Review Article

Molecular mechanisms of programmed cell death-1 dependent T cell suppression: relevance for immunotherapy

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Abstract: Programmed cell death-1 (PD1) has become a significant target for cancer immunotherapy. PD1 and its receptor programmed cell death 1 ligand 1 (PDL1) are key regulatory physiological immune checkpoints that maintain self-tolerance in the organism by regulating the degree of activation of T and B cells amongst other immune cell types. However, cancer cells take advantage of these immunosuppressive regulatory mechanisms to escape T and B cell-mediated immunity. PD1 engagement on T cells by PDL1 on the surface of cancer cells dramatically interferes with T cell activation and the acquisition of effector capacities. Interestingly, PD1-targeted therapies have demonstrated to be highly effective in rescuing T cell anti-tumor effector functions. Amongst these the use of anti-PD1/PDL1 monoclonal antibodies are particularly efficacious in human therapies. Furthermore, clinical findings with PD1/PDL1 blockers over several cancer types demonstrate clinical benefit. Despite the successful results, the molecular mechanisms by which PD1-targeted therapies rescue T cell functions still remain elusive. Therefore, it is a key issue to uncover the molecular pathways by which these therapies exert its function in T cells. A profound knowledge of PDL1/PD1 mechanisms will surely uncover a new array of targets susceptible of therapeutic intervention. Here, we provide an overview of the molecular events underlying PD1-dependent T cell suppression in cancer.

Keywords: Cancer; immune checkpoint inhibitors; immunotherapy

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Introduction

After decades of extensive research in the development of cancer immunotherapies, during the last ten years these therapies have achieved clinical success. Among the most promising approaches is the blockade of immune checkpoints that regulate immune responses. T cell antigen recognition is highly regulated by co-stimulatory positive and negative signals. Under normal immune responses, negative signals called immune checkpoints are critical to maintain peripheral tolerance and protection from autoimmunity. However, inhibitory ligands and receptors of these immune checkpoints are frequently up-regulated in tumors. Programmed cell death 1 ligand 1 (PDL1)-programmed cell death-1 (PD1) together with CD80-CTLA4 interactions are one of the best known by their clinical relevance. PD1 is a type 1 transmembrane protein expressed by many effector immune cells. In fact,

PD1 expression is up-regulated following T and B cell activation. Its receptor PDL1, a member of the B7 family of co-stimulatory/co-inhibitory molecules is expressed by many cell types including antigen presenting cells such as dendritic cells and cancer cells (1). Importantly, PDL1 is commonly overexpressed in several cancer types as an immune resistance mechanism (2,3). PDL1 on the surface of cancer cell binding to PD1 on T cells causes T inactivation within the tumor microenvironment (4).

The application of PDL1/PD1 monoclonal antibody immunotherapy has demonstrated efficacious clinical responses in diverse human cancers. An increasing number of clinical trials demonstrate that PDL1/PD1 blockade is remarkably more effective than conventional therapies in many cases, with durable clinical responses and milder side effects (5). This therapy enhances T cell responses toward cancer cells while surprisingly sparing non-transformed cells. Nevertheless, a significant number of patients are intrinsically resistant to these therapies. The uncovering of the molecular mechanisms in which the efficacy of PD1/PDL1 targeted therapies relies may identify non-responder patients.

Mechanisms of antigen presentation to T cells

T cell activation is highly regulated at multiple levels especially during antigen presentation to ensure a proper immune response. T cells recognize peptides derived from antigens through their surface T cell receptor (TCR). This recognition relies on the establishment of an immune synapsis established by the TCR with antigenic peptide-major histocompatibility molecules (p-MHC), and stabilized by a range of positive and negative interactions between the T cell and the antigen presenting cell (*Figure 1A*). The relative contribution of co-stimulatory/co-inhibitory ligand-receptor interactions determines the activation state and the type of effector T cell responses (6,7). The main positive regulatory interaction is provided by CD80 on the surface of APC which binds to CD28. On the other hand, negative regulatory signals (immune-checkpoints) modulate this recognition to ensure self-tolerance and protection against exacerbated immune responses (8). Cytotoxic T-lymphocyte-associated antigen 4 (CTLA4) and PD1 on T cell surface regulate negatively immune responses binding to CD80 and PDL1, respectively. Finally, cytokine-dependent signals regulate T cell differentiation and effector capacities.

At the molecular level, when the TCR α and β chains associated to CD3 molecules recognize the p-MHC together with CD4 or CD8 clustering, a complex signaling pathway is started (*Figure 1B*). The signaling events begin with the recruitment and activation of Src/like tyrosine kinases such as LCK into the signaling complex. These kinases phosphorylate the TCR and CD28 intracellular chains allowing the TCR signal transduction to proceed through the recruitment of ZAP-70 and PI3K to the CD3 and CD28 molecules, respectively (9). With the phosphorylation of additional T cell molecules such as LAT and p38, signal transduction continues through the recruitment of other molecules such as GRB2, VAV and SOS, culminating with the activation of MAPKs ERK and JNK (10-12). PLCγ1 also gets activated causing the release of Ca2+ ions from the cell endoplasmic reticulum and NFAT (Nuclear factor of activated T-cells) and CREB translocation to the nucleus. PKC isoforms also get activated which participate in the nuclear factor NF-kappa-B (NF-kB) pathway (13). These events induce also the activation of RasGRP1 leading to ERK activation (14).

The co-stimulatory interaction between CD80 and CD28 reinforces T cell activation signaling. CD80-CD28 association recruits PI3K to CD28 intracytoplasmic domain and then gets activated producing phosphatidylinositol [3,4,5]-triphosphate (PIP_3) which is required for AKT and PKCΘ activation and therefore Bcl-x expression. AKT through the mTOR pathway rescues T cells from anergy (15), while PKCΘ activates NF-kβ and MKK7 required to Il-2 production.

Mechanisms of pd1-dependent T cell suppression

During antigen presentation PDL1-PD1 engage within the immunological synapse blocking T cell activation, proliferation and acquisition of effector capacities (16) by strongly inhibiting TCR signal transduction and CD28-CD80 costimulation (17). However, PD1 plays also critical roles in physiological conditions by fine-tuning the activation state of T cells following their activation. Therefore, PD1 is up-regulated in naïve T cells following antigen presentation (18). PD1 consists of a single N-terminal immunoglobulin variable region-like domain, a transmembrane domain, and a cytoplasmic domain containing tyrosine-based signal motifs. These includes an immunoreceptor tyrosine based inhibitory motif (ITIM) and an immunoreceptor tyrosine-based switch motif (ITSM) (*Figure 2A*). All the data point to the ITSM motif

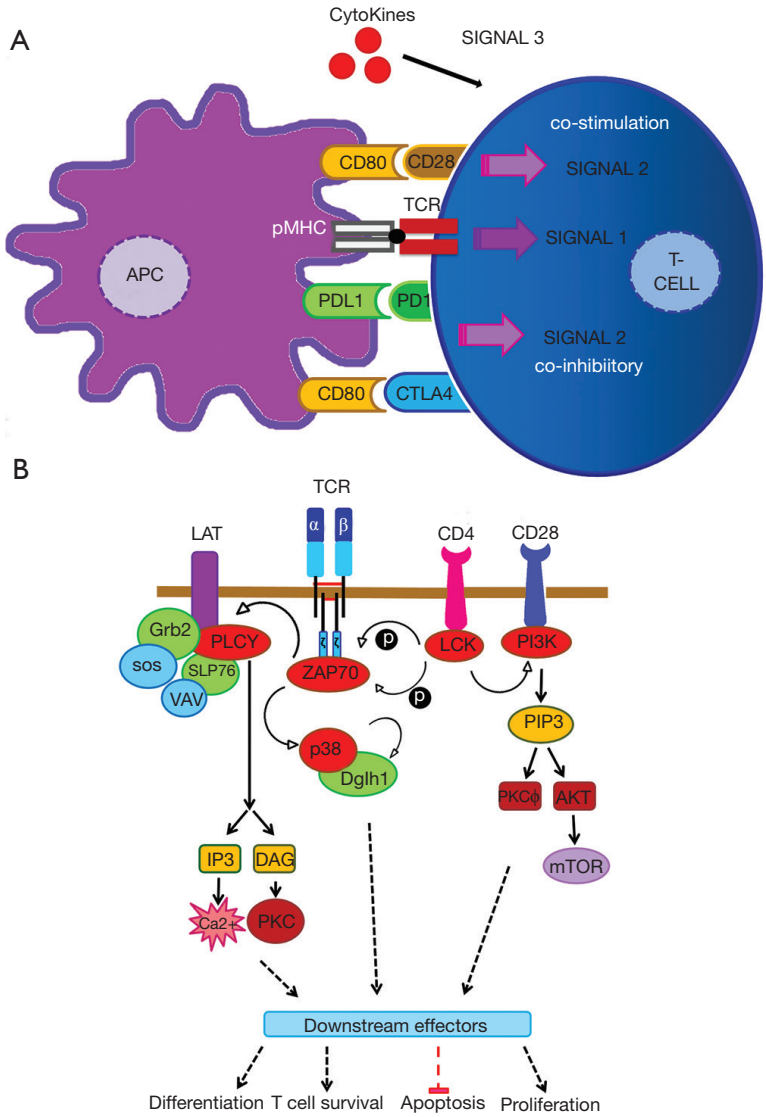

Figure 1 Antigen presentation and T cell activation. (A) T cells (right on the picture) get activated through antigen recognition on the surface of antigen presenting cells (APCs) (left of the picture) upon three signals. APCs present antigenic peptides in MHC molecules (pMHC) to T cells through binding to their T cell receptor (TCR). This interaction delivers signal 1 as indicated within the T cell. T cells simultaneously receive additional co-stimulatory and co-inhibitory signals through ligand-receptor interactions within the immunological synapse. On the top, it is represented the CD80-CD28 co-stimulatory interaction, and below two inhibitory interactions between PDL1-PD1 and CD80-CTLA4. The integration of these signals delivers a second signal which drives regulation of T cell activation. A third signal is also provided by cytokine secretion. (B) The figure is represented TCR molecules and associated components which form TCR signalosome. This complex drives T cell activation signaling pathways. TCR signaling initiation depends on LCK activation that phosphorylates TCR-CD3 and CD28 cytoplasmic domains. ZAP70 then binds to CD3ζ and phosphorylates LAT and p38. Phosphorylated LAT recruits other adaptor and signaling molecules as shown which will trigger calcium-dependent and MAPK-dependent pathways. When CD28 associates to CD80 on the surface of the APC, PI3K generates PIP_3 leading to activation of AKT-mTOR pathways which induce T cell proliferation and survival. CD28 engagement also prevents apoptosis and acts synergistically with CD3-dependent signals to ensure correct T cell proliferation, differentiation and growth. In green, adaptor molecules. In red, kinases and in green adaptor molecules.

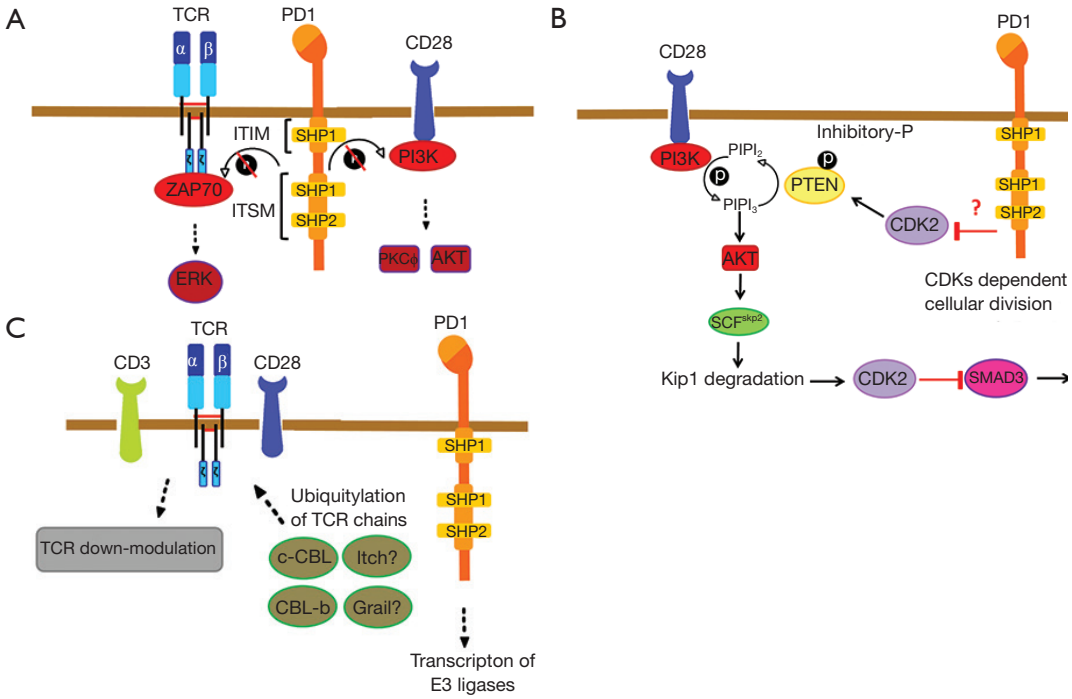

Figure 2 PD1-dependent inhibitory mechanisms. (A) The figure represents PD1-dependent proximal inhibitory mechanisms, which depend on the recruitment of SHP1 and SHP2 phosphatases. These phosphatases inhibit ZAP70 and PI3K activities (blue arrows). Consequently, downstream intracellular pathways are also terminated, as exemplified in the figure with, AKT, ERK and PKCΘ. (B) Indirect inhibitory mechanisms over TCR signaling and T cell proliferation are shown trigged by the regulation of CK2 expression. The PI3K-dependent signaling pathway activates CDK2 and inhibits SMAD3-depending gene expression as shown. Briefly, PIP_3 activates AKT which enhances ubiquitin ligase SCF^{skp2} that degrades the CDK2 inhibitor $p27^{Kip1}$. Activated CDK2 triggers cell cycle progression and inactivates SMAD3 by phosphorylation. These pathways are negatively regulated by the PTEN phosphatase that degrades PIP_3. During TCR activation CK2 phosphorylates PTEN with resulting decrease in its activities. When PD1 is engaged PTEN phosphatase activity is active dephosphorylating PIP3, and therefore CK2 expression and activities decrease. (C) Regulation of TCR surface expression by PD1 engagement. PD1 engagement promotes expression of E3 ubiquitin ligases of the CBL family, as shown. These ubiquitin ligases ubiquitylate TCR chains and associated kinases, such as ZAP-70 and PI3K, leading to TRC signaling termination and the removal of TCRs from the T cell surface.

as the mediator of PD1 inhibitory signaling activities (19). PD1 exerts its immunosuppressive activities by recruiting phosphatases containing SH2 domains, SHP1 and SHP2 to the tyrosine-based motifs (20). During T cell antigen recognition, when TCR signal transduction is activated these motifs undergo phosphorylation by LCK. If in that situation PD1 binds to its receptor PDL1, the TCR signal transduction terminates through several mechanisms. The first one includes the SHP-mediated dephosphorylation of the TCR signalosome components (4). More specifically, the dephosphorylation of CD3ζ, ZAP70 and PI3K kinases resulting in the deactivation of downstream signaling targets (21,22). Although both SHP1 and SHP2 interact with ITSM motif (19) it has been shown that only SHP2 binds to the ITSM during T cell activation (21). Moreover, SHP2 but not SHP1 has been found associated to PD1 in microclusters (23). Therefore, most of the evidence points to SHP2 as the main mediator of PD1 inhibitory effects, while the participation of SHP1 is still under debate.

Apart from proximal inhibitory effects over the TCR signalosome, PD1 can also suppress T cell activation through indirect pathways mostly affecting T cell proliferation. Upon T cell activation, TCR signal transduction increases CK2 expression that phosphorylates the regulatory domain of PTEN which inhibits its phosphatase activity over PIP_3 produced by PI3K (24)

(*Figure 2B*). If PD1 is engaged, CK2 kinase expression is down-regulated and PTEN can terminate PI3K activities by dephosphorylating PIP_3 (*Figure 2B*) (25). Furthermore, PD1 can also inhibit cyclin-dependent kinase (CDKs) resulting in the arrest of T cell proliferation (*Figure 2B*). PD1 achieves this by inhibiting the transcription of the SCF^{skp2} following AKT and ERK inhibition. SCF^{skp2} is a ubiquitin ligase that targets the CDK2 inhibitor $p27^{Kip1}$ for proteosomal degradation. In the absence of SCF^{skp2} the CDK2 inhibitor accumulates stopping cell division (22). This mechanism is also reinforced through the absence of the CDK2-dependent SMAD3 inhibiting phosphorylation. SMAD3 activities transactivates genes that encode CDK4, CDK6 and CDC25A transcriptional repressors (26). All in all, PD1-dependent T cell function inhibition is a complex mechanism that implicates several signaling pathways (27).

PD1 can also control the surface expression levels of the TCR, avoiding the recognition of pMHC by the T cell. Although the molecular mechanisms leading to TCR down-modulation are still unclear, most of the studies point to E3 ubiquitin ligases as the main mediators of PD1-depedent control of TCR expression. Particularly, the E3 ubiquitin ligases of the CBL family plays a critical role in regulating TCR expression levels and antigen-induced TCR down-modulation that takes place during antigen presentation. CBL knockout mice demonstrated that E3-ubiquitin ligases CBL-B, c-CBL and ITCH up-regulation induce the termination of TCR signal transduction (8,28,29) (*Figure 2C*). CBL-B and ITCH have been shown to cause the ubiquitination of CD3 and CD28 chains preventing their phosphorylation and the association of TCR signalosome kinases such as ZAP-70 and PI3K (30,31). PDL1-PD1 interactions during antigen presentation by dendritic cells to T cells cause the strong up-regulation of CBL-b and c-CBL which contributes to antigen-induced TCR down-modulation (8,28). It is highly likely that this process is also taking place within the tumor environment, as PD1 expression is up-regulated in tumor-infiltrating T cells and these T cells usually show low expression levels of surface TCR (32,33).

Upon activation T cells undergo a metabolic reprogramming to cover high energy needs. While quiescent T cells use lipid degradation to obtain the energy, aerobic glycolysis becomes the dominant energy source by effector T cells. An increment of glutamine uptake and catabolism is also required. When PD1 is engaged a suppression of oxygen consume takes place and aerobic glycolysis is no longer used. In this situation, fatty-lipid oxidation is the main energy source (34,35) (*Figure 3*). Moreover, accumulation of polyunsaturated fatty acids is another characteristic of the suppressed T cells (36). PI3K-AKT and ERK are known to induce the expression of glycolysis genes, so the resulting metabolism pattern change might be mediated by PD1-dependent inhibition of this signaling axis (35). As a consequence, PD1-engaged T cells also increase production of reactive oxidative species derived from fatty acid oxidation contributing to create an oxidative environment (37). Overall, PD1 engagement causes a shift on the metabolic reprogramming from an effector T phenotype to a memory-like phenotype. Interestingly enough, this metabolic changes might have an important contribution in PD1-dependent T cell suppression in cancer (38).

Functional consequences of PDL1-PD1 disruption

Cancer cells upregulate PDL1 surface expression to escape from host immune responses. This upregulation is mediated by inflammatory cytokines such as interferon γ (39). In addition, PDL1 expression is also regulated by oncogenic pathways including AKT and PTEN (40). When there is lymphocyte infiltration in tumors, these are mainly PD1high memory T cells specific for tumor-antigens (7,32). PDL1-PD1 engagement inactivates these tumor-specific T cells (41), and blockade of this interaction rescues these T cells from inhibition. Recent studies have demonstrated that PD1 blockade selectively increase CD8 memory T cell numbers within the tumor microenvironment with a concurrent increase in INF-γ production. This infiltration positively correlates with therapeutic efficacy. For example, in melanoma patients with PD1 CD8 T cells in the tumor before the treatment, and the increase of this population following treatment correlates with tumor regression (42). For this reason, CD8 T cell infiltration can be used as a biomarker for therapeutic efficacy. Actually, tumor infiltration with PD1high and CTLA4high exhausted CD8+ T cells has been proposed as an accurate predictor of responses to anti-PD1 therapy in melanoma (43).

Recently published studies in lung cancer have shown that T cells expanding in peripheral blood during anti-PD1 therapy are predominately CD28+ CD8 T cells. These findings points suggest that CD28-coestimulation might contribute to the reactivation of exhausted CD8 T cells in the tumor (44). Thus, it assigns to CD28 a possible function as a biomarker to predict PD1-antibody therapy efficacy.

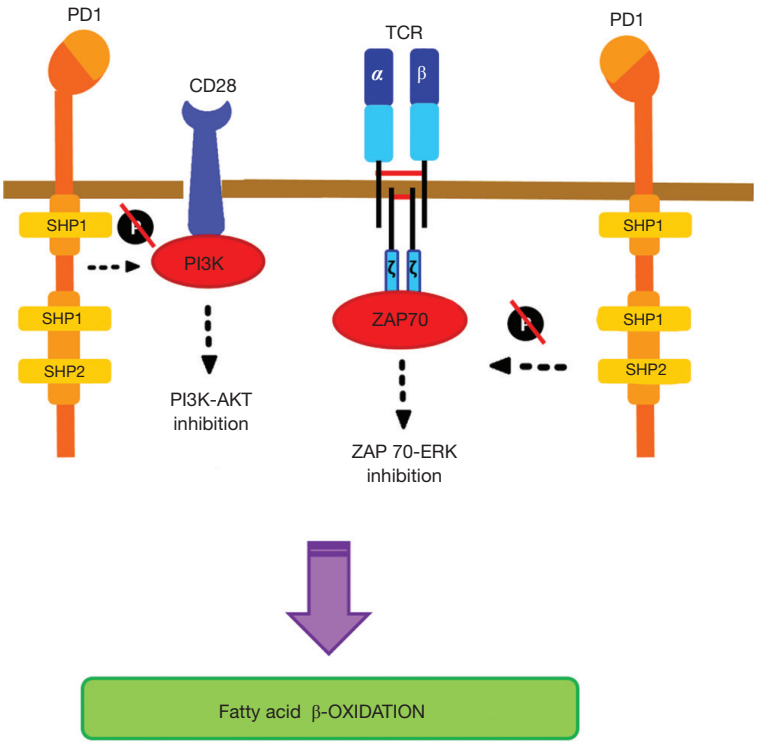

Figure 3 Metabolic consequences of PD1 engagement. PD1 engagement generated a shift on T cell metabolism from glycolysis to β-oxidation by inhibition of ERK and PI3K-AKT activities. PD1-stimulated T cells would then metabolically resemble long-lived memory T cells.

Nevertheless, it is still not clear where this co-stimulation takes place, either within the tumor driven by cancer cells co-expressing CD80, or systemically in secondary lymphoid organs. In fact, PDL1-PD1 interactions have a key role in the maintenance of peripheral tolerance. Since PDL1/PD1 blockade therapies are administrated systematically, such disruption must have a range of effects in the immunological synapse in peripheral tissues. When PDL1 is silenced in the peripheral tissues there is in fact an expansion of polyclonal CD8 T cells (43). Moreover, disruption of PDL1-PD1 interactions abrogates TCR down-modulation leading to the expansion of hyperactivated TCR^{high} T cells. The differentiation of this pool of hyperactivated T cells might strongly contribute to anti-tumor responses in PDL1-PD1 blockade therapy, explaining in addition the role of CD80-CD28 interactions for therapeutic efficacy. As a consequence, a pool of these cells in some patients may manifest auto-reactive inflammatory adverse events which cause damage to tissues and organs (28,44).

The clinical application of PDL1-PD1 blockade therapies in patients with a wide range of cancers has demonstrated important therapeutic efficacies and long-lasting responses overtime. However, there is still a significant group of patients who do not respond to these therapies. Therefore, a key issue nowadays is the identification of predictive biomarkers for these therapies. It is assumed that PDL1 expression in cancer cells would directly correlate with therapeutic efficiency. However, patients with either PDL1-positve or PDL1-negative tumors can respond to these treatments (45). The presence of inactivating mutations in JAK1, JAK2 and beta2-microglobulin genes in cancer cells correlates with lack of response (46). Furthermore, there is also a correlation between tumor mutation burden with better clinical response. For example, non-squamous lung cancer (NSCLC) and melanoma are cancer types with a high number of somatic mutations. These cancers are frequently clinically responsive to anti-PD1/PDL1 therapy (47). Similarly, patients with microsatellite instability colorectal cancer show good responses while patients with mismatch repair-proficient colorectal cancer do not (48).

As mentioned before, the lymphocyte infiltration and its location within the tumor seems a good prognostic marker for immunotherapies (42). Furthermore, T cells exhibiting

a Th1 phenotype in the tumor infiltrate also correlate with good responses and survival in some human cancers (49).

Conclusions

The therapeutic application of PDL1/PD1 blocking antibodies has revolutionized cancer treatments and care. Their efficacies and durable effects have opened a new line of first line treatments. PDL1-PD1 antibody-mediated blockade was recently approved by FDA for metastatic melanoma, NSCLC, head and neck, kidney and urothelial carcinoma. Moreover, several clinical trials undergoing with several cancer types have promising responses. However, this therapy is still refractory for a significant number of patients and the molecular bases of this unresponsiveness are still unclear. PDL1-PD1 interactions mediates immunosuppression by several mechanisms that are still under study. The uncovering of the molecular mechanisms governing their actions is relevant for a better understanding of therapeutic responses. Such discoveries will reveal novel biomarkers to predict whether patients will benefit or not from anti-PD1/PDL1 therapies. Furthermore, it will also provide new targets to enhance therapeutic intervention for those patients who will not respond.

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Footnote

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