# From chemotherapy to biological therapy: A review of novel concepts to reduce the side effects of systemic cancer treatment (Review)

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**Abstract.** The side effects of systemic chemotherapy used to treat cancer are often severe. For decades, oncologists have focused on treating the tumor, which may result in damage to the tumor-bearing host and its immune system. Recently, much attention has been paid to the immune system of patients and its activation via biological therapies. Biological therapies, including immunotherapy and oncolytic virus (OV) therapy, are often more physiological and well tolerated. The present review elucidated how these therapies work and why these therapies may be better tolerated: i) In contrast to chemotherapy, immunotherapies induce a memory function of the adaptive immunity system; ii) immunotherapies aim to specifically activate the immune system against cancer; side effects are low due to immune tolerance mechanisms, which maintain the integrity of the body in the presence of B and T lymphocytes with their antigen-receptor specificities and; iii) the type I interferon response, which is evoked by OVs, is an ancient innate immune defense system. Biological and physiological therapies, which support the immune system, may therefore benefit cancer treatment. The present review focused on immunotherapy, with the aim of reducing side effects and increasing long-lasting efficacy in cancer therapy.

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*Abbreviations:* AE, adverse event; APC, antigen-presenting cell; ATV-NDV, autologous tumor vaccine modified by infection with NDV; CAR, chimeric antigen-specific receptor; CRC, colorectal cancer; CTL, cytotoxic T lymphocyte; DAMP, damage-associated molecular pattern; DC, dendritic cell; DKFZ, Deutsches Krebsforschungszentrum; FasL, Fas ligand; FDA, Food and Drug Administration; GBM, glioblastoma multiforme; GM-CSF, granulocyte-macrophage colony-stimulating factor; GMP, good manufacturing practice; GvL, graft-versus-leukemia; HMGB1, high mobility group box 1; HN, hemagglutinin-neuraminidase; HSP, heatshock protein; HSV, herpes simplex virus; ICB, immune checkpoint blockade; ICD, immunogenic cell death; IFN, interferon; IOZK, Immunological and Oncological Center Cologne; IT, immunotherapy; LDI, low-dose γ irradiation; MAb, monoclonal antibody; MHC, major histocompatibility complex; MTC, memory T cell; mTEC, medullary thymic epithelial cell; NCI, National Cancer Institute; NDV, Newcastle disease virus; NK, natural killer; OS, overall survival; OV, oncolytic virus; OVT, OV therapy; PAMP, pathogen-associated molecular pattern; PCD, programmed cell death; RIG-I, retinoic acid-induced gene I; SMI, small molecule inhibitor; TAA, tumor-associated antigen; TIL, tumor-infiltrating lymphocyte; TC, tumor cell; TCR, T cell receptor; TMZ, temozolomide; TRAIL, tumor necrosis factor-related apoptosis-inducing ligand; Treg, regulatory T cell; VEGF, vascular endothelial growth factor; VOL-DC, viral oncolysate-pulsed DC vaccine; WHO, World Health Organization

*Key words:* cancer metastasis, chemotherapy, immunotherapy, oncolytic viruses, immunological self-tolerance, immunological memory

## Contents



## 1. Introduction

Examples of cancer treatments from the early 19th century include radical, super-radical and ultra-radical surgery, as propagated by William S Halstedt (1). In particular, radical mastectomy was used to treat breast cancer for ~90 years, between 1891 and 1981. However, in 1981, Bernhard Fisher

published a study that disproved radical surgery for the treatment of cancer (2). The use of radical surgery was immediately reduced after it was confirmed that systemic adjuvant therapy, in combination with local surgery, produced similar results (2). Systemic adjuvant therapies include radiation and the application of cytostatic drugs, and are required for the treatment of cancer dissemination and metastasis.

The present review focused on cancer chemotherapy, which is a type of standard cancer therapy, and on modern biological types of targeted therapy, which are not yet part of standard care. In the late 1970s, bleomycin, vinblastine and cisplatin were novel drugs used in chemotherapy; however, they induced severe side effects, such as vomiting ~12 times per day. Since antiemetic drugs were not available at this time, patients were expected to tolerate the side effects of aggressive chemotherapy (3).

The US National Cancer Institute (NCI) is involved in research into cytostatic drugs, thus resulting in the generation of diverse medication mixtures and study plans for the treatment of cancer. Until 1979, the NCI had constructed a network of 20 cancer centers, which were involved in executing new studies of cancer treatment. Clinical boards, which were involved in the approval and coordination of studies involving human subjects, aimed to accelerate the process of approval. However, since these studies often tested treatments on humans first, without testing their efficacy on animal tumors, errors were often made. In addition, some cytostatic drugs were approved despite their low effectivity and severe side effects (3).

Gradually, the scientific basis of cancer metastasis has been explored in animal tumor models. Furthermore, over the last 60 years, discoveries have been made in molecular biology, immunology and virology, and novel types of cancer treatment have been developed (4). These include targeted therapies via small molecule inhibitors (SMIs) or monoclonal antibodies (MAbs). In recent years, two novel types of immunotherapy have had a marked impact on oncology: Checkpoint inhibitory MAbs and chimeric antigen-specific receptor (CAR)-transfected T-cells (CAR-T cells) (4). Cancer immunotherapy has been demonstrated to be capable of producing durable responses in numerous types of cancer. Antigen-specific immune responses can be markedly effective, even in late stage disease. In addition, two other types of biological therapy, antitumor vaccines and oncolytic viruses (OVs), have been developed in recent decades. These types of therapy are physiological and well tolerated (5).

The present review aimed to focus on the side effects of treatment in general. The World Health Organization (WHO) has defined side effects as grades 0-4 (6). The present review noted that not only cytostatic drugs, but also several of the novel drugs of the last decade, can generate severe adverse events (AEs).

Since it is important to understand the difference between therapies with grade 3 and 4 side effects and those with grade 0-2 side effects, this review detailed the functioning of the immune system. The immune system has been optimized by evolution, including mechanisms of immunological tolerance towards self-antigens, mechanisms of memory function and the anti-viral type I interferon (IFN) response system. This review also provided overviews regarding the various types of cancer therapy, the mechanisms of self-tolerance in T and B lymphocytes, and the important parameters that differentiate chemotherapy, immunotherapy and OV therapy.

## 2. Approved cytostatic drugs have relatively low tumor specificity and high toxicity

Cytostatic drugs target the cell cycle. They can be grouped according to their type; examples include alkylating agents, alkaloids, antibiotics and antimetabolites. These drugs interfere with cell proliferation by targeting cellular DNA or RNA and their metabolism. Antimetabolites target purine or pyrimidine metabolic enzymes, whereas alkaloids target the cytoskeleton (β-tubulin) and mitosis (6).

Objective criteria for the evaluation of therapeutic effects, as defined by the WHO, include the extent of tumor remission (tumor response), and the determination of remission time, survival and toxicity. Notably, chemotherapy is known to be effective in the treatment of various types of lymphoma. However, in other types of cancer, including carcinoma, chemotherapy often does not have curative effects, but it may prolong overall survival (OS). An OS benefit of <5% has been achieved in the adjuvant treatment of breast, colon, and head and neck cancers (7). Often, chemotherapy is given mainly due to palliative effects.

In 2004, a literature search of randomized clinical trials reporting a 5-year survival benefit attributable solely to cytotoxic chemotherapy in adult malignancies was performed. It included 154,971 patients with cancer from the USA and 72,903 patients with cancer from Australia. The overall contribution of chemotherapy to 5-year survival was estimated to be only 2.3% in Australia and 2.1% in the USA. The five most 'chemo-sensitive' cancers, namely testicular cancer, Hodgkin's and non-Hodgkin's lymphoma, cervical cancer and ovarian cancer, accounted for 8.4% of the total cancer incidence in Australia in 1998 (7).

Chemotherapy is associated with numerous severe side effects, which include immediate signs of toxicity and late signs of chronic toxicity (4,5). Their intensity can be mild (grade 1), moderate (grade 2), severe (grade 3), or life-threatening or disabling (grade 4), according to the WHO classification. Immediate effects can be observed on skin and hair, bone marrow and blood, the gastrointestinal tract and the kidneys. All organs of the body can be affected, including essential organs, such as the heart, lungs and brain. Grade 3 and 4 neurotoxicity can induce somnolence, paresthesia, paralysis, ataxia, spasms and coma. In addition, the chronic effects of chemotherapy include drug resistance, carcinogenicity and infertility.

In its 2014 report, the WHO provided data on cancer incidence and mortality for 2012 (8). Worldwide, there were 14 million new cases of cancer, and 8.2 million patients with cancer succumbed to the disease. In Europe, there were 3.4 million new cases of cancer, and 1.8 million patients with cancer succumbed to the disease. Of the 1.8 million cases of mortality, lung cancer had the highest rate (20%), followed by colorectal cancer (CRC; 12.2%), breast carcinoma (7.5%) and stomach cancer (6.1%).

These figures are disappointing and indicate that standard therapy cannot completely control cancer; however, some

Table I. Overview of chemotherapy and biological cancer therapy.

| Type of therapy | C or B | Mechanism of action | Physiological | Side effects |
|---|---|---|---|---|
| 1. Cytostatic drugs | C | Interfere with cell proliferation | No | Grade 1-4 |
| 2. Small molecule inhibitors[a] | C | Targeted therapy: Interfere with oncogenic signal transduction | Yes | Grade 1-4 |
| 3. Antitumor MAbs[b] | B | Targeted immunotherapy | Yes | Grade 1-3 |
| 4. Anti-angiogenesis MAbs[c] | B | Inhibit angiogenesis | Yes | Grade 1-3 |
| 5. Checkpoint inhibitor MAbs[d] | B | Immune regulation | No | Grade 1-4 |
| 6. CAR-T cells | B | Targeted cytotoxic T lymphocytes | No | Grade 1-3 |
| 7. Antitumor vaccines | B | Active specific vaccination | Yes | Grade 0-2 |
| 8. Oncolytic viruses[e] | B | Oncolysis, induction of immunogenic cell death | Yes | Grade 0-2 |

[a]e.g. KIT inhibitors, such as sunitinib, imatinib, sorafenib and lapatinib; [b]e.g. cetuximab, trastuzumab, panitumumab (targets include HER-1, HER-2 and RAS); [c]e.g. bevacizumab (Avastin; targets VEGF-L), ramucirumab (Cyramza; targets VEGF receptor 2); [d]e.g. ipilimumab (targets cytotoxic T-lymphocyte-associated protein 4), nivolumab (targets programmed cell death protein 1), atezolizumab and durvalumab (targets programmed death-ligand 1); [e]e.g. RNA viruses, including Newcastle Disease Virus from attenuated natural wild type strains. B, biological therapy; C, chemoteherapy; HER, human epidermal growth factor receptor; VEGF, vascular endothelial growth factor.

changes have recently become apparent. The American Cancer Society reported that, for 2018, cancer mortality was reduced by 1.7% (9). This may be due to steady reductions in smoking, and advances in early detection and treatment. The reduction in mortality rates includes major cancer types, namely lung, breast, prostate and CRC.

## 3. Discoveries of the last 60 years have paved the way for novel targeted therapies

Tumor virologists, molecular biologists and cell biologists have revealed that oncogenes and tumor suppressor genes often function via affecting signal transduction through cellular growth factor receptors. Previous studies have also led to novel insights into the cell cycle, including its function and control. Notably, the decision within a cell concerning growth or quiescence, cellular senescence or programmed cell death (PCD) is taken at restriction point R, during G1 phase of the cell cycle (4).

An overview of the novel types of cancer therapy that have been developed in recent decades is presented in Table I. Briefly, the following eight types of agent are listed: i) Chemotherapeutic cytostatic drugs, e.g. docetaxel, capecitabine, gemcitabine, irinitocan, ixabepilone or pemetrexed. ii) Chemically synthesized SMIs, such as imatinib or sunitinib, which target KIT oncogenic signal transduction pathways with specific targets in cancer, such as gastrointestinal stromal cancer or chronic myelogenous leukemia. iii) MAbs that target tumor cells, e.g. those expressing growth factor receptors [human epidermal growth factor receptor (HER)-1 and HER-2] or oncogenes (RAS). iv) MAbs that target vascular endothelium to inhibit tumor-associated angiogenesis. v) MAbs that target inhibitory receptors on T-cells and interfere with immune regulation. vi) Genetically modified T-cells for adoptive T-cell therapy; these are autologous but express an artificial CAR composed of an antibody-binding site and a T-cell signaling chain. vii) Antitumor vaccines for active specific immunotherapy. viii) OVs, which exhibit tumor selectivity and induce tumor cell death (oncolysis); these agents positively affect the immune system of patients by inducing immunogenic cell death (ICD) (4).

Chemotherapeutic cytostatic drugs and SMIs are chemical therapies, whereas the other therapies are biological. The mechanisms of action and side effects of these drugs are presented in Table I. Notably, the side effects of newly approved drugs from the majority of these therapy types, whether chemical or biological, were severe (grade 1-4). Only antitumor vaccines and OVs are well tolerated with side effects between grades 0 and 2.

The distinction between chemical or biological types of cancer therapy may therefore not be sufficient to explain why a therapy is well tolerated or not. Therefore, it may be suggested that another parameter be introduced, namely whether or not a therapy is physiological. Physiological means according to the function of the human body as a complex process at multiple levels (cells, tissues, organs and organ systems, including the cardiovascular, respiratory, gastrointestinal, renal, endocrine, reproductive or the nervous system) (4,5). However, it is not so easy to define whether a therapy is physiological or not; side effects may be useful in this definition, as they can be used to determine when a therapy is not physiological. Table I includes a column as to whether the therapies are considered 'physiological'. With regard to SMIs, it was concluded that they were physiological, since SMIs represent a tumor-targeted approach; nevertheless, normal cells can also be affected. With regards to checkpoint inhibitory MAbs, it was concluded that they were not physiological, because interference with immune regulation also interferes with autoimmune reactivity. With regards to CAR-T cells, it was concluded that they were not physiological, because the receptor is artificial and all cells have the same receptor.

The distinction between physiological and non-physiological therapies appears of great importance. Notably, meta-analyses of the toxicity of novel drugs approved by the Food and Drug Administration (FDA) between 2000 and 2010 were conducted. The novel drugs were approved

based on reasonably certain estimates of benefit but less certain estimates of harm (10-12). The analyses revealed that the novel drugs were associated with a significantly higher risk of harm than in the control groups treated with standard therapy; this was true for toxic death, treatment discontinuation and grade 3 or 4 AEs. The most common severe side effects were fatigue, diarrhea, nausea/vomiting, febrile neutropenia and rash. One analysis, including 74 studies and >48,000 patients, concluded that immunotherapy appears to have a better safety and tolerability compared with other therapies (12).

## 4. Cancer and the immune system: Physiological facts and principles

*Immunosurveillance of cancer.* As shown in Table I, antitumor vaccines and OVs are better tolerated than the majority of other therapies. It is therefore important to elucidate the reasons for this, which are based on immunological and physiological grounds. Until recently, the association between cancer and the host immune system was unclear. However, there is now known to be a link between cancer and host immunity. Notably, an intact immune system is important, not only for defense against foreign invaders, but also for immunosurveillance and defense against malignant cells of the host organism. In the case of destruction of cluster of differentiation (CD)$4^+$ T lymphocytes by human immunodeficiency virus, there is an increased risk for the development of cancer, such as lymphoma, Kaposi's sarcoma and cervix carcinoma (13). In addition, kidney or liver transplant patients who receive immunosuppressive drugs have a threefold increased risk of developing cancer (14). However, even people with an intact immune system can develop cancer.

The activity of the immune system with regards to cancer is an important factor of prognosis in patients with cancer. In a study of 100 patients with ovarian carcinoma a significant correlation was determined between survival and the infiltration of tumor tissue by T lymphocytes. The 5-year survival of patients with tumor-infiltrating T cells (TILs) was 38%, compared with 4.5% of patients with tumors without TILs (15). Similar correlations have been detected in patients with malignant melanoma (16), bladder cancer (17) and CRC (18).

Bone marrow from untreated patients with breast cancer has been reported to contain spontaneously induced cancer-reactive memory T-cells (19), which are capable of transferring protective antitumor immunity in an autologous tumor xenotransplant model (20). Furthermore, a successful standard active immunotherapy procedure exists with regards to superficial bladder carcinoma; treatment with Bacillus Calmette Guérin vaccine has been reported to exert superior effects to chemotherapy (21).

To avoid attack by the immune system, tumors develop various strategies, including antigenic variation, downregulation of major histocompatibility (MHC) molecules, antigen shedding or secretion of immunosuppressive molecules (22). One of these tumor immune escape strategies uses physiological immune regulatory mechanisms. One example is the targeting of cytotoxic T-lymphocyte-associated protein 4 (CTLA-4) or programmed cell death protein-1 (PD-1) receptors on T-cells, thus shutting down a T-cell-mediated immune response. Another example is the expression of Fas ligand (FasL, CD95) by tumor cells to induce apoptosis of T-cells expressing the corresponding Fas receptor (22).

Immunoediting is a term introduced to describe the dynamic interaction between a tumor and its host immune system over time. This mechanism continuously shapes the phenotype of the tumor (23,24).

Biological and physiological cancer therapies follow principles that differ from those of cytostatic drugs. The most important difference is the dose-response curve. Whilst in the case of cytostatic drugs this is linear, in the case of biological and physiological therapies it is bell-shaped (4).

*Complementarity of molecular interactions.* One of the potential reasons for the bell-shaped dose response curve detected in response to biological and physiological therapies is the complementarity of molecular interactions. More than 100 years ago, Paul Ehrlich titrated the reaction between a bacterial toxin and its complementary antibody, and reported on the reproducibility with which a point of equivalence could be determined (25); at this equivalence point, the maximum number of precipitated immune complexes was detected. In 1940, Linus Pauling confirmed the then already existing lock-and-key theory and demonstrated that the interaction between antibodies and antigens depends on their shape, rather than on their chemical composition (26). Two protein molecules may attract each other through various interactions, including electronic van der Waals attraction, Coulomb's attraction of groups with opposite electric charges, attraction of electric dipoles or multipoles, and hydrogen-bond formation. It has been reported that the forces of attraction increase as the molecules approach one another, and that the bond between the molecules reaches its maximum strength when the molecules are as close together as possible (26).

Molecular complementarity is also a principle employed by chemists designing SMIs. These inhibitors have to exactly fit into the groove of an enzyme involved in signal transduction along a key molecular pathway that leads to tumor progression. Imatinib mesylate (Gleevec) is a prototype example for this. There are still numerous weaknesses and challenges of SMIs that need to be solved, e.g. off-target side effects and lack of tumor specificity of the target.

T-cell-mediated immune responses also depend on complementarity of molecular interactions. These, however, occur between cells and not in cell-free solution. Instead of two, there are three participants in the molecular interaction: An antigenic fragment (peptide) forms a complex with a presenter molecule (MHC) and this complex is recognized by a recognizer molecule, the T-cell receptor (TCR). Cognate interactions between antigen-presenting cells (APCs) and T-cells with the corresponding antigen-specific TCR depend on numerous molecular interactions at the surfaces of interacting cells: Peptide-MHC (pMHC) complexes on APCs interacting with TCR $\alpha$ and $\beta$ binding sites to transmit to T-cells the so-called signal 1; CD80/CD86 molecules on APCs interacting with CD28 receptors on T-cells to transmit the so-called signal 2 or costimulatory signal. Other interactions of adhesion molecules within the immunological synapse regulate the stability and duration of the communication contact between the T-cell and its APC. How TCRs bind MHCs, peptides and coreceptors has previously been described in detail (27).

Some further information is of importance for biological cancer therapy: i) The detection limit for T-cell triggering is very low: Four pMHC per TCR cluster (28). The vast majority of the 10,000 presented peptides of an APC *in vivo* are in fact normal 'self' peptides, with only a few from foreign antigens (29). ii) Lack of a costimulatory molecule of a tumor cell, expressing a tumor-associated antigen (TAA), leads to a hyporesponsive state in a TAA-specific responding T-cell, which is known as T-cell anergy (30). Cancer vaccines are designed in such a way as to provide TAAs in association with costimulatory molecules, in order to be immunogenic and to overcome T-cell non-responsiveness (anergy) in patients with cancer.

Surveillance of the surface of an APC by a cognate T-cell for maximal key-lock fits might explain the low detection limit for T-cell triggering. *In vivo* imaging of cytotoxic T lymphocyte (CTL) infiltration and elimination of a solid tumor revealed that CTLs infiltrate tumors in depth only when these express the cognate antigen. In tumors that do not express the cognate antigen, CTL infiltration is restricted to peripheral regions, and lymphocytes neither stop moving nor do they kill tumor cells (31). Intravital two-photon microscopy also allowed single cell visualization and tracking of a physiological antiviral CD8+ T-cell response. A low dose of 1,000 antigen-specific TCR transgenic naïve T-cells was revealed to be sufficient to transfer the response into virus-infected recipients, where the cells expanded strongly in the spleen and bone marrow within 10 days post-infection (32).

*Design of anticancer vaccines*. The aim of therapeutic dendritic cell (DC) vaccines in cancer immunotherapy is to activate CTLs to recognize and attack tumors. Even when the T-cell response is already initiated by antigen engagement, it is the complex balance between costimulatory and co-inhibitory signals on DCs that results in either T-cell activation or T-cell tolerance (33). Immunosuppressive cues in the tumor microenvironment are major factors currently hampering the application of DC vaccination. It has been proposed that four different types of tumor microenvironment exist based on the presence or absence of TILs and PD-L1 expression (34). Ideal combination cancer therapies based on tumor immunology have to find an optimal niche between maximal antitumor immunity and minimal autoimmunity. This is particularly true for the application of checkpoint inhibitors, which interfere with immune regulation.

In the case of OV therapy, an optimal niche has to be found between maximal antitumor immunity and minimal antiviral immunity. In the 1960s, Lindenmann *et al* (35), and Cassel and Garrett (36), discovered that viral oncolysis in peritoneal tumor ascites of mice is induced only with an intermediate dose of virus; too low and too high doses are considered inefficient. Similar to these early observations, we observed in 1986 that a post-operative immunotherapeutic effect against metastases in an animal model could be obtained with a virus-infected tumor cell vaccine, only when the ratio of virus particle per tumor cell was ~10; lower and higher ratios were far less effective (37). The same rule was later found to be true for stimulation of an antitumor T-cell response *ex vivo* (38,39) by autologous tumor vaccine modified by infection with Newcastle disease virus (ATV-NDV).

Principles of biological and physiological cancer therapies aim at achieving a balance between different entities: Antitumor immunity, autoimmunity and antiviral immunity. Our previous study described tumor dormancy in the bone marrow as a balance between proliferating tumor cells and tumor-reactive $CD8^+$ T-cell-mediated memory T-cells (40). This balance provides the basis for long-term protective antitumor immunity (41). Another balance is known in the clinic as stable disease, i.e. a tumor that does not change in volume over a long period of time; often stable disease is a sign of successful interaction between the tumor and the immune system of the tumor-bearing host, thereby preventing further tumor growth.

*Low dose stimulation versus high dose inhibition*. Hormesis is a biological principle of interest not only for toxicologists. It describes a dose-response relationship to stressors (e.g. carcinogens, toxins, irradiation) with low dose stimulation and high dose inhibition. A recent study revealed that low-dose γ irradiation (LDI), but not high dose, was able to affect the barrier in the tumor microenvironment that prevents efficient T-cell infiltration. LDI programs macrophage differentiation to a certain phenotype (i.e. inducible nitric oxide $synthase^+$/M1) that then can orchestrate effective T-cell immunotherapy (42).

## 5. TAAs: Targets for specific cancer immunotherapy

TAAs (43) can be cell surface macromolecules that are detected by antibodies, or they can be pMHC complexes from intracellular proteins that are detected by T lymphocytes.

Every tumor may contain hundreds of mutations in coding regions of the genome. In addition, deletions, amplifications and chromosomal rearrangements can result in new genetic sequences. The vast majority of these mutations occur in intracellular proteins. Therefore, such 'neoantigens' (44) are not readily recognized and targeted by antibodies. Fortunately, pMHC molecules and the system that transports these to the cell surface for T-cell recognition have developed during evolution, as aforementioned. It has been estimated that roughly one third of the mutations identified from genome sequencing of breast and colon cancers are capable of binding to common MHC human leukocyte antigen alleles. However, the hallmarks of cancer are not only genetic (45) but are also epigenetic (46).

In the 1990s, Coulie *et al* in Thierry Boon's group described for the first time the molecular nature of a human TAA (47). This was possible by means of novel technology: Gene cloning and transfer in combination with a read-out system using CTLs. Upon cognate interaction with TAA-expressing target-cells, CTLs execute a process called PCD. One TAA was a peptide derived from a mutated intron sequence, which was recognized by CTLs as a pMHC complex at the tumor cell surface (47). Hundreds of TAAs have now been identified as pMHC complexes. They can be divided into the following categories (4): i) TAAs that arise from common oncogene/tumor suppressor gene mutations. Such mutations can be individually specific or can be shared. Examples of the latter are Kras G12A (colon and pancreatic cancer), Braf V599E (melanoma) and p53 G249T (hepatoma) mutations. ii) Cancer-testis antigens represent examples of

Table II. Self-tolerance in T and B lymphocytes.

| Feature | T lymphocytes | B lymphocytes |
|---|---|---|
| Sites of induction | Thymus (cortex) and periphery | Bone marrow and periphery |
| Stage of maturation | $CD4^+CD8^+$ thymocyte. | Immature $IgM^+IgD^-$ B cell. |
| Stimuli | Central: High-avidity recognition of antigens in the thymus. Peripheral: Antigen presentation by antigen-presenting cells lacking costimulators. | Central: Recognition of multivalent antigens in bone marrow. Peripheral: Antigen recognition without T-cell help or second signals. |
| Principle mechanisms of tolerance | Central: Deletion or regulatory T cells. Peripheral: Anergy, apoptosis, suppression. | Central: Deletion or receptor editing. Peripheral: Block in signal transduction, failure to enter lymphoid follicles. |

Modified from Abbas *et al* (104). CD, cluster of differentiation; Ig, immunoglobulin.

widely shared TAAs whose expression is restricted to tumors. The most commonly explored antigens in human vaccine trials are the cancer testis antigens melanoma-associated antigen 3 and NY-ESO-1. iii) Other human TAAs are upregulated via epigenetic mechanisms, including carcinoembryonic antigen (CEA; gastrointestinal cancer), Wilms tumor-1 (Wilms tumor, leukemia and lymphoma), mesothelin (pancreatic cancer, ovarian cancer and mesothelioma) and Her2/Neu (breast and ovarian cancer). iv) Tissue-restricted antigens expressed by tumors represent another category of shared TAAs. They have been popular targets for cancer vaccination. Examples include tyrosinase (melanoma), MART1/Melan A (melanoma), gp100 (melanoma), prostate-specific antigen (prostate) and prostatic acid phosphatase (prostate). v) Another category of TAAs are viral antigens for virus-associated cancers or precancerous lesions. Examples are human papilloma virus E6/E7 (cervical cancer), Epstein-Barr nuclear antigen 1, latent membrane protein (LMP)-1 and LMP-2 (Hodgkin's lymphoma and nasopharyngeal cancer).

Comparing this list of TAAs as targets of immunotherapy with the aforementioned targets of cytostatic drugs (5,6), it may be suggested that immunotherapy possesses higher tumor specificity.

## 6. The immune system avoids attacking the body, maintains integrity and retains a memory of successful defenses

*Inventions by nature: Immunological self-tolerance.* Originally, it was hypothesized that each cell type and organ expresses its own characteristic set of genes. Therefore, it was difficult to understand how self-antigens of several different organs could be presented by APCs in the thymus to lead to deletion of auto-reactive cells. In a series of publications, the group of Bruno Kyewski and colleagues described a cell type in the thymus, namely medullary thymic epithelial cells (mTECs), which are able to express a huge repertoire of organ-specific proteins (48). The ectopic gene expression in this cell type could be associated with a distinct transcription factor, namely autoimmune regulator gene (49). A few hundred mTECs express a certain set of self-antigens which are taken up and presented by thymic DCs. This then leads to deletion of immature self-reactive T-cells, a process designated as negative selection; however, not all self-reactive T-cells are deleted. Some are turned into regulatory T-cells (Tregs), which exert their function in the periphery. Thymic APCs exhibit marked heterogeneity. Cortical thymic epithelial cells exert autophagy for intracellular antigen sampling and mediate positive selection of thymic precursor T-cells with receptors specific for non-self antigens. These positively selected non-self reactive mature T lymphocytes are then allowed to leave the thymus to exert their functions in the periphery.

The key factor determining the choice between positive and negative selection in the thymus is the strength of antigen recognition, with low-avidity recognition leading to positive selection and high avidity recognition leading to negative selection. Peptides bound to MHC molecules on thymic epithelial cells serve an essential role in positive selection (48).

Delacher *et al* recently described tissue-restricted Tregs based on genome-wide DNA methylation analysis (50); the described epigenetic mechanisms allow Tregs to adapt to specific tissue sites. Tissue-restricted Tregs thus help to maintain self-tolerance and organ homeostasis. A summary of the many mechanisms of self-tolerance in T and B lymphocytes is presented in Table II. These findings confirm the central importance of immunological tolerance towards self-antigens during evolution for a functional immune system.

In the case of partial defects of central tolerance, T-cell responses against self-antigens with restricted tissue distribution can lead to organ-specific autoimmune diseases, such as myasthenia gravis, type 1 diabetes and multiple sclerosis.

The sophisticated mechanisms of self-tolerance in T and B lymphocytes might explain why side effects of classical immunotherapy approaches, such as active immunization with cancer vaccines, have very low side effects. Therapeutic vaccinations in chronic diseases, including cancer, are likely to work best in a post-operative adjuvant situation as prophylaxis against metastases. The indication in this situation is that the tumor burden is low and the immune system competence is high.

*Inventions by nature: Immunological long-term memory.* At the end of an antigen-specific T-cell response, the majority of

the clonally expanded cells die, leaving a small population of memory T-cells (MTCs). These can be maintained for long periods of time, even in the absence of antigens. Several subsets, such as central and effector MTCs, have been distinguished (51). Prophylactic vaccination campaigns against diphtheria (1950), polio (1960) and measles (1970) have been very successful and depend on long-term immunological memory.

Bone marrow contains niches not only for hematopoietic stem cells (HSCs), but also for B and T lymphocyte-derived MTCs. T-cell niches, rich in interleukin (IL)-7 and IL-15, allow for optimal T-cell maintenance (52). Bone marrow contains white adipose tissue, which has been reported to serve as reservoir for MTCs with a distinct metabolic profile, ready to promote antigen-specific MTC responses (53). Epigenetic profiling of human $CD4^+$ T-cells suggests a linear differentiation of MTCs and allows for identification of molecular regulators of MTC development (54). Notably, bone marrow-derived MTCs reveal a unique epigenetic profile in comparison to circulating MTCs (54). This is reminiscent of the epigenetic profiling of Tregs (50).

A human MTC subset has been described with stem cell-like properties. This is a long-lived T-cell population with enhanced capacity for self-renewal (55). MTCs exhibit distinct gene expression profiles and share a transcriptional program of self-renewal with long-term HSCs (56,57). A longitudinal analysis of human memory $CD8^+$ T-cells following vaccination against yellow fever virus revealed the following: The MTC pool originated from $CD8^+$ T-cells that divided extensively during the first 2 weeks post-infection and was maintained by quiescenT-cells that divide less than once every year. These long-lived virus-specific MTCs did not express effector molecules, but an open chromatin profile at effector genes was maintained in cells isolated even a decade after vaccination (58).

## 7. Successful cancer immunotherapy

The progress made in immunotherapy, OV treatment and targeted therapies with SMIs has previously been described in detail (4). There are numerous examples of successful cancer immunotherapy, including: i) The development of MAbs, which are products of B lymphocytes. One of the first FDA-approved MAbs was trastuzumab (Herceptin), which targets the cell surface receptor HER2 that can be expressed by cancer cells, including breast cancer cells. At present, dozens of therapeutic MAbs are available for application in patients with various types of cancer.

ii) The recent development of MAbs targeting immune regulatory receptors (checkpoints) on T-cells, such as CTLA-4 and PD-1. Such receptors deliver negative signals to activated T-cells to stop their activity at the end of their antigenic response. Tumors often use this physiological regulatory mechanism for immune escape, thereby shutting off antitumor reactivity of TILs. The clinical application of checkpoint inhibitory MAbs, which interfere with this tumor immune escape mechanism, has resulted in an improvement of long-term survival in a proportion of patients with melanoma (59) and carcinoma (60).

In 2018, the Nobel Prize for Physiology or Medicine was awarded to James P. Allison and Tasuko Honjo for their work in cancer immunotherapy. The group of JP Allison discovered the TCR in 1982 and went on to develop the field of checkpoint blockade (61); this led to the breakthrough drug ipilimumab (62). The group of Honjo discovered an enzyme important for class-switch recombination of antibodies, as well as PD1 and the mechanism underlying PD1 checkpoint protein blockade (63).

Checkpoint inhibitors interfere with immune regulation; therefore, the side effects of this type of immunotherapy are more severe than those of conventional immunotherapies. Immune-associated AEs (e.g. autoimmune reactions) can affect any organ system. Early recognition and quick intervention, for instance with corticosteroids, is essential. A previous comprehensive review focused on the role of cosignaling (costimulatory or co-inhibitory) receptors and Treg homeostasis in autoimmunity and tumor immunity (64).

iii) Adoptive T-cell therapies. An example of which involves the transfer of allogeneic peripheral blood-derived donor cells to achieve graft-versus-leukemia (GvL) effects in patients with leukemia. In 1995, a GvL animal model was developed for advanced metastasized cancer (65). Normally, immunotherapy works in early stage, but rarely in late-stage disease. In this model, instead of normal T-cells, tumor-immune MTCs were used. Following a single transfer of allogeneic MTCs into 5 Gy irradiated cachectic mice with large tumor burden and metastases in the liver and kidney, complete cancer remission was observed (65). Later, it was observed in this model that tumor-immune MTCs from bone marrow are superior to those from the spleen; they exert GvL without inducing graft-versus-host reactivity (66). The mechanism underlying this effective immunotherapy was elucidated over 10 years and has recently been summarized (67).

In 2001, Feuerer *et al* (20) described the treatment of human tumors in NOD/SCID mice with patient-derived reactivated MTCs from bone marrow. A single intraperitoneal transfer of such cells induced regression of subcutaneous autologous tumor xenotransplants. Tumor regression was associated with infiltration by human T-cells and DCs, and with tumor cell apoptosis and necrosis. Reactivated T-cells from the peripheral blood of the same patients demonstrated much lower antitumor reactivity. Shortly thereafter, Feuerer *et al* demonstrated T-cell priming in the bone marrow in response to blood-borne antigens (68). This phenomenon has the potential for inducing long-lasting protective antitumor immunity (69).

A novel development involves modern gene transfer technologies, which allow the production of T-cells with TAA-specific TCRs (4) or with CAR, which consist of antibody binding sites fused to TCR signaling chains (70). Such T-cells can further be engineered with T-cell redirected universal cytokine killing, in order to allow inducible cytokines to modulate the tumor stroma (71).

iv) Antitumor vaccination in combination with OVs. A vaccine developed at Deutsches Krebsforschungszentrum (DKFZ, Heidelberg, Germany), known as ATV-NDV, is an autologous-irradiated NDV-infected live tumor cell vaccine (72). It was developed in metastatic animal tumors and was then transferred into the clinic. Elucidation of the effects of NDV on immune system cells revealed molecular evidence for its stimulatory effects with regard to macrophages, natural killer (NK) cells, DCs, $CD4^+$ T helper cells and $CD8^+$ CTLs. In all of these cell types, NDV was revealed to stimulate or

costimulate (in the case of T cells) their immune activity without inducing toxic effects. Oncolytic NDV has been used in the clinic for the treatment of patients with cancer for ≤50 years (73); therefore, much experience has been gained. NDV can exert oncolytic activity against hypoxic cancer cells (74); because of this, and of other properties, NDV has been suggested to be capable to negatively affect cancer therapeutic resistance of various kinds (75).

The results of clinical trials pertaining to ATV-NDV are summarized as follows; ATV-NDV was applied post-operatively following approval by local ethics committees: i) In a phase I study of primary operated breast cancer, the results revealed feasibility and immunogenicity of the vaccine. Immunogenicity of the vaccine required that the tumor cell number and tumor cell viability of the individually produced vaccine fulfilled defined parameters (72).

ii) Another clinical study worth mentioning is a post-operative ATV-NDV vaccination study in patients with glioblastoma multiforme (GBM), which was conducted to assess feasibility, safety and clinical benefit. The progression-free survival of vaccinated patients (n=23) was 40 weeks compared with 26 weeks in 87 non-vaccinated control patients from the same time period and the same clinic. The median OS was 100 weeks compared with 49 weeks in the control patient group (P<0.001). In the vaccinated group, immune monitoring revealed significant increases in skin delayed-type hypersensitivity reactivity, numbers of tumor-reactive MTCs in the blood and in the number of $CD8^+$ tumor-infiltrating T-cells in frozen tissue slices from GBM recurrences. In addition, there was one complete remission of non-resectable brain tumor (76).

iii) A prospectively randomized clinical trial in CRC investigated the efficacy of ATV-NDV vaccination following liver resection for hepatic metastases as a tertiary prevention method. A total of 25 patients with stage IV CRC were vaccinated and compared with a similar number of non-vaccinated comparable control patients. After a long follow-up period of 9-10 years there was no significant difference between the vaccinated and control groups. However, when stratified for tumor localization, there were significant differences between the vaccinated patients with colon and rectum carcinoma. Vaccination exhibited a significant benefit only in patients with colon cancer: in the control arm, 78.6% of patients succumbed to the disease, whereas in the vaccinated arm only 30.8% succumbed. These trial results from 2009 (77) provide clinical evidence for the value and potential for long-term improvement of OS in response to ATV-NDV. The potential mechanism of function has been discussed in a separate review (78).

iv) The Immunological and Oncological Center Cologne (IOZK, Cologne, Germany) has developed a second-generation ATV-NDV vaccine [consisting of three components: Autologous DCs, TAAs and NDV (79)]. The latter two components were obtained from an oncolysate of autologous NDV-infected tumor cells. The IOZK succeeded in producing these components to the highest quality level (good manufacturing practice, GMP). IOZK was the first worldwide institution to produce NDV according to GMP standards. Therefore, this institution obtained official approval for its products on a compassionate use basis.

Since 2015, the IOZK has offered a viral oncolysate-pulsed DC vaccine (VOL-DC). In addition, it offers a novel multimodal strategy of cancer immunotherapy combining hyperthermia/oncolytic NDV pretreatment with specific autologous antitumor vaccination applying the vaccine VOL-DC (79). Hyperthermia is included because it enhances immune activity in response to cancer (80).

Interim results from the treatment of patients with GBM are promising and similar to those recently published by a group from the Duke University Medical Center (Durham, NC, USA). This previous trial treated 61 patients with recurrent GBM by intratumoral delivery of a recombinant nonpathogenic polio-rhinovirus chimera (81). Notably, and similar to our observations (82), survival, compared with historical controls, reached a plateau at ~20% at 2 years, which was sustained >3 years.

The efficiency of multimodal immunotherapy was studied at IOZK as part of first line treatment for patients with GBM. With a median follow up of 17 months, median OS was not reached, and estimated OS at 30 months was 58% (82). Maintenance temozolomide (TMZ) chemotherapy targets dividing tumor cells, whereas NDV and ICD target dividing and non-dividing tumor cells. These data suggested that the additional induction of ICD via NDV/hyperthermia during maintenance TMZ chemotherapy is beneficial in improving OS.

Taken together, there are various successful types of cancer immunotherapy. The design of currently popular T-cell-based immunotherapies has been described as follows (44):

i) Release the brakes, checkpoint blockade; ii) boost instruction via antigens, cancer vaccines;

iii) boost instruction via cell transfer and bypassing presentation, adoptive DC therapy; iv) boost recognition via cell transfer and bypassing instruction, adoptive T-cell therapy; v) boost recognition via cell transfer and bypassing instruction and MHC presentation. At present, IOZK offers products (the oncolytic virus NDV, the antitumor vaccine VOL-DC and checkpoint inhibitors) that boost instruction and release the brakes.

With regards to cancer vaccines, a distinction exists between TAA vaccines and neo-epitope antigen vaccines. The strengths and weaknesses of the latter, and of future novel technologies, are described in a previous review (44).

## 8. OVs interact with the immune system and have few side effects

*OVs and tumor selectivity*. Cancer therapy using OVs is an emerging biological treatment modality, which uses replication-competent viruses to destroy cancer cells. OVs selectively replicate in cancer cells and damage cancerous tissue without causing harm to normal tissue. OVs have been developed from various virus families, including *Herpesviridae*, *Adenoviridae*, *Paramyxoviridae*, *Rhabdoviridae*, *Poxviridae* or *Retroviridae*. Herpes simplex virus (HSV), adenovirus and poxvirus are DNA viruses, whereas the others are RNA viruses. Often the viruses have to be genetically modified to achieve tumor selectivity, oncolysis, safety and few side effects. The first approved OV was T-VEC, which is a recombinant HSV with the transgene granulocyte-macrophage

colony-stimulating factor (GM-CSF) (83). Some viruses can be developed from natural wild type strains, particularly RNA viruses (84); an example of this is avian NDV.

*OV-induced immunogenic tumor cell death*. The main molecular markers of OV-induced cellular responses and ICD are pathogen-associated molecular patterns (PAMPs), damage-associated molecular patterns (DAMPs) and cytokines. PAMPs can be viral capsids, viral DNA, viral double-stranded or single-stranded RNA, and viral proteins. Examples of DAMPs are heat shock proteins (HSPs), high mobility group box 1 (HMGB1) protein, calreticulin, ATP and uric acid. Examples of cytokines are type I IFNs, tumor necrosis factor (TNF), IFNγ, IL-12 and GM-CSF.

Oncolytic NDV has been applied to patients with cancer for >50 years (73); therefore, it is considered the OV with the longest history of application in humans. OV research dates back to the 1960s, when inefficient results from chemotherapy necessitated the search for alternatives (85).

NDV has a high safety profile, is very well tolerated and its side effects are mild (grade 0-2). Oncolysis following tumor cell infection by NDV involves ICD (86,87). It starts with immunogenic apoptosis with translocation to the plasma membrane of calreticulin, HSPs, and of viral proteins hemagglutinin-neuraminidase (HN) and fusion protein. This is followed by necrosis with the release of HMGB1 protein, cytokines and chemokines. HN at the cell surface can be recognized by NK cells via the cytotoxicity inducing receptor NKp46 (88), and contributes to the process of ICD. Macrophages in contact with NDV polarize into the M1 phenotype, produce nitric oxide, express TNF-related apoptosis-inducing ligand (TRAIL) and exhibit antitumor cytotoxic effects (73,75).

There are various means and concepts of application of OVs. They can be applied into the tumor itself (i.e. intratumoral administration) (89), into the tissue environment (i.e. locoregional administration) or systemically. OVs can be combined with carrier cells (90) or with bispecific antibodies (91) to improve tumor targeting. The combination of OVs with TAAs in vaccines is a specialty of the IOZK (79).

OVs are also well suited for combinatorial treatments. One example is the combination of OVT with immune checkpoint blockade (ICB). Intratumoral application of NDV in animal models has been reported to reduce therapeutic resistance to checkpoint inhibitory antibodies (92) and to mediate systemic antitumor effects. Early clinical trials of intratumoral T-VEC administration in patients with melanoma combined with ICB reported augmentation of intratumoral T-cell infiltration and an objective response rate of 62% (93). OV therapy can be combined not only with ICB but also with hyperthermia, chemotherapy, SMIs, radiotherapy and adoptive T-cell immunotherapy.

*OV-induced type I IFN response*. The avian virus NDV induces in man a strong type I IFN response. This has an early phase, which is induced in the cytoplasm of infected cells when the receptor retinoic acid-induced gene I (RIG-I) recognizes foreign uncapped (5'-triphosphate) viral RNA. The induced type I IFNs, α and β, are released from the cell and bind to IFN-α/β receptors (IFNARs), which are expressed by cells of all lineages, with the exception of mature erythrocytes. This initiates the later phase (8-18 h), which is a feedback loop response in the infected cells that greatly amplifies the type I IFN response. The released IFN can also bind to IFNARs on other cells, thereby initiating an antiviral response (73,75).

Tumor cells often have defects in the IFN pathway (94); this is why they are susceptible to infection by certain OVs, including the avian virus NDV (95).

The importance of signaling through RIG-I and IFNAR is particularly evident when comparing NDV with the Ebola virus. Whereas human infection with NDV leads to IFN signaling-induced immune activation, human infection with Ebola leads to evasion of the type I IFN response via viral proteins that specifically target RIG-I and IFNAR signaling (96). As a consequence, there is no interference with viral replication within infected cells and there is no immune response to prevent viral spread within the organism; this explains the high lethality of Ebola. The relevance of this finding is that it demonstrates the importance of the type I IFN response for activation of the immune system and for surviving a viral infection.

The type I IFN response can explain the mild side effects observed in response to OVs, such as NDV. During evolution, the type I IFN response developed as an important defense against viral infection. There are >300 IFN-stimulated genes, transcription factors and stimulated gene products (IFN-regulated proteins), which confer antiviral activity to cells. The type I IFNs not only protect normal cells, but also activate innate and adaptive immunity. Some IFN-induced proteins (e.g. TRAIL, FasL, IFN regulatory factor 1, RNAse L, 2'-5'-oligoadenylate synthetase and protein kinase R) contribute to apoptosis, others [e.g. MHC, LMP-2, LMP-7, transporter associated with antigen processing, CEA, tumor-associated glycoprotein 72, CC chemokines, CXC chemokines and CXC ligand (CXCL) chemokines] stimulate the immune response, and others (e.g. basic fibroblast growth factor, vascular endothelial growth factor, IL-8, CXCL-9 and CXCL-10) inhibit angiogenesis (97).

NDV-induced genes are involved in polarization of human DCs towards type 1 DCs; this has been reported to involve a genetic programming process that takes 18 h. This process involves 24 transcription factors that lead to upregulation of 779 genes (98). Interaction of naïve T-cells with such polarized APCs, which are present in the viral oncolysate-pulsed vaccine VOL-DC, leads to polarization of the T-cell response towards T helper 1. In addition, NDV-induced IFN-α enhances antigen cross-presentation in human DCs by modulating proteasome activity, antigen survival, endocytic routing and processing (99,100).

## 9. Important parameters of chemotherapy, immunotherapy and OV therapy

An overview of the important parameters of chemotherapy, immunotherapy and OV therapy is presented in Table III. Tumor specificity is high for immunotherapy and OVT, but low for chemotherapy. While all three therapies exhibit toxicity towards proliferating tumor cells, non-proliferating tumor cells (e.g. tumor stem cells and dormant tumor cells) can be lysed only by immunotherapy and OV therapy. Conversely, chemotherapy exerts unwanted toxic activity towards normal proliferating cells within the body (e.g. bone marrow and

Table III. Important parameters of CT, IT and OVT.

| Parameter | CT | IT | OVT |
|---|---|---|---|
| Tumor specificity | Low | High | High |
| Toxicity towards proliferating TCs | + | + | + |
| Toxicity towards non-proliferating TCs | - | + | + |
| Toxicity towards normal proliferating cells | + | - | - |
| Effects on the immune system | Negative | Positive | Positive |
| Optimized by evolution | No | Yes | Yes |
| Associated with self-tolerance | No | Yes | Yes |
| Associated with a memory function | No | Yes | Yes |
| Approved for application in patients with cancer | Yes[a] | No[b] | No[c] |

[a]Since the 1970s. [b]With the exception of monoclonal antibodies and checkpoint inhibitors in certain cases. [c]With the exception of T-VEC approved for melanoma immunotherapy and Newcastle disease virus in Germany with a permit for compassionate use. CT, chemotherapy; IT, immunotherapy; OVT, oncolytic virus therapy; TCs, tumor cells.

endothelia), whereas this is not the case with immunotherapy and OV therapy.

Chemotherapy exerts negative effects on the immune system, whereas immunotherapy and OV therapy have positive effects on the immune system. Immunotherapy and OV therapy are based on mechanisms optimized by evolution, whereas chemotherapy was originally invented by chemists. The immune system has developed sophisticated mechanisms of self-tolerance to prevent autoimmune reactions and to maintain integrity of the body. Chemotherapy is toxic to normal cells thereby causing severe AEs. Furthermore, the immune system has a memory function, which is important for achieving long-term therapeutic effects; this is lacking with chemotherapy.

In spite of the advantages of immunotherapy and OV therapy, these forms of cancer therapy are not yet part of standard therapy; there are only a few exceptions.

## 10. Discussion

In spite of its severe side effects, chemotherapy remains a main treatment option for cancer. As early as 1963, the disappointing efficacy of chemotherapy was reported (4). However, between 1984 and 1985, at the peak of aggressive chemotherapy, >6,000 articles were published in medical journals regarding treatment of cancer with chemotherapy; none of these studies reported on novel strategies that could cure advanced solid tumors in combination with chemotherapy (3). Grade 3 and 4 side effects can be life threatening. One of the many types of cytostatic drug that produce such side effects are molecular derivatives of nitrogen mustard, which is a toxin that was used during World War I. Examples, still in use, are melphalan, chlorambucil, cyclophosphamide, ifosfamid and others.

Evidence-based medicine is currently the gold standard for the approval of novel drugs. The quality of criteria for clinical studies has steadily increased since the introduction of cytostatic drugs; however, some drugs originally approved many years ago are still in use. Recommendations for updates of standard therapy come from medical oncology societies. There is no guarantee, however, that such recommendations are devoid of conflicts of interest; therefore, it remains the individual responsibility of a medical oncologist to decide which drugs to apply or not. Medical ethics should be respected.

To change the direction of cancer therapy is not easy, as healthcare is a huge market. At present, immunological products, such as MAbs and checkpoint inhibitors have successfully entered the market; however, this is only a small portion of the potential of immunotherapy. In the future, immunotherapy may be a discipline in its own right, including immune diagnosis, immunotherapy, immune monitoring and immunological follow-up. Furthermore, two cancer vaccines, ATV-NDV and VOL-DC, which combine OVs with TAAs, have been entered into clinical application. After >30 years, integrating OVs into cancer immunotherapy may soon become mainstream (101).

## 11. Conclusions

The present review compared chemotherapy and biological therapies, including immunotherapy and OV therapy.

Systemic forms of cancer treatment are necessary at the transition phase of cancer, when it turns from a localized into a systemic form of disease with metastases. Systemic forms of cancer treatment can be prophylactic (e.g. in a post-operative situation) or therapeutic. The primary aim of chemotherapy is to reduce tumor burden, whereas the aim of immunotherapy is to generate systemic protective anticancer immunity. The focus is either on the tumor or on the tumor-bearing host organism and its immune system.

The aim of this review was to present novel concepts, which may reduce side effects from systemic cancer treatment. This is necessary because chemotherapy often exhibits relatively low tumor specificity and high toxicity. Targeted therapy with chemically designed SMIs has higher tumor specificity than conventional chemotherapy; however, the side effects are similar. The majority of novel concepts are derived from biological types of therapy (Tables I-III); some of these biological therapies exert considerable side effects. Conversely, conventional immunotherapy, including vaccination and OV therapy, exerts only mild side effects and is well tolerated. It is suggested that the reason for this difference is physiological: Immunological self-tolerance and immunological memory.

An important difference between chemotherapy and immunotherapy or OV therapy is the dose-response curve. While in the case of cytostatic drugs the curve is linear, in the case of biological and physiological therapies it is bell-shaped. The reason for this difference appears to be due to the complementarity of specific cognate molecular lock-and-key interactions. This is exemplified with interactions between antigens and antibodies, as well as between pMHC and TCR in cases of T-cell-mediated immune responses.

Notably, a combination of cancer immunotherapy and OV therapy was successful in a randomized controlled

study (77,78). This previous study evaluated the efficacy of post-operative vaccination with ATV-NDV in patients with stage IV CRC following resection of liver metastases. The results revealed that in patients with colon cancer a significant 10-year survival benefit of as much as 30% was detected. The magnitude of the effect is similar to that obtained in patients with melanoma treated with ICB (59). The side effects of these two approaches, however, were different: Grade 0-2 for the vaccination study compared with grade 1-4 for the ICB study.

With regards to future developments, it has been suggested to combine vaccines, OVs and immune checkpoint inhibitors to prime, expand and facilitate effective tumor immunotherapy (102,103). The main conclusions of this review are: i) It may be beneficial for immunotherapy to be included in standard care. Rules of evidence-based medicine should be adjusted to the needs of individualized immunotherapy studies, as well as to multimodal therapy studies in general. ii) Recommendations for the use of cytostatic drugs that produce severe side effects and low efficacy should be reviewed by societies of internal medicine.

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**References**

1. Halstedt WS: Surgical Papers. Burket WC (ed). Vol 2. Baltimore, 1924.
2. Fisher B and Wolmark N: The current status of systemic adjuvant therapy in the management of primary breast cancer. Surg Clin North Am 61: 1347-1360, 1981.
3. Mukherjee S: The Emperor of All Maladies: A Biography of Cancer. Scribner, a Division of Simon and Schuster, Inc., New York, 2010.
4. Schirrmacher V: Quo Vadis Cancer Therapy? Fascinating discoveries of the last 60 years. Lambert Academic Publishing, pp1-353, 2017.
5. Koeppen BM and Stanton BA (eds): Berne and Levy Physiology. 7th edition. Elsevier, Amsterdam, p880, 2018.
6. Seeber S and Schütte J (eds). Therapiekonzepte Onkologie. Springer-Verlag, Berlin, Heidelberg, 1993.
7. Morgan G, Ward R and Barton M: The contribution of cytotoxic chemotherapy to 5-year survival in adult malignancies. Clin Oncol (R Coll Radiol) 16: 549-560, 2004.
8. Steward BW and Wild CW (eds): World Cancer Report 2014. IARC Press, Lyon, 2014.
9. American Cancer Society: Cancer Facts and Figures 2018. American Cancer Society, Inc., Atlanta, GA, 2018.
10. Niraula S, Seruga B, Ocana A, Shao T, Goldstein R, Tannock IF and Amir E: The price we pay for progress: A meta-analysis of harms of newly approved anticancer drugs. J Clin Oncol 30: 3012-3019, 2012.
11. Niraula S, Amir E, Vera-Badillo F, Seruga B, Ocana A and Tannock IF: Risk of incremental toxicities and associated costs of new anticancer drugs: A meta-analysis. J Clin Oncol 32: 3634-3642, 2014.
12. Barnes TA, Amir E, Templeton AJ, Gomez-Garcia S, Navarro B, Seruga B and Ocana A: Efficacy, safety, tolerability and price of newly approved drugs in solid tumors. Cancer Treat Rev 56: 1-7, 2017.
13. Reid E, Suneja G, Ambinder RF, Ard K, Baiocchi R, Barta SK, Carchman E, Cohen A, Gupta N, Johung KL, *et al*: Cancer in people living with HIV, version 1.2018, NCCN clinical practice guidelines in oncology. J Natl Compr Canc Netw 16: 986-1017, 2018.
14. Gutierrez-Dalmau A and Campistol JM: Immunosuppressive therapy and malignancy in organ transplant recipients: A systematic review. Drugs 67: 1167-1198, 2007.
15. Zhang L, Conejo-Garcia JR, Katsaros D, Gimotty PA, Massobrio M, Regnani G, Makrigiannakis A, Gray H, Schlienger K, Liebman MN, *et al*: Intratumoral T cells, recurrence, and survival in epithelial ovarian cancer. N Engl J Med 348: 203-213, 2003.
16. Clemente CG, Mihm MC Jr, Bufalino R, Zurrida S, Collini P and Cascinelli N: Prognostic value of tumor infiltrating lymphocytes in the vertical growth phase of primary cutaneous melanoma. Cancer 77: 1303-1310, 1996.
17. Lipponen PK, Eskelinen MJ, Jauhiainen K, Harju E and Terho R: Tumour infiltrating lymphocytes as an independent prognostic factor in transitional cell bladder cancer. Eur J Cancer 29A: 69-75, 1992.
18. Naito Y, Saito K, Shiiba K, Ohuchi A, Saigenji K, Nagura H and Ohtani H: $CD8^+$ T cells infiltrated within cancer cell nests as a prognostic factor in human colorectal cancer. Cancer Res 58: 3491-3494, 1998.
19. Sommerfeldt N, Schütz F, Sohn C, Förster J, Schirrmacher V and Beckhove P: The shaping of a polyvalent and highly individual T-cell repertoire in the bone marrow of breast cancer patients. Cancer Res 66: 8258-8265, 2006.
20. Feuerer M, Beckhove P, Bai L, Solomayer EF, Bastert G, Diel IJ, Pedain C, Oberniedermayr M, Schirrmacher V and Umansky V: Therapy of human tumors in NOD/SCID mice with patient-derived reactivated memory T cells from bone marrow. Nat Med 7: 452-458, 2001.
21. Böhle A and Brandau S: Immune mechanisms in bacillus Calmette-Guerin immunotherapy for superficial bladder cancer. J Urol 170: 964-969, 2003.
22. Khong HT and Restifo NP: Natural selection of tumor variants in the generation of 'tumor escape' phenotypes. Nat Immunol 3: 999-1005, 2002.
23. Teng MW, Galon J, Fridman WH and Smyth MJ: From mice to humans: Developments in cancer immunoediting. J Clin Invest 125: 3338-3346, 2015.
24. Zhang AW, McPherson A, Milne K, Kroeger DR, Hamilton PT, Miranda A, Funnell T, Little N, de Souza CPE, Laan S, *et al*: Interfaces of malignant and immunologic clonal dynamics in ovarian cancer. Cell 173: 1755-1769.e22, 2018.
25. Bäumler E: Paul Ehrlich. Forscher für das Leben. Bastei-Lübbe-Taschenbuch 61, 163, 1989.
26. Pauling L and Delbrück M: The nature of the intermolecular forces operative in biological processes. Science 92: 77-79, 1940.

27. Rudolph MG, Stanfield RL and Wilson IA: How TCRs bind MHCs, peptides, and coreceptors. Annu Rev Immunol 24: 419-466, 2006.
28. Manz BN, Jackson BL, Petit RS, Dustin ML and Groves J: T-cell triggering thresholds are modulated by the number of antigen within individual T-cell receptor clusters. Proc Natl Acad Sci USA 108: 9089-9094, 2011.
29. Reinherz EL: αβ TCR-mediated recognition: Relevance to tumor-antigen discovery and cancer immunotherapy. Cancer Immunol Res 3: 305-312, 2015.
30. Crespo J, Sun H, Welling TH, Tian Z and Zou W: T cell anergy, exhaustion, senescence, and stemness in the tumor microenvironment. Curr Opin Immunol 25: 214-221, 2013.
31. Boissonnas A, Fetler L, Zeelenberg IS, Hugues S and Amigorena S: In vivo imaging of cytotoxic T cell infiltration and elimination of a solid tumor. J Exp Med 204: 345-356, 2007.
32. Otto L, Zelinskyy G, Schuster M, Dittmer U and Gunzer M: Imaging of cytotoxic antiviral immunity while considering the 3R principle of animal research. J Mol Med (Berl) 96: 349-360, 2018.
33. Vasaturo A, Di Blasio S, Peeters DG, de Koning CC, de Vries JM, Figdor CG and Hato SV: Clinical implications of co-inhibitory molecule expression in the tumor microenvironment for DC vaccination: A game of stop and go. Front Immunol 4: 417, 2013.
34. Teng MW, Ngiow SF, Ribas A and Smyth MJ: Classifying cancers based on T-cell infiltration and PD-L1. Cancer Res 75: 2139-2145, 2015.
35. Lindenmann J: Viral oncolysis with host survival. Proc Soc Exp Biol Med 113: 85-91, 1963.
36. Cassel WA and Garrett RE: Tumor immunity after viral oncolysis. J Bacteriol 92: 792, 1966.
37. Heicappell R, Schirrmacher V, von Hoegen P, Ahlert T and Appelhans B: Prevention of metastatic spread by postoperative immunotherapy with virally modified autologous tumor cells. I. Parameters for optimal therapeutic effects. Int J Cancer 37: 569-577, 1986.
38. Ertel C, Millar NS, Emmerson PT, Schirrmacher V and von Hoegen P: Viral hemagglutinin augments peptide-specific cytotoxic T cell responses. Eur J Immunol 23: 2592-2596, 1993.
39. Schirrmacher V, Haas C, Bonifer R and Ertel C: Virus potentiation of tumor vaccine T-cell stimulatory capacity requires cell surface binding but not infection. Clin Cancer Res 3: 1135-1148, 1997.
40. Khazaie K, Prifti S, Beckhove P, Griesbach A, Russell S, Collins M and Schirrmacher V: Persistence of dormant tumor cells in the bone marrow of tumor cell-vaccinated mice correlates with long-term immunological protection. Proc Natl Acad Sci USA 91: 7430-7434, 1994.
41. Müller M, Gounari F, Prifti S, Hacker HJ, Schirrmacher V and Khazaie K: EblacZ tumor dormancy in bone marrow and lymph nodes: Active control of proliferating tumor cells by $CD8^+$ immune T cells. Cancer Res 58: 5439-5446, 1998.
42. Klug F, Prakash H, Huber PE, Seibel T, Bender N, Halama N, Pfirschke C, Voss RH, Timke C, Umansky L, *et al*: Low-dose irradiation programs macrophage differentiation to an $iNOS^+$/M1 phenotype that orchestrates effective T cell immunotherapy. Cancer Cell 24: 589-602, 2013.
43. Gires O and Seliger B (eds): Tumor-Associated Antigens. Wiley-Blackwell, Hoboken, NJ, 2009.
44. Tokuyasu TA and Huang JD: A primer on the recent developments in cancer immunotherapy, with a focus on neoantigen vaccines. J Cancer Metastasis Treat 4: 2-24, 2018.
45. Hanahan D and Weinberg RA: The hallmarks of cancer. Cell 100: 57-70, 2000.
46. Flavahan WA, Gaskell E and Bernstein BE: Epigenetic plasticity and the hallmarks of cancer. Science 357: 2380, 2017.
47. Coulie PG, Lehmann F, Lethé B, Herman J, Lurquin C, Andrawiss M and Boon T: A mutated intron sequence codes for an antigenic peptide recognized by cytolytic T lymphocytes on a human melanoma. Proc Natl Acad Sci USA 92: 7976-7980, 1995.
48. Derbinski J and Kyewski B: How thymic antigen presenting cells sample the body's self-antigens. Curr Opin Immunol 22: 592-600, 2010.
49. Kyewski B and Peterson P: Aire, master of many trades. Cell 140: 24-26, 2010.
50. Delacher M, Imbusch CD, Weichenhan D, Breiling A, Hotz-Wagenblatt A, Träger U, Hofer AC, Kägebein D, Wang Q, Frauhammer F, *et al*: Genome-wide DNA-methylation landscape defines specialization of regulatory T cells in tissues. Nat Immunol 18: 1160-1172, 2017.
51. Sallusto F, Geginat J and Lanzavecchia A: Central memory and effector memory T cell subsets: Function, generation, and maintenance. Annu Rev Immunol 22: 745-763, 2004.
52. Di Rosa F and Pabst R: The bone marrow: A nest for migratory memory T cells. Trends Immunol 26: 360-366, 2005.
53. Han SJ, Glatman Zaretsky A, Andrade-Oliveira V, Collins N, Dzutsev A, Shaik J, Morais da Fonseca D, Harrison OJ, Tamoutounour S, Byrd AL, *et al*: White adipose tissue is a reservoir for memory T cells and promotes protective memory responses to infection. Immunity 47: 1154-1168.e6, 2017.
54. Durek P, Nordström K, Gasparoni G, Salhab A, Kressler C, de Almeida M, Bassler K, Ulas T, Schmidt F, Xiong J, *et al*; DEEP Consortium: Epigenomic profiling of human $CD4^+$ T cells supports a linear differentiation model and highlights molecular regulators of memory development. Immunity 45: 1148-1161, 2016.
55. Gattinoni L, Lugli E, Ji Y, Pos Z, Paulos CM, Quigley MF, Almeida JR, Gostick E, Yu Z, Carpenito C, *et al*: A human memory T cell subset with stem cell-like properties. Nat Med 17: 1290-1297, 2011.
56. Luckey CJ, Bhattacharya D, Goldrath AW, Weissman IL, Benoist C and Mathis D: Memory T and memory B cells share a transcriptional program of self-renewal with long-term hematopoietic stem cells. Proc Natl Acad Sci USA 103: 3304-3309, 2006.
57. Gattinoni L, Speiser DE, Lichterfeld M and Bonini C: T memory stem cells in health and disease. Nat Med 23: 18-27, 2017.
58. Akondy RS, Fitch M, Edupuganti S, Yang S, Kissick HT, Li KW, Youngblood BA, Abdelsamed HA, McGuire DJ, Cohen KW, *et al*: Origin and differentiation of human memory CD8 T cells after vaccination. Nature 552: 362-367, 2017.
59. Wolchok JD, Kluger H, Callahan MK, Postow MA, Rizvi NA, Lesokhin AM, Segal NH, Ariyan CE, Gordon RA, Reed K, *et al*: Nivolumab plus ipilimumab in advanced melanoma. N Engl J Med 369: 122-133, 2013.
60. Fehrenbacher L, Spira A, Ballinger M, Kowanetz M, Vansteenkiste J, Mazieres J, Park K, Smith D, Artal-Cortes A, Lewanski C, *et al*; POPLAR Study Group: Atezolizumab versus docetaxel for patients with previously treated non-small-cell lung cancer (POPLAR): A multicentre, open-label, phase 2 randomised controlled trial. Lancet 387: 1837-1846, 2016.
61. Allison JP: Checkpoints. Cell 162: 1202-1205, 2015.
62. Oiseth SJ and Aziz MS: Cancer immunotherapy: A brief review of the history, possibilities, and challenges ahead. J Cancer Metastasis Treat 3: 250-261, 2017.
63. Chamoto K, Al-Habsi M and Honjo T: Role of PD-1 in immunity and diseases. Curr Top Microbiol Immunol 410: 75-97, 2017.
64. Kumar P, Bhattacharya P and Prabhakar BS: A comprehensive review on the role of co-signaling receptors and Treg homeostasis in autoimmunity and tumor immunity. J Autoimmun: Aug 30, 2018 (Epub ahead of print). doi: 10.1016/j.jaut.2018.08.007.
65. Schirrmacher V, Beckhove P, Krüger A, Rocha M, Umansky V, Fichtner K, Hull W, Zangemeisterwittke U, Griesbach A, Jurianz K, *et al*: Effective immune rejection of advanced metastasized cancer. Int J Oncol 6: 505-521, 1995.
66. Schirrmacher V, Beckhove P, Choi C, Griesbach A and Mahnke Y: Tumor-immune memory T cells from the bone marrow exert GvL without GvH reactivity in advanced metastasized cancer. Int J Oncol 27: 1141-1149, 2005.
67. Schirrmacher V: Complete remission of cancer in late-stage disease by radiation and transfer of allogeneic MHC-matched immune T cells: Lessons from GvL studies in animals. Cancer Immunol Immunother 63: 535-543, 2014.
68. Feuerer M, Beckhove P, Garbi N, Mahnke Y, Limmer A, Hommel M, Hämmerling GJ, Kyewski B, Hamann A, Umansky V, *et al*: Bone marrow as a priming site for T-cell responses to blood-borne antigen. Nat Med 9: 1151-1157, 2003.
69. Schirrmacher V, Feuerer M, Fournier P, Ahlert T, Umansky V and Beckhove P: T-cell priming in bone marrow: The potential for long-lasting protective anti-tumor immunity. Trends Mol Med 9: 526-534, 2003.
70. Newick K, O'Brien S, Moon E and Albelda SM: CAR T cell therapy of solid tumors. Annu Rev Med 68: 139-152, 2017.
71. Chmielewski M, Hombach AA and Abken H: Of CARs and TRUCKs: Chimeric antigen receptor (CAR) T cells engineered with an inducible cytokine to modulate the tumor stroma. Immunol Rev 257: 83-90, 2014.
72. Ahlert T, Sauerbrei W, Bastert G, Ruhland S, Bartik B, Simiantonaki N, Schumacher J, Häcker B, Schumacher M and Schirrmacher V: Tumor-cell number and viability as quality and efficacy parameters of autologous virus-modified cancer vaccines in patients with breast or ovarian cancer. J Clin Oncol 15: 1354-1366, 1997.

73. Schirrmacher V: Fifty years of clinical application of Newcastle disease virus: Time to celebrate! Biomedicines 4: E16, 2016.
74. Ch'ng WC, Stanbridge EJ, Yusoff K and Shafee N: The oncolytic activity of Newcastle disease virus in clear cell carcinoma cells in normoxic and hypoxic conditions: The interplay between VHL and interferon beta signaling. J Interferon Cytokine Res 33: 346-354, 2013.
75. Schirrmacher V: Oncolytic Newcastle disease virus as a prospective anti-cancer therapy. A biological agent with potential to break therapy resistance. Exp Opon Biol Ther 15: 1-15, 2015.
76. Steiner HH, Bonsanto MM, Beckhove P, Brysch M, Geletneky K, Ahmadi R, Schuele-Freyer R, Kremer P, Ranaie G, Matejic D, *et al*: Antitumor vaccination of patients with glioblastoma multiforme: A pilot study to assess feasibility, safety, and clinical benefit. J Clin Oncol 22: 4272-4281, 2004.
77. Schulze T, Kemmner W, Weitz J, Wernecke KD, Schirrmacher V and Schlag PM: Efficiency of adjuvant active specific immunization with Newcastle disease virus modified tumor cells in colorectal cancer patients following resection of liver metastases: Results of a prospective randomized trial. Cancer Immunol Immunother 58: 61-69, 2009.
78. Schirrmacher V, Fournier P and Schlag P: Autologous tumor cell vaccines for post-operative active-specific immunotherapy of colorectal carcinoma: Long-term patient survival and mechanism of function. Expert Rev Vaccines 13: 117-130, 2014.
79. Schirrmacher V, Lorenzen D, Van Gool SW and Stuecker W: A new strategy of cancer immunotherapy combining hyperthermia/oncolytic virus pretreatment with specific autologous anti-tumor vaccination - A review. Austin Oncol Case Rep 2: 1006, 2017.
80. Yagawa Y, Tanigawa K, Kobayashi Y and Yamamoto M: Cancer immunity and therapy using hyperthermia with immunotherapy, radiotherapy, chemotherapy, and surgery. J Cancer Metastasis Treat 3: 218-230, 2017.
81. Desjardins A, Gromeier M, Herndon JE II, Beaubier N, Bolognesi DP, Friedman AH, Friedman HS, McSherry F, Muscat AM, Nair S, *et al*: Recurrent glioblastoma treated with recombinant poliovirus. N Engl J Med 379: 150-161, 2018.
82. VanGool SW, Makalowsky J, Feyen O, Prix L, Schirrmacher V and Stuecker W: The induction of immunogenic cell death (ICD) during maintenance chemotherapy and subsequent multimodal immunotherapy for glioblastoma (GBM). Austin Oncol Case Rep 3: 1010, 2018.
83. Watanabe D and Goshima F: Oncolytic Virotherapy by HSV. Adv Exp Med Biol 1045: 63-84, 2018.
84. Russell SJ: RNA viruses as virotherapy agents. Cancer Gene Ther 9: 961-966, 2002.
85. Cassel WA and Garrett RE: Newcastle disease virus as an antineoplastic agent. Cancer 18: 863-868, 1965.
86. Kroemer G, Galluzzi L, Kepp O and Zitvogel L: Immunogenic cell death in cancer therapy. Annu Rev Immunol 31: 51-72, 2013.
87. Koks CA, Garg AD, Ehrhardt M, Riva M, Vandenberk L, Boon L, De Vleeschouwer S, Agostinis P, Graf N and Van Gool SW: Newcastle disease virotherapy induces long-term survival and tumor-specific immune memory in orthotopic glioma through the induction of immunogenic cell death. Int J Cancer 136: E313-E325, 2015.
88. Jarahian M, Watzl C, Fournier P, Arnold A, Djandji D, Zahedi S, Cerwenka A, Paschen A, Schirrmacher V and Momburg F: Activation of natural killer cells by newcastle disease virus hemagglutinin-neuraminidase. J Virol 83: 8108-8121, 2009.
89. Zamarin D, Holmgaard RB, Subudhi SK, Park JS, Mansour M, Palese P, Merghoub T, Wolchok JD and Allison JP: Localized oncolytic virotherapy overcomes systemic tumor resistance to immune checkpoint blockade immunotherapy. Sci Transl Med 6: 226ra32, 2014.
90. Sampath P, Li J, Hou W, Chen H, Bartlett DL and Thorne SH: Crosstalk between immune cell and oncolytic vaccinia therapy enhances tumor trafficking and antitumor effects. Mol Ther 21: 620-628, 2013.
91. Fournier P and Schirrmacher V: Bispecific antibodies and trispecific immunocytokines for targeting the immune system against cancer: Preparing for the future. BioDrugs 27: 35-53, 2013.
92. Ribas A, Dummer R, Puzanov I, VanderWalde A, Andtbacka RH, Michielin O, Olszanski AJ, Malvehy J, Cebon J, Fernandez E, *et al*: Oncolytic virotherapy promotes intratumoral T cell infiltration and improves anti-PD1 immunotherapy. Cell 170: 1109-1119.e10, 2017.
93. Harrington KJ, Puzanov I, Hecht JR, Hodi FS, Szabo Z, Murugappan S and Kaufman HL: Clinical development of talimogene laherparepvec (T-VEC): A modified herpes simplex virus type-1-derived oncolytic immunotherapy. Expert Rev Anticancer Ther 15: 1389-1403, 2015.
94. Stojdl DF, Lichty B, Knowles S, Marius R, Atkins H, Sonenberg N and Bell JC: Exploiting tumor-specific defects in the interferon pathway with a previously unknown oncolytic virus. Nat Med 6: 821-825, 2000.
95. Fournier P, Wilden H and Schirrmacher V: Importance of retinoic acid-inducible gene I and of receptor for type I interferon for cellular resistance to infection by Newcastle disease virus. Int J Oncol 40: 287-298, 2012.
96. Schirrmacher V: Signaling through RIG-I and type I interferon receptor: Immune activation by Newcastle disease virus in man versus immune evasion by Ebola virus (Review). Int J Mol Med 36: 3-10, 2015.
97. Ivashkiv LB and Donlin LT: Regulation of type I interferon responses. Nat Rev Immunol 14: 36-49, 2014.
98. Zaslavsky E, Hershberg U, Seto J, Pham AM, Marquez S, Duke JL, Wetmur JG, Tenoever BR, Sealfon SC and Kleinstein SH: Antiviral response dictated by choreographed cascade of transcription factors. J Immunol 184: 2908-2917, 2010.
99. Tough DF: Type I interferon as a link between innate and adaptive immunity through dendritic cell stimulation. Leuk Lymphoma 45: 257-264, 2004.
100. Lattanzi L, Rozera C, Marescotti D, D'Agostino G, Santodonato L, Cellini S, Belardelli F, Gavioli R and Ferrantini M: IFN-α boosts epitope cross-presentation by dendritic cells via modulation of proteasome activity. Immunobiology 216: 537-547, 2011.
101. Bommareddy PK, Shettigar M and Kaufman HL: Integrating oncolytic viruses in combination cancer immunotherapy. Nat Rev Immunol 18: 498-513, 2018.
102. Collins JM, Redman JM and Gulley JL: Combining vaccines and immune checkpoint inhibitors to prime, expand, and facilitate effective tumor immunotherapy. Expert Rev Vaccines 17: 697-705, 2018.
103. van Willigen WW, Bloemendal M, Gerritsen WR, Schreibelt G, de Vries IJ and Bol KF: Dendritic cell cancer therapy: Vaccinating the right patient at the right time. Front Immunol 9: 2265, 2018.
104. Abbas KA, Lichtman AH and Pillai S (eds): Cellular and Molecular Immunology. 6th Edition. Saunders Elsevier, Oxford, p261, 2010.