Mechanisms regulating T-cell infiltration and activity in solid tumors

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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 T cells 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 (CTLA-4) 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].

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...
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
A variety of tumor antigens can be recognized by T cells. Examples include peptides derived from mutated proteins (i.e. neo-epitopes), 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 structural mutations [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+ CD8α+ CD11b+ 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 BRAFV600E 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 epithelial 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]. Naive 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-γamma (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], 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 downregulation 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 (PGE2), specifically induces apoptosis of Fas-expressing CD8+ cells.
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], as well as secrete soluble inhibitory molecules including IL-6, PGE2, 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, adenosine, and PGE2. 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.
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 (TcM) 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.

**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 unresectable 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-Lipoplexes) to DCs have been recently shown capable of inducing strong adaptive and type 1 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 by the 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 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 30% objective response rate with a tolerable safety profile [242], and T-VEC plus anti-PD-1 mAb in a phase 1b/III trial (NCT0263508) 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], ETAR 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], and proinflammatory cytokines such as IL-1ß 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 (1400206/AdG-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.

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