Cellular Immunology 343 (2019) 103730

Contents lists available at ScienceDirect

Cellular Immunology

journal homepage: www.elsevier.com/locate/ycimm

Research paper

The tumor microenvironment: Thousand obstacles for effector T cells

Chiara Massa, Barbara Seliger*

Institute for Medical Immunology, Martin Luther University Halle-Wittenberg, Halle (Saale), Germany

ARTICLE INFO

Keywords:
Effector T cells
Immune check point
Tumor microenvironment

ABSTRACT

The immune system is endowed with the capability to recognize and destroy transformed cells, but even in the presence of an immune infiltrate many tumors do progress. In the last decades new discoveries have shed light into (some of) the underlying mechanisms. Immune effector cells are not only under the influence of immune suppressive cell subsets, but also intrinsically regulated by immune check point molecules that under physiological condition avoid attach of healthy tissue. Moreover, tumor cells are modifying the surrounding microenvironment through secretion of immune modulators as well as via their own metabolism, thus further impairing the development of immune effector functions. Different approaches are currently being evaluated in the clinic to overcome those regulatory mechanisms and to unleash effector T cells.

1. Introduction

Since the demonstration that T cells can recognize tumor associated antigens (TAA), which lead to the eradication of cancer cells, efforts have been made to increase the understanding of the interaction between T cells and tumor cells and to improve the use of this knowledge not only to enhance the ability to cure cancer patients with novel immune based therapies, but also to select the best therapy for each tumor patient.

During the last two decades different strategies have been identified through which tumor cells can protect themselves against antigen specific $CD8^+$ cytotoxic T lymphocytes (CTLs). Some of them are "normal" negative feedback mechanisms of the immune system, which are required under physiologic conditions to shut down a successful immune response in order to avoid damage to bystander healthy tissues. These include the induction of negative regulators on the effector cells to inhibit their response, but also the recruitment/polarization of immune suppressive cells. In addition, the tumor is converting the microenvironment into a hostile environment for T cells and their ability to perform effector functions, which is related to the tumor metabolism that not only deplete important nutrients for the T cells, but also induce accumulation of "waste products", which could further impair T cell function.

In the following sections we will discuss in more detail all these distinct mechanisms summarized in Fig. 1 and the implementation of this information to improve immunotherapeutic strategies in preclinical experimental models and in the clinic (Fig. 2).

2. Immune check point as an intrinsic shut down mechanism

In order to keep a balance between the elimination of dangerous entities and protection of healthy tissues, different negative feedback mechanisms exist in the immune system. Thus, directly after recognizing their target structure and performing their effector activity, T cells upregulate one or more negative feedback process(es) leading to a shut-down of the response, once the danger is eliminated. The prototype of this mechanism encompass immune check point (ICP) molecules or co-inhibitory receptors that are upregulated upon T cell stimulation and when engaged by their ligand impair different signaling pathways resulting in a reduced proliferation, secretion of cytokines and/or development of cytotoxic activity. Evaluation of tumor infiltrating lymphocytes (TILs) highlights an upregulation of many of these ICP

Abbreviations: A, anti; Ab, antibody; ACT, adoptive cell therapy; AML, acute myeloid leukemia; APC, antigen presenting cell; CAR, chimeric antigen receptor; CEACAM1, carcinoembryonic antigen related adhesion molecule 1; CPI, check point inhibitor; CTL, cytotoxic T lymphocyte; CTLA-4, cytotoxic T lymphocyte associated protein-4; CXCL, CXC-motive chemokine; EC, endothelial cell; gal, galectin; DC, dendritic cell; GITR, glucocorticoid induced TNF-receptor related gene; HCC, hepatocellular carcinoma; HMGB1, high mobility group protein B1; ICOS, inducible T-cell co-stimulatory; ICP, immune check point; IDO, indoleamine 2,3 dioxygenase; IFN, interferon; IHC, immunohistochemistry; IL, interleukin; iNOS, inducible nitric oxide synthase; LAG-3, lymphocyte activation gene-3; MDSC, myeloid derived suppressor cell; MHC, major histocompatibility complex; MSI, multispectral imaging; NK, natural killer; PBMNC, peripheral blood mononuclear cell; PD-1, programmed death-1; PD-L, programmed death ligand; ROS, reactive oxygen species; TAA, tumor associated antigen; TAF, tumor associated fibroblast; TAN, tumor associated neutrophil; TCR, T cell receptor; TIL, tumor infiltrating lymphocyte; Teff, T effector cell; TGF, transforming growth factor; TIGIT, T cell immunoglobulin and immunoreceptor tyrosine-based inhibitory motif; Tim-3, T-cell immunoglobulin domain and mucin domain-3; TME, tumor microenvironment; Treg, regulatory T cell; VISTA, V-domain Ig suppressor of T cells activation

* Corresponding author at: Institute for Medical Immunology, Martin Luther University Halle-Wittenberg, Magdeburger str 2, 06112 Halle (Saale), Germany.
E-mail address: Barbara.seliger@uk-halle.de (B. Seliger).

https://doi.org/10.1016/j.cellimm.2017.12.004
Received 17 August 2017; Received in revised form 4 December 2017; Accepted 7 December 2017
Available online 08 December 2017
0008-8749/

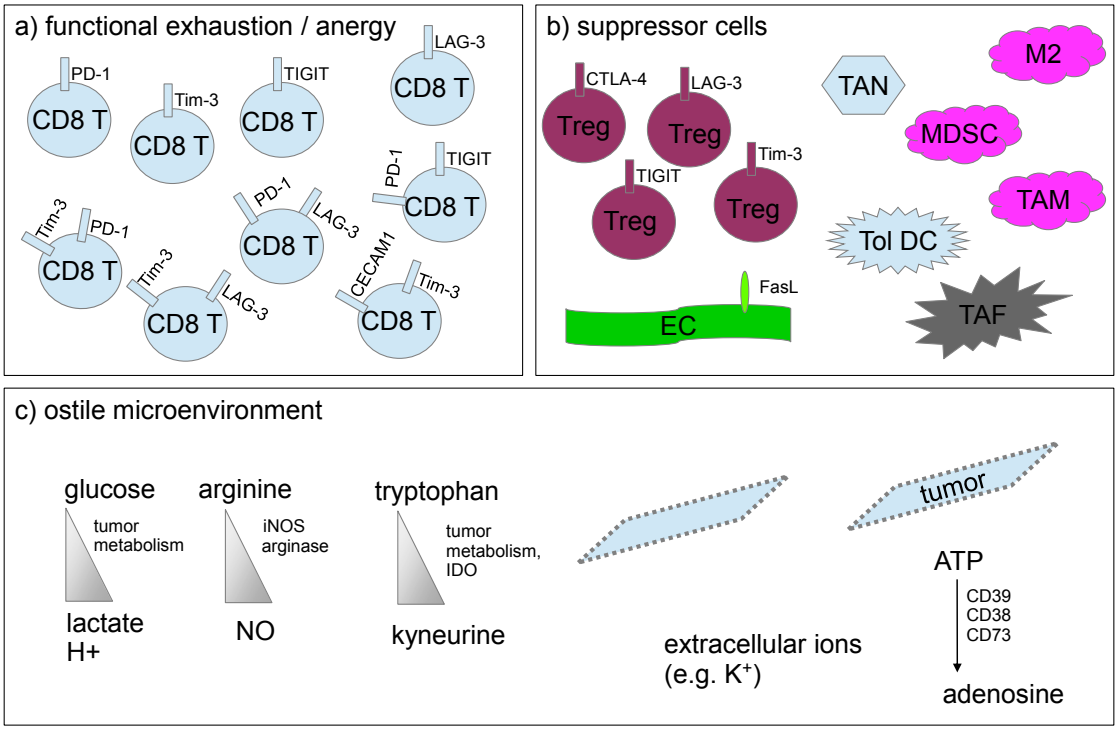

Fig. 1. Multiple mechanisms inhibit antigen specific effector cells within the tumor microenvironment. Antigen specific CD8$^+$ T cells that infiltrate the tumor are characterized by an enhanced expression of co-inhibitory receptors, either alone or in combination, and by low levels of co-stimulatory molecules, a phenotype correlating with functional exhaustion or anergy. In addition to an intrinsic impairment, the TME is also characterized by the presence of many immune and stromal cells that can directly suppress Teff functions in a cell contact dependent and independent manner as well as by a depletion of nutrients and accumulation of metabolites that further impair their activity and/or survival.

molecules on antigen specific T cell, which is accompanied by an either reversible or irreversible dysfunctional state. In the next sections the basic data on these ICPs, their relation to cancer progression and therapeutic targeting are outlined.

2.1. CTLA-4

The first identified co-inhibitory receptor has been CTLA-4 (cytotoxic T lymphocyte associated protein-4 or CD152) that is upregulated shortly after T cell receptor (TCR) triggering and due to its higher affinity for CD80/B7.1 and CD86/B7.2 than CD28 it can dampen its co-stimulatory signal and thus turn off T cell activation. In light of the absence of an own intracellular signaling domain and the difficulties in identifying a signaling partner, such inhibition might be mainly due to competition and not to direct signaling of CTLA-4. Moreover, the effect can also be cell extrinsic, since CTLA-4 can induce a depletion of co-stimulatory molecules from professional antigen presenting cells (APCs) via trogocytosis [1]. High CTLA-4 expression levels have been found on CD4$^+$ regulatory T cells (Tregs), which are frequently used as a marker together with CD25^{hi} to identify Tregs when intracellular staining of Foxp3 cannot be performed. Furthermore, CTLA-4 is not exclusively expressed on T cells, since its expression was found on a sub population of suppressive dendritic cells (DCs) in hepatocellular carcinoma (HCC) [2].

Being the first identified negative regulator of T cell responses, it was also the first one to be targeted in the clinic with an approval of the anti-(a)CTLA-4 antibody (Ab) ipilimumab in 2011 by the Food and Drug Administration for the treatment of unresectable or metastatic melanoma, whereas the second aCTLA-4 antibody tremelimumab was approved in 2015 for the treatment of mesothelioma, but both antibodies have been also tested in other tumor histotypes. In addition to the direct promotion of effector cell functionality [3], aCTLA-4 therapy works by depleting Tregs [4] and can also possibly influence migration of effector cells [5].

2.2. PD-1

PD-1 (programmed death-1 or CD279) is an inhibitory receptor that is transiently upregulated after TCR triggering and that upon binding to PD-L1 (B7-H1 or CD274) or PD-L2 (B7-DC or CD273) block the TCR signaling and T cell effector functions. Constitutive expression of the receptor is associated with conditions of chronic and/or suboptimal antigen stimulation and correlate with a state of functional exhaustion. High levels of PD-1$^+$ T cells in the tumor infiltrate can have a good prognostic value since they postulate the presence of an existing antigen specific immune response [6]. Different antibodies targeting either PD-1 (nivolumab and pembrolizumab, to cite the most used) or its major ligand PD-L1 (avelumab, atezolizumab and durvalumab) have been implemented in clinical trials in order to “remove the brake” and unleash the immune response against the tumor resulting in an increased clinical outcome in different tumor settings [7].

Evaluation of the response to aPD-1 therapy in relation to the expression of PD-L1 in the tumor biopsy has provided contrasting results. Indeed, despite patients with PD-L1$^+$ tumors have a higher probability to respond to the therapy, there are some responding patients that lack PD-L1expression suggesting a role of PD-L2 [8] or even a direct effect of the antibody on a small population of PD-1$^+$ melanoma cells [9]. Alternative explanations can also be related to technical issues, like the

clone of antibody implemented for the staining, the cut off value used to define a tumor as positive and/or the discrimination between membrane versus cytosolic staining or the expression by tumor versus infiltrating cells. Finally, distinct mechanisms leading to the expression of PD-L1 could be responsible. In some cases PD-L1 is constitutively expressed as a result of oncogenic signaling and is not associated with the immune infiltrate that can be targeted by the blocking antibody. Similarly, even when PD-L1 expression is induced as escape mechanism against an immune response, its expression can vary over time, and a "one time point" evaluation can provide a misleading value (reviewed in [10]).

2.3. Tim-3

Tim-3 (T-cell immunoglobulin domain and mucin domain-3) expression was originally described on activated $CD4^+$ and $CD8^+$ type 1 effector cells [11], but it was also detected on Tregs [12,13], where it plays an important role in their suppressive function [14]. Mechanistically, it has been demonstrated that chronic exposure to interleukin (IL)-12 or stimulation with IL-27 are responsible for the induction of Tim-3 expression and the dysfunctional phenotype [15,16].

Triggering by its canonical ligand galectin (gal)-9, expressed by tumor cells and also by Tregs, induce impaired T cell responses and can also lead to cell death [17]. In addition, Tim-3 can bind to the "danger associated molecule" high mobility group protein B1 (HMGB1) thereby suppressing immune responses by limiting its availability [18] and to carcinoembryonic antigen related adhesion molecule 1 (CEACAM1) that, when expressed in *cis*, can promote Tim-3 surface expression and mark cells with a more dysfunctional effector status [19,20].

In tumor patients infiltrating lymphocytes are enriched in $Tim\text{-}3^+$ cells when compared to peripheral counterparts and can also co-express PD-1 [21–23]. Analogous to PD-1, Tim-3 expression is considered as a marker for antigen specific T cells and a target for tumor immunotherapy. Therefore, different phase I clinical trials using Tim-3 blocking Abs are currently ongoing in patients with solid tumors as well as with acute myeloid leukemia (AML) (NCT02817633, NCT03099109, NCT02608268, NCT03066648 on www.clinicaltrials.gov).

Clinical targeting of Tim-3 can have also effects independent of the adaptive immunity. Different tumor cells have been shown to express Tim-3, which is directly involved in their tumorigenic potential *in vitro* [24–26]. Furthermore, cells from the innate immunity, like natural killer (NK) cells, express Tim-3, which could lead by gal-9 triggering to inhibition of their cytotoxic activity, but also to improved secretion of interferon (IFN)γ [27,28]. The secreted IFNγ can have positive antitumor effects, but can also induce an upregulation of other inhibitory feedback mechanisms. The role of Tim-3 on APC is more complex. On monocytes/macrophages it favors their polarization toward a tumor promoting M2 phenotype [29] and can boost immune suppression via interaction with $gal\text{-}9^+$ Tregs [30]. Due to its ability to bind to phosphatidylserine, Tim-3 plays also a role in the removal of apoptotic bodies and can induce antigen cross presentation in murine DC [31].

2.4. LAG-3

LAG-3 (lymphocyte activation gene-3 or CD223) is upregulated on activated $CD4^+$ and $CD8^+$ T cells in response to treatment with different cytokines [32]. It shares homology with CD4, but displays a higher affinity to major histocompatibility complex (MHC) class II molecules and therefore can act as a decoy receptor impairing CD4 mediated TCR co-stimulation. Despite its interaction with MHC class II molecules LAG-3 is able to directly inhibit $CD8^+$ T cells in a $CD4^+$ T cell independent manner [33]. LAG-3 plays an important role in the suppressive activity of Tregs [34] as well as in the sensitivity of naïve cells toward their suppressive activity [35].

Upregulated expression of LAG-3 in $CD8^+$ TILs was found in different tumor settings, both alone [36,37] and in combination with PD-1 [38,39], Tim-3 [40] or 4-1BB [41].

Binding of LAG-3 to MHC class II antigen can induce a reverse signaling into accessory cells, whose effect seems to depend on the "form" of LAG-3. While soluble LAG-3 can promote DC maturation [42,43], the interaction with transmembrane LAG-3 expressed by Tregs induce a semi-mature, and thus tolerogenic DC phenotype [44,45]. A reverse signaling by LAG-3 has also been described for MHC class II positive melanoma cells that became resistant to apoptosis upon interaction with a soluble as well as cell associated LAG-3 [46].

Immunotherapeutic approaches aiming at blocking the LAG-3 signaling use either classical inhibitory Abs or a soluble form of LAG-3. Whereas for the former no published results yet exist, absence of toxicity and some hints of functional activity have already been demonstrated for the latter [47–49].

2.5. TIGIT

TIGIT (T cell immunoglobulin and immunoreceptor tyrosine-based inhibitory motif [ITIM] domain) is a co-inhibitory receptor that bind to nectin molecules like CD155 (or Polio virus receptor), nectin-2 (CD112) and nectin-3 (CD113) and thus can directly transduce inhibitory signals into the effector cells as well as compete with DNAM-1 (CD226) and CD96 for their binding to these ligands and thus dampen their signaling pathway. It is highly expressed on Tregs and cells expressing TIGIT are also more prone to become regulatory cells characterized by the ability to secrete IL-10 and induce M2 polarization of macrophages [50].

In melanoma a direct role of TIGIT in inhibiting killing of CD155 overexpressing cells was reported *in vitro* [51] and in patients it is frequently co-expressed with PD-1 and can thus be responsible for the impaired response to ligand positive tumors [52]. A phase I clinical trial (NCT03119428) using a TIGIT blocking Ab is currently recruiting patients.

2.6. VISTA

VISTA (V-domain Ig suppressor of T cell activation, or programmed death-1 homolog, PD-1H) is a co-inhibitory molecule belonging to the CD28/B7 family that is expressed both on APC and on naïve T cells, leading to an inhibition of effector T cells (Teffs) and the induction of $Foxp3^+$ Tregs [53]. In the human setting its expression has been found in patients with gastric cancer but with no correlation to their survival [54], whereas in oral squamous carcinoma high VISTA expression levels combined with paucity of $CD8^+$ T cells is a predictor of poor survival [55]. Preclinical evaluation in mouse models suggests potential synergy with the blockade of PD-1 and/or CTLA-4 [56,57], and the increase of $VISTA^+$ cells in ipilimumab treated patients [58] suggests that these combinations might be required also in the human setting. Currently, two ongoing clinical trials are targeting VISTA either with an Ab (NCT02671955) or with the small molecule inhibitor CA-170, which target VISTA, PD-L1 and PD-L2 (NCT02812875).

3. Co-stimulatory molecules

Comparison of the phenotype of Teffs and Tregs from TILs and peripheral blood mononuclear cells (PBMNCs) as well as from cancer patients and healthy donors highlights low levels of co-stimulatory

molecules on effector cells, whereas higher levels are found on Tregs [59]. Since in most cases triggering of the co-stimulatory molecule on Tregs reduces their suppressive activity and/or induces their depletion, different clinical attempts are utilizing agonists of such co-stimulatory molecules to reduce the immune suppression rather than boosting effector cells [60,61]. In the following paragraph basic information on the various co-stimulatory molecules and their possible implementation for tumor immunotherapy are provided.

3.1. OX40

OX40 (or CD134) is a co-stimulatory receptor expressed transiently on activated T cells, but constitutively expressed on Tregs. Experiments performed *in vitro* indicated that OX40 triggering on Tregs reduces the expression of Foxp3 and their suppressive activity [62,63]. In experimental murine models, combination of OX40 triggering with blockade of CTLA-4 [64,65] or PD-1 [66] as well as with triggering of other co-stimulatory molecules like 4-1BB [67] or ICOS [68] is currently being investigated. OX40 triggering can also induce regression of murine sarcoma in the absence of T cells via the activation of endothelial cells and endogenous DCs [69] further promoting the implementation of OX40 for tumor immunotherapy.

After a first clinical trial with a single dose of a murine Ab [70], many humanized Abs have been produced [71,72] and phase I clinical trials are currently recruiting patients.

3.2. GITR

GITR (glucocorticoid induced TNF-receptor related gene or CD357) is expressed in infiltrating Tregs at higher levels than in the circulating PBMNCs and upon triggering with soluble ligand *in vitro* their suppressive activity is reduced [73]. *In vivo* experiments using mice as well as humanized NSG mice indicate that the GITR-specific Ab induces tumor regression by inhibition of Tregs and activation of Teffs [74,75].

As for the other co-stimulatory molecules, combinations with IFNα [76] or aPD-1 therapy, with or without additional chemotherapy or peptide vaccination [77–79] are currently evaluated in mouse models, whereas a combination with aCTLA-4 therapy has been tested in mice as well as *in vitro* with PBMCs from cancer patients [80,81].

Different triggering antibodies like MK4166 [82], but also AMG228, TRX518 and INCAGNO1876 as well as a trimeric form of the extracellular domain of GITR-L, MEDI1873 [83] are currently studied in various phase I clinical trials.

3.3. 4-1BB

CD137 (or 4-1BB) is a co-stimulatory receptor upregulated shortly after TCR triggering and is thus considered a marker of antigen specific activation [84]. A 4-1BB mAb has been used in the setting of adoptive cell therapy (ACT) both to purify [85] and to specifically stimulate antigen specific cells *in vitro* [86–89].

The receptor is also expressed on Tregs, but in contrast to the other co-stimulatory molecules, its *in vitro* triggering with the soluble ligand induces expansion of Tregs without affecting their suppressive capacity [90]. Furthermore, stimulation of $CD4^+$ and $CD8^+$ Teffs via 4-1BB renders them less susceptible to Treg suppressive activity [90,91].

While also new antibodies are being developed and tested in phase I clinical trials, the first results obtained by administering *in vivo* the same antibody that has been used for *in vitro* expansion, Urelumab or BMS663513, displayed limited toxicity together with some immunological functions as indicated by the induction of different cytokines in the patients sera [92].

3.4. ICOS

ICOS (inducible T cell costimulator or CD278) is a co-stimulatory receptor belonging to the family of CD28. Cancer patients harbor high levels of $ICOS^+$ Tregs that can be expanded both by the tumor cells themselves and by $ICOS\text{-}L^+$ plasmacytoid DC and their presence can correlate with poor prognosis [93–96]. In contrast, high levels of ICOS expression on effector cells are found in patients responding to treatment with aCTLA-4 [97–101] but for example not to treatment with anti PD-1[101]. Furthermore, mouse experiments suggest a direct role of such effectors for tumor rejection [102] thus paving the way for the preclinical combination of blocking aCTLA-4 mAb and a cell vaccine overexpressing ICOS-L [103]. In the murine setting an important role of $ICOS^+$ effector cells has also been found in response to OX40 agonist therapy [68]. Currently, two different phase I trials are evaluating ICOS triggering Abs in lymphoma (NCT02520791) and in solid cancer (NCT02723955).

4. Immune suppressive cells

Tumor cells are able to subvert immune cells and to polarize them toward tumor-promoting and/or immune suppressing types. In addition, cellular components of the tumor microenvironment (TME), like tumor associated fibroblasts (TAFs), endothelial cells (ECs) and tumor associated neutrophils (TANs) can be manipulated by the tumor for protection against an immune response.

4.1. $CD4^{\pm}$ and $CD8^{\pm}$ regulatory T cells

T lymphocytes have a high degree of functional plasticity and include also cells with immune regulatory/suppressive activities whose function is to maintain self-tolerance and avoid immune toxicity on bystander healthy cells. Tregs can act either in a contact dependent way via an APC bridge by downregulating immune responses in an antigen specific way or via the secretion of immune suppressive cytokines, like e.g. IL10, that dampen immune responses independently of contact and antigen specificity.

Despite most of the studies in the end of last century have focused on $CD4^+$ Tregs the existence of $CD8^+$ Tregs has been re-proposed in the last decade and their presence has been identified in the infiltrate of different tumor histotypes [104–107]. The induction and/or expansion of those suppressive cells can be directly mediated by the tumor or via the induction of tolerogenic APCs [94,108].

4.2. Accessory cells

In addition to lymphocytes, many other immune cells can infiltrate the tumor, where they can be subverted to a pro-tumorigenic phenotype not only directly promoting tumor cell proliferation and metastasis formation, but also protecting them from an immune response. Indeed, tumor derived factors can promote the differentiation of monocytes versus myeloid derived suppressor cells (MDSC) [109] and the polarization of macrophages and neutrophils toward a type 2, immuno suppressive phenotype [110]. Moreover, myeloid as well as plasmacytoid DCs infiltrating the TME have a semi-mature or tolerogenic phenotype [111,112]. All those pro-tumorigenic accessory cells are characterized by expression of low levels of co-stimulatory molecules together with the upregulation of different suppressive mechanisms. As further discussed below, those cells can upregulate different enzymes like inducible nitric oxide synthase (iNOS), indoleamine 2,3 dioxygenase (IDO) and arginase that deplete the microenvironment from nutrients required for the cytotoxic function of effector cells. Moreover,

they can also upregulate ligands for different immune checkpoints. Indeed, myeloid cells can increase PD-L1 in response to co-culture with T cells [113] and such expression can protect a PD-L1 negative tumor from immune-mediated rejection [114]. Similarly, myeloid cells, and in particularly MDSC express the highest levels of VISTA and are thus the major mediators of T cells inhibition [115].

4.3. Stromal cells: endothelial cells and fibroblasts

One of the first obstacles for anti-tumor T cells is to penetrate into the tumor mass. This is frequently caused by the missing vasculature and the abnormal pressure flow, but also by delimiting ECs, which are modified by the tumor itself. ECs not only express low levels of adhesion molecules, thus impairing adhesion and extravasation, but they can also upregulate death ligands like FasL thus inducing $CD8^+$ T cell death [116]. Due to this fact, attempts to combine immunotherapy with a normalization of the tumor vasculature have been implemented [117].

TAFs composing the stroma within the tumor mass also exhibit immune suppressive activity. They can directly suppress Teff function via the transforming growth factor (TGF)β and their ability to penetrate into the tumor via the CXC-motive chemokine (CXCL)-12 [118,119]. Moreover, TAF can foster the immune suppressive condition by induction of regulatory DCs and M2 polarized macrophages [120,121]. In experimental mouse models different strategies have been implemented to eliminate such TAFs, but with discrepant results reporting either a positive or a negative outcome on tumor growth, respectively [122–124]. In the human setting strategies targeting TAFs are still under evaluation. Interestingly, TAFs obtained from lung cancer patients were neither affected in their suppressive activity nor in their survival by radiation therapy *in vitro* [125].

5. Ostile microenvironment

In addition to the above mentioned immune mechanisms, also other "general" aspects of the TME modulate the composition of the tumor infiltrating immune cell repertoire as well as the activity of immune cells.

5.1. Metabolic competition

Whereas the tumor metabolism has a long history with the description of the so called "Warburg effect", a link between different metabolic pathways and immune cell subset differentiation and functional activity has only recently started to be dissected.

While Tregs mainly depend on oxidative phosphorylation and the availability of fatty acids, Teffs rely on glycolysis and thus on glucose to mount their effector functions. Since the tumor is depleting the TME of glucose to sustain its energetic requirement, different metabolic sensors or check points induce a functional inactivation of T cells in response to glucose deprivation [126,127]. This link between the inability to shift to mitochondrial respiration and exhaustion of Teffs, which has been also described upon virus infection [128], is currently re-evaluated, since experiments performed with human PBMCs comparing glycolysis inhibition via 2-deoxy-glucose (with which most of the previous studies were performed) with real glucose deprivation highlighted a level of metabolic plasticity that might be important in therapeutic settings [129]. To further sustain the role of metabolic competition between tumor and immune cells, an inverse correlation between tumor metabolism and T infiltration was found both in renal and in squamous cell carcinoma [130,131].

In addition to glucose, the TME is also depleted of different amino acids. On one side tumor cells utilize them for their anabolism, not only for building up proteins, but also as precursors for nucleotide biosynthesis, while on the other side immune suppressor cells, like MDSCs, are upregulating IDO or arginase enzymes that induce the degradation of tryptophan and arginine, respectively, thereby further reducing the availability of such amino acids [132].

5.2. Suppressive "waste products"

The metabolism of tumor cells not only deprives the TME of nutrients, but it also fills it with degradation or by-products, some of which display immune suppressive activities.

Degradation of glucose into lactate results not only in high concentration of extracellular lactate, but also in an acidification of the microenvironment that further suppresses Teff functions [133–136], whereas Tregs are less affected [137].

Similarly, the enhanced degradation of tryptophan by IDO results in the release of 3-hydroxyanthranilic acid that inhibits T cells either directly or via DC inhibition [138,139]. Moreover, despite glycolysis produces less oxygen reactive species (ROS) than the mitochondrial respiration, there is also an increase in various ROS species within the TME. Once the detoxifying mechanisms have been saturated, ROS can induce downregulation of functional proteins like the CD3zeta chain, but also cell death [140,141].

5.3. Released cellular content

Upon cell death different compounds are released, which negatively interfere with Teff functions. This includes e.g. ATP, which is characteristic of immunogenic cell death due to its ability to enhance DC maturation. On the contrary, its release can have immune suppressive effects in the TME, since Tregs, MDSCs, but also stromal and tumor cells could express CD39 and/or CD73, two enzymes that progressively degrade ATP into adenosine that can then trigger the inhibitory receptors Adora2A and Adora2B. This directly affects T cells and their migratory capabilities [142], but also inhibits APCs like DCs and promotes induction of Tregs [143]. Different compounds targeting those receptors are being evaluated in the clinic.

Upon cell death there is also release of intracellular ions resulting in alterations of the gradient between intracellular and extracellular space. For example excessive extracellular levels of potassium impair T cell functions if they are not able to keep the required gradient [144] and in the TME T cells with the highest levels of the Kv1,3 channel are the most functional ones [145].

6. Clinical translation

Against all the above described mechanisms of immune evasion exploited by the cancer, different attempts to overcome them therapeutically have been undertaken (Fig. 2). As stated above, therapy with CPIs has provided some good responses, but only in 20–40% of patients and some of them develop resistances during treatment [146–148]. Therefore the search for criteria to stratify patients (see below), but also to develop even more potent treatment options are urgently required. Recently, combinations among different CPIs have been employed, such as the combination of CTLA-4 and PD-1, but despite an improvement [149] the side effects and the associated costs led to the evaluation of other combinations, mostly based on the inhibition of the PD-1/PD-L1 axis [52,150]. CPIs have also been combined with vaccinations [151–153], chemotherapy [154], radiation [155] and with modulators of the TME [132,156].

The increased knowledge has also been translated in ACT. Studies performed on the role of different co-stimulatory molecules in influencing T cell metabolism and fate decision are translated into second and third generation chimeric antigen receptors (CARs) that are endowed with different signaling domain(s) [157,158], whereas cytokine

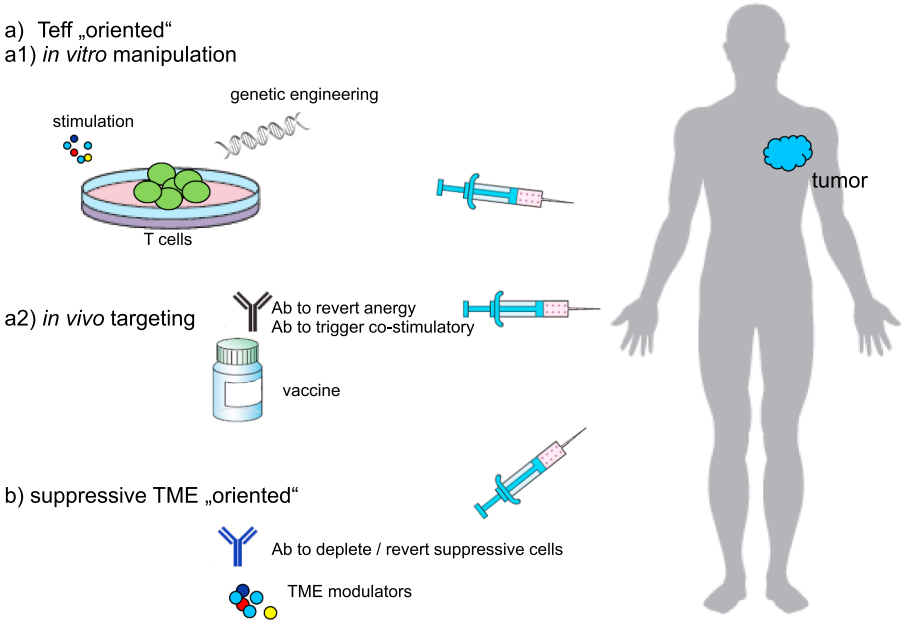

Fig. 2. Therapeutic strategies to rescue tumor infiltrating effector cells. Different immunotherapeutic approaches have been developed to fight cancer, either focusing directly at the antigen specific effector cells or on other components of the TME. The first approach includes ACT, vaccination and CPI that act by blocking the inhibitory signal in the Teffs and thus by reversing their exhaustion or anergy, while the latter comprises antibodies or small molecules that can deplete or reverse the polarization of the immune suppressor cells as well as those that act on the mediators of immune suppressive conditions of the TME like cytokines and metabolites.

induced killer cells have been transfected with the full ICOS co-stimulatory receptor [159]. Similarly, CAR or TCR transgenic cells are further modified with genes that will render them less susceptible to immune suppressive pathways [160–163], high levels of ROS [164,165] or also to cope better with the nutrient situation of the TME [158,166].

7. The quest for the holy grail: immune markers with prognostic and/or predictive value

The above data demonstrate that just the presence of an immune cell infiltrate cannot be a sufficient marker for cancer patients. For that reason many laboratories have taken/are undergoing the difficult task of identifying biomarkers correlated with disease progression and patients' survival, both spontaneous (i.e. prognostic markers) and in response to therapy (i.e. predictive markers), in order to stratify patients accordingly and thus being able to perform a personalized therapy, selecting for each patient the most promising therapeutic approach, and/or to quickly evaluate if the therapy is working or the patient is developing resistances to the treatment regimen.

For this purpose the immune system of tumor patients with many different cancer types has been deeply characterized and correlated with clinical parameters, such as tumor grading, staging, and patients' outcome. Different approaches have been used for such characterization, like immunohistochemistry (IHC), flow cytometry and deep sequencing, each with advantages and disadvantages.

IHC requires a highly invasive intervention to obtain the tumor biopsies, and is therefore mainly used as one time evaluation, but can provide information about the localization of the immune cells within the tumor. This has been shown to be of relevance and led to the development of the immunoscore or immune contexture [167]: evaluation of the presence of combinations of immune markers like CD3, CD8, CD45RO, CD103 and/or CD163 in the invasive margin and/or the core of the tumor can identify patients with better survival chances [55,168–174]. Recently, with the technical improvement both in the staining techniques that allow multiple parameters to be analyzed in parallel and in the software for imaging evaluation, multispectral imaging (MSI) could be employed to evaluate the reciprocal location and even the topology and distance between different cell types, like Teffs and Tregs within the tumor, which is of potential value for prognosis as shown for oral squamous carcinoma [175]. Evaluation by conventional flow cytometry allows the analysis of a higher number of parameters in parallel (up to 32), a number that can be further expanded by mass cytometry due to the introduction of isotope- instead of fluorochrome-labeled Abs. On the downside, these analyses give only information about the cellular composition and their activation status, but all information on the cellular localization are lost, since they are performed in cell suspension. However, a huge advantage of the implementation of these flow cytometric based technologies is their application on liquid biopsies like PBMNCs or ascites that can be obtained with less invasive techniques than tumor biopsies and thus allow multiple evaluations over time that are essential for the determination of predictive biomarkers for responsiveness to therapy, such as e.g. the enhanced levels of $ICOS^+$ effector cells or the reduction in MDSC found following aCTLA-4 therapy [176–178]. Despite the increased knowledge obtained using these technology on liquid biopsies, it is noteworthy that circulating cells are not a faithful representation of TILs and that small changes in the TME correlating with therapy response might be lost when peripheral lymphocytes are evaluated.

More recently, with the advances in sequencing techniques, tumor biopsies have also been characterized from the genomic and transcriptomic point of view not only to evaluate the mutation load of the neoplastic cells, that can correlate with its immunogenicity and have prognostic value, but also to characterize its cellular composition and thus the "status" of the immune infiltrate. For example, response to aPD-1 therapy has been positively associated with the presence of an IFNγ signature in the pre-operative biopsy [179], whereas a negative correlation was found with high expression levels of metabolic enzymes [180]. Similarly, expression levels of genes associated with antigen processing, immunomodulatory molecules and effector as well as suppressor cells has been converted into an immunophenoscore that when applied to biopsies from clinical trials with aCTLA-4 or aPD-1 blockade was able to predict response to therapy [181]. An additional tool that has been "created" to help stratifying patients for therapy selection is the immunogram. Initially theorized by Blank et al. [182] on the basis

of the cancer immunity cycle [183] it has then been "created" and experimentally applied to lung cancer patients by Karasaki and co-authors [184].

8. Conclusion

In the last decade a greater understanding of the interaction between tumor and immune cells has been obtained and its translation into clinical practice has resulted in some great clinical responses. Despite this improvement, a long way has still to be undertaken in order to expand the patients that can really take advantages from such therapies, both by enhancing their efficacy and by finding reliable markers to initially select the best therapy for each patient and for monitoring the possible development of resistance(s) in order to change the therapeutic approach to be applied. In this way it will be possible not only to reduce the side effects to the patients, but also to better use the economical resources by (quickly) selecting the best possible therapeutic strategy(ies) for each patient.

Acknowledgment

The work was supported by a grant the Mildred Scheel Stiftung to CM and BS (111105).

References

[1] O.S. Qureshi, Y. Zheng, K. Nakamura, K. Attridge, C. Manzotti, E.M. Schmidt, J. Baker, L.E. Jeffery, S. Kaur, Z. Briggs, T.Z. Hou, C.E. Futter, G. Anderson, L.S. Walker, D.M. Sansom, Trans-endocytosis of CD80 and CD86: a molecular basis for the cell-extrinsic function of CTLA-4, Science 332 (2011) 600–603.

[2] Y. Han, Z. Chen, Y. Yang, Z. Jiang, Y. Gu, Y. Liu, C. Lin, Z. Pan, Y. Yu, M. Jiang, W. Zhou, X. Cao, Human CD14+ CTLA-4+ regulatory dendritic cells suppress T-cell response by cytotoxic T-lymphocyte antigen-4-dependent IL-10 and indoleamine-2,3-dioxygenase production in hepatocellular carcinoma, Hepatology 59 (2014) 567–579.

[3] S. Khan, D.J. Burt, C. Ralph, F.C. Thistlethwaite, R.E. Hawkins, E. Elkord, Tremelimumab (anti-CTLA4) mediates immune responses mainly by direct activation of T effector cells rather than by affecting T regulatory cells, Clin. Immunol. 138 (2011) 85–96.

[4] T.R. Simpson, F. Li, W. Montalvo-Ortiz, M.A. Sepulveda, K. Bergerhoff, F. Arce, C. Roddie, J.Y. Henry, H. Yagita, J.D. Wolchok, K.S. Peggs, J.V. Ravetch, J.P. Allison, S.A. Quezada, Fc-dependent depletion of tumor-infiltrating regulatory T cells co-defines the efficacy of anti-CTLA-4 therapy against melanoma, J. Exp. Med. 210 (2013) 1695–1710.

[5] T. Pentcheva-Hoang, T.R. Simpson, W. Montalvo-Ortiz, J.P. Allison, Cytotoxic T lymphocyte antigen-4 blockade enhances antitumor immunity by stimulating melanoma-specific T-cell motility, Cancer Immunol. Res. 2 (2014) 970–980.

[6] A. Gros, P.F. Robbins, X. Yao, Y.F. Li, S. Turcotte, E. Tran, J.R. Wunderlich, A. Mixon, S. Farid, M.E. Dudley, K. Hanada, J.R. Almeida, S. Darko, D.C. Douek, J.C. Yang, S.A. Rosenberg, PD-1 identifies the patient-specific CD8(+) tumor-reactive repertoire infiltrating human tumors, J. Clin. Invest. 124 (2014) 2246–2259.

[7] Y. Iwai, J. Hamanishi, K. Chamoto, T. Honjo, Cancer immunotherapies targeting the PD-1 signaling pathway, J. Biomed. Sci. 24 (2017) 26.

[8] J.H. Yearley, C. Gibson, N. Yu, C. Moon, E. Murphy, J. Juco, J. Lunceford, J. Cheng, L.Q.M. Chow, T.Y. Seiwert, M. Handa, J.E. Tomassini, T. McClanahan, PD-L2 expression in human tumors: relevance to Anti-PD-1 therapy in cancer, Clin. Cancer Res. 23 (2017) 3158–3167.

[9] S. Kleffel, C. Posch, S.R. Barthel, H. Mueller, C. Schlapbach, E. Guenova, C.P. Elco, N. Lee, V.R. Juneja, Q. Zhan, C.G. Lian, R. Thomi, W. Hoetzenecker, A. Cozzio, R. Dummer, M.C. Mihm Jr., K.T. Flaherty, M.H. Frank, G.F. Murphy, A.H. Sharpe, T.S. Kupper, T. Schatton, Melanoma cell-intrinsic PD-1 receptor functions promote tumor growth, Cell 162 (2015) 1242–1256.

[10] M.W. Teng, S.F. Ngiow, A. Ribas, M.J. Smyth, Classifying cancers based on T-cell infiltration and PD-L1, Cancer Res. 75 (2015) 2139–2145.

[11] L. Monney, C.A. Sabatos, J.L. Gaglia, A. Ryu, H. Waldner, T. Chernova, S. Manning, E.A. Greenfield, A.J. Coyle, R.A. Sobel, G.J. Freeman, V.K. Kuchroo, Th1-specific cell surface protein Tim-3 regulates macrophage activation and severity of an autoimmune disease, Nature 415 (2002) 536–541.

[12] J. Yan, Y. Zhang, J.P. Zhang, J. Liang, L. Li, L. Zheng, Tim-3 expression defines regulatory T cells in human tumors, PLoS One 8 (2013) e58006.

[13] X. Gao, Y. Zhu, G. Li, H. Huang, G. Zhang, F. Wang, J. Sun, Q. Yang, X. Zhang, B. Lu, TIM-3 expression characterizes regulatory T cells in tumor tissues and is associated with lung cancer progression, PLoS One 7 (2012) e30676.

[14] M. Bu, Y. Shen, W.L. Seeger, S. An, R. Qi, J.A. Sanderson, Y. Cai, Ovarian carcinoma-infiltrating regulatory T cells were more potent suppressors of CD8(+) T cell inflammation than their peripheral counterparts, a function dependent on TIM3 expression, Tumour Biol. 37 (2016) 3949–3956.

[15] Z.Z. Yang, D.M. Grote, S.C. Ziesmer, T. Niki, M. Hirashima, A.J. Novak, T.E. Witzig, S.M. Ansell, IL-12 upregulates TIM-3 expression and induces T cell exhaustion in patients with follicular B cell non-Hodgkin lymphoma, J. Clin. Invest. 122 (2012) 1271–1282.

[16] C. Zhu, K. Sakuishi, S. Xiao, Z. Sun, S. Zaghouani, G. Gu, C. Wang, D.J. Tan, C. Wu, M. Rangachari, T. Pertel, H.T. Jin, R. Ahmed, A.C. Anderson, V.K. Kuchroo, An IL-27/NFIL3 signalling axis drives Tim-3 and IL-10 expression and T-cell dysfunction, Nat. Commun. 6 (2015) 6072.

[17] C. Zhu, A.C. Anderson, A. Schubart, H. Xiong, J. Imitola, S.J. Khoury, X.X. Zheng, T.B. Strom, V.K. Kuchroo, The Tim-3 ligand galectin-9 negatively regulates T helper type 1 immunity, Nat. Immunol. 6 (2005) 1245–1252.

[18] J.S. Dolina, T.J. Braciale, Y.S. Hahn, Liver-primed CD8+ T cells suppress antiviral adaptive immunity through galectin-9-independent T-cell immunoglobulin and mucin 3 engagement of high-mobility group box 1 in mice, Hepatology 59 (2014) 1351–1365.

[19] Y.H. Huang, C. Zhu, Y. Kondo, A.C. Anderson, A. Gandhi, A. Russell, S.K. Dougan, B.S. Petersen, E. Melum, T. Pertel, K.L. Clayton, M. Raab, Q. Chen, N. Beauchemin, P.J. Yazaki, M. Pyzik, M.A. Ostrowski, J.N. Glickman, C.E. Rudd, H.L. Ploegh, A. Franke, G.A. Petsko, V.K. Kuchroo, R.S. Blumberg, CEACAM1 regulates TIM-3-mediated tolerance and exhaustion, Nature 517 (2015) 386–390.

[20] Y. Zhang, P. Cai, L. Li, L. Shi, P. Chang, T. Liang, Q. Yang, Y. Liu, L. Wang, L. Hu, Co-expression of TIM-3 and CEACAM1 promotes T cell exhaustion in colorectal cancer patients, Int. Immunopharmacol. 43 (2017) 210–218.

[21] P. Shen, R. Yue, J. Tang, H. Si, L. Shen, C. Guo, L. Zhang, H. Han, H.K. Song, P. Zhao, N. Wang, Z. Song, C. Guo, Preferential Tim-3 expression on Treg and CD8(+) T cells, supported by tumor-associated macrophages, is associated with worse prognosis in gastric cancer, Am. J. Transl. Res. 8 (2016) 3419–3428.

[22] J. Fourcade, Z. Sun, M. Benallaoua, P. Guillaume, I.F. Luescher, C. Sander, J.M. Kirkwood, V. Kuchroo, H.M. Zarour, Upregulation of Tim-3 and PD-1 expression is associated with tumor antigen-specific CD8+ T cell dysfunction in melanoma patients, J. Exp. Med. 207 (2010) 2175–2186.

[23] C. Granier, C. Dariane, P. Combe, V. Verkarre, S. Urien, C. Badoual, H. Roussel, M. Mandavit, P. Ravel, M. Sibony, L. Biard, C. Radulescu, E. Vinatier, N. Benhamouda, M. Peyromaure, S. Oudard, A. Mejean, M.O. Timsit, A. Gey, E. Tartour, Tim-3 expression on tumor-infiltrating PD-1+CD8+ T cells correlates with poor clinical outcome in renal cell carcinoma, Cancer Res. 77 (2017) 1075–1082.

[24] M. Yu, B. Lu, Y. Liu, Y. Me, L. Wang, H. Li, Interference with Tim-3 protein expression attenuates the invasion of clear cell renal cell carcinoma and aggravates anoikis, Mol. Med. Rep. 15 (2017) 1103–1108.

[25] B. Shan, H. Man, J. Liu, L. Wang, T. Zhu, M. Ma, Z. Xv, X. Chen, X. Yang, P. Li, TIM-3 promotes the metastasis of esophageal squamous cell carcinoma by targeting epithelial-mesenchymal transition via the Akt/GSK-3beta/Snail signaling pathway, Oncol. Rep. 36 (2016) 1551–1561.

[26] M. Yu, B. Lu, Y. Liu, Y. Me, L. Wang, P. Zhang, Tim-3 is upregulated in human colorectal carcinoma and associated with tumor progression, Mol. Med. Rep. 15 (2017) 689–695.

[27] L.C. Ndhlovu, S. Lopez-Verges, J.D. Barbour, R.B. Jones, A.R. Jha, B.R. Long, E.C. Schoeffler, T. Fujita, D.F. Nixon, L.L. Lanier, Tim-3 marks human natural killer cell maturation and suppresses cell-mediated cytotoxicity, Blood 119 (2012) 3734–3743.

[28] M.K. Gleason, T.R. Lenvik, V. McCullar, M. Felices, M.S. O'Brien, S.A. Cooley, M.R. Verneris, F. Cichocki, C.J. Holman, A. Panoskaltsis-Mortari, T. Niki, M. Hirashima, B.R. Blazar, J.S. Miller, Tim-3 is an inducible human natural killer cell receptor that enhances interferon gamma production in response to galectin-9, Blood 119 (2012) 3064–3072.

[29] X. Jiang, T. Zhou, Y. Xiao, J. Yu, S. Dou, G. Chen, R. Wang, H. Xiao, C. Hou, W. Wang, Q. Shi, J. Feng, Y. Ma, B. Shen, Y. Li, G. Han, Tim-3 promotes tumor-promoting M2 macrophage polarization by binding to STAT1 and suppressing the STAT1-miR-155 signaling axis, Oncoimmunology 5 (2016) e1211219.

[30] X. Li, Y. Chen, X. Liu, J. Zhang, X. He, G. Teng, D. Yu, Tim3/Gal9 interactions between T cells and monocytes result in an immunosuppressive feedback loop that inhibits Th1 responses in osteosarcoma patients, Int. Immunopharmacol. 44 (2017) 153–159.

[31] M. Nakayama, H. Akiba, K. Takeda, Y. Kojima, M. Hashiguchi, M. Azuma, H. Yagita, K. Okumura, Tim-3 mediates phagocytosis of apoptotic cells and cross-presentation, Blood 113 (2009) 3821–3830.

[32] D. Bruniquel, N. Borie, S. Hannier, F. Triebel, Regulation of expression of the human lymphocyte activation gene-3 (LAG-3) molecule, a ligand for MHC class II, Immunogenetics 48 (1998) 116–124.

[33] J.F. Grosso, C.C. Kelleher, T.J. Harris, C.H. Maris, E.L. Hipkiss, A. De Marzo, R. Anders, G. Netto, D. Getnet, T.C. Bruno, M.V. Goldberg, D.M. Pardoll, C.G. Drake, LAG-3 regulates CD8+ T cell accumulation and effector function in murine self- and tumor-tolerance systems, J. Clin. Invest. 117 (2007) 3383–3392.

[34] C. Camisaschi, C. Casati, F. Rini, M. Perego, A. De Filippo, F. Triebel, G. Parmiani, F. Belli, L. Rivoltini, C. Castelli, LAG-3 expression defines a subset of CD4(+)CD25(high)Foxp3(+) regulatory T cells that are expanded at tumor sites, J. Immunol. 184 (2010) 6545–6551.

[35] N.M. Durham, C.J. Nirschl, C.M. Jackson, J. Elias, C.M. Kochel, R.A. Anders, C.G. Drake, Lymphocyte Activation Gene 3 (LAG-3) modulates the ability of CD4 T-cells to be suppressed in vivo, PLoS One 9 (2014) e109080.

[36] F.J. Li, Y. Zhang, G.X. Jin, L. Yao, D.Q. Wu, Expression of LAG-3 is coincident with the impaired effector function of HBV-specific CD8(+) T cell in HCC patients, Immunol. Lett. 150 (2013) 116–122.

[37] Z. Liu, Q. Meng, J. Bartek Jr., T. Poiret, O. Persson, L. Rane, E. Rangelova, C. Illies, I.H. Peredo, X. Luo, M.V. Rao, R.A. Robertson, E. Dodoo, M. Maeurer, Tumor-

infiltrating lymphocytes (TILs) from patients with glioma, Oncoimmunology 6 (2017) e1252894.
[38] G. Bottai, C. Raschioni, A. Losurdo, L. Di Tommaso, C. Tinterri, R. Torrisi, J.S. Reis-Filho, M. Roncalli, C. Sotiriou, A. Santoro, A. Mantovani, S. Loi, L. Santarpia, An immune stratification reveals a subset of PD-1/LAG-3 double-positive triple-negative breast cancers, Breast Cancer Res. 18 (2016) 121.
[39] A.K. Mishra, T. Kadoishi, X. Wang, E. Driver, Z. Chen, X.J. Wang, J.H. Wang, Squamous cell carcinomas escape immune surveillance via inducing chronic activation and exhaustion of CD8+ T Cells co-expressing PD-1 and LAG-3 inhibitory receptors, Oncotarget 7 (2016) 81341–81356.
[40] N.A. Giraldo, E. Becht, Y. Vano, F. Petitprez, L. Lacroix, P. Validire, R. Sanchez-Salas, A. Ingels, S. Oudard, A. Moatti, B. Buttard, S. Bourass, C. Germain, X. Cathelineau, W.H. Fridman, C. Sautes-Fridman, Tumor-infiltrating and peripheral blood T-cell immunophenotypes predict early relapse in localized clear cell renal cell carcinoma, Clin. Cancer Res. (2017).
[41] J.B. Williams, B.L. Horton, Y. Zheng, Y. Duan, J.D. Powell, T.F. Gajewski, The EGR2 targets LAG-3 and 4–1BB describe and regulate dysfunctional antigen-specific CD8+ T cells in the tumor microenvironment, J. Exp. Med. 214 (2017) 381–400.
[42] S. Andreae, F. Piras, N. Burdin, F. Triebel, Maturation and activation of dendritic cells induced by lymphocyte activation gene-3 (CD223), J. Immunol. 168 (2002) 3874–3880.
[43] C. Casati, C. Camisaschi, L. Novellino, A. Mazzocchi, F. Triebel, L. Rivoltini, G. Parmiani, C. Castelli, Human lymphocyte activation gene-3 molecules expressed by activated T cells deliver costimulation signal for dendritic cell activation, J. Immunol. 180 (2008) 3782–3788.
[44] B. Liang, C. Workman, J. Lee, C. Chew, B.M. Dale, L. Colonna, M. Flores, N. Li, E. Schweighoffer, S. Greenberg, V. Tybulewicz, D. Vignali, R. Clynes, Regulatory T cells inhibit dendritic cells by lymphocyte activation gene-3 engagement of MHC class II, J. Immunol. 180 (2008) 5916–5926.
[45] J. Bayry, F. Triebel, S.V. Kaveri, D.F. Tough, Human dendritic cells acquire a semimature phenotype and lymph node homing potential through interaction with CD4+CD25+ regulatory T cells, J. Immunol. 178 (2007) 4184–4193.
[46] P. Hemon, F. Jean-Louis, K. Ramgolam, C. Brignone, M. Viguier, H. Bachelez, F. Triebel, D. Charron, F. Aoudjit, R. Al-Daccak, L. Michel, MHC class II engagement by its ligand LAG-3 (CD223) contributes to melanoma resistance to apoptosis, J. Immunol. 186 (2011) 5173–5183.
[47] A. Wang-Gillam, S. Plambeck-Suess, P. Goedegebuure, P.O. Simon, J.B. Mitchem, J.R. Hornick, S. Sorscher, J. Picus, R. Suresh, A.C. Lockhart, B. Tan, W.G. Hawkins, A phase I study of IMP321 and gemcitabine as the front-line therapy in patients with advanced pancreatic adenocarcinoma, Invest. New Drugs 31 (2013) 707–713.
[48] C. Brignone, M. Gutierrez, F. Mefti, E. Brain, R. Jarcau, F. Cvitkovic, N. Bousetta, J. Medioni, J. Gligorov, C. Grygar, M. Marcu, F. Triebel, First-line chemoimmunotherapy in metastatic breast carcinoma: combination of paclitaxel and IMP321 (LAG-3Ig) enhances immune responses and antitumor activity, J. Transl. Med. 8 (2010) 71.
[49] C. Brignone, B. Escudier, C. Grygar, M. Marcu, F. Triebel, A phase I pharmacokinetic and biological correlative study of IMP321, a novel MHC class II agonist, in patients with advanced renal cell carcinoma, Clin. Cancer Res. 15 (2009) 6225–6231.
[50] N. Joller, E. Lozano, P.R. Burkett, B. Patel, S. Xiao, C. Zhu, J. Xia, T.G. Tan, E. Sefik, V. Yajnik, A.H. Sharpe, F.J. Quintana, D. Mathis, C. Benoist, D.A. Hafler, V.K. Kuchroo, Treg cells expressing the coinhibitory molecule TIGIT selectively inhibit proinflammatory Th1 and Th17 cell responses, Immunity 40 (2014) 569–581.
[51] T. Inozume, T. Yaguchi, J. Furuta, K. Harada, Y. Kawakami, S. Shimada, Melanoma cells control antimelanoma CTL responses via interaction between TIGIT and CD155 in the effector phase, J. Invest. Dermatol. 136 (2016) 255–263.
[52] J.M. Chauvin, O. Pagliano, J. Fourcade, Z. Sun, H. Wang, C. Sander, J.M. Kirkwood, T.H. Chen, M. Maurer, A.J. Korman, H.M. Zarour, TIGIT and PD-1 impair tumor antigen-specific CD8(+) T cells in melanoma patients, J. Clin. Invest. 125 (2015) 2046–2058.
[53] J.L. Lines, E. Pantazi, J. Mak, L.F. Sempere, L. Wang, S. O'Connell, S. Ceeraz, A.A. Suriawinata, S. Yan, M.S. Ernstoff, R. Noelle, VISTA is an immune checkpoint molecule for human T cells, Cancer Res. 74 (2014) 1924–1932.
[54] C. Boger, H.M. Behrens, S. Kruger, C. Rocken, The novel negative checkpoint regulator VISTA is expressed in gastric carcinoma and associated with PD-L1/PD-1: a future perspective for a combined gastric cancer therapy? Oncoimmunology 6 (2017) e1293215.
[55] L. Wu, W.W. Deng, C.F. Huang, L.L. Bu, G.T. Yu, L. Mao, W.F. Zhang, B. Liu, Z.J. Sun, Expression of VISTA correlated with immunosuppression and synergized with CD8 to predict survival in human oral squamous cell carcinoma, Cancer Immunol. Immunother. 66 (2017) 627–636.
[56] Y. Kondo, T. Ohno, N. Nishii, K. Harada, H. Yagita, M. Azuma, Differential contribution of three immune checkpoint (VISTA, CTLA-4, PD-1) pathways to antitumor responses against squamous cell carcinoma, Oral Oncol. 57 (2016) 54–60.
[57] J. Liu, Y. Yuan, W. Chen, J. Putra, A.A. Suriawinata, A.D. Schenk, H.E. Miller, I. Guleria, R.J. Barth, Y.H. Huang, L. Wang, Immune-checkpoint proteins VISTA and PD-1 nonredundantly regulate murine T-cell responses, Proc. Natl. Acad. Sci. U.S.A. 112 (2015) 6682–6687.
[58] J. Gao, J.F. Ward, C.A. Pettaway, L.Z. Shi, S.K. Subudhi, L.M. Vence, H. Zhao, J. Chen, H. Chen, E. Efstathiou, P. Troncoso, J.P. Allison, C.J. Logothetis, I.I. Wistuba, M.A. Sepulveda, J. Sun, J. Wargo, J. Blando, P. Sharma, VISTA is an inhibitory immune checkpoint that is increased after ipilimumab therapy in patients with prostate cancer, Nat. Med. 23 (2017) 551–555.
[59] P. Baruah, M. Lee, T. Odutoye, P. Williamson, N. Hyde, J.C. Kaski, I.E. Dumitriu, Decreased levels of alternative co-stimulatory receptors OX40 and 4–1BB characterise T cells from head and neck cancer patients, Immunobiology 217 (2012) 669–675.
[60] A.E. Moran, M. Kovacsovics-Bankowski, A.D. Weinberg, The TNFRs OX40, 4–1BB, and CD40 as targets for cancer immunotherapy, Curr. Opin. Immunol. 25 (2013) 230–237.
[61] M.F. Sanmamed, F. Pastor, A. Rodriguez, J.L. Perez-Gracia, M.E. Rodriguez-Ruiz, M. Jure-Kunkel, I. Melero, Agonists of co-stimulation in cancer immunotherapy directed against CD137, OX40, GITR, CD27, CD28, and ICOS, Semin. Oncol. 42 (2015) 640–655.
[62] N. Kitamura, S. Murata, T. Ueki, E. Mekata, R.T. Reilly, E.M. Jaffee, T. Tani, OX40 costimulation can abrogate Foxp3+ regulatory T cell-mediated suppression of antitumor immunity, Int. J. Cancer 125 (2009) 630–638.
[63] C. Lai, S. August, A. Albibas, R. Behar, S.Y. Cho, M.E. Polak, J. Theaker, A.S. MacLeod, R.R. French, M.J. Glennie, A. Al-Shamkhani, E. Healy, OX40+ Regulatory T cells in cutaneous squamous cell carcinoma suppress Effector T-cell responses and associate with metastatic potential, Clin. Cancer Res. 22 (2016) 4236–4248.
[64] W.L. Redmond, S.N. Linch, M.J. Kasiewicz, Combined targeting of costimulatory (OX40) and coinhibitory (CTLA-4) pathways elicits potent effector T cells capable of driving robust antitumor immunity, Cancer Immunol. Res. 2 (2014) 142–153.
[65] S.N. Linch, M.J. Kasiewicz, M.J. McNamara, I.F. Hilgart-Martiszus, M. Farhad, W.L. Redmond, Combination OX40 agonism/CTLA-4 blockade with HER2 vaccination reverses T-cell anergy and promotes survival in tumor-bearing mice, Proc. Natl. Acad. Sci. U.S.A. 113 (2016) E319–327.
[66] Z. Guo, X. Wang, D. Cheng, Z. Xia, M. Luan, S. Zhang, PD-1 blockade and OX40 triggering synergistically protects against tumor growth in a murine model of ovarian cancer, PLoS One 9 (2014) e89350.
[67] P. Mittal, M.C. St Rose, X. Wang, J.M. Ryan, J.S. Wasser, A.T. Vella, A.J. Adler, Tumor-unrelated CD4 T cell help augments CD134 plus CD137 dual costimulation tumor therapy, J. Immunol. 195 (2015) 5816–5826.
[68] T.C. Metzger, H. Long, S. Potluri, T. Pertel, S.L. Bailey-Bucktrout, J.C. Lin, T. Fu, P. Sharma, J.P. Allison, R.M. Feldman, ICOS promotes the function of CD4+ effector T cells during anti-OX40-mediated tumor rejection, Cancer Res. 76 (2016) 3684–3689.
[69] A.D. Pardee, D. McCurry, S. Alber, P. Hu, A.L. Epstein, W.J. Storkus, A therapeutic OX40 agonist dynamically alters dendritic, endothelial, and T cell subsets within the established tumor microenvironment, Cancer Res. 70 (2010) 9041–9052.
[70] B.D. Curti, M. Kovacsovics-Bankowski, N. Morris, E. Walker, L. Chisholm, K. Floyd, J. Walker, I. Gonzalez, T. Meeuwsen, B.A. Fox, T. Moudgil, W. Miller, D. Haley, T. Coffey, B. Fisher, L. Delanty-Miller, N. Rymarchyk, T. Kelly, T. Crocenzi, E. Bernstein, R. Sanborn, W.J. Urba, A.D. Weinberg, OX40 is a potent immune-stimulating target in late-stage cancer patients, Cancer Res. 73 (2013) 7189–7198.
[71] K.S. Voo, L. Bover, M.L. Harline, L.T. Vien, V. Facchinetti, K. Arima, L.W. Kwak, Y.J. Liu, Antibodies targeting human OX40 expand effector T cells and block inducible and natural regulatory T cell function, J. Immunol. 191 (2013) 3641–3650.
[72] D. Zhang, M.V. Goldberg, M.L. Chiu, Fc engineering approaches to enhance the agonism and effector functions of an anti-OX40 antibody, J. Biol. Chem. 291 (2016) 27134–27146.
[73] A. Pedroza-Gonzalez, C. Verhoef, J.N. Ijzermans, M.P. Peppelenbosch, J. Kwekkeboom, J. Verheij, H.L. Janssen, D. Sprengers, Activated tumor-infiltrating CD4+ regulatory T cells restrain antitumor immunity in patients with primary or metastatic liver cancer, Hepatology 57 (2013) 183–194.
[74] A.D. Cohen, D.A. Schaer, C. Liu, Y. Li, D. Hirschhorn-Cymmerman, S.C. Kim, A. Diab, G. Rizzuto, F. Duan, M.A. Perales, T. Merghoub, A.N. Houghton, J.D. Wolchok, Agonist anti-GITR monoclonal antibody induces melanoma tumor immunity in mice by altering regulatory T cell stability and intra-tumor accumulation, PLoS One 5 (2010) e10436.
[75] A.E. Mahne, S. Mauze, B. Joyce-Shaikh, J. Xia, E.P. Bowman, A.M. Beebe, D.J. Cua, R. Jain, Dual roles for regulatory T-cell depletion and costimulatory signaling in agonistic GITR targeting for tumor immunotherapy, Cancer Res. 77 (2017) 1108–1118.
[76] K. Aida, R. Miyakawa, K. Suzuki, K. Narumi, T. Udagawa, Y. Yamamoto, T. Chikaraishi, T. Yoshida, K. Aoki, Suppression of Tregs by anti-glucocorticoid induced TNF receptor antibody enhances the antitumor immunity of interferon-alpha gene therapy for pancreatic cancer, Cancer Sci. 105 (2014) 159–167.
[77] L. Lu, X. Xu, B. Zhang, R. Zhang, H. Ji, X. Wang, Combined PD-1 blockade and GITR triggering induce a potent antitumor immunity in murine cancer models and synergizes with chemotherapeutic drugs, J. Transl. Med. 12 (2014) 36.
[78] D.O. Villarreal, D. Chin, M.A. Smith, L.L. Luistro, L.A. Snyder, Combination GITR targeting/PD-1 blockade with vaccination drives robust antigen-specific antitumor immunity, Oncotarget 8 (2017) 39117–39130.
[79] G. Nocentini, L. Cari, S. Ronchetti, C. Riccardi, Modulation of tumor immunity: a patent evaluation of WO2015026684A1, Expert Opin. Ther. Pat. 26 (2016) 417–425.
[80] J. Mitsui, H. Nishikawa, D. Muraoka, L. Wang, T. Noguchi, E. Sato, S. Kondo, J.P. Allison, S. Sakaguchi, L.J. Old, T. Kato, H. Shiku, Two distinct mechanisms of augmented antitumor activity by modulation of immunostimulatory/inhibitory signals, Clin. Cancer Res. 16 (2010) 2781–2791.
[81] A. Pedroza-Gonzalez, G. Zhou, S.P. Singh, P.P. Boor, Q. Pan, D. Grunhagen, J. de Jonge, T.K. Tran, C. Verhoef, J.N. IJzermans, H. Janssen, K. Biermann, J. Kwekkeboom, D. Sprengers, GITR engagement in combination with CTLA-4 blockade completely abrogates immunosuppression mediated by human liver tumor-derived regulatory T cells ex vivo, Oncoimmunology 4 (2015) e1051297.

[82] S. Sukumar, D.C. Wilson, Y. Yu, J. Wong, S. Naravula, G. Ermakov, R. Riener, B. Bhagwat, A.S. Necheva, J. Grein, T. Churakova, R. Mangadu, P. Georgiev, D. Manfra, E.M. Pinheiro, V. Sriram, W.J. Bailey, D. Herzyk, T.K. McClanahan, A. Willingham, A.M. Beebe, S. Sadekova, Characterization of MK-4166, a clinical agonistic antibody that targets human GITR and inhibits the generation and suppressive effects of T regulatory cells, Cancer Res. (2017).
[83] N.J. Tigue, L. Bamber, J. Andrews, S. Ireland, J. Hair, E. Carter, S. Sridharan, J. Jovanovic, D.G. Rees, J.S. Springall, E. Solier, Y.M. Li, M. Chodorge, D. Perez-Martinez, D.R. Higazi, M. Oberst, M. Kennedy, C.M. Black, L. Yan, M. Schwickart, S. Maguire, J.A. Cann, L. de Haan, L.L. Young, T. Vaughan, R.W. Wilkinson, R. Stewart, MEDI1873, a potent, stabilized hexameric agonist of human GITR with regulatory T-cell targeting potential, Oncoimmunology 6 (2017) e1280645.
[84] Q. Ye, D.G. Song, M. Poussin, T. Yamamoto, A. Best, C. Li, G. Coukos, D.J. Powell Jr., CD137 accurately identifies and enriches for naturally occurring tumor-reactive T cells in tumor, Clin. Cancer Res. 20 (2014) 44–55.
[85] M. Wolfl, J. Kuball, W.Y. Ho, H. Nguyen, T.J. Manley, M. Bleakley, P.D. Greenberg, Activation-induced expression of CD137 permits detection, isolation, and expansion of the full repertoire of CD8+ T cells responding to antigen without requiring knowledge of epitope specificities, Blood 110 (2007) 201–210.
[86] J.A. Hernandez-Chacon, Y. Li, R.C. Wu, C. Bernatchez, Y. Wang, J.S. Weber, P. Hwu, L.G. Radvanyi, Costimulation through the CD137/4-1BB pathway protects human melanoma tumor-infiltrating lymphocytes from activation-induced cell death and enhances antitumor effector function, J. Immunother. 34 (2011) 236–250.
[87] J.A. Chacon, R.C. Wu, P. Sukhumalchandra, J.J. Molldrem, A. Sarnaik, S. Pilon-Thomas, J. Weber, P. Hwu, L. Radvanyi, Co-stimulation through 4–1BB/CD137 improves the expansion and function of CD8(+) melanoma tumor-infiltrating lymphocytes for adoptive T-cell therapy, PLoS One 8 (2013) e60031.
[88] M. Harao, M.A. Forget, J. Roszik, H. Gao, G.V. Babiera, S. Krishnamurthy, J.A. Chacon, S. Li, E.A. Mittendorf, S.M. DeSnyder, K.F. Rockwood, C. Bernatchez, N.T. Ueno, L.G. Radvanyi, L. Vence, C. Haymaker, J.M. Reuben, 4–1BB-enhanced expansion of CD8+ TIL from triple-negative breast cancer unveils mutation-specific CD8+ T cells, Cancer Immunol. Res. 5 (2017) 439–445.
[89] J.A. Chacon, A.A. Sarnaik, J.Q. Chen, C. Creasy, C. Kale, J. Robinson, J. Weber, P. Hwu, S. Pilon-Thomas, L. Radvanyi, Manipulating the tumor microenvironment ex vivo for enhanced expansion of tumor-infiltrating lymphocytes for adoptive cell therapy, Clin. Cancer Res. 21 (2015) 611–621.
[90] K.G. Elpek, E.S. Yolcu, D.D. Franke, C. Lacelle, R.H. Schabowsky, H. Shirwan, Ex vivo expansion of CD4+CD25+FoxP3+ T regulatory cells based on synergy between IL-2 and 4–1BB signaling, J. Immunol. 179 (2007) 7295–7304.
[91] H.B. Barsoumian, E.S. Yolcu, H. Shirwan, 4–1BB signaling in conventional T cells drives IL-2 production that overcomes CD4+CD25+FoxP3+ T regulatory cell suppression, PLoS One 11 (2016) e0153088.
[92] N.H. Segal, T.F. Logan, F.S. Hodi, D. McDermott, I. Melero, O. Hamid, H. Schmidt, C. Robert, V. Chiarion-Sileni, P.A. Ascierto, M. Maio, W.J. Urba, T.C. Gangadhar, S. Suryawanshi, J. Neely, M. Jure-Kunkel, S. Krishnan, H. Kohrt, M. Sznol, R. Levy, Results from an integrated safety analysis of urelumab, an agonist anti-CD137 monoclonal antibody, Clin. Cancer Res. 23 (2017) 1929–1936.
[93] C. Conrad, J. Gregorio, Y.H. Wang, T. Ito, S. Meller, S. Hanabuchi, S. Anderson, N. Atkinson, P.T. Ramirez, Y.J. Liu, R. Freedman, M. Gilliet, Plasmacytoid dendritic cells promote immunosuppression in ovarian cancer via ICOS costimulation of Foxp3(+) T-regulatory cells, Cancer Res. 72 (2012) 5240–5249.
[94] J. Faget, N. Bendriss-Vermare, M. Gobert, I. Durand, D. Olive, C. Biota, T. Bachelot, I. Treilleux, S. Goddard-Leon, E. Lavergne, S. Chabaud, J.Y. Blay, C. Caux, C. Menetrier-Caux, ICOS-ligand expression on plasmacytoid dendritic cells supports breast cancer progression by promoting the accumulation of immunosuppressive CD4+ T cells, Cancer Res. 72 (2012) 6130–6141.
[95] J. Faget, V. Sisirak, J.Y. Blay, C. Caux, N. Bendriss-Vermare, C. Menetrier-Caux, ICOS is associated with poor prognosis in breast cancer as it promotes the amplification of immunosuppressive CD4+ T cells by plasmacytoid dendritic cells, Oncoimmunology 2 (2013) e23185.
[96] K.S. Le, M.L. Thibult, S. Just-Landi, S. Pastor, F. Gondois-Rey, S. Granjeaud, F. Broussais, R. Bouabdallah, R. Colisson, C. Caux, C. Menetrier-Caux, D. Leroux, L. Xerri, D. Olive, Follicular B lymphomas generate regulatory T cells via the ICOS/ICOSL pathway and are susceptible to treatment by anti-ICOS/ICOSL therapy, Cancer Res. 76 (2016) 4648–4660.
[97] D. Ng Tang, Y. Shen, J. Sun, S. Wen, J.D. Wolchok, J. Yuan, J.P. Allison, P. Sharma, Increased frequency of ICOS+ CD4 T cells as a pharmacodynamic biomarker for anti-CTLA-4 therapy, Cancer Immunol. Res. 1 (2013) 229–234.
[98] B.C. Carthon, J.D. Wolchok, J. Yuan, A. Kamat, D.S. Ng Tang, J. Sun, G. Ku, P. Troncoso, C.J. Logothetis, J.P. Allison, P. Sharma, Preoperative CTLA-4 blockade: tolerability and immune monitoring in the setting of a presurgical clinical trial, Clin. Cancer Res. 16 (2010) 2861–2871.
[99] H. Chen, C.I. Liakou, A. Kamat, C. Pettaway, J.F. Ward, D.N. Tang, J. Sun, A.A. Jungbluth, P. Troncoso, C. Logothetis, P. Sharma, Anti-CTLA-4 therapy results in higher CD4+ICOShi T cell frequency and IFN-gamma levels in both nonmalignant and malignant prostate tissues, Proc. Natl. Acad. Sci. U.S.A. 106 (2009) 2729–2734.
[100] C.I. Liakou, A. Kamat, D.N. Tang, H. Chen, J. Sun, P. Troncoso, C. Logothetis, P. Sharma, CTLA-4 blockade increases IFNgamma-producing CD4+ICOShi cells to shift the ratio of effector to regulatory T cells in cancer patients, Proc. Natl. Acad. Sci. U.S.A. 105 (2008) 14987–14992.
[101] S.C. Wei, J.H. Levine, A.P. Cogdill, Y. Zhao, N.A.S. Anang, M.C. Andrews, P. Sharma, J. Wang, J.A. Wargo, D. Pe'er, J.P. Allison, Distinct cellular mechanisms underlie anti-CTLA-4 and Anti-PD-1 checkpoint blockade, Cell 170 (2017) 1120–1133 e1117.
[102] T. Fu, Q. He, P. Sharma, The ICOS/ICOSL pathway is required for optimal anti-tumor responses mediated by anti-CTLA-4 therapy, Cancer Res. 71 (2011) 5445–5454.
[103] X. Fan, S.A. Quezada, M.A. Sepulveda, P. Sharma, J.P. Allison, Engagement of the ICOS pathway markedly enhances efficacy of CTLA-4 blockade in cancer immunotherapy, J. Exp. Med. 211 (2014) 715–725.
[104] J. Li, Z.F. Huang, G. Xiong, H.Y. Mo, F. Qiu, H.Q. Mai, Q.Y. Chen, J. He, S.P. Chen, L.M. Zheng, C.N. Qian, Y.X. Zeng, Distribution, characterization, and induction of CD8+ regulatory T cells and IL-17-producing CD8+ T cells in nasopharyngeal carcinoma, J. Transl. Med. 9 (2011) 189.
[105] B.M. Olson, E. Jankowska-Gan, J.T. Becker, D.A. Vignali, W.J. Burlingham, D.G. McNeel, Human prostate tumor antigen-specific CD8+ regulatory T cells are inhibited by CTLA-4 or IL-35 blockade, J. Immunol. 189 (2012) 5590–5601.
[106] Y. Kiniwa, Y. Miyahara, H.Y. Wang, W. Peng, G. Peng, T.M. Wheeler, T.C. Thompson, L.J. Old, R.F. Wang, CD8+ Foxp3+ regulatory T cells mediate immunosuppression in prostate cancer, Clin. Cancer Res. 13 (2007) 6947–6958.
[107] S. Wei, I. Kryczek, L. Zou, B. Daniel, P. Cheng, P. Mottram, T. Curiel, A. Lange, W. Zou, Plasmacytoid dendritic cells induce CD8+ regulatory T cells in human ovarian carcinoma, Cancer Res. 65 (2005) 5020–5026.
[108] V. Sisirak, J. Faget, M. Gobert, N. Goutagny, N. Vey, I. Treilleux, S. Renaudineau, G. Poyet, S.I. Labidi-Galy, S. Goddard-Leon, I. Durand, I. Le Mercier, A. Bajard, T. Bachelot, A. Puisieux, I. Puisieux, J.Y. Blay, C. Menetrier-Caux, C. Caux, N. Bendriss-Vermare, Impaired IFN-alpha production by plasmacytoid dendritic cells favors regulatory T-cell expansion that may contribute to breast cancer progression, Cancer Res. 72 (2012) 5188–5197.
[109] D. Marvel, D.I. Gabrilovich, Myeloid-derived suppressor cells in the tumor microenvironment: expect the unexpected, J. Clin. Invest. 125 (2015) 3356–3364.
[110] M.R. Galdiero, C. Garlanda, S. Jaillon, G. Marone, A. Mantovani, Tumor associated macrophages and neutrophils in tumor progression, J. Cell. Physiol. 228 (2013) 1404–1412.
[111] A. da Cunha, M.A. Michelin, E.F. Murta, Pattern response of dendritic cells in the tumor microenvironment and breast cancer, World J. Clin. Oncol. 5 (2014) 495–502.
[112] S. Demoulin, M. Herfs, P. Delvenne, P. Hubert, Tumor microenvironment converts plasmacytoid dendritic cells into immunosuppressive/tolerogenic cells: insight into the molecular mechanisms, J. Leukoc. Biol. 93 (2013) 343–352.
[113] M. Ballbach, A. Dannert, A. Singh, D.M. Siegmund, R. Handgretinger, L. Piali, N. Rieber, D. Hartl, Expression of checkpoint molecules on myeloid-derived suppressor cells, Immunol. Lett. 192 (2017) 1–6.
[114] J.W. Kleinovink, K.A. Marijt, M.J.A. Schoonderwoerd, T. van Hall, F. Ossendorp, M.F. Fransen, PD-L1 expression on malignant cells is no prerequisite for checkpoint therapy, Oncoimmunology 6 (2017) e1294299.
[115] L. Wang, R. Rubinstein, J.L. Lines, A. Wasiuk, C. Ahonen, Y. Guo, L.F. Lu, D. Gondek, Y. Wang, R.A. Fava, A. Fiser, S. Almo, R.J. Noelle, VISTA, a novel mouse Ig superfamily ligand that negatively regulates T cell responses, J. Exp. Med. 208 (2011) 577–592.
[116] G.T. Motz, S.P. Santoro, L.P. Wang, T. Garrabrant, R.R. Lastra, I.S. Hagemann, P. Lal, M.D. Feldman, F. Benencia, G. Coukos, Tumor endothelium FasL establishes a selective immune barrier promoting tolerance in tumors, Nat. Med. 20 (2014) 607–615.
[117] L.Y. Tan, C. Martini, Z.G. Fridlender, C.S. Bonder, M.P. Brown, L.M. Ebert, Control of immune cell entry through the tumour vasculature: a missing link in optimising melanoma immunotherapy? Clin. Transl. Immunol. 6 (2017) e134.
[118] D.T. Fearon, The carcinoma-associated fibroblast expressing fibroblast activation protein and escape from immune surveillance, Cancer Immunol. Res. 2 (2014) 187–193.
[119] M.R. Nazareth, L. Broderick, M.R. Simpson-Abelson, R.J. Kelleher Jr., S.J. Yokota, R.B. Bankert, Characterization of human lung tumor-associated fibroblasts and their ability to modulate the activation of tumor-associated T cells, J. Immunol. 178 (2007) 5552–5562.
[120] J.T. Cheng, Y.N. Deng, H.M. Yi, G.Y. Wang, B.S. Fu, W.J. Chen, W. Liu, Y. Tai, Y.W. Peng, Q. Zhang, Hepatic carcinoma-associated fibroblasts induce IDO-producing regulatory dendritic cells through IL-6-mediated STAT3 activation, Oncogenesis 5 (2016) e198.
[121] N. Cohen, O. Shani, Y. Raz, Y. Sharon, D. Hoffman, L. Abramovitz, N. Erez, Fibroblasts drive an immunosuppressive and growth-promoting microenvironment in breast cancer via secretion of Chitinase 3-like 1, Oncogene (2017).
[122] D. Liao, Y. Luo, D. Markowitz, R. Xiang, R.A. Reisfeld, Cancer associated fibroblasts promote tumor growth and metastasis by modulating the tumor immune microenvironment in a 4T1 murine breast cancer model, PLoS One 4 (2009) e7965.
[123] C. Feig, J.O. Jones, M. Kraman, R.J. Wells, A. Deonarine, D.S. Chan, C.M. Connell, E.W. Roberts, Q. Zhao, O.L. Caballero, S.A. Teichmann, T. Janowitz, D.I. Jodrell, D.A. Tuveson, D.T. Fearon, Targeting CXCL12 from FAP-expressing carcinoma-associated fibroblasts synergizes with anti-PD-L1 immunotherapy in pancreatic cancer, Proc. Natl. Acad. Sci. U.S.A. 110 (2013) 20212–20217.
[124] B.C. Ozdemir, T. Pentcheva-Hoang, J.L. Carstens, X. Zheng, C.C. Wu, T.R. Simpson, H. Laklai, H. Sugimoto, C. Kahlert, S.V. Novitskiy, A. De Jesus-Acosta, P. Sharma, P. Heidari, U. Mahmood, L. Chin, H.L. Moses, V.M. Weaver, A. Maitra, J.P. Allison, V.S. LeBleu, R. Kalluri, Depletion of carcinoma-associated fibroblasts and fibrosis induces immunosuppression and accelerates pancreas cancer with reduced survival, Cancer Cell 25 (2014) 719–734.
[125] L. Gorchs, T. Hellevik, J.A. Bruun, K.A. Camilio, S. Al-Saad, T.B. Stuge, I. Martinez-Zubiaurre, Cancer-associated fibroblasts from lung tumors maintain their immunosuppressive abilities after high-dose irradiation, Front. Oncol. 5 (2015) 87.
[126] P.C. Ho, J.D. Bihuniak, A.N. Macintyre, M. Staron, X. Liu, R. Amezquita, Y.C. Tsui,

G. Cui, G. Micevic, J.C. Perales, S.H. Kleinstein, E.D. Abel, K.L. Insogna, S. Feske, J.W. Locasale, M.W. Bosenberg, J.C. Rathmell, S.M. Kaech, phosphoenolpyruvate is a metabolic checkpoint of anti-tumor T cell responses, Cell 162 (2015) 1217–1228.
[127] C.H. Chang, J.D. Curtis, L.B. Maggi Jr., B. Faubert, A.V. Villarino, D. O'Sullivan, S.C. Huang, G.J. van der Windt, J. Blagih, J. Qiu, J.D. Weber, E.J. Pearce, R.G. Jones, E.L. Pearce, Posttranscriptional control of T cell effector function by aerobic glycolysis, Cell 153 (2013) 1239–1251.
[128] A. Schurich, L.J. Pallett, D. Jajbhay, J. Wijngaarden, I. Otano, U.S. Gill, N. Hansi, P.T. Kennedy, E. Nastouli, R. Gilson, C. Frezza, S.M. Henson, M.K. Maini, Distinct metabolic requirements of exhausted and functional virus-specific CD8 T cells in the same host, Cell Rep 16 (2016) 1243–1252.
[129] K. Renner, A.L. Geiselhoringer, M. Fante, C. Bruss, S. Farber, G. Schonhammer, K. Peter, K. Singer, R. Andreesen, P. Hoffmann, P. Oefner, W. Herr, M. Kreutz, Metabolic plasticity of human T cells: preserved cytokine production under glucose deprivation or mitochondrial restriction, but 2-deoxy-glucose affects effector functions, Eur. J. Immunol. 45 (2015) 2504–2516.
[130] C.H. Ottensmeier, K.L. Perry, E.L. Harden, J. Stasakova, V. Jenei, J. Fleming, O. Wood, J. Woo, C.H. Woelk, G.J. Thomas, S.M. Thirdborough, Upregulated glucose metabolism correlates inversely with CD8+ T-cell infiltration and survival in squamous cell carcinoma, Cancer Res. 76 (2016) 4136–4148.
[131] K. Singer, M. Kastenberger, E. Gottfried, C.G. Hammerschmied, M. Buttner, M. Aigner, B. Seliger, B. Walter, H. Schlosser, A. Hartmann, R. Andreesen, A. Mackensen, M. Kreutz, Warburg phenotype in renal cell carcinoma: high expression of glucose-transporter 1 (GLUT-1) correlates with low CD8(+) T-cell infiltration in the tumor, Int. J. Cancer 128 (2011) 2085–2095.
[132] G. Mondanelli, S. Ugel, U. Grohmann, V. Bronte, The immune regulation in cancer by the amino acid metabolizing enzymes ARG and IDO, Curr. Opin. Pharmacol. 35 (2017) 30–39.
[133] A. Calcinotto, P. Filipazzi, M. Grioni, M. Iero, A. De Milito, A. Ricupito, A. Cova, R. Canese, E. Jachetti, M. Rossetti, V. Huber, G. Parmiani, L. Generoso, M. Santinami, M. Borghi, S. Fais, M. Bellone, L. Rivoltini, Modulation of microenvironment acidity reverses anergy in human and murine tumor-infiltrating T lymphocytes, Cancer Res. 72 (2012) 2746–2756.
[134] S.Y. Choi, C.C. Collins, P.W. Gout, Y. Wang, Cancer-generated lactic acid: a regulatory, immunosuppressive metabolite? J. Pathol. 230 (2013) 350–355.
[135] V. Huber, C. Camisaschi, A. Berzi, S. Ferro, L. Lugini, T. Triulzi, A. Tuccitto, E. Tagliabue, C. Castelli, L. Rivoltini, Cancer acidity: an ultimate frontier of tumor immune escape and a novel target of immunomodulation, Semin. Cancer Biol. 43 (2017) 74–89.
[136] A. Brand, K. Singer, G.E. Koehl, M. Kolitzus, G. Schoenhammer, A. Thiel, C. Matos, C. Bruss, S. Klobuch, K. Peter, M. Kastenberger, C. Bogdan, U. Schleicher, A. Mackensen, E. Ullrich, S. Fichtner-Feigl, R. Kesselring, M. Mack, U. Ritter, M. Schmid, C. Blank, K. Dettmer, P.J. Oefner, P. Hoffmann, S. Walenta, E.K. Geissler, J. Pouyssegur, A. Villunger, A. Steven, B. Seliger, S. Schreml, S. Haferkamp, E. Kohl, S. Karrer, M. Berneburg, W. Herr, W. Mueller-Klieser, K. Renner, M. Kreutz, LDHA-associated lactic acid production blunts tumor immunosurveillance by T and NK cells, Cell Metab. 24 (2016) 657–671.
[137] A. Angelin, L. Gil-de-Gomez, S. Dahiya, J. Jiao, L. Guo, M.H. Levine, Z. Wang, W.J. Quinn 3rd, P.K. Kopinski, L. Wang, T. Akimova, Y. Liu, T.R. Bhatti, R. Han, B.L. Laskin, J.A. Baur, I.A. Blair, D.C. Wallace, W.W. Hancock, U.H. Beier, Foxp3 reprograms T cell metabolism to function in low-glucose, high-lactate environments, Cell Metab. 25 (2017) 1282–1293 e1287.
[138] S.M. Lee, Y.S. Lee, J.H. Choi, S.G. Park, I.W. Choi, Y.D. Joo, W.S. Lee, J.N. Lee, I. Choi, S.K. Seo, Tryptophan metabolite 3-hydroxyanthranilic acid selectively induces activated T cell death via intracellular GSH depletion, Immunol. Lett. 132 (2010) 53–60.
[139] W.S. Lee, S.M. Lee, M.K. Kim, S.G. Park, I.W. Choi, I. Choi, Y.D. Joo, S.J. Park, S.W. Kang, S.K. Seo, The tryptophan metabolite 3-hydroxyanthranilic acid suppresses T cell responses by inhibiting dendritic cell activation, Int. Immunopharmacol. 17 (2013) 721–726.
[140] S. Bhattacharyya, J. Saha, Tumour oxidative stress and host T cell response: cementing the dominance, Scand. J. Immunol. 82 (2015) 477–488.
[141] X. Chen, M. Song, B. Zhang, Y. Zhang, Reactive oxygen species regulate T cell immune response in the tumor microenvironment, Oxid. Med. Cell. Longevity 2016 (2016) 1580967.
[142] A.A. Chimote, P. Hajdu, V. Kucher, N. Boiko, Z. Kuras, O. Szilagyi, Y.H. Yun, L. Conforti, Selective inhibition of KCa3.1 channels mediates adenosine regulation of the motility of human T cells, J. Immunol. 191 (2013) 6273–6280.
[143] A. Ohta, R. Kini, A. Ohta, M. Subramanian, M. Madasu, M. Sitkovsky, The development and immunosuppressive functions of CD4(+) CD25(+) FoxP3(+) regulatory T cells are under influence of the adenosine-A2A adenosine receptor pathway, Front. Immunol. 3 (2012) 190.
[144] R. Eil, S.K. Vodnala, D. Clever, C.A. Klebanoff, M. Sukumar, J.H. Pan, D.C. Palmer, A. Gros, T.N. Yamamoto, S.J. Patel, G.C. Guittard, Z. Yu, V. Carbonaro, K. Okkenhaug, D.S. Schrump, W.M. Linehan, R. Roychoudhuri, N.P. Restifo, Ionic immune suppression within the tumour microenvironment limits T cell effector function, Nature 537 (2016) 539–543.
[145] A.A. Chimote, P. Hajdu, A.M. Sfyris, B.N. Gleich, T. Wise-Draper, K.A. Casper, L. Conforti, Kv1.3 channels mark functionally competent CD8+ tumor-infiltrating lymphocytes in head and neck cancer, Cancer Res. 77 (2017) 53–61.
[146] D.S. Shin, J.M. Zaretsky, H. Escuin-Ordinas, A. Garcia-Diaz, S. Hu-Lieskovan, A. Kalbasi, C.S. Grasso, W. Hugo, S. Sandoval, D.Y. Torrejon, N. Palaskas, G.A. Rodriguez, G. Parisi, A. Azhdam, B. Chmielowski, G. Cherry, E. Seja, B. Berent-Maoz, I.P. Shintaku, D.T. Le, D.M. Pardoll, L.A. Diaz Jr., P.C. Tumeh, T.G. Graeber, R.S. Lo, B. Comin-Anduix, A. Ribas, Primary resistance to PD-1 blockade mediated by JAK1/2 mutations, Cancer Discov 7 (2017) 188–201.
[147] R.Y. Huang, A. Francois, A.R. McGray, A. Miliotto, K. Odunsi, Compensatory upregulation of PD-1, LAG-3, and CTLA-4 limits the efficacy of single-agent checkpoint blockade in metastatic ovarian cancer, Oncoimmunology 6 (2017) e1249561.
[148] G. Shayan, R. Srivastava, J. Li, N. Schmitt, L.P. Kane, R.L. Ferris, Adaptive resistance to anti-PD1 therapy by Tim-3 upregulation is mediated by the PI3K-Akt pathway in head and neck cancer, Oncoimmunology 6 (2017) e1261779.
[149] J.D. Wolchok, H. Kluger, M.K. Callahan, M.A. Postow, N.A. Rizvi, A.M. Lesokhin, N.H. Segal, C.E. Ariyan, R.A. Gordon, K. Reed, M.M. Burke, A. Caldwell, S.A. Kronenberg, B.U. Agunwamba, X. Zhang, I. Lowy, H.D. Inzunza, W. Feely, C.E. Horak, Q. Hong, A.J. Korman, J.M. Wigginton, A. Gupta, M. Sznol, Nivolumab plus ipilimumab in advanced melanoma, N. Engl. J. Med. 369 (2013) 122–133.
[150] K. Sakuishi, L. Apetoh, J.M. Sullivan, B.R. Blazar, V.K. Kuchroo, A.C. Anderson, Targeting Tim-3 and PD-1 pathways to reverse T cell exhaustion and restore anti-tumor immunity, J. Exp. Med. 207 (2010) 2187–2194.
[151] S.K. Pruitt, D. Boczkowski, N. de Rosa, N.R. Haley, M.A. Morse, D.S. Tyler, J. Dannull, S. Nair, Enhancement of anti-tumor immunity through local modulation of CTLA-4 and GITR by dendritic cells, Eur. J. Immunol. 41 (2011) 3553–3563.
[152] A. Ribas, B. Comin-Anduix, B. Chmielowski, J. Jalil, P. de la Rocha, T.A. McCannel, M.T. Ochoa, E. Seja, A. Villanueva, D.K. Oseguera, B.R. Straatsma, A.J. Cochran, J.A. Glaspy, L. Hui, F.M. Marincola, E. Wang, J.S. Economou, J. Gomez-Navarro, Dendritic cell vaccination combined with CTLA4 blockade in patients with metastatic melanoma, Clin. Cancer Res. 15 (2009) 6267–6276.
[153] J. Rosenblatt, B. Glotzbecker, H. Mills, B. Vasir, D. Tzachanis, J.D. Levine, R.M. Joyce, K. Wellenstein, W. Keefe, M. Schickler, R. Rotem-Yehudar, D. Kufe, D. Avigan, PD-1 blockade by CT-011, anti-PD-1 antibody, enhances ex vivo T-cell responses to autologous dendritic cell/myeloma fusion vaccine, J. Immunother. 34 (2011) 409–418.
[154] J.J. Luke, P.A. Ott, Kinase inhibitors and immune check-point blockade for the treatment of metastatic melanoma and advanced cancer: synergistic or antagonistic? Expert Opin. Pharmacother. 14 (2013) 2457–2462.
[155] S. Takamori, G. Toyokawa, K. Takada, F. Shoji, T. Okamoto, Y. Maehara, Combination therapy of radiotherapy and anti-PD-1/PD-L1 treatment in non-small-cell lung cancer: a mini-review, Clin. Lung Cancer (2017).
[156] P.A. Beavis, N. Milenkovski, M.A. Henderson, L.B. John, B. Allard, S. Loi, M.H. Kershaw, J. Stagg, P.K. Darcy, Adenosine receptor 2A blockade increases the efficacy of anti-PD-1 through enhanced antitumor T-cell responses, Cancer Immunol. Res. 3 (2015) 506–517.
[157] C.M. Suryadevara, R. Desai, S.H. Farber, P.C. Gedeon, A. Swartz, D. Snyder, J. Herndon, P. Healy, B.D. Choi, P.E. Fecci, L. Sanchez-Perez, J.H. Sampson, 369 Chimeric antigen receptors deficient in Lck signaling require 4–1BB costimulation to expand in vivo, resist regulatory T-Cell suppression, and treat solid tumors in immune-intact hosts, Neurosurgery 63 (Suppl. 1) (2016) 209–210.
[158] M. Irving, R. Vuillefroy de Silly, K. Scholten, N. Dilek, G. Coukos, Engineering chimeric antigen receptor T-cells for racing in solid tumors: don't forget the fuel, Front. Immunol. 8 (2017) 267.
[159] M. He, Y. Wang, W.J. Shi, S.J. Wang, H.F. Sha, J.X. Feng, J. Wang, Immunomodulation of inducible co-stimulator (ICOS) in human cytokine-induced killer cells against cholangiocarcinoma through ICOS/ICOS ligand interaction, J. Dig. Dis. 12 (2011) 393–400.
[160] S. Mohammed, S. Sukumaran, P. Bajgain, N. Watanabe, H.E. Heslop, C.M. Rooney, M.K. Brenner, W.E. Fisher, A.M. Leen, J.F. Vera, Improving Chimeric antigen receptor-modified T cell function by reversing the immunosuppressive tumor microenvironment of pancreatic cancer, Mol. Ther. 25 (2017) 249–258.
[161] K. Newick, S. O'Brien, J. Sun, V. Kapoor, S. Maceyko, A. Lo, E. Pure, E. Moon, S.M. Albelda, Augmentation of CAR T-cell trafficking and antitumor efficacy by blocking protein kinase A localization, Cancer Immunol. Res. 4 (2016) 541–551.
[162] L. Wang, W. Wen, J. Yuan, B. Helfand, Y. Li, C. Shi, F. Tian, J. Zheng, F. Wang, L. Chen, L. Liang, L. Zhou, C. Lee, Z. Chen, Y. Guo, H. Wang, Q. Zhang, W. Qin, Immunotherapy for human renal cell carcinoma by adoptive transfer of autologous transforming growth factor beta-insensitive CD8+ T cells, Clin. Cancer Res. 16 (2010) 164–173.
[163] G.M. Bendle, C. Linnemann, L. Bies, J.Y. Song, T.N. Schumacher, Blockade of TGF-beta signaling greatly enhances the efficacy of TCR gene therapy of cancer, J. Immunol. 191 (2013) 3232–3239.
[164] T. Ando, K. Mimura, C.C. Johansson, M.G. Hanson, D. Mougiakakos, C. Larsson, T. Martins da Palma, D. Sakurai, H. Norell, M. Li, M.I. Nishimura, R. Kiessling, Transduction with the antioxidant enzyme catalase protects human T cells against oxidative stress, J. Immunol. 181 (2008) 8382–8390.
[165] M.A. Ligtenberg, D. Mougiakakos, M. Mukhopadhyay, K. Witt, A. Lladser, M. Chmielewski, T. Riet, H. Abken, R. Kiessling, Coexpressed catalase protects chimeric antigen receptor-redirected T cells as well as bystander cells from oxidative stress-induced loss of antitumor activity, J. Immunol. 196 (2016) 759–766.
[166] L. Chaudhuri, R.K. Srivastava, F. Kos, P.A. Shrikant, Uncoupling protein 2 regulates metabolic reprogramming and fate of antigen-stimulated CD8+ T cells, Cancer Immunol. Immunother. 65 (2016) 869–874.
[167] H. Angell, J. Galon, From the immune contexture to the Immunoscore: the role of prognostic and predictive immune markers in cancer, Curr. Opin. Immunol. 25 (2013) 261–267.
[168] F. Pages, A. Kirilovsky, B. Mlecnik, M. Asslaber, M. Tosolini, G. Bindea, C. Lagorce, P. Wind, F. Marliot, P. Bruneval, K. Zatloukal, Z. Trajanoski, A. Berger, W.H. Fridman, J. Galon, In situ cytotoxic and memory T cells predict outcome in patients with early-stage colorectal cancer, J. Clin. Oncol. 27 (2009) 5944–5951.
[169] J.R. Webb, K. Milne, B.H. Nelson, Location, location, location: CD103 demarcates

intraepithelial, prognostically favorable CD8+ tumor-infiltrating lymphocytes in ovarian cancer, Oncoimmunology 3 (2014) e27668.
[170] J.R. Webb, K. Milne, P. Watson, R.J. Deleeuw, B.H. Nelson, Tumor-infiltrating lymphocytes expressing the tissue resident memory marker CD103 are associated with increased survival in high-grade serous ovarian cancer, Clin. Cancer Res. 20 (2014) 434–444.
[171] C. Sun, J. Xu, J. Song, C. Liu, J. Wang, C. Weng, H. Sun, H. Wei, W. Xiao, R. Sun, Z. Tian, The predictive value of centre tumour CD8(+) T cells in patients with hepatocellular carcinoma: comparison with Immunoscore, Oncotarget 6 (2015) 35602–35615.
[172] A. Gabrielson, Y. Wu, H. Wang, J. Jiang, B. Kallakury, Z. Gatalica, S. Reddy, D. Kleiner, T. Fishbein, L. Johnson, E. Island, R. Satoskar, F. Banovac, R. Jha, J. Kachhela, P. Feng, T. Zhang, A. Tesfaye, P. Prins, C. Loffredo, J. Marshall, L. Weiner, M. Atkins, A.R. He, Intratumoral CD3 and CD8 T-cell densities associated with relapse-free survival in HCC, Cancer Immunol. Res. 4 (2016) 419–430.
[173] H.C. Bosmuller, P. Wagner, J.K. Peper, H. Schuster, D.L. Pham, K. Greif, C. Beschorner, H.G. Rammensee, S. Stevanovic, F. Fend, A. Staebler, Combined Immunoscore of CD103 and CD3 identifies long-term survivors in high-grade serous ovarian cancer, Int. J. Gynecol. Cancer 26 (2016) 671–679.
[174] S.P. Fortis, M. Sofopoulos, N.N. Sotiriadou, C. Haritos, C.K. Vaxevanis, E.A. Anastasopoulou, N. Janssen, N. Arnogiannaki, A. Ardavanis, G. Pawelec, S.A. Perez, C.N. Baxevanis, Differential intratumoral distributions of CD8 and CD163 immune cells as prognostic biomarkers in breast cancer, J. Immunother. Cancer 5 (2017) 39.
[175] Z. Feng, D. Bethmann, M. Kappler, C. Ballesteros-Merino, A. Eckert, R.B. Bell, A. Cheng, T. Bui, R. Leidner, W.J. Urba, K. Johnson, C. Hoyt, C.B. Bifulco, J. Bukur, C. Wickenhauser, B. Seliger, B.A. Fox, Multiparametric immune profiling in HPV- oral squamous cell cancer, JCI Insight 2 (2017).
[176] R. Danielli, O. Cutaia, C. Fazio, E. Bertocci, E. Fonsatti, M. Maio, L. Calabro, ICOS expression as immunologic marker in immune activating monoclonal antibodies, Methods Mol. Biol. 1393 (2016) 133–139.
[177] F. Pages, C. Granier, A. Kirilovsky, C. Elsissy, E. Tartour, Bull. Cancer 103 (Suppl. 1) (2016) S151–S159.
[178] Y.P. de Coana, M. Wolodarski, I. Poschke, Y. Yoshimoto, Y. Yang, M. Nystrom, U. Edback, S.E. Brage, A. Lundqvist, G.V. Masucci, J. Hansson, R. Kiessling, Ipilimumab treatment decreases monocytic MDSCs and increases CD8 effector memory T cells in long-term survivors with advanced melanoma, Oncotarget 8 (2017) 21539–21553.
[179] M. Ayers, J. Lunceford, M. Nebozhyn, E. Murphy, A. Loboda, D.R. Kaufman, A. Albright, J.D. Cheng, S.P. Kang, V. Shankaran, S.A. Piha-Paul, J. Yearley, T.Y. Seiwert, A. Ribas, T.K. McClanahan, IFN-gamma-related mRNA profile predicts clinical response to PD-1 blockade, J. Clin. Invest. 127 (2017) 2930–2940.
[180] M.L. Ascierto, T.L. McMiller, A.E. Berger, L. Danilova, R.A. Anders, G.J. Netto, H. Xu, T.S. Pritchard, J. Fan, C. Cheadle, L. Cope, C.G. Drake, D.M. Pardoll, J.M. Taube, S.L. Topalian, The intratumoral balance between metabolic and immunologic gene expression is associated with anti-PD-1 response in patients with renal cell carcinoma, Cancer Immunol. Res. 4 (2016) 726–733.
[181] P. Charoentong, F. Finotello, M. Angelova, C. Mayer, M. Efremova, D. Rieder, H. Hackl, Z. Trajanoski, Pan-cancer immunogenomic analyses reveal genotype-immunophenotype relationships and predictors of response to checkpoint blockade, Cell Rep. 18 (2017) 248–262.
[182] C.U. Blank, J.B. Haanen, A. Ribas, T.N. Schumacher, CANCER IMMUNOLOGY. The “cancer immunogram”, Science 352 (2016) 658–660.
[183] D.S. Chen, I. Mellman, Oncology meets immunology: the cancer-immunity cycle, Immunity 39 (2013) 1–10.
[184] T. Karasaki, K. Nagayama, H. Kuwano, J.I. Nitadori, M. Sato, M. Anraku, A. Hosoi, H. Matsushita, Y. Morishita, K. Kashiwabara, M. Takazawa, O. Ohara, K. Kakimi, J. Nakajima, An immunogram for the cancer-immunity cycle: towards personalized immunotherapy of lung cancer, J. Thorac. Oncol. 12 (2017) 791–803.