Published in final edited form as:
Clin Dermatol. 2013 ; 31(3): 251–256. doi:10.1016/j.clindermatol.2012.08.010.

Strategies to reverse melanoma-induced T-cell dysfunction

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Abstract

Patients with advanced melanoma can develop spontaneous cellular and humoral responses to tumor antigens. Understanding the failure of spontaneous or vaccine-induced tumor antigen-specific T cell responses to promote the immunological clearance of melanomas is critical. Multiple mechanisms of melanoma-induced immune escape, which are likely to cause the failure of the spontaneous or vaccine-induced immune responses to promote tumor regression in humans have been elucidated. In addition, in the tumor microenvironment, a number of negative factors dampens anti-tumor immune responses, including cytokines (like TGF-β or IL-10), suppressive cells (regulatory T cells and myelosuppressive dendritic cells), defective antigen presentation by tumor cells (HLA or TA expression loss, antigen processing machinery defects), amino-acid catabolizing enzymes (indoleamine-2-3 dioxygenase, arginase) and immune inhibitory pathways (like CTLA-4/CD28, PD-1/PD-L1). Based on this information, a number of therapies to specifically target these negative regulators of anti- melanoma immune responses have been developed in order to enhance tumor antigen-specific immune responses and to increase the likelihood of clinical benefits in patients with advanced melanoma.

Human melanoma express tumor antigens (TAs) recognized by T cells present at the periphery and at tumor sites [1, 2]. There is now ample evidence that patients with advanced melanoma can develop spontaneous cellular and humoral responses to TAs [2]. In addition, a number of melanoma vaccines have successfully induced high frequencies of TA-specific T cells in patients with advanced melanoma without evidence of clinical benefits [3]. Understanding the failure of spontaneous or vaccine- induced TA-specific T cell responses to promote the immunological clearance of melanomas appears critical for the design of novel therapeutic interventions to overcome the tumor evasion of host immune responses.

We now have a better understanding of the multiple mechanisms of melanoma-induced immune escape, which are likely to cause the failure of the spontaneous or vaccine- induced immune responses to promote tumor regression in humans. In the tumor microenvironment (TME), a number of negative factors dampens anti- tumor immune responses, including cytokines (like TGF-β or IL-10), suppressive cells (regulatory T cells and myelosuppressive

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Conflict of interest: The authors have no conflicting financial interests.

dendritic cells), defective antigen presentation by tumor cells (HLA or TA expression loss, antigen processing machinery defects), amino-acid catabolizing enzymes (indoleamine-2-3 dioxygenase, arginase) and immune inhibitory pathways (like CTLA-4/CD28, PD-1/PD-L1)[4-6]. As a consequence, a number of therapies to specifically target these negative regulators of anti- melanoma immune responses have been developed in order to enhance TA-specific immune responses and to increase the likelihood of clinical benefits in patients with advanced melanoma.

Inhibitory receptors and pathways involved in melanoma-induced T cell dysfunction

T cell responses to TAs are regulated by co-stimulatory and co- inhibitory signals. The co-stimulatory receptors/ligands (such as CD28/CD80/CD86, ICOS/ICOSL or 4-1BB/4-1BBL) play a critical role in promoting T cell optimal activation and the development of T cell effector function. Co-inhibitory receptors including CTLA-4, PD-1, BTLA, Tim-3, LAG-3 and CD160 negatively regulate T cell functions and expansion. These immune checkpoints play an important role in preventing the hyperactivation of T cells, which may cause immune- mediated pathologies and autoimmune reactions.

Three major mechanisms support the inhibitory effects of these immune checkpoint molecules [7].

1. Inhibitory receptors compete with co-stimulatory molecules for their shared ligands. For example, CTLA-4 and CD28 compete to bind to CD80 (B7-1) and CD86 (B7-2), while PD-L1 binds to CD80 in addition to PD-1, sequestering CD80 away from CD28[8, 9Butte, 2007 #5513}].
2. The majority of inhibitory receptors use intracellular immunoreceptor tyrosine-based inhibititory motifs (ITIMs) to disrupt T cell signaling following activation.
3. Inhibitory pathways can lead to the up regulation of genes involved in T cell dysfunction. One example is the up regulation of the basic leucine zipper transcription factor, activating transcription factor-like (BATF) in exhausted T cells, upon activation of PD-1 pathway[10].

It is now well established that inhibitory pathways play a critical role in melanoma-induced T cell dysfunction [11-14]. In the context of chronic antigen stimulation by TAs, T cells become dysfunctional/exhausted and up regulate multiple inhibitory receptors while in the TME, antigen presenting cells (APCs) and melanoma cells express inhibitory ligands (such as PD-L1) either spontaneously or upon exposure to cytokines like IFN-γ [15, 16]. Through inhibitory receptor- ligand interactions, melanoma cells can activate negative regulatory pathways of T cells and promote tumor- induced T cell dysfunction. These observations have led to the use of monoclonal antibodies (mAbs) to block T cell immune checkpoints and reverse melanoma- induced T cell dysfunction.

CTLA-4 is an inhibitory receptor expressed by activated T cells and Tregs. It acts as a negative regulator of T-cell activation, serving as a checkpoint blockade to prevent excessive T- cell proliferation and immune- mediated damage to normal tissues [17]. CTLA-4 binds to B7 molecules expressed by APCs with a higher affinity than CD28, also a ligand for B7 molecules. Treatment with anti-CTLA-4 mAb led to tumor rejection in mice [18]. A dose response phase clinical trial with the fully humanized anti-CTLA-4 mAb ipilumumab at three dose levels (0.3, 3 and 10mg/kg) in 217 patients with unresectable melanoma has shown evidence of clinical responses [19]. The higher response of 11% was observed in the 10mg/kg cohort with a median overall survival of 14 months. A large phase III randomized trial of ipilimumab (3mg/kg) in combination with or without a gp100 peptide vaccine,

versus the peptide vaccine alone in stage IV melanoma patients, demonstrated that ipilimumab improved overall survival with evidence of durable clinical responses among the responders [20]. This led to the US FDA approval of the 3mg/kg dose of ipilumumab for metastatic melanoma. A fraction of patients with advanced melanoma appears to benefit from anti-CTLA-4 mAb therapy. Therefore, it appears critical to define biomarkers predicting clinical responses in order to avoid exposing a large number of melanoma patients who will not benefit from anti-CTLA-4 mAb therapy to its serious side effects. Such biomarker studies may help elucidating the immune mechanisms supporting the clinical efficacy of anti-CTLA-4-mAb directly responsible for the improved clinical outcome. Notably, a number of patients did not respond immediately to anti-CTLA-4-mAb therapy, but exhibited either late or slow responses over time, suggesting that the evaluation of objective clinical responses over a short period of time may not correctly predict the response to immune checkpoint blockade therapy. These observations have supported the proposition of novel, immune-related response criteria (irRC) to avoid the premature exclusion of patients who may initially progress before responding to immunotherapy [21].

Studies in animals [15, 16] and *in vitro* [22] have suggested the role of PD-1/PD- L1 interactions in inhibiting the effector functions of TA-specific CD8+ T cells. PD-1 is a co-inhibitory receptor expressed by activated T and B cells [23-26]. PD-1 binds to two known ligands: PD-L1 (B7-H1) [24, 27] and PD-L2 (B7-DC) [28, 29]. PD-1 negatively regulates T -cell functions through the engagement of PD-L1, which is expressed by a wide variety of tissues [24, 26, 27]. PD-L1 is also expressed by human tumors, either constitutively or after treatment with IFN-γ [15, 16]. In the context of chronic antigen stimulation and high antigen load, T cells become dysfunctional /exhausted T-cells and upregulate PD-1. In chronic viral infections, blockade of the PD-1/PD-L1 pathway with mAb increased T cell expansion cytokine production and proliferation, resulting in a significant reduction of the viral load [30]. In patients with advanced melanoma, TA-specific CTLs present in peripheral blood lymphocytes (PBLs) or at tumor sites upregulate PD-1 expression [11, 31]. PD-1 blockade using anti-PD-1 mAb increased T cell expansion, cytokine production and proliferation, indicating that PD-1 plays a critical role in regulating the expansion and function of TA-specific CD8+ T cells [31].

A number of clinical trials with blocking anti-PD-1 and anti-PD- L1 mAbs have been implemented in patients with cancers. MDX-1106 (BMS) is a fully humanized anti-PD-1 IgG4 antibody, which has been tested in phase I dose escalation trial of 39 patients with solid tumors [32]. No major adverse event was observed even at the highest dose tested (10mg/kg) and there was some evidence of objective clinical responses (1 complete response, 2 partial responses and 2 mixed responses). An additional trial with multiple doses of anti-PD-1 mAbs has shown evidence of clinical activity and durable clinical responses in patients with advanced solid tumors, including melanoma, non small cell lung cancer and renal cancers [33]. A number of additional anti-PD-1 (CT-011/Curetech, MK3475/ Merck) and anti-PD-L1 (MDX-1105/BMS) blocking mAbs are currently under clinical investigation. In sharp contrast with anti-CTLA-4 mAb therapy, the absence of major auto-immune side effects observed to date following anti-PD-1 treatment was unexpected because of the role of the PD-1 pathway in promoting immune tolerance. Although a number of studies correlating PD-L1 expression with clinical prognosis in patients with melanoma had contradictory conclusions [34, 35], there is recent evidence that cell surface expression of PD-L1 may represent a biomarker predicting clinical response to PD-1 blockade [36].

In addition to the CTLA-4 and PD-1, a number of additional inhibitory receptors appear to play a significant role in dampening antitumor T cell responses. One of them is B and T cell attenuator (BTLA). BTLA, like PD-1, is an immunoglobulin- like molecule that belongs to the B7/CD28 family and is expressed by different cell types, including T cells, B cells, NK

cells and DCs. In mice, BTLA inhibits proliferation of T cells and negatively regulates the homeostasis of CD8+ T cells and generation of memory T cells in vivo. BTLA plays a role in the induction of peripheral tolerance mediated by T cells in vivo [37]. In cancer immunology, BTLA expression is upregulated by TA-specific CD8+ T cells isolated from peripheral blood of melanoma patients. The engagement of BTLA by its ligand HVEM expressed on melanoma cells limits the expansion and functions of TA-specific CD8+ T cells [38]. Blocking anti- human BTLA mAbs are being tested in preclinical models.

Lymphocyte activation gene 3 (LAG-3) is another inhibitory receptor, which is a CD4 homolog type I membrane protein and is expressed on activated CD4+ and CD8+ T cells. At tumor sites, LAG-3 is expressed by TILs, NKT, NK cells and Tregs. LAG-3 binds to MHC class II present on APCs and melanoma cells. LAG-3 negatively regulates TCR signal transduction impeding T cell proliferation and function. There is also evidence that LAG-3 promotes melanoma progression through enhanced resistance of tumors to T cell responses and apoptosis [39].

Tim-3 is a transmembrane protein whose constitutive expression was first observed in Th-1 cells and induced T cell death upon ligation with its ligand galectin-9 [40]. In mice, blocking Tim-3 pathway leads to the hyperproliferation of Th-1 cells and abrogates the induction of peripheral tolerance [41, 42]. Galectin-9 was identified as a ligand for Tim-3 and it was observed that Tim-3 engagement by galectin-9 interaction induces T cell death [40]. Galectin-9 is expressed by APCs such as monocytes, macrophages and DCs and by some tumors, including melanoma cells [43]. In patients with metastatic melanoma, TA-specific CD8+ T cells upregulate Tim-3 at periphery and at tumor sites [44]. In particular, the upregulation of PD-1 and Tim-3 labels a highly dysfunctional TA-specific CD8+ T cell subset in patients advanced melanoma [14].

Targeting multiple inhibitory pathways

Dysfunctional/exhausted TA-specific T cells present in peripheral blood and at tumor sites co-express multiple inhibitory receptors [44, 45]. The implications of this important finding are two- fold.

1. Multiple subsets of TA-specific T cells can be identified in patients with advanced melanoma that exhibit variable levels of T cell dysfunction.
2. This observation supports the implementation of combinatorial therapies aiming at blocking multiple inhibitory pathways to enhance TA-specific immune responses and reverse tumor- induced T cell dysfunction. One example is the combination of anti-CTLA-4 and anti-PD-1 mAbs, which appears promising in experimental animal models [46] and is currently evaluated in the clinic.

A number of studies have suggested the potency of other combinatorial immune checkpoint blockades targeting PD-1, Tim-3 and BTLA. A subset of highly dysfunctional TA-specific CD8+ T cells isolated from patients with advanced melanoma upregulate both PD-1 and Tim-3 [47]. PD-1 and Tim-3 blockade strongly enhanced TA-specific CD8+ T cell expansion and function. Accordingly, targeting PD-1 and Tim-3 in *vivo* induced melanoma regression in mice [48]. The combination of PD-1 and Tim-3 blockade either alone or in combination with cancer vaccines appears to be a promising potent approach to reverse melanoma-induced T cell dysfunction. Recently, a subset of TA-specific CD8+ T cells with intermediate T cell dysfunction isolated from peripheral blood of melanoma patients appears to upregulate both BTLA and PD-1 [45]. Added with PD-1 and Tim-3 pathway blockades, BTLA blockade enhanced the expansion, proliferation and cytokine production of TA-specific CD8+ T cells isolated from patients with advanced melanoma. A number of studies in animal and cancer patients have suggested that T cell subsets present in TILs upregulate

PD-1 and LAG-3 and that dual PD-1 and LAG-3 blockade enhance T cell expansion and functions [49]. Such findings will need to be evaluated in patients with advanced melanoma.

Targeting other inhibitors of anti-melanoma T cell immune responses in the TME

A growing number of soluble or membrane-bound inhibitors have been identified in the TME. As a consequence, a number of targeted therapies with mAbs or small molecules are being developed to impede their negative regulatory effects. The TME contains soluble factors that inhibit T cell immune responses to melanoma such as TGF-β, IL-10 and nitric oxide (NO). TGF-β is secreted in large amounts by melanoma cells, and exerts immunosuppressive properties [50]. TGF-β inhibits the proliferation and effector functions of T cells, impedes their differentiation into CTLs or Th cells and disrupts antigen presentation by APCs [51]. Furthermore, TGF-ß can directly act on CTLs to down regulate their intracellular expression of granzymes and perforin as well as the expression of Fas-L, molecules that are collectively responsible for their cytotoxic functions [52].

IL-10 is an immunosuppressive cytokine secreted by many types of cancer cells and its production level by melanoma cells correlates with a poor prognosis [53]. IL-10 can indirectly suppress melanoma antigen-specific T-cell responses by inducing the down regulation of MHC molecules on the surface of cancer cells [54], as well as the decreased expression of proteins involved in the presentation of antigens to CD8+ T cells [55]. IL-10 also inhibits the production of pro- inflammatory cytokines such as TNF, IFN-γ and IL-2 by T cells [56]. Available anti-IL-10 or anti-IL-10R blocking antibodies remains to be evaluated in patients with advanced melanoma.

Indoleamine-2, 3-dioxygenase (IDO) is an enzyme produced in the TME by APCs and melanoma cells. IDO induces tryptophan degradation into kynurenines that are T-cell immunosuppressive agents [57]. IDO inhibitors are being evaluated in the clinic. In addition, there is evidence that TCR-CD8 dissociation observed on dysfunctional TA-specific T cells in TILs isolated from melanoma patients is due to a reduced mobility of the TCRs, which are trapped in a lattice of glycoproteins clustered by extracellular galectin-3, a lectin abundantly secreted by macrophages and various types of tumor cells [58, 59]. There is further evidence that intratumoral galectin-3 impairs TA-specific T-cell function and that galectin-3 ligands improve antitumor immunity *in vivo* [60]. Galectin 3 ligands are being tested in the clinic to reverse tumor- induced T cell dysfunction/anergy.

Conclusions

With the evidence of clinical responses induced by blockade of a single inhibitory pathway, it is expected that combinatorial approaches aiming at blocking multiple inhibitory receptors with or without targeting other inhibitory molecules present in the TME may further lead to improved clinical benefits and survival for patients with advanced melanoma. Immune checkpoint blockades will also need to be evaluated in combination with cancer vaccines, in particular in melanoma patients who develop no or low frequenc ies of TA-specific T cells. A number of experimental models have suggested the potency of such combination in vivo. Such an approach may likely increase the likelihood that vaccine- induced T cells lead to significant tumor regression and improved survival in patients with advanced melanoma. Finally, the identification of biomarkers predicting clinical response to inhibitory receptor blockades appears critical to select and tailor combinatorial blockades to patients most likely to respond and avoid exposing non-responder patients to potential serious immune-related adverse events.

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