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Immunotherapy – The New Era of Oncology

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ABSTRACT

In the field of immunotherapy, essential progress was achieved over the past years partially demonstrating long-lasting therapeutic responses in different tumor entities. A better understanding of the interactions between the tumor and the immune system as well as the integration of immunotherapeutic approaches into clinical routine were the foundations for this development. The different approaches intervene on multiple levels of the immune response and directly or indirectly mount the patient's own immune defense against tumor cells. Immunotherapeutic approaches are represented by cytokine therapies, vaccinations, the use of oncocytic viruses, and monoclonal antibody therapies as well as adoptive cell transfer strategies.

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1. Introduction

Especially in the last years, the field of immunotherapy was determined by significant progress although the idea to use the endogenous immune system for fighting against cancer was not new. Already in 1891, William Coley stimulated the immune system of sarcoma patients with bacterial fragments and achieved a short-term tumor reduction in some of his patients [1]. So why could this initial immunotherapeutic approach not prevail in the beginning? The reasons are manifold: the immune system is a very complexly regulated and balanced system, which on one hand may respond to pathogens due

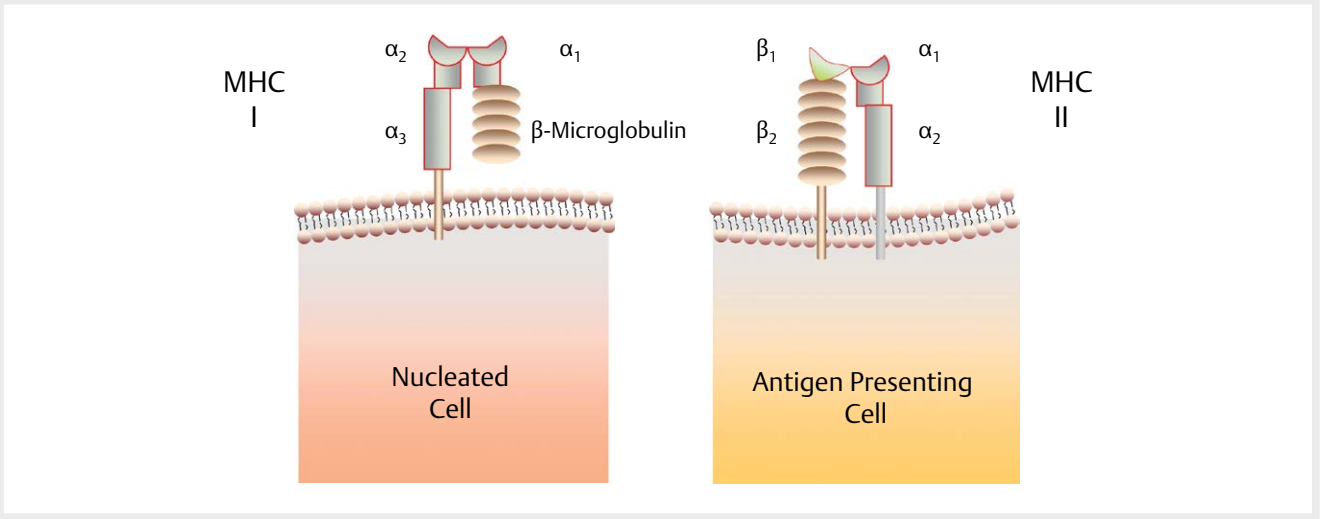
to stimulating and inhibiting components, on the other hand, however, it avoids excessive reaction and thus does not attack the own body. Furthermore, tumors are very heterogenic since they develop individually, and their properties depend on the individual patient and the tissue of origin. This situation is even aggravated by the fact that the original tissue of the tumor is not exogenous, and so important mechanisms of immune response, as they might work with the identification of exogenous pathogens, do not apply. Since Coley could only describe an unspecific reaction that was not directed against tumor antigens, the therapeutic effect was only temporary. Those mentioned aspects are the reason for initial difficulties and deceiving results oncological immunotherapy had and has to cope with. But what has finally changed? Why are there currently such high investments and efforts undertaken in the development of new therapeutic modalities with regard to tumor immunology? One crucial step was certainly the possibility to intervene specifically in the tumor development on the molecular level with new monoclonal antibodies (mAb). For many years, efforts were made to develop immunotherapies in the sense of immune activation; however, for some time now it has become obvious that antagonizing or influencing immunological blockades, checkpoints, and immunosuppressive mechanisms are of even higher importance. This was first achieved in the context of malignant melanoma by applying cytotoxic T lymphocyte-associated protein 4 (CTLA4) [2] and programmed cell death 1 (PD1) specific antibodies [3]. The results were convincing so that Science ennobled this type of immunotherapy as breakthrough of the year [4]. In addition, the scientific progress allows focusing the endogenous immune components on specific (tumor) antigens as it is the case for example with adoptive T cell transfer or in the context of vaccinations. Many of those strategies are relevant and innovative, however, they are at the very beginning of their (further) development. In the following, the chances and risks of immunotherapy will be discussed. For this purpose, first immunological basics of tumor interaction with the immune system will be explained in order to present different therapeutic approaches afterwards. This includes an overview of already existing therapeutic modalities as well as an outlook to future developments.

2. Tumor-immunological basics

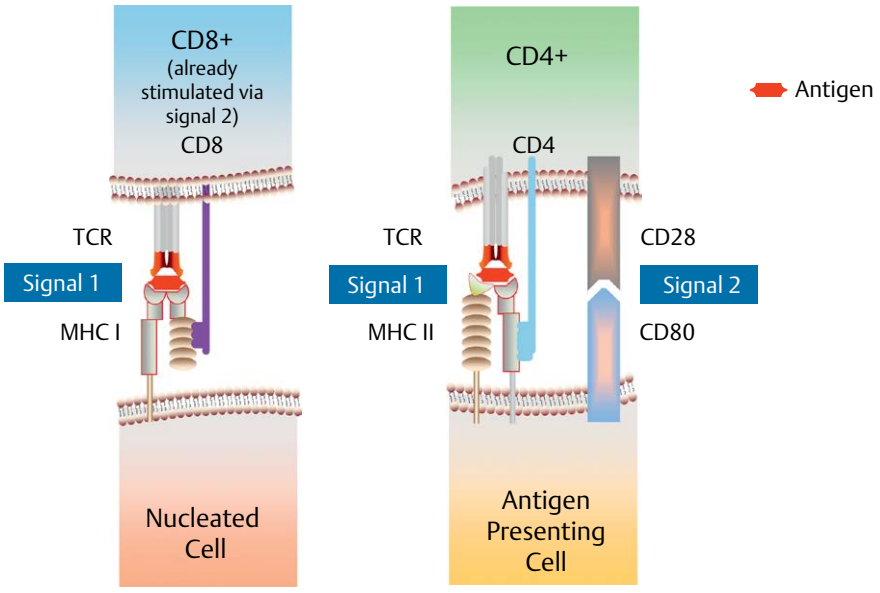
Based on history and function, the immune system can be divided into 2 branches: the innate (native) immunity is the first front of immune defense and identifies, fights, and removes – mostly successfully – foreign pathogens in a rapid and effective way. However, the innate immunity is neither antigen-specific nor capable of learning (adaptive). Those properties belong to the so-called acquired (adaptive) immunity. It adapts to specific antigens and may thus generate a long-lasting, specifically adapted immune response. Both arms are not autonomous but interact intensively. Additionally, it becomes more and more obvious that the distinction between the innate and the adaptive immune system is not entirely clear.

2.1. Innate immune response

The innate immune response includes physiological barriers such as humoral and cellular components. The cellular parts are characterized mainly by their ability to migrate into the tissue and to initiate the immune response there and at the same time to attract further components of the immune system. Many cells of the innate immune response have the ability of phagocytosis, i. e., they actively take in pathogens, process them, and present – according to the cell type – parts of them on their surface on molecules of the major histocompatibility complex II (MHC II; ▶ **Fig. 1**). The cellular components of the innate immune response include granulocytes, macrophages, dendritic cells (DC), and the natural killer cells (NK cells). Macrophages are able to process antigens absorbed by phagocytosis and present them efficiently to other cells via MHC II in order to trigger an antigen-specific response. So they are an important interface between the innate and the adaptive immune response. Depending on



▶ **Fig. 1** MHC molecules. MHC molecules are expressed on nucleated, endogenous cells (MHC I) and antigen-presenting cells (MHC II). MHC I molecules consist of an α and a β subunit. The α subunit contains three domains, α1 and α2 are responsible for antigen presentation and α3 secures anchoring in the cell membrane. β2 microglobulin is the fourth soluble domain of MHC I molecules. The MHC II molecules consist of 2 subunits both anchored in the cell membrane. One subunit (α or β) consists of 2 domains each, α1 and α2 or β1 and β2, respectively; α1 and β1 domains are responsible for antigen presentation. (MHC: main histocompatibility complex)

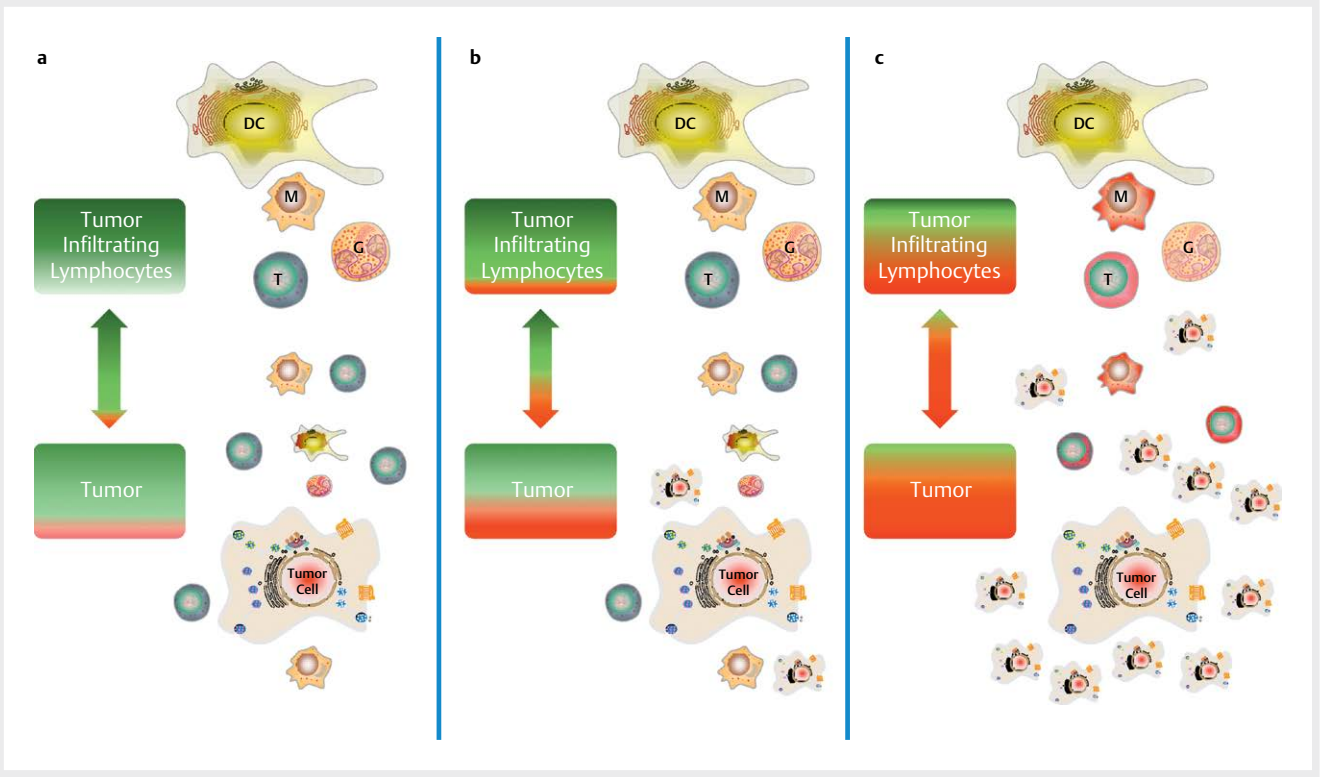


▶ **Fig. 2** Antigen presentation and antigen identification. CD8+ T cells identify antigens that are presented on MHC I molecules. CD4+ T cells recognize antigens of antigen presenting cells on MHC II molecules. MHC I molecules are charged with peptide fragments from the cellular cytosol that permanently develop from degradation products of proteasomes within the cell. This mechanism controls if the cell produces endogenous or exogenous proteins. MHC II molecules are exclusively expressed on antigen presenting cells and present foreign, extracellular peptide fragments that entered into the cell via phagocytosis or endocytosis in order to activate receptors of antigen-specific cells. In addition to activation via the TCR/MHC complex (signal 1) the T cells require a co-stimulating signal (CD80, signal 2) to be activated. (MHC: main histocompatibility complex; TCR: T cell receptor).

the environment, generally 2 phenotypes of macrophages are differentiated, the M1 and the M2 phenotype. Whereas the M1 phenotype is typically activated by interferon γ (IFNγ) or parts of bacteria such as lipopolysaccharides (LPS), the M2 phenotype results mainly from a stimulation by the anti-inflammatory interleukin (IL) 4. M1 macrophages are polarized by IFNγ, LPS, GM-CSF, and TNF; they produce mainly pro-inflammatory cytokines such as IL1, IL6, IL12, IL23, and TNFα and induce in this way a T helper cell response that is directed against the tumor (T_H1 response). M2 macrophages are mainly polarized by IL4, IL10, and IL13, they produce themselves much IL19 and TGFβ but less IL1, IL6, IL12, and TNF and thus cause T_H1 suppression, T_H2 activation as well as immunosuppression and promote wound healing and tissue regeneration. Due to immunosuppressive influences in the tumor environment, tumor-associated macrophages (TAM) mostly polarize in direction of the M2 phenotype [5]. Their number in the tumor often correlates with angiogenesis, the development of metastases, and tumor progression [6]. Natural killer cells (NK cells) identify pathologically changed cells and may directly lyse them. For this purpose, they dispose of different receptors such as NKG2 (natural killer group 2) and KIR (killer cell immunoglobulin-like receptors). They interact with ligands on the tumor cells and send stimulating or inhibitory signals. NK cells do not need to be activated, however, their activity can only be increased by cytokines such as IL12, IFNα, and IFNβ. NK cells produce themselves IFNγ and initiate in this way a direct stimulation of the components in the tumor environment contributing to tumor defense. Even if the innate immune response plays a major role especially in the detection of foreign pathogens, its effect – in particular of the cellular components – on tumor development and progress is increasingly in the focus of research.

2.2. Adaptive immune response

The adaptive immune response completes the innate immune response and allows the development of persisting and antigen-specific immune reaction. The principle is based on antigen presentation. Antibodies represent the humoral components of adaptive immune response. They act by opsonization, induction of antibody-related cytotoxicity, neutralization, activation of the complement system, and agglutination of the pathogens. Antibodies are produced and secreted by B cells. In addition to B cells, the T cells are responsible for the adaptive immune response. Cytotoxic T cells (CD8+) require different signals for activation: first, the specific T cell receptor (TCR) has to be connected to an according antigen (▶ **Fig. 2**). Second, co-stimulating receptors of the T cells have to be activated in order to achieve proliferation as well as cytotoxic cell activity. Because of their ability to act highly specifically in a cytotoxic way, CD8+ T cells are in the particular focus of new oncological therapy approaches: on the one hand in the field of adaptive T cell therapy where tumor antigen-specific T cells are multiplied and (re-)applied to the patient [7], on the other hand in the field of checkpoint inhibitors where specific inhibitory receptors of the T cells are blocked in order to impede T cell anergy [8]. T helper cells (CD4+) contribute in particular to regulatory processes of immune defense. They mostly do not dispose of own cytotoxic effects but transmit them via partner cells, e.g., cytotoxic T cells or NK cells. The antigen presentation is performed via MHC II molecules. In contrast to CD8+ T cells, CD4+ T cells need more frequent antigen contact to get stimulated. Even with regard to CD4+ cells, different phenotypes are known: the T_H1 phenotype secretes IL2 as well as IFNγ and stimulates the antitumor immune response whereas T_H2 cells produce IL4 and IL10 that have an



▶ **Fig. 3** Immunoediting. **a** Elimination phase. In the elimination phase, the adaptive and innate immune system identify and destroy tumor cells. **b** Equilibrium phase. In the equilibrium phase, the immune system maintains the control over tumor cells, complete elimination does no longer take place. **c** Escape phase. In the escape phase, the immune system loses control over the tumor cells leading to tumor progress. The transition from the elimination phase via the equilibrium phase to the escape phase is called immunoediting. (DC: dendritic cells; T: T cells; G: granulocytes; M: macrophages).

inhibitory effect on the immune system in the tumor environment. The consideration of those basic phenotypes and their impact on the tumor environment is crucial for immunotherapy.

2.3. Tumor development and tumor evasion

In the middle of the 20th century, Burnet and Thomas established the hypothesis of immuno-surveillance [9]. It consists of the assumption that during lifetime the incidence of non-hereditary, genetic changes in the cells increases. Since this phenomenon fosters the development of malignancy, a surveillance mechanism has to exist with an immunological background in order to eliminate or activate those mutated cells [10]. The hypothesis of immuno-surveillance has meanwhile been extended to the model of "Cancer Immuno-Editing". Chronological aspects of tumor development are considered and different phases of the balance between defense and progress are described (▶ **Fig. 3**) as "elimination", "equilibrium", and "escape" phases [11]. In the elimination phase, the adaptive and the innate immune system can identify and destroy tumor cells. The equilibrium phase represents the transition between elimination and escape when the immune system controls the tumor cells. It is an equilibrium between the interleukins IL12 (immuno-stimulation) and IL23 (immunosuppression) [12]. In the escape phase, the immune system loses control over the tumor resulting in tumor progress: tumor cells achieve progress by mechanisms that reduce their identification by the immune system [13–18], that lead to an increased resistance of the tumor cells [19, 20], and that cause inactivation of the antitumor effector cells [21]. In addition, the tumor environment is influenced in favor of immunosuppressive signaling pathways.

2.4. Tumor environment

The progress in the cancer treatment of the last decades is mainly due to a more profound knowledge of the interacting influences in the tumor environment. It is clear that tumor cells are influenced by the environment and vice versa influence the tumor environment. It becomes more and more obvious that the interactions between involved cells is highly complex and that a differentiation between the origin and the effect of the tumor environment is an important scientific challenge. So, an exact assessment of the involved (cellular) structures and signaling pathways is strongly required. In the tumor environment, tumor cells encounter cells and structures of the surrounding tissue. In addition to the cells of the immune system, they contain components of the extracellular matrix, vascular structures, stroma cells, and fibroblasts. The tumor cells interact with many, partly changing partners. Because of the heterogenic composition, the tumor environment is different from patient to patient. In addition to the inter-individual variation, there is an intra-individual dynamism. This factor is often still neglected in the analysis of tumor/patient specimens.

2.4.1. Extracellular matrix

The extracellular matrix (ECM) describes the space outside the cellular plasma membranes and is formed by interstitial macromolecu-

les. Those macromolecules include glycoproteins and polysaccharides. Additionally, the ECM contains mainly water, electrolytes, and nutrients. It is influenced by surrounding cells. So the composition of the ECM is not unchangeable but subject to changing processes of metabolism and production as well as degradation of macromolecules. Beside important influences on the tissue properties of shape, elasticity, and the contents of water, the ECM also plays a major role in the induction of immune reactions and wound healing as well as signal transduction and binding of signal receptors. In this way, the intracellular gene expression may be influenced which has an effect on the adhesion, migration, and proliferation of the surrounding cells. All this explains the important impact of the ECM on the surrounding tissue. Even in tumors, the ECM acts on the tumor environment and the tumor development [22]. In this context, the deregulation of the ECM plays a crucial role. The increased collagen implementation in the ECM may induce an integrin-mediated cell proliferation [23]. Due to anti-apoptotic effects [24] and support of oncogenic cell transformations [25], the ECM may be the basis for tumor progress. Additionally, tumor progress may be caused by an inhibiting impact of the ECM on the immune system [26]. The proliferation of T cells may be impeded, for example by binding LAIR (leukocyte-associated Ig-like receptor) by means of collagen [27] or by compromising antigen-presenting cells [28].

2.4.2. Vascular supply

Such as all metabolically active tissues, especially tumor cells with their highly active cell division and the significantly activated metabolism depend on the supply with nutrients. Also the oxygen consumption and the transportation of metabolites are important reasons for the dependence of tumors on an adequate vascular supply. Thus, angiogenesis in the tumor environment is important for tumor growth, invasive and metastatic behavior [29, 30]. Angiogenesis describes the process of new vascular formation developing from the already existing vascular bed. Among others, angiogenesis is induced by hypoxia, a low pH value, hypoglycemia, stress, and inflammation. In this context, endothelial cells are activated by growth factors that migrate and proliferate. Those growth factors are the vascular endothelial growth factors (VEGF), fibroblast growth factors (FGF), platelet derived growth factors (PDGF), hepatocyte growth factors (HGF), and angiopoietin 1 and 2 [30]. The receptors of those growth factors, mainly tyrosine kinase receptors, can not only be stimulated by their ligands but also by hormones, neurotransmitters, and lymphokines. Especially the latter play a major role in angiogenesis because the involved messenger substances also contribute to inflammation and immune cell migration [29]. For example, mast cells and macrophages can be recruited by tumor cells and stimulated to secrete angiogenetic cytokines.

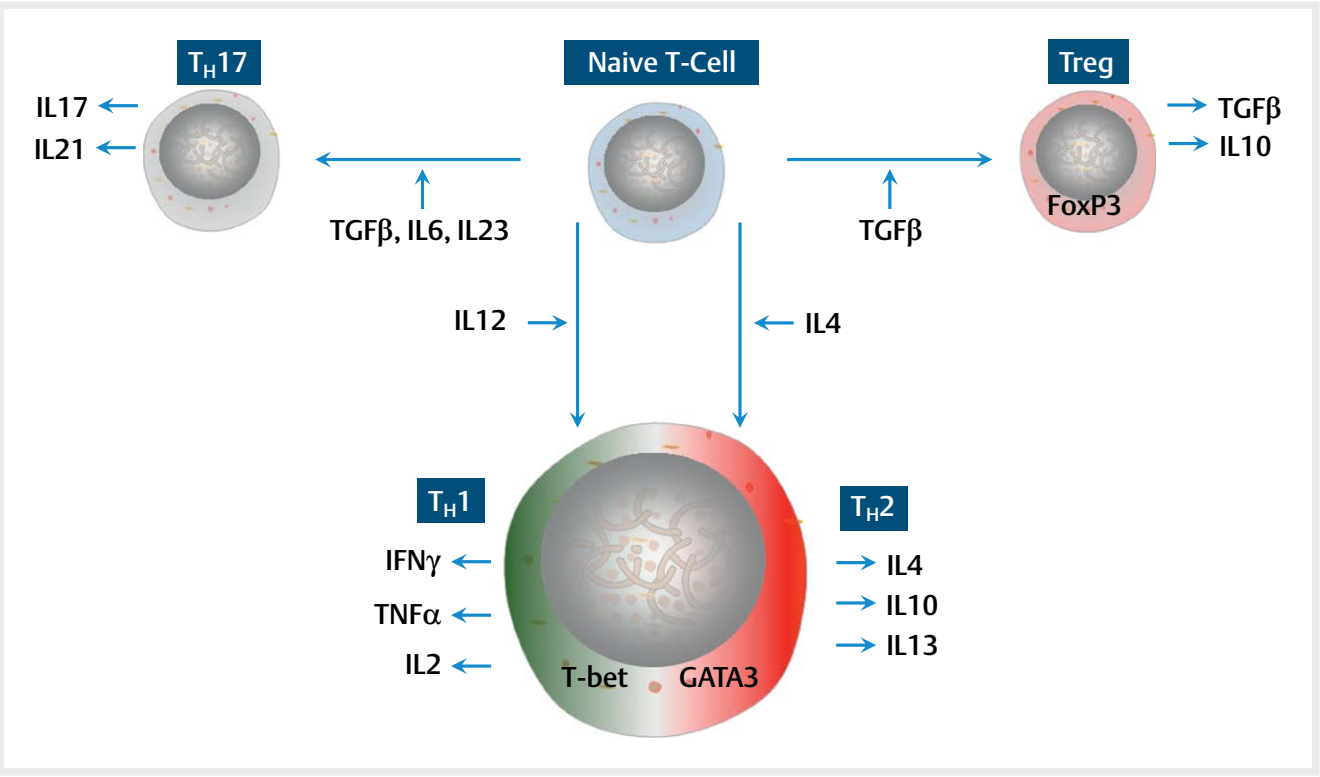
2.4.3. Fibroblasts

Fibroblasts have a mesenchymal origin and are involved in the development of ECM as cells of the connective tissue. They secrete in particular collagen, fibronectin, and growth factors. At the same time, however, they contribute to the re-structuring of the ECM and can produce matrix metalloproteinases (MMP) that solve the peptide connections of ECM structures and thus play an important role in angiogenesis, wound healing, and tumor growth. As cells with mesenchymal origin, they are also part of the tumor environment of solid tumors. In the tumor environment, mesenchymal stroma cells may differentiate to so-called cancer-associated fibroblasts (CAF). They are characterized by a clearly higher activity compared to other fibroblasts [31]. They secrete TGFβ and other growth factors that promote tumor growth [32]. Even metabolic processes in tumor cells can be supported by CAFs [33]. Additionally, the MMPs secreted by CAF are associated with increased invasiveness and metastasis [32, 34]. CAF have an effect on the immune system by chronic cytokine secretion that is one reason of chronic inflammatory reaction in the tumor environment. The mobilized immune cells, as for example macrophages, are converted by chronic stimulation of the CAF and other immuno-modulating influences of the tumor environment in the context of chronic inflammatory reaction into the tumor promoting phenotype (M2) [35, 36].

2.4.4. Suppressive immune cells

In addition to the initially described cellular parts of the immune system, there are further immune cells that are present in the tumor environment: regulatory T cells (Treg), tumor-associated macrophages (TAM), and myeloid-derived suppressor cells (MDSC).

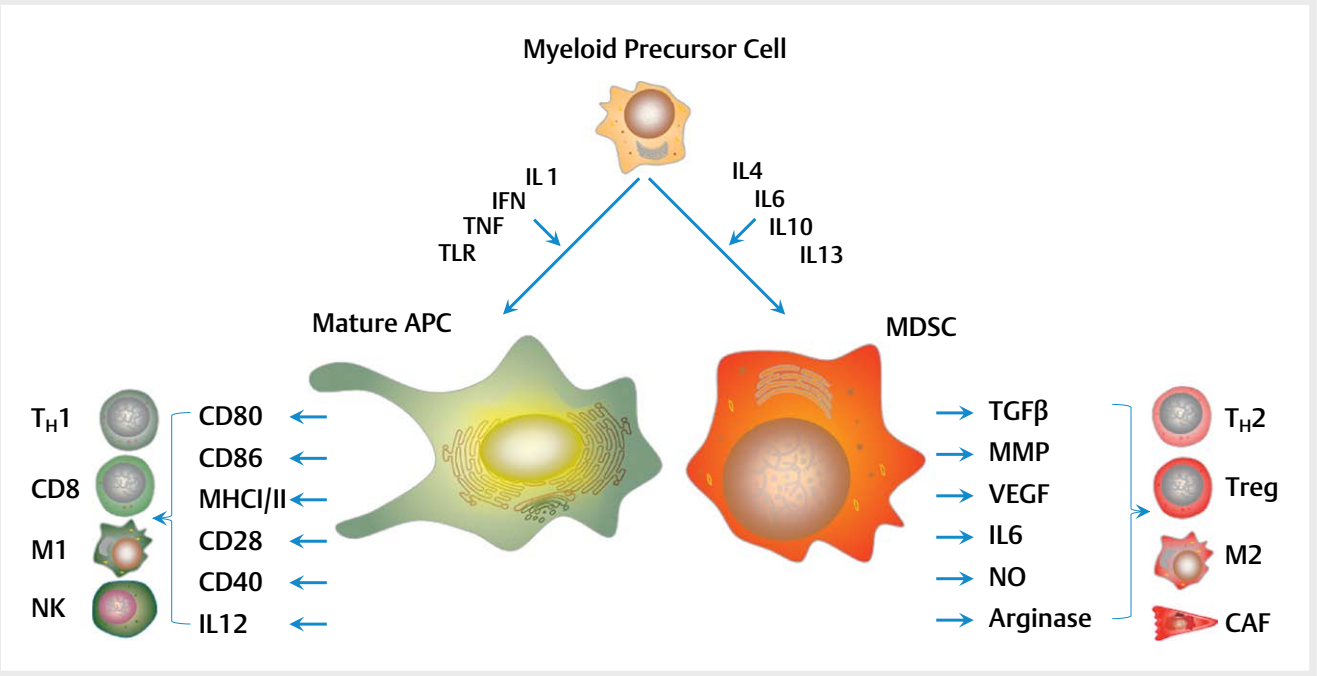
2.4.1 Treg Treg are mainly a subpopulation of CD4 + T cells that are responsible for the maintenance of self-tolerance as so-called suppressor cells. This is achieved by inhibiting activated T cells. Treg is characterized by the expression of CD4 and CD25 (sub-unit of the IL2 receptor) and the transcription factor FOXP3 (forkhead box protein 3). Up to now, more than 4 different CD4 + subpopulations of regulatory cells have been described. The different subpopulations may possibly be responsible for the partly contradictory research results. Nonetheless, there is a broad consensus that Treg have immunosuppressive properties in the tumor environment [37]. They do not only have this effect due to secretion of specific interleukins and other cytokines but they need direct cell-to-cell contact for part of their functions [38]. The involved cytokines are mainly IL4, IL10, IL35, and TGFβ. ▶ **Fig. 4** shows an overview of cytokine influence on CD4 + T cells. In the direct cell-to-cell contact, Treg transmit immunosuppressive properties via the expression of so-called checkpoint molecules, in particular CTLA4 and LAG3 (lymphocyte activation gene 3) as well as surface-bound enzymes such as CD39. The checkpoint molecules work via competitive binding in the cell-to-cell contact with co-stimulating molecules of antigen presenting cells and thus impede effective antigen presentation. Additionally, the maturation of the antigen presenting cells, especially of dendritic cells, is prevented. The enzymatic function of CD39, an ectonucleotidase, plays a significant role in the processing of ATP to AMP. In this way, the pro-inflammatory properties of extracellular ATP are inhibited. Beside dendritic cells, Treg have an inhibitory effect on activated CD4 + and CD8 + T cells. They significantly contribute to the development of T cell anergy [39]. Under anergic conditions, T cells can no longer be stimulated and do not respond to new antigen presentation. Even the proliferation of T cells is prevented via an inhibitory effect on the cell cycle [39]. Furthermore, Treg compete with other T cells regarding IL2, which is required for the proliferation and activation of Treg as well as other T cells in the tumor environment. An inhibitory effect of Treg could also be confirmed on NK cells. The secretion of TGFβ causes a reduced expression of NKG2D, an activating NK cell receptor [40]. The abilities of Treg to influence the immune response are manifold and appear significantly in the tumor environment [41].



▶ **Fig. 4** The influence of the cytokine environment on the differentiation of naïve CD4+ T cells. CD4+ T cells are influenced by different cytokines for differentiation into T_H1 and T_H2 as well as T_H17 and Treg cells. Especially TGFβ induces the development of Treg. Treg itself influence the tumor environment by means of TGFβ and IL10. Additionally, the development of T cells is differentiated into T_H1, T_H2, and T_H17 cells. IL12 stimulates a differentiation into T_H1 cells that are associated with an anticancer response in the tumor environment. IL4 is responsible for induction of T_H2 cells that play a pro-tumor role in the tumor environment by means of IL4, IL10, and IL13 secretion. The role of T_H17 cells in the tumor environment is still controversially discussed, they are stimulated by IL6 and IL23 and TGFβ. (green: mainly anticancer effect; red: mainly pro-tumor effect; IL: interleukin; TGF: transforming growth factor; IFN: interferon; TNF: tumor necrosis factor; T-bet: T-box transcription factor; GATA3: GATA transcription factor; FoxP3: F box protein 3 transcription factor).

2.4.4.2. MDSC MDSC is used as an umbrella term for a subgroup of suppressor cells of myeloid origin. They are defined by the expression of specific surface molecules (CD11b+, CD33+, and CD34+) and could be identified in most solid tumors. They are characterized mainly by protumoral differentiation. In contrast to mature dendritic cells, MDSC suppress the immune response in the tumor environment (▶ **Fig. 5**). MDSC are stimulated by pro-inflammatory signals and so they are relevant for controlling the immune response in the tumor environment [42]. Similar to Treg, MDSC exercise their immunosuppressing effect in many areas of adaptive and innate immune response. For this purpose, they use different mechanisms: The antigen detection of T cells is disturbed by nitrading the T cell receptor. Furthermore, they inhibit T cell activation by the consumption of cysteine, which is an essential amino acid for T cells [43]. Also the proliferation of T cells is controlled via inhibition of IL2 production. The production of arginase and oxygen radicals impedes the antigen detection and T cell activation [44]. Additionally, the increased differentiation to MDSC from progenitor cells of myeloid origin influences the adaptive immune response via a reduction of antigen presenting cells in favor of MDSC. Further, MDSC support the development of Tregs by increasingly producing IL10, TGFβ, and arginase [42]. Parts of the innate immune response are inhibited by MDSC, in particular NK cells and M1 macrophages that are disturbed in their functionality by an increased production of IL10 and a reduction of the IL12 secretion [45]. The development of MDSC is influenced by different cytokines, among others VEGF, granulocyte monocyte colony stimulating factor (GM-CSF), IL6, IL1β, PGE2 (prostaglandin E2), and complement C5a.

2.4.4.3 TAM Due to their versatility, mobility, and the fact that they belong to the innate immune system, macrophages are active in many pathways of the immune defense – including wound healing and inflammatory processes. This also includes the tumor environment where macrophages are involved in angiogenesis, leukocyte infiltration, mutation of ECM, and immunosuppression [46]. With regard to the tumor environment, a heterogenic group of macrophages has been described in the last years that is defined as tumor-associated macrophages. It must be taken into account that some authors describe mainly the M2 phenotype with regard to tumors, others, however, differentiate even in TAM between M1 and M2 phenotypes because of the high plasticity of the macrophages (▶ **Fig. 6**). In the majority of the tumors (except colon cancer) TAM correlate with a poor prognosis [47]. They may represent up to one third of the cellular components of the tumor environment [48]. TAM are recruited into the tumor environment via chemokines (e. g., chemokine ligand 2, CCL2, and CCL5) that are mostly secreted by tumor or stroma cells. In addition, VEGF, PDGF, M-CSF, and TGFβ are involved



▶ **Fig. 5** Myeloid suppressor cells and antigen presenting cells in the tumor environment. MDSC are stimulated by pro-inflammatory signals and suppress the immune response in the tumor environment. They induce the development of Treg and T_H2 cells and promote the differentiation to M2 phenotypes. Mature antigen presenting cells can stimulate the anticancer immune response by supporting CD8 + T cells, CD4 + T_H1 cells and NK cells by means of antigen presenting and co-stimulation. (green: mainly anticancer effect; red: mainly pro-tumor effect; IL: interleukin; TGF: transforming growth factor; IFN: interferon; TNF: tumor necrosis factor; M1: M1 phenotype; NK: natural killer cells; TLR: toll-like receptor stimulation; MMP: matrix metalloproteinase; VEGF: vascular endothelial growth factor; NO: nitric oxide).

in the macrophages recruiting process [49]. M1 macrophages are polarized by IFNγ, LPS, GM-CSF, and TNF; they produce much IL1, IL6, IL12, IL23, TNF, and lower levels of IL19 and trigger a T_H1 response, tissue destruction, and immune stimulation. M2 macrophages are mainly polarized by IL4, IL10, and IL13, they produce much IL10, TGFβ, and few IL1, IL6, IL12, TNF and cause T_H1 suppression, T_H2 activation, immunosuppression and promote wound healing as well as tissue regeneration. Since macrophages have a high plasticity and strongly depend on influences of the tumor environment, TAM are rather allotted to the M2 phenotype [5]. So in the tumor they correlate with angiogenesis, the development of metastases, and tumor progress [6].

2.4.5. Cancer stem cells

Cancer stem cells (CSC) are tumor cells with stem cell properties. Those properties include the self-renewal as well as the potential of differentiation [50]. The hypothesis of cancer stem cells is controversially discussed [51], however, there are more and more hints that different tumor entities contain tumor cells with stem cell characteristics [52]. Cancer stem cells are responsible for example for the development of therapy resistances [53]. In connection with chemotherapy, a conversion of glioblastoma cells into cancer stem cells could be confirmed [54]. Another important property of cancer stem cells is the low immunogenicity [55]. This ability to avoid the immune system is based on different properties. On the one hand, CSC express inhibitory ligands such as for example FasL and inhibitory NK ligands, on the other hand anti-apoptotic molecules such as Bcl2 and survivin. Furthermore, they secrete the classic immunosuppressing cytokines like TGFβ, IL4, IL6, IL10, and PGE2. Additionally, CSC inhibit the T cell proliferation in a STAT3-mediated way [56].

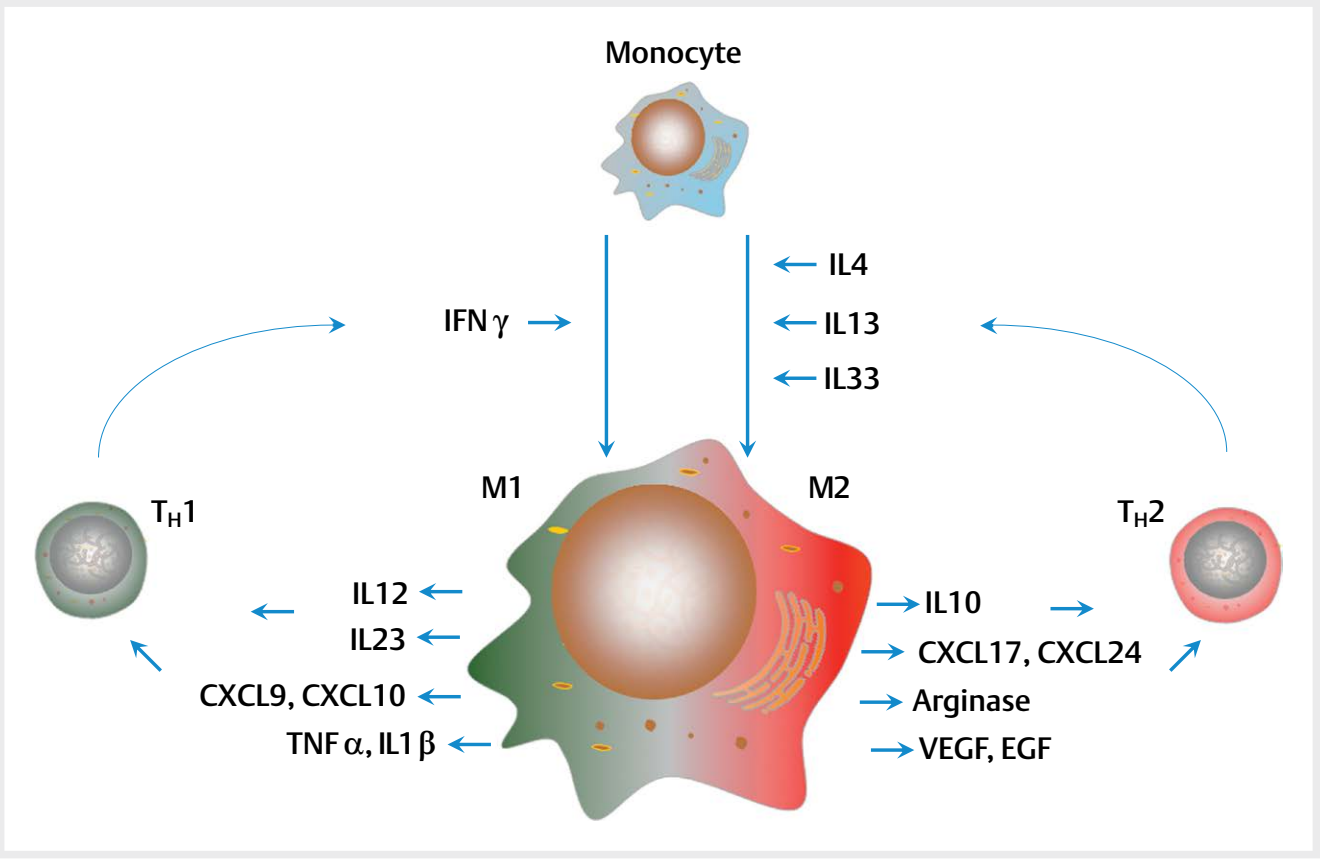
3. Immunotherapies

The objective of different immunotherapies is to possibly target the activity of the immune system against the tumor, to inhibit protumoral effects, and to allow tumor elimination (▶ **Fig. 7**). The basics of pro- and antitumoral processes and the involved cellular and noncellular structures have been described in the previous chapters. In the following, single therapeutic approaches will be discussed more in detail regarding their modes of action.

3.1. Cytokine therapies

Cytokines are proteins, which regulate the activity, migration, and differentiation of cells. In the tumor environment, the cytokine secretion is a relevant communication pathway between tumor and immune cells. The significance of the cytokine environment becomes clear considering the fact that for example the differentiation – and thus the function – of CD4 + T cells is mainly controlled by cytokines. They influence if a T_H1 cell with anti-tumoral activity develops or a Treg cell with immunosuppressive function [57]. The group of cytokines includes interleukins, chemokines, tumor necrosis factors (TNF), interferons, and the colony stimulating factors (CSF).

Interleukins are peptide hormones belonging to the cytokines that influence cell growth and differentiation and that are generated by all cells of the immune system. They may have a stimulating as



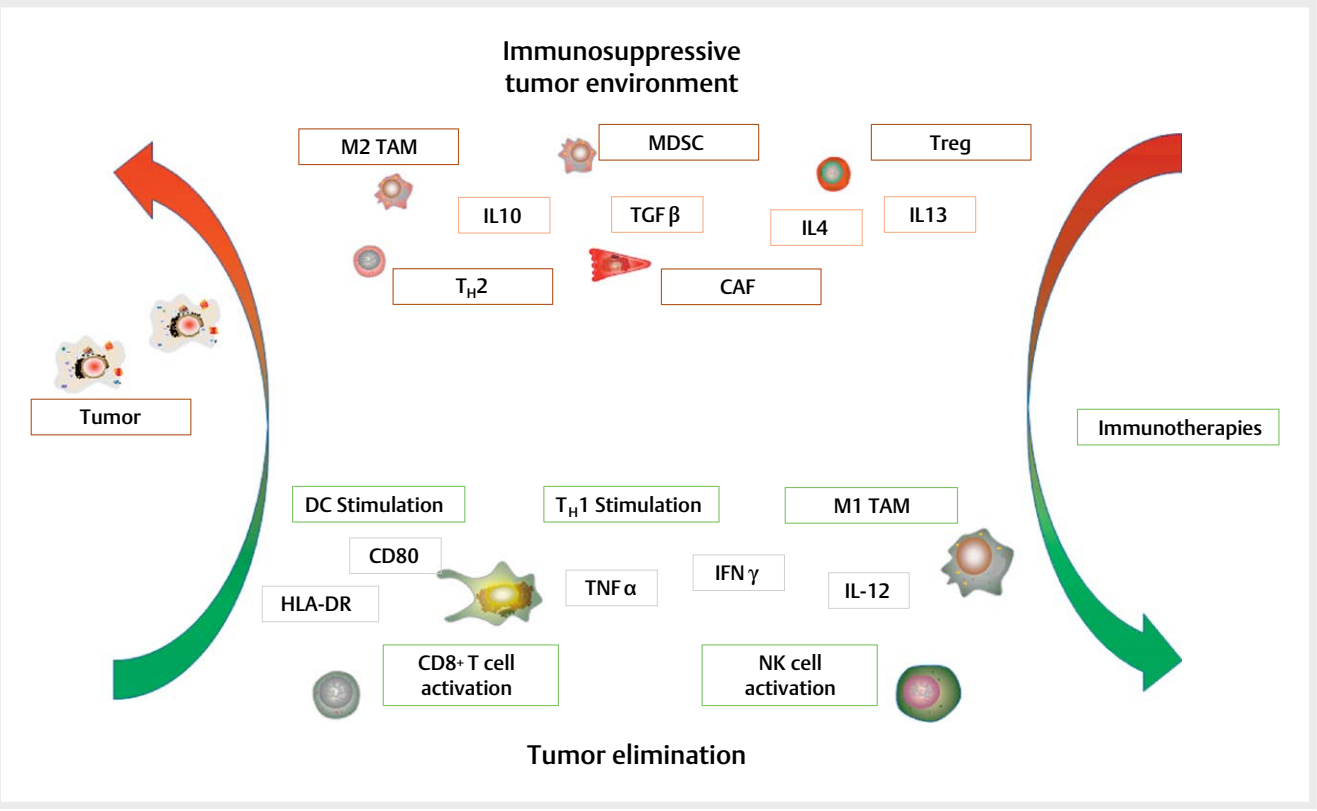
▶ **Fig. 6** Monocytes in the tumor environment. In the tumor environment, monocytes differentiate into M1 and M2 macrophages depending on the predominant influences. M1 macrophages stimulate the T_H1 response via IL12 and are associated with an anticancer effect. M2 macrophages stimulate a T_H2 response and have a pro-tumor effect by developing IL10, VEGF, and arginase in the tumor environment. (green: mainly anticancer effect; red: mainly pro-tumor effect; IL: interleukin; IFN: interferon; TNF: tumor necrosis factor, M1: M1 phenotype; CXCL: chemokine; VEGF: vascular endothelial growth factor; EGF: epidermal growth factor).

well as inhibiting effect on growth, division, and especially differentiation of other immune cells. Because of their mostly pleiotropic effects, interleukins influence several phenotypic characteristics. Meanwhile, more than 40 interleukins with different physiological functions are known that are numbered chronologically according to their discovery. In the tumor environment, interleukins mostly work in a paracrine way.

Due to their function, interleukins are appropriate for therapeutic use in order to influence the communication and the control of the immune system. During the last decades, several immunotherapeutic approaches were developed on the basis of interleukin therapies [58]. They include the interleukins IL2, IL7, IL12, IL18, and IL21 [57]. One of the most important interleukins applied in therapeutic approaches is IL2. As already mentioned, it plays a major role in the activation and proliferation of T cells. It is the first interleukin that has been applied as anticancer drug in humans and that could achieve therapeutic success. Historically, IL2 as T cell growth factor was discovered in 1976 [59]; in 1994 it was approved as drug for treatment of metastatic renal cell carcinoma and in 1998 in the USA for metastatic malignant melanoma. The systemic application of IL2 especially for metastatic tumors bears the disadvantage of significant side effects. Under certain circumstances, IL2 may lead to the so-called vascular leak syndrome, i. e., the permeability of the vascular walls is strongly increased because of endothelial cell mediated hyperpermeability. This leads to extravasation that might limit treatment [60]. For a small part (8 %; 33 of 409) of the treated patients, however, a complete long-lasting (median observation interval > 7 years) remission of metastatic renal cell carcinomas (9.3 %) and melanomas (6.6 %) could be achieved [61]. Further interleukins are currently investigated in clinical trials [62].

Chemokines have a chemotactic effect on other cells and are increasingly released in cases of inflammation or other acute events. Among others, chemokines seem to be associated with increased angiogenesis [63]. Interestingly, the chemokine receptor expression in tumor-infiltrating lymphocytes is down-regulated which is explained by an increased internalization of the receptors due to the strong concentration in the tumor environment [64]. Clinical trials also investigated monoclonal antibodies against chemokine receptors in patients with T cell malignoma [65]. In addition, a current trial deals with the chemokine modulation in patients with colon cancer (NCT01545141).

Tumor necrosis factors (TNFα and TNFβ) contribute to the proliferation, differentiation, apoptosis, necrosis, angiogenesis, and activation of the immune system. TNF further influence the fat metabolism; insulin resistance, and endothelial cells work systemically via fever and may cause cachexia (TNFα was formerly called cachexin).

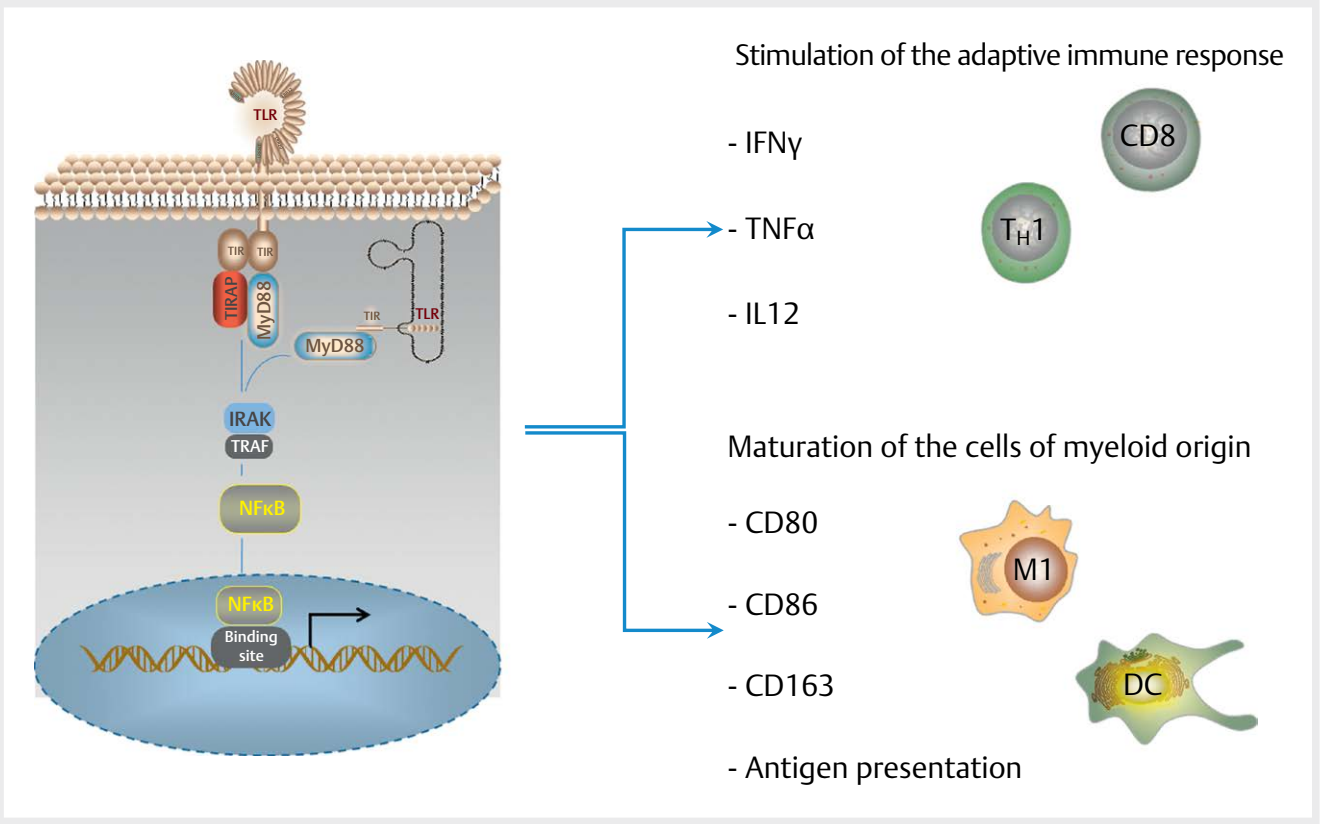


▶ **Fig. 7** Overview about pro-tumor and anticancer influences in the tumor environment. Based on the tumor, a pro-tumor environment develops stimulating cell types that themselves promote tumor development. The different approaches of immunotherapy support the development of an anticancer environment with the intention of eliminating the tumor. (green: mainly anticancer effect; red: mainly pro-tumor effect; IL: interleukin; IFN: interferon; TNF: tumor necrosis factor; M1: M1 phenotype; M2: M2 phenotype; MDSC: myeloid suppressor cell; Treg: regulatory T cell; NK: natural killer cell; DC: dendritic cell; CD: cluster of differentiation; HLA-DR: human leukocyte antigen DR, CAF: cancer-associated fibroblast; TGF: transforming growth factor).

In cancer therapy, TNFα is applied for malignant melanoma and soft tissue sarcomas [66]. In the tumor itself, it induces hyperpermeability of the vessels with resulting hemorrhagic necrosis and destruction of the vascular structures [67]. However, the initial expectations to broadly apply TNFα-based cancer therapy could not be confirmed. This is sometimes explained by the pleiotropic characteristic of TNFα and the different mode of action depending on the timely course.

Interferons are pleiotropic cytokines that are generated from stroma cells as well as immune cells and that have a broad immunostimulating effect by activating transcription proteins (Jak-STAT signaling pathway) and an increased expression of components of antigen presentation (e. g., MHC molecules). Physiologically, the interferon generation occurs mainly after activation by viral or bacterial antigens. In humans, IFNα, IFNβ (type I interferon), and IFNγ (type II interferon) are distinguished. IFNα and IFNβ enhance the MHC I expression, activate dendritic cells, T cells and NK cells [68] and inhibit the development of immunosuppressive Treg and MDSC [69, 70]. Additionally, they have an effect on tumor cells and lead to increased differentiation and increased tumor antigen presentation [71]. IFNγ mainly activates CD8 + T cells and enhances the MHC II expression as well as the development of macrophages of the M1 phenotype [68]. On the other hand, also an interferon-dependent immunosuppressive effect in the tumor environment was observed in the last years [72]. It is based on an increased expression of a checkpoint receptor ligand (programmed death receptor ligand 1; PDL1) after interferon stimulation. Thus, also inhibiting effects are increasingly observed [73]. In this context, the duration of the stimulation may play an important role and make the difference between an acute inflammatory reaction with tumor inhibiting properties and a chronic inflammatory reaction with tumor progressing effect. This observation has significant consequences for the clinical application. The immune activating properties of interferons are applied for therapy in order to treat for example viral hepatitis (IFNα). But also in cancer therapy, the treatment with interferons is applied against specific tumor entities (e. g., special lymphomas, leukemia, Kaposi sarcomas, or malignant melanoma). Also hereby, it must be taken into account that partly severe side effects may limit the treatment. Many efforts are undertaken to optimize the effect and at the same time reduce the side effects [74]. In the context of malignant melanoma, a marginally significant impact on the progression-free survival after resection of stage III melanoma could be confirmed in comparison to clinical observation strategy [75]. The application of interferon in cancer therapy depends from the tumor entity, the spectrum of side effects, and the associated patients' compliance so that this treatment has to the discussed individually with the patient.

Colony stimulating factors are glycoproteins that influence the proliferation and differentiation of cells originating from the hematopoietic system. Several CSF are classified: G-CSF (granulocytes),

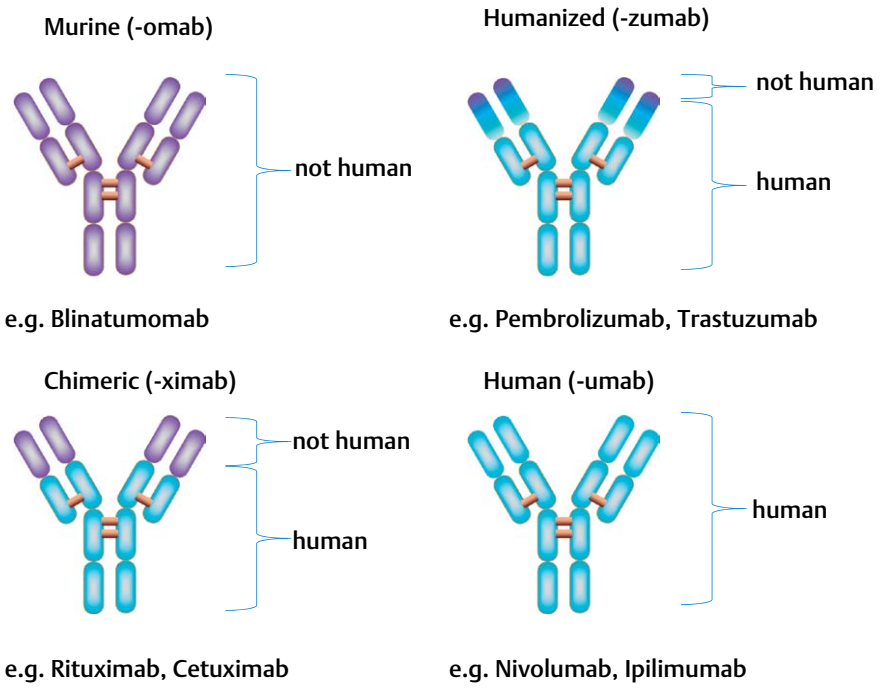


▶ **Fig. 8** Toll-like receptors. Toll-like receptors may be bound on the cell surface as well as with the cells. Their activation leads to a signal cascade that mainly initiates a production of IFNγ, TNFα, and IL12 via MyD88 and NFKB. Additionally, cell differentiation is stimulated. (NFKB: transcription factor; MyD88: myeloid differentiation protein 88; TIR: toll/IL-1 R homology domain, TIRAP: adaptor molecule; IRAK: interleukin-1 receptor-associated kinase; TRAF: TNF receptor-associated factor; IFN: interferon; TNF: tumor necrosis factor).

M-CSF (monocytes), GM-CSF (granulocytes and monocytes), Meg-CSF (megakaryocytes), and the SCF (stem cell factor). Furthermore, some interleukins, e. g., IL2 as well as erythropoietin, range among CSF because they also act on the proliferation and differentiation of cells of the hematopoietic system. The generation and secretion are performed in the bone marrow, the stroma, and in immune cells (B and T cells, macrophages etc.). In oncology, CSF are applied as adjuvant agents in order to achieve restitution of the hematopoietic cell lines after suppression. However, CSF are also investigated in active tumor treatment. As already described, the number of intratumoral TAM is associated with a poor prognosis. Efforts are undertaken to block the receptor of M-CSF to impede the survival of macrophages and thus reduce the number of TAM. Phase I and II studies show a limited to moderate effect in the monotherapy with moderate side effect profile [76, 77]. GM-CSF is also applied in oncological immunotherapy because it could be shown that differentiation of the dendritic cells, suppression of the MDSC and development of the M1 phenotype were induced [78, 79].

3.2. Toll-like receptor stimulation

Pattern recognition receptors (PRR) recognize molecules that are associated with pathogens such as for example viruses and bacteria (pathogen-associated molecular patterns, PAMP). Those receptors initiate a defense reaction and belong to the epigenetically oldest components of immune response. Toll-like receptors (TLR), a main group of PRR, are widespread within the different species. They were discovered in the middle of the 1990ies for the first time in drosophila melanogaster. Since then, 10 different TLR subtypes were identified in humans. The activation of TLR leads to an intracellular signaling cascade (▶ **Fig. 8**) that causes mainly a differentiation of cells of myeloid origin and stimulates their maturation and proliferation. In addition, also cells of adaptive defense are activated. The immunologically activating effect of single TLR antagonists was examined in different cancer entities and led to the approval of single immunotherapeutics of this substance class. Among those are the TLR2/4 agonists Bacillus Calmette-Guérin (BCG, inactivated mycobacterium bovis) for bladder cancer, the TLR4 agonist Picibanil for head and neck cancer [80], or the TLR7 agonist Imiquimod for basal cell cancer. Resiquimod, which is a potent TLR7 and TLR8 agonist, is currently clinically developed as successor product. Another TLR8 agonist that is currently under clinical investigation, is Motolimod for head and neck cancer and other solid tumors (NCT01836029). Furthermore, TLR agonists are meanwhile frequently combined with vaccinations because of the promotion of maturing and differentiating antigen presenting cells in order to support antigen presentation [81].



▶ **Fig. 9** Scheme of the monoclonal antibody. Monoclonal antibodies are classified according to their composition and the human percentage.

3.3. Oncolytic viruses

Oncolytic viruses may directly or indirectly destroy tumor cells. In a narrow sense, oncolytic viruses infect tumor cells and lyse them. However, there are other possibilities to attack tumor cells by means of virus-based methods. Among those, there is the inclusion of tumor suppressor genes and toxins into the tumor cell or the generation of an immune response with subsequent tumor cell destruction. So oncolytic viruses are applied that may directly cause tumor cell death as well as stimulate the systemic immune response [82]. Trials analyzing the application of oncolytic viruses, are already performed in larger phase II and III studies. A phase III study in patients with advanced malignant melanoma could reveal improved response rates with herpes simplex based viral therapy (talimogene laherparepvec, T-VEC) [83]. Furthermore, trials are currently performed with regard to hepatocellular carcinomas (phase II, pexastimogene devacirepvec, Posavec, NCT01387555) and to head and neck cancer (phase III, pelareorep, Reolysin combined with chemotherapy, NCT01166542) [84].

3.4. Monoclonal antibodies

In 1975, Kohler and Milstein developed a technology allowing the production of nearly unlimited quantities of a single, epitope-specific antibody [85]. By merging an antibody-producing B cell with an immortal "myeloma cell" a 'hybridoma' results that produces an antibody of an original B cell: it is then called a monoclonal antibody. In 1984, Kohler and Milstein were awarded the Nobel Prize in Physiology or Medicine together with Niels Jerne for their achievements in this context. This award honored in particular the application options to produce highly specific antibodies in theoretically unlimited quantities. This was the breakthrough for the application of antibodies in research and clinic because now antibodies were available in sufficient quantities in order to be able to influence cellular signaling pathways on a molecular level.

In the beginning, the therapeutic application of monoclonal antibodies was suitable only to a limited extent. Since they were generally harvested in mice, they were "foreign" for humans and induced an immune response called HAMA reaction (human antibodies against mouse antibodies). Meanwhile, antibodies can be produced that are partly or even completely of human origin. The terminology of therapeutic antibodies provides information about the origin and composition. The suffix -omab defines antibodies of murine origin; -imab means antibodies with origin of primate species; -ximab describes chimeric antibodies (variable percentage of murine origin, remaining part of human origin); -zumab defines humanized antibodies (murine antigen binding, remaining part of human origin), and -umab describes completely human antibodies (▶ **Fig. 9**).

The therapeutic spectrum where mAb are meanwhile applied is very broad. It ranges from autoimmune diseases such as rheumatoid arthritis via hematological diseases such as hemophilia up to oncological diseases such as malignant melanoma, bronchial cancer, or even head and neck cancer [86]. Oncology is the main focus of development, research, and application of monoclonal antibodies that are mostly targeted against tumor antigens or checkpoint receptors.

3.4.1. mAb against tumor antigens

Tumor antigens are protein structures that are produced by tumor cells and may trigger an immune response. Tumor-specific antigens (TSA) and tumor-associated antigens (TAA) are currently known. TSA are produced exclusively by tumors and develop for example based on mutations in a protein-coding gene. In contrast, TAA are also pre-

▶ **Table 1** Tumor antigen mAb.

Tumor antigen	mAb	Indication
CA-125	Oregovomab	Ovarian cancer
CD19	Blinatumomab	Acute lymphatic leukemia (ALL)
CD20	Ofatumumab	Chronic lymphatic leukemia (CLL)
CD20	Obinutuzumab	CLL, Non Hodgkin lymphoma (NHL)
CD20	Rituximab	NHL
CD20	Ibritumomab	NHL
CD20	Tositumomab	NHL
CD22	Inotuzumab	AML
CD22	Epratuzumab	NHL, ALL
CD33	Gemtuzumab	AML
CD38	Daratumumab	Multiple myeloma
CD4	Zanolimumab	T cell lymphoma
CD52	Alemtuzumab	ALL, CLL, T cell lymphoma
DLL3 (delta-like protein3)	Rovalpituzumab	Small-cell lung cancer (SCLC)
EGFR	Necitumumab	Non-small-cell lung cancer (NSCLC), stomach cancer
EGFR	Cetuximab	Head and neck cancer, colon cancer
EGFR	Panitumumab	Solid EGFR+ tumors
EpCAM antigen	Catumaxomab	Malignant ascites
Her2/neu receptor	Trastuzumab	Breast cancer, stomach cancer
Her2/neu, Her2/neu receptor	Pertuzumab	Ovarian cancer, breast cancer, lung cancer, prostate cancer

sent on healthy cells but they are expressed to a clearly higher amount on tumor cells. Examples in this context are TAA of the ErbB family, a receptor family of 4 tyrosine kinase receptors. Two members of this family, ErbB-1 (EGFR) and ErbB-2 (Her2) are of high relevance in oncology. EGFR is overexpressed in most head and neck cancers, non-small-cell lung cancer (NSCLC), and colorectal carcinomas, Her2 in breast and ovarian carcinomas [87]. Antibodies against EGFR and Her2 have been applied for several years in clinical routine (Cetuximab, Trastuzumab, ▶ **Table 1**) [88]. Unfortunately, the response rates to those therapies are significantly below the expression rates so the new developments focus on combined therapies in order to avoid tumor cell resistances more effectively.

3.4.2. mAb against checkpoint molecules

A new possibility to intervene specifically into the signaling pathways of the immune system results from the introduction of mAb that are able to stimulate or block checkpoint molecules. Checkpoint molecules are mainly receptors on cells of the immune system, mostly lymphocytes, that have a stimulating or inhibiting effect.

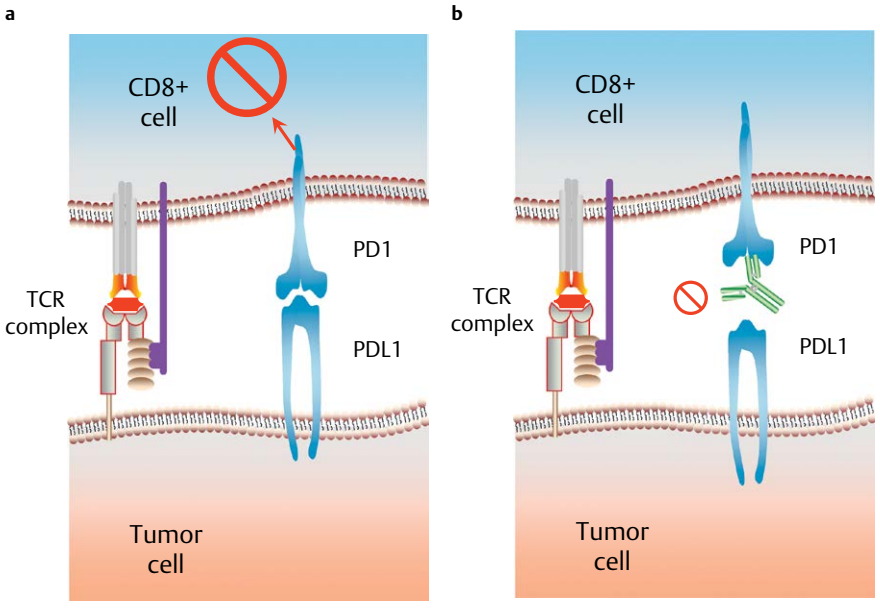
Especially the molecules CTLA4 and PD1 have to be emphasized among the group of checkpoint molecules because in the last years significant therapeutic success could be achieved in different tumor entities with therapeutic antibodies that are targeted against those molecules (▶ **Table 2**). PD1 is a receptor for the ligands of PDL1 and PDL2. Especially binding PDL1 leads to T cell anergy (▶ **Fig. 10**), i. e., the IFNγ secretion and proliferation of T cells are suppressed. In the tumor environment an expansion of activated, tumor-reactive T lymphocytes is inhibited [8]. Interestingly, PD1 is also strongly expressed on Treg, but here it leads to an increased functionality [89]. PD1 blockage with the mAb Nivolumab was first applied successfully in 2010 in a clinical phase I study [90]. Since then, long-term success could be achieved with even sometimes complete regression of malignant melanoma, renal cell carcinoma, or colorectal cancer over 3 years [91].

CTLA4 is expressed on T cells and induces immunosuppression via 2 relevant mechanisms. On the one hand it acts as competitive binding partner for the surface molecules CD80 and CD86 on antigen presenting cells because of its structural similarity to the co-stimulating molecule CD28 and thus impedes the activation of T cells (▶ **Fig. 11**). On the other hand the binding of CTLA4 directly inhibits T cells, also by inactivating the T cell receptors. Anti CTLA4 mAb were the first immune checkpoint mAb that were clinically tested [92] and that achieved significant, long-lasting therapeutic success [2].

2.4.2.1. Immuno-stimulating checkpoint molecules The immuno-stimulating checkpoint molecules include CD27, CD28, CD40, CD122, CD134 (OX49), CD137, CD278 (ICOS) and GITR (glucocorticoid-induced TNFR family related gene) (▶ **Table 3**) [93]. CD 27 is important for the induction of memory T cells and supports the T cell expansion. CD28 is a relevant co-stimulating T cell receptor that is necessary for activation of CD4+ T cells and binds CD80 and CD86 of antigen presenting cells. CD40 is expressed on antigen presenting cells and is activated by its ligand CD40L that is expressed on CD4+ T cells. CD122 is expressed on CD8+ T cells and supports their proliferation. CD134 (OX40) is expressed on CD4+ and CD8+ T cells and also enhances proliferation. In addition, stimulation by means of OX40 inhibits the development of Treg cells. CD137 is expressed on

▶ **Table 2** Checkpoint receptor mAB.

Checkpoint receptor	mAb	Indication
CTLA4	Tremelimumab	Lung cancer, mesothelioma
CTLA4	Ipilimumab	Malignant melanoma
PD1	Nivolumab	Malignant melanoma, non-small-cell lung cancer (NSCLC)
PD1	Pembrolizumab	Malignant melanoma, mesothelioma, NSCLC
PDL1	Atezolizumab	Bladder cancer
PDL1	Avelumab	Bladder cancer, NSCLC, Merkel cell carcinoma
PDL1	Durvalumab	Lung cancer

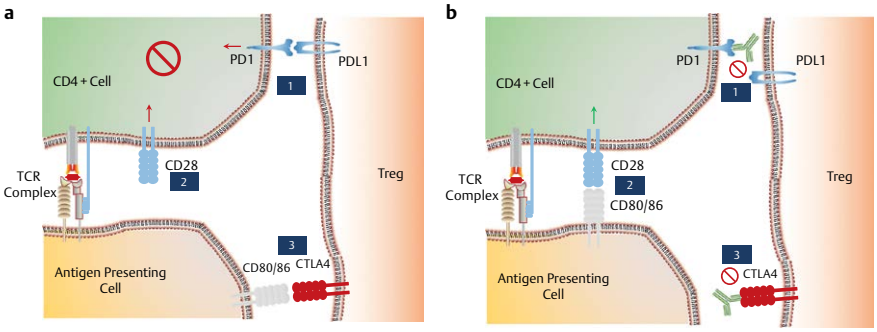


▶ **Fig. 10** Effect of the checkpoint molecules and the checkpoint blockade. a The CD8+ T cell is inhibited via the binding of the PD1 receptor by PDL1 expressed by the tumor cells. The receptor binding leads to T cell anergy by inhibiting the proliferation and the T cell function. b The inhibiting effect is eliminated by the checkpoint blockade. (TCR: T cell receptor complex; PD1: programmed death receptor 1; PDL1: PD1 ligand).

CD8+ T cells, it stimulates the proliferation and inhibits the activation-related induction of apoptosis. CD278 (inducible T cell co-stimulator, ICOS) is expressed on activated T cells and interacts with antigen presenting cells and B cells. GITR stimulates the expansion of T cells and is also stimulated by antigen presenting cells. For many of the stimulating checkpoint receptors, currently clinical trials are conducted in order to generate an enhanced, long-lasting immune response via pharmaceutic ligands.

2.4.2.2. Inhibiting checkpoint molecules The inhibiting checkpoint molecules that could be identified up to now include the receptors A2AR (adenosine A2A receptor), B7-H3, B7-H4, BTLA (B and T lymphocyte attenuator), CTLA4, KIR, LAG3, PD1, TIM3 (T cell immunoglobulin and mucin domain 3), and VISTA (V-domain Ig suppressor of T cell activation) (▶ **Table 4**). Via adenosine binding in the tumor environment, A2AR mediates a suppressing activity on cells of the immune system [94]. B7-H3 and B7-H4 are expressed on tumor cells, inhibit T cells and promote tumor migration [95]. BTLA is expressed on T cells and leads to inhibition of the T cell activity after binding [96]. KIR are particularly expressed on NK cells and may have an inhibiting effect on the NK cell function [97]. LAG3 acts especially over the MHC II binding on CD4+ T cells in an immunosuppressive way and is an important molecule in the suppressing function of Treg cells [98]. TIM3 is responsible for the activation of macrophages, at the same time, apoptosis of T_H1 cells can be induced by binding galectin-9 [99]. VISTA was identified as another immuno-regulatory checkpoint in refractory melanoma patients [100].

Other mAb are targeted against cytokines or growth factors (▶ **Table 5**). The intra- and extracellular signaling pathways of many of the described molecules are not yet completely clarified – so that several effective mechanisms are not yet identified. A clear classification can only be made in a carefully restricted way because many signaling pathways in immunology may induce stimulating as well



▶ **Fig. 11** Effect of checkpoint molecules and checkpoint blockade. **a** The CD4+ T cells is inhibited on several levels. Binding of the PD1 receptor by PDL1 leads to T cell anergy (1). The missing co-stimulatory signal by the antigen presenting cell also causes inhibition (2). CTLA4 expressed by the Treg competitively binds the co-stimulatory ligands of the antigen presenting cell (3) and thus impedes the co-stimulatory signal. **b** The inhibiting effect of the checkpoint blockade is eliminated in (1). The co-stimulatory ligand (CD80/86) is again available for the co-stimulatory signal (2) because the competitive binding partner CTLA4 is also blocked by an antibody (3). (TCR: T cell receptor complex; PD1: programmed death receptor 1; PDL1: PD1 ligand; CTLA4: cytotoxic T lymphocyte-associated protein; CD28: costimulatory receptor; CD80/86: co-stimulatory ligand).

▶ **Table 3** Immuno-stimulating checkpoint molecules.

Molecule	Expressed on	Effect
CD27	(memory) T cells	T cell expansion
CD28	CD4+/CD8+ T cells	Essential signal for T cell activation
CD40	APC	Binds with CD40L on T cells, stimulates their activity
CD122	CD8+	Proliferation
CD134 (OX40)	CD4+/CD8+ T cells	Proliferation
CD137 (4-1BB)	CD8+	Protection against apoptosis, proliferation
CD278 (ICOS)	CD4+/CD8+ T cells	Interaction with APC and B cells
GITR	CD4+/CD8+ T cells	Proliferation

▶ **Table 4** Immune inhibiting checkpoint molecules.

Molecule	Expressed on	Effect
A2AR	T cells	T cell inhibition, TGFβ induction
B7-H3	Tumor cells	T cell inhibition, tumor migration
B7-H4	Tumor cells	T cell inhibition, tumor migration
BTLA	T cells	T cell inhibition
CTLA4	Treg, CD4+/CD8+ T cells	T cell inhibition, APC inhibition
KIR	NK cells	NK cell inhibition
LAG3	Treg, CD4+/CD8+ T cells	T cell inhibition
PD1	Treg, CD4+/CD8+ T cells	T cell anergy of CD4+/CD8+ T cells
TIM3	CD4+/CD8+ T cells	T cell apoptosis
VISTA	MDSC, Treg	T cell inhibition

as inhibiting effects. This may not only lead to contradictory pre-clinical findings but also to various therapy responses. So it is even more important to analyze the effect of the signaling pathways already in pre-clinical trials and to critically evaluate and verify the in vitro results with valid in vivo data.

3.5. Vaccination

In oncology, 2 vaccination strategies have to be differentiated: On the one hand, a preventive vaccination before the development of cancer may be applied. This is possible in the context of vaccination against oncogenic viruses and can have a protective effect against virus-associated carcinomas. On the other hand, efforts are undertaken to establish therapeutic vaccination in other, i. e., not exclusively viral, tumors.

Preventive vaccination to avoid infections with oncogenic viruses turned out to be highly effective to avoid virus-associated cancer. The development of vaccinations against high-risk virus subtypes of oncogenic human papilloma viruses (HPV) 16 and 18 could achieve a protection against HPV infections [101]. For his research that led to the discovery of a correlation between HPV infections and cervical cancer, Harald zur Hausen received the Nobel Prize in Physiology or Medicine in 2008 [102]. So there is meanwhile the recommendation for young women to vaccinate against the high-risk subtypes, possible before first sexual contact. Meanwhile it is known that oncogenic HPV infections in the western world are not only responsible for almost all cervical carcinomas, but also for more than 90 % of anal carcinomas, 70 % of oropharyngeal carcinomas, 70 % of vagina carcinomas, 40 % of vulva carcinomas, and 50 % of penile carcinomas [101]. Because of the high incidence of HPV16-positive oropharyngeal cancers, investigations are performed if vaccination might have a protective effect with regard to the incidence of head and neck cancer [103]. So also the vaccination of boys and young men is discussed [104]. The impact on those epidemiologic rates in the context of vac-

▶ **Table 5** Other onco-therapeutic mAb.

Target structure	mAb	Indication
CCR4	Mogamulizumab	Adult T cell leukemia, NHL
HLA-DR	Apolizumab	Acute lymphatic leukemia (ALL), chronic lymphatic leukemia (CLL), Non Hodgkin lymphoma (NHL), solid tumors
IgG1 on PDGF receptor-α (platelet-derived growth factor receptor α)	Olaratumab	Sarcoma
IL6 receptor	Tocilizumab	Cytokine storm after CART cell therapy
IL6	Siltuximab	Multiple myeloma
RANK ligand (receptor activator of NF-κB ligands)	Denosumab	Bone metastases
Signaling lymphocyte activation molecule (SLAMF7)	Elotuzumab	Multiple myeloma
VEGF (vascular endothelial growth factor)	Bevacizumab	Colon cancer, breast cancer, non-small-cell lung cancer (NSCLC)
VEGF receptor	Ramucirumab	Lung cancer, stomach cancer

cination of young women that had started in the last years will have to be analyzed in the upcoming years.

In contrast to preventive vaccination that immunize against viral oncogenic structures, also therapeutic vaccinations against tumor cell antigens are currently developed. Unlike the therapy with mAb, the immune system is intended to be activated against tumor antigens in order to mobilize specific T cells, antibodies, and other components especially of the adaptive immune response against tumor cells. This activation is based on the interaction of dendritic cells and T cells. In this context, different techniques exist how dendritic cells are confronted with tumor antigens. Either the DC are charged with tumor lysates, proteins, or peptides or they are transfected with DNA or RNA [105].

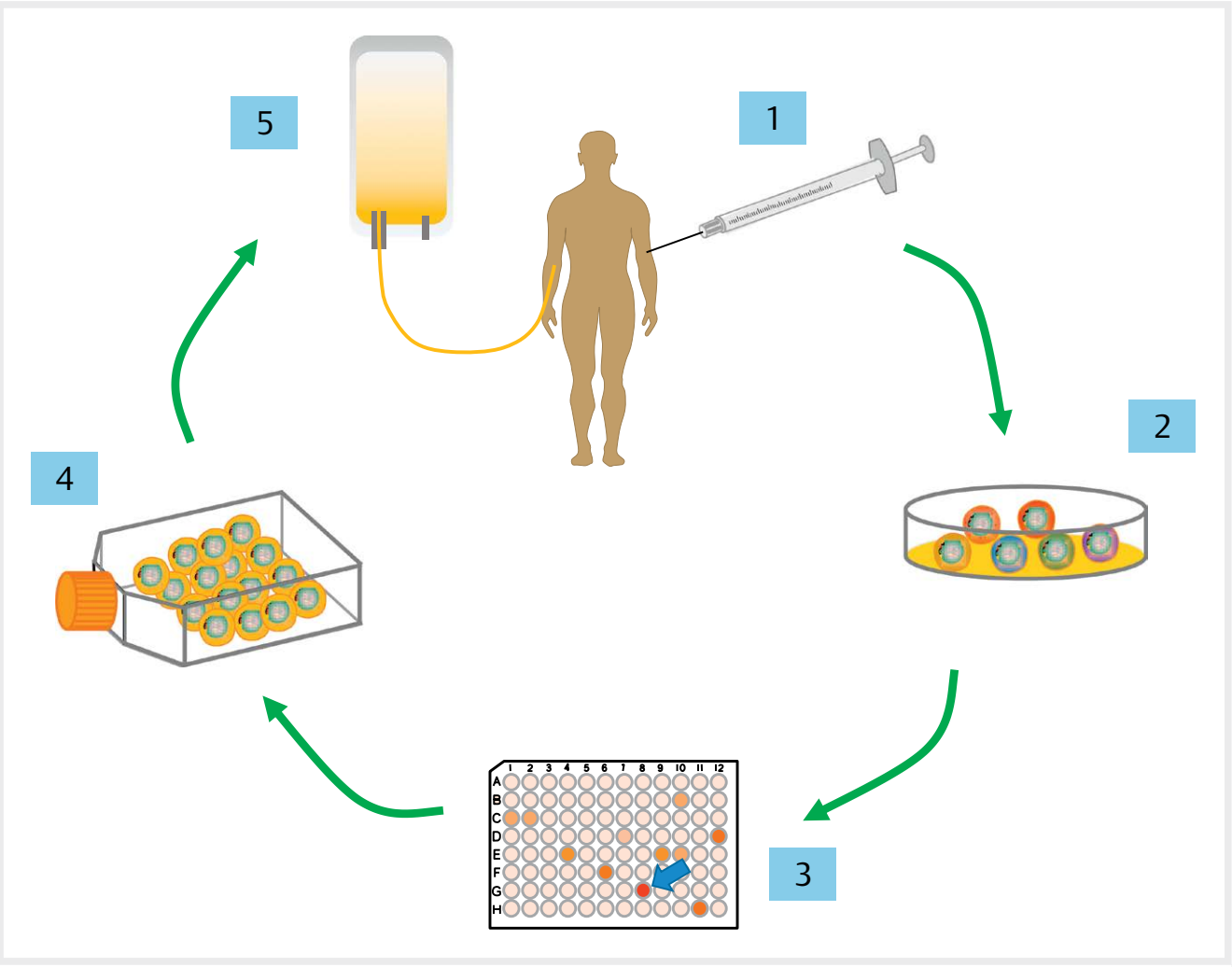
Active oncologically effective vaccinations have not been broadly established in the clinic. Especially for in situ carcinomas, however, clinical studies could achieve substantial success. In cases of intraepithelial neoplasms, e. g., the vulvar intraepithelial neoplasm (VIN), vaccination against HPV16 oncoproteins led to regression of the lesion and the therapy response correlated directly with the T cell response induced by vaccination [106–108]. Similar results were revealed by Czerniecki and colleagues for ductal carcinoma in situ (DCIS) with vaccination targeted against Her2/neu [109]. Morse and colleagues confirmed a significantly higher total survival in patients with resected colon carcinoma metastases after vaccination against the tumor antigens CEA and MUC1 [110]. In a phase I study about vaccination against the tumor antigen p53, head and neck cancer patients showed a 2-year-survival rate of 88 % [111].

One difficulty that might also influence the effectiveness of vaccination is the immunosuppressive tumor environment. The development of Treg, TAM, and MDSC promotes immunosuppressing signaling pathways that complicate the expansion of tumor antigen specific T cells. To avoid this, Mould and colleagues described for example a therapeutic efficiency enhancement by using multiple locations for vaccination [112]. Other strategies focus on the combination with other immune stimulants such as for example toll-like receptor agonists [113].

3.6. Adoptive cell transfer

In the context of adoptive cell transfer, lymphocytes (mainly T cells, but also dendritic cells, NK cells and others) are isolated from the patients' peripheral blood. Afterwards, tumor antigen specific (T) cells may be produced or expanded that are then re-infused to the patient [114, 115] (▶ **Fig. 12**). An advantage of the adoptive cell transfer is that lymphocytes can be influenced and expanded outside the immunosuppressive tumor environment [115].

First clinical trials on adoptive cell transfer were already conducted in the 1990ies [61]. Hereby, a therapy response could be achieved in about 30 % of the melanoma patients. Meanwhile, remission rates of up to 90 % of the patients are reported in the context of specific tumor entities, e. g., patients with acute CD19 + leukemia [116]. "Pre-conditioning" contributes relevantly, consisting of a pre-treatment with lymphatic depletion therapy [117]. In this way, (also) suppressive immune cells such as regulatory T cells or MDSC are reduced that would inhibit the effectiveness of re-infused antigen-specific T cells. Antigen-presenting cells are needed for successful antigen-specific expansion of the cells. APC that are used for adoptive cell transfer for antigen presentation include natural dendritic cells, artificial cells, or "beads" charged with antigens. T cells may be gained from peripheral blood or from the tumor. Then expansion of tumor antigen-specific cells is performed ex vivo with different methods to a cell number of more than 10^9 to 10^{11} cells [118]. Selection of the tumor antigen-specific T cells is performed after isolation of single T cell lines that are then tested with regard to their reactivity against different tumor antigens. In this way, T cell lines are expanded that reveal the highest reactivity against the presented tumor antigens. Modern methods use a genetically determined antigen specificity of T cells and can thus be adapted more precisely and variably to specific tumor antigens. Beside the advantage to select extracorporal antigen-specific T cells and to expand them in high quantities, this expansion is not influenced by the immunosuppressive tumor environment. T cells can be expanded in a functional stage. In this context, high-quality expansion of the T cells has to be assured in order to preserve the tumor antigen specificity of



▶ **Fig. 12** Adoptive cell transfer. (1) Lymphocytes are taken from the patient either from the peripheral blood or the tumor itself. (2) Then the lymphocytes are isolated and modified if necessary. (3) Selection of the lymphocytes with the highest specificity is performed. (4) The selected lymphocytes are expanded and then re-infused (5).

the T cells. So not only the differentiation of the T cells is important but also the cellular metabolic processes [119]. In order to stimulate the re-infused expanded T cells, cytokines are applied [120].

The use of a chimeric antigen receptor (CAR) instead of a tumor antigen-specific T cell receptor is called CAR-T cell transfer. The chimeric antigen receptors consist of an antigen-binding component – for example an antibody – as well as another T cell activating, co-stimulating component to increase the effectiveness of the T cell response [121]. This co-stimulating component influences the cytokine secretion and may stimulate T cell proliferation. Regarding CAR-T cells of the third generation, 2 or more immuno-stimulating domains are integrated in the receptor. The applied co-stimulating molecules include CD28, OX-40 (CD134), or 4-1BB (CD137). A particular effectiveness of those therapeutic methods could be revealed for hematological diseases. Especially in B cell malignomas, a good response rate and clinical effectiveness were observed (NCT02345849) [116, 122]. In solid tumors, convincing results of this dimension are not yet available, which is mainly due to the fact that the identification of specific tumor antigens is more complicated. Up to now, trials on CAR-T cell therapies were performed for Her2 + tumors, EGFR + tumors, CEA + (carcinoembryonic antigen) tumors, and mesothelin + tumors [123–126].

4. Chances and risks

One major task of medical activity is to weigh the potential benefit against the risks of therapeutic measures. This applies especially in the context of oncology because hereby therapeutic measures may strongly impair the patients' quality of life. So it is important to consider also the side effects and possible risks of different immunotherapeutic procedures.

Since the immunotherapeutic principle is based on stimulating the immune system and on eliminating inhibiting effects of the tumor environment, many side effects arise because of excessive autoimmune processes. Typically, the profile of the side effects depends on the spectrum of the immunotherapeutic approach. The more

pluripotent the influenced signaling pathways are, the higher is the risk of possible side effects.

This is especially true for cytokine-based immunotherapies. So IL2 may be associated with side effects like nausea, vomiting, gastrointestinal complaints, severe malaise, increased capillary permeability, cardiac damage, and low blood pressure [61]. Many of those side effects are so severe that they require discontinuation of the therapy. Regarding IL21, similar side effects were described, especially fever, chills, liver damage, and effects on the hematopoietic system with neutropenia and thrombocytopenia [127]. Beside leukopenia, interferons may induce vertigo, anorexia, and depression [68].

With regard to monoclonal antibodies targeted against tumor antigens, especially 2 aspects influence the development and the profile of side effects: on one hand there is the above-mentioned composition of the antibody. An increasing "human" percentage reduces the foreign body reaction and allergic reaction against medication is avoided. Meanwhile, this may be achieved by molecular biological modification of the antibody. On the other hand, it is also important to know if the target molecule is present on healthy cells. The more unspecific the tumor antigen is, the severer are the observed side effects. Additionally, a high tumor load may lead to an uncontrolled release of cytokines by cytolysis (cytokine release syndrome). mAb acting against tumor antigen may cause fever, chills, respiratory complaints, and rashes. In rare cases, treatment with Rituximab (anti-CD20 mAb) may lead to progressive multifocal leukencephalopathy (PML) [128]. One typical side effect that may be caused by the anti-EGFR mAb Cetuximab is rashes, of which the etiology is still not completely clarified [129]. The anti-Her2 mAb Trastuzumab may cause in particular cardiac side effects that require regular controls of the cardiac function during and after therapy [130]. Bevacizumab, however, often causes gastrointestinal complaints as well as arterial hypertension and proteinuria [131].

mAb that are directed against checkpoint molecules increase the immune reaction. This might lead to inflammatory reactions in the body. Nivolumab, an anti-PD1 mAb, may also cause arthritis, colitis, and in particular pneumonitis. Ipilimumab, an anti-CTLA4 mAb, is associated with side effects that concern among others skin, liver, eyes, gut, and the pituitary gland.

Also vaccination therapies may lead to vaccination reactions and other side effects. Even the specific T cell receptor selection in the context of the adoptive cell transfer may cause antigen-related severe side effects. So currently methods are being developed that reduce the probability of such complications. Kunert and colleagues suggest specific methods to allow a risk assessment with regard to the toxicity by means of TCR selection [132]. Among others, this includes the analysis of comprehensive genomic databases regarding the observation if target antigens also appear in other healthy tissues and organs. Another approach is the development of combined therapies that allow a synergistic therapeutic effect with at the same time reduction of the single dose and associated dose-related side effects.

5. Current development

Especially the checkpoint inhibitors achieved an outstanding clinical relevance in the context of immunotherapy in the last years. Up to now, CTLA4, PD1, and PDL1 inhibitors are approved for oncological therapy. The anti-CTLA4 mAb Ipilimumab (Yervoy®, Bristol-Myers Squibb) has been approved in Europe for the treatment of advanced metastatic or non-resectable malignant melanoma since 2011. Since 2015, PD1 inhibitors are available for several tumor entities for treatment of advanced metastatic or non-resectable malignant melanoma. As second-line therapy, it is additionally approved for the treatment of advanced non-small-cell bronchial carcinomas, advanced renal cell carcinomas, and advanced Hodgkin lymphomas. Furthermore, refractory head and neck cancer and urothelial carcinomas wait for approval as indication of anti-PD1 therapy. The already approved anti-PD1 mAb include Nivolumab (Opdivo®, Bristol-Myers Squibb) and Pembrolizumab (Keytruda®, Merck Sharp & Dohme). In March 2017, the FDA approved the anti-PDL1 mAb Avelumab (Bavenico®, Merck KGaA, Pfizer and Eli Lilly and Company) for the treatment of metastatic Merkel cell carcinoma in the USA. Atezolizumab (Tecentriq®, Genentech/Roche), also a PDL1 mAb, has been approved in the USA since 2016 for treatment of advanced or metastatic urothelial carcinomas and pre-treated metastatic non-small-cell lung carcinomas. The number of clinical studies conducted with regard to checkpoint modulators is tremendous. For head and neck cancer alone, more than 45 clinical phase I–III studies have been performed since 2010 [133]. The majority of the studies investigates medications that influence the PD1/PDL1 signaling pathway. But also other immuno-stimulating and inhibiting checkpoint molecules are in the focus of research. In a phase I study, one mAb for stimulation of OX40 revealed regression of metastatic findings in one third of the patients (NCT01644968, [134]). Also other stimulatory molecules such as CD137 or inhibitory molecules such as LAG3 are clinically investigated (NCT02658981).

5.1. Biomarkers

Not all patients benefit from newly established therapies; the reasons are mostly unknown. The already mentioned side effects and high therapy costs require the identification of immunotherapeutic predictive biomarkers that allow prognosis regarding the therapeutic response and thus therapy selection. However, investigations show a very heterogenic picture. Especially PDL1 expression was evaluated in different trials with partly contradictory results. This is explained by the fact that different standards for PDL1 determination exist. Additionally, different limit values are set for positivity. Furthermore, the PDL1 expression as biomarker depends on the tumor entity. The PDL1 expression is established as biomarker for non-small-cell bronchial carcinomas rather than for head and neck cancer [135]. A therapy response after inhibition of the PD1/PDL1 signaling pathway was observed also in PDL1 negative patients [136]. Up to now, reliable biomarkers are missing in the field of checkpoint immunotherapy. Recently published investigations of patients with head and neck cancer indicate that the level of PD1 expression of CD8+ cells is associated with the cellular functionality and the overall survival of the patients [137].

The response rates of immunotherapy do not only depend on the medication and the tumor entities but also on the individual patient. Since the response rates vary and amount to less than 50 % in cases of monotherapies, combined therapies are increasingly applied in order to achieve therapeutic success in as many patients as possible. On one hand, this increases the chance that patient-specifically relevant signaling pathways can be influenced; on the other hand, potentially synergistic therapeutic effects may be achieved.

5.2. Combination therapies

For combination, especially checkpoint receptor therapies are very interesting because they directly approach antitumor-specific cells and block a mechanism that possibly impedes the effectiveness of other therapeutic procedures. This is true for classic therapeutic procedures such as radiotherapy that induces PDL1 expression [138] as well as for other immunotherapeutic approaches. The checkpoint receptor therapy is also applied in combination with cytokine therapies and vaccination strategies.

Pre-clinical studies investigated for example the combination of a GM-CSF modified prostate carcinoma vaccine with a PDL1 blockade [139] because the effectiveness of current vaccination therapies had been suppressed by PDL1 induction. In this murine trial a strong increase of CD4 + and CD8 + T cells with enhanced IFNγ secretion could be observed, which was associated with tumor regression. A similar result could be achieved with the oncolytic virus Onkovex in combination with GM-CSF and anti-CTLA4 [140]. In this model, a systemic effect due to treatment success in not directly injected tumors (50 %) could be observed as well as a locally higher effectiveness (100 %) in directly injected tumors. In addition to direct oncolytic therapy in the tumor, CD8 + T cell reaction was observed in the non-injected tumor.

In a clinical study, Hodi and colleagues investigated the combination of GM-CSF (Sargramostim) with the anti-CTLA4 mAb Ipilimumab in patients with non-resectable malignant melanoma stage III or IV [141] and compared this therapy with Ipilimumab monotherapy. In the context of this study, no significant difference could be observed with regard to the progression-free survival but a significant increase of the overall survival and a reduction of the side effects due to combination therapy.

Currently the expectations of adoptive T cell therapy together with checkpoint molecules are very high. Based on promising results, the first CAR-T cell therapy was approved in August 2017 in the USA. CTL019 (Tisagenlecleucel®, Novartis) is applied in children and adolescents for therapy of acute refractory B cell leukemia. For the future, a significant increase of approvals and the associated indications is expected. A combination of adoptive T cell therapy intervening in the PD1 signaling pathway of the CAR-T cells is currently investigated in a phase I study in patients with metastatic non-small-cell bronchial carcinoma (NCT02793856). By means of modern technique of the CRISPR/Cas method, the genome of the T cells is specifically and exactly modified and the PD1 expression is eliminated. T cells modified in this way are then re-infused after selection and proliferation.

6. Summary and outlook

The interactions in the tumor environment are highly complex and very challenging regarding oncological therapy approaches. Nevertheless, oncological immunotherapy could achieve relevant progress in the last years. For some tumor entities successful results could be observed with long-term therapy response rates, sometimes even with complete remission. But not all patients benefit from those developments. For many tumor entities the response rates are limited in the context of monotherapeutic approaches. However, progress in basic and clinical research is the main precondition for an improved understanding of the interaction between tumor and immune system. This allows new therapeutic combinations in order to use synergistic effects. Especially the development of checkpoint-specific antibodies eliminating the blockade of immune inhibiting signaling pathways seems to be very promising and allows combinations with other therapeutic strategies that up to now have not been successful because of this inhibition. At the same time, immuno-stimulating signaling pathways may enhance cells that have been suppressed by the tumor environment leading to an improved immune response. Establishing predictive markers and thus improving the patient selection for different therapeutic modalities will gain in importance in the future. Because immune therapy in oncology has such a potential, critical and reflected assessment of the study results and therapeutic options is essential in order to implement their current and future importance in clinical routine.

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Conflict of interest

The authors declare no conflict of interest.

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