Post-translational Modification of PD-1: Potential Targets for Cancer Immunotherapy

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ABSTRACT

Activation of effector T cells leads to upregulation of PD-1, which can inhibit T-cell activity following engagement with its ligand PD-L1. Post-translational modifications (PTM), including glycosylation, phosphorylation, ubiquitination, and palmitoylation, play a significant role in regulating PD-1 protein stability, localization, and interprotein interactions. Targeting PTM of PD-1 in T cells has emerged as a potential strategy to overcome PD-1–mediated immunosuppression in cancer and enhances antitumor immunity. The regulatory signaling pathways that induce PTM of PD-1 can be suppressed with small-molecule inhibitors, and mAbs can directly target PD-1 PTMs. Preliminary outcomes from exploratory studies suggest that focusing on the PTM of PD-1 has strong therapeutic potential and can enhance the response to anti-PD-1.

Introduction

The advent of immune checkpoint blockades has ushered in a new era of cancer immunotherapy (1). Extensive research has been devoted to studying PD-1 and PD-L1 signaling in the area of immune checkpoints over the past decades (2). The introduction of inhibitory antibodies targeting the PD-1/PD-L1 axis has garnered significant attention due to their remarkable and long-lasting efficacy. The FDA has approved five mAbs for targeting PD-1 in more than 20 cancer types, including retifanlimab (NCT03599713), which received approval in March 2023 (3, 4). These antibodies block immunosuppressive pathways, restoring T-cell functionality and anticancer immunity by interrupting the interaction of PD-1 with PD-L1 or PD-L2 (5). Significant clinical benefits are observed in a subset of patients with cancer like melanoma, in which objective response rate showed 40% and 52%, with the treatment of nivolumab and pembrolizumab, respectively (6, 7). However, the response rates are usually below 40% in most types of cancer, such as advanced non–small cell lung carcinoma (30%) (8), malignant mesothelioma (11%; ref. 9), and liver cancer (15.4; ref. 10), highlighting the need to develop optimal therapeutic strategies. Therefore, thoroughly comprehending the molecular interaction between immunosuppressive cells and T cells in the primary and metastatic tumor microenvironments (TME) is of great interest.

Immunosuppression can be instigated by factors originating from intracellular oncogenic signaling or TME (11, 12). In recent years, researchers have unveiled that PD-1 expression levels are stringently regulated via epigenetics, transcriptional and post-translational mechanisms (13–15). Nuclear factor of activated T cells (NFAT) 2 and activator protein-1 (AP-1) are common T-cell receptor (TCR) downstream transcription factors that can be triggered following T-cell activation (16). They bind to conserved region C (*CR-C)* and B (*CR-B)* of *Pdcd1*, respectively, representing an initial step in activating PD-1 expression. Furthermore, during chronic antigen stimulation, AKT and mTOR signaling pathways can be activated, thereby inducing transcriptional factor forkhead box protein O1 (FoxO1) to sustain PD-1 expression on CTL (17). Conversely, T-box expressed in T cells (T-bet) and inhibitory factor B lymphocyte-induced maturation protein-1 (BLIMP1), which are involved in terminal T-cell differentiation and functional memory formation, suppress *Pdcd1* gene expression in acute antigen stimulation (18). In addition to transcriptional regulation, epigenetic modifications are necessary for PD-1 expression as well. DNA methylation at the CpG sites in transcriptional enhancers or promoters leads to gene silencing (19). Two CpG sites located upstream of the *Pdcd1* transcription start site were hypermethylated in resting naïve $CD8^+$ T cells. However, the methylation status is lost 4 days after the viral infection and restored on day 8. Surprisingly, compared with acute stimulation, the CpG sites remain unmethylated at day 8 during chronic infection (20), suggesting that differential machinery is involved depending on the exposure conditions.

While *Pdcd1* regulation has been well documented, post-translational modifications (PTM) have emerged as crucial factors in understanding the interactions of PD-1 and PD-L1. Previous studies have unveiled numerous PTMs on PD-L1, playing essential roles in modulating stability and directly impacting T-cell functionality (21, 22). Among these PTMs, glycosylation of PD-L1 emerges as a critical determinant of its interaction with PD-1, having a great impact on T cell–mediated immune responses (23). Moreover, recent research has uncovered the significance of B3GNT3-mediated glycosylation of PD-L1 in the context of triple-negative breast cancer, underlining it as a promising target for glyco-specific antibody-based interventions (24). Furthermore, various modifications such as phosphorylation (25, 26), ubiquitination and deubiquitination (27, 28), and palmitoylation (29, 30) have been disclosed to contribute to the

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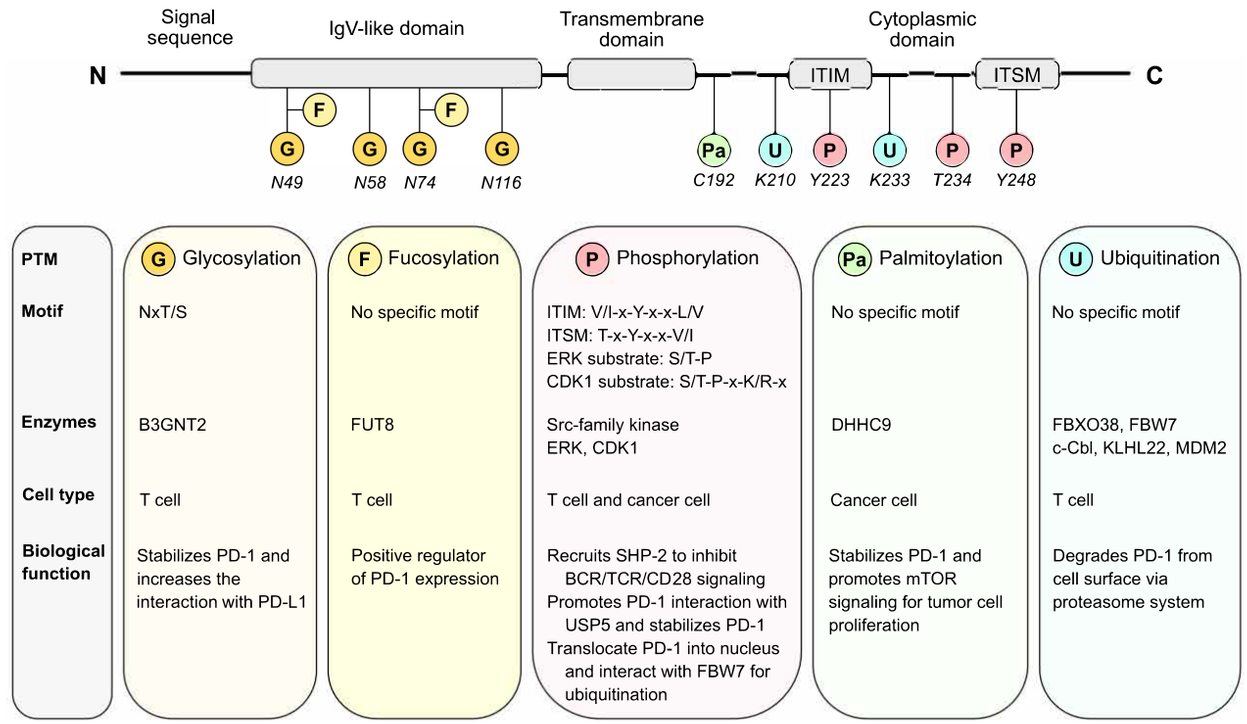
stability, subcellular localization, and overall functionality of molecules. These findings raise the possibility that PD-1 may undergo similar PTMs with potential implications for its stability and function. Indeed, recent findings have uncovered that PD-1 expression levels and activity are tightly regulated by protein glycosylation, phosphorylation, palmitoylation, and ubiquitination in T cells or cancer cells (**Fig. 1**). Given the immense promise of pharmacologically targeting PTMs in cancer therapy, an in-depth analysis of PTMs of PD-1 would provide novel insights to augment the current immune checkpoint blockade therapy. This review aims to shed light on recent discoveries regarding PD-1 PTMs and their potential impact on cancer immunotherapy.

The Biological Function of PD-1 on Different Cell Types

Immune cells

PD-1 is a well-characterized immune checkpoint receptor predominantly expressed on the surface of activated T cells (2). As a negative regulator, the engagement of PD-1 with PD-L1 or PD-L2 often transduces inhibitory signals during antigen-stimulated T-cell responses and facilitates peripheral immune tolerance (31). In $CD8^+$ T cells, the PD-1/PD-L1 axis dampens proliferation, cytokine production, and cytotoxicity capacity by antagonizing the downstream signaling pathways of TCR and CD28 (32, 33). In addition, PD-1 expression often remains constitutively high in conditions characterized by persistent antigen presentation, leading to the exhaustion of tumor-specific $CD8^+$ T cells (2). However, PD-1 signaling has also been identified as a priority T-cell activation marker for tumor-reactive $CD8^+$ T cells in melanomas (5), suggesting a dual role for PD-1 in both $CD8^+$ T cells activation and exhaustion. Regarding $CD4^+$ Th cells, PD-1 signaling leads to the loss of effector function and modulates the differentiation of regulatory T cells (Treg) by reducing STAT activation in Th1 cells through SHP1/2 (34). Notably, in human solid cancer, tumor-infiltrating $CD4^+$ T cells coexpressing PD-1 and inducible T-cell costimulator have demonstrated the capacity to recognize both tumor-associated and tumor-specific antigens, indicating the relevance of PD-1 serves as a necessary marker for intratumor $CD4^+$ T cells (35). In addition, PD-1 signaling plays a role in the localization of follicular Th cells to germinal centers (GC), thereby enhancing the stringency of GC affinity selection (36). In Treg, PD-1 induces proliferation and amplifies their immunosuppressive effect via stabilizing Foxp3 expression, while some studies have reported contrasting results of restraining Treg suppressive activity (33, 37). From various perspectives, additional evidence indicates that the expression of PD-1 on Treg can initiate interactions between Treg and PD-L1–expressed lymphatic endothelial cells, facilitating the migration of Treg to draining lymph nodes. The application of anti-PD-1 treatment targeting PD-1-expressing Treg, consequently restricting Treg within the tumor and promoting tumor regression (38). Furthermore, PD-1 was exhibited to be a potent mediator in Treg development because antigen-presenting cells depleted of PD-L1 impairs the production of Treg differentiated from $CD4^+$ T cells (39), highlighting the diverse functions of PD-1 in Treg.



PTM	G Glycosylation	F Fucosylation	P Phosphorylation	Pa Palmitoylation	U Ubiquitination
Motif	NxT/S	No specific motif	ITIM: V/I-x-Y-x-x-L/V ITSM: T-x-Y-x-x-V/I ERK substrate: S/T-P CDK1 substrate: S/T-P-x-K/R-x	No specific motif	No specific motif
Enzymes	B3GNT2	FUT8	Src-family kinase ERK, CDK1	DHHC9	FBXO38, FBW7 c-Cbl, KLHL22, MDM2
Cell type	T cell	T cell	T cell and cancer cell	Cancer cell	T cell
Biological function	Stabilizes PD-1 and increases the interaction with PD-L1	Positive regulator of PD-1 expression	Recruits SHP-2 to inhibit BCR/TCR/CD28 signaling Promotes PD-1 interaction with USP5 and stabilizes PD-1 Translocate PD-1 into nucleus and interact with FBW7 for ubiquitination	Stabilizes PD-1 and promotes mTOR signaling for tumor cell proliferation	Degrades PD-1 from cell surface via proteasome system

Figure 1.

Summary of PD-1 PTMs. Top, PD-1 domains with different sites of PTMs are illustrated. Bottom, the table summarizes the characterizations of five PTMs found on PD-1, including glycosylation, fucosylation, phosphorylation, palmitoylation, and ubiquitination. B3GNT2, β-1,3-N-acetylglucosaminyltransferase 2; c-Cbl, casitas B-lineage lymphoma; CDK1, cyclin-dependent kinase 1; DHHC9, aspartate-histidine-histidine-cysteine acyltransferases 9; FBW7, F-box and WD repeat domain containing 7; FBXO38, F-box only protein 38; FUT8, fucosyltransferase 8; ITIM, immunoreceptor tyrosine-based inhibitory motif; ITSM, immunoreceptor tyrosine-based switch motif; KLHL22, Kelch-like family member 22; MDM2, mouse double minute 2; SHP2, Src homology-2 domain-containing protein tyrosine phosphatase-2; USP5, ubiquitin specific peptidase 5.

Beyond its prominent role in T cells, PD-1 has been detected in various cell types across the adaptive and innate immune systems. Among B cells, PD-1 is expressed on specific subsets and has been shown to induce dephosphorylation of B-cell receptor signaling through SHP1/2, resulting in functional impairment (40). Moreover, elevated PD-1 expression in B cells can lead to T-cell dysfunction in the TME through an IL10-dependent mechanism (41). Within the innate lymphoid cells (ILC) population, abundant PD-1 expression level is found in the ILC2, ILC3, and lymphoid tissue inducer. PD-1 induced by TNFα and IL33 in the ILC2 population has been implicated in metabolic dysregulation in an obese mice model (42). In addition, PD-1 in ILC3 may alleviate the inflammatory microenvironment by impeding the antitumor function of this subset (43). In the context of nature killer (NK) cells, PD-1 expression is observed upon encountering certain cancer types and has been associated with the decreased cytotoxic activity of NK cells. Moreover, the PD-1 expression level is positively correlated with the dysfunction of tumor-infiltrating NK cells (44). Turning to myeloid cells, PD-1 significantly impacts tumor-associated macrophages (TAM) by arresting their phagocytotic ability and promoting a phenotype transition from M1 to M2, thereby exerting a protumor effect (45). Similarly, in dendritic cells (DC), PD-1 expression may hinder $CD8^+$ T cells functionality and antitumor immunity (46). Besides, PD-1 could also constrain DC effector functions, including cytokine secretion and antigen presentation, resulting in impaired T-cell priming (47). However, despite its renowned inhibitory role, PD-1 on DC may induce T-cell activation through *cis* ligation with PD-L1 (48). Furthermore, specific PD-1 ablation on myeloid cells has been found to reduce the population of GMPs and myeloid-derived suppressive cells (MDSC) and promote the differentiation of effector macrophages and DC, thus underscoring the suppressive role of PD-1 in antitumor immunity (49). In summary, PD-1 expression is a widespread mechanism that regulates immunity within the innate and adaptive immune systems. While its primary function is inhibitory, it can also play essential roles in immune activation, highlighting the multifaceted nature of PD-1 (Supplementary Fig. S1).

Cancer cells

PD-1 expression extends beyond immune cells and is also detectable in various cancer types, including melanoma, lung cancer, pancreatic cancer, head and neck cancer, breast cancer, and hepatocellular carcinoma (HCC; ref. 50). Interestingly, tumor-intrinsic PD-1 exhibits divergent functions, either promoting or suppressing tumor progression through the modulation of different signal transductions. In contrast to the functions in immune cells, tumor-intrinsic PD-1 promotes cancer progression by activating the mTOR signaling pathway (51–53). Mechanistically, intrinsic PD-1 in melanoma has been identified to modulate ribosomal protein S6 phosphorylation, resulting in the induction of tumor growth (52). Similarly, in HCC, PD-1 interacts with the downstream molecules of the mTOR pathway, eukaryotic initiation factor 4E and ribosomal protein S6, promoting their phosphorylation and tumor progression (51). In the context of pancreatic cancer, tumor cell-intrinsic PD-1 engages in an interaction with MOB1, leading to the reduction of MOB1 phosphorylation, which in turn triggers the activation of downstream genes associated with the Hippo pathway, including cysteine-rich angiogenic inducer 61 (CYR61) and connective tissue growth factor, thus promoting tumor proliferation in an immune-independent manner (54). On the other hand, intrinsic PD-1 acts as a tumor suppressor in lung cancer cell lines by prohibiting canonical pathways such as the AKT and ERK1/2 signaling (55). A similar condition has been observed in PD-1+ human colon cancer, where intrinsic PD-1 significantly quashes tumor proliferation and prompts apoptosis. Remarkably, treatment with anti-PD-1 rescues tumors from apoptosis and increases proliferation, conferring the protection of PD-1+ tumor cells from chemotherapy/radiotherapy (56). Conclusively, PD-1 is not only expressed in the immune system, but is found intrinsically in multiple cancer types (Supplementary Fig. S1). The dual effects of intrinsic PD-1 in tumor cells may have critical implications for cancer immunotherapy.

PTM

N-linked glycosylation

Glycosylation constitutes one of the most intricate PTM and plays a pivotal role in the modification of glycoproteins. In particular, glycosylation of membrane receptors assumes significance in mediating protein–protein interactions, such as ligand-receptor binding, and has been shown to impact anticancer immunity (57, 58). Moreover, aberrant glycosylation has been implicated in cancer cell progression and is associated with differential expressions of enzymes, including glycosyltransferase and glycosidases (59). Previous studies have elucidated extensive glycosylation of PD-1, specifically involving B3GNT2-mediated GlcNAc-β-1,3-Gal (LacNAc) linkages at asparagine 49 (N49), N58, N74, and N116 sites (60, 61). PD-1 purified from activated T cells exhibits heightened levels of poly-LacNAc (recognized by wheat germ agglutinin lectin) and core fucose (recognized by lens culinaris agglutinin lectin). Upon triggering TCR signaling, poly-LacNAc glycosylation is initiated on PD-1, leading to alterations in the intensity of PD-1–specific glycoforms. These findings suggest that, beyond transcriptional upregulation of PD-1 expression, T-cell activation also likely modulates PD-1 functions through glycosylation. Removal of PD-1 glycan lattices by PNGaseF diminishes the interaction between PD-1 and PD-L1 (24). The absence of these glycans leads to the accumulation of ubiquitination on PD-1, destabilizing its presence on the T-cell surface and reducing the binding affinity of PD-L1 (60). In addition, it was observed that N49 and N74 underwent fucosylation in a FUT8-dependent manner, and the core fucosylation could be interrupted by 2-fluoro-L-fucose on PD-1, leading to decreased expression on the surface of T cells. The attenuation of PD-1 levels via a fucosylation inhibitor strengthens T-cell activation, thereby enhancing the ability for tumor eradication. (61). These collective findings underscore the critical biological functions of N-linked glycans on PD-1 in T cell–mediated immunosuppression in TME.

Glycosylation of immune checkpoint receptors has emerged as a promising and intriguing anticancer strategy with implications for both basic science and translational research. Previous study has demonstrated that the N58 glycosylation site on the BC loop of PD-1 strongly interacts with cemiplimab (anti-PD-1), which is tightly associated with the binding and blocking efficacy of cemiplimab (62). This evidence underlines the critical importance of comprehending the role that PD-1 glycosylation plays in the development of cancer immunotherapies. Nevertheless, the complexity of glycosylation arises from the diversity of glycans and the myriad ways in which they are assembled within cells, posing inherent challenges for developing therapeutics targeting the heterogenous protein glycosylation. In 2014, a unique technology, STGlyTarget, was developed as an optimized platform for identifying and developing glycosylation-specific antibodies. Through this platform, a glyco-specific PD-1 antibody was successfully isolated (60). Enriched glycosylated PD-1 (gPD-1) on antigen-specific $CD8^+$ T cells provides unique glycan motifs that stabilize PD-1, reinforce PD-L1 engagement and dampen T-cell

activity. The mAb against gPD-1 specifically recognizes PD-1 on the tumor-infiltrating lymphocytes (TIL) but not on the circulating T cells, B cells, NK cells, and DCs. Of note, in humanized breast cancer mouse models, STM418-induced T-cell proliferation and activation surpass the effects of FDA-approved PD-1 antibodies such as pembrolizumab and nivolumab (60). However, previous studies have suggested that glycosylated PD-L1 might cause issues with IHC detection, potentially leading to false-negative results in cancer patient biopsies during anti-PD-1/PD-L1 treatment. Lee and colleagues demonstrated the improvement of biomarker detection by removing glycosylation from PD-L1, establishing a positive correlation with the response to anti-PD-1/PD-L1 therapy (63). On the basis of the premise that deglycosylated PD-L1 could serve as a predictive biomarker for monoclonal therapy, the notion of deglycosylated PD-1 as a potential strategy emerges. This concept is promising for becoming a valuable immunotherapy biomarker, offering a similar potential for clinical observation and patient stratification. Collectively, unraveling the intricate role of glycosylation in modulating immune responses will enhance our understanding of interplays in cancer immunotherapy interactions and pave the way for developing next-generation therapeutic interventions with improved efficacy.

Tyrosine/serine phosphorylation

Tyrosine phosphorylation and the subsequent signal transduction constitute critical regulatory processes governing the inhibitory function of PD-1. Specifically, tyrosine phosphorylation of PD-1 on the immunoreceptor tyrosine-based inhibitory motif (ITIM; Y223) and the immunoreceptor tyrosine-based switch motif (ITSM; Y248) by the Src-family kinases, such as Fyn and Lck, facilitates recruitment and activation of the phosphatase SHP-2 signaling (64). This downstream cascade exerts immunosuppressive effects through two distinct mechanisms. First, SHP-2 signaling directly impairs the activated signaling pathways in various immune cells. Several studies uncovered that PD-1 disrupts the activated signaling of TCR and costimulatory molecules (CD28), such as RAS/ERK, PI3K/AKT, and calcium-mediated signaling, thus curtailing the activation, proliferation, and cytokine production of activated T cells (65, 66). Intriguingly, a small population of PD-1+T-cell acute lymphoblastic leukemia stem cells has been identified to exploit the PD-1/SHP-2 signaling as a protective mechanism against acute activation-induced apoptosis by mitigating phosphorylation of ERK and AKT downstream of the TCR (67). In addition to T cells, the PD-1/SHP-2 signaling in myeloid cells could also hamper the activated GMCSF/homeobox A10 (HOXA10)/interferon regulatory factor 8 (IRF8) signaling, thereby modulating the differentiation of MDSCs and TAMs (68). This PD-1/SHP-2 signaling in myeloid cells has also been validated to have the ability to manipulate antitumor immunity in the TME. The second immunosuppressive mechanism of PD-1 phosphorylation involves SHP-2–mediated activation of the downstream transcription factors, such as the basic leucine zipper ATF-like transcription factor, which regulates other gene signatures. For example, PD-1 signaling can transcriptionally affect the metabolic reprogramming of T cells by downregulating the expressions of genes involved in glycolysis while upregulating those associated with the tricarboxylic acid cycle and fatty acid β-oxidation, impeding the metabolic fitness required for T effector cell differentiation (69). An additional phosphorylation site at serine 261 (S261) on PD-1 has been identified as an important regulator of PD-1 translocation into the nucleus in a CDK1-dependent manner. The translocation facilitates the interaction between PD-1 and the E3 ligase F-box and WD repeat domain-containing 7 (FBW7), which controls the stability and degradation of PD-1 (70).

Threonine phosphorylation

Besides the tyrosine phosphorylation, recent research has unveiled the previously unexplored realm of threonine phosphorylation in the regulatory network of PD-1. The 234-threonine (T234) phosphorylation of PD-1 by ERK emerged as a significant PTM, conferring a recognizable site for a deubiquitinase ubiquitin-specific peptidase 5 (USP5). Such engagement subsequently protects PD-1 from ubiquitination-mediated protein degradation, culminating in an increment of PD-1 stability (71). Consequently, the underlying mechanism of ERK-mediated phosphorylation on PD-1 appears to align seamlessly with clinical observations. A recent IHC analysis has substantiated the prognostic value of ERK1/2 phosphorylation as an indicator for the overall survival of patients with glioblastoma after anti-PD-1 treatment (72). While the precise mechanism governing ERK1/2 phosphorylation of PD-1 in cancer cells remains unclear, the improved prognostic outcomes observed in patients with elevated pERK levels undergoing anti-PD-1 treatment suggest a potential connection between ERK activation and the stabilization of PD-1 within cancer cells. This stabilization process may involve USP5-mediated mechanisms, likely raising the efficacy of anti-PD-1 therapies. This finding uncovers the importance of threonine phosphorylation on PD-1 but further deepens our understanding of the mechanism regulating PD-1 PTM with regard to therapeutic interventions.

Ubiquitination and deubiquitination

Ubiquitination is a crucial biochemical process in which ubiquitin molecules are conjugated to specific target proteins via the E3 ligase machinery (73). This PTM leads to the proteasomal degradation of the ubiquitinated protein substrate. In the case of PD-1, potential ubiquitination sites have been delineated at lysine 78 (K78), K210, and K233. Several E3 ligases, including murine double minute 2 (MDM2; ref. 74), Kelch-like family member 22 (KLHL22; ref. 75), F-Box protein 38 (FBXO38; ref. 76), F-box and WD repeat domain-containing 7 (FBW7; ref. 70), and Casitas B-lineage lymphoma (c-Cbl; ref. 77), mediate the ubiquitination of PD-1. Earlier investigations have elucidated that MDM2 facilitates the interaction between PD-1 and glycosidase N-glycanase 1 (NGLY1), which catalyzes the process of delycosylation of PD-1. MDM2 also serves as an essential E3 ligase, managing the deglycosylation of PD-1 and reducing its cell membrane expression levels (74). The other research has provided insights into the function of KLHL22, an adaptor of Cul3-based E3 ligase, that orchestrates PD-1 ubiquitination at K210 and K233 in T cells. The expression of *KLHL22* was subdued in TILs derived from patients with colorectal cancer, demonstrating that 5-fluorouracil raises PD-1 expression by suppressing *KLHL22* transcription (75) FBW7, an E3 ligase fostering K48-linked polyubiquitination at the K233 site of PD-1, is contingent upon phosphorylation at S261 by CDK1. This interaction induces PD-1 degradation and thus elicits better antitumor immunity *in vivo* (70). Similarly, FBXO38 is an E3 ligase that facilitates K48-linked polyubiquitination at K233 of PD-1. The diminished expression of *FBXO38* and *Fbxo38* was exhibited in both human and mouse TILs, resulting in the upregulation of PD-1. Mainly, the administration of IL2 restores *Fbxo38* expression, effectuating PD-1 downregulation within murine T cells (76). Finally, c-Cbl, a RING-domain containing E3 ligase, governs ubiquitination dynamics in tumor-associated T cells and macrophages, and enhanced PD-1 expression is revealed in c-Cbl knockout mice (77). Augmenting the expression or catalytic efficacy of these E3 ligases targeting PD-1 can potentially enhance T-cell activity against malignancies. For instance, oridonin upregulates the expression of FBW7, leading to PD-1 degradation in T cells. The concomitant

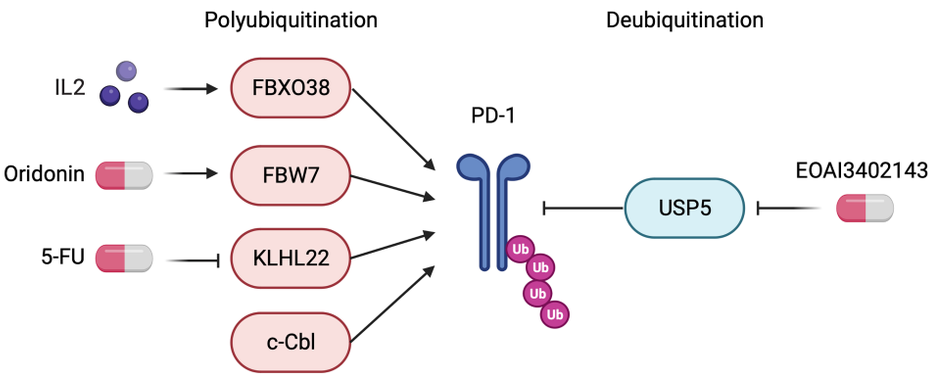


Figure 2.
Schematic of PD-1 polyubiquitination and deubiquitination. Regulation of PD-1 ubiquitination by E3 ligases, including FBXO38, FBW7, KLHL22, and c-Cbl. Destabilization of PD-1 by IL2 inducing FBXO38-mediated PD-1 ubiquitination (left), whereas EOA13402143 inhibits deubiquitinating enzyme USP5 to degrade PD-1 for immune activation (right). (Created with BioRender.com.)

treatment of oridonin and anti-PD-1 antibodies significantly suppresses tumor growth in immunocompetent mice (**Fig. 2**; ref. 70).

Conversely, deubiquitination serves as a regulatory mechanism by dismantling ubiquitin moieties from ubiquitinated proteins, restoring them to their native state, and exerting influence over protein stability, localization, and activity. A study revealed that phosphorylation of T234 on PD-1 by ERK enhances its interaction with USP5, a deubiquitinase that counteracts ubiquitination and stabilizes PD-1 expression in T cells (**Fig. 2**; ref. 71). Inhibition of USP5 activity results in decreased PD-1 expression. EOAI3402143, a small-molecule inhibitor targeting the enzymatic activity of USP5, disrupts its interaction with PD-1, ultimately promoting PD-1 degradation. The combination therapy of trametinib or anti-CTLA-4 with EOAI3402143 yields promising outcomes in restraining mouse tumor growth (71). Overall, manipulating the ubiquitination and deubiquitination processes on PD-1 presents viable strategies for modulating T-cell activity in cancer treatment.

Palmitoylation

Palmitoylation represents a significant PTM process involving the attachment of the fatty acid palmitate to cysteine residues of a protein. This PTM is pivotal in dictating various aspects of protein behavior, including protein subcellular localization, structural stability, intracellular trafficking, and intermolecular interactions (78). Recent investigative efforts have illuminated that PD-1 undergoes palmitoylation at cysteine 192 (C192) within a tumor cell line in a DHHC9-dependent manner, thereby endowing PD-1 with enhanced stability on the surface of tumor cells. In addition, Rab11, a crucial regulator of intracellular vesicular trafficking, was identified to interact with palmitoylated PD-1, facilitating the recycling of PD-1 to the cell surface and concomitantly activating the mTOR signaling pathway, contributing to tumor progression (79). To attenuate such signaling cascade regarding this phenomenon, the group developed a peptide to antagonize DHHC9 and thereby reduce the expression of PD-1 on the tumor cell surface (79). This novel molecular target may potentially pave a new avenue in the landscape of cancer therapy, further furnishing a foundation for developing combinational strategies in immunotherapy.

Therapeutic Strategies Targeting PTM of PD-1

Glycosylation has been an attractive target for developing new inhibitors or antibodies against cancer (80). Inhibition of glycosyltransferase emerges as a promising strategy for targeting glycosylation on PD-1, as deglycosylation may selectively impact coinhibitory but not coactivatory signals (24). To date, several N-linked glycosylation inhibitors, such as tunicamycin, swainsonine, castanospermine, and 2-deoxy-D-glucose (2DG), have been shown to reduce PD-1 glycosylation (24, 60). Regarding safety considerations, 2DG showed tolerable adverse effects in patients with advanced solid tumors during a phase I trial (81). While the clinical efficacy and safety profiles of other compounds require further confirmation, the notable decrease in PD-1 expression following treatment highlights the importance of investigating the antitumor responses induced by these compounds. In contrast, inhibitors targeting O-linked glycosylation, such as benzyl-GalNAc, do not exhibit any discernible effects on PD-1 expression patterns (24). In addition, inhibitors of oligosaccharyltransferase such as NGI-1 (STT3A inhibitor) can reduce PD-L1 or PD-1 glycosylation and thus increase sensitivity to immunotherapy. To reduce the off-target effects of glycosylation inhibitors, an antibody–drug conjugate has been developed to reduce specific protein glycosylation (82). Furthermore, as mentioned before, the glycospecific antibody for PD-1 is a novel strategy for targeting tumors because the TCR activation signaling alters the components of glycans, therefore binding more precisely to tumor-infiltrating T cells rather than circulating T cells (60). Altogether, targeting glycosylation on PD-1 may provide a new route to explore next-generation cancer drugs and immunotherapy.

Aside from glycosylation, a multitude of drugs targeting PD-1 phosphorylation and SHP-2 activity are currently in development and under clinical evaluation for their potential in treating autoimmune diseases and cancers (83). Encouraging preliminary safety and efficacy profiles have emerged from ongoing phase I/II clinical trials investigating the small-molecule allosteric SHP-2 inhibitors, such as RMC-4630 and TNO155, for various tumor types (Supplementary Table S1; refs. 83–85). In addition, multiple clinical trials for the combinational strategy of anti-PD-1 and SHP-2–specific inhibitors are actively progressing (Supplementary Table S2). The other PD-1 phosphorylation kinase, ERK, is also a potential target for combinational therapy. Since the ERK activation may sustain PD-1 on the surface via deubiquitinase USP5 (71), inhibition of ERK may have the insight to reduce immunosuppression caused by PD-1/PD-L1 axis and, in the meantime, introducing anti-PD-1 as the supporting therapeutic strategy (Supplementary Table S2).

Finally, the proteolysis-targeting chimeras (PROTAC), a bifunctional molecule that facilitates protein degradation by recruiting protein of interest to E3 ligases (86), may take advantage of ubiquitination on PD-1 to reduce the expression level in a proteasome-dependent manner. Studies have shown that PROTACs targeting PD-L1 degradation can enhance immunotherapy in human cancer. The PROTAC molecule 21a mediates the degradation of PD-L1

across various cancer cell lines, leading to improved antitumor activity in C57BL/6 mice inoculated with MC-38 cells (87). Furthermore, the utility of PROTACs extends to regulate PD-1 expression on T cells by recruiting PD-1 and associated E3 ligases, including FBXO38, FBW7, KLHL22, and c-Cbl, thereby facilitating PD-1 proteasomal degradation. This process may ultimately reduce PD-1 expression on T cells and enhance T-cell activity. In a recent study, peptide-based PROTACs target the PD-1/PD-L1 complex, engaging their E3 ligase for degradation (88). This observation highlighted the potential of PROTACS to augment the efficacy of PD-1/PD-L1 blockade in cancer immunotherapy synergistically. Altogether, PTMs are necessary for the biological function and stability of PD-1, thereby becoming one of the promising strategies in the clinical aspect.

Future Perspective

Evasion of immune surveillance stands as a fundamental hallmark of cancer malignancy. Despite the clinical achievement of anti-PD-1 therapy, there remains room for improving response rates. The forefront of innovative immunotherapy concentrates on marker-guided immunotherapy and synergistic approaches. Many combinatory strategies can be rationally formulated on the basis of the mechanisms underlying the post-translational regulation of PD-1. Given the significant post-translational regulation affecting PD-1 and PD-L1, future studies can focus on generating a cell-specific, common degradation strategy targeting PD-L1 in cancer cells and PD-1 in T cells, which may achieve a synergistic impact. With more and more PTM of PD-L1 and PD-1 being identified, comprehensively understanding the molecular mechanism of cancer immunosuppression could create a new era for next-generation immunotherapy.

Authors' Disclosures

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References

1. Pardoll DM. The blockade of immune checkpoints in cancer immunotherapy. Nat Rev Cancer 2012;12:252–64.
2. Sharpe AH, Pauken KE. The diverse functions of the PD1 inhibitory pathway. Nat Rev Immunol 2018;18:153–67.
3. Marin-Acevedo JA, Kimbrough EO, Lou Y. Next generation of immune checkpoint inhibitors and beyond. J Hematol Oncol 2021;14:45.
4. Mirza MR, Chase DM, Slomovitz BM, dePont Christensen R, Novak Z, Black D, et al. Dostarlimab for primary advanced or recurrent endometrial cancer. N Engl J Med 2023;388:2145–58.
5. Simon S, Labarriere N. PD-1 expression on tumor-specific T cells: friend or foe for immunotherapy? Oncoimmunology 2017;7:e1364828.
6. Hodi FS, Kluger H, Sznol M, Carvajal R, Lawrence D, Atkins M, et al. Durable, long-term survival in previously treated patients with advanced melanoma (MEL) who received nivolumab (NIVO) monotherapy in a phase I trial [abstract]. In: Proceedings of the 107th Annual Meeting of the American Association for Cancer Research; 2016 Apr 16–20; New Orleans, LA. Philadelphia (PA): AACR; Cancer Res 2016;76(14 Suppl):Abstract nr CT001.
7. Hamid O, Robert C, Daud A, Hodi F, Hwu W, Kefford R, et al. Five-year survival outcomes for patients with advanced melanoma treated with pembrolizumab in KEYNOTE-001. Ann Oncol 2019;30:582–8.
8. Gettinger SN, Shepherd FA, Antonia SJ, Brahmer JR, Chow LQM, Juergens RA, et al. First-line nivolumab (anti-PD-1; BMS-936558, ONO-4538) monotherapy in advanced NSCLC: safety, efficacy, and correlation of outcomes with PD-L1 status. J Clin Oncol 32: 15s, 2014 (suppl; asbtr 8024).
9. Fennell DA, Ewings S, Ottensmeier C, Califano R, Hanna GG, Hill K, et al. Nivolumab versus placebo in patients with relapsed malignant mesothelioma (CONFIRM): a multicentre, double-blind, randomised, phase 3 trial. Lancet Oncol 2021;22:1530–40.
10. Yau T, Park JW, Finn RS, Cheng AL, Mathurin P, Edeline J, et al. Nivolumab versus sorafenib in advanced hepatocellular carcinoma (CheckMate 459): a randomised, multicentre, open-label, phase 3 trial. Lancet Oncol 2022;23:77–90.
11. Setlai BP, Hull R, Bida M, Durandt C, Mulaudzi TV, Chatziioannou A, et al. Immunosuppressive signaling pathways as targeted cancer therapies. Biomedicines 2022;10:682.
12. Labani-Motlagh A, Ashja-Mahdavi M, Loskog A. The tumor microenvironment: a milieu hindering and obstructing antitumor immune responses. Front Immunol 2020;11:940.
13. Bally AP, Austin JW, Boss JM. Genetic and epigenetic regulation of PD-1 expression. J Immunol 2016;196:2431–7.
14. Dai X, Gao Y, Wei W. Post-translational regulations of PD-L1 and PD-1: mechanisms and opportunities for combined immunotherapy. Semin Cancer Biol 2022;85:246–52.
15. Zhou S, Zhu J, Xu J, Gu B, Zhao Q, Luo C, et al. Anti-tumour potential of PD-L1/PD-1 post-translational modifications. Immunology 2022;167:471–81.
16. Courtney AH, Lo WL, Weiss A. TCR signaling: mechanisms of initiation and propagation. Trends Biochem Sci 2018;43:108–23.
17. Staron MM, Gray SM, Marshall HD, Parish IA, Chen JH, Perry CJ, et al. The transcription factor FoxO1 sustains expression of the inhibitory receptor PD-1 and survival of antiviral CD8(+) T cells during chronic infection. Immunity 2014;41:802–14.
18. Fu SH, Yeh LT, Chu CC, Yen BL, Sytwu HK. New insights into Blimp-1 in T lymphocytes: a divergent regulator of cell destiny and effector function. J Biomed Sci 2017;24:49.
19. Moore LD, Le T, Fan G. DNA methylation and its basic function. Neuropsychopharmacology 2013;38:23–38.
20. Youngblood B, Oestreich KJ, Ha SJ, Duraiswamy J, Akondy RS, West EE, et al. Chronic virus infection enforces demethylation of the locus that encodes PD-1 in antigen-specific CD8(+) T cells. Immunity 2011;35:400–12.
21. Yu X, Li W, Young KH, Li Y. Posttranslational modifications in PD-L1 turnover and function: from cradle to grave. Biomedicines 2021;9:1702.
22. Hsu JM, Li CW, Lai YJ, Hung MC. Posttranslational modifications of PD-L1 and their applications in cancer therapy. Cancer Res 2018;78:6349–53.
23. Li CW, Lim SO, Xia W, Lee HH, Chan LC, Kuo CW, et al. Glycosylation and stabilization of programmed death ligand-1 suppresses T-cell activity. Nat Commun 2016;7:12632.
24. Li CW, Lim SO, Chung EM, Kim YS, Park AH, Yao J, et al. Eradication of triple-negative breast cancer cells by targeting glycosylated PD-L1. Cancer Cell 2018;33:187–201.
25. Zhao X, Wei Y, Chu YY, Li Y, Hsu JM, Jiang Z, et al. Phosphorylation and stabilization of PD-L1 by CK2 suppresses dendritic cell function. Cancer Res 2022;82:2185–95.
26. Chan LC, Li CW, Xia W, Hsu JM, Lee HH, Cha JH, et al. IL-6/JAK1 pathway drives PD-L1 Y112 phosphorylation to promote cancer immune evasion. J Clin Invest 2019;129:3324–38.
27. Lim SO, Li CW, Xia W, Cha JH, Chan LC, Wu Y, et al. Deubiquitination and stabilization of PD-L1 by CSN5. Cancer Cell 2016;30:925–39.
28. De S, Holvey-Bates EG, Mahen K, Willard B, Stark GR. The ubiquitin E3 ligase FBXO22 degrades PD-L1 and sensitizes cancer cells to DNA damage. Proc Natl Acad Sci U S A 2021;118:e2112674118.

29. Yao H, Lan J, Li C, Shi H, Brosseau JP, Wang H, et al. Inhibiting PD-L1 palmitoylation enhances T-cell immune responses against tumours. Nat Biomed Eng 2019;3:306–17.
30. Yang Y, Hsu JM, Sun L, Chan LC, Li CW, Hsu JL, et al. Palmitoylation stabilizes PD-L1 to promote breast tumor growth. Cell Res 2019;29:83–6.
31. Yao S, Chen L. PD-1 as an immune modulatory receptor. Cancer J 2014;20: 262–4.
32. Riley JL. PD-1 signaling in primary T cells. Immunol Rev 2009;229:114–25.
33. Kim MJ, Ha SJ. Differential role of PD-1 expressed by various immune and tumor cells in the tumor immune microenvironment: expression, function, therapeutic efficacy, and resistance to cancer immunotherapy. Front Cell Dev Biol 2021;9: 767466.
34. Cai J, Wang D, Zhang G, Guo X. The role Of PD-1/PD-L1 axis in Treg development and function: implications for cancer immunotherapy. Onco Targets Ther 2019;12:8437–45.
35. Duhen R, Fesneau O, Samson KA, Frye AK, Beymer M, Rajamanickam V, et al. PD-1 and ICOS coexpression identifies tumor-reactive CD4+ T cells in human solid tumors. J Clin Invest 2022;132:e156821.
36. Shi J, Hou S, Fang Q, Liu X, Liu X, Qi H. PD-1 controls follicular T helper cell positioning and function. Immunity 2018;49:264–74.
37. Qin W, Hu L, Zhang X, Jiang S, Li J, Zhang Z, et al. The diverse function of PD-1/ PD-L pathway beyond cancer. Front Immunol 2019;10:2298.
38. Piao W, Li L, Saxena V, Iyyathurai J, Lakhan R, Zhang Y, et al. PD-L1 signaling selectively regulates T cell lymphatic transendothelial migration. Nat Commun 2022;13:2176.
39. Gianchecchi E, Fierabracci A. Inhibitory receptors and pathways of lymphocytes: the role of PD-1 in treg development and their involvement in autoimmunity onset and cancer progression. Front Immunol 2018;9:2374.
40. Yokosuka T, Takamatsu M, Kobayashi-Imanishi W, Hashimoto-Tane A, Azuma M, Saito T. Programmed cell death 1 forms negative costimulatory microclusters that directly inhibit T cell receptor signaling by recruiting phosphatase SHP2. J Exp Med 2012;209:1201–17.
41. Ren Z, Peng H, Fu YX. PD-1 shapes B cells as evildoers in the tumor microenvironment. Cancer Discov 2016;6:477–8.
42. Oldenhove G, Boucquey E, Taquin A, Acolty V, Bonetti L, Ryffel B, et al. PD-1 is involved in the dysregulation of type 2 innate lymphoid cells in a murine model of obesity. Cell Rep 2018;25:2053–60.
43. Mallett G, Laurence A, Amarnath S. Programmed cell death-1 receptor (PD-1)-mediated regulation of innate lymphoid cells. Int J Mol Sci 2019;20:2836.
44. Quatrini L, Mariotti FR, Munari E, Tumino N, Vacca P, Moretta L. The immune checkpoint PD-1 in natural killer cells: expression, function and targeting in tumour immunotherapy. Cancers 2020;12:3285.
45. Gordon SR, Maute RL, Dulken BW, Hutter G, George BM, McCracken MN, et al. PD-1 expression by tumour-associated macrophages inhibits phagocytosis and tumour immunity. Nature 2017;545:495–9.
46. Lim TS, Chew V, Sieow JL, Goh S, Yeong JP, Soon AL, et al. PD-1 expression on dendritic cells suppresses CD8(+) T cell function and antitumor immunity. Oncoimmunology 2016;5:e1085146.
47. Laba S, Mallett G, Amarnath S. The depths of PD-1 function within the tumor microenvironment beyond CD8(+) T cells. Semin Cancer Biol 2022;86:1045–55.
48. Zhao Y, Harrison DL, Song Y, Ji J, Huang J, Hui E. Antigen-presenting cell-intrinsic PD-1 neutralizes PD-L1 in cis to attenuate PD-1 signaling in T cells. Cell Rep 2018;24:379–90.
49. Strauss L, Mahmoud MAA, Weaver JD, Tijaro-Ovalle NM, Christofides A, Wang Q, et al. Targeted deletion of PD-1 in myeloid cells induces antitumor immunity. Sci Immunol 2020;5:eaay1863.
50. Zheng H, Ning Y, Zhan Y, Liu S, Wen Q, Fan S. New insights into the important roles of tumor cell-intrinsic PD-1. Int J Biol Sci 2021;17:2537–47.
51. Li H, Li X, Liu S, Guo L, Zhang B, Zhang J, et al. Programmed cell death-1 (PD-1) checkpoint blockade in combination with a mammalian target of rapamycin inhibitor restrains hepatocellular carcinoma growth induced by hepatoma cell-intrinsic PD-1. Hepatology 2017;66:1920–33.
52. Kleffel S, Posch C, Barthel SR, Mueller H, Schlapbach C, Guenova E, et al. Melanoma cell-intrinsic PD-1 receptor functions promote tumor growth. Cell 2015;162:1242–56.
53. Yao H, Wang H, Li C, Fang JY, Xu J. Cancer cell-intrinsic PD-1 and implications in combinatorial immunotherapy. Front Immunol 2018;9:1774.
54. Pu N, Gao S, Yin H, Li JA, Wu W, Fang Y, et al. Cell-intrinsic PD-1 promotes proliferation in pancreatic cancer by targeting CYR61/CTGF via the Hippo pathway. Cancer Lett 2019;460:42–53.
55. Wang X, Yang X, Zhang C, Wang Y, Cheng T, Duan L, et al. Tumor cell-intrinsic PD-1 receptor is a tumor suppressor and mediates resistance to PD-1 blockade therapy. Proc Natl Acad Sci U S A 2020;117:6640–50.
56. Ierano C, Righelli D, D'Alterio C, Napolitano M, Portella L, Rea G, et al. In PD-1+ human colon cancer cells NIVOLUMAB promotes survival and could protect tumor cells from conventional therapies. J Immunother Cancer 2022; 10:e004032.
57. Sun R, Kim AMJ, Lim SO. Glycosylation of immune receptors in cancer. Cells 2021;10:1100.
58. Cadena AP, Cushman TR, Welsh JW. Glycosylation and antitumor immunity. Int Rev Cell Mol Biol 2019;343:111–27.
59. Kurosu M. Inhibition of N-Glycosylation towards novel anti-cancer chemotherapeutics. J Mol Pharm Org Process Res 2018;6:141.
60. Sun L, Li CW, Chung EM, Yang R, Kim YS, Park AH, et al. Targeting glycosylated PD-1 induces potent antitumor immunity. Cancer Res 2020;80:2298–310.
61. Okada M, Chikuma S, Kondo T, Hibino S, Machiyama H, Yokosuka T, et al. Blockage of core fucosylation reduces cell-surface expression of PD-1 and promotes anti-tumor immune responses of T cells. Cell Rep 2017;20:1017–28.
62. Lu D, Xu Z, Zhang D, Jiang M, Liu K, He J, et al. PD-1 N58-glycosylation-dependent binding of monoclonal antibody cemiplimab for immune checkpoint therapy. Front Immunol 2022;13:826045.
63. Lee HH, Wang YN, Xia W, Chen CH, Rau KM, Ye L, et al. Removal of N-linked glycosylation enhances PD-L1 detection and predicts anti-PD-1/PD-L1 therapeutic efficacy. Cancer Cell 2019;36:168–78.
64. Patsoukis N, Duke-Cohan JS, Chaudhri A, Aksoylar HI, Wang Q, Council A, et al. Interaction of SHP-2 SH2 domains with PD-1 ITSM induces PD-1 dimerization and SHP-2 activation. Commun Biol 2020;3:128.
65. Hui E, Cheung J, Zhu J, Su X, Taylor MJ, Wallweber HA, et al. T cell costimulatory receptor CD28 is a primary target for PD-1-mediated inhibition. Science 2017;355:1428–33.
66. Li K, Yuan Z, Lyu J, Ahn E, Davis SJ, Ahmed R, et al. PD-1 suppresses TCR-CD8 cooperativity during T-cell antigen recognition. Nat Commun 2021;12:2746.
67. Xu X, Zhang W, Xuan L, Yu Y, Zheng W, Tao F, et al. PD-1 signalling defines and protects leukaemic stem cells from T cell receptor-induced cell death in T cell acute lymphoblastic leukaemia. Nat Cell Biol 2023;25:170–82.
68. Christofides A, Katopodi XL, Cao C, Karagkouni D, Aliazis K, Yenyuwadee S, et al. SHP-2 and PD-1-SHP-2 signaling regulate myeloid cell differentiation and antitumor responses. Nat Immunol 2023;24:55–68.
69. Patsoukis N, Bardhan K, Chatterjee P, Sari D, Liu B, Bell LN, et al. PD-1 alters T-cell metabolic reprogramming by inhibiting glycolysis and promoting lipolysis and fatty acid oxidation. Nat Commun 2015;6:6692.
70. Liu J, Wei L, Hu N, Wang D, Ni J, Zhang S, et al. FBW7-mediated ubiquitination and destruction of PD-1 protein primes sensitivity to anti-PD-1 immunotherapy in non-small cell lung cancer. J Immunother Cancer 2022;10:e005116.
71. Xiao X, Shi J, He C, Bu X, Sun Y, Gao M, et al. ERK and USP5 govern PD-1 homeostasis via deubiquitination to modulate tumor immunotherapy. Nat Commun 2023;14:2859.
72. Arrieta VA, Chen AX, Kane JR, Kang SJ, Kassab C, Dmello C, et al. ERK1/2 phosphorylation predicts survival following anti-PD-1 immunotherapy in recurrent glioblastoma. Nat Cancer 2021;2:1372–86.
73. Swatek KN, Komander D. Ubiquitin modifications. Cell Res 2016;26:399–422.
74. Wu Z, Cao Z, Yao H, Yan X, Xu W, Zhang M, et al. Coupled deglycosylation-ubiquitination cascade in regulating PD-1 degradation by MDM2. Cell Rep 2023; 42:112693.
75. Zhou XA, Zhou J, Zhao L, Yu G, Zhan J, Shi C, et al. KLHL22 maintains PD-1 homeostasis and prevents excessive T cell suppression. Proc Natl Acad Sci U S A 2020;117:28239–50.
76. Meng X, Liu X, Guo X, Jiang S, Chen T, Hu Z, et al. FBXO38 mediates PD-1 ubiquitination and regulates anti-tumour immunity of T cells. Nature 2018;564: 130–5.
77. Lyle C, Richards S, Yasuda K, Napoleon MA, Walker J, Arinze N, et al. c-Cbl targets PD-1 in immune cells for proteasomal degradation and modulates colorectal tumor growth. Sci Rep 2019;9:20257.
78. Linder ME, Deschenes RJ. Palmitoylation: policing protein stability and traffic. Nat Rev Mol Cell Biol 2007;8:74–84.
79. Yao H, Li C, He F, Song T, Brosseau JP, Wang H, et al. A peptidic inhibitor for PD-1 palmitoylation targets its expression and functions. RSC Chem Biol 2021;2: 192–205.
80. Costa AF, Campos D, Reis CA, Gomes C. Targeting glycosylation: a new road for cancer drug discovery. Trends Cancer 2020;6:757–66.

81. Raez LE, Papadopoulos K, Ricart AD, Chiorean EG, Dipaola RS, Stein MN, et al. A phase I dose-escalation trial of 2-deoxy-D-glucose alone or combined with docetaxel in patients with advanced solid tumors. Cancer Chemother Pharmacol 2013;71:523–30.
82. Huang HC, Lai YJ, Liao CC, Yang WF, Huang KB, Lee IJ, et al. Targeting conserved N-glycosylation blocks SARS-CoV-2 variant infection *in vitro*. EBioMedicine 2021;74:103712.
83. Stanford SM, Bottini N. Targeting protein phosphatases in cancer immunotherapy and autoimmune disorders. Nat Rev Drug Discov 2023;22:273–94.
84. Brana I, Shapiro G, Johnson ML, Yu HA, Robbrecht D, Tan DS-W, et al. Initial results from a dose finding study of TNO155, a SHP2 inhibitor, in adults with advanced solid tumors. J Clin Oncol 39:15s, 2021 (suppl; abstr 3005).
85. LaMarche MJ, Acker M, Argintaru A, Bauer D, Boisclair J, Chan H, et al. Identification of TNO155, an Allosteric SHP2 inhibitor for the treatment of cancer. J Med Chem 2020;63:13578–94.
86. Gadd MS, Testa A, Lucas X, Chan KH, Chen W, Lamont DJ, et al. Structural basis of PROTAC cooperative recognition for selective protein degradation. Nat Chem Biol 2017;13:514–21.
87. Wang Y, Zhou Y, Cao S, Sun Y, Dong Z, Li C, et al. In vitro and in vivo degradation of programmed cell death ligand 1 (PD-L1) by a proteolysis targeting chimera (PROTAC). Bioorg Chem 2021;111:104833.
88. Cheng B, Ren Y, Cao H, Chen J. Discovery of novel resorcinol diphenyl ether-based PROTAC-like molecules as dual inhibitors and degraders of PD-L1. Eur J Med Chem 2020;199:112377.