ESMO GOOD SCIENCE BETTER MEDICINE BEST PRACTICE

Annals of Oncology 28 (Supplement 12): xii18–xii32, 2017
doi:10.1093/annonc/mdx238
Published online 21 September 2017

REVIEW

Mechanisms regulating T-cell infiltration and activity in solid tumors

E. Lanitis[1], D. Dangaj[1], M. Irving[1] & G. Coukos[1,2]*

[1]The Ludwig Branch for Cancer Research of the University of Lausanne, Epalinges; [2]Department of Oncology, University Hospital of Lausanne (CHUV), Lausanne, Switzerland

Correspondence to: George Coukos, Director, Department of Oncology, Chief, Immuno-Oncology Service, University Hospital of Lausanne (CHUV); Director, Lausanne Branch, Ludwig Institute for Cancer Research, Rue du Bugnon 46-BH09-701, CASE POSTALE 5, 1011 Lausanne, Switzerland. Tel: +41 21 314 06 27; E-mail: george.coukos@chuv.ch

T-lymphocytes play a critical role in cancer immunity as evidenced by their presence in resected tumor samples derived from long-surviving patients, and impressive clinical responses to various immunotherapies that reinvigorate them. Indeed, tumors can upregulate a wide array of defense mechanisms, both direct and indirect, to suppress the ability of Tcells to reach the tumor bed and mount curative responses upon infiltration. In addition, patient and tumor genetics, previous antigenic experience, and the microbiome, are all important factors in shaping the T-cell repertoire and sensitivity to immunotherapy. Here, we review the mechanisms that regulate T-cell homing, infiltration, and activity within the solid tumor bed. Finally, we summarize different immunotherapies and combinatorial treatment strategies that enable the immune system to overcome barriers for enhanced tumor control and improved patient outcome.

Key words: T cells, immunotherapy, cancer, tumor microenvironment

Introduction

Retrospective studies of most solid tumor types have demonstrated a correlation between the presence of tumor-infiltrating lymphocytes (TILs) and progression-free survival as well as overall patient survival, thus pointing to a central role for T cells in tumor immunity [1–5]. This assertion is further supported by the durable responses of some patients to high-dose interleukin-2 (IL-2) as well as to the adoptive transfer of autologous *ex vivo* expanded TILs (TIL therapy), both of which were pioneered for the treatment of advanced metastatic melanoma patients [6–8]. More recently, the advent of checkpoint blockade therapy with monoclonal antibodies (mAbs) targeting cytotoxic T lymphocyte associated protein 4 (CLTA-4), as well as the programmed cell death protein 1 (PD-1) and its ligand (PD-L1), has enabled T cell-mediated tumor regression for a range of malignancies including melanoma [9, 10], ovarian [11], lung [12], bladder [13], renal-cell carcinoma [14], Hodgkin's lymphoma [15], as well as colorectal, gastrointestinal and endometrial cancers having DNA mismatch repair defects [16].

PD-1 inhibition alone leads to response rates in about 20%–30% of patients with different solid tumor types, but when combined with CTLA-blockade, which promotes T-cell priming [17], this can increase up to 57% for advanced metastatic melanoma [18]. Why some patients respond to checkpoint therapy and others not remains unclear, but the existence of TILs at the onset of therapy is a key factor. Responses to PD-1 inhibition are highly correlated to the presence of $CD8^+$ T cells at the invasive margin and within the tumor bed [19], which define the so-called hot tumors, but not all patients with inflamed tumors respond to checkpoint blockade (Figure 1A and B). There also exist tumors that exclude T cells (Figure 1C), and others that are completely devoid of immune infiltrate, often referred to as cold tumors, or immune deserts (Figure 1D) [20, 21]. The immunoscore, first proposed to classify malignant colorectal tumors based on their level of immune infiltrate, is emerging as a more important predictor of cancer progression than tumor stage or its pathological grade [3]. Moreover, the presence of tertiary lymphoid structures (TLS) in lung tumors [22], characterized by the association of T cells, mature DCs, a follicular center with follicular DCs, proliferating B cells, and high endothelial venules, is favorable for patient prognosis [23], and increased densities of TLS are associated with increased $CD4^+$ T-cell receptor (TCR) repertoire clonality [24].

Elucidating factors regulating T-cell infiltration and functionality once within the tumor is critical for the development of

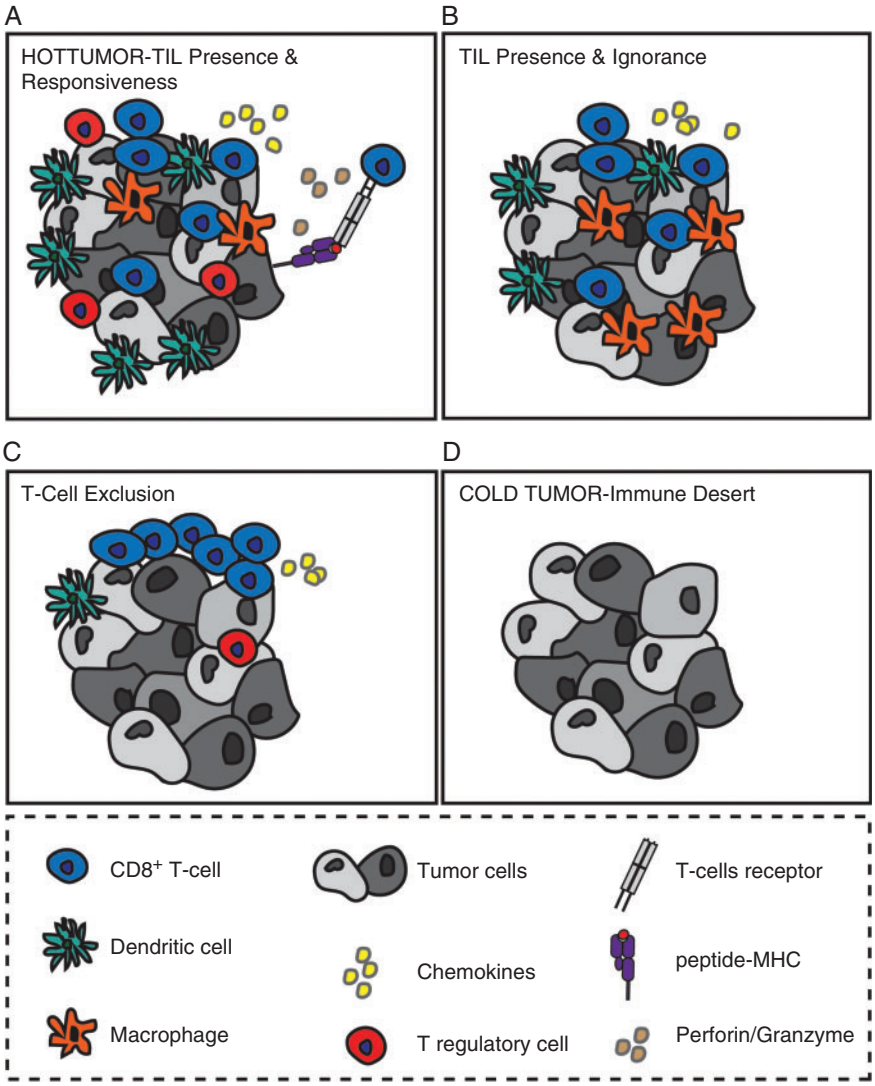

Figure 1. Classification of tumors based on their immune cell infiltrate. Tumors infiltrated by T cells are often referred to as hot tumors. It has been observed, however, that some T-cell-inflamed tumors respond to checkpoint blockade therapies (A) and others not (B). There also exist tumors for which immune cells are excluded at the periphery (C), as well as tumors that are completely devoid of immune infiltrate, and having a so-called desert immune landscape (D).

novel combinatorial strategies conferring improved patient response rates to immunotherapy. Here, we review our current understanding of the mechanisms leading to T-cell inflamed versus noninflamed tumors, forces regulating TIL function in the tumor microenvironment (TME), and combinatorial therapies being used to re-program the TME and enhance T-cell homing and activity.

Patient and tumor intrinsic properties that govern T lymphocyte responses against tumors

T cells are educated in the thymus to distinguish self from nonself peptides in the context of major histocompatibility complex (pMHC) molecules; T cells having TCRs of either too low or too high affinity for pMHC are eliminated during positive and negative thymic selection, respectively [25]. Having passed this process, naïve T cells circulate secondary lymphoid tissues with the quest of being primed by an activated antigen-presenting cell (APC) displaying cognate pMHC. In the context of cancer, this may take place in tumor-draining lymph nodes (TdLN) by dendritic cells (DCs) that have already sampled the tumor and been activated (Figure 2). Several groups have linked the presence of $CD8^+$ TILs to a type I interferon (IFN) signature [26, 27] resulting from of a subset of $CD103^+$ $CD8^+$ DCs driven by the transcription factor Batf3 in the TdLNs [28–30]. Moreover, the activation of these DCs appears to be largely mediated through sensing of cytosolic DNA through the cGAS-Sting pathway [31–33]. Such DCs represent ~1% of the total tumor infiltrate [30]. Indeed, DCs isolated from cancer patients are oftentimes functionally impaired, having low expression levels of the costimulatory ligands CD80, CD86, and CD40, as well as the cytokine IL-12, while upregulating genes associated with T-cell inhibition including PD-L1, T-cell immunoglobulin mucin

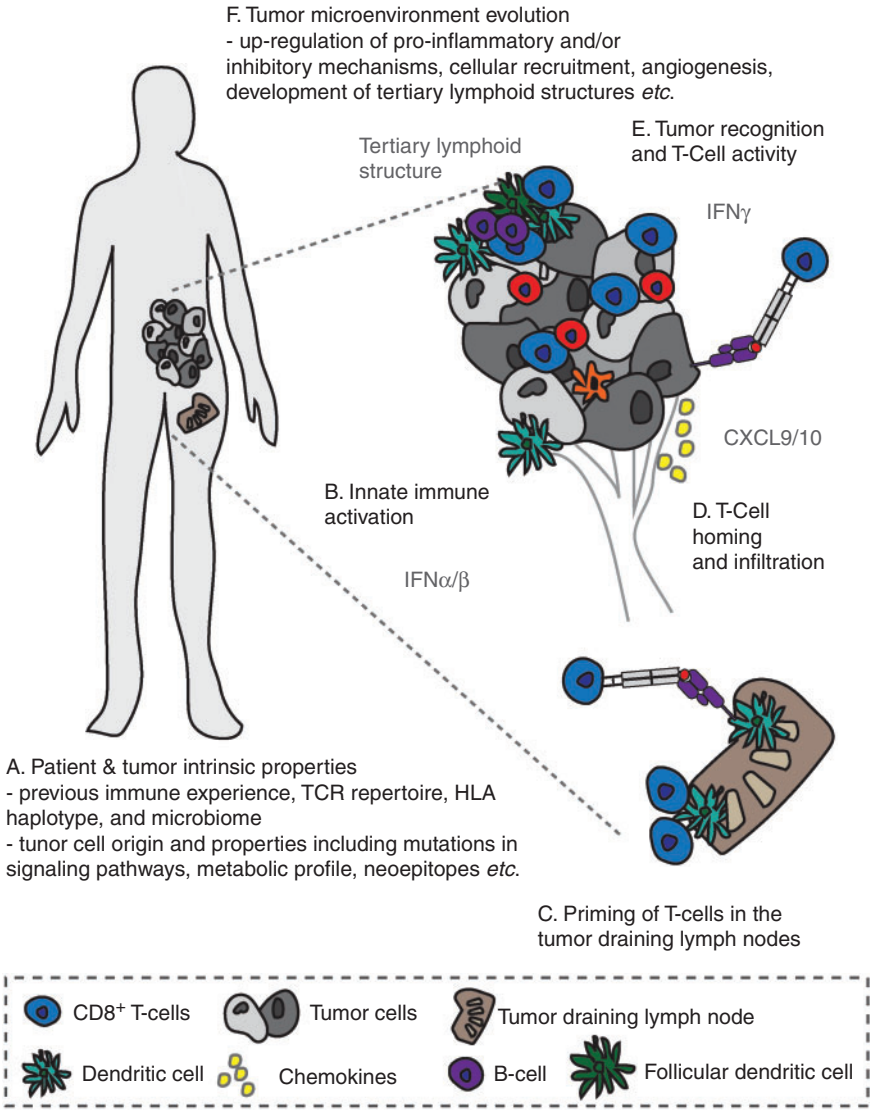

Figure 2. An overview of the steps and variables driving T-cell recruitment, infiltration, and activity in tumors. Both patient and tumor intrinsic properties can affect anti-tumor T-cell responses. Examples include previous immune experience of the patient and mutations of the tumor cells (A). Early innate immune activation of DCs that produce type I IFN is critical (B) for the recruitment and priming of T cells in the tumor draining lymph nodes (TdLNs) (C). T cells activated in the TdLNs must chemotactically navigate an aberrant tumor vasculature and overcome various barriers in the stroma to gain entry into the tumor bed (D). Once in the tumor, T cells must be able to recognize and bind to specific pMHC complexes and then begin effector functions such as IFNγ secretion and killing (E). The secretion of IFNγ by activated T cells will trigger a series of events in the tumor including the up-regulation of PD-L1, while the development of tertiary lymphoid structures in the tumor can help promote local adaptive immunity (F). Barriers at many of these steps can potentially abolish T-cell homing, infiltration and/or activity in the TME.

receptor 3 (Tim-3), interleukin 10 (IL-10), and indoleamine 2–3, dioxygenase-1 (IDO-1) [34].

A variety of tumor antigens can be recognized by T cells. Examples include peptides derived from mutated proteins (i.e. neoeptiopes), tissue differentiation antigens, oncofetal antigens like carcinoembryonic antigen, oncogenic viral antigens such as from human papilloma virus, cancer testis antigens (CTAs), and proteins that are highly overexpressed in tumor cells such as tyrosinase in melanoma [35]. Because many of these antigens are self, tumor-directed TCR may be of lower affinity than those directed against viral epitopes, for example [36, 37]. An association between neoepitope-specific T cells and sensitivity to checkpoint blockade, as well as their abundance in melanoma TIL therapy, has led to speculation that neoepitopes are critical for tumor immunogenicity [38–42]. Moreover, acquired resistance in non-small cell lung cancer (NSCLC) patients to immune checkpoint blockade has recently been associated with neoantigen loss through the elimination of tumor subclones or through the deletion of chromosomal regions [43]. Interestingly, however, comparable levels of differentiated, germline, and mutated antigens are expressed by T-cell inflamed and noninflamed melanoma tumors [44]. In addition, Merkel-cell carcinoma patients showed similar responses to PD-1 blockade regardless of whether their cancer had been UV-induced and was highly mutated, or was caused by Merkel cell polyomavirus and had

a lower mutational burden [45, 46], thus underlying the importance of the quality of the epitope in the generation of robust TILs.

TCR-pMHC binding is the central event in mounting an anti-tumor T-cell response, but what is presented and what can be seen by the immune system varies from patient to patient (Figure 2). The Human Leukocyte Antigen (HLA) genes (encoding MHC) are highly polymorphic [47]. Moreover, the circulating TCR repertoire diversity and frequency, generated from V(D)J recombinations in the thymus, depends upon both patient genetics and previous antigenic exposure [48]. A common cause of poor tumor immunogenicity is the loss or down-regulation of HLA class I [49–51], as well as of the tumor antigen processing and presentation machinery in tumor cells, due to either genetic or epigenetic alterations [52]. HLA class I alterations are defined as being soft, if they are regulatory in nature, including the downregulation of genes encoding the HLA complex or components of the antigen processing/presentation machinery, and hard if they involve mutational events and chromosomal abnormalities affecting the HLA class I heavy chain or β2m genes [51]. In the absence of pMHC expression T cells are ignorant to tumors.

An inverse correlation was recently demonstrated between activation of the WNT/β-catenin signaling pathway and CD8α and PD-L1 expression in non-T-cell inflamed metastatic melanoma [53]. In a murine model such tumors were shown to have reduced expression of the chemokine CCL4, they were nonresponsive to checkpoint blockade, and they lacked $CD103^+$ $CD8^+$ $batf3^+$ DCs, indicating that a defect in early innate immune priming caused the lack of T-cell infiltrate. PTEN loss-of-function mutations [54] have also been shown to limit T-cell recruitment. Notably, the $BRAF_{V600E}$ oncogene that can be found in metastatic melanoma gives rise to a highly glycolytic phenotype [55] due to dysfunctional oxidative phosphorylation, thereby limiting glucose availability to T cells and diminishing effector capabilities. In addition, aberrant epidermal growth factor receptor (EGFR)/RAS signaling has been shown to suppress CCL27 production, thus inhibiting T-cell homing and accelerating tumor outgrowth [56]. In contrast, mutations in BRCA1/2 [57], and POLE3 [58], as well as microsatellite instability [59], all of which result in genomically unstable tumors, are characterized by a higher T-cell content. Finally, immune priming of DCs, T-cell activation, and responses to cancer therapy, including CpG oligonucleotide, oxaliplatin [60], cyclophosphamide [61], and PD-L1 [62] or CTLA-4 [63] blockade, are influenced by the commensal gut microbiota profile of the patient.

T-cell homing and overcoming stromal barriers

Chemokine networks for T-cell tumor homing

Chemokines can be functionally classified as being homeostatic or inflammatory, corresponding to expression that is constitutive or inducible, respectively. Homeostatic cytokines regulate T-cell trafficking during thymic selection, as well as the physiological movement of immune cells (i.e. chemokinesis) through secondary lymphoid organs and peripheral tissues under routine conditions of immune surveillance. Inflammatory chemokines, on the other hand, play a key role in the recruitment of immune cells to peripheral tissue in response to antigenic challenge [64, 65]. Naïve T cells are supported by chemokines CCL19, CCL21, and CXCL12 secreted by fibroblast reticular cells and stromal cells, as well as by lymphocyte function-associated antigen-1 (LFA-1) interactions with intercellular adhesion molecule-1 (ICAM-1) on the surface of DCs [66–68]. Activated and memory T cells upregulate a variety of chemokine receptors such as CCR5 and CXCR3 [69–73] to enable rapid chemotaxis toward inflamed regions to detect and respond to infected or transformed cells.

Chemokines regulate the trafficking of immune cells into tumors and have been implicated in tumor development, progression, and angiogenesis [74]. In melanoma, the presence of TILs has been shown to correlate with the expression of CCL2, CCL3, CCL4, CCL5, CXCL9, and CXCL10 [26, 75]. The IFN-gamma (IFNγ)-inducible chemokines CXCL9 and CXCL10, for example, which can be secreted by local myeloid and stromal cells, recruit $CXCR3^+$ memory $CD8^+$ T cells [76, 77] and are strongly associated with a Th1 immune response [72, 78–83], as well as favorable outcome to chemotherapy and immunotherapy [79, 84]. In addition, CXCR3 signaling contributes to the transendothelial migration of T cells into the tumor bed [85] (Figures 2 and 3).

Most tumors, however, alter local chemokine networks to attract immune-inhibitory, tumor-promoting infiltrate like tumor-associated macrophages (TAMs) [86, 87], myeloid-derived suppressor cells (MDSCs) [74, 88–90], and regulatory T cells (Tregs) that are associated with poor patient prognosis [91–93]. CCL28, for example, a chemokine ligand that is upregulated in response to hypoxia, recruits Tregs that in turn promote tumor tolerance and angiogenesis [94]. CXCL12 recruits $CXCR4^+$ stromal cells [74], and promotes growth, metastasis (to CXCL12-expressing organs), and angiogenesis. CXCR4 blockade by siRNA or pharmacologic inhibition slows tumor growth by increased apoptosis and reduces the metastatic potential [95, 96]. Alternatively, tumors such as ovarian can use epigenetic mechanisms to silence the T-cell attracting chemokines CXCL9 and CXCL10 [97]. In addition, nitrosylation by reactive oxygen species (ROS) in the TME abrogates the ability of CCL2 to attract T cells [98], while altered proteolytic processing of CXCL11 impairs its binding-induced signaling, thereby reducing the recruitment of $CXCR3^+$ effector T cells [99].

The tumor vasculature barrier

Tumors rely upon a vasculature that is tortuous, leaky and lacking proper pericyte coverage, to supply themselves with oxygen and nutrients as well as for waste removal (Figure 3). These aberrant vessels are also the gateway for immune infiltrate that must adhere to the endothelium by chemokine-dependent and -independent mechanisms [100]—lymphocytes require integrin interactions with endothelial cell adhesion molecules to extravasate into the tumor [101, 102]. Several inhibitory mechanisms limiting T-cell transendothelial migration have been described [103]. For example, the down-regulation of ICAM-1 by the proangiogenic vascular endothelial growth factor A (VEGF-A) and basic fibroblast growth factor [104, 105], as well as overexpression and signaling *via* the endothelin-1/endothelin B-receptor axis [106–108], help tumors evade T-cell attack [109]. The upregulation of Fas ligand in response to tumor-derived factors including VEGF-A, IL-10, and prostaglandin E2 (PGE_2), specifically induces apoptosis of Fas-expressing $CD8^+$

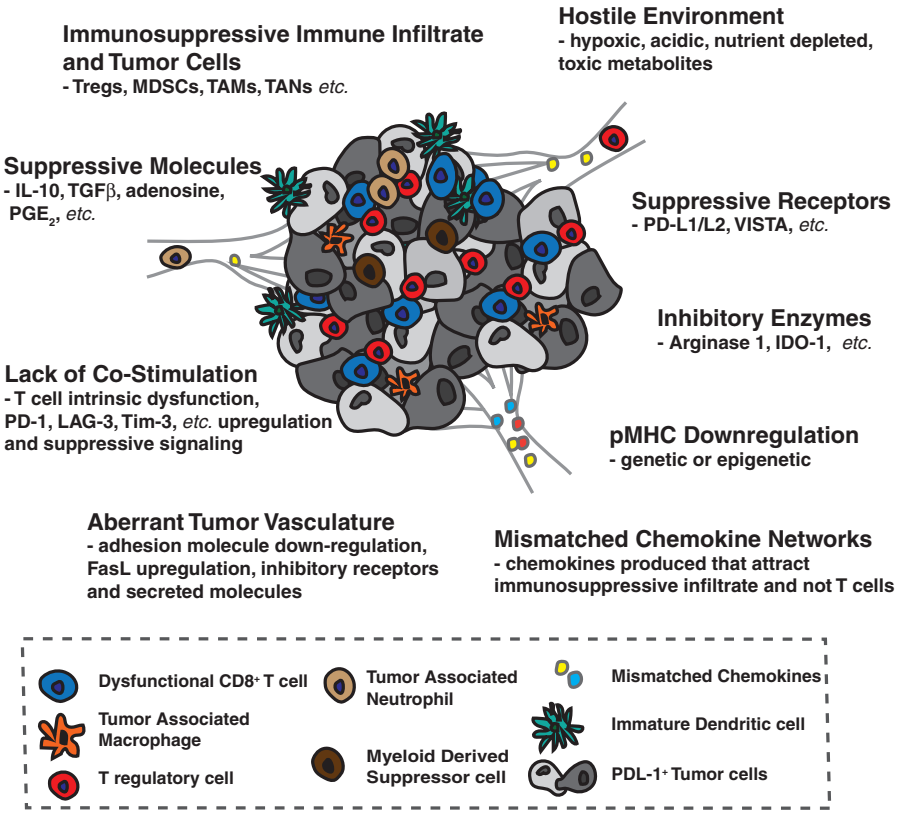

Figure 3. Barriers to T-cell activity in solid tumors. T cells that are able to overcome chemokine mismatches and the aberrant vasculature, and have gained entry into the tumor bed, will secrete IFNγ upon activation that can trigger the upregulation of immunosuppressive mechanisms like PD-L1 and IDO-1. Immunosuppressive immune cells can also be chemotactically attracted to the tumor by chemokines expressed in response to hypoxia that in turn express inhibitory receptors and secrete a range of molecules that can block effector T-cell activity as well as DC maturation, and/or promote the activities of suppressor cells. Examples of inhibitory immune infiltrate include MDSCs, Tregs, TANs, and TAMs. Examples of suppressive molecules include VEGF, IL-10, TGFβ, adenosine, and PGE_2. Tumor cells can also down-regulate pMHC expression so that they can no longer be detected by T cells. Metabolic competition for glucose and amino acids, as well as toxic metabolites, hypoxia, and acidity of the TME can also diminish effector T-cell function. In response to chronic activation in the absence of sufficient T-cell co-stimulation, a variety of T-cell intrinsic inhibitory mechanisms are upregulated. T cells in the TME are often anergic or exhausted.

T cells while leaving Tregs unharmed [110, 111]. Finally, tumor endothelial cells can upregulate a variety of inhibitory receptors such as B7-H3 [112–114], PD-L1 and PD-L2 [115, 116], Tim-3 [117, 118] and B7-H4 [119], as well as secrete soluble inhibitory molecules including IL-6, PGE_2, IL-10, and TGFβ [120–123]. In addition to the vasculature, other components of the tumor stroma, including its dense matrix [124] and cancer-associated fibroblasts [125], can suppress T-cell function and block their entry into the tumor bed. There is now a strong body of evidence that cross-talk between tumor cells and its stroma influences cancer progression and metastasis [126].

Immunometabolic obstacles in the tumor bed

Immunosuppressive tumor cells and immune infiltrate

T cells that successfully home and extravasate via the vasculature face further challenges to both their function and survival in the tumor bed (summarized in Figure 3). Tumor cells can upregulate a variety of inhibitory receptors like PD-L1, and secrete molecules including IL-10, TGFβ [127], and PGE_2 [128, 129], that can directly block T-cell function and/or attract and activate immunosuppressive immune cells including Tregs, MDSCs, TAMs, and tumor-associated neutrophils (TANs) [130]. Notably, inhibitory mechanisms like PD-L1 and IDO-1 expression are adaptive rather than tumor cell-intrinsic as they are induced by IFNγ secreted by activated T cells [131, 132]. There is also co-operative action amongst suppressive immune cells. For example, MDSCs in addition to directly inhibiting effector T cells can also induce Tregs [130]. Tregs in turn produce a range of inhibitory molecules such as adenosine *via* CD39/CD73 [133], compete with effector T cells for IL-2 [134], and can abrogate DC maturation and activity [135]. Moreover, many immunosuppressive molecules are pleiotropic. As an example, PGE_2, a small molecule derivative of arachidonic acid produced by the inducible cyclooxygenase 2 enzyme, is produced by both tumor cells and macrophages and can inhibit DC maturation, and selectively suppress Th1, cytotoxic T lymphocytes, and NK-mediated immunity while promoting Th2, Th17, and Treg responses [128, 129].

Chronic antigen exposure in the absence of sufficient T-cell co-stimulation

Another important challenge faced by T cells in the TME is chronic antigen exposure in the absence of sufficient co-stimulation. As previously mentioned, DCs in the tumor are oftentimes immature and lacking in ligands CD80 and CD86 [136] which must be engaged by CD28 to provide signal 2 of T-cell activation in order to avoid tolerance or the induction of anergy [137]. TILs are typically characterized by high expression levels of one or more inhibitory receptors including PD1, CTLA-4, lymphocyte activation gene 3 (LAG-3), Tim-3, T-cell Ig, and ITIM domain (TIGIT) [138], and/or B- and T-lymphocyte attenuator [139], which relay signals to dampen or block T-cell function [136]. In this context, inhibitory intracellular signaling molecules are also upregulated including the phosphatase SHP-1, a negative regulator of T-cell signaling [140, 141], the ubiquitin ligase Cbl-b which negatively regulates activation signals derived from the TCR and co-stimulatory molecules and increases the sensitivity of effector T cells to Treg inhibition [142], and the enzyme diacylglycerol kinase which attenuates the production of effector cytokines including IL-2 and IFNγ as well as T-cell proliferation [143]. Also upregulated is the transcription factor Ikaros, which imposes a barrier to $CD8^+$ T-cell differentiation by restricting autocrine IL-2 production [144], along with T-bet, EOMES, and BLIMP1, which are implicated in T-cell terminal differentiation and exhaustion [145].

Metabolic competition and harsh living conditions

Tumors present challenging living conditions to T cells as they are typically hypoxic, acidic, nutrient depleted and they accumulate toxic metabolites (Figure 3). Tumor cells use the process of aerobic glycolysis despite sufficient oxygen to undertake oxidative phosphorylation, the so-called Warburg effect, to support their high energy and biosynthetic needs [146, 147]. In doing so they deplete nutrient supplies (glucose, glutamine, etc.) also required by effector T cells [148, 149], and they pump high levels of metabolites into the TME including lactate from the fermentation process that can cause T-cell dysfunction [150–152]. In addition, tumors limit T-cell activity by producing enzymes including arginase-1 (Arg-1) and IDO-1, which degrade the essential amino acids arginine and tryptophan, respectively [153]. T cells deprived of L-arginine downregulate CD3ζ chain expression and go into cell cycle arrest [154–157]. In the absence of tryptophan, T-cell responses are also blunted, but the effects of IDO-1 are compounded by tryptophan derivatives like kynurenine, which can further block T-cell proliferation and promote Treg activity [158–161]. In addition, engagement of PD-1 and CTLA-4 by their respective ligands can attenuate aerobic glycolysis of activated T cells by inhibition of the PI3K/Akt/mTOR pathway [162, 163]. Thus, there is an important interplay between the metabolic status of T cells and checkpoint pathways (i.e. immunometabolism) that is a critical consideration in the development of personalized combinatorial immunotherapy.

Therapeutic interventions

T cells play a critical role in tumor immunity but in some instances they are unable to reach and penetrate the tumor bed, or they gain access but their activity is inhibited by a plethora of immunosuppressive mechanisms. Checkpoint blockade as a single intervention is successful in a proportion of patients with solid tumors [164], while others do not respond to this therapy despite having T-cell-inflamed tumors. The underlying mechanisms of therapeutic resistance remain unclear, but are likely related to excessive suppression by a number of additional immunometabolic barriers, along with patient and tumor intrinsic properties as previously described. Next, we consider different clinical treatments that can either directly promote T-cell activity, and/or that help to re-program the TME to potentiate T-cell homing and anti-tumor activity.

Adoptive T-cell transfer

Arguably the most direct means of promoting T-cell presence in the tumor bed is by adoptive cell therapy (ACT) for which there are two main approaches. The first, as previously described, is the *ex vivo* expansion and reinfusion of autologous TILs into a lymphodepleted patient, along with high-dose IL-2 [165]. Traditionally, the TILs are cultured with high levels of IL-2 and then rapidly expanded in the presence of anti-CD3 Ab and allogeneic feeder cells prior to transfer [166]. In order to promote a less differentiated, central memory (T_{CM}) phenotype, T cells have been cultured with artificial APCs in the presence of the alternative common gamma chain cytokine IL-15, for example, and shown to mediate objective clinical responses [167, 168]. In the second approach to ACT, peripheral blood T cells can be gene-engineered to express a tumor-directed TCR [169] or a so-called chimeric antigen receptor (CAR) [170].

CARs are synthetic receptors, typically comprising a tumor antigen-specific single chain Ab fragment (scFv) fused to a linker, transmembrane region, and various combinations of endodomains associated with T-cell activation; CD3ζ is used to provide signal 1, and one or more co-stimulatory endodomains, such as from CD28 or 4-1BB, are included for signal 2 [171]. Unlike TCRs that are HLA-restricted, CARs can bind virtually any cell surface-expressed molecule [172] and they represent a critical treatment strategy against cold tumors having defects in antigen presentation by MHC. While CD19-targeted CAR T cells [173] have demonstrated unprecedented clinical results for the treatment of several B-cell malignancies, including up to 90% complete response rates in acute lymphoblastic leukemia patients [174], solid tumors remain an important challenge. One limitation is identifying tumor-restricted antigens to ensure that CAR T cells do not cause on-target/off-tumor toxicity [175–177]. To enhance safety, a variety of suicide genes [178], split signaling approaches [179], and novel druggable intracellular on-switches [180], have been developed. Probably the greatest challenge facing CAR T cells, however, relates to overcoming the same barriers to T-cell homing, engraftment, and function that endogenous T cells face [126, 156, 181, 182]. Indeed, CAR therapy can be enhanced by checkpoint blockade [183, 184], as well as by various engineering strategies [185] such as the co-expression of the

chemokine receptors CCR4 and CCR2 [186, 187], IL-12 [188], and CD40L [189].

Immune checkpoint blockade

As previously described, checkpoint blockade, first with anti-CTLA-4 [9] and then with anti-PD1/PD-L1 has brought impressive responses for advanced metastatic melanoma patients, and clinical trials for several other cancer-types have yielded similar results [9, 11, 12–16, 164, 190–193] . While CTLA-4 blockade reduces the activation threshold required for T-cell priming [164], PD1/PD-L1 blockade can reverse, at least in part and for some T-cell subsets [194, 195], immune defects such as exhaustion [196, 197], thus enabling synergy when the treatments are combined [18]. Abs against other inhibitory receptors including LAG-3, TIGIT, Tim-3, and VISTA have shown promise in preclinical models, both as monotherapies and in various combinatorial strategies [138, 198–200]. Tim-3 and LAG-3 blockade are currently being explored in early phase clinical studies (NCT02817633, NCT01968109), alone or in combination with anti-PD1 mAb, for the treatment of solid tumors.

Agonistic mAbs targeting T cells

Insufficient co-stimulation in the TME can cause critical T-cell dysfunction, but the provision of agonistic Abs of the tumor necrosis factor receptor superfamily (TNFRS) helps to reverse this phenomena. Such agonistic Abs have been shown to enhance T-cell effector function, proliferation and survival, as well as boost memory $CD8^+$ T-cell differentiation and overcome Treg suppression [201–203]. In preclinical models synergy has been demonstrated with vaccination, checkpoint blockade, and ACT [202, 204, 205]. A phase 1 clinical trial with an anti-4-1BB mAb demonstrated activity (NCT00309023), but a follow-up phase 2 study was terminated due to toxicity. Lower doses are now being assessed in combination with PD1 blockade (NCT02253992). Agonistic anti-OX40 mAb has been assessed on its own (NCT01644968), and is currently being tested in combination with either anti-CTLA-4 or anti-PD1 (NCT02205333). Agonistic mAbs targeting CD27 and glucocorticoid-induced TNF-related protein (GITR; TNFRSF18) have shown efficacy in preclinical models [206, 207] and have recently entered clinical trials. Because agonistic mAbs targeting co-stimulatory receptors can trigger systemic inflammation and toxicity in vital organs, dose escalation and careful patient monitoring are critical.

Agonistic mAbs targeting APCs

CD40, another member of the TNFRS, is expressed broadly on APCs including DCs, B cells and monocytes, as well as by nonimmune cells and a wide range of tumors [208, 209]. Agonistic Abs targeting CD40 promote DC maturation and efficient cross-presentation of antigen to T cells [210, 211]. In addition, they can induce apoptosis of tumor cells and TAM conversion to M1-like macrophages [212, 213]. Pre-clinical studies have demonstrated synergy with chemotherapy, checkpoint blockade, vaccines, radiotherapy (RT), and cytokine treatment [214]. Phase I clinical trials of agonistic CD40-targeting mAbs in combination with gemcitabine have shown promising systemic immune responses in pancreatic cancer patients [215]. Agonistic anti-CD40 mAbs are currently being tested in solid tumors as a monotherapy (NCT02482168), and in combination with anti-PD1 mAb (NCT02304393).

Macrophage reprogramming

TAMs are highly immunosuppressive and Ab blockade of the receptor for colony stimulating factor 1 (CSF1), also known as macrophage colony-stimulating factor (M-CSF), highly expressed by TAMs, can re-program them toward an M1 phenotype. These M1-like macrophages have enhanced antigen presentation, promote stronger anti-tumor T-cell responses, and synergize with checkpoint blockade [216]. MDSCs can also be targeted by CSF1-R blockade to sensitize IDO-expressing tumors to immunotherapy [217], and improve efficacy of RT in preclinical models of prostate cancer [218]. A multicenter clinical trial is ongoing to evaluate the impact of a CSF1-R inhibitor in combination with anti-PD1 mAb in various solid tumors (NCT02452424). Macrophage polarization to an M1 phenotype has also been reported for TNFα treatment [219].

IDO-1 inhibition

Tumor cells compete with T cells for essential nutrients including glucose and amino acids [150–152]. In addition, they can upregulate IDO-1, an important immunomodulatory enzyme that catabolizes tryptophan to kynurenine and 3-hydroxyanthranilic acid, in order to inhibit T-cell activity and promote Tregs [158–161, 220]. IDO-1 inhibitors hold great promise in combination with chemotherapy, RT, and immunotherapy [221] and are being assessed in the clinic against many tumor types [222]. IDO-1 inhibition is currently being clinically tested in combination with anti-CTLA-4 for metastatic melanoma patients (NCT01604889). Previously, in combination with anti-PD1, it led to objective response rates of 53% for unresectabe stage 3 or stage 4 melanoma patients (NCT02073123).

DC vaccines

DC vaccines can be used to enhance tumor antigen presentation to, and priming of, T cells. For this therapy, DCs are generated *ex vivo* and pulsed with specific peptides, protein or whole tumor lysate, or transfected with RNA encoding tumor-specific epitopes [223], before being re-infused into the patient. We have combined whole tumor lysate DC vaccines with anti-VEGF mAb for the treatment of ovarian cancer (NCT01132014) [224, 225], while others have treated patients with DC vaccines and checkpoint blockade (anti-CTLA-4), and observed more durable responses for the combination therapy than single agents [226]. More recently, personalized DC vaccines have been developed with tumor-specific mutated epitopes [227], yielding diverse neoantigen specific TCR repertoires in treated patients [228]. Finally, lipid carriers for systemic RNA delivery (RNA-Lipolexes) to DCs have been recently shown capable of inducing strong adaptive and type I IFN-mediated innate immune responses [229]. This may be a powerful approach for turning some cold tumors hot.

Immunogenic chemotherapy

Several cytostatic drugs including anthracyclines and oxaliplatin promote the so-called immunogenic cell death (ICD) characterized the by secretion of damage-associated molecular patterns

(DAMPS), the activation of DCs, and ultimately the recruitment and activation of TILs [230, 231]. ICD is a multi-step process including the release of find-me signals from apoptotic tumor cells such as ATP, nucleotides and fractalkine, eat-me signals from phosphatidylserine and calreticulin [232], and finally the release of danger signals from DAMPs like high-mobility group box protein 1 (HMGB1) that act via pattern recognition receptors including Toll-like receptors 2 and 4 expressed on DCs to enhance antigen presentation [233, 234]. ICD can also induce digest me signals that enhance the capacity of caspases to cut and release apoptotic antigenic fragments from tumor cells that can be cross-presented by DCs to TILs via the class I-processing pathways. Finally, immunogenic chemotherapies can induce the expression of T-cell attracting chemokines including CCL5, CXCL9, and CXCL10 [79], and have been shown to synergize with checkpoint blockade therapy in an innate-immune sensing dependent manner [235].

Oncolytic viruses

Oncolytic viruses (OVs), a new class of immunotherapy drugs, promote anti-tumor responses through both direct tumor cell killing and the induction of innate and adaptive anti-tumor immunity through ICD. In response to infection by OVs, tumor cells release ROS and type I IFNs, and, upon subsequent lysis, DAMPs and pathogen-associated molecular patterns. Furthermore, the necrotic tumor cells provoke the spreading of tumor-associated antigens and neoantigens that can be cross-presented by DCs to TILs [236, 237]. OVs have been gene-engineered to integrate immunomodulatory genes including cytokines, chemokines, and T-cell costimulatory molecules [238]. One of the most widely used cytokines is GM-CSF that can promote the differentiation and recruitment of DCs into the tumor bed and TdLNs [239]. Oncolytic virotherapy can promote checkpoint blockade [240, 241]. In a phase 1b trial for melanoma patients, the combination of Talimogene laherparepvec (T-VEC; an OV engineered to express GM-CSF) with anti-CTLA-4 mAb resulted in a 50% objective response rate with a tolerable safety profile [242], and T-VEC plus anti-PD-1 mAb in a phase 1b/III trial (NCT02263508) also provided clinical benefit, and a phase II trial is planned (NCT02965716).

Targeting the tumor vasculature

Although anti-angiogenesis monotherapies have yielded only modest survival benefit in the clinic, an important observation that some of them can normalize the vasculature was made [103, 243]. Tumor vasculature normalization describes a transient state induced by the blockade of angiogenic signaling during which the vessels are more permissive to tissue perfusion and delivery of oxygen, drugs (e.g. chemotherapies), and Abs [244–247], as well as T-cell infiltration following vaccination and ACT [248–250]. Normalization is characterized by the upregulation of the leukocyte adhesion molecules ICAM-1 and VCAM-1 on tumor endothelial cells [251] and has been reported for anti-VEGFR and anti-VEGF-A mAbs (at low doses), various tyrosine kinase inhibitors [252], ET_BR blockade [109], vessel-targeted TNFα [219, 253–256], and agonistic anti-4-1BB mAb [257]. More recently, the phenomena of vascular promotion have been described [258]. Low-dose cilengitide, verapamil, and gemcitabine have been combined, for example, in the pre-clinical treatment of pancreatic cancer [259] to increase tumor blood vessel density and leakiness, and decrease hypoxia. Vessel promotion thereby confers improved drug delivery and decreased drug resistance, resulting in impaired tumor progression and metastasis.

In the clinic, anti-angiogenic therapies have been shown to synergize with checkpoint blockade [260]. For example, anti-VEGF in combination with CTLA-4 blockade conferred a disease control rate of almost 70% in metastatic melanoma patients [261]. This combination has been shown to upregulate ICAM-1 and VCAM-1, as well as the production of various cytokines and chemokines, including IL-1α, TNFα, and CXCL10, leading to increased lymphocyte infiltration [262]. Anti-VEGF and anti-PD-L1 yielded a 40% response rate in metastatic renal-cell carcinoma patients—the combination therapy increased lymphocyte trafficking and intra-tumoral MHC-I levels, and conferred gene-signatures associated with Th1 chemokines such as CX3CL1 [263].

Targeting the cancer epigenome

In addition to genomic mutations that directly effect tumorigenesis, mutations can occur in chromatin-regulating genes leading to epigenetic abnormalities that cause cancer [264]. There are two main categories of drugs for targeting the cancer epigenome, broad reprogrammers that reverse genome-wide cancer-specific gene expression patterns [265, 266], and targeted ones that are directed against specific enzymes involved in epigenetic pathways. Broad reprogrammers include DNA methyltransferase inhibitors (DNMTi), histone deacetylase inhibitors (HDACi), and inhibitors of the bromodomain and extra-terminal motif proteins (iBETs). Targeted therapies have been developed, for example, against the EZH2 H3K27 histone *N*-methyltransferase that is activated by mutations in lymphomas [267, 268], and against the tricarboxylic acid cycle genes *IDH1* and *IDH2* that are mutated in gliomas and acute myeloid leukemia resulting in aberrant hypermethylation due to the production of a metabolite that inhibits DNA and histone demethylation [269, 270].

DNMTi including 5-azacytidine and its deoxy derivative decitabine (also known as 5-aza-2′-deoxycytidine) have been shown to upregulate tumor antigens including melanoma-associated antigen 1 (MAGE1) and CTAs [271–273]. Moreover, DNMTi treatment of tumor cells upregulates endogenous retroviral sequences that are sensed by the tumor cell-autonomous nucleic acid sensing machinery causing type I IFN signaling, characterized by potent cytokine and chemokine production [274, 275] and leading to enhanced tumor immunogenicity. The combination of DNMT1 and EZH2 inhibitors has been shown to sensitize ovarian cancer to checkpoint blockade, release CXCL9 and CXCL10 from epigenetic silencing, and improve the trafficking of adoptively transferred T cells [97].

Radiotherapy

Low-dose RT is immunostimulatory and synergizes with immunotherapy to enhance anti-tumor responses by a variety of mechanisms [276]. For example, it can induce ICD and the release of DAMPs such as IFN I/II, which in turn promote DC maturation and cross-presentation to T cells [277, 278].

Furthermore, it can upregulate MHC and tumor antigens [279], chemokine ligands including CXCL10 and CXCL16 [280, 281], Fas [282], and proinflammatory cytokines such as IL-1β and TNFα. In addition, it has been shown to inhibit Tregs [283], upregulate adhesion molecules including ICAM-1, VCAM-1, and E-selectin on tumor endothelial cells (i.e. normalize the tumor vasculature) [281, 284–286], and promote macrophage differentiation to an immunostimulatory iNOS+/M1 phenotype [287, 288], all of which support anti-tumor T-cell responses.

Several pre-clinical studies have demonstrated benefit in combining local irradiation with checkpoint blockade [289–291], and clinical trials are underway (NCT02298946, NCT02303990). Notably, a phase 1 clinical trial treating advanced melanoma patients with RT and anti-CTLA-4 yielded only 18% partial responses, possibly due to PD-L1 upregulation [292, 293] and suggesting that a regimen targeting both CTLA-4 and the PD1/PD-L1 axis may be required [294]. Like chemotherapy, total body irradiation can be used for intense lymphodepletion of cancer patients prior to ACT to improve T-cell engraftment [6]. RT has also been shown to synergize with anti-OX40 and anti-CD40 agonistic Abs [222, 295], ACT [279, 283, 296], and vaccines [282, 297]. Many parameters must be optimized when combining RT with immunotherapy including optimal dose, fractionation, the treatment site, and timing, and results may vary depending on several parameters including tumor burden and the degree and types of immunosuppression [276, 298, 299]. Overall, RT, even at low doses, is a very promising approach for TME reprogramming and helping to turn cold tumors hot.

Discussion

Concluding remarks

Some patients undergoing immunotherapy achieve robust and durable anti-tumor responses as a result of reinvigorated T-cell activity and changes to the immune balance in favor of protective rather than suppressive activities. Elucidating why and how these patients benefit from treatment, while others do not, is critical to advance the field of immunotherapy. As we gain a deeper understanding of tumor escape mechanisms and how to reverse them, and as technology advances enabling deep TME characterization on a patient-to-patient basis, personalized, combinatorial immunotherapies will improve responses and lead to more patients being cured.

Funding

European Research Council Advanced Grant to GC (1400206AdG-322875); Leenaards Foundation (no grant numbers apply).

This supplement was sponsored by F. Hoffmann-La Roche.

Disclosure

The authors have declared no conflicts of interest.

References

1. Jass JR. Lymphocytic infiltration and survival in rectal cancer. J Clin Pathol 1986; 39: 585–589.
2. Zhang L, Conejo-Garcia JR, Katsaros D et al. Intratumoral T cells, recurrence, and survival in epithelial ovarian cancer. N Engl J Med 2003; 348: 203–213.
3. Galon J, Costes A, Sanchez-Cabo F et al. Type, density, and location of immune cells within human colorectal tumors predict clinical outcome. Science 2006; 313: 1960–1964.
4. Fridman WH, Pages F, Sautes-Fridman C, Galon J. The immune contexture in human tumours: impact on clinical outcome. Nat Rev Cancer 2012; 12: 298–306.
5. Hwang WT, Adams SF, Tahirovic E et al. Prognostic significance of tumor-infiltrating T cells in ovarian cancer: a meta-analysis. Gynecol Oncol 2012; 124: 192–198.
6. Rosenberg SA, Yang JC, Sherry RM et al. Durable complete responses in heavily pretreated patients with metastatic melanoma using T-cell transfer immunotherapy. Clin Cancer Res 2011; 17: 4550–4557.
7. Restifo NP, Dudley ME, Rosenberg SA. Adoptive immunotherapy for cancer: harnessing the T cell response. Nat Rev Immunol 2012; 12: 269–281.
8. Rosenberg SA, Restifo NP. Adoptive cell transfer as personalized immunotherapy for human cancer. Science 2015; 348: 62–68.
9. Hodi FS, O'Day SJ, McDermott DF et al. Improved survival with ipilimumab in patients with metastatic melanoma. N Engl J Med 2010; 363: 711–723.
10. Topalian SL, Hodi FS, Brahmer JR et al. Safety, activity, and immune correlates of anti-PD-1 antibody in cancer. N Engl J Med 2012; 366: 2443–2454.
11. Hamanishi J, Mandai M, Ikeda T et al. Safety and antitumor activity of anti-PD-1 antibody, nivolumab, in patients with platinum-resistant ovarian cancer. J Clin Oncol 2015; 33: 4015–4022.
12. Brahmer J, Reckamp KL, Baas P et al. Nivolumab versus docetaxel in advanced squamous-cell non-small-cell lung cancer. N Engl J Med 2015; 373: 123–135.
13. Powles T, Eder JP, Fine GD et al. MPDL3280A (anti-PD-L1) treatment leads to clinical activity in metastatic bladder cancer. Nature 2014; 515: 558–562.
14. Motzer RJ, Escudier B, McDermott DF et al. Nivolumab versus everolimus in advanced renal-cell carcinoma. N Engl J Med 2015; 373: 1803–1813.
15. Ansell SM, Lesokhin AM, Borrello I et al. PD-1 blockade with nivolumab in relapsed or refractory Hodgkin's lymphoma. N Engl J Med 2015; 372: 311–319.
16. Le DT, Uram JN, Wang H et al. PD-1 blockade in tumors with mismatch-repair deficiency. N Engl J Med 2015; 372: 2509–2520.
17. Zang X, Allison JP. The B7 family and cancer therapy: costimulation and coinhibition. Clin Cancer Res 2007; 13: 5271–5279.
18. Larkin J, Chiarion-Sileni V, Gonzalez R et al. Combined nivolumab and ipilimumab or monotherapy in untreated melanoma. N Engl J Med 2015; 373: 23–34.
19. Tumeh PC, Harview CL, Yearley JH et al. PD-1 blockade induces responses by inhibiting adaptive immune resistance. Nature 2014; 515: 568–571.
20. Chen DS, Mellman I. Oncology meets immunology: the cancer-immunity cycle. Immunity 2013; 39: 1–10.
21. Kim JM, Chen DS. Immune escape to PD-L1/PD-1 blockade: seven steps to success (or failure). Ann Oncol 2016; 27: 1492–1504.
22. Dieu-Nosjean MC, Goc J, Giraldo NA et al. Tertiary lymphoid structures in cancer and beyond. Trends Immunol 2014; 35: 571–580.
23. Goc J, Fridman WH, Hammond SA et al. Tertiary lymphoid structures in human lung cancers, a new driver of antitumor immune responses. Oncoimmunology 2014; 3: e28976.
24. Zhu W, Germain C, Liu Z et al. A high density of tertiary lymphoid structure B cells in lung tumors is associated with increased CD4+ T cell receptor repertoire clonality. Oncoimmunology 2015; 4: e1051922.
25. Starr TK, Jameson SC, Hogquist KA. Positive and negative selection of T cells. Annu Rev Immunol 2003; 21: 139–176.

26. Harlin H, Meng Y, Peterson AC et al. Chemokine expression in melanoma metastases associated with CD8+ T-cell recruitment. Cancer Res 2009; 69: 3077–3085.
27. Salerno EP, Olson WC, McSkimming C et al. T cells in the human metastatic melanoma microenvironment express site-specific homing receptors and retention integrins. Int J Cancer 2014; 134: 563–574.
28. Gajewski TF, Schreiber H, Fu YX. Innate and adaptive immune cells in the tumor microenvironment. Nat Immunol 2013; 14: 1014–1022.
29. Fuertes MB, Kacha AK, Kline J et al. Host type I IFN signals are required for antitumor CD8+ T cell responses through CD8{alpha}+ dendritic cells. J Exp Med 2011; 208: 2005–2016.
30. Broz ML, Binnewies M, Boldajipour B et al. Dissecting the tumor myeloid compartment reveals rare activating antigen-presenting cells critical for T cell immunity. Cancer Cell 2014; 26: 638–652.
31. Woo SR, Fuertes MB, Corrales L et al. STING-dependent cytosolic DNA sensing mediates innate immune recognition of immunogenic tumors. Immunity 2014; 41: 830–842.
32. Corrales L, Glickman LH, McWhirter SM et al. Direct Activation of STING in the Tumor Microenvironment Leads to Potent and Systemic Tumor Regression and Immunity. Cell Rep 2015; 11: 1018–1030.
33. Woo SR, Corrales L, Gajewski TF. The STING pathway and the T cell-inflamed tumor microenvironment. Trends Immunol 2015; 36: 250–256.
34. Tran Janco JM, Lamichhane P, Karyampudi L, Knutson KL. Tumor-infiltrating dendritic cells in cancer pathogenesis. J Immunol 2015; 194: 2985–2991.
35. Coulie PG, Van den Eynde BJ, van der Bruggen P, Boon T. Tumour antigens recognized by T lymphocytes: at the core of cancer immunotherapy. Nat Rev Cancer 2014; 14: 135–146.
36. Irving M, Zoete V, Hebeisen M et al. Interplay between T cell receptor binding kinetics and the level of cognate peptide presented by major histocompatibility complexes governs CD8+ T cell responsiveness. J Biol Chem 2012; 287: 23068–23078.
37. Vonderheide RH, June CH. Engineering T cells for cancer: our synthetic future. Immunol Rev 2014; 257: 7–13.
38. Snyder A, Makarov V, Merghoub T et al. Genetic basis for clinical response to CTLA-4 blockade in melanoma. N Engl J Med 2014; 371: 2189–2199.
39. Rizvi NA, Hellmann MD, Snyder A et al. Cancer immunology. Mutational landscape determines sensitivity to PD-1 blockade in non-small cell lung cancer. Science 2015; 348: 124–128.
40. Gubin MM, Zhang X, Schuster H et al. Checkpoint blockade cancer immunotherapy targets tumour-specific mutant antigens. Nature 2014; 515: 577–581.
41. van Rooij N, van Buuren MM, Philips D et al. Tumor exome analysis reveals neoantigen-specific T-cell reactivity in an ipilimumab-responsive melanoma. J Clin Oncol 2013; 31: e439–e442.
42. Tran E, Turcotte S, Gros A et al. Cancer immunotherapy based on mutation-specific CD4+ T cells in a patient with epithelial cancer. Science 2014; 344: 641–645.
43. Anagnostou V, Smith KN, Forde PM et al. Evolution of neoantigen landscape during immune checkpoint blockade in non-small cell lung cancer. Cancer Discov 2017; 7: 264–276.
44. Spranger S, Luke JJ, Bao R et al. Density of immunogenic antigens does not explain the presence or absence of the T-cell-inflamed tumor microenvironment in melanoma. Proc Natl Acad Sci USA 2016; 113: E7759–E7768.
45. Nghiem PT, Bhatia S, Lipson EJ et al. PD-1 blockade with pembrolizumab in advanced merkel-cell carcinoma. N Engl J Med 2016; 374: 2542–2552.
46. Lipson EJ, Vincent JG, Loyo M et al. PD-L1 expression in the Merkel cell carcinoma microenvironment: association with inflammation, Merkel cell polyomavirus and overall survival. Cancer Immunol Res 2013; 1: 54–63.
47. Williams TM. Human leukocyte antigen gene polymorphism and the histocompatibility laboratory. J Mol Diagn 2001; 3: 98–104.
48. Bassing CH, Swat W, Alt FW. The mechanism and regulation of chromosomal V(D)J recombination. Cell 2002; 109 Suppl: S45–S55.
49. Seliger B, Cabrera T, Garrido F, Ferrone S. HLA class I antigen abnormalities and immune escape by malignant cells. Semin Cancer Biol 2002; 12: 3–13.
50. Marincola FM, Jaffee EM, Hicklin DJ, Ferrone S. Escape of human solid tumors from T-cell recognition: molecular mechanisms and functional significance. Adv Immunol 2000; 74: 181–273.
51. Garrido F, Cabrera T, Aptsiauri N. "Hard" and "soft" lesions underlying the HLA class I alterations in cancer cells: implications for immunotherapy. Int J Cancer 2010; 127: 249–256.
52. Schreiber RD, Old LJ, Smyth MJ. Cancer immunoediting: integrating immunity's roles in cancer suppression and promotion. Science 2011; 331: 1565–1570.
53. Spranger S, Bao R, Gajewski TF. Melanoma-intrinsic beta-catenin signalling prevents anti-tumour immunity. Nature 2015; 523: 231–235.
54. Peng W, Chen JQ, Liu C et al. Loss of PTEN promotes resistance to T cell-mediated immunotherapy. Cancer Discov 2016; 6: 202–216.
55. Hall A, Meyle KD, Lange MK et al. Dysfunctional oxidative phosphorylation makes malignant melanoma cells addicted to glycolysis driven by the (V600E)BRAF oncogene. Oncotarget 2013; 4: 584–599.
56. Pivarcsi A, Muller A, Hippe A et al. Tumor immune escape by the loss of homeostatic chemokine expression. Proc Natl Acad Sci USA 2007; 104: 19055–19060.
57. Strickland KC, Howitt BE, Shukla SA et al. Association and prognostic significance of BRCA1/2-mutation status with neoantigen load, number of tumor-infiltrating lymphocytes and expression of PD-1/PD-L1 in high grade serous ovarian cancer. Oncotarget 2016; 7: 13587–13598.
58. van Gool IC, Eggink FA, Freeman-Mills L et al. POLE proofreading mutations elicit an antitumor immune response in endometrial cancer. Clin Cancer Res 2015; 21: 3347–3355.
59. Prall F, Duhrkop T, Weirich V et al. Prognostic role of CD8+ tumor-infiltrating lymphocytes in stage III colorectal cancer with and without microsatellite instability. Hum Pathol 2004; 35: 808–816.
60. Iida N, Dzutsev A, Stewart CA et al. Commensal bacteria control cancer response to therapy by modulating the tumor microenvironment. Science 2013; 342: 967–970.
61. Viaud S, Saccheri F, Mignot G et al. The intestinal microbiota modulates the anticancer immune effects of cyclophosphamide. Science 2013; 342: 971–976.
62. Sivan A, Corrales L, Hubert N et al. Commensal Bifidobacterium promotes antitumor immunity and facilitates anti-PD-L1 efficacy. Science 2015; 350: 1084–1089.
63. Vetizou M, Pitt JM, Daillere R et al. Anticancer immunotherapy by CTLA-4 blockade relies on the gut microbiota. Science 2015; 350: 1079–1084.
64. Krummel MF, Bartumeus F, Gerard A. T cell migration, search strategies and mechanisms. Nat Rev Immunol 2016; 16: 193–201.
65. Griffith JW, Sokol CL, Luster AD. Chemokines and chemokine receptors: positioning cells for host defense and immunity. Annu Rev Immunol 2014; 32: 659–702.
66. Woolf E, Grigorova I, Sagiv A et al. Lymph node chemokines promote sustained T lymphocyte motility without triggering stable integrin adhesiveness in the absence of shear forces. Nat Immunol 2007; 8: 1076–1085.
67. Katakai T, Habiro K, Kinashi T. Dendritic cells regulate high-speed interstitial T cell migration in the lymph node via LFA-1/ICAM-1. J Immunol 2013; 191: 1188–1199.
68. Bajenoff M, Egen JG, Koo LY et al. Stromal cell networks regulate lymphocyte entry, migration, and territoriality in lymph nodes. Immunity 2006; 25: 989–1001.
69. Mueller SN, Hosiawa-Meagher KA, Konieczny BT et al. Regulation of homeostatic chemokine expression and cell trafficking during immune responses. Science 2007; 317: 670–674.
70. Ferguson AR, Engelhard VH. CD8 T cells activated in distinct lymphoid organs differentially express adhesion proteins and coexpress multiple chemokine receptors. J Immunol 2010; 184: 4079–4086.
71. Sallusto F, Kremmer E, Palermo B et al. Switch in chemokine receptor expression upon TCR stimulation reveals novel homing potential for recently activated T cells. Eur J Immunol 1999; 29: 2037–2045.

72. Groom JR, Richmond J, Murooka TT et al. CXCR3 chemokine receptor-ligand interactions in the lymph node optimize CD4+ T helper 1 cell differentiation. Immunity 2012; 37: 1091–1103.
73. Hugues S, Scholer A, Boissonnas A et al. Dynamic imaging of chemokine-dependent CD8+ T cell help for CD8+ T cell responses. Nat Immunol 2007; 8: 921–930.
74. Allavena P, Germano G, Marchesi F, Mantovani A. Chemokines in cancer related inflammation. Exp Cell Res 2011; 317: 664–673.
75. Gonzalez-Martin A, Gomez L, Lustgarten J et al. Maximal T cell-mediated antitumor responses rely upon CCR5 expression in both CD4(+) and CD8(+) T cells. Cancer Res 2011; 71: 5455–5466.
76. Sung JH, Zhang H, Moseman EA et al. Chemokine guidance of central memory T cells is critical for antiviral recall responses in lymph nodes. Cell 2012; 150: 1249–1263.
77. Kastenmuller W, Brandes M, Wang Z et al. Peripheral prepositioning and local CXCL9 chemokine-mediated guidance orchestrate rapid memory CD8+ T cell responses in the lymph node. Immunity 2013; 38: 502–513.
78. Kurachi M, Kurachi J, Suenaga F et al. Chemokine receptor CXCR3 facilitates CD8(+) T cell differentiation into short-lived effector cells leading to memory degeneration. J Exp Med 2011; 208: 1605–1620.
79. Hong M, Puaux AL, Huang C et al. Chemotherapy induces intratumoral expression of chemokines in cutaneous melanoma, favoring T-cell infiltration and tumor control. Cancer Res 2011; 71: 6997–7009.
80. Hu JK, Kagari T, Clingan JM, Matloubian M. Expression of chemokine receptor CXCR3 on T cells affects the balance between effector and memory CD8 T-cell generation. Proc Natl Acad Sci USA 2011; 108: E118–E127.
81. Wendel M, Galani IE, Suri-Payer E, Cerwenka A. Natural killer cell accumulation in tumors is dependent on IFN-gamma and CXCR3 ligands. Cancer Res 2008; 68: 8437–8445.
82. Hensbergen PJ, Wijnands PG, Schreurs MW et al. The CXCR3 targeting chemokine CXCL11 has potent antitumor activity in vivo involving attraction of CD8+ T lymphocytes but not inhibition of angiogenesis. J Immunother 2005; 28: 343–351.
83. Peng W, Liu C, Xu C et al. PD-1 blockade enhances T-cell migration to tumors by elevating IFN-gamma inducible chemokines. Cancer Res 2012; 72: 5209–5218.
84. Ulloa-Montoya F, Louahed J, Dizier B et al. Predictive gene signature in MAGE-A3 antigen-specific cancer immunotherapy. J Clin Oncol 2013; 31: 2388–2395.
85. Mikucki ME, Fisher DT, Matsuzaki J et al. Non-redundant requirement for CXCR3 signalling during tumoricidal T-cell trafficking across tumour vascular checkpoints. Nat Commun. 2015; 6: 7458.
86. Allavena P, Mantovani A. Immunology in the clinic review series; focus on cancer: tumour-associated macrophages: undisputed stars of the inflammatory tumour microenvironment. Clin Exp Immunol 2012; 167: 195–205.
87. Lewis CE, Pollard JW. Distinct role of macrophages in different tumor microenvironments. Cancer Res 2006; 66: 605–612.
88. Schlecker E, Stojanovic A, Eisen C et al. Tumor-infiltrating monocytic myeloid-derived suppressor cells mediate CCR5-dependent recruitment of regulatory T cells favoring tumor growth. J Immunol 2012; 189: 5602–5611.
89. Liu J, Zhang N, Li Q et al. Tumor-associated macrophages recruit CCR6+ regulatory T cells and promote the development of colorectal cancer via enhancing CCL20 production in mice. PLoS One 2011; 6: e19495.
90. Qian BZ, Pollard JW. Macrophage diversity enhances tumor progression and metastasis. Cell 2010; 141: 39–51.
91. Choi HS, Ha SY, Kim HM et al. The prognostic effects of tumor infiltrating regulatory T cells and myeloid derived suppressor cells assessed by multicolor flow cytometry in gastric cancer patients. Oncotarget 2016; 7: 7940–7951.
92. Shang B, Liu Y, Jiang SJ, Liu Y. Prognostic value of tumor-infiltrating FoxP3+ regulatory T cells in cancers: a systematic review and meta-analysis. Sci Rep 2015; 5: 15179.
93. Colvin EK. Tumor-associated macrophages contribute to tumor progression in ovarian cancer. Front Oncol 2014; 4: 137.
94. Facciabene A, Peng X, Hagemann IS et al. Tumour hypoxia promotes tolerance and angiogenesis via CCL28 and T(reg) cells. Nature 2011; 475: 226–230.
95. Righi E, Kashiwagi S, Yuan J et al. CXCL12/CXCR4 blockade induces multimodal antitumor effects that prolong survival in an immunocompetent mouse model of ovarian cancer. Cancer Res 2011; 71: 5522–5534.
96. Wang Z, Ma Q, Liu Q et al. Blockade of SDF-1/CXCR4 signalling inhibits pancreatic cancer progression in vitro via inactivation of canonical Wnt pathway. Br J Cancer 2008; 99: 1695–1703.
97. Peng D, Kryczek I, Nagarsheth N et al. Epigenetic silencing of TH1-type chemokines shapes tumour immunity and immunotherapy. Nature 2015; 527: 249–253.
98. Molon B, Ugel S, Del Pozzo F et al. Chemokine nitration prevents intratumoral infiltration of antigen-specific T cells. J Exp Med 2011; 208: 1949–1962.
99. Proost P, Mortier A, Loos T et al. Proteolytic processing of CXCL11 by CD13/aminopeptidase N impairs CXCR3 and CXCR7 binding and signaling and reduces lymphocyte and endothelial cell migration. Blood 2007; 110: 37–44.
100. Hogg N, Patzak I, Willenbrock F. The insider's guide to leukocyte integrin signalling and function. Nat Rev Immunol 2011; 11: 416–426.
101. Adams DH, Shaw S. Leucocyte-endothelial interactions and regulation of leucocyte migration. Lancet 1994; 343: 831–836.
102. Butcher EC, Picker LJ. Lymphocyte homing and homeostasis. Science 1996; 272: 60–66.
103. Lanitis E, Irving M, Coukos G. Targeting the tumor vasculature to enhance T cell activity. Curr Opin Immunol 2015; 33: 55–63.
104. Bouzin C, Feron O. Targeting tumor stroma and exploiting mature tumor vasculature to improve anti-cancer drug delivery. Drug Resist Updat 2007; 10: 109–120.
105. Griffioen AW, Damen CA, Martinotti S et al. Endothelial intercellular adhesion molecule-1 expression is suppressed in human malignancies: the role of angiogenic factors. Cancer Res 1996; 56: 1111–1117.
106. Demunter A, De Wolf-Peeters C, Degreef H et al. Expression of the endothelin-B receptor in pigment cell lesions of the skin. Evidence for its role as tumor progression marker in malignant melanoma. Virchows Arch 2001; 438: 485–491.
107. Egidy G, Eberl LP, Valdenaire O et al. The endothelin system in human glioblastoma. Lab Invest 2000; 80: 1681–1689.
108. Tanaka T, Sho M, Takayama T et al. Endothelin B receptor expression correlates with tumour angiogenesis and prognosis in oesophageal squamous cell carcinoma. Br J Cancer 2014; 110: 1027–1033.
109. Buckanovich RJ, Facciabene A, Kim S et al. Endothelin B receptor mediates the endothelial barrier to T cell homing to tumors and disables immune therapy. Nat Med 2008; 14: 28–36.
110. Motz GT, Santoro SP, Wang LP et al. Tumor endothelium FasL establishes a selective immune barrier promoting tolerance in tumors. Nat Med 2014; 20: 607–615.
111. Yu JS, Lee PK, Ehtesham M et al. Intratumoral T cell subset ratios and Fas ligand expression on brain tumor endothelium. J Neurooncol 2003; 64: 55–61.
112. Zang X, Sullivan PS, Soslow RA et al. Tumor associated endothelial expression of B7-H3 predicts survival in ovarian carcinomas. Mod Pathol 2010; 23: 1104–1112.
113. Kraan J, van den Broek P, Verhoef C et al. Endothelial CD276 (B7-H3) expression is increased in human malignancies and distinguishes between normal and tumour-derived circulating endothelial cells. Br J Cancer 2014; 111: 149–156.
114. Qin X, Zhang H, Ye D et al. B7-H3 is a new cancer-specific endothelial marker in clear cell renal cell carcinoma. Onco Targets Ther 2013; 6: 1667–1673.
115. Mazanet MM, Hughes CC. B7-H1 is expressed by human endothelial cells and suppresses T cell cytokine synthesis. J Immunol 2002; 169: 3581–3588.

116. Rodig N, Ryan T, Allen JA et al. Endothelial expression of PD-L1 and PD-L2 down-regulates CD8+ T cell activation and cytolysis. Eur J Immunol 2003; 33: 3117–3126.
117. Wu FH, Yuan Y, Li D et al. Endothelial cell-expressed Tim-3 facilitates metastasis of melanoma cells by activating the NF-kappaB pathway. Oncol Rep 2010; 24: 693–699.
118. Huang X, Bai X, Cao Y et al. Lymphoma endothelium preferentially expresses Tim-3 and facilitates the progression of lymphoma by mediating immune evasion. J Exp Med 2010; 207: 505–520.
119. Krambeck AE, Thompson RH, Dong H et al. B7-H4 expression in renal cell carcinoma and tumor vasculature: associations with cancer progression and survival. Proc Natl Acad Sci USA 2006; 103: 10391–10396.
120. Mulligan JK, Young MR. Tumors induce the formation of suppressor endothelial cells in vivo. Cancer Immunol Immunother 2010; 59: 267–277.
121. Pirtskhalaishvili G, Nelson JB. Endothelium-derived factors as paracrine mediators of prostate cancer progression. Prostate 2000; 44: 77–87.
122. Casos K, Siguero L, Fernandez-Figueras MT et al. Tumor cells induce COX-2 and mPGES-1 expression in microvascular endothelial cells mainly by means of IL-1 receptor activation. Microvasc Res 2011; 81: 261–268.
123. Taflin C, Favier B, Baudhuin J et al. Human endothelial cells generate Th17 and regulatory T cells under inflammatory conditions. Proc Natl Acad Sci USA 2011; 108: 2891–2896.
124. Lider O, Mekori YA, Miller T et al. Inhibition of T lymphocyte heparanase by heparin prevents T cell migration and T cell-mediated immunity. Eur J Immunol 1990; 20: 493–499.
125. Kalluri R, Zeisberg M. Fibroblasts in cancer. Nat Rev Cancer 2006; 6: 392–401.
126. Joyce JA, Fearon DT. T cell exclusion, immune privilege, and the tumor microenvironment. Science 2015; 348: 74–80.
127. Kano A. Tumor cell secretion of soluble factor(s) for specific immunosuppression. Sci Rep 2015; 5: 8913.
128. Nakanishi M, Rosenberg DW. Multifaceted roles of PGE2 in inflammation and cancer. Semin Immunopathol 2013; 35: 123–137.
129. Kalinski P. Regulation of immune responses by prostaglandin E2. J Immunol 2012; 188: 21–28.
130. Marvel D, Gabrilovich DI. Myeloid-derived suppressor cells in the tumor microenvironment: expect the unexpected. J Clin Invest 2015; 125: 3356–3364.
131. Mandai M, Hamanishi J, Abiko K et al. Dual faces of IFNgamma in cancer progression: a role of PD-L1 induction in the determination of pro- and antitumor immunity. Clin Cancer Res 2016; 22: 2329–2334.
132. Spranger S, Spaapen RM, Zha Y et al. Up-regulation of PD-L1, IDO, and T(regs) in the melanoma tumor microenvironment is driven by CD8(+) T cells. Sci Transl Med 2013; 5: 200ra116.
133. Deaglio S, Dwyer KM, Gao W et al. Adenosine generation catalyzed by CD39 and CD73 expressed on regulatory T cells mediates immune suppression. J Exp Med 2007; 204: 1257–1265.
134. Pandiyan P, Zheng L, Ishihara S et al. CD4+CD25+Foxp3+ regulatory T cells induce cytokine deprivation-mediated apoptosis of effector CD4+ T cells. Nat Immunol 2007; 8: 1353–1362.
135. Liang B, Workman C, Lee J et al. Regulatory T cells inhibit dendritic cells by lymphocyte activation gene-3 engagement of MHC class II. J Immunol 2008; 180: 5916–5926.
136. Chen L, Flies DB. Molecular mechanisms of T cell co-stimulation and co-inhibition. Nat Rev Immunol 2013; 13: 227–242.
137. Staveley-O'Carroll K, Sotomayor E, Montgomery J et al. Induction of antigen-specific T cell anergy: An early event in the course of tumor progression. Proc Natl Acad Sci USA 1998; 95: 1178–1183.
138. Johnston RJ, Comps-Agrar L, Hackney J et al. The immunoreceptor TIGIT regulates antitumor and antiviral CD8(+) T cell effector function. Cancer Cell 2014; 26: 923–937.
139. Sledzinska A, Menger L, Bergerhoff K et al. Negative immune checkpoints on T lymphocytes and their relevance to cancer immunotherapy. Mol Oncol 2015; 9: 1936–1965.
140. Dolton GM, Sathish JG, Matthews RJ. Protein tyrosine phosphatases as negative regulators of the immune response. Biochm Soc Trans 2006; 34: 1041–1045.
141. Lorenz U. SHP-1 and SHP-2 in T cells: two phosphatases functioning at many levels. Immunol Rev 2009; 228: 342–359.
142. Lutz-Nicoladoni C, Wolf D, Sopper S. Modulation of immune cell functions by the E3 ligase Cbl-b. Front Oncol 2015; 5: 58.
143. Riese MJ, Moon EK, Johnson BD, Albelda SM. Diacylglycerol kinases (DGKs): novel targets for improving T cell activity in cancer. Front Cell Dev Biol 2016; 4: 108.
144. O'Brien S, Thomas RM, Wertheim GB et al. Ikaros imposes a barrier to CD8+ T cell differentiation by restricting autocrine IL-2 production. J Immunol 2014; 192: 5118–5129.
145. Wherry EJ, Kurachi M. Molecular and cellular insights into T cell exhaustion. Nat Rev Immunol 2015; 15: 486–499.
146. Vander Heiden MG, Cantley LC, Thompson CB. Understanding the Warburg effect: the metabolic requirements of cell proliferation. Science 2009; 324: 1029–1033.
147. Warburg O. On respiratory impairment in cancer cells. Science 1956; 124: 269–270.
148. MacIver NJ, Michalek RD, Rathmell JC. Metabolic regulation of T lymphocytes. Annu Rev Immunol 2013; 31: 259–283.
149. Wang R, Green DR. Metabolic reprogramming and metabolic dependency in T cells. Immunol Rev 2012; 249: 14–26.
150. Chang CH, Qiu J, O'Sullivan D et al. Metabolic competition in the tumor microenvironment is a driver of cancer progression. Cell 2015; 162: 1229–1241.
151. Zhao E, Maj T, Kryczek I et al. Cancer mediates effector T cell dysfunction by targeting microRNAs and EZH2 via glycolysis restriction. Nat Immunol 2016; 17: 95–103.
152. Fischer K, Hoffmann P, Voelkl S et al. Inhibitory effect of tumor cell-derived lactic acid on human T cells. Blood 2007; 109: 3812–3819.
153. Kouidhi S, Noman MZ, Kieda C et al. Intrinsic and tumor microenvironment-induced metabolism adaptations of T cells and impact on their differentiation and function. Front Immunol 2016; 7: 114.
154. Bronte V, Zanovello P. Regulation of immune responses by L-arginine metabolism. Nat Rev Immunol 2005; 5: 641–654.
155. Rodriguez PC, Quiceno DG, Ochoa AC. L-arginine availability regulates T-lymphocyte cell-cycle progression. Blood 2007; 109: 1568–1573.
156. Raber P, Ochoa AC, Rodriguez PC. Metabolism of L-arginine by myeloid-derived suppressor cells in cancer: mechanisms of T cell suppression and therapeutic perspectives. Immunol Invest 2012; 41: 614–634.
157. Rodriguez PC, Quiceno DG, Zabaleta J et al. Arginase I production in the tumor microenvironment by mature myeloid cells inhibits T-cell receptor expression and antigen-specific T-cell responses. Cancer Res 2004; 64: 5839–5849.
158. Friberg M, Jennings R, Alsarraj M et al. Indoleamine 2,3-dioxygenase contributes to tumor cell evasion of T cell-mediated rejection. Int J Cancer 2002; 101: 151–155.
159. Frumento G, Rotondo R, Tonetti M et al. Tryptophan-derived catabolites are responsible for inhibition of T and natural killer cell proliferation induced by indoleamine 2,3-dioxygenase. J Exp Med 2002; 196: 459–468.
160. Katz JB, Muller AJ, Prendergast GC. Indoleamine 2,3-dioxygenase in T-cell tolerance and tumoral immune escape. Immunol Rev 2008; 222: 206–221.
161. Baban B, Chandler PR, Sharma MD et al. IDO activates regulatory T cells and blocks their conversion into Th17-like T cells. J Immunol 2009; 183: 2475–2483.
162. Patsoukis N, Bardhan K, Chatterjee P et al. PD-1 alters T-cell metabolic reprogramming by inhibiting glycolysis and promoting lipolysis and fatty acid oxidation. Nat Commun 2015; 6: 6692.
163. Parry RV, Chemnitz JM, Frauwirth KA et al. CTLA-4 and PD-1 receptors inhibit T-cell activation by distinct mechanisms. Mol Cell Biol 2005; 25: 9543–9553.

164. Topalian SL, Drake CG, Pardoll DM. Immune checkpoint blockade: a common denominator approach to cancer therapy. Cancer Cell 2015; 27: 450–461.
165. Dudley ME, Wunderlich JR, Shelton TE et al. Generation of tumor-infiltrating lymphocyte cultures for use in adoptive transfer therapy for melanoma patients. J Immunother 2003; 26: 332–342.
166. Topalian SL, Muul LM, Solomon D, Rosenberg SA. Expansion of human tumor infiltrating lymphocytes for use in immunotherapy trials. J Immunol Methods 1987; 102: 127–141.
167. Butler MO, Lee JS, Ansen S et al. Long-lived antitumor CD8+ lymphocytes for adoptive therapy generated using an artificial antigen-presenting cell. Clin Cancer Res 2007; 13: 1857–1867.
168. Butler MO, Friedlander P, Milstein MI et al. Establishment of antitumor memory in humans using in vitro-educated CD8+ T cells. Sci Transl Med 2011; 3: 80ra34.
169. Rapoport AP, Stadtmauer EA, Binder-Scholl GK et al. NY-ESO-1-specific TCR-engineered T cells mediate sustained antigen-specific antitumor effects in myeloma. Nat Med 2015; 21: 914–921.
170. Hinrichs CS, Rosenberg SA. Exploiting the curative potential of adoptive T-cell therapy for cancer. Immunol Rev 2014; 257: 56–71.
171. Sadelain M, Brentjens R, Riviere I. The basic principles of chimeric antigen receptor design. Cancer Discov 2013; 3: 388–398.
172. Gill S, Maus MV, Porter DL. Chimeric antigen receptor T cell therapy: 25years in the making. Blood Rev 2016; 30: 157–167.
173. Maus MV, Grupp SA, Porter DL, June CH. Antibody-modified T cells: CARs take the front seat for hematologic malignancies. Blood 2014; 123: 2625–2635.
174. Maude SL, Frey N, Shaw PA et al. Chimeric antigen receptor T cells for sustained remissions in leukemia. N Engl J Med 2014; 371: 1507–1517.
175. Lamers CH, Sleijfer S, Vulto AG et al. Treatment of metastatic renal cell carcinoma with autologous T-lymphocytes genetically retargeted against carbonic anhydrase IX: first clinical experience. J Clin Oncol 2006; 24: e20–e22.
176. Lamers CH, Sleijfer S, van Steenbergen S et al. Treatment of metastatic renal cell carcinoma with CAIX CAR-engineered T cells: clinical evaluation and management of on-target toxicity. Mol Ther 2013; 21: 904–912.
177. Morgan RA, Yang JC, Kitano M et al. 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: 843–851.
178. Gargett T, Brown MP. The inducible caspase-9 suicide gene system as a "safety switch" to limit on-target, off-tumor toxicities of chimeric antigen receptor T cells. Front Pharmacol 2014; 5: 235.
179. Lanitis E, Poussin M, Klattenhoff AW et al. Chimeric antigen receptor T Cells with dissociated signaling domains exhibit focused antitumor activity with reduced potential for toxicity in vivo. Cancer Immunol Res 2013; 1: 43–53.
180. Wu CY, Roybal KT, Puchner EM et al. Remote control of therapeutic T cells through a small molecule-gated chimeric receptor. Science 2015; 350: aab4077.
181. Beatty GL, Moon EK. Chimeric antigen receptor T cells are vulnerable to immunosuppressive mechanisms present within the tumor microenvironment. Oncoimmunology 2014; 3: e970027.
182. Moon EK, Wang LC, Dolfi DV et al. Multifactorial T-cell hypofunction that is reversible can limit the efficacy of chimeric antigen receptor-transduced human T cells in solid tumors. Clin Cancer Res 2014; 20: 4262–4273.
183. Cherkassky L, Morello A, Villena-Vargas J et al. Human CAR T cells with cell-intrinsic PD-1 checkpoint blockade resist tumor-mediated inhibition. J Clin Invest 2016; 126: 3130–3144.
184. John LB, Devaud C, Duong CP et al. Anti-PD-1 antibody therapy potently enhances the eradication of established tumors by gene-modified T cells. Clin Cancer Res 2013; 19: 5636–5646.
185. Khalil DN, Smith EL, Brentjens RJ, Wolchok JD. The future of cancer treatment: immunomodulation, CARs and combination immunotherapy. Nat Rev Clin Oncol 2016; 13: 273–290.
186. Di Stasi A, De Angelis B, Rooney CM et al. T lymphocytes coexpressing CCR4 and a chimeric antigen receptor targeting CD30 have improved homing and antitumor activity in a Hodgkin tumor model. Blood 2009; 113: 6392–6402.
187. Moon EK, Carpenito C, Sun J et al. Expression of a functional CCR2 receptor enhances tumor localization and tumor eradication by retargeted human T cells expressing a mesothelin-specific chimeric antibody receptor. Clin Cancer Res 2011; 17: 4719–4730.
188. Chmielewski M, Hombach AA, Abken H. Of CARs and TRUCKs: chimeric antigen receptor (CAR) T cells engineered with an inducible cytokine to modulate the tumor stroma. Immunol Rev 2014; 257: 83–90.
189. Curran KJ, Seinstra BA, Nikhamin Y et al. Enhancing antitumor efficacy of chimeric antigen receptor T cells through constitutive CD40L expression. Mol Ther 2015; 23: 769–778.
190. Pardoll DM. The blockade of immune checkpoints in cancer immunotherapy. Nat Rev Cancer 2012; 12: 252–264.
191. Weinstock M, McDermott D. Targeting PD-1/PD-L1 in the treatment of metastatic renal cell carcinoma. Ther Adv Urol 2015; 7: 365–377.
192. Califano R, Kerr K, Morgan RD et al. Immune checkpoint blockade: a new era for non-small cell lung cancer. Curr Oncol Rep 2016; 18: 59.
193. Mahoney KM, Freeman GJ, McDermott DF. The next immune-checkpoint inhibitors: PD-1/PD-L1 blockade in melanoma. Clin Ther 2015; 37: 764–782.
194. Im SJ, Hashimoto M, Gerner MY et al. Defining CD8+ T cells that provide the proliferative burst after PD-1 therapy. Nature 2016; 537: 417–421.
195. Utzschneider DT, Charmoy M, Chennupati V et al. T cell factor 1-expressing memory-like CD8(+) T cells sustain the immune response to chronic viral infections. Immunity 2016; 45: 415–427.
196. Wherry EJ. T cell exhaustion. Nat Immunol 2011; 12: 492–499.
197. Okazaki T, Honjo T. PD-1 and PD-1 ligands: from discovery to clinical application. Int Immunol 2007; 19: 813–824.
198. Grosso JF, Goldberg MV, Getnet D et al. Functionally distinct LAG-3 and PD-1 subsets on activated and chronically stimulated CD8 T cells. J Immunol 2009; 182: 6659–6669.
199. Sakuishi K, Apetoh L, Sullivan JM et al. Targeting Tim-3 and PD-1 pathways to reverse T cell exhaustion and restore anti-tumor immunity. J Exp Med 2010; 207: 2187–2194.
200. Le Mercier I, Chen W, Lines JL et al. VISTA regulates the development of protective antitumor immunity. Cancer Res 2014; 74: 1933–1944.
201. Yonezawa A, Dutt S, Chester C et al. Boosting cancer immunotherapy with anti-CD137 antibody therapy. Clin Cancer Res 2015; 21: 3113–3120.
202. Linch SN, McNamara MJ, Redmond WL. OX40 agonists and combination immunotherapy: putting the pedal to the metal. Front Oncol 2015; 5: 34.
203. Moran AE, Kovacsovics-Bankowski M, Weinberg AD. The TNFRs OX40, 4-1BB, and CD40 as targets for cancer immunotherapy. Curr Opin Immunol 2013; 25: 230–237.
204. Weigelin B, Bolanos E, Rodriguez-Ruiz ME et al. Anti-CD137 monoclonal antibodies and adoptive T cell therapy: a perfect marriage? Cancer Immunol Immunother 2016; 65: 493–497.
205. Bartkowiak T, Curran MA. 4-1BB agonists: multi-potent potentiators of tumor immunity. Front Oncol 2015; 5: 117.
206. Roberts DJ, Franklin NA, Kingeter LM et al. Control of established melanoma by CD27 stimulation is associated with enhanced effector function and persistence, and reduced PD-1 expression of tumor infiltrating CD8(+) T cells. J Immunother 2010; 33: 769–779.
207. Cohen AD, Diab A, Perales MA et al. Agonist anti-GITR antibody enhances vaccine-induced CD8(+) T-cell responses and tumor immunity. Cancer Res 2006; 66: 4904–4912.
208. Eliopoulos AG, Young LS. The role of the CD40 pathway in the pathogenesis and treatment of cancer. Curr Opin Pharmacol 2004; 4: 360–367.
209. Grewal IS, Flavell RA. CD40 and CD154 in cell-mediated immunity. Annu Rev Immunol 1998; 16: 111–135.
210. Vonderheide RH, Glennie MJ. Agonistic CD40 antibodies and cancer therapy. Clin Cancer Res 2013; 19: 1035–1043.
211. Ma DY, Clark EA. The role of CD40 and CD154/CD40L in dendritic cells. Semin Immunol 2009; 21: 265–272.

212. Mangsbo SM, Broos S, Fletcher E et al. The human agonistic CD40 antibody ADC-1013 eradicates bladder tumors and generates T-cell-dependent tumor immunity. Clin Cancer Res 2015; 21: 1115–1126.
213. Beatty GL, Chiorean EG, Fishman MP et al. CD40 agonists alter tumor stroma and show efficacy against pancreatic carcinoma in mice and humans. Science 2011; 331: 1612–1616.
214. Weiss JM, Wiltout RH. Multifaceted antitumor responses to activating anti-CD40 antibody therapy combined with immunomodulatory or targeted agents. Oncoimmunology 2014; 3: e954483.
215. Beatty GL, Torigian DA, Chiorean EG et al. A phase I study of an agonist CD40 monoclonal antibody (CP-870,893) in combination with gemcitabine in patients with advanced pancreatic ductal adenocarcinoma. Clin Cancer Res 2013; 19: 6286–6295.
216. Zhu Y, Knolhoff BL, Meyer MA et al. CSF1/CSF1R blockade reprograms tumor-infiltrating macrophages and improves response to T-cell checkpoint immunotherapy in pancreatic cancer models. Cancer Res 2014; 74: 5057–5069.
217. Holmgaard RB, Zamarin D, Lesokhin A et al. Targeting myeloid-derived suppressor cells with colony stimulating factor-1 receptor blockade can reverse immune resistance to immunotherapy in indoleamine 2,3-dioxygenase-expressing tumors. EBioMedicine 2016; 6: 50–58.
218. Xu J, Escamilla J, Mok S et al. CSF1R signaling blockade stanches tumor-infiltrating myeloid cells and improves the efficacy of radiotherapy in prostate cancer. Cancer Res 2013; 73: 2782–2794.
219. Johansson A, Hamzah J, Payne CJ, Ganss R. Tumor-targeted TNFalpha stabilizes tumor vessels and enhances active immunotherapy. Proc Natl Acad Sci USA 2012; 109: 7841–7846.
220. Becker JC, Andersen MH, Schrama D, Thor Straten P. Immune-suppressive properties of the tumor microenvironment. Cancer Immunol Immunother 2013; 62: 1137–1148.
221. Platten M, von Knebel Doeberitz N, Oezen I et al. Cancer immunotherapy by targeting IDO1/TDO and their downstream effectors. Front Immunol 2014; 5: 673.
222. Honeychurch J, Glennie MJ, Johnson PW, Illidge TM. Anti-CD40 monoclonal antibody therapy in combination with irradiation results in a CD8 T-cell-dependent immunity to B-cell lymphoma. Blood 2003; 102: 1449–1457.
223. Palucka K, Banchereau J. Cancer immunotherapy via dendritic cells. Nat Rev Cancer 2012; 12: 265–277.
224. Kandalaft LE, Chiang CL, Tanyi J et al. A Phase I vaccine trial using dendritic cells pulsed with autologous oxidized lysate for recurrent ovarian cancer. J Transl Med 2013; 11: 149.
225. Chiang CL, Kandalaft LE, Tanyi J et al. A dendritic cell vaccine pulsed with autologous hypochlorous acid-oxidized ovarian cancer lysate primes effective broad antitumor immunity: from bench to bedside. Clin Cancer Res 2013; 19: 4801–4815.
226. Ribas A, Comin-Anduix B, Chmielowski B et al. Dendritic cell vaccination combined with CTLA4 blockade in patients with metastatic melanoma. Clin Cancer Res 2009; 15: 6267–6276.
227. Tureci O, Vormehr M, Diken M et al. Targeting the heterogeneity of cancer with individualized neoepitope vaccines. Clin Cancer Res 2016; 22: 1885–1896.
228. Carreno BM, Magrini V, Becker-Hapak M et al. Cancer immunotherapy. A dendritic cell vaccine increases the breadth and diversity of melanoma neoantigen-specific T cells. Science 2015; 348: 803–808.
229. Kranz LM, Diken M, Haas H et al. Systemic RNA delivery to dendritic cells exploits antiviral defence for cancer immunotherapy. Nature 2016; 534: 396–401.
230. Galluzzi L, Buque A, Kepp O et al. Immunological effects of conventional chemotherapy and targeted anticancer agents. Cancer Cell 2015; 28: 690–714.
231. Zitvogel L, Apetoh L, Ghiringhelli F, Kroemer G. Immunological aspects of cancer chemotherapy. Nat Rev Immunol 2008; 8: 59–73.
232. Obeid M, Tesniere A, Ghiringhelli F et al. Calreticulin exposure dictates the immunogenicity of cancer cell death. Nat Med 2007; 13: 54–61.
233. Palombo F, Focaccetti C, Barnaba V. Therapeutic implications of immunogenic cell death in human cancer. Front Immunol 2014; 4: 503.
234. Sistigu A, Yamazaki T, Vacchelli E et al. Cancer cell-autonomous contribution of type I interferon signaling to the efficacy of chemotherapy. Nat Med 2014; 20: 1301–1309.
235. Pfirschke C, Engblom C, Rickelt S et al. Immunogenic chemotherapy sensitizes tumors to checkpoint blockade therapy. Immunity 2016; 44: 343–354.
236. Kaufman HL, Kohlhapp FJ, Zloza A. Oncolytic viruses: a new class of immunotherapy drugs. Nat Rev Drug Discov 2015; 14: 642–662.
237. Aurelian L. Oncolytic viruses as immunotherapy: progress and remaining challenges. Onco Targets Ther 2016; 9: 2627–2637.
238. Lichty BD, Breitbach CJ, Stojdl DF, Bell JC. Going viral with cancer immunotherapy. Nat Rev Cancer 2014; 14: 559–567.
239. Johnson DB, Puzanov I, Kelley MC. Talimogene laherparepvec (T-VEC) for the treatment of advanced melanoma. Immunotherapy 2015; 7: 611–619.
240. Rojas JJ, Sampath P, Hou W, Thorne SH. Defining effective combinations of immune checkpoint blockade and oncolytic virotherapy. Clin Cancer Res 2015; 21: 5543–5551.
241. Zamarin D, Holmgaard RB, Subudhi SK et al. Localized oncolytic virotherapy overcomes systemic tumor resistance to immune checkpoint blockade immunotherapy. Sci Transl Med 2014; 6: 226ra232.
242. Puzanov I, Milhem MM, Minor D et al. Talimogene laherparepvec in combination with ipilimumab in previously untreated, unresectable stage IIIB-IV melanoma. J Clin Oncol 2016; 34: 2619–2626.
243. Jain RK. Normalization of tumor vasculature: an emerging concept in antiangiogenic therapy. Science 2005; 307: 58–62.
244. Carmeliet P, Jain RK. Angiogenesis in cancer and other diseases. Nature 2000; 407: 249–257.
245. Winkler F, Kozin SV, Tong RT et al. Kinetics of vascular normalization by VEGFR2 blockade governs brain tumor response to radiation: role of oxygenation, angiopoietin-1, and matrix metalloproteinases. Cancer Cell 2004; 6: 553–563.
246. Tong RT, Boucher Y, Kozin SV et al. Vascular normalization by vascular endothelial growth factor receptor 2 blockade induces a pressure gradient across the vasculature and improves drug penetration in tumors. Cancer Res 2004; 64: 3731–3736.
247. Dirkx AE, oude Egbrink MG, Castermans K et al. Anti-angiogenesis therapy can overcome endothelial cell anergy and promote leukocyte-endothelium interactions and infiltration in tumors. FASEB J 2006; 20: 621–630.
248. Li B, Lalani AS, Harding TC et al. Vascular endothelial growth factor blockade reduces intratumoral regulatory T cells and enhances the efficacy of a GM-CSF-secreting cancer immunotherapy. Clin Cancer Res 2006; 12: 6808–6816.
249. Manning EA, Ullman JG, Leatherman JM et al. A vascular endothelial growth factor receptor-2 inhibitor enhances antitumor immunity through an immune-based mechanism. Clin Cancer Res 2007; 13: 3951–3959.
250. Shrimali RK, Yu Z, Theoret MR et al. Antiangiogenic agents can increase lymphocyte infiltration into tumor and enhance the effectiveness of adoptive immunotherapy of cancer. Cancer Res 2010; 70: 6171–6180.
251. Kandalaft LE, Motz GT, Busch J, Coukos G. Angiogenesis and the tumor vasculature as antitumor immune modulators: the role of vascular endothelial growth factor and endothelin. Curr Top Microbiol Immunol 2011; 344: 129–148.
252. Miller DW, Vosseler S, Mirancea N et al. Rapid vessel regression, protease inhibition, and stromal normalization upon short-term vascular endothelial growth factor receptor 2 inhibition in skin carcinoma heterotransplants. Am J Pathol 2005; 167: 1389–1403.
253. Sacchi A, Gasparri A, Gallo-Stampino C et al. Synergistic antitumor activity of cisplatin, paclitaxel, and gemcitabine with tumor vasculature-targeted tumor necrosis factor-alpha. Clin Cancer Res 2006; 12: 175–182.
254. Curnis F, Sacchi A, Borgna L et al. Enhancement of tumor necrosis factor alpha antitumor immunotherapeutic properties by targeted delivery to aminopeptidase N (CD13). Nat Biotechnol 2000; 18: 1185–1190.
255. Curnis F, Sacchi A, Corti A. Improving chemotherapeutic drug penetration in tumors by vascular targeting and barrier alteration. J Clin Invest 2002; 110: 475–482.

256. Calcinotto A, Grioni M, Jachetti E et al. Targeting TNF-alpha to neoangiogenic vessels enhances lymphocyte infiltration in tumors and increases the therapeutic potential of immunotherapy. J Immunol 2012; 188: 2687–2694.
257. Palazon A, Teijeira A, Martinez-Forero I et al. Agonist anti-CD137 mAb act on tumor endothelial cells to enhance recruitment of activated T lymphocytes. Cancer Res 2011; 71: 801–811.
258. Wong PP, Bodrug N, Hodivala-Dilke KM. Exploring novel methods for modulating tumor blood vessels in cancer treatment. Curr Biol 2016; 26: R1161–R1166.
259. Wong PP, Demircioglu F, Ghazaly E et al. Dual-action combination therapy enhances angiogenesis while reducing tumor growth and spread. Cancer Cell 2015; 27: 123–137.
260. Ott PA, Hodi FS, Buchbinder EI. Inhibition of immune checkpoints and vascular endothelial growth factor as combination therapy for metastatic melanoma: an overview of rationale, preclinical evidence, and initial clinical data. Front Oncol 2015; 5: 202.
261. Hodi FS, Lawrence D, Lezcano C et al. Bevacizumab plus ipilimumab in patients with metastatic melanoma. Cancer Immunol Res 2014; 2: 632–642.
262. Wu X, Giobbie-Hurder A, Liao X et al. VEGF neutralization plus CTLA-4 blockade alters soluble and cellular factors associated with enhancing lymphocyte infiltration and humoral recognition in melanoma. Cancer Immunol Res 2016; 4: 858–868.
263. Wallin JJ, Bendell JC, Funke R et al. Atezolizumab in combination with bevacizumab enhances antigen-specific T-cell migration in metastatic renal cell carcinoma. Nat Commun 2016; 7: 12624.
264. Jones PA, Issa JP, Baylin S. Targeting the cancer epigenome for therapy. Nat Rev Genet 2016; 17: 630–641.
265. Bhadury J, Nilsson LM, Muralidharan SV et al. BET and HDAC inhibitors induce similar genes and biological effects and synergize to kill in Myc-induced murine lymphoma. Proc Natl Acad Sci USA 2014; 111: E2721–E2730.
266. Yang X, Han H, De Carvalho DD et al. Gene body methylation can alter gene expression and is a therapeutic target in cancer. Cancer Cell 2014; 26: 577–590.
267. Morin RD, Johnson NA, Severson TM et al. Somatic mutations altering EZH2 (Tyr641) in follicular and diffuse large B-cell lymphomas of germinal-center origin. Nat Genet 2010; 42: 181–185.
268. McCabe MT, Ott HM, Ganji G et al. EZH2 inhibition as a therapeutic strategy for lymphoma with EZH2-activating mutations. Nature 2012; 492: 108–112.
269. Lu C, Ward PS, Kapoor GS et al. IDH mutation impairs histone demethylation and results in a block to cell differentiation. Nature 2012; 483: 474–478.
270. Turcan S, Rohle D, Goenka A et al. IDH1 mutation is sufficient to establish the glioma hypermethylator phenotype. Nature 2012; 483: 479–483.
271. Weber J, Salgaller M, Samid D et al. Expression of the MAGE-1 tumor antigen is up-regulated by the demethylating agent 5-aza-2'-deoxycytidine. Cancer Res 1994; 54: 1766–1771.
272. Karpf AR, Jones DA. Reactivating the expression of methylation silenced genes in human cancer. Oncogene 2002; 21: 5496–5503.
273. Odunsi K, Matsuzaki J, James SR et al. Epigenetic potentiation of NY-ESO-1 vaccine therapy in human ovarian cancer. Cancer Immunol Res 2014; 2: 37–49.
274. Chiappinelli KB, Strissel PL, Desrichard A et al. Inhibiting DNA methylation causes an interferon response in cancer via dsRNA including endogenous retroviruses. Cell 2016; 164: 1073.
275. Roulois D, Loo Yau H, Singhania R et al. DNA-demethylating agents target colorectal cancer cells by inducing viral mimicry by endogenous transcripts. Cell 2015; 162: 961–973.
276. Herrera FG, Bourhis J, Coukos G. Radiotherapy combination opportunities leveraging immunity for the next oncology practice. CA Cancer J Clin 2017; 67: 65–85.
277. Kaur P, Asea A. Radiation-induced effects and the immune system in cancer. Front Oncol 2012; 2: 191.
278. Reynders K, De Ruysscher D. Radiotherapy and immunotherapy: improving cancer treatment through synergy. Prog Tumor Res 2015; 42: 67–78.
279. Reits EA, Hodge JW, Herberts CA et al. Radiation modulates the peptide repertoire, enhances MHC class I expression, and induces successful antitumor immunotherapy. J Exp Med 2006; 203: 1259–1271.
280. Matsumura S, Demaria S. Up-regulation of the pro-inflammatory chemokine CXCL16 is a common response of tumor cells to ionizing radiation. Radiat Res 2010; 173: 418–425.
281. Lugade AA, Sorensen EW, Gerber SA et al. Radiation-induced IFN-gamma production within the tumor microenvironment influences antitumor immunity. J Immunol 2008; 180: 3132–3139.
282. Chakraborty M, Abrams SI, Coleman CN et al. External beam radiation of tumors alters phenotype of tumor cells to render them susceptible to vaccine-mediated T-cell killing. Cancer Res 2004; 64: 4328–4337.
283. Wei S, Egenti MU, Teitz-Tennenbaum S et al. Effects of tumor irradiation on host T-regulatory cells and systemic immunity in the context of adoptive T-cell therapy in mice. J Immunother 2013; 36: 124–132.
284. Quarmby S, Hunter RD, Kumar S. Irradiation induced expression of CD31, ICAM-1 and VCAM-1 in human microvascular endothelial cells. Anticancer Res 2000; 20: 3375–3381.
285. Lugade AA, Moran JP, Gerber SA et al. Local radiation therapy of B16 melanoma tumors increases the generation of tumor antigen-specific effector cells that traffic to the tumor. J Immunol 2005; 174: 7516–7523.
286. Hallahan D, Kuchibhotla J, Wyble C. Cell adhesion molecules mediate radiation-induced leukocyte adhesion to the vascular endothelium. Cancer Res 1996; 56: 5150–5155.
287. Klug F, Prakash H, Huber PE et al. Low-dose irradiation programs macrophage differentiation to an iNOS(+)/M1 phenotype that orchestrates effective T cell immunotherapy. Cancer Cell 2013; 24: 589–602.
288. De Palma M, Coukos G, Hanahan D. A new twist on radiation oncology: low-dose irradiation elicits immunostimulatory macrophages that unlock barriers to tumor immunotherapy. Cancer Cell 2013; 24: 559–561.
289. Demaria S, Kawashima N, Yang AM et al. Immune-mediated inhibition of metastases after treatment with local radiation and CTLA-4 blockade in a mouse model of breast cancer. Clin Cancer Res 2005; 11: 728–734.
290. Dewan MZ, Galloway AE, Kawashima N et al. Fractionated but not single-dose radiotherapy induces an immune-mediated abscopal effect when combined with anti-CTLA-4 antibody. Clin Cancer Res 2009; 15: 5379–5388.
291. Deng L, Liang H, Burnette B et al. Irradiation and anti-PD-L1 treatment synergistically promote antitumor immunity in mice. J Clin Invest 2014; 124: 687–695.
292. Twyman-Saint Victor C, Rech AJ, Maity A et al. Radiation and dual checkpoint blockade activate non-redundant immune mechanisms in cancer. Nature 2015; 520: 373–377.
293. PD-L1 upregulation drives escape from anti-CTLA4 and radiation therapy. Cancer Discov 2015; 5: OF13.
294. Leavy O. Immunotherapy: a triple blow for cancer. Nat Rev Cancer 2015; 15: 258–259.
295. Yokouchi H, Yamazaki K, Chamoto K et al. Anti-OX40 monoclonal antibody therapy in combination with radiotherapy results in therapeutic antitumor immunity to murine lung cancer. Cancer Sci 2008; 99: 361–367.
296. Chakraborty M, Abrams SI, Camphausen K et al. Irradiation of tumor cells up-regulates Fas and enhances CTL lytic activity and CTL adoptive immunotherapy. J Immunol 2003; 170: 6338–6347.
297. Witek M, Blomain ES, Magee MS et al. Tumor radiation therapy creates therapeutic vaccine responses to the colorectal cancer antigen GUCY2C. Int J Radiat Oncol Biol Phys 2014; 88: 1188–1195.
298. Postow MA, Callahan MK, Barker CA et al. Immunologic correlates of the abscopal effect in a patient with melanoma. N Engl J Med 2012; 366: 925–931.
299. Demaria S, Coleman CN, Formenti SC. Radiotherapy: changing the game in immunotherapy. Trends Cancer 2016; 2: 286–294.