.
55. International Human Genome Sequencing Consortium. Finishing the euchromatic sequence of the human genome. *Nature*. 2004;431:931–45.
56. 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.