

Cancer immunotherapy through the prism of adaptation: Will Achilles catch the tortoise?

Igor Y. Malyshev^{a,b,*}, Larisa V. Kuznetsova^a, Stefania Mardente^c

^a Laboratory of Cellular Biotechnologies, Scientific Research Institute of Medicine and Dentistry, A. I. Yevdokimov Moscow State University of Medicine and Dentistry, Delegatskaya Ulitsa, 20/1, Moscow 127473, Russia

^b Department of Pathophysiology, General Medicine Faculty, A. I. Yevdokimov Moscow State University of Medicine and Dentistry, Delegatskaya Ulitsa, 20/1, Moscow 127473, Russia

^c Department of Experimental Medicine, University Sapienza, Piazzale Aldo Moro 5, 00185 Rome, Italy

ABSTRACT

There is no secret that despite the rapid development of new methods of cancer therapy, we still are not able to completely destroy the tumor. Every time we attack the tumor, the tumor neutralizes our attempts. Carcinogenesis can be presented as a tree whose branches are different pro-tumor mechanisms and whose trunk is a biological phenomenon that “feeds” those branches. A tree can be destroyed in two ways: either by cutting a branch for a branch without a guarantee that new branches will not grow, or cutting down the trunk and letting the branches wither away. To cut down the trunk, it is necessary to understand the nature of the biological phenomenon, which helps the tumor to avoid attack by the immune system, drugs and immunotherapy. The clue is that the pro-tumor mechanisms are united by one goal – to increase the resistance of the tumor cell to immune factors and drugs. A phenomenon that improves cell resistance is well known in biology – adaptation. If the immunity does not immediately destroy the tumor cell, the cell begins to adapt to it. Our hypothesis is that short range adaptation to immune factors plays a role in the formation of tumor tolerance for immunity and immunotherapy. This gives rise to the idea of reducing the survival of tumor cells by disrupting adaptation mechanisms. Indeed, “turning off” the immune system for a period of time before therapy and applying immunotherapy only to tumor cells that have lost their increased resistance could be a new approach to increase the effectiveness of immunotherapy.

In 2018, 9.6 million people died of cancer [1] – exactly the same as the number of soldiers who died during the First World War [2]. This number is not going down. The task of developing effective ways of treating cancer requires an understanding of the mechanisms of carcinogenesis. Discovery of the role of immunity in tumor growth has given rise to a new method of cancer treatment – immunotherapy. However, immunotherapy does not always help and does not help everybody. Here we look at the interaction of immunity and tumors and types of immunotherapy and explore how to increase the effectiveness of cancer treatment.

The cancer-immunity cycle

When tumor cells appear, the immune system forms an immune cycle against cancer. This cycle is described in the review by Daniel S. Chen and Ira Mellman [3]. Here we briefly describe the main stages of the cycle (Fig. 1).

At the first stage, the tumor, as a result of necrosis, releases tumor antigens. At the second, antigen-presenting cells (APC) seize antigens and expose them to the surface using the Major Histocompatibility

Complex (MHC). IL-1, TNF- α , INF- γ , CD40, Toll-like receptor ligands and adjuvants stimulate the process, and IL-4, IL-10, IL-13 inhibit it [4]. Furthermore, APCs with antigen migrate to the lymph nodes.

There, at the third stage, tumor antigens associated with APCs bind to the T cell receptor (TCR) of CD8 + T cells and stimulate differentiation of T cells into cytotoxic T lymphocytes (CTL) [5]. At the same time, APCs present tumor antigen in association with MHC II molecules to T CD4 lymphocytes. Presentation of antigens in association with MHC II molecules to CD4 + T cells help to increase CD8 + T cell responses.

The interaction of molecules B7-1 or B7-2 with CD28, CD137 and CD137L, OX40 and OX40L, CD27 and CD70, as well as the effect of IL-2 and IL-12, stimulate this process, while interactions of CTLA-4 with B7.1, PDL-1 with B7.1 or with PD-1 inhibit it (Fig. 2) [3].

At the fourth stage, CTLs enter the bloodstream and reach the organ where the tumor is located with blood flow. The CTLs movement is guided by the CX3CL1, CXCL9, CXCL10 and CCL5 [6].

At the fifth stage, CTLs from the bloodstream penetrate the vascular wall and infiltrate the tumor [7]. The number of tumor infiltrating lymphocytes (TIL) depends on the relationship between the stimulants

* Corresponding author at: Department of Pathophysiology, Laboratory of Cellular Biotechnologies, Moscow State University of Medicine and Dentistry, Delegatskaya Ulitsa, 20/1, Moscow 127473, Russia.

E-mail address: MALYSHEV_IU@msmsu.ru (I.Y. Malyshev).

<https://doi.org/10.1016/j.mehy.2019.109545>

Received 15 September 2019; Received in revised form 14 December 2019; Accepted 31 December 2019

0306-9877/ © 2020 The Authors. Published by Elsevier Ltd. This is an open access article under the CC BY-NC-ND license (<http://creativecommons.org/licenses/by-nc-nd/4.0/>).

ANTI-CANCER CYCLE AND ITS PRO-TUMOR IMMUNOEDITING

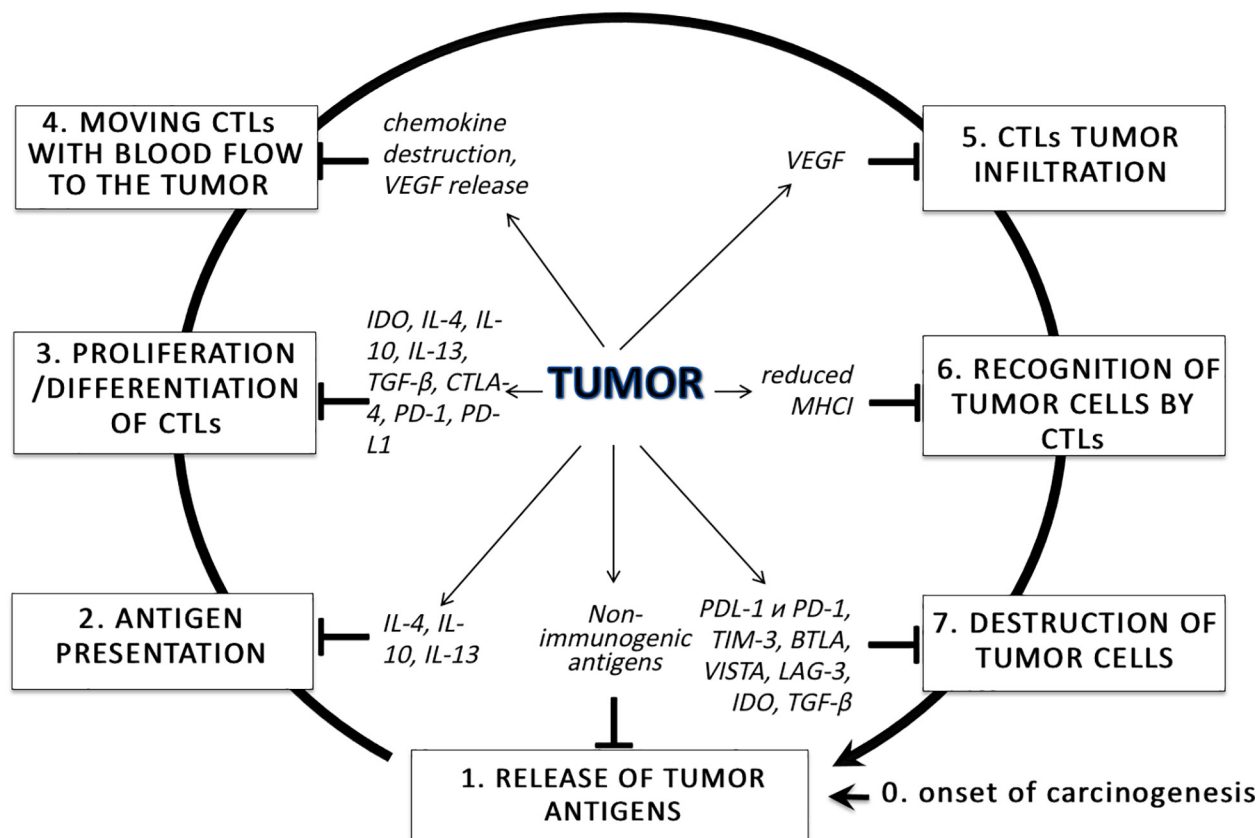


Fig. 1. Immunological cycle against cancer. Methods of pro-tumor immunoeediting aimed at this stage of the cycle are indicated in italics. Adapted from [3].

of the process — LFA1, ICAM1 and selectin, and inhibitors — VEGF and the endothelin B receptor [8].

At the sixth stage, CTLs interact with cancer cells by binding MHC I presented antigens with their specific TCR [4].

At the seventh stage, CTLs destroy cancer cells [5]. Destroyed cancer cells release a new batch of antigens (step 1). As a result, the immune cycle is re-activated to produce the next turn to destroy the tumor.

INF- γ enhances an efficient anti-tumoral immune response, while PDL-1 and PD-1 molecules, TIM-3, BTLA, VISTA, LAG-3, IDO, arginase, TGF- β , T regulatory cells (Treg), myeloid-derived suppressor cells (MDSC) and M2 macrophages inhibit the function of CTL [4].

The cycle against cancer is regulated in order to prevent both excessive cycle activation and the development of an excessive inflammatory reaction, as well as weak activation and a decrease in anti-tumor activity. The former problem is solved by co-inhibitory molecules, and the latter by co-stimulating ones. Co-stimulating receptors CD28 on T cells interact with CD80/B7-1 or CD86/B7-2 on APC and activate the formation of CTL from T cells. A functional pair of a co-inhibitory receptor with a ligand was called a checkpoint. Checkpoints that form CTLA-4 (cytotoxic T-lymphocyte – associated antigen-4) and PD-1 (programmed cell death-1) have been studied the most. CTLA-4 controls the antigen presentation at stage 3 of the cycle, and PD-1 controls the activity of CTL at stage 7 (Figs. 1 and 2).

CTLA-4 on T and T regulatory (Treg) cells inhibits T cell activation due to: i. “Winning” in competition with the co-stimulatory receptor CD28 for binding CD80 and CD86 on APC (Fig. 2); ii. reduced signalling from TCR and CD28; iii. removing CD80 and CD86 on APC using transendocytosis; iv. suppressor effects of Treg bearing CTLA-4 and v. induction of tryptophan-splitting enzyme IDO (indoleamin 2,3-deoxygenase) [9].

PD-1 binds PD-L1 and PD-L2 on immune and tumor cells [10]. PD-1 inhibits CTL function by: i. reduced TCR phosphorylation; ii. Oppression division of CTL; iii. induction of apoptosis of CTL; iv. decrease in the mobility of CTLs and the time of their interaction with APC and tumor cells; and v. stimulating suppressor Treg [9].

Understanding the immune mechanisms of carcinogenesis is the cornerstone of cancer immunotherapy development

The number of oncological diseases is increasing and this undermines faith in the effectiveness of immunity. The absence of an increase in the frequency of induced tumors in mice without T cells has further discredited the immune defense. The concept of immuno-editing, which united the opposite roles of the immune system in cancer, has acted as an “advocate” for anti-tumor immunity [11]. In one context, the immune system recognizes a tumor and forms a cycle against cancer. Spontaneous tumor regressions confirm the clinical significance of immunity. In another context, the tumor “edits” the anti-cancer cycle and survives.

Immune-editing of cycle against cancer

Protumor editing occurs at each stage of the immune cycle (Fig. 1). At the first stage of the immune cycle, the tumor releases its antigens, and APC will recognize them. However, a tumor often carries normal tissue antigens. For example, in prostate cancer (PC), these are prostatic acid phosphatase (PAP), prostate specific antigen (PSA) and prostatic specific membrane antigen (PSMA) [12]. Therefore, APC and T cells, recognizing these antigens, interfere with the immune response to the tumor. In addition, the death of tumor cells can be apoptotic without releasing antigens.

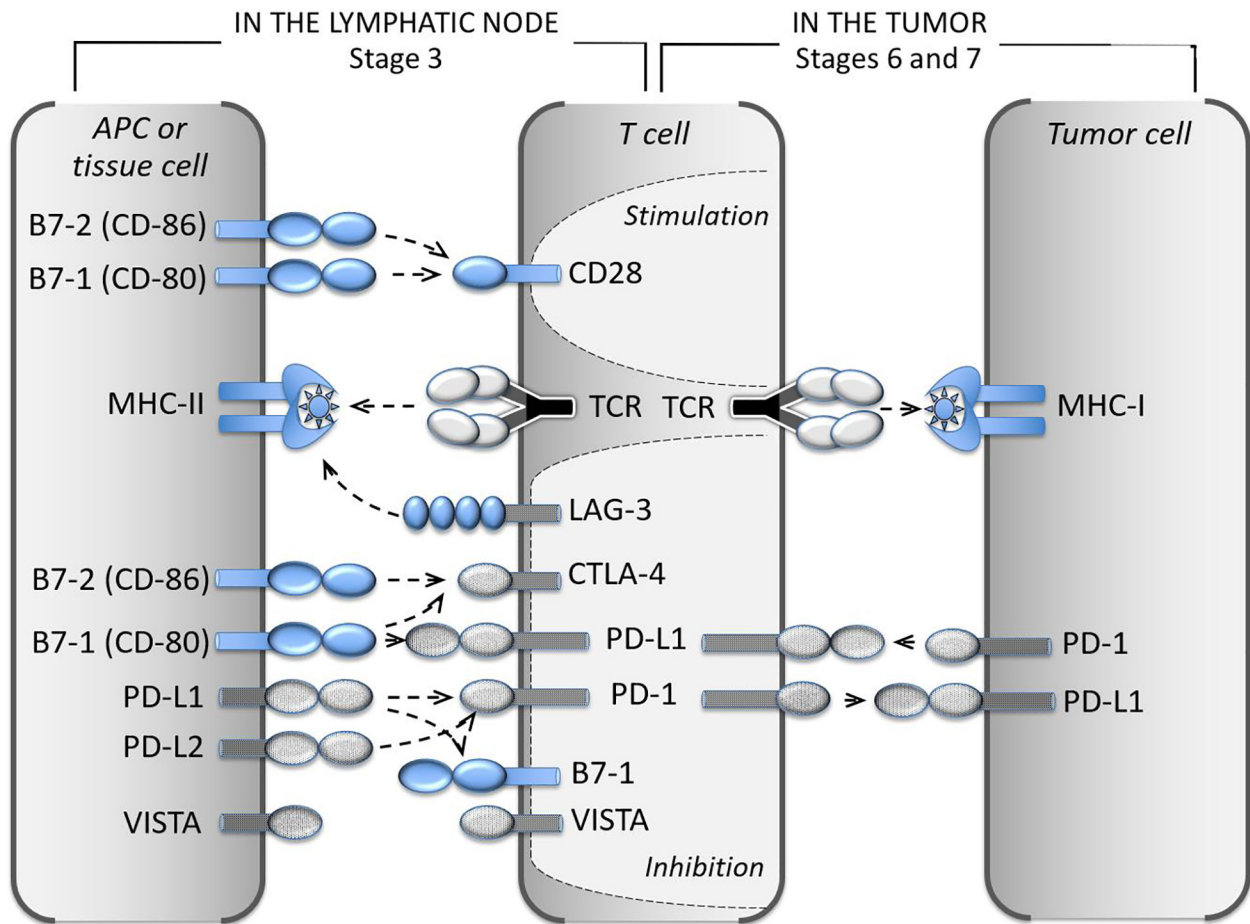


Fig. 2. Stimulating and inhibiting regulators of antigen presentation and interaction of T cells with a tumor.

At the second stage, when the APC captures the antigens, the molecular tools for immuno-editing are secreted by the tumor and tumor-associated macrophage IL-4, IL-10, IL-13. These cytokines violate the capture of antigens by APC [12].

At the fourth stage, the tumor interferes with CTL migration through the endothelial barrier with i. chemokine disruption that attracts CTLs [6], and ii. the release of VEGF, which inhibits the expression of adhesion molecules on the vascular endothelium [8].

At the fifth stage, due to the production of VEGF, the tumor and M2 macrophages prevent the extravasation of CTLs [8] and reduce the number of CTLs in the tumor [3].

At the sixth stage, when antigens are recognized, the number of MHC I associated with tumor antigens decreases. The ability to recognize cancer cells by cognate TCR CTLs is reduced [4].

At the seventh stage of the anti-cancer cycle, when the CTLs begin to destroy cancer cells, the tumor secretes IDO, which depletes tryptophan. Tryptophan is necessary for the survival of CTLs. The number of CTLs is thus reduced [13]. Interestingly, in prostate cancer, IDO production is activated by IFN- γ and TNF- α [14] secreted by Th 1 and CTLs to activate innate response. This is a shining example of protumor immuno-editing. In addition, many cancers increase the expression of PD-L1, which inhibits CTL activity and contributes to tumor progression [10].

Immune cells are also the tools of pro-tumor immune editing. For example, a tumor reprograms anti-tumor M1 macrophages into pro-tumor M2 macrophages that promote tumor growth and metastasis [3,15].

Tumor microenvironment is an important determinant of immune-editing and, as a result, of disease prognosis and success of therapy [16]. The microenvironment may form either “hot” or “cold” tumors. If

tumor microenvironment is rich of T cells and myeloid cells, the tumor is “hot”. The tumor infiltrating T cells are not able to kill a “hot” tumor due to checkpoint-induced T cell inactivation. However, these T cells can be activated by checkpoint inhibitors. “Hot” tumors often produce neoantigens that can cause a strong immune response. “Hot” tumors are often found in cancers of the bladder, head and neck, kidney, liver, melanoma and non-small cell lung cancer, as well as cancers with high microsatellite instability [17]. A microenvironment with a large number of regulatory T cells, myeloid suppressor cells and M2 macrophages, but with a low content of T cells, forms “cold” tumors. Cells of cold tumors create an immunosuppressive environment and therefore an immune response against these tumors is very difficult to induce. Cold tumors include glioblastoma, cancers of the ovaries, prostate and pancreas [18].

Understanding of protumor immuno-editing is necessary in order to develop an effective cancer therapy. Cancer immunotherapy uses immunity-stimulating technologies using vaccines and CTLs, technologies of mimicking anti-cancer cycle stages using modified TCR and CAR-T cell lymphocytes, and PD-1 and CTLA-4 inhibitors to eliminate immunosuppression.

Technologies to stimulate anti-tumor immunity: vaccines and CTLs

The idea behind vaccines based on dendritic cells was to remove APC with a antigen from the tumor suppressive effect. To do this, monocytes are isolated from the patient’s blood, and tumor antigens are extracted from the tumor biopsy or exosomes. Then the monocytes are differentiated to dendritic cells and loaded with antigen. Such cells are a vaccine capable of presenting a tumor antigen. When the vaccine is administered to a patient, “trained” APCs stimulate the formation of

CTLs. The first vaccine on dendritic cells Sipuleucel-T presented the PAP antigen. Sipuleucel-T improved the survival of patients with metastatic prostate cancer by an average of 4 months and increased the probability of survival for 3 years by 10% compared with the placebo group [19].

Another type of vaccine is obtained using a viral vector that contains the tumor antigen gene. The vector is introduced into the body, enters the dendritic cells and embeds its DNA into their genome. As a result of gene expression of the antigen, the dendritic cell receives a tumor antigen and exposes it to the surface. This was how the PROSTVAC vaccine was created with the PSA gene. In clinical trials, PROSTVAC increased the life expectancy of patients with metastatic prostate cancer by more than 8 months [20].

UV1 vaccine based on telomerase reverse transcriptase peptides is an example of a peptide vaccine. After intradermal injection, UV1 induced an immune response in more than 85% of patients with metastatic prostate cancer. In 64% of these patients, PSA decreased to normal values, and in 45% of patients MRI did not reveal prostate tumors [21].

DNA vaccine is another way to stimulate the cycle against cancer. This vaccine is a bacterial plasmid containing the tumor antigen gene under the control of a eukaryotic promoter. After entering the cell, the plasmid produces tumor antigens and, as a result, causes an antitumor response. In clinical studies, DNA vaccines showed promising results [22]. Compared with other vaccines, they are safer, easier to manufacture and have high stability.

The appearance of CTL in the tumor gave rise to the idea of adoptive cell therapy (ACT) with the help of CTLs isolated from the tumor. The ACT technology includes: isolation of CTLs from tumor biopsy material, in vitro CTL expansion and reinfusion of a large amount of CTLs into the patient. In clinical studies, ACT CTL caused a many-year remission in most patients with metastatic melanoma [23]. However, for cancer of the ovaries, breast, colon, cervix and kidney, ACT, CTL only resulted in moderate improvements.

Technologies to mimic key stages of the anti-cancer cycle: lymphocytes with modified TCR and CAR-T cells

Tumor antigen specific T cell are obtained by introducing genes encoding either the antigen specific TCR or the chimeric antigen receptor (CAR).

The binding of the TCR to an antigen represented by MHC/HLA on APC or a tumor cell activates T cells. A TCR consists of α - and β -chains linked to CD3 on the surface of T cells (Fig. 3). A new pair of α - and β -chains specific for a tumor antigen increases the specificity of T cells. The technology for modifying T cells includes: isolating an antigen-specific TCR from a tumor, sequencing its α - and β - chains; insertion of the α and β chains of the antigen-specific TCR into viral vectors; transduction of T cells from patient's blood with ex vivo vectors; expansion of modified T cells and infusion of these cells into patients.

Therapy with TCRs with modified TCR for melanoma antigen MART-1 resulted in prolonged regression of metastatic melanoma in at least 10% of patients [25]. Side effects manifested in the form of: (i) toxicity, which occurs when T cells recognize MART-1 autoantigens inherent in both melanoma cells and normal melanocytes, nerve cells and cardiomyocytes, and (ii) an acute release syndrome of inflammatory cytokines. Good results were also obtained when using modified TCR to MAGE-A4 for esophageal cancer [26].

CAR-T cells are T cells with a genetically modified antigen receptor (Fig. 3). The first-generation CAR consists of the antigen-binding domain of a tumor-specific antibody fused to the intracellular domain of the TCR. The second-generation CAR contains the additional costimulatory domain CD28 or 4-1BB. Third-generation CARs include another costimulatory domain. Additional domains enhance the activation of CAR-T cells.

The production of CAR-T cells begins with the isolation of T cells

from the patient's blood. Then, using viral vectors, the CAR gene is introduced into the T cell genome and proliferation of CAR-T cells is stimulated, increasing to the required number. After that, the end product is administered to the patient [27].

CAR-T cells recognize surface tumor antigens. Unlike vaccines, they do not require immunization, and unlike T cells with modified TCR, they do not need MHC to bind to the antigen. Due to its independence from MHC, CAR-T cells recognize tumor cells that, having lost MHC, become "invisible" to T cells with native or modified TCR [28]. It is estimated that each CAR-T cell and its progeny kill 1000 tumor cells. Evidence of the clinical significance of CAR-T cells was found in acute lymphoblastic leukemia. CAR-T cells targeting a CD19 tumor antigen increased the life expectancy of patients by 13–20 months [29]. However, CAR-T cell therapy was accompanied by a neurotoxic effect and severe cytokine release syndrome.

The effectiveness of CAR-T cells in prostate cancer was demonstrated by doctors at Boston University in 2016 [30]. CAR-T cells, targeting the PSMA antigen, reduced the level of the PSA tumor growth marker by 70% in 40% of patients. There were no side effects. However, in many patients with solid cancers, CAR-T cells did not provide long-term clinical improvement [31].

PD-1 and CTLA-4-checkpoint inhibitors

Immunotherapy of the third group eliminates the immunosuppression created by the checkpoint [9]. For inhibition of CTLA-4 checkpoint, the antibody preparation ipilimumab [32] was used, and for the inhibition of PD-1 checkpoint, a drug preparation of antibodies, nivolumab and pembrolizumab, was used [33–35] (Fig. 4).

Blocking CTLA-4 on T and Treg cells caused prolonged regression of metastatic melanoma. The survival curve for patients reached a plateau of 21% over 3 years and remained stable for 10 years [37]. With advanced lung cancer, hepatocellular carcinoma, colorectal cancer, mesothelioma and prostate cancer, the effectiveness of ipilimumab was low, although in some patients there was an improvement and even sustained remission [38]. Ipilimumab caused undesirable inflammation in the gastrointestinal tract, skin, and endocrine glands, but these complications were eliminated with the help of corticosteroids [32,39].

In preclinical models, PD-1 or PD-L1 blockade increased cytotoxicity, production of inflammatory cytokines and CTL proliferation, contributing to tumor destruction [34]. In 2014–2015, the FDA approved nivolumab (anti-PD-1) and pembrolizumab (anti-PD-1) drugs for the treatment of melanoma, lung cancer, and head and neck cancer.

In metastatic melanoma and in metastatic renal cell carcinoma, the anti-tumor efficacy of nivolumab exceeds the effectiveness of chemotherapy [33,40]. Nivolumab showed unprecedented long-lasting effects, maintaining the anti-tumor effect for melanoma up to 117 weeks. Pembrolizumab was more effective than ipilimumab in patients with progressive melanoma [35] and showed anti-tumor activity in prostate cancer [41]. Side effects of PD-1 checkpoint inhibitors responded well to treatment with steroids [32].

However, four out of five patients are resistant to anti-PD1/PDL1 therapy. There are also patients who, after an initial good response to therapy, eventually develop resistance to PD1/PDL1 inhibitors and tumor growth resumes [42]. Therefore, there is an urgent need to study resistance mechanisms, identify immunotherapy targets and novel approaches to improve patient care.

As a rule, pro-tumor immuno-editing affects not one, but several stages of the cycle against cancer. Therefore, the simultaneous correction of several stages of the cycle is likely to be more effective in the treatment of cancer than monotherapy. Given that the cycle against cancer consists of seven stages, the number of combinations in combination therapy can reach 7 factorial, or 5040 variants. This is a huge reserve for future developments. The task of researchers is to choose the most effective options.

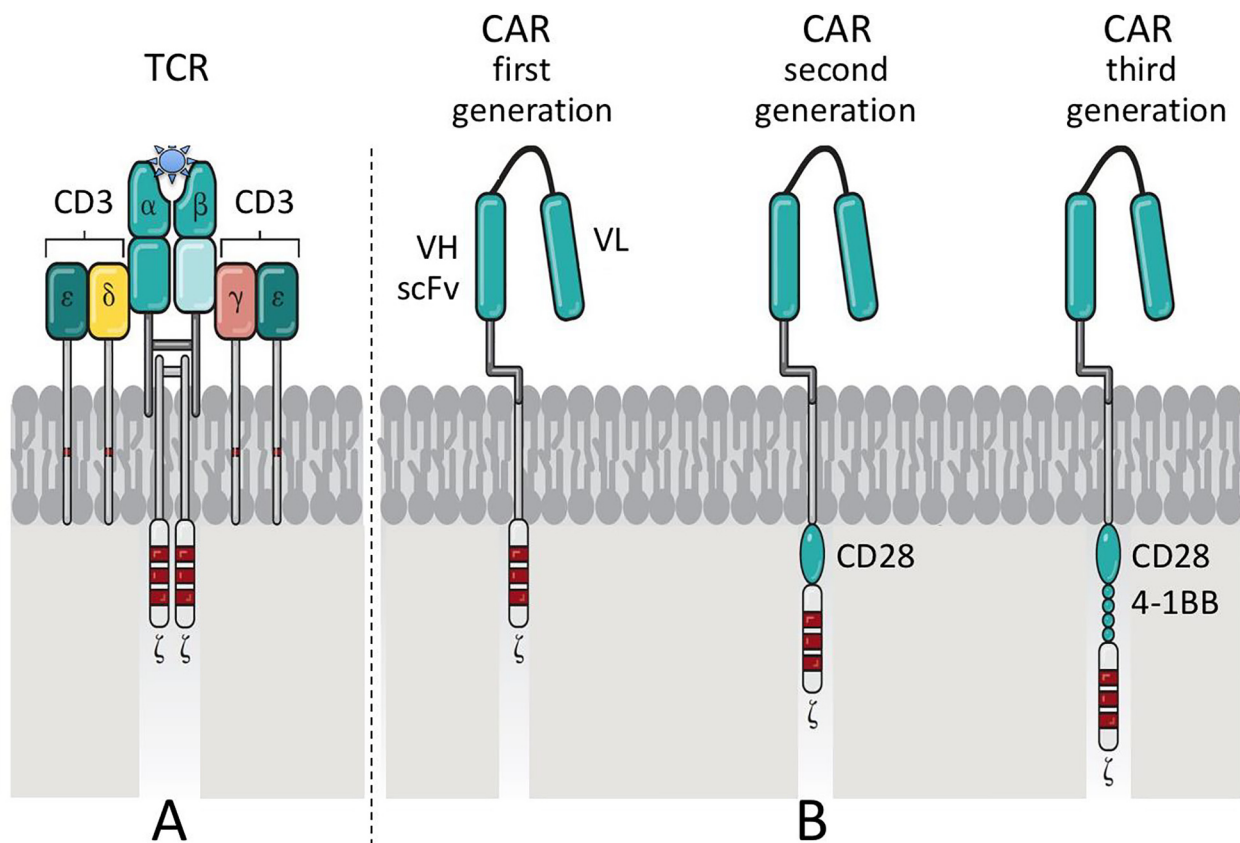


Fig. 3. Genetically modified T cells. (A) T cells recognize an antigen by means of a TCR, which consists of α and β chains and CD3 ζ , which transmits a signal to activate T cells. T cells are modified by expressing new α - and β -chains with the desired specificity. (B) CAR consists of an antigen binding domain (scFv) fused to the transmembrane and intracellular domain of CD3 ζ from TCR. 1st generation CARs contain CD3 ζ , 2nd generation CARs have one costimulatory CD28 or 4-1BB domain fused to CD3 ζ , and 3rd generation CAR has two costimulatory domains associated with CD3 ζ . VH – variable heavy chain; VL – variable light chain. Adapted from [24].

Adaptation is a way for tumor cells to survive, and their maladaptation is a way for immunotherapy to kill them (hypothesis)

As immunotherapy methods developed, serious problems began to arise concerning the way to achieve a reliable anti-tumor effect in patients. Immunotherapy and other methods could not guarantee 100% success in cancer treatment. Why, despite the rapid development of new methods of cancer therapy, can we not catch up with and completely destroy the tumor? Every time the immunity or the doctor attacks the tumor, the tumor neutralizes their attempts. When a surgeon excises a tumor, the tumor metastasizes and develops elsewhere. When the immune system recognizes tumor antigens, the tumor masks them or resets or leaves the normal tissue antigens on its surface, so that when CTL starts attacking a tumor cell, Treg lymphocytes perceive it as an attack on their own cells, and suppress CTL activity [43]. When macrophages infiltrate a tumor, the tumor reprograms these cells to the pro-tumor phenotype [44]. When CTL or CAR-T cells attack a tumor, the tumor creates an immunosuppressive environment [10,45–47]. When the drug penetrates the tumor cells, the tumor synthesizes new channels and takes the drug out [48]. It seems that the tumor is a unique pathology, which, unlike other pathologies, has a dynamically changing pathogenesis in response to treatment and the action of the immune system.

New hopes, noted in 2018 by the award of Nobel Prize for Medicine to James P. Allison and Tasuku Honjo were pinned on PD-1 and CTLA-4 inhibitors. However, in response to the inhibition of these checkpoints, the tumor synthesizes molecules of other checkpoints [49]. This has already generated considerable pessimism about this type of

immunotherapy.

The situation resembles the ancient Greek philosopher Zeno's paradox of the unsuccessful efforts of Achilles to catch up with the tortoise – by the time Achilles gets to where the tortoise was, the tortoise has moved a fraction ahead of him. Paradoxically, this is so, if we assume that Achilles runs discretely. Antitumor therapies are being developed “discretely” according to the principle of “once a pro-tumor mechanism is discovered, develop a medicine to inhibit it.” Checkpoint inhibitors are the latest example. However, as soon as the doctor inhibits PD-1 and CTLA-4, the tumor synthesizes other checkpoints, for example VISTA [49]. Zenon's paradox suggests that the goal of therapy need to go beyond the capabilities of the tumor to level the therapeutic effect. For this to succeed, you need to understand which biological phenomena make the tumor resistant to various aggressive factors and help it to avoid the immune system prosecution.

Carcinogenesis can be represented as a tree whose branches are different pro-tumor mechanisms and whose trunk is a biological phenomenon that “feeds” those branches. A tree can be destroyed in two ways: either by cutting a branch for a branch without a guarantee that new branches will not grow, or cutting down the trunk and letting the branches wither away. Immunotherapy uses the first method, for example by blocking a checkpoint. But soon the tumor synthesizes other checkpoints and survives. To cut down the trunk, it is necessary to understand the nature of the biological phenomenon, which helps the tumor to avoid attack by the immune system, drugs and immunotherapy. The clue is that the pro-tumor mechanisms are united by one goal – to increase the resistance of the tumor cell to immune factors and drugs. A phenomenon that improves cell resistance is well known in biology – adaptation.

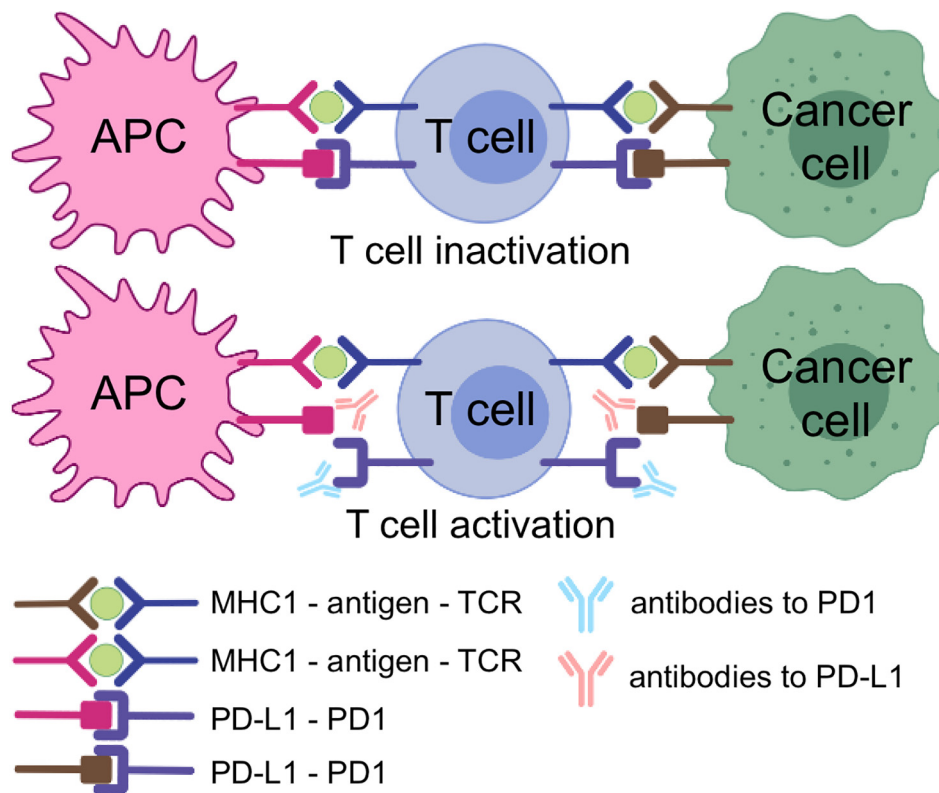


Fig. 4. Checkpoint inhibitors eliminate T cell immunosuppression. APC: antigen presenting cell; PD-1: programmed cell death protein 1; PD-L1: programmed death ligand 1; TCR: T cell receptors. Adapted from [36].

On the basis of their nature and role in evolution, the adaptations were classified into two categories: short range (temporary) and long range (permanent) adaptations. The main principles of the concept of short range adaptation were formulated by Felix Z. Meerson [50,51]:

1. Periodic action of a damaging factor increases the resistance of cells to this factor of a greater, previously intolerable force, i.e. forms adaptation. When adapting to one damaging factor, the resistance of cells to another factor that the cell has not even encountered may increase.
2. Adaptation to a damaging factor of a long enough duration is provided by a structural trace. A structural trace is a complex of changes in cells, such as gene expression, an increase in the number of ribosomes and mitochondria, which synthesize the proteins, ATP.
3. After the termination of the adaptive factor, the structural trace disappears together with increased stability. This process is called maladaptation.

The first principal of the concept of adaptation suggests that tumor survives due to adaptation to aggressive factors of the microenvironment, such as hypoxia, free radicals and inflammatory cytokines [52]. Cancer cells increase glucose uptake, neutralize free radicals, produce many anti-inflammatory cytokines [52,53] and thereby increase their resistance to hypoxia, oxidative stress and inflammation. In addition, tumor anti-inflammatory cytokines reprogram macrophages into the pro-tumor phenotype and inhibit CTL [15]. These processes reflect the formation of the adaptation of tumor cells to the aggressive microenvironment, which creates hypoxia and the immune system.

Thus, if the immunity does not immediately destroy the tumor cell, the cell begins to adapt to it. If the doctor, with the help of immunotherapy, fails to increase the strength of the immune attack to a level that kills the tumor, this leads to additional adaptation of the tumor cell and increases its resistance. “What doesn’t kill us makes us stronger” – an adaptation formula that Nietzsche himself unknowingly

used. This survival strategy was well known in Medieval France. French kings, who took non-lethal doses of poison from childhood, over time became immune to lethal doses. Similarly, an adapted tumor becomes tolerant to the action of immune factors and immunotherapy. Probably, therefore, immunotherapy is more effective in the early stages of tumor development [19], when the adaptation of the tumor is not yet complete than in the later stages, when the tumor has already adapted. The weak immunogenicity of the tumor and the weakened immunity of the elderly, causing weak immune responses, may contribute to the adaptation of the tumor.

Immuno-editing often involves several stages of the anti-cancer immune cycle. Therefore, it is reasonable to assume that combined strategies will more effectively overcome tumor adaptation than monotherapies. Indeed, the combined therapy with PD-1/CTLA-4 inhibitors increased the effectiveness of treatment in patients with melanoma compared to monotherapy with any of the blockers [54].

Target selection plays an important role in combined therapy. In particular, in contrast to the PD1/CTLA4 combination [54], the therapy with PD1 and IDO (epacadostat) inhibitors did not provide an increased benefit in advanced melanoma [55] whereas the combined treatment with IDO inhibitor and radiotherapy prevented the T cell exhaustion and suppressed tumor growth [56].

The “regenerative potential” of the tumor defense mechanisms and the development of resistance is another problem of combined therapy. In response to the inhibition of CTLA-4 checkpoint, the tumor synthesizes other checkpoints such as VISTA and “regenerates” the immunosuppressive mechanism [49] and resistance often develops in response to PD-1 checkpoint inhibition and tumor growth resumes [42].

With a large number of potential therapeutic targets and individual characteristics of carcinogenesis, the possibility of activating one defense mechanism in response to the inhibition of another one and the likelihood of resistance development, a special algorithm is required to select an effective combination of targets. An example of such an algorithm is a biomarker approach based on the analysis of the genomic

and immunologic landscape of a tumor. In particular, this approach allowed to identify biomarkers of the HPV-positive head and neck squamous cell carcinoma (HNSCC) such as viral oncoproteins E6 and E7, p53 degradation and functional inactivation of Rb and HPV-negative HNSCC such as mutation in the TP53 and CDKN2A genes [57]. Understanding the genomic and immunologic landscape of a tumor will help translational immune-oncologists to select targets for combination therapies, more effectively.

Why does the immunity, which is designed to neutralize the tumor, begins to promote its adaptation and survival? Tumor cells divide rapidly and, as a result of mutations, expose non-host antigens. The situation resembles the development of the embryo. Probably, for reasons of biological expediency, immunity uses adaptation mechanisms to protect dividing tumor cells without noticing the difference from the embryo.

Our hypothesis that adaptation to immune factors plays a role in the formation of tumor tolerance for immunity and immunotherapy gives rise to the idea of reducing the survival of tumor cells by disrupting adaptation mechanisms.

The second principal of the concept of adaptation involves the prevention of adaptation. This can be achieved by inhibition of the activation of genes and proteins of the structural trace of adaptation in tumor cells. Genomic and proteomic analysis can identify critical components of the structural trace and show which genes to block. Prevention of the formation of a structural trace in tumor cells could increase the effectiveness of immunotherapy.

The above “biomarker approach”, as well as for prognosis [57], could be used in a broader sense, to identify a specific structural trace of the adaptation of a particular tumor, and in the end address an individualized therapeutic approach.

The third principal of the concept of adaptation addresses what to do when the structural trace has already been formed – the termination of the adaptive factor leads to the disappearance of the structural trace and the loss of acquired stability. Within the context of cancer immunotherapy, this gives rise to the idea of “turning off” the immune system for a period before the onset of tumor maladaptation, and then applying immunotherapy only to tumor cells that have lost their increased resistance. This hypothesis is consistent with the fact that lymphodepletion is a critical step to create a favorable immune environment in patients prior to adoptive cell therapy [58,59]. It is possible that maladaptation of tumor cells as a result of the removal of lymphocyte-derived mediators contribute to this effect.

The fact that therapy in the early stages of tumor formation is more effective than in the later ones can be partly explained by the fact that the formation of a systemic structural trace requires time, and in the early stages there is still no adaptive increase in the resistance of tumor cells. Another indirect example of the involvement of tumor adaptation in the formation of tumor resistance is given by a bridging therapy prior to CAR-T cells. Recently, DeSelm et al. showed that radiation sensitizes tumors to immune rejection by CAR T cells as a result of the mitigate antigen escape [60]. It can be assumed that antigen escape is a component of the systemic structural trace of tumor cell adaptation and its violation by radiation makes CAR T cell therapy more effective. Deep understanding of the components of the systemic structural trace of adaptation will result in more effective inhibition of tumor cell adaptive resistance and will make tumor cells more vulnerable to both traditional and new cancer treatment methods.

A look at the problem of cancer from the positions of the concept of adaptation allowed us to outline a new approach to increase the effectiveness of immunotherapy. This is the destruction of the structural trace of adaptation in tumor cells using inhibitors, or disadaptation of tumor cells by shutting down the immune system before the start of immunotherapy.

A good theory should have predictive power, reveal the mechanisms and have practical application. Within this context, it is interesting to discuss the concept of adaptation for hot and cold tumors. In “hot”

tumors, T cells express PD-1 and tumor cells express PD-L1. The PD-1/PD-L1 interaction blocks the cytotoxic activity of T cells. Therefore, PD1/PD-L1 checkpoint inhibitors are very effective [61]. Solid “cold” tumors, due to low immunogenicity, immunosuppressive micro-environment and absence of T cells, do not respond to checkpoint inhibitors. To increase the effectiveness of checkpoint inhibitors, it has been suggested to convert cold tumors into “hot” ones [62].

The concept of adaptation can help to understand the nature of hot and cold tumors and contribute to more effective therapy. The first fact to pay attention to is that the hot microenvironment contains molecular and cellular factors of anti-tumor immunity, and these factors are assumed to act on the tumor cell, form a systemic structural trace and make the tumor adapt to anti-tumor conditions. In particular, one of the components of the systemic structural trace is an increase in the expression of PD-L1. Therefore, a violation of one of the adaptation mechanisms with checkpoint inhibitors is a reasonable strategy for the immunotherapy of hot tumors.

The microenvironment of a “cold” tumor impedes the action of immune anti-tumor factors and is supposed to protect the tumor. However, as a result of such protection, there is no adaptation to anti-tumor immune factors and so, a systemic structural trace does not form and consequently there is no an increase in the ligands of checkpoints. Therefore, checkpoint inhibitors in absence of acquired adaptation are not effective. A protective microenvironment can be considered as both the strength and weakness of a cold tumor. It protects the tumor from an aggressive immune response but it does not allow adaptation.

If our hypothesis is true, then tumor cells isolated from a “hot” tumor should be more resistant to anti-tumor immune factors compared to tumor cells isolated from a cold tumor. Therefore, it could be suggested that in the case of “hot” tumors, the therapeutic target is an adapted tumor cell, whereas in the case of “cold” tumors, the micro-environment should become the target. After reprogramming the micro-environment of a cold tumor with cytotoxic lymphocytes, lymphocytes with modified T cell receptors or CAR-T cells, will effectively destroy the non-adapted tumor cell. How to reprogram the micro-environment of a cold tumor is a very important question, but it is beyond the scope of this review. Here, it is suggested that the micro-environment consists of immunosuppressive cells, that can become targets of adaptation/maladaptation approaches.

The concept of adaptation within the context of the anti-tumor immunotherapy allows a new vision of the problem of carcinogenesis and the development of new therapeutic strategies.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

The article has been written with the support of the Ministry of Health of the Russian Federation (State Order No. 056-00149-18-00). There has been no role of the sponsor in the collection, analysis and interpretation of data; in the writing of the manuscript; and in the decision to submit the manuscript for publication.

Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.mehy.2019.109545>.

References

- [1] World Health Organization. Cancer. World Health Organization 2018. <https://www.who.int/news-room/fact-sheets/detail/cancer> (accessed January 9, 2019).

- [2] Keegan J. *The First World War*. London: Hutchinson; 1998.
- [3] Chen DS, Mellman I. Oncology meets immunology: the cancer-immunity cycle. *Immunity* 2013;39:1–10. <https://doi.org/10.1016/j.immuni.2013.07.012>.
- [4] Mellman I, Coukos G, Dranoff G. Cancer immunotherapy comes of age. *Nature* 2011;480:480–9. <https://doi.org/10.1038/nature10673>.
- [5] Chraa D, Naim A, Olive D, Badou A. T lymphocyte subsets in cancer immunity: friends or foes. *J Leukoc Biol* 2019;105:243–55. <https://doi.org/10.1002/JLB.MR0318-097R>.
- [6] Loveridge CJ, Mui EJ, Patel R, et al. Increased T-cell infiltration elicited by Erk5 deletion in a Pten-deficient mouse model of prostate carcinogenesis. *Cancer Res* 2017;77:3158–68. <https://doi.org/10.1158/0008-5472.CAN-16-2565>.
- [7] Franciszkiewicz K, Le Floch A, Boutet M, Vergnon I, Schmitt A, Mami-Chouaib F. CD103 or LFA-1 engagement at the immune synapse between cytotoxic T cells and tumor cells promotes maturation and regulates T-cell effector functions. *Cancer Res* 2013;73:617–28. <https://doi.org/10.1158/0008-5472.CAN-12-2569>.
- [8] Modena A, Massari F, Ciccarese C, et al. Targeting met and VEGFR axis in metastatic castration-resistant prostate cancer: 'Game Over'? *Target Oncol* 2016;11:431–46. <https://doi.org/10.1007/s11523-015-0412-7>.
- [9] Baumeister SH, Freeman GJ, Dranoff G, Sharpe AH. Coinhibitory pathways in immunotherapy for cancer. *Annu Rev Immunol* 2016;34:539–73. <https://doi.org/10.1146/annurev-immunol-032414-112049>.
- [10] Gevensleben H, Dietrich D, Golletz C, et al. The immune checkpoint regulator PD-L1 is highly expressed in aggressive primary prostate cancer. *Clin Cancer Res* 2016;22:1969–77. <https://doi.org/10.1158/1078-0432.CCR-15-2042>.
- [11] O'Donnell JS, Teng MWL, Smyth MJ. Cancer immunoeediting and resistance to T cell-based immunotherapy. *Nat Rev Clin Oncol* 2019;16:151–67. <https://doi.org/10.1038/s41571-018-0142-8>.
- [12] Fujita K, Hayashi T, Matsushita M, Uemura M, Nonomura N. Obesity, inflammation, and prostate cancer. *J Clin Med* 2019;8:E201. <https://doi.org/10.3390/jcm8020201>.
- [13] Prendergast GC, Smith C, Thomas S, et al. Indoleamine 2,3-dioxygenase pathways of pathogenic inflammation and immune escape in cancer. *Clin Immunol Immunother* 2014;63:721–35. <https://doi.org/10.1007/s00262-014-1549-4>.
- [14] Banzola I, Mengus C, Wyler S, et al. Expression of indoleamine 2,3-dioxygenase induced by IFN- γ and TNF- α as potential biomarker of prostate cancer progression. *Front Immunol* 2018;9:1051. <https://doi.org/10.3389/fimmu.2018.01051>.
- [15] Malyshev IY, Manukhina EB, Malyshev YI. Physiological organization of immune response based on the homeostatic mechanism of matrix reprogramming: Implication in tumor and biotechnology. *Med Hypotheses* 2014;82:754–65. <https://doi.org/10.1016/j.mehy.2014.03.019>.
- [16] Sevenich L. Turning "Cold" into "Hot" tumors—opportunities and challenges for radio-immunotherapy against primary and metastatic brain cancers. *Front Oncol* 2019;9. <https://doi.org/10.3389/fonc.2019.00163>.
- [17] Niemann J, Woller N, Brooks J, et al. Molecular retargeting of antibodies converts immune defense against oncolytic viruses into cancer immunotherapy. *Nat Commun* 2019;10:3236. <https://doi.org/10.1038/s41467-019-1137-5>.
- [18] Pikor LA, Bell JC, Diallo J-S. Oncolytic viruses: exploiting cancer's deal with the devil. *Trends Cancer* 2015;1:266–77. <https://doi.org/10.1016/j.trecan.2015.10.004>.
- [19] Kantoff PW, Higano CS, Shore ND, et al. Sipuleucel-T immunotherapy for castration-resistant prostate cancer. *N Engl J Med* 2010;363:411–22. <https://doi.org/10.1056/NEJMoa1001294>.
- [20] Kantoff PW, Schuetz TJ, Blumenstein BA, et al. Overall survival analysis of a phase II randomized controlled trial of a poxviral-based PSA-targeted immunotherapy in metastatic castration-resistant prostate cancer. *J Clin Oncol* 2010;28:1099–105. <https://doi.org/10.1200/JCO.2009.25.0597>.
- [21] Lilleby W, Gaudernack G, Brunsvig PF, et al. Phase I/IIa clinical trial of a novel hTERT peptide vaccine in men with metastatic hormone-naïve prostate cancer. *Cancer Immunol Immunother* 2017;66:891–901. <https://doi.org/10.1007/s00262-017-1994-y>.
- [22] Colluru VT, Johnson LE, Olson BM, McNeel DG. Preclinical and clinical development of DNA vaccines for prostate cancer. *Urol Oncol Semin Orig Investig* 2016;34:193–204. <https://doi.org/10.1016/j.urolonc.2013.09.014>.
- [23] Andersen R, Donia M, Ellebaek E, et al. Long-Lasting complete responses in patients with metastatic melanoma after adoptive cell therapy with tumor-infiltrating lymphocytes and an attenuated iL2 regimen. *Clin Cancer Res* 2016;22:3734–45. <https://doi.org/10.1158/1078-0432.CCR-15-1879>.
- [24] June CH, O'Connor RS, Kawalekar OU, Ghassemi S, Milone MC. CAR T cell immunotherapy for human cancer. *Science* (80-) 2018;359:1361–5. doi:10.1126/science.aar6711.
- [25] Morgan RA, Dudley ME, Wunderlich JR, et al. Cancer regression in patients after transfer of genetically engineered lymphocytes. *Science* (80-) 2006;314:126–9. doi:10.1126/science.1129003.
- [26] Kageyama S, Ikeda H, Miyahara Y, et al. Adoptive transfer of MAGE-A4 T-cell receptor gene-transduced lymphocytes in patients with recurrent esophageal cancer. *Clin Cancer Res* 2015;21:2268–77. <https://doi.org/10.1158/1078-0432.CCR-14-1559>.
- [27] Thibodeaux SR, Milone MC. Immunotherapy using chimeric antigen receptor-engineered T cells: a novel cellular therapy with important implications for the clinical laboratory. *Clin Chem* 2019;65:519–29. <https://doi.org/10.1373/clinchem.2016.258988>.
- [28] Garrido F, Aptsiauri N, Doorduyn EM, Garcia Lora AM, van Hall T. The urgent need to recover MHC class I in cancers for effective immunotherapy. *Curr Opin Immunol* 2016;39:44–51. <https://doi.org/10.1016/j.coi.2015.12.007>.
- [29] Park JH, Rivière I, Gonen M, et al. Long-term follow-up of CD19 CAR therapy in acute lymphoblastic leukemia. *N Engl J Med* 2018;378:449–59. <https://doi.org/10.1056/NEJMoa1709919>.
- [30] Junghans RP, Ma Q, Rathore R, et al. Phase I trial of anti-PSMA designer CAR-T cells in prostate cancer: possible role for interacting interleukin 2-T cell pharmacodynamics as a determinant of clinical response. *Prostate* 2016;76:1257–70. <https://doi.org/10.1002/pros.23214>.
- [31] Srivastava S, Riddell SR. Chimeric antigen receptor T cell therapy: challenges to bench-to-bedside efficacy. *J Immunol* 2018;200:459–68. <https://doi.org/10.4049/jimmunol.1701155>.
- [32] Hodi FS, O'Day SJ, McDermott DF, et al. Improved survival with ipilimumab in patients with metastatic melanoma. *N Engl J Med* 2010;363:711–23. <https://doi.org/10.1056/NEJMoa1003466>.
- [33] Robert C, Long GV, Brady B, et al. Nivolumab in previously untreated melanoma without BRAF mutation. *N Engl J Med* 2015;372:320–30. <https://doi.org/10.1056/NEJMoa1412082>.
- [34] Chen DS, Irving BA, Hodi FS. Molecular pathways: Next-generation immunotherapy-inhibiting programmed death-ligand 1 and programmed death-1. *Clin Cancer Res* 2012;18:6580–7. <https://doi.org/10.1158/1078-0432.CCR-12-1362>.
- [35] Robert C, Schachter J, Long GV, et al. Pembrolizumab versus ipilimumab in advanced melanoma. *N Engl J Med* 2015;372:2521–32. <https://doi.org/10.1056/NEJMoa1503093>.
- [36] Jung CY, Antonia SJ. Tumor immunology and immune checkpoint inhibitors in non-small cell lung cancer. *Tuberc Respir Dis (Seoul)* 2018;81:29–41. <https://doi.org/10.4046/trd.2017.0120>.
- [37] Schadendorf D, Hodi FS, Robert C, et al. Pooled analysis of long-term survival data from phase II and phase III trials of ipilimumab in unresectable or metastatic melanoma. *J Clin Oncol* 2015;33:1889–94. <https://doi.org/10.1200/JCO.2014.56.2736>.
- [38] Cabel L, Loir E, Gravis G, et al. Long-term complete remission with ipilimumab in metastatic castrate-resistant prostate cancer: case report of two patients. *J Immunother Cancer* 2017;5:31. <https://doi.org/10.1186/s40425-017-0232-7>.
- [39] Iwama S, De Remigis A, Callahan MK, Slovin SF, Wolchok JD, Caturegli P. Pituitary expression of CTLA-4 mediates hypophysitis secondary to administration of CTLA-4 blocking antibody. *Sci Transl Med* 2014;6:230ra45. <https://doi.org/10.1126/scitranslmed.3008002>.
- [40] Motzer RJ, Escudier B, McDermott DF, et al. Nivolumab versus everolimus in advanced renal-cell carcinoma. *N Engl J Med* 2015;373:1803–13. <https://doi.org/10.1056/NEJMoa1510665>.
- [41] Graff JN, Alunkal JJ, Drake CG, et al. First evidence of significant clinical activity of PD-1 inhibitors in metastatic, castration resistant prostate cancer (mCRPC). *Ann Oncol* 2016;27:7190. <https://doi.org/10.1093/annonc/mdw372.03>.
- [42] O'Donnell JS, Long GV, Scolyer RA, Teng MWL, Smyth MJ. Resistance to PD1/PDL1 checkpoint inhibition. *Cancer Treat Rev* 2017;52:71–81. <https://doi.org/10.1016/j.ctrv.2016.11.007>.
- [43] Najafi M, Farhood B, Mortezaee K. Contribution of regulatory T cells to cancer: a review. *J Cell Physiol* 2019;234:7983–93. <https://doi.org/10.1002/jcp.27553>.
- [44] Choi J, Gyamfi J, Jang H, Koo JS. The role of tumor-associated macrophage in breast cancer biology. *Histol Histopathol* 2018;33:133–45. <https://doi.org/10.14670/HH-11-916>.
- [45] Gowrishanker K, Birtwistle L, Micklethwaite K. Manipulating the tumor micro-environment by adoptive cell transfer of CAR T-cells. *Mamm Genome* 2018;29:739–56. <https://doi.org/10.1007/s00335-018-9756-5>.
- [46] Akbay EA, Koyama S, Carretero J, et al. Activation of the PD-1 pathway contributes to immune escape in EGFR-driven lung tumors. *Cancer Discov* 2013;3:1355–63. <https://doi.org/10.1158/2159-8290.CD-13-0310>.
- [47] Abiko K, Matsumura N, Hamanishi J, et al. IFN- γ from lymphocytes induces PD-L1 expression and promotes progression of ovarian cancer. *Br J Cancer* 2015;112:1501–9. <https://doi.org/10.1038/bjc.2015.101>.
- [48] Pluchino KM, Hall MD, Goldsborough AS, Callaghan R, Gottesman MM. Collateral sensitivity as a strategy against cancer multidrug resistance. *Drug Resist Updat* 2012;15:98–105. <https://doi.org/10.1016/j.drug.2012.03.002>.
- [49] Gao J, Ward JF, Pettaway CA, et al. VISTA is an inhibitory immune checkpoint that is increased after ipilimumab therapy in patients with prostate cancer. *Nat Med* 2017;23:551–5. <https://doi.org/10.1038/nm.4308>.
- [50] Meerson FZ. *Essentials of adaptive medicine: protective effects of adaptation*. Moscow: Hypoxia Medical LTD; 1994.
- [51] Meerson FZ, Malyshev IY. *Phenomenon of adaptive stabilization of structures and protection of the heart*. Moscow: Nauka; 1993.
- [52] Chen M, Xie S. Therapeutic targeting of cellular stress responses in cancer. *Thorax* 2018;9:1575–82. <https://doi.org/10.1111/1759-7714.12890>.
- [53] Lippitz BE. Cytokine patterns in patients with cancer: a systematic review. *Lancet Oncol* 2013;14:e218–28. [https://doi.org/10.1016/S1470-2045\(12\)70582-X](https://doi.org/10.1016/S1470-2045(12)70582-X).
- [54] Postow MA, Chesney J, Pavlick AC, et al. Nivolumab and ipilimumab versus ipilimumab in untreated melanoma. *N Engl J Med* 2015;372:2006–17. <https://doi.org/10.1056/NEJMoa1414428>.
- [55] Muller AJ, Manfredi MG, Zakharia Y, Prendergast GC. Inhibiting IDO pathways to treat cancer: lessons from the ECHO-301 trial and beyond. *Semin Immunopathol* 2019;41:41–8. <https://doi.org/10.1007/s00281-018-0702-0>.
- [56] Liu M, Li Z, Yao W, et al. IDO inhibitor synergized with radiotherapy to delay tumor growth by reversing T cell exhaustion. *Mol Med Rep* 2020;21:445–53. <https://doi.org/10.3892/mmr.2019.10816>.
- [57] Solomon B, Young RJ, Rischin D. Head and neck squamous cell carcinoma: genomics and emerging biomarkers for immunomodulatory cancer treatments. *Semin Cancer Biol* 2018;52:228–40. <https://doi.org/10.1016/j.semcancer.2018.01.008>.
- [58] Ludwig D, Dawicki W, Allen K, Dadachova E. Modeling targeted lymphodepletion with 177Lu-radiolabeled CD45 antibody as a preparative regimen prior to adoptive cell therapy. *J Nucl Med* 2019;60:1420.

- [59] Goff SL, Dudley ME, Citrin DE, et al. Randomized, prospective evaluation comparing intensity of lymphodepletion before adoptive transfer of tumor-infiltrating lymphocytes for patients with metastatic melanoma. *J Clin Oncol* 2016;34:2389–97. <https://doi.org/10.1200/JCO.2016.66.7220>.
- [60] DeSelm C, Palomba ML, Yahalom J, et al. Low-dose radiation conditioning enables CAR T cells to mitigate antigen escape. *Mol Ther* 2018;26:2542–52. <https://doi.org/10.1016/j.ymthe.2018.09.008>.
- [61] Chen DS, Mellman I. Elements of cancer immunity and the cancer-immune set point. *Nature* 2017;541:321–30. <https://doi.org/10.1038/nature21349>.
- [62] Rodallec A, Sicard G, Fanciullino R, et al. Turning cold tumors into hot tumors: harnessing the potential of tumor immunity using nanoparticles. *Expert Opin Drug Metab Toxicol* 2018;14:1–9. <https://doi.org/10.1080/17425255.2018.1540588>.