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Review

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The immune system and cancer evasion strategies: therapeutic concepts

S. Muenst[1], H. Läubli[2,3], S. D. Soysal[4], A. Zippelius[2,3], A. Tzankov[1] & S. Hoeller[1]

From the [1]Institute of Pathology, University Hospital Basel; [2]Division of Medical Oncology, University Hospital Basel; [3]Department of Biomedicine, Cancer Immunology Laboratory, University of Basel; and [4]Department of Surgery, University Hospital Basel, Basel, Switzerland

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The complicated interplay between cancer and the host immune system has been studied for decades. New insights into the human immune system as well as the mechanisms by which tumours evade immune control have led to the new and innovative therapeutic strategies that are considered amongst the medical breakthroughs of the last few years. Here, we will review the current understanding of cancer immunology in general, including immune surveillance and immunoediting, with a detailed look at immune cells (T cells, B cells, natural killer cells, macrophages and dendritic cells), immune checkpoints and regulators, sialic acid-binding immunoglobulin-like lectins (Siglecs) and other mechanisms. We will also present examples of new immune therapies able to reverse immune evasion strategies of tumour cells. Finally, we will focus on therapies that are already used in daily oncological practice such as the blockade of immune checkpoints cytotoxic T-lymphocyte antigen 4 (CTLA-4) and programmed death-1 (PD-1) in patients with metastatic melanoma or advanced lung cancer, or therapies currently being tested in clinical trials such as adoptive T-cell transfer.

Keywords: immune checkpoints, immune regulators, immunoediting, immunosurveillance, siglecs.

Introduction to immune recognition of tumours

Immune surveillance and immunoediting

The concept of cancer immunosurveillance was first proposed in 1909 by Ehrlich [1] who suggested that evolving tumours are constantly identified and eradicated by the host immune system even before clinical manifestations occur. This concept was refined by Burnet in 1970 with their proposal that genetic changes leading to malignancy are common in somatic cells and that the immune system is responsible for eliminating or inactivating these potentially dangerous mutant cells [2]. This concept has now been experimentally confirmed, primarily through demonstration of the increased incidence of malignant tumours in immune-deficient mice or humans [3, 4]. Studies have shown that severely immunocompromised mice, with deficiencies in the innate and the adaptive immune system, have a significantly increased incidence of tumours, suggesting that immunosurveillance is essential to control the gradual development of tumours [4]. These mice are also more susceptible to carcinogen-induced tumours such as fibrosarcomas [5]. In addition, patients receiving immunosuppressive therapy after organ transplantation and HIV-positive patients display a high incidence of malignancies [4].

However, the fact that malignant tumours also develop in patients with a fully functional immune system suggests that immunosurveillance is only a part of the process, and as a consequence, the concept of immunosurveillance has been adapted and refined over the last 15 years into a theory termed 'immunoediting' [6]. It is widely accepted that immunoediting is a dynamic process that not only involves tumour prevention, but also shapes the immunogenicity of developing tumours. Three separate steps of cancer immunoediting have been proposed: elimination, equilibrium and escape [6] (Fig. 1). However, these are not in fact separate phases, but rather represent a continuum of the interplay between tumour and immune system, shifting between elimination, equilibrium and escape depending on the state of the immune system and genuine or acquired properties of the tumour cells.

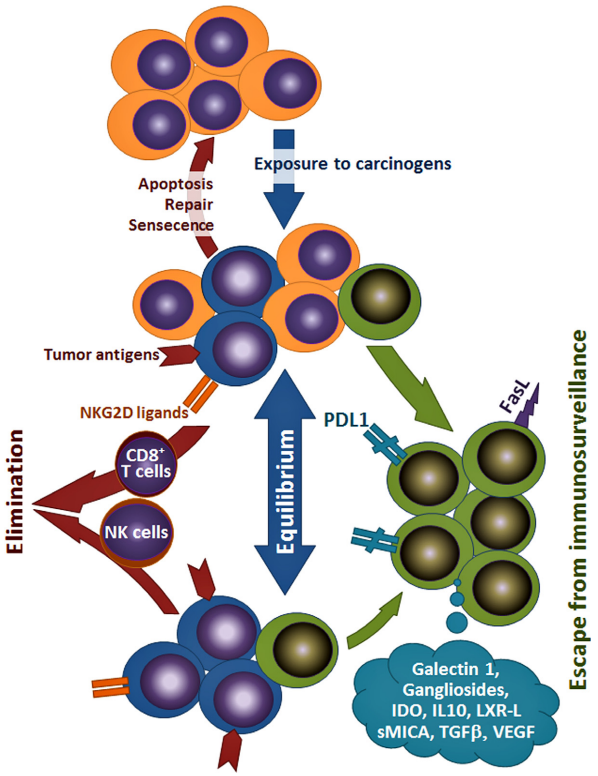

Fig. 1 *Schematic of immunoediting of cancer. Yellow cells - unaffected cells; Blue cells - highly immunogenic (pre)-cancer cells; Green cells - poorly immunogenic (pre)-cancer cells or (pre)-cancer cells with acquired immunosuppressive potential; Red cells - immune-cell.*

Elimination, representing a modern view of immunosurveillance, means that evolving tumours are successfully rejected by the innate and the adaptive immune systems by various mechanisms [6]. In cases in which the evolving tumour is completely destroyed, elimination represents the end-point of immunoediting. However, if tumour cells are not completely eliminated, they may enter the equilibrium state in which the immune system controls tumour outgrowth and tumour cells can enter a dormant state for many years, as demonstrated in experimental models [7] and suggested by data from transplantation patients [8]. Finally, tumour cells may escape from immune control and proliferate in an unrestricted manner, leading to clinically apparent tumours [6]. This escape can be mediated through various mechanisms, such as reduced immune recognition, increased resistance to attack by immune cells or the development of an immunosuppressive tumour microenvironment [6]. Selection pressure by the immune system can lead to tumour cells that are less immunogenic and/or more resistant to lysis and consequently survive [9]. There is also increasing evidence that tumours are able to create an immunosuppressive microenvironment and recruit specific immune cells that favour tumour growth and progression [10, 11].

The initial evidence for cancer immunoediting was provided by the observation that primary carcinomas from immunodeficient animals were immunogenic and regressed in immunocompetent hosts, whereas primary carcinomas from immunocompetent hosts were poorly immunogenic and escaped immune responses when transplanted into immunocompetent hosts [12]. In multiple mouse models, various immune cell types, along with a number of effector molecules, have been implicated in the processes of immunoediting [5]. However, observations in mouse models remain an indirect read-out of tumour elimination, and the success of immunoediting varies amongst experimental systems [5]. In humans, a number of clinical observations provide evidence to support the concept of immunosurveillance (elimination) [5]. Patients receiving chronic immunosuppressive therapy after organ transplantation or those with HIV infection have a significantly increased incidence of malignancies, both virally and nonvirally induced [4]. Additionally, the phenomenon of spontaneously regressing melanomas with accompanying clonal expansion of T cells provides strong evidence that existing tumours can be eliminated as a part of immunoediting in humans [5].

The discovery of T cells reactive to autologous premalignant cells in patients with monoclonal gammopathy of unknown significance, which are not detectable in patients with multiple myeloma, suggests an initial T-cell response holding premalignant cells in check (equilibrium), followed by the eventual failure of this control (escape) and the resulting transition to multiple myeloma [5]. Furthermore, there is a clinical evidence that tumours can remain dormant in patients for many years, and cases of relapse after long periods are well known [5]. Also supporting the concept of the equilibrium and escape phases in tumour immune editing is the fact that transmission of tumours from organ donor to (immunosuppressed) organ recipient has been described [5]. Finally, studies showing that treatment with a vaccine targeting the cancer/testis antigen NY-ESO-1 in patients with melanoma led to the outgrowth or relapse of NY-ESO-1-negative tumour cells [13, 14] suggest that cancer immunoediting also occurs as a consequence of immunotherapy [6].

Immunoediting is now considered to be a 'hallmark of cancer' [15], and understanding its mechanisms has provided a basis for the development of multiple new immunotherapies against malignant tumours. Here, we discuss the specific mechanisms of immunoediting and examine the interplay between different immune cell types, receptors, ligands and tumour cells, as well as the potential clinical utilization of these mechanisms. We will focus on immunomodulatory therapies that influence immunoediting and aim to shift the balance from the escape phase towards the equilibrium/elimination phase. An overview of the different immune escape mechanisms, their consequences and possible clinical significance is provided in Table 1.

The immune system and its therapeutic targeting in anticancer treatment

Immune cells

T cells

T cells play a critical role in both natural and therapeutically induced immunoediting [16]. These cells include regulatory T cells (Tregs), which co-express CD4, CD25 and the transcription factor Foxp3, and effector and memory T cells, which express CD4 or CD8 [16]. Effective cancer immunosurveillance requires the expression of tumour-specific antigens (TSAs) exclusively expressed on tumour cells and encoded by mutant genes, and tumour-associated antigens (TAAs) shared by normal and tumour cells that are capable of stimulating T-cell expansion [4, 16, 17]. For TSAs, the sensitivity and specificity of T-cell recognition allow CD8+ T cells to distinguish tumour-specific peptides with single amino acid changes [18]. However, TSAs naturally vary for every tumour, and their identification requires extensive sequencing for tumour-specific mutations and identification of immunogenic epitopes [19]. Studies have shown the responsiveness of melanoma and other solid cancers such as lung and bladder cancer to a variety of immunotherapies, suggesting that tumour-infiltrating lymphocytes (TILs) may specifically target TSAs [20]. In support of this, another study showed that increased mutational epitopes were associated with increased patient survival and were scarce in tumours without evidence of cytotoxic TILs [21].

Table 1 *Overview of immune escape mechanisms, their consequences and possible clinical solutions*

General tumour escape strategies	Tumour escape mechanisms	Clinical significance
Reduced immune recognition	Absence of strong tumour antigens	Experimental: enhancement via dendritic cell manipulation/vaccination strategies
	Loss of MHC class I (e.g. loss of chromosome 6), class I-like and co-stimulatory factors	Experimental: *ex vivo*-stimulated NK-/T-cell transfer [93] or stimulation of NK cells via fully humanized anti-KIR antibodies [94]
Increased resistance or survival	Increased expression of STAT3 (↑ proliferation, ↓ apoptosis) or of BCL-2 (↓ apoptosis), etc	Limited: drugs targeting STAT3 [203] or BCL-2 [204]
Development of an immunosuppressive tumour environment	Expression of cytokines: VEGF, IL-10 or TGFβ	Limited: see section on NK and T cells and macrophages
	Expression of immunoregulatory molecules: IDO, B7 family checkpoint molecules (PD-1/PD-L1, CTLA-4, VISTA, B7-H4, BTLA), TIM3/galectin9, LAG-3, sMICA, etc	Promising: B7 family checkpoint molecules (see text)
	Expression of CD73, adenosine receptors (limiting antitumour T-cell immunity)	Experimental: anti-CD73/anti-CD39 monoclonal antibodies [205]

Adapted from Mittal D *et al.* (2014) [6]. MHC, major histocompatibility complex; VEGF, vascular endothelial growth factor; IL, interleukin; TGFβ, transforming growth factor-beta; IDO, indoleamine-2,3-dioxygenase; PD, programmed death; CTLA, cytotoxic T-lymphocyte antigen 4; NK, natural killer; CD, cluster of differentiation; KIR, killer immunoglobulin-like receptors.

Regarding TAAs, studies in patients with melanoma have shown that the immune system can recognize TAAs, such as NY-ESO-1 or the mutant form of p53, and generate both tumour-specific CD4+ and CD8+ T cells and antibodies against TAAs. This recognition of TAAs by T cells may be due to an overabundance of antigen or its enhanced presentation as a result of tumour cell death [22]. However, because cancer originates from normal cells, the same immune mechanisms that lead to self-tolerance also restrict the development of an efficient antitumour immune response [18]. These immunoregulatory mechanisms include Tregs as well as expression of immune checkpoints and enable malignant tumours to escape immune control and proliferate.

Under physiological circumstances, Tregs protect against autoimmune diseases by suppressing self-reactive T cells; in the tumour microenvironment, the presence of Tregs may result in inhibition of effective antitumour immune responses [23]. For example, Tregs specific for NY-ESO-1 are common in the blood of metastatic patients with melanoma [24], and the proportion of peripheral Tregs in the blood is significantly increased in patients with a wide range of cancers [24]. It is probable that Tregs are selected by their T-cell receptor (TCR) affinity for peptide – major histocompatibility complex (MHC) class II complexes that are of intermediate strength between positive selection and clonal deletion [25].

Tregs are capable of suppressing a wide range of immune cells, including CD8+ T, natural killer (NK), B and antigen-presenting cells (APCs) [26], and are attracted to tumour tissues by the secretion of the chemokine (C-C motif) ligand 22 (CCL22) by tumour cells and macrophages. CCL22 binds to the C-C chemokine receptor type 4 (CCR4) on Tregs [27]. In the tumour microenvironment, Tregs become activated through the recognition of TAAs or self-antigens released by dying tumour cells [27]. Once activated and expanded, Tregs are able to selectively suppress the activation of TAA-specific effector T cells through various mechanisms, including interleukin (IL)-10 and transforming growth factor (TGF)-β production, and consequently prevent tumour destruction [22, 27]. As an example, high TGF-β expression by lung cancer is associated with poor outcome [28]. Moreover, TGF-β secretion by tumour cells can convert effector T cells into Tregs, which in turn suppress other effector T cells [29].

The fact that experimental tumour models lacking Tregs show both a robust antitumour immune response and rejection of transplanted or primary tumours underscores the critical role of Tregs in immunoediting [30]. In humans, an increase in the number of tumour-infiltrating Tregs is associated with poor prognosis in ovarian, breast and gastric cancers [31–33], but with better prognosis in colorectal cancer and some lymphomas [34, 35]. This finding suggests that the function of Tregs in tumours may be context dependent [18].

On the other hand, CD8+ effector T cells are thought to prevent local tumour growth through direct cytolytic killing of tumour cells or through secretion of effector cytokines such as interferon (IFN)-γ or tumour necrosis factor (TNF)-α [9, 18]. Accordingly, a high number of CD8+ effector T cells infiltrating the tumour are associated with a favourable prognosis in melanoma as well as in breast, ovarian and colorectal cancers [36–39]. However, the potency of CD8+ T cells is regulated by the balance between co-stimulatory and co-inhibitory signals at the so-called immune checkpoints (see section on immune checkpoints) [40].

CD8+ memory T cells are involved in controlling tumour cells. Studies in patients with colorectal cancer show that a high percentage of CD8+ memory T cells protects against metastatic recurrence, suggesting that the local CD8+ memory T-cell response is particularly effective in controlling the emigration of potential metastatic tumour cells [41]. Similar findings have been reported in a variety of malignant tumours, such as head and neck, prostate, lung and urothelial cancers [42–46]. This has led to the development of an immunoscore to assess the amount and location of CD8+ memory T cells in patients with colorectal cancer; the prognostic value of this score has been confirmed in a limited series of patients [47]. However, recent studies suggest that other inflammatory and angiogenic components of the tumour microenvironment, such as dendritic cells (DCs) and vascular endothelial growth factor (VEGF), modulate the impact of this immunoscore [48].

Therapeutic administration of T cells

The first successful cellular cancer immunotherapy was performed by E. Donall Thomas and his team at the Fred Hutchinsons Cancer Center in Seattle between 1950 and 1970 [49]. Allogeneic haematopoietic stem cell transplantation (AHSCT) led to significant prognostic improvement for many

patients with haematological malignancies, including acute and chronic leukaemia [49]. One of the main results of AHSCT, and the reason for its success, is the graft-versus-leukaemia effect, mainly executed by Th1 T-cell-mediated immune responses [49]. Whilst AHSCT is effective for different haematological cancers, it has not been shown to be successful for solid cancers.

Isolation and expansion of TILs, which are then activated *ex vivo* and re-introduced into the patient, can be used in a therapy termed adoptive T-cell transfer [50, 51]. This treatment relies on the concept that removal of the TILs from their immunosuppressive microenvironment and stimulation *ex vivo* make it possible to overcome tumour-induced T-cell dysfunction [50]. The initial trials of adoptive T-cell transfer date back to the 1960s [50, 52]. Recent approaches have included whole exome sequencing of tumour tissues from a patient and identification of neoantigens [53]. Based on this approach, expanded CD4+ T cells from a lung metastasis, which were specific for a neoantigen due to a nonsynonymous mutation in the gene for *ERBB2IP*, were experimentally used for the treatment of cholangiocellular carcinoma [53]. Although this is a very interesting concept, it remains to be determined whether it is an approach that can be used for a broader patient population.

Autologous immune cells can also be genetically manipulated to enhance their function or to specifically target tumour antigens [50, 54]. T cells can be genetically engineered to express either conventional TCRs that specifically recognize tumour antigens or chimeric antigen receptors [(CARs) constructs of antibody domains fused to T-cell signalling domains] [50, 54]. Transduction of T cells with conventional TCRs targeting MART-1, gp-100, NY-ESO-1 or p53 and subsequent adoptive transfer induced tumour regression in some patients [55]. As another example of T cells engineered to express conventional TCRs, Robbins *et al.* recently described the treatment of HLA-*0201 patients with NY-ESO-1-expressing metastatic synovial cell sarcoma and melanoma through an adoptive transfer of T cells retrovirally transduced with a TCR recognizing NY-ESO-1 [56]. The introduction of a CAR into patient-derived T cells before re-infusion was shown to be highly successful in patients with refractory B-cell leukaemia [57, 58]. Thirty children and adults with acute B-cell leukaemia were treated with autologous T cells that were transduced with a CAR lentiviral vector coding for a CAR specific for CD19 [57, 58]. In 27 patients, complete responses to the infusion of CAR T cells (CARTs) were observed, including in 22 patients who showed no or minimal residual disease [57]. Seven patients with complete remission relapsed, whilst 19 remained in remission [57]. In another recently published trial, T cells with a slightly differently engineered CAR against CD19 were used successfully in patients with pre-B-cell acute lymphoblastic leukaemia [59]. Moreover, anti-CD19 CARTs were also used to treat advanced B-cell lymphomas [60]. CARTs targeting other tumour epitopes are currently in development [56].

Although CART therapy seems to be effective for CD19-positive malignancies, other therapies against shared epitopes can be quite toxic and the choice of the antigen to be targeted remains a central issue in the development of CARTs [50, 61]. An additional challenge is the persistence of CARTs after lentiviral transduction and the possibility of random insertion of transduced DNA into the genomic DNA of T cells, which may be circumvented by the use of RNA electroporation. For example, in patients with pancreatic cancer, electroporation of T cells with RNA coding for a CAR directed against mesothelin resulted in activity with the limited toxicity [62]. In general, toxicity of CART therapy is due to cytokine release and off-target effects as a result of the expression of the targeted antigen on other tissues. A case report of a patient with metastatic colorectal cancer treated with CARTs targeting human epidermal growth factor receptor 2 (HER2) demonstrated an off-target effect due to low-level expression of HER2 on lung epithelial cells with a cytokine storm leading to the death of the patient [63]. Table 2 provides an overview of adoptive cell transfer for cancer immunotherapy.

A significant step forward that improved the efficacy of adoptive cell transfer was the development of better conditioning regimens. Conditioning with nonmyeloablative but lymphodepleting chemotherapy with cyclophosphamide and fludarabine led to an important objective response rate of 51% (18/35 patients) to TIL therapy together with high-dose IL-2 in patients with melanoma [64]. Such regimens cause less immune depression and less competition for cytokines such as IL-7 or IL-15 [65].

Recent approaches to mobilize antitumour T cells also include engineering of bivalent antibodies with one variable region targeting an antigen on the tumour and another targeting CD3, as the

Table 2 *Overview of adoptive cell transfer for cancer immunotherapy*

Method	TAAs	Cancer types	References
TILs, *ex vivo* expanded	Unselected, various different epitopes (neoepitopes, tissue-differentiation antigens, cancer–testis antigens, viral antigens)	Melanoma, leukaemia, cervical cancer	[51, 206–211]
Tumour-antigen-specific expanded TIL	Neoepitopes (ERBB2IP), cancer–testis antigen (NY-ESO-1), tissue-differentiation antigens (WT-1)	Cholangiocarcinoma, melanoma, leukaemia	[53, 212, 213]
Engineered TCR with autologous T cells	Tissue-differentiation antigens (MART1), cancer–testis antigen (NY-ESO-1)	Melanoma, synovial cell sarcomas	[55, 56, 214, 215]
CAR T cells	CD19, GD2, mesothelin	ALL, CLL, B-cell lymphoma, malignant pleural mesothelioma, pancreatic cancer	[57, 59, 60, 216–221]

TAAs, tumour-associated antigens, TIL, tumour-infiltrating lymphocyte, TCR, T-cell receptor, CAR, chimeric antigen receptor, ALL, acute lymphoblastic leukaemia, CLL, chronic lymphocytic leukaemia.

engagement of T cells in close proximity to tumour cells is believed to induce or enhance a tumour-specific immune response [66]. The 'trifunctional' bispecific antibody catumaxumab is a mouse IgG2a anti-EpCAM (epidermal cell adhesion molecule) hemi-antibody paired with a rat IgG2b anti-CD3 hemi-antibody [66]. Catumaxomab is approved to treat malignant ascites in patients with epithelial cancers [66]. Other technologies, such as the bispecific T-cell engager (BITE) method employed by Amgen, have been used to design bispecific antibodies wholly lacking Fc [66]. Blinatumomab is a BITE antibody that was recently tested in a Phase III trial in patients with refractory or relapsed B-precursor lymphoblastic leukaemia [67, 68].

Reduction of immuno-inhibitory Tregs could be an alternative approach to improve antitumour immune responses [69]. Initially, anti-CD25 antibodies were used in a number of studies to deplete Tregs in patients with cancer, but because CD25 is also an activation marker on activated antitumour T cells, these trials were not successful [27]. In another approach, chemokine receptor antibodies against CCR4 in combination with vaccination against NY-ESO-1 were used to reduce infiltration of Tregs into tumour tissues [70]. The cytotoxic drug cyclophosphamide might also, to some extent, selectively target Tregs, as low-dose cyclophosphamide was shown to reduce Tregs in tumours [71]. Finally, cytotoxic T-lymphocyte antigen 4 (CTLA-4) on regulatory T cells can also be targeted by checkpoint blockade with anti-CTLA-4 antibodies [69].

NK cells

NK cells were the first innate lymphoid cells discovered [72] and are capable of spontaneously lysing tumour cells without requiring prior activation and without MHC restriction [72], properties that appear to play an important role in tumour immunosurveillance. This notion is supported by data from a longitudinal study from Japan in which subjects with a low natural cytotoxicity of NK cells had a significantly higher incidence of cancer over an 11-year period [73]. Moreover, studies on a variety of solid tumours, such as lung, gastric, colorectal and head and neck cancers, have shown that high intratumour NK-cell infiltration is associated with a better prognosis (reviewed in [74, 75]).

Cytolytic activity of NK cells is highly controlled and is activated through a variety of cell surface receptors, the so-called natural cytotoxicity receptors (NCRs), which bind to ligands that are primarily upregulated and expressed by 'stressed' cells [72]. The best example of this is the recognition of NKG2D ligands expressed by target cells via NKG2D expressed on NK cells [76]. In recent years, several tumour-specific cell ligands for NCRs have been identified. One example is B7-H6 [72], which is expressed in a variety of malignant neoplasms, such as lymphomas, leukaemias, melanomas and carcinomas [76].

NK cells also express a variety of inhibitory receptors specific for MHC class I molecules [72]. As a consequence, NK cells effectively lyse target cells that have lost MHC I molecules, a phenomenon

that frequently occurs in tumour cells [72]. Through this mechanism, NK cells kill tumour cells that have escaped the control of CD8+ cytotoxic T cells [77]. It has been shown that cancer stem cells of colorectal carcinoma express the reduced levels of MHC I and increased levels of NK-activating ligands, and are thus preferentially attacked by NK cells [78]. Through this mechanism, NK cells might also modulate metastatic disease by killing the cancer stem cells responsible for metastatic dissemination [72].

Tumour cells escape the antitumour function of NK cells through the two main mechanisms: (i) suppression of the effector NK-cell function and (ii) evasion through editing of poorly immunogenic tumour cells [79]. Suppression of NK cells is achieved by downregulation of NK-attracting chemokines such as CXCL2 in the tumour microenvironment [72, 80]. As a consequence, the number of NK cells is substantially reduced in tumour tissues compared with healthy tissues [72]. The cytolytic function of NK cells is also inhibited by mediators such as TGF-β, produced by the tumour cells themselves, or by tumour stromal cells that downregulate surface expression of NCRs [77]. Another important phenomenon is NK-cell exhaustion through continuous exposure to certain target antigens, similar to the situation in T cells, leading to tolerance towards the continuously expressed tumour antigens [79]. The hypoxic milieu in the tumour microenvironment also acts as a suppressive factor, as it significantly reduces the expression of NCRs on NK cells, resulting in an impaired ability to kill tumour cells [81].

In terms of immunoediting, tumour cells are able to reduce the expression of ligands such as NKG2D ligand to impair NK-cell recognition, whilst simultaneously inhibiting the expression of NCR [79, 82]. Moreover, tumour cells can increase the expression of MHC I to inhibit NK cytotoxic functions [82], a mechanism employed by melanoma cells [83]. Another mechanism is regulation of NK cells by Tregs, which limit the availability of IL-2 to NK cells [82]; this competition for IL-2 between Tregs and NK cells seems to be a significant regulatory mechanism for both cell subsets [82].

In certain cancers, such as squamous cell carcinoma of the lung and colorectal and breast cancers, the tumour seems to have a direct effect on NK-cell phenotype, repressing cytolytic functions and polarizing the cells towards a proangiogenic phenotype [72]. These altered NK cells express high levels of VEGF, which results in tumour promotion rather than tumour inhibition [72]. In addition to direct cell interactions, it is likely that this polarization is also induced by high levels of TGF-β and hypoxia in the tumour microenvironment. There is evidence that these altered NK cells directly reduce the number of T cells in the tumour microenvironment, thus also inhibiting the immune response to tumours [82]. The notion of a possible tumour-promoting function of NK cells is supported by the finding of an association between increased NK-cell infiltration and poorer outcome in metastatic ovarian carcinoma and invasive ductal carcinoma of the breast [84, 85].

Therapeutic administration of NK cells and NK/T cells

The use of NK cells for cellular immunotherapy currently focuses on haematological malignancies [86–88]. Treatment with alloreactive NK cells dependent on their killer immunoglobulin-like receptors (KIRs) in an early study after *ex vivo* expansion with IL-2 led to a beneficial response in some patients [89]. In a prospective Phase II study at two different centres, alloreactive NK cells were infused into 16 patients with high-risk leukaemias after AHSCT; this treatment was associated with survival of four patients at 5 years [90]. NK cells were also used, apart from in the transplantation context, in a study of haploidentical NK-cell infusion in patients with acute myelogenous leukaemia (AML) [91]. NK/T cells can be activated with alpha-galactosylceramide [87], but direct injection of this glycolipid did not result in clinically meaningful outcomes [92]. However, combined infusion of *ex vivo*-stimulated NK/T cells together with alpha-galactosylceramide-pulsed DCs led to a clinical beneficial response in patients with head and neck tumours [93].

Another approach to mobilize NK cells against tumours is the manipulation of activating or inhibitory receptors on NK cells using fully humanized anti-KIR antibodies [94]. The 1-7F9 antibody blocks KIR2DL 1, 2 and 3 receptors and showed an antitumor effect in a mouse model [94]. A Phase I trial has shown good tolerability in patients with AML and multiple myeloma [95, 96], and a Phase II trial of this antibody in patients with AML and a Phase I trial of combinations with other immunotherapies, including checkpoint inhibition with nivolumab, are ongoing (NCT01687387 and

NCT01714739). Finally, activation of NK cells via bispecific antibodies, which bind to a target epitope on cancer cells and to an activating NK-cell receptor (similar to bispecific antibodies for T cells that bind to CD3 and to a tumour epitope such as EpCAM in the case of catumaxumab), is under development [97].

Macrophages

Tumour-associated macrophages (TAMs) are particularly abundant amongst the inflammatory cells of the tumour microenvironment, representing up to 50% of the tumour mass, and are present at all stages of tumour progression [79]. They originate from circulating monocytes recruited to the tumour site through tumour- or stroma-derived chemotactic factors such as CCL2, or from tissue-resident macrophages [79, 98]. Additionally, tumour hypoxia promotes macrophage recruitment [98], probably through factors such as VEGF.

In general, macrophages can be classified into two subsets: (i) 'classical' M1 macrophages, which produce Th1 cytokines such as IL-12 and TNF-α and promote antitumour responses, and (ii) 'alternative' M2 macrophages, which produce Th2 cytokines such as IL-6, IL-10 and TGF-β, associated with tissue remodelling, wound healing and angiogenesis [72]. TAMs predominantly show an M2-like profile, which is favoured by factors secreted by immune cells such as DCs and Tregs, and also by the hypoxic milieu surrounding the tumour [79]. It has been shown that, during tumour progression, TAMs can switch from an M1 to an M2 phenotype and in turn provide a favourable microenvironment for tumour growth and angiogenesis by secreting VEGF [98]. This polarization of macrophages towards an M2-like phenotype occurs through a number of factors, including IL-4 synthesized by T cells and growth factors such as colony-stimulating factor (CSF)-1 produced by tumour cells [99]. M2-like TAMs are characterized by poor antigen-presenting capabilities and suppress T-cell immune responses by releasing immunosuppressive factors such as IL-10 and TGF-β [98]. It has been shown that TAMs express programmed death ligand (PD-L)-1 and are thus able to directly inhibit T-cell signalling [99]. TAMs can also suppress T-cell activity by the depletion of L-arginine in the tumour microenvironment through the secretion of arginase I [99].

Additionally, TAMs have been implicated in tumour cell invasion and the formation of metastases, as TAM-derived proteases degrade the surrounding extracellular matrix and allow cancer cells to migrate [98]. For example, matrix metalloproteases (MMPs) have been implicated in tumour progression due to their capacity to degrade the basement membrane and enhance angiogenesis [10]. Macrophages recruited to the metastatic site also enhance extravasation of tumour cells by expression of VEGF, causing vascular permeability [99]. Accordingly, studies have shown that increased TAM infiltration is associated with worse clinical outcome in a variety of malignant neoplasms [100–103]. By contrast, other studies have suggested that the prognostic significance of TAMs can be controversial, because they have been associated with a better prognosis in colorectal cancer [104] and diffuse large B-cell lymphoma. This effect seems to largely depend on whether patients have been treated with rituximab and anthracyclines, but could also be related to their polarization status [105, 106].

Therapeutic manipulation of macrophages

Macrophage polarization to an M2 phenotype within the tumour stroma is a predictor of poor prognosis in some cancers. The use of antitumour therapies, such as radiotherapy, chemotherapy or immunotherapy, can influence macrophage polarization, and TAMs may therefore mediate resistance to such treatments [107]. In preclinical models, M-CSF (macrophage colony-stimulating factor) binding to its receptor CSF-R1 [99] was shown to be pivotal for the recruitment and polarization of M2 macrophages. Blockade of this interaction by small molecules was demonstrated in a mouse model of glioblastoma [108].

Antibodies directed against cancer-specific molecules are important tools in clinical oncology. Although some newer type II antibodies also directly induce apoptosis of cancer cells, type I antibodies such as rituximab rely on antibody-dependent cellular cytotoxicity and complement-dependent cytotoxicity. In addition to NK cells, macrophages are important mediators of cellular cytotoxicity, and approaches that increase macrophage-mediated cytotoxicity and improve macrophage-mediated phagocytosis could thus enhance antibody therapy[107]. CD47 has been identified as a 'don't eat me' signal by Irving Weissman and collaborators, and subsequent studies demonstrated the achievement of improved antibody-mediated cytotoxicity by targeting CD47 [109]. In the light of these findings, clinical trials with an

anti-CD47 antibody are currently planned (together with Celgene, e.g. NCT02367196).

DCs

DCs play an important role in the induction of antitumour responses due to their ability to cross-present antigens to CD4+ and CD8+ T cells [110]. However, an effective antitumour immune response by T cells requires efficient antigen presentation by mature DCs. The maturation of DCs depends on the local microenvironment and is influenced by various factors [110, 111]. In the tumour microenvironment, DCs often show an immature/tolerogenic phenotype, which is induced by factors such as VEGF, IL-8 and IL-10, released by tumour cells or TAMs [79]. Tolerogenic DCs themselves produce IL-10, TGF-β and indoleamine-2,3-dioxygenase (IDO) and have a poor ability to stimulate T cells [79]. They also induce an expansion of Tregs and can directly suppress T-cell responses by inducing T-cell anergy [112]. Additionally, these tumour-associated DCs produce proangiogenic factors and enhance endothelial cell migration, thus actively promoting tumour growth [113]. This proangiogenic property is suppressed by DC maturation [113]. In fact, it has been shown that infiltration of mature DCs into primary tumour lesions is associated with fewer metastases and better clinical outcome [114]. Collectively, tumours can reprogramme DCs into immunosuppressive/tolerogenic and proangiogenic cells, favouring tumour growth [112].

DCs can also activate NK cells and thus elicit a potent cytotoxic immune response against tumour cells [115]. Moreover, emerging evidence suggests that DCs can also adopt a direct cytotoxic effector function against cancer cells [115]. However, the high effector:target ratios required for the detection of cytotoxic activity of DCs support a more predominant role as APCs rather than as effector cells [115].

DCs infiltrate a wide variety of tumours, including skin, lung, colorectal and ovarian cancers, but their effect on prognosis is not conclusive [115].

DCs and vaccinations

The ultimate goal of any approach in cancer immunotherapy is to induce a strong and lasting antitumour immune response to achieve long-term control of the cancer and eventually cure the patient. Active immunization by vaccination is commonly used to prevent infection by pathogens. As TAAs and TSAs are recognized by the adaptive immune system, the use of different vaccination methods to mobilize the immune system against cancer and induce immune memory was also tested [116]. Preparing a successful tumour vaccine requires careful consideration of various factors: the correct antigen needs to be selected and, subsequently, the correct adjuvants to overcome immune suppression within the cancer, as well as the optimal delivery vehicle and administration route [116]. Whilst the expression pattern in the tumour and the healthy tissue should be considered for antigen selection, the adjuvants chosen should induce a strong Th1 response, in contrast to immunization for pathogens that usually requires a Th2 response. The vaccine can be delivered by injection, via an attenuated virus or, logistically more challenging, via APCs, including DCs. As many clinical trials have been conducted, often with ambiguous results, only a few exemplary vaccination strategies can be discussed here (for review, see [116]).

Sipuleucel-T (Provenge) is a vaccine approved in the USA by the Food and Drug Administration (FDA), but not in Europe, for the treatment of castration-refractory prostate cancer (CRPC) [117, 118]. Sipuleucel-T is based on patient-derived monocytes that are activated *ex vivo* with the fusion protein PA2024, which consists of an antigen, prostate-specific phosphatase and granulocyte–monocyte CSF that acts as activator [117, 118]. In a recent clinical trial, CRPC patients with asymptomatic bone metastasis, a Gleason score of 7 or less and no visceral metastasis were treated with Sipuleucel-T. The median survival was improved in the Sipuleucel-T arm to 25.8 months compared to 21.7 months in the placebo arm [117].

Tecemotide (L-BLP25), a vaccine consisting of mucin 1 lipopeptide and monophosphoryl A, was used in the START trial to treat patients with unresectable stage III nonsmall-cell lung cancer who had received radiochemotherapy [119]. Although no difference was seen for the entire cohort, improved survival was noted in a preplanned analysis of a subgroup of patients receiving concurrent radiochemotherapy (overall survival of 30.8 months vs. 20.6 months), but not in the subgroup receiving sequential radiochemotherapy [119].

In the DERMA trial, patients with resected stage IIIB and C melanoma received a MAGE-A3-based vaccine together with the immune adjuvant AS15

(GSK1572932A) [120]. Although the primary endpoint of prolonging disease-free survival was not met, a gene signature that predicts response to the therapy was identified [120].

Algenpantucel-L is a combination of two allogeneic pancreatic cancer cell lines transduced with murine alpha-1,3-galactosyltransferase which has been tested in patients with pancreatic cancer [121]. In a Phase II study, the survival of patients treated with adjuvant chemotherapy after resection was improved when they also received algenpantucel-L, compared to historical controls [121].

Taken together, despite the testing of various vaccination strategies, clinicial success to date has been limited. One explanation for the limited success is the lack of potent immunostimulating agents that would overcome the immunosuppressive microenvironment. In this regard, the success of blocking antibodies directed against immune checkpoints on T cells could lead to an improved immune response to vaccines. Another problem could be vaccination with a single antigen, even if this antigen is expressed strongly within the cancer tissue. The development of an escape clone that cannot be controlled by the immune system is very likely, due to the genetic instability of tumour cells.

Ligands and receptors

Immune checkpoints

Upon recognition of an antigen presented on the surface of an APC, T cells require at least two signals to become fully activated [122]. The initial signal through the TCR is antigen specific, and the second is antigen independent and transduced by specific co-receptors on the T cells belonging to the B7/CD28 protein family, which can be either stimulatory or inhibitory [122]. Co-stimulatory receptors such as CD28 promote T-cell activation, whilst co-inhibitory receptors such as programmed death-1 (PD-1), CTLA-4 or B and T-lymphocyte attenuator, also termed 'immune checkpoint molecules', inhibit T-cell function, preventing inappropriately directed immune reactions and limiting the extent and duration of immune responses [123–127].

Regarding its structure, PD-1 is a type I transmembrane protein of the immunoglobulin superfamily [128]. It is composed of an extracellular domain, similar to the variable region of an immunoglobulin, with a transmembrane region and a cytoplasmic tail [129]. In contrast to CTLA-4, which forms homodimers, PD-1 exists as a monomer on the cell surface. The cytoplasmic tail contains an immunoreceptor tyrosine-based inhibitory motif (ITIM) and an immunoreceptor tyrosine-based switch motif (ITSM) that are essential for the transmission of inhibitory signals [123, 130]. Upon TCR stimulation and ligation with either PD-L1 or PD-L2, the ITSM and ITIM undergo phosphorylation, leading to recruitment of the phosphatases SHP-1 and SHP-2, which, in turn, leads to dephosphorylation of downstream signalling molecules with resulting inhibition of the phosphatidylinositol 3-kinase [122, 123]. This is followed by a net blockade of Akt signalling leading to decreased cytokine production, T-cell proliferation and survival. Additionally, interaction between PD-1 on T cells and PD-L1 on APCs and endothelium inhibits the production of several cytokines (IFNγ, IL-2 and TNF-α). Simultaneously, T-cell apoptosis is promoted through inhibition of the survival factor B-cell lymphoma-extra large [131].

A negative feedback loop is created by cytokines produced after T-cell activation, such as IFNγ and IL-4, which leads to upregulation of PD-1 ligands that attenuates immune responses and limits immune-mediated bystander tissue damage [132].

PD-1 is constitutively expressed on a subset of thymic T cells and becomes upregulated on activated NK, T and B cells, monocytes and DCs, and is particularly highly expressed on CD4+ follicular helper T cells [132–135]. It binds two ligands, PD-L1 (B7-H1, CD274) and PD-L2 (B7-DC, CD273), which belong to the B7 protein family. PD-L1 seems to restrain effector T-cell function primarily in nonlymphoid organs and may play a role in protecting immune-privileged sites such as the placenta, testis or eye from T-cell immune responses, whereas PD-L2 functions primarily in lymphoid organs [133, 136–139]. Evidence from multiple studies has shown that binding of PD-L2 by PD-1 preferentially inhibits Th2 responses, which could explain potential differences in the clinical activity and toxicity profile of antibodies against PD-L1 compared to those directed against PD-1, such as decreased pulmonary toxicity for anti-PD-L1 versus anti-PD-1 blockade. The PD-1/PD-L1 pathway seems, therefore, to represent a critical T-cell resistance mechanism in human malignancies. PD-1 is significantly upregulated on cancer-specific T cells, suggesting functional exhaustion of these cells, and PD-L1 is expressed

by a variety of epithelial cancers and haematological malignancies, suggesting that these malignancies may use the PD-1/PD-L1 signalling pathway to attenuate or escape antitumour T-cell immunity and thus facilitate tumour progression.

PD-1 is expressed on several lymphoma subtypes (Fig. 2), such as angioimmunoblastic T-cell lymphoma, small lymphocytic lymphoma and diffuse large B-cell lymphoma [132, 140–143]. However, in breast cancer, melanoma, classical Hodgkin's lymphoma and follicular lymphoma, PD-1 has also been found on TILs [142, 144–148]. PD-L1 expression has been demonstrated in carcinomas of the lung, breast, kidney, bladder, ovary and cervix as well as melanoma, glioblastoma and various lymphomas and leukaemias [142, 144, 145].

It has been shown that PD-L1 in tumour cells can induce resistance to T-cell-mediated killing and inhibit tumour cell apoptosis induced by antigen-specific T cells [149]. Accordingly, PD-L1 expression on tumour cells is associated with poorer prognosis in various cancers, such as kidney, oesophageal, bladder, ovarian, breast, gastric and pancreatic cancers [138, 139, 146, 150–152], which might partially explain why induction of cancer-specific T cells in many trials of adoptive cell therapies has not inhibited tumour growth [40, 153].

Clinical targeting of immune checkpoints

Ipilimumab (Yervoy), a fully human IgG1 blocking antibody targeting CTLA-4, was approved in 2011 by the FDA as a 'first-in-class' drug to treat metastatic melanoma progressing after first-line chemotherapy with dacarbazine. After initial trials showed CTLA-4 blockade [154, 155], two subsequent pivotal studies led to the approval of ipilimumab [156, 157]. One of these Phase III trials investigated the effect of ipilimumab with or without a gp100 vaccine in patients with metastatic

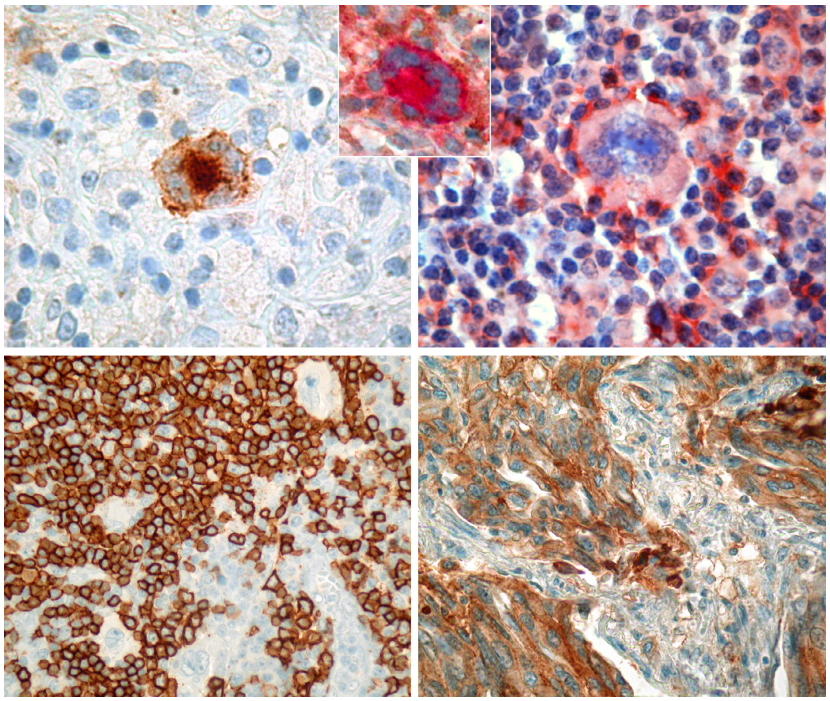

Fig. 2 *In situ PD1 and PDL1 expression in different types of cancer. Upper left: Reed-Sternberg cell of classical Hodgkin lymphoma strongly expressing PDL1 (clone E1L3N); DAB staining, 400x. Insert: The "immune check" - Reed-Sternberg cell of classical Hodgkin lymphoma strongly expressing PDL1 (red color), surrounded by PD1+ T cells (brown color), 400x. Upper right: Rosetting ("microscopic immunosuppressive wall") around a Reed-Sternberg cell of classical Hodgkin lymphoma by PD1+ T cells; AEC staining, 400x. Lower left: plenty of tumor-infiltrating PD1+ T cells in a case of a T cell-rich B-cell lymphoma; note the few negative large tumor B cells; DAB staining, 200x. Lower right: Expression of PDL1 by a squamous cell lung cancer; DAB staining, 200x.*

melanoma who had progressed after first-line chemotherapy [156]. Patients who received ipilimumab with or without gp100 had a median survival of 10 months compared to 6.4 months for patients treated with gp100 vaccination alone [156]. In the other Phase III trial, the concurrent administration of ipilimumab and dacarbazine was analysed in patients with untreated unresectable or metastatic melanoma [157]. Survival was significantly improved in patients who received ipilimumab in combination with dacarbazine compared to those treated with dacarbazine alone (11.2 vs. 9.1 months) [157]. More importantly, an analysis of the 10 years of experience from ipilimumab trials has demonstrated that about 20% of patients achieved long-term remission, which suggests that sustained immune control of metastatic cancers can be achieved [158]. This result is even more impressive as ipilimumab was used only during the first 3 months of treatment in many of these trials. The activity of ipilimumab in the adjuvant setting was also proved by the findings of a trial in which the drug was analysed as an additional therapy after resection of high-risk melanoma [159]. The findings of a recent study suggested the possibility of identifying patients likely to respond to CTLA-4 blockade [160], as an analysis of the mutational landscape of responders showed that melanoma patients with a high frequency of somatic mutations responded better to treatment with ipilimumab [160]. *In silico* prediction of class I neoepitopes also identified a set of antigens predictive of a good response, defined as remission or stabilization for more than 6 months [160]. Further analysis will be needed before implementation of such analyses in clinical practice. However, the efficacy of CTLA-4 blockade is accompanied by immune-mediated side effects (the so-called 'immune-related adverse events'), which are primarily autoimmune reactions of the skin, intestines and liver in ipilimumab-treated patients [156, 157]. Combinations with tyrosine kinase inhibitors for *BRAF*-mutated melanoma were initially halted due to liver toxicity [161], but more careful dosing regimens and novel BRAF and MEK inhibitors are currently being tested (e.g. NCT01767454).

The second immuno-inhibitory pathway targeted by FDA-approved drugs is the PD-1/PD-L pathway. Nivolumab (Optivo) and pembrolizumab (Keytruda), both fully human IgG4 blocking antibodies against PD-1, were approved by the FDA in 2014 for the treatment of malignant melanoma progressing after treatment with ipilimumab and, in the case of *BRAF* V600 mutations, after treatment with a BRAF inhibitor. Moreover, nivolumab was approved in March 2015 for the treatment of refractory or relapsed squamous nonsmall-cell lung cancer. Nivolumab was successfully tested in melanoma patients with progressive disease after treatment with ipilimumab in a Phase III trial (CheckMate-037), with resulting objective response rates of 31.7% [162]. Only 5% of patients receiving nivolumab experienced serious adverse events with increased lipase and/or aminotransferase levels, fatigue and anaemia [162]. Nivolumab was also tested as a first-line treatment for patients with non-*BRAF*-mutated metastatic melanoma [163]. The overall survival at 1 year was 72.9% in the nivolumab group compared to 42.1% in the dacarbazine group [163] [164]. The CheckMate-017 Phase III study comparing nivolumab as a second-line treatment against docetaxel chemotherapy in patients with squamous nonsmall-cell lung cancer was stopped and led to the approval of nivolumab by the FDA due to a significant overall survival benefit prior to publication of the study results [165]. Nivolumab was also successfully tested in patients with nonsquamous nonsmall-cell lung cancer in the CheckMate-057 trial [166] It has also been demonstrated in a Phase II trial that nivolumab is active in patients with renal cell cancer; overall response rates of 20–22% were observed in 168 patients after the treatment with at least one tyrosine kinase inhibitor [167]. Recently, a Phase III trial showed a significantly longer survival of patients with metastatic clear cell carcinoma of the kidney with nivolumab compared to everolimus in second line [168]. Strinking results were also obtained with nivolumab in a Phase I trial including 23 patients with relapsed or refractory classical Hodgkin's lymphoma. In this trial, an objective response rate of 87% was achieved, and none of the patients showed progressive disease during nivolumab therapy [169]. One explanation of this impressive efficacy of nivolumab could be the intrinsic upregulation of PD-L1 in classical Hodgkin's lymphoma [169].

Combination therapy of CTLA-4 blockade with ipilimumab and PD-1 blockade with nivolumab in patients with melanoma during a Phase I trial led to an impressive objective response rate in 53% of patients with tumour reduction of 80% or more [170]. Recently released results from the trials in which nivolumab together with ipilimumab was compared with ipilimumab alone in patients with

treatment-naïve metastatic melanoma [171] showed a response rate of 61% in the combination therapy compared to 11% in the ipilimumab group. However, the fact that more than 50% of patients also experienced severe adverse events suggests that this combination therapy is probably the best reserved for relatively younger and generally healthier patients. Many trials that include immunotherapy with nivolumab for the treatment of other cancers are currently ongoing.

Pembrolizumab was recently approved for use in patients with melanoma. Analysis of an expansion cohort of the Keynote-001 Phase I trial [172] showed an overall response rate of 26% in patients progressing after ipilimumab therapy [172]. Pembrolizumab was also found to be more effective than ipilimumab as first-line therapy in metastatic melanoma patients, with a 6-month progression-free survival of 47% vs. 26% [173]. Additionally, pembrolizumab is active in other solid tumours, such as lung, head and neck and triple-negative breast cancer and renal cell carcinoma [171]. The efficacy of pembrolizumab was recently evaluated in another large expansion cohort of 495 patients with non-small-cell lung cancer of the Keynote-001 trial and showed an overall response rate of 19%, reaching 45% in patients with tumours with high PD-L1 expression [174].

Blockade of PD-L1 is also currently being investigated as an immunotherapy for various tumour types [175, 176]. MPDL3280A, a PD-L1 blocking, human IgG1 antibody with an engineered Fc portion, was tested in a Phase I trial in patients with urothelial cancer [176]. Overall response rates were 43% with a mild toxicity profile [176]. This treatment could become an important tool in hard-to-treat patient cohorts. PD-L1 blockade is also being tested in patients with advanced lung cancer (e.g. NCT02031458) and in various combination therapies. For example, the combination of obinutuzumab (a type II anti-CD20 antibody) and MPDL3280A for patients with relapsed and refractory follicular lymphoma is interesting (NCT02220842). An overview of relevant clinical trials of inhibitors for CTLA-4 and PD-1/PD-L1 is provided in Table 3.

It has been suggested that the presence of PD-L1 on tumour and stromal cells within the tumour microenvironment represents a predictive biomarker for the response to PD-1/PD-L1 blocking therapy [177]. However, because it can also be upregulated upon stimulation with IFNγ, PD-L1 does not represent a static value and should therefore not be used as a sole indicator for PD1/PD-L1 blockade. Furthermore, accurate determination of PD-L1 expression by immunohistochemistry is restricted by the fact that there are no fully validated antibody assays and no clearly defined cut-off values for PD-L1 positivity. Another predictor of response to therapy is the presence of CD8+ T cells at the invasive border of the cancer [178].

As other immunomodulatory receptors that influence antitumour immune responses in preclinical models have also been identified [179], several

Table 3 *Overview of relevant clinical trials of inhibitors of CTLA-4 and PD-1/PD-L1*

Agent	Target	Cancer types	References
Ipilimumab (Yervoy, BMS)	CTLA-4	Melanoma	[156, 157]
Pembrolizumab (Keytruda, MSD)	PD-1	Melanoma, NSCLC, mismatch repair-deficient cancers (CRC, etc), etc	[172–174, 222]
Nivolumab (Opdivo, BMS)	PD-1	Melanoma, NSCLC, RCC, Hodgkin's lymphoma, HCC, etc	[162, 163, 165, 167, 169], J Clin Oncol 33, 2015, suppl; abstr LBA101)
Pidilizumab (CureTech)	PD-1	NHL	[223, 224]
Atezolizumab (MPDL3280A, Roche)	PD-L1	Bladder cancer, NSCLC, melanoma, RCC, etc.	[175, 176]
Nivolumab and ipilimumab	PD-1 and CTLA-4	Melanoma	[171, 225]

PD, programmed death; PD-L1, programmed death ligand; CTLA-4, cytotoxic T-lymphocyte antigen 4; CRC, colorectal cancer; NSCLC, nonsmall-cell lung cancer; RCC, renal cell carcinoma; HCC, hepatocellular carcinoma; NHL, non-Hodgkin lymphoma.

trials to investigate immune stimulation, via blockade of inhibitory receptors such as LAG-3 (e.g. NCT01968109) or engaging activating receptors such as OX40 or CD137, are ongoing [179]. In addition to studies to determine the most efficient combination therapy for immune stimulation in cancer, further studies are needed to understand why immune stimulation via current checkpoint inhibitors is ineffective in some tumours, such as prostate and colorectal cancers.

Sialic acids and sialic acid-binding immunoglobulin-like lectins

During cancer progression, mutations and epigenetic changes not only affect the protein composition within cancers, but also induce profound changes in glycosylation [180–183]. One of the hallmarks of altered glycosylation is the upregulation of terminal sialic acids on secreted and cell surface glycoconjugates. Moreover, increased incorporation of the nonhuman sialic acid N-glycolyl-neuraminic acid (Neu5Gc), but not the normal human sialic acid N-acetyl-neuraminic acid, is observed in various human carcinomas including breast and colorectal cancers [184]. This differential and increased sialylation has important implications for cancer progression, including upregulation of selectin ligands that influence metastatic colonization [185], modulation of the interaction with factor H and complement-mediated tumour cell killing [186] and enhancement of tumour-related inflammation by interactions between anti-Neu5Gc antibodies and Neu5Gc on tumour cells [184, 187]. Moreover, recent experimental findings suggest that interactions between hypersialylated ligands on tumour cells and immunomodulatory sialic acid-binding immunoglobulin-like lectins (Siglecs) on myeloid cells and NK cells have a role in regulation of cancer immunosurveillance and cancer-associated immune suppression [188–191]. Upregulation of ligands for Siglecs was observed in different types of cancer, including melanoma and breast, prostate and nonsmall cell lung cancers [188–190]. In *in vitro* and mouse models, engagement of inhibitory Siglecs on NK cells or neutrophils can mediate immune evasion by cancer cells [188–190], and binding of inhibitory Siglecs on macrophages influences their polarization to M2 macrophages [190]. Of note, improved early survival of nonsmall-cell lung cancer patients with a polymorphism that reduces the binding of Siglec-9 to sialylated ligands was observed, indicating that Siglec-9 represents a potential target in such patients [190]. Additional analyses to investigate which cell types express Siglecs and to determine their exact function during different phases of cancer progression are needed.

Immune regulators (IDO and IL)

IDO is one of the enzymes that control the metabolism of tryptophan to kynurenine. In general, IDO1 activity is low, with no or minor physiological effects. However, under pathological conditions (such as cancer, allergic inflammation or infection), IDO1 is overexpressed in response to inflammatory cytokines, including IFN-α, IFN-γ, lipopolysaccharide, IL-1 and TNF. In cancer, high IDO1 expression stimulates an immunosuppressive feedback loop that maintains the immunosuppressive microenvironment via subsequent inhibition of T-cell responses [192, 193].

ILs are cytokines that mediate the complex cross-talk between immune cells. Every IL has its own spectrum of capacity, and many regulatory functions thereof remain to be identified. Many ILs also play a role in immunosurveillance and maintenance of the microenvironment in tumours, which is beyond the scope of this review. However, IL-2, a cytokine regulating the growth and differentiation of T cells and certain B cells, has recently attracted attention in cancer immunotherapy (see below).

Therapeutic manipulation of immune regulators

Targeting of IDO1 by small molecules such as tryptophan-analogon d-1-methyl-tryptophan is being studied in several clinical trials (e.g. NCT01042535), and the generation of novel inhibitors is ongoing [193]. After successful testing in preclinical models, second-generation inhibitors such as INCB024360 are also being investigated in various cancers, either alone or in combination with vaccination strategies or checkpoint blockade (e.g. NCT02178722, NCT01961115 and NCT02042430) [194].

IL-2 has long been used in cancer immunotherapy, the first results having been published in 1985 [195, 196]. To date, it is primarily patients with metastatic renal cell carcinoma who receive high doses of IL-2, with a small subset of patients achieving long-term remission [197, 198]. However, its toxicity is quite high, and only young patients with a good performance status can be treated with this modality. Recent findings suggest that careful selection of patients and well-planned

therapies can significantly reduce the side effects of IL-2 therapy, thus rendering it a valid option for an increased number of patients [199]. IL-2 is also used to induce rapid expansion of T cells in adoptive cell transfer therapies [20].

Stimulation of the immune system by treatment with INF-α is within the guidelines for the adjuvant treatment of locally advanced malignant melanoma [200]. The ECOG 1684 trial was the first to show an improvement in overall survival using INF-α, with a 5-year survival rate of 46% for patients receiving 1 year of high-dose INF-α versus 37% for patients in the placebo arm [201]. In an another trial, treatment with pegylated INF-α 2b for 5 years improved disease-free and distant metastasis-free survival in melanoma patients with microscopic lymph node metastasis (N1a), but not in those with macroscopic lymph node metastasis (N1b) [202].

Summary

The interaction between the immune system and cancer cells is complex, and the cancer microenvironment is far from being fully understood. Accordingly, we have not covered all aspects of this complicated interplay but focused on potential therapeutically useful pathways and mechanisms to guide clinicians through the array of new therapy approaches that will soon be available in clinical practice.

Conflict of interest statement

The authors declare no conflict of interest.

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References

1 Ehrlich P. Über den jetzigen Stand der Karzinomforschung. *Ned Tijdschr Geneeskd* 1909; **5:** 273–90.
2 Burnet FM. The concept of immunological surveillance. *Prog Exp Tumor Res* 1970; **13:** 1–27.
3 Dunn GP, Old LJ, Schreiber RD. The immunobiology of cancer immunosurveillance and immunoediting. *Immunity* 2004; **21:** 137–48.
4 Mapara MY, Sykes M. Tolerance and cancer: mechanisms of tumor evasion and strategies for breaking tolerance. *J Clin Oncol* 2004; **22:** 1136–51.
5 Swann JB, Smyth MJ. Immune surveillance of tumors. *J Clin Investig* 2007; **117:** 1137–46.
6 Mittal D, Gubin MM, Schreiber RD, Smyth MJ. New insights into cancer immunoediting and its three component phases–elimination, equilibrium and escape. *Curr Opin Immunol* 2014; **27:** 16–25.
7 Koebel CM, Vermi W, Swann JB, Zerafa N, Rodig SJ, Old LJ, *et al.* Adaptive immunity maintains occult cancer in an equilibrium state. *Nature* 2007; **450:** 903–7.
8 Strauss DC, Thomas JM. Transmission of donor melanoma by organ transplantation. *Lancet Oncol* 2010; **11:** 790–6.
9 Fridman WH, Remark R, Goc J, Giraldo NA, Becht E, Hammond SA, *et al.* The immune microenvironment: a major player in human cancers. *Int Arch Allergy Immunol* 2014; **164:** 13–26.
10 Galdiero MR, Bonavita E, Barajon I, Garlanda C, Mantovani A, Jaillon S. Tumor associated macrophages and neutrophils in cancer. *Immunobiology* 2013; **218:** 1402–10.
11 Joyce JA, Fearon DT. T cell exclusion, immune privilege, and the tumor microenvironment. *Science* 2015; **348:** 74–80.
12 Shankaran V, Ikeda H, Bruce AT, White JM, Swanson PE, Old LJ, *et al.* IFNgamma and lymphocytes prevent primary tumour development and shape tumour immunogenicity. *Nature* 2001; **410:** 1107–11.
13 Nicholaou T, Chen W, Davis ID, Jackson HM, Dimopoulos N, Barrow C, *et al.* Immunoediting and persistence of antigen-specific immunity in patients who have previously been vaccinated with NY-ESO-1 protein formulated in ISCOMATRIX. *Cancer Immunol, Immunother* 2011; **60:** 1625–37.
14 von Boehmer L, Mattle M, Bode P, Landshammer A, Schafer C, Nuber N, *et al.* NY-ESO-1-specific immunological pressure and escape in a patient with metastatic melanoma. *Cancer Immunity* 2013; **13:** 12.
15 Hanahan D, Weinberg RA. Hallmarks of cancer: the next generation. *Cell* 2011; **144:** 646–74.
16 Schreiber RD, Old LJ, Smyth MJ. Cancer immunoediting: integrating immunity's roles in cancer suppression and promotion. *Science* 2011; **331:** 1565–70.
17 Schumacher TN, Schreiber RD. Neoantigens in cancer immunotherapy. *Science* 2015; **348:** 69–74.
18 Savage PA, Leventhal DS, Malchow S. Shaping the repertoire of tumor-infiltrating effector and regulatory T cells. *Immunol Rev* 2014; **259:** 245–58.
19 Manzo T, Heslop HE, Rooney CM. Antigen-specific T cell therapies for cancer. *Hum Mol Genet* 2015; **15:** R67–73.
20 Rosenberg SA, Restifo NP. Adoptive cell transfer as personalized immunotherapy for human cancer. *Science* 2015; **348:** 62–8.
21 Brown SD, Warren RL, Gibb EA, Martin SD, Spinelli JJ, Nelson BH, *et al.* Neo-antigens predicted by tumor genome meta-analysis correlate with increased patient survival. *Genome Res* 2014; **24:** 743–50.

22 Vesely MD, Kershaw MH, Schreiber RD, Smyth MJ. Natural innate and adaptive immunity to cancer. *Annu Rev Immunol* 2011; **29:** 235–71.
23 Allen M, Louise Jones J. Jekyll and Hyde: the role of the microenvironment on the progression of cancer. *J Pathol* 2011; **223:** 162–76.
24 Ebert LM, MacRaild SE, Zanker D, Davis ID, Cebon J, Chen W. A cancer vaccine induces expansion of NY-ESO-1-specific regulatory T cells in patients with advanced melanoma. *PLoS ONE* 2012; **7:** e48424.
25 Hogquist KA, Jameson SC. The self-obsession of T cells: how TCR signaling thresholds affect fate 'decisions' and effector function. *Nat Immunol* 2014; **15:** 815–23.
26 Jiang X, Shapiro DJ. The immune system and inflammation in breast cancer. *Mol Cell Endocrinol* 2014; **382:** 673–82.
27 Nishikawa H, Sakaguchi S. Regulatory T cells in cancer immunotherapy. *Curr Opin Immunol* 2014; **27:** 1–7.
28 Sterlacci W, Wolf D, Savic S, Hilbe W, Schmid T, Jamnig H, *et al.* High transforming growth factor beta expression represents an important prognostic parameter for surgically resected non-small cell lung cancer. *Hum Pathol* 2012; **43:** 339–49.
29 Sakaguchi S, Wing K, Onishi Y, Prieto-Martin P, Yamaguchi T. Regulatory T cells: how do they suppress immune responses? *Int Immunol* 2009; **21:** 1105–11.
30 Teng MW, Swann JB, von Scheidt B, Sharkey J, Zerafa N, McLaughlin N, *et al.* Multiple antitumor mechanisms downstream of prophylactic regulatory T-cell depletion. *Cancer Res* 2010; **70:** 2665–74.
31 Bohling SD, Allison KH. Immunosuppressive regulatory T cells are associated with aggressive breast cancer phenotypes: a potential therapeutic target. *Mod Pathol* 2008; **21:** 1527–32.
32 Preston CC, Maurer MJ, Oberg AL, Visscher DW, Kalli KR, Hartmann LC, *et al.* The ratios of CD8+ T cells to CD4+CD25+ FOXP3+ and FOXP3- T cells correlate with poor clinical outcome in human serous ovarian cancer. *PLoS ONE* 2013; **8:** e80063.
33 Shen Z, Zhou S, Wang Y, Li RL, Zhong C, Liang C, *et al.* Higher intratumoral infiltrated Foxp3+ Treg numbers and Foxp3+/CD8+ ratio are associated with adverse prognosis in resectable gastric cancer. *J Cancer Res Clin Oncol* 2010; **136:** 1585–95.
34 Huang Y, Liao H, Zhang Y, Yuan R, Wang F, Gao Y, *et al.* Prognostic value of tumor-infiltrating FoxP3+ T cells in gastrointestinal cancers: a meta analysis. *PLoS ONE* 2014; **9:** e94376.
35 Tzankov A, Meier C, Hirschmann P, Went P, Pileri SA, Dirnhofer S. Correlation of high numbers of intratumoral FOXP3+ regulatory T cells with improved survival in germinal center-like diffuse large B-cell lymphoma, follicular lymphoma and classical Hodgkin's lymphoma. *Haematologica* 2008; **93:** 193–200.
36 Bachmayr-Heyda A, Aust S, Heinze G, Polterauer S, Grimm C, Braicu EI, *et al.* Prognostic impact of tumor infiltrating CD8+ T cells in association with cell proliferation in ovarian cancer patients–a study of the OVCAD consortium. *BMC Cancer* 2013; **13:** 422.
37 Clemente CG, Mihm MC Jr, Bufalino R, Zurrida S, Collini P, Cascinelli N. Prognostic value of tumor infiltrating lymphocytes in the vertical growth phase of primary cutaneous melanoma. *Cancer* 1996; **77:** 1303–10.
38 Prall F, Duhrkop T, Weirich V, Ostwald C, Lenz P, Nizze H, *et al.* Prognostic role of CD8+ tumor-infiltrating lymphocytes in stage III colorectal cancer with and without microsatellite instability. *Hum Pathol* 2004; **35:** 808–16.
39 Mahmoud SM, Paish EC, Powe DG, Macmillan RD, Grainge MJ, Lee AH, *et al.* Tumor-infiltrating CD8+ lymphocytes predict clinical outcome in breast cancer. *J Clinic Oncol* 2011; **29:** 1949–55.
40 Muenst S, Soysal SD, Tzankov A, Hoeller S. The PD-1/PD-L1 pathway: biological background and clinical relevance of an emerging treatment target in immunotherapy. *Expert Opin Ther Targets* 2015; **19:** 201–11.
41 Mlecnik B, Tosolini M, Kirilovsky A, Berger A, Bindea G, Meatchi T, *et al.* Histopathologic-based prognostic factors of colorectal cancers are associated with the state of the local immune reaction. *J Clinic Oncol* 2011; **29:** 610–8.
42 Al-Shibli KI, Donnem T, Al-Saad S, Persson M, Bremnes RM, Busund LT. Prognostic effect of epithelial and stromal lymphocyte infiltration in non-small cell lung cancer. *Clinic Cancer Res* 2008; **14:** 5220–7.
43 Leffers N, Gooden MJ, de Jong RA, Hoogeboom BN, ten Hoor KA, Hollema H, *et al.* Prognostic significance of tumor-infiltrating T-lymphocytes in primary and metastatic lesions of advanced stage ovarian cancer. *Cancer Immunol, Immunother* 2009; **58:** 449–59.
44 Sharma P, Shen Y, Wen S, Yamada S, Jungbluth AA, Gnjatic S, *et al.* CD8 tumor-infiltrating lymphocytes are predictive of survival in muscle-invasive urothelial carcinoma. *Proc Natl Acad Sci USA* 2007; **104:** 3967–72.
45 Shibuya TY, Nugyen N, McLaren CE, Li KT, Wei WZ, Kim S, *et al.* Clinical significance of poor CD3 response in head and neck cancer. *Clinic Cancer Res* 2002; **8:** 745–51.
46 Vesalainen S, Lipponen P, Talja M, Syrjanen K. Histological grade, perineural infiltration, tumour-infiltrating lymphocytes and apoptosis as determinants of long-term prognosis in prostatic adenocarcinoma. *Eur J Cancer* 1994; **30A:** 1797–803.
47 Pages F, Kirilovsky A, Mlecnik B, Asslaber M, Tosolini M, Bindea G, *et al.* In situ cytotoxic and memory T cells predict outcome in patients with early-stage colorectal cancer. *J Clin Oncol* 2009; **27:** 5944–51.
48 Remark R, Alifano M, Cremer I, Lupo A, Dieu-Nosjean MC, Riquet M, *et al.* Characteristics and clinical impacts of the immune environments in colorectal and renal cell carcinoma lung metastases: influence of tumor origin. *Clinic Cancer Res* 2013; **19:** 4079–91.
49 Copelan EA. Hematopoietic stem-cell transplantation. *N Engl J Med* 2006; **354:** 1813–26.
50 Restifo NP, Dudley ME, Rosenberg SA. Adoptive immunotherapy for cancer: harnessing the T cell response. *Nat Rev Immunol* 2012; **12:** 269–81.
51 Dudley ME, Wunderlich JR, Robbins PF, Yang JC, Hwu P, Schwartzentruber DJ, *et al.* Cancer regression and autoimmunity in patients after clonal repopulation with antitumor lymphocytes. *Science* 2002; **298:** 850–4.
52 Rosenberg SA, Terry WD. Passive immunotherapy of cancer in animals and man. *Adv Cancer Res* 1977; **25:** 323–88.
53 Tran E, Turcotte S, Gros A, Robbins PF, Lu YC, Dudley ME, *et al.* Cancer immunotherapy based on mutation-specific

CD4+ T cells in a patient with epithelial cancer. *Science* 2014; **344:** 641–5.
54 June CH, Maus MV, Plesa G, Johnson LA, Zhao Y, Levine BL, *et al.* Engineered T cells for cancer therapy. *Cancer Immunol, Immunother* 2014; **63:** 969–75.
55 Morgan RA, Dudley ME, Wunderlich JR, Hughes MS, Yang JC, Sherry RM, *et al.* Cancer regression in patients after transfer of genetically engineered lymphocytes. *Science* 2006; **314:** 126–9.
56 Robbins PF, Kassim SH, Tran TL, Crystal JS, Morgan RA, Feldman SA, *et al.* A pilot trial using lymphocytes genetically engineered with an NY-ESO-1-reactive T-cell receptor: long-term follow-up and correlates with response. *Clinic Cancer Res* 2015; **21:** 1019–27.
57 Maude SL, Frey N, Shaw PA, Aplenc R, Barrett DM, Bunin NJ, *et al.* Chimeric antigen receptor T cells for sustained remissions in leukemia. *N Engl J Med* 2014; **371:** 1507–17.
58 Grupp SA, Kalos M, Barrett D, Aplenc R, Porter DL, Rheingold SR, *et al.* Chimeric antigen receptor-modified T cells for acute lymphoid leukemia. *N Engl J Med* 2013; **368:** 1509–18.
59 Lee DW, Kochenderfer JN, Stetler-Stevenson M, Cui YK, Delbrook C, Feldman SA, *et al.* T cells expressing CD19 chimeric antigen receptors for acute lymphoblastic leukaemia in children and young adults: a phase 1 dose-escalation trial. *Lancet* 2015; **385:** 517–28.
60 Kochenderfer JN, Dudley ME, Kassim SH, Somerville RP, Carpenter RO, Stetler-Stevenson M, *et al.* Chemotherapy-refractory diffuse large B-cell lymphoma and indolent B-cell malignancies can be effectively treated with autologous T cells expressing an anti-CD19 chimeric antigen receptor. *J Clinic Oncol* 2015; **33:** 540–9.
61 Maus MV, Haas AR, Beatty GL, Albelda SM, Levine BL, Liu X, *et al.* T cells expressing chimeric antigen receptors can cause anaphylaxis in humans. *Cancer Immunol Res* 2013; **1:** 26–31.
62 Zhao Y, Moon E, Carpenito C, Paulos CM, Liu X, Brennan AL, *et al.* Multiple injections of electroporated autologous T cells expressing a chimeric antigen receptor mediate regression of human disseminated tumor. *Cancer Res* 2010; **70:** 9053–61.
63 Morgan RA, Yang JC, Kitano M, Dudley ME, Laurencot CM, Rosenberg SA. Case report of a serious adverse event following the administration of T cells transduced with a chimeric antigen receptor recognizing ERBB2. *Mol Ther* 2010; **18:** 843–51.
64 Dudley ME, Wunderlich JR, Yang JC, Sherry RM, Topalian SL, Restifo NP, *et al.* Adoptive cell transfer therapy following non-myeloablative but lymphodepleting chemotherapy for the treatment of patients with refractory metastatic melanoma. *J Clinic Oncol* 2005; **23:** 2346–57.
65 Dudley ME, Yang JC, Sherry R, Hughes MS, Royal R, Kammula U, *et al.* Adoptive cell therapy for patients with metastatic melanoma: evaluation of intensive myeloablative chemoradiation preparative regimens. *J Clinic Oncol* 2008; **26:** 5233–9.
66 Kontermann RE, Brinkmann U. Bispecific antibodies. *Drug Discov Today* 2015; **20:** 838–47.
67 Topp MS, Gokbuget N, Stein AS, Zugmaier G, O'Brien S, Bargou RC, *et al.* Safety and activity of blinatumomab for adult patients with relapsed or refractory B-precursor acute lymphoblastic leukaemia: a multicentre, single-arm, phase 2 study. *Lancet Oncol* 2015; **16:** 57–66.
68 Topp MS, Gokbuget N, Zugmaier G, Klappers P, Stelljes M, Neumann S, *et al.* Phase II trial of the anti-CD19 bispecific T cell-engager blinatumomab shows hematologic and molecular remissions in patients with relapsed or refractory B-precursor acute lymphoblastic leukemia. *J Clinic Oncol* 2014; **32:** 4134–40.
69 Colombo MP, Piconese S. Regulatory-T-cell inhibition versus depletion: the right choice in cancer immunotherapy. *Nat Rev Cancer* 2007; **7:** 880–7.
70 Sugiyama D, Nishikawa H, Maeda Y, Nishioka M, Tanemura A, Katayama I, *et al.* Anti-CCR4 mAb selectively depletes effector-type FoxP3+CD4+ regulatory T cells, evoking anti-tumor immune responses in humans. *Proc Natl Acad Sci USA* 2013; **110:** 17945–50.
71 Le DT, Jaffee EM. Regulatory T-cell modulation using cyclophosphamide in vaccine approaches: a current perspective. *Cancer Res* 2012; **72:** 3439–44.
72 Bruno A, Ferlazzo G, Albini A, Noonan DM. A think tank of TINK/TANKs: tumor-infiltrating/tumor-associated natural killer cells in tumor progression and angiogenesis. *J Natl Cancer Inst* 2014; **106:** dju200.
73 Imai K, Matsuyama S, Miyake S, Suga K, Nakachi K. Natural cytotoxic activity of peripheral-blood lymphocytes and cancer incidence: an 11-year follow-up study of a general population. *Lancet* 2000; **356:** 1795–9.
74 Burke S, Lakshmikanth T, Colucci F, Carbone E. New views on natural killer cell-based immunotherapy for melanoma treatment. *Trends Immunol* 2010; **31:** 339–45.
75 Larsen SK, Gao Y, Basse PH. NK cells in the tumor microenvironment. *Crit Rev Oncog* 2014; **19:** 91–105.
76 Matta J, Baratin M, Chiche L, Forel JM, Cognet C, Thomas G, *et al.* Induction of B7-H6, a ligand for the natural killer cell-activating receptor NKp30, in inflammatory conditions. *Blood* 2013; **122:** 394–404.
77 Moretta L, Montaldo E, Vacca P, Del Zotto G, Moretta F, Merli P, *et al.* Human natural killer cells: origin, receptors, function, and clinical applications. *Int Arch Allergy Immunol* 2014; **164:** 253–64.
78 Tallerico R, Todaro M, Di Franco S, Maccalli C, Garofalo C, Sottile R, *et al.* Human NK cells selective targeting of colon cancer-initiating cells: a role for natural cytotoxicity receptors and MHC class I molecules. *J Immunol* 2013; **190:** 2381–90.
79 Vitale M, Cantoni C, Pietra G, Mingari MC, Moretta L. Effect of tumor cells and tumor microenvironment on NK-cell function. *Eur J Immunol* 2014; **44:** 1582–92.
80 Gross E, Sunwoo JB, Bui JD. Cancer immunosurveillance and immunoediting by natural killer cells. *Cancer J* 2013; **19:** 483–9.
81 Balsamo M, Manzini C, Pietra G, Raggi F, Blengio F, Mingari MC, *et al.* Hypoxia downregulates the expression of activating receptors involved in NK-cell-mediated target cell killing without affecting ADCC. *Eur J Immunol* 2013; **43:** 2756–64.
82 Sungur CM, Murphy WJ. Positive and negative regulation by NK cells in cancer. *Crit Rev Oncog* 2014; **19:** 57–66.
83 Balsamo M, Vermi W, Parodi M, Pietra G, Manzini C, Queirolo P, *et al.* Melanoma cells become resistant to NK-cell-mediated killing when exposed to NK-cell numbers compatible with NK-cell infiltration in the tumor. *Eur J Immunol* 2012; **42:** 1833–42.
84 Dong HP, Elstrand MB, Holth A, Silins I, Berner A, Trope CG, *et al.* NK- and B-cell infiltration correlates with worse

outcome in metastatic ovarian carcinoma. *Am J Clin Pathol* 2006; **125:** 451–8.
85 Rathore AS, Goel MM, Makker A, Kumar S, Srivastava AN. Is the tumor infiltrating natural killer cell (NK-TILs) count in infiltrating ductal carcinoma of breast prognostically significant? *Asian Pac J Cancer Prev* 2014; **15:** 3757–61.
86 Leung W. Infusions of allogeneic natural killer cells as cancer therapy. *Clinic Cancer Res* 2014; **20:** 3390–400.
87 Vivier E, Ugolini S, Blaise D, Chabannon C, Brossay L. Targeting natural killer cells and natural killer T cells in cancer. *Nat Rev Immunol* 2012; **12:** 239–52.
88 Terme M, Ullrich E, Delahaye NF, Chaput N, Zitvogel L. Natural killer cell-directed therapies: moving from unexpected results to successful strategies. *Nat Immunol* 2008; **9:** 486–94.
89 Koehl U, Sorensen J, Esser R, Zimmermann S, Gruttner HP, Tonn T, *et al.* IL-2 activated NK cell immunotherapy of three children after haploidentical stem cell transplantation. *Blood Cells Mol Dis* 2004; **33:** 261–6.
90 Stern M, Passweg JR, Meyer-Monard S, Esser R, Tonn T, Soerensen J, *et al.* Pre-emptive immunotherapy with purified natural killer cells after haploidentical SCT: a prospective phase II study in two centers. *Bone Marrow Transplant* 2013; **48:** 433–8.
91 Miller JS, Soignier Y, Panoskaltsis-Mortari A, McNearney SA, Yun GH, Fautsch SK, *et al.* Successful adoptive transfer and in vivo expansion of human haploidentical NK cells in patients with cancer. *Blood* 2005; **105:** 3051–7.
92 Giaccone G, Punt CJ, Ando Y, Ruijter R, Nishi N, Peters M, *et al.* A phase I study of the natural killer T-cell ligand alpha-galactosylceramide (KRN7000) in patients with solid tumors. *Clinic Cancer Res* 2002; **8:** 3702–9.
93 Kunii N, Horiguchi S, Motohashi S, Yamamoto H, Ueno N, Yamamoto S, *et al.* Combination therapy of in vitro-expanded natural killer T cells and alpha-galactosylceramide-pulsed antigen-presenting cells in patients with recurrent head and neck carcinoma. *Cancer Sci* 2009; **100:** 1092–8.
94 Romagne F, Andre P, Spee P, Zahn S, Anfossi N, Gauthier L, *et al.* Preclinical characterization of 1-7F9, a novel human anti-KIR receptor therapeutic antibody that augments natural killer-mediated killing of tumor cells. *Blood* 2009; **114:** 2667–77.
95 Thielens A, Vivier E, Romagne F. NK cell MHC class I specific receptors (KIR): from biology to clinical intervention. *Curr Opin Immunol* 2012; **24:** 239–45.
96 Alici E. IPH-2101, a fully human anti-NK-cell inhibitory receptor mAb for the potential treatment of hematological cancers. *Curr Opin Mol Ther* 2010; **12:** 724–33.
97 Knorr DA, Bachanova V, Verneris MR, Miller JS. Clinical utility of natural killer cells in cancer therapy and transplantation. *Semin Immunol* 2014; **26:** 161–72.
98 Chanmee T, Ontong P, Konno K, Itano N. Tumor-associated macrophages as major players in the tumor microenvironment. *Cancers (Basel)* 2014; **6:** 1670–90.
99 Noy R, Pollard JW. Tumor-associated macrophages: from mechanisms to therapy. *Immunity* 2014; **41:** 49–61.
100 Kubler K, Ayub TH, Weber SK, Zivanovic O, Abramian A, Keyver-Paik MD, *et al.* Prognostic significance of tumor-associated macrophages in endometrial adenocarcinoma. *Gynecol Oncol* 2014; **135:** 176–83.
101 Nabeshima A, Matsumoto Y, Fukushi J, Iura K, Matsunobu T, Endo M, *et al.* Tumour-associated macrophages correlate with poor prognosis in myxoid liposarcoma and promote cell motility and invasion via the HB-EGF-EGFR-PI3K/Akt pathways. *Br J Cancer* 2015; **112:** 547–55.
102 Yuan ZY, Luo RZ, Peng RJ, Wang SS, Xue C. High infiltration of tumor-associated macrophages in triple-negative breast cancer is associated with a higher risk of distant metastasis. *Onco Targets Ther* 2014; **7:** 1475–80.
103 Tzankov A, Matter MS, Dirnhofer S. Refined prognostic role of CD68-positive tumor macrophages in the context of the cellular micromilieu of classical Hodgkin lymphoma. *Pathobiology* 2010; **77:** 301–8.
104 Gulubova M, Ananiev J, Yovchev Y, Julianov A, Karashmalakov A, Vlaykova T. The density of macrophages in colorectal cancer is inversely correlated to TGF-beta1 expression and patients' survival. *J Mol Histol* 2013; **44:** 679–92.
105 Riihijarvi S, Fiskvik I, Taskinen M, Vajavaara H, Tikkala M, Yri O, *et al.* Prognostic influence of macrophages in patients with diffuse large B-cell lymphoma: a correlative study from a Nordic phase II trial. *Haematologica* 2015; **100:** 238–45.
106 Hannesdottir L, Tymoszuk P, Parajuli N, Wasmer MH, Philipp S, Daschil N, *et al.* Lapatinib and doxorubicin enhance the Stat1-dependent antitumor immune response. *Eur J Immunol* 2013; **43:** 2718–29.
107 Mantovani A, Allavena P. The interaction of anticancer therapies with tumor-associated macrophages. *J Exp Med* 2015; **212:** 435–45.
108 Pyonteck SM, Akkari L, Schuhmacher AJ, Bowman RL, Sevenich L, Quail DF, *et al.* CSF-1R inhibition alters macrophage polarization and blocks glioma progression. *Nat Med* 2013; **19:** 1264–72.
109 Weiskopf K, Ring AM, Ho CC, Volkmer JP, Levin AM, Volkmer AK, *et al.* Engineered SIRPalpha variants as immunotherapeutic adjuvants to anticancer antibodies. *Science* 2013; **341:** 88–91.
110 da Cunha A, Michelin MA, Murta EF. Pattern response of dendritic cells in the tumor microenvironment and breast cancer. *World J Clin Oncol* 2014; **5:** 495–502.
111 Steinman RM, Hawiger D, Nussenzweig MC. Tolerogenic dendritic cells. *Annu Rev Immunol* 2003; **21:** 685–711.
112 Benencia F, Muccioli M, Alnaeeli M. Perspectives on reprograming cancer-associated dendritic cells for anti-tumor therapies. *Front Oncol* 2014; **4:** 72.
113 Fainaru O, Almog N, Yung CW, Nakai K, Montoya-Zavala M, Abdollahi A, *et al.* Tumor growth and angiogenesis are dependent on the presence of immature dendritic cells. *FASEB J* 2010; **24:** 1411–8.
114 Lotze MT. Getting to the source: dendritic cells as therapeutic reagents for the treatment of patients with cancer. *Ann Surg* 1997; **226:** 1–5.
115 Tel J, Anguille S, Waterborg CE, Smits EL, Figdor CG, de Vries IJ. Tumoricidal activity of human dendritic cells. *Trends Immunol* 2014; **35:** 38–46.
116 Melero I, Gaudernack G, Gerritsen W, Huber C, Parmiani G, Scholl S, *et al.* Therapeutic vaccines for cancer: an overview of clinical trials. *Nat Rev Clin Oncol* 2014; **11:** 509–24.
117 Kantoff PW, Higano CS, Shore ND, Berger ER, Small EJ, Penson DF, *et al.* Sipuleucel-T immunotherapy for castration-resistant prostate cancer. *N Engl J Med* 2010; **363:** 411–22.
118 Higano CS, Small EJ, Schellhammer P, Yasothan U, Gubernick S, Kirkpatrick P, *et al.* Sipuleucel-T. *Nat Rev Drug Discov* 2010; **9:** 513–4.

119 Butts C, Socinski MA, Mitchell PL, Thatcher N, Havel L, Krzakowski M, *et al.* Tecemotide (L-BLP25) versus placebo after chemoradiotherapy for stage III non-small-cell lung cancer (START): a randomised, double-blind, phase 3 trial. *Lancet Oncol* 2014; **15:** 59–68.

120 Ulloa-Montoya F, Louahed J, Dizier B, Gruselle O, Spiessens B, Lehmann FF, *et al.* Predictive gene signature in MAGE-A3 antigen-specific cancer immunotherapy. *J Clinic Oncol* 2013; **31:** 2388–95.

121 Hardacre JM, Mulcahy M, Small W, Talamonti M, Obel J, Krishnamurthi S, *et al.* Addition of algenpantucel-L immunotherapy to standard adjuvant therapy for pancreatic cancer: a phase 2 study. *J Gastrointest Surg* 2013; **17:** 94–100; discussion p -1.

122 Pedoeem A, Azoulay-Alfaguter I, Strazza M, Silverman GJ, Mor A. Programmed death-1 pathway in cancer and autoimmunity. *Clin Immunol* 2014; **153:** 145–52.

123 Intlekofer AM, Thompson CB. At the bench: preclinical rationale for CTLA-4 and PD-1 blockade as cancer immunotherapy. *J Leukoc Biol* 2013; **94:** 25–39.

124 Walunas TL, Lenschow DJ, Bakker CY, Linsley PS, Freeman GJ, Green JM, *et al.* CTLA-4 can function as a negative regulator of T cell activation. *Immunity* 1994; **1:** 405–13.

125 Watanabe N, Gavrieli M, Sedy JR, Yang J, Fallarino F, Loftin SK, *et al.* BTLA is a lymphocyte inhibitory receptor with similarities to CTLA-4 and PD-1. *Nat Immunol* 2003; **4:** 670–9.

126 Sznol M, Chen L. Antagonist antibodies to PD-1 and B7-H1 (PD-L1) in the treatment of advanced human cancer. *Clinic Cancer Res* 2013; **19:** 1021–34.

127 Topalian SL, Drake CG, Pardoll DM. Immune checkpoint blockade: a common denominator approach to cancer therapy. *Cancer Cell* 2015; **27:** 450–61.

128 Ishida Y, Agata Y, Shibahara K, Honjo T. Induced expression of PD-1, a novel member of the immunoglobulin gene superfamily, upon programmed cell death. *EMBO J* 1992; **11:** 3887–95.

129 Zhang X, Schwartz JC, Guo X, Bhatia S, Cao E, Lorenz M, *et al.* Structural and functional analysis of the costimulatory receptor programmed death-1. *Immunity* 2004; **20:** 337–47.

130 Dai S, Jia R, Zhang X, Fang Q, Huang L. The PD-1/PD-Ls pathway and autoimmune diseases. *Cell Immunol* 2014; **290:** 72–9.

131 Sznol M, Chen L. Antagonist antibodies to PD-1 and B7-H1 (PD-L1) in the treatment of advanced human cancer–response. *Clinic Cancer Res* 2013; **19:** 5542.

132 Shi L, Chen S, Yang L, Li Y. The role of PD-1 and PD-L1 in T-cell immune suppression in patients with hematological malignancies. *J Hematol Oncol* 2013; **6:** 74.

133 Jin HT, Ahmed R, Okazaki T. Role of PD-1 in regulating T-cell immunity. *Curr Top Microbiol Immunol* 2011; **350:** 17–37.

134 Latchman Y, Wood CR, Chernova T, Chaudhary D, Borde M, Chernova I, *et al.* PD-L2 is a second ligand for PD-1 and inhibits T cell activation. *Nat Immunol* 2001; **2:** 261–8.

135 Kitazawa Y, Fujino M, Wang Q, Kimura H, Azuma M, Kubo M, *et al.* Involvement of the programmed death-1/programmed death-1 ligand pathway in CD4+CD25+ regulatory T-cell activity to suppress alloimmune responses. *Transplantation* 2007; **83:** 774–82.

136 Fife BT, Pauken KE. The role of the PD-1 pathway in autoimmunity and peripheral tolerance. *Ann N Y Acad Sci* 2011; **1217:** 45–59.

137 Konishi J, Yamazaki K, Azuma M, Kinoshita I, Dosaka-Akita H, Nishimura M. B7-H1 expression on non-small cell lung cancer cells and its relationship with tumor-infiltrating lymphocytes and their PD-1 expression. *Clinic Cancer Res* 2004; **10:** 5094–100.

138 Nomi T, Sho M, Akahori T, Hamada K, Kubo A, Kanehiro H, *et al.* Clinical significance and therapeutic potential of the programmed death-1 ligand/programmed death-1 pathway in human pancreatic cancer. *Clinic Cancer Res* 2007; **13:** 2151–7.

139 Ohigashi Y, Sho M, Yamada Y, Tsurui Y, Hamada K, Ikeda N, *et al.* Clinical significance of programmed death-1 ligand-1 and programmed death-1 ligand-2 expression in human esophageal cancer. *Clinic Cancer Res* 2005; **11:** 2947–53.

140 Chen BJ, Chapuy B, Ouyang J, Sun HH, Roemer MG, Xu ML, *et al.* PD-L1 expression is characteristic of a subset of aggressive B-cell lymphomas and virus-associated malignancies. *Clinic Cancer Res* 2013; **19:** 3462–73.

141 Muenst S, Hoeller S, Willi N, Dirnhofera S, Tzankov A. Diagnostic and prognostic utility of PD-1 in B cell lymphomas. *Dis Markers* 2010; **29:** 47–53.

142 Dorfman DM, Brown JA, Shahsafaei A, Freeman GJ. Programmed death-1 (PD-1) is a marker of germinal center-associated T cells and angioimmunoblastic T-cell lymphoma. *Am J Surg Pathol* 2006; **30:** 802–10.

143 Xerri L, Chetaille B, Serriari N, Attias C, Guillaume Y, Arnoulet C, *et al.* Programmed death 1 is a marker of angioimmunoblastic T-cell lymphoma and B-cell small lymphocytic lymphoma/chronic lymphocytic leukemia. *Hum Pathol* 2008; **39:** 1050–8.

144 Dong H, Strome SE, Salomao DR, Tamura H, Hirano F, Flies DB, *et al.* Tumor-associated B7-H1 promotes T-cell apoptosis: a potential mechanism of immune evasion. *Nat Med* 2002; **8:** 793–800.

145 Muenst S, Schaerli AR, Gao F, Daster S, Trella E, Droeser RA, *et al.* Expression of programmed death ligand 1 (PD-L1) is associated with poor prognosis in human breast cancer. *Breast Cancer Res Treat* 2014; **146:** 15–24.

146 Thompson RH, Gillett MD, Cheville JC, Lohse CM, Dong H, Webster WS, *et al.* Costimulatory B7-H1 in renal cell carcinoma patients: Indicator of tumor aggressiveness and potential therapeutic target. *Proc Natl Acad Sci USA* 2004; **101:** 17174–9.

147 Ishida M, Iwai Y, Tanaka Y, Okazaki T, Freeman GJ, Minato N, *et al.* Differential expression of PD-L1 and PD-L2, ligands for an inhibitory receptor PD-1, in the cells of lymphohematopoietic tissues. *Immunol Lett* 2002; **84:** 57–62.

148 Iwai Y, Ishida M, Tanaka Y, Okazaki T, Honjo T, Minato N. Involvement of PD-L1 on tumor cells in the escape from host immune system and tumor immunotherapy by PD-L1 blockade. *Proc Natl Acad Sci USA* 2002; **99:** 12293–7.

149 Azuma T, Yao S, Zhu G, Flies AS, Flies SJ, Chen L. B7-H1 is a ubiquitous antiapoptotic receptor on cancer cells. *Blood* 2008; **111:** 3635–43.

150 Hamanishi J, Mandai M, Iwasaki M, Okazaki T, Tanaka Y, Yamaguchi K, *et al.* Programmed cell death 1 ligand 1 and tumor-infiltrating CD8+ T lymphocytes are prognostic factors of human ovarian cancer. *Proc Natl Acad Sci USA* 2007; **104:** 3360–5.

151 Nakanishi J, Wada Y, Matsumoto K, Azuma M, Kikuchi K, Ueda S. Overexpression of B7-H1 (PD-L1) significantly associates with tumor grade and postoperative prognosis

in human urothelial cancers. *Cancer Immunol, Immunother* 2007; **56:** 1173–82.
152 Wu C, Zhu Y, Jiang J, Zhao J, Zhang XG, Xu N. Immunohistochemical localization of programmed death-1 ligand-1 (PD-L1) in gastric carcinoma and its clinical significance. *Acta Histochem* 2006; **108:** 19–24.
153 Blank C, Gajewski TF, Mackensen A. Interaction of PD-L1 on tumor cells with PD-1 on tumor-specific T cells as a mechanism of immune evasion: implications for tumor immunotherapy. *Cancer Immunol, Immunother* 2005; **54:** 307–14.
154 Hodi FS, Mihm MC, Soiffer RJ, Haluska FG, Butler M, Seiden MV, *et al.* Biologic activity of cytotoxic T lymphocyte-associated antigen 4 antibody blockade in previously vaccinated metastatic melanoma and ovarian carcinoma patients. *Proc Natl Acad Sci USA* 2003; **100:** 4712–7.
155 Phan GQ, Yang JC, Sherry RM, Hwu P, Topalian SL, Schwartzentruber DJ, *et al.* Cancer regression and autoimmunity induced by cytotoxic T lymphocyte-associated antigen 4 blockade in patients with metastatic melanoma. *Proc Natl Acad Sci USA* 2003; **100:** 8372–7.
156 Hodi FS, O'Day SJ, McDermott DF, Weber RW, Sosman JA, Haanen JB, *et al.* Improved survival with ipilimumab in patients with metastatic melanoma. *N Engl J Med* 2010; **363:** 711–23.
157 Robert C, Thomas L, Bondarenko I, O'Day S, Weber J, Garbe C, *et al.* Ipilimumab plus dacarbazine for previously untreated metastatic melanoma. *N Engl J Med* 2011; **364:** 2517–26.
158 Schadendorf D, Hodi FS, Robert C, Weber JS, Margolin K, Hamid O, *et al.* Pooled analysis of long-term survival data from Phase II and Phase III trials of ipilimumab in unresectable or metastatic melanoma. *J Clinic Oncol* 2015; **10:** 1889–94.
159 Eggermont AM, Chiarion-Sileni V, Grob JJ, Dummer R, Wolchok JD, Schmidt H, *et al.* Adjuvant ipilimumab versus placebo after complete resection of high-risk stage III melanoma (EORTC 18071): a randomised, double-blind, phase 3 trial. *Lancet Oncol* 2015; **16:** 522–30.
160 Snyder A, Makarov V, Merghoub T, Yuan J, Zaretsky JM, Desrichard A, *et al.* Genetic basis for clinical response to CTLA-4 blockade in melanoma. *N Engl J Med* 2014; **371:** 2189–99.
161 Ribas A, Hodi FS, Callahan M, Konto C, Wolchok J. Hepatotoxicity with combination of vemurafenib and ipilimumab. *N Engl J Med* 2013; **368:** 1365–6.
162 Weber JS, D'Angelo SP, Minor D, Hodi FS, Gutzmer R, Neyns B, *et al.* Nivolumab versus chemotherapy in patients with advanced melanoma who progressed after anti-CTLA-4 treatment (CheckMate 037): a randomised, controlled, open-label, phase 3 trial. *Lancet Oncol* 2015; **16:** 375–84.
163 Robert C, Long GV, Brady B, Dutriaux C, Maio M, Mortier L, *et al.* Nivolumab in previously untreated melanoma without BRAF mutation. *N Engl J Med* 2015; **372:** 320–30.
164 Rizvi NA, Mazieres J, Planchard D, Stinchcombe TE, Dy GK, Antonia SJ, *et al.* Activity and safety of nivolumab, an anti-PD-1 immune checkpoint inhibitor, for patients with advanced, refractory squamous non-small-cell lung cancer (CheckMate 063): a phase 2, single-arm trial. *Lancet Oncol* 2015; **16:** 257–65.
165 Brahmer J, Reckamp KL, Baas P, Crino L, Eberhardt WE, Poddubskaya E, *et al.* Nivolumab versus Docetaxel in Advanced Squamous-Cell Non-Small-Cell Lung Cancer. *N Engl J Med* 2015; **373:** 123–35.
166 Borghaei H, Paz-Ares L, Horn L, Spigel DR, Steins M, Ready NE, *et al.* Nivolumab versus Docetaxel in Advanced Nonsquamous Non-Small-Cell Lung Cancer. *N Engl J Med* 2015; **373:** 1627–39.
167 Motzer RJ, Rini BI, McDermott DF, Redman BG, Kuzel TM, Harrison MR, *et al.* Nivolumab for metastatic renal cell carcinoma: results of a randomized Phase II trial. *J Clinic Oncol* 2014; **1:** 1430–7.
168 Motzer RJ, Escudier B, McDermott DF, George S, Hammers HJ, Srinivas S, *et al.* Nivolumab versus Everolimus in Advanced Renal-Cell Carcinoma. *N Engl J Med* 2015; **373:** 1803–13.
169 Ansell SM, Lesokhin AM, Borrello I, Halwani A, Scott EC, Gutierrez M, *et al.* PD-1 blockade with nivolumab in relapsed or refractory Hodgkin's lymphoma. *N Engl J Med* 2015; **372:** 311–9.
170 Wolchok JD, Kluger H, Callahan MK, Postow MA, Rizvi NA, Lesokhin AM, *et al.* Nivolumab plus ipilimumab in advanced melanoma. *N Engl J Med* 2013; **369:** 122–33.
171 Postow MA, Callahan MK, Wolchok JD. Immune checkpoint blockade in cancer therapy. *J Clinic Oncol* 2015; **10:** 1974–82.
172 Robert C, Ribas A, Wolchok JD, Hodi FS, Hamid O, Kefford R, *et al.* Anti-programmed-death-receptor-1 treatment with pembrolizumab in ipilimumab-refractory advanced melanoma: a randomised dose-comparison cohort of a phase 1 trial. *Lancet* 2014; **384:** 1109–17.
173 Robert C, Schachter J, Long GV, Arance A, Grob JJ, Mortier L, *et al.* Pembrolizumab versus Ipilimumab in Advanced Melanoma. *N Engl J Med* 2015; **25:** 2521–32.
174 Garon EB, Rizvi NA, Hui R, Leighl N, Balmanoukian AS, Eder JP, *et al.* Pembrolizumab for the Treatment of Non-Small-Cell Lung Cancer. *N Engl J Med* 2015; **21:** 2018–28.
175 Topalian SL, Hodi FS, Brahmer JR, Gettinger SN, Smith DC, McDermott DF, *et al.* Safety, activity, and immune correlates of anti-PD-1 antibody in cancer. *N Engl J Med* 2012; **366:** 2443–54.
176 Powles T, Eder JP, Fine GD, Braiteh FS, Loriot Y, Cruz C, *et al.* MPDL3280A (anti-PD-L1) treatment leads to clinical activity in metastatic bladder cancer. *Nature* 2014; **515:** 558–62.
177 Herbst RS, Soria JC, Kowanetz M, Fine GD, Hamid O, Gordon MS, *et al.* Predictive correlates of response to the anti-PD-L1 antibody MPDL3280A in cancer patients. *Nature* 2014; **515:** 563–7.
178 Tumeh PC, Harview CL, Yearley JH, Shintaku IP, Taylor EJ, Robert L, *et al.* PD-1 blockade induces responses by inhibiting adaptive immune resistance. *Nature* 2014; **515:** 568–71.
179 Chen DS, Mellman I. Oncology meets immunology: the cancer-immunity cycle. *Immunity* 2013; **39:** 1–10.
180 Rabinovich GA, Croci DO. Regulatory circuits mediated by lectin-glycan interactions in autoimmunity and cancer. *Immunity* 2012; **36:** 322–35.
181 Fuster MM, Esko JD. The sweet and sour of cancer: glycans as novel therapeutic targets. *Nat Rev Cancer* 2005; **5:** 526–42.
182 Boligan KF, Mesa C, Fernandez LE, von Gunten S. Cancer intelligence acquired (CIA): tumor glycosylation and

sialylation codes dismantling antitumor defense. *Cell Mol Life Sci* 2015; **72:** 1231–48.
183 Varki A, Kannagi R, Toole BP. Glycosylation Changes in Cancer. In: Varki A, Cummings RD, Esko JD, Freeze HH, Stanley P, Bertozzi CR, *et al.*, eds. *Essentials of Glycobiology*. 2nd ed. NY Cold Spring Harbor Laboratory Press 2009 Chapter 44.
184 Samraj AN, Laubli H, Varki N, Varki A. Involvement of a non-human sialic Acid in human cancer. *Front Oncol* 2014; **4:** 33.
185 Laubli H, Borsig L. Selectins promote tumor metastasis. *Semin Cancer Biol* 2010; **20:** 169–77.
186 Shi WX, Chammas R, Varki NM, Powell L, Varki A. Sialic acid 9-O-acetylation on murine erythroleukemia cells affects complement activation, binding to I-type lectins, and tissue homing. *J Biol Chem* 1996; **271:** 31526–32.
187 Samraj AN, Pearce OM, Laubli H, Crittenden AN, Bergfeld AK, Banda K, *et al.* A red meat-derived glycan promotes inflammation and cancer progression. *Proc Natl Acad Sci USA* 2015; **112:** 542–7.
188 Jandus C, Boligan KF, Chijioke O, Liu H, Dahlhaus M, Demoulins T, *et al.* Interactions between Siglec-7/9 receptors and ligands influence NK cell-dependent tumor immunosurveillance. *J Clin Investig* 2014; **124:** 1810–20.
189 Laubli H, Alisson-Silva F, Stanczak MA, Siddiqui SS, Deng L, Verhagen A, *et al.* Lectin galactoside-binding soluble 3 binding protein (LGALS3BP) is a tumor-associated immunomodulatory ligand for CD33-related Siglecs. *J Biol Chem* 2014; **289:** 33481–91.
190 Laubli H, Pearce OM, Schwarz F, Siddiqui SS, Deng L, Stanczak MA, *et al.* Engagement of myelomonocytic Siglecs by tumor-associated ligands modulates the innate immune response to cancer. *Proc Natl Acad Sci USA* 2014; **111:** 14211–6.
191 Hudak JE, Canham SM, Bertozzi CR. Glycocalyx engineering reveals a Siglec-based mechanism for NK cell immunoevasion. *Nat Chem Biol* 2014; **10:** 69–75.
192 Austin CJ, Rendina LM. Targeting key dioxygenases in tryptophan-kynurenine metabolism for immunomodulation and cancer chemotherapy. *Drug Discov Today* 2014; **20:** 609–17.
193 Platten M, von Knebel Doeberitz N, Oezen I, Wick W, Ochs K. Cancer Immunotherapy by Targeting IDO1/TDO and Their Downstream Effectors. *Front Immunol* 2014; **5:** 673.
194 Liu X, Shin N, Koblish HK, Yang G, Wang Q, Wang K, *et al.* Selective inhibition of IDO1 effectively regulates mediators of antitumor immunity. *Blood* 2010; **115:** 3520–30.
195 Rosenberg SA. IL-2: the first effective immunotherapy for human cancer. *J Immunol* 2014; **192:** 5451–8.
196 Rosenberg SA, Lotze MT, Muul LM, Leitman S, Chang AE, Ettinghausen SE, *et al.* Observations on the systemic administration of autologous lymphokine-activated killer cells and recombinant interleukin-2 to patients with metastatic cancer. *N Engl J Med* 1985; **313:** 1485–92.
197 Yang JC, Topalian SL, Parkinson D, Schwartzentruber DJ, Weber JS, Ettinghausen SE, *et al.* Randomized comparison of high-dose and low-dose intravenous interleukin-2 for the therapy of metastatic renal cell carcinoma: an interim report. *J Clinic Oncol* 1994; **12:** 1572–6.
198 Fyfe GA, Fisher RI, Rosenberg SA, Sznol M, Parkinson DR, Louie AC. Long-term response data for 255 patients with metastatic renal cell carcinoma treated with high-dose recombinant interleukin-2 therapy. *J Clinic Oncol* 1996; **14:** 2410–1.
199 Shablak A, Sikand K, Shanks JH, Thistlethwaite F, Spencer-Shaw A, Hawkins RE. High-dose interleukin-2 can produce a high rate of response and durable remissions in appropriately selected patients with metastatic renal cancer. *J Immunother* 2011; **34:** 107–12.
200 Dummer R, Hauschild A, Guggenheim M, Keilholz U, Pentheroudakis G, Group EGW. Cutaneous melanoma: ESMO Clinical Practice Guidelines for diagnosis, treatment and follow-up. *Ann Oncol* 2012; **23(Suppl 7):** vii86–91.
201 Kirkwood JM, Strawderman MH, Ernstoff MS, Smith TJ, Borden EC, Blum RH. Interferon alfa-2b adjuvant therapy of high-risk resected cutaneous melanoma: the Eastern Cooperative Oncology Group Trial EST 1684. *J Clinic Oncol* 1996; **14:** 7–17.
202 Eggermont AM, Suciu S, Santinami M, Testori A, Kruit WH, Marsden J, *et al.* Adjuvant therapy with pegylated interferon alfa-2b versus observation alone in resected stage III melanoma: final results of EORTC 18991, a randomised phase III trial. *Lancet* 2008; **372:** 117–26.
203 Li Y, Rogoff HA, Keates S, Gao Y, Murikipudi S, Mikule K, *et al.* Suppression of cancer relapse and metastasis by inhibiting cancer stemness. *Proc Natl Acad Sci USA* 2015; **112:** 1839–44.
204 Bai L, Wang S. Targeting apoptosis pathways for new cancer therapeutics. *Annu Rev Med* 2014; **65:** 139–55.
205 Hausler SF, Del Barrio IM, Diessner J, Stein RG, Strohschein J, Honig A, *et al.* Anti-CD39 and anti-CD73 antibodies A1 and 7G2 improve targeted therapy in ovarian cancer by blocking adenosine-dependent immune evasion. *Am J Transl Res* 2014; **6:** 129–39.
206 Rosenberg SA, Packard BS, Aebersold PM, Solomon D, Topalian SL, Toy ST, *et al.* Use of tumor-infiltrating lymphocytes and interleukin-2 in the immunotherapy of patients with metastatic melanoma. A preliminary report. *N Engl J Med* 1988; **319:** 1676–80.
207 Rosenberg SA, Yannelli JR, Yang JC, Topalian SL, Schwartzentruber DJ, Weber JS, *et al.* Treatment of patients with metastatic melanoma with autologous tumor-infiltrating lymphocytes and interleukin 2. *J Natl Cancer Inst* 1994; **86:** 1159–66.
208 Rosenberg SA, Yang JC, Sherry RM, Kammula US, Hughes MS, Phan GQ, *et al.* Durable complete responses in heavily pretreated patients with metastatic melanoma using T-cell transfer immunotherapy. *Clinic Cancer Res* 2011; **17:** 4550–7.
209 Pilon-Thomas S, Kuhn L, Ellwanger S, Janssen W, Royster E, Marzban S, *et al.* Efficacy of adoptive cell transfer of tumor-infiltrating lymphocytes after lymphopenia induction for metastatic melanoma. *J Immunother* 2012; **35:** 615–20.
210 Besser MJ, Shapira-Frommer R, Itzhaki O, Treves AJ, Zippel DB, Levy D, *et al.* Adoptive transfer of tumor-infiltrating lymphocytes in patients with metastatic melanoma: intent-to-treat analysis and efficacy after failure to prior immunotherapies. *Clinic Cancer Res* 2013; **19:** 4792–800.
211 Stevanovic S, Draper LM, Langhan MM, Campbell TE, Kwong ML, Wunderlich JR, *et al.* Complete regression of metastatic cervical cancer after treatment with human papillomavirus-targeted tumor-infiltrating T cells. *J Clinic Oncol* 2015; **33:** 1543–50.

212 Hunder NN, Wallen H, Cao J, Hendricks DW, Reilly JZ, Rodmyre R, *et al.* Treatment of metastatic melanoma with autologous CD4+ T cells against NY-ESO-1. *N Engl J Med* 2008; **358:** 2698–703.
213 Chapuis AG, Ragnarsson GB, Nguyen HN, Chaney CN, Pufnock JS, Schmitt TM, *et al.* Transferred WT1-reactive CD8+ T cells can mediate antileukemic activity and persist in post-transplant patients. *Sci Transl Med* 2013; **5:** 174ra27.
214 Johnson LA, Morgan RA, Dudley ME, Cassard L, Yang JC, Hughes MS, *et al.* Gene therapy with human and mouse T-cell receptors mediates cancer regression and targets normal tissues expressing cognate antigen. *Blood* 2009; **114:** 535–46.
215 Robbins PF, Morgan RA, Feldman SA, Yang JC, Sherry RM, Dudley ME, *et al.* Tumor regression in patients with metastatic synovial cell sarcoma and melanoma using genetically engineered lymphocytes reactive with NY-ESO-1. *J Clinic Oncol* 2011; **29:** 917–24.
216 Kochenderfer JN, Wilson WH, Janik JE, Dudley ME, Stetler-Stevenson M, Feldman SA, *et al.* Eradication of B-lineage cells and regression of lymphoma in a patient treated with autologous T cells genetically engineered to recognize CD19. *Blood* 2010; **116:** 4099–102.
217 Kalos M, Levine BL, Porter DL, Katz S, Grupp SA, Bagg A, *et al.* T cells with chimeric antigen receptors have potent antitumor effects and can establish memory in patients with advanced leukemia. *Sci Transl Med* 2011; **3:** 95ra73.
218 Brentjens RJ, Davila ML, Riviere I, Park J, Wang X, Cowell LG, *et al.* CD19-targeted T cells rapidly induce molecular remissions in adults with chemotherapy-refractory acute lymphoblastic leukemia. *Sci Transl Med* 2013; **5:** 177ra38.
219 Davila ML, Riviere I, Wang X, Bartido S, Park J, Curran K, *et al.* Efficacy and toxicity management of 19-28z CAR T cell therapy in B cell acute lymphoblastic leukemia. *Sci Transl Med* 2014; **6:** 224ra25.
220 Louis CU, Savoldo B, Dotti G, Pule M, Yvon E, Myers GD, *et al.* Antitumor activity and long-term fate of chimeric antigen receptor-positive T cells in patients with neuroblastoma. *Blood* 2011; **118:** 6050–6.
221 Beatty GL, Haas AR, Maus MV, Torigian DA, Soulen MC, Plesa G, *et al.* Mesothelin-specific chimeric antigen receptor mRNA-engineered T cells induce anti-tumor activity in solid malignancies. *Cancer Immunol Res* 2014; **2:** 112–20.
222 Le DT, Uram JN, Wang H, Bartlett BR, Kemberling H, Eyring AD, *et al.* PD-1 blockade in tumors with mismatch-repair deficiency. *N Engl J Med* 2015; **372:** 2509–20.
223 Westin JR, Chu F, Zhang M, Fayad LE, Kwak LW, Fowler N, *et al.* Safety and activity of PD1 blockade by pidilizumab in combination with rituximab in patients with relapsed follicular lymphoma: a single group, open-label, phase 2 trial. *Lancet Oncol* 2014; **15:** 69–77.
224 Armand P, Nagler A, Weller EA, Devine SM, Avigan DE, Chen YB, *et al.* Disabling immune tolerance by programmed death-1 blockade with pidilizumab after autologous hematopoietic stem-cell transplantation for diffuse large B-cell lymphoma: results of an international phase II trial. *J Clinic Oncol* 2013; **31:** 4199–206.
225 Larkin J, Chiarion-Sileni V, Gonzalez R, Grob JJ, Cowey CL, Lao CD, *et al.* Combined Nivolumab and Ipilimumab or Monotherapy in Untreated Melanoma. *N Engl J Med* 2015; **373:** 23–34.

Correspondence: Sylvia Hoeller, MD, Institute of Pathology, Schoenbeinstrasse 40 4031 Basel, Switzerland.
(fax: 0041/61-2652950; e-mail: Sylvia.Hoeller@usb.ch).