

# Posttranslational Modifications of PD-L1 and Their Applications in Cancer Therapy

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## Abstract

Posttranslational modifications (PTM) of PD-L1 have emerged as important regulatory mechanisms that modulate immunosuppression in patients with cancer. In exposure to inflammatory cytokines, cancer cells and antigen-presenting cells, such as macrophages and dendritic cells, express PD-L1 to inhibit the activity of effector T cells through PD-1 engagement. Recent studies suggested that glycosylation, phosphorylation, ubiquitination, sumoylation, and acetylation play important roles in the regulation of PD-L1 protein stability and translocation and protein-protein interactions. Aberrant

alterations of PTMs directly influence PD-L1-mediated immune resistance. On the basis of the newly identified regulatory signaling pathways of PD-L1 PTMs, researchers have investigated the cancer therapeutic potential of natural food compounds, small-molecule inhibitors, and mAbs by targeting PD-L1 PTMs. Results of these preclinical studies demonstrated that targeting PTMs of PD-L1 yields promising antitumor effects and that clinical translation of these therapeutic strategies is warranted. *Cancer Res*; 78(22); 6349–53. ©2018 AACR.

## Introduction

The field of immunotherapy has dramatically reshaped the landscape of cancer therapy since the development of immune checkpoint blockade (1). The FDA has approved mAbs targeting immune checkpoint ligands and receptors such as PD-L1 and PD-1 for treatment of more than 25 types of cancer (2). Despite PD-1/PD-L1 blockade therapy having shown remarkable clinical benefit, response rates to date have rarely exceeded 40% across multiple cancer types (3, 4). Identifying the optimal therapeutic strategy that enhances the efficacy of immune checkpoint blockade has become a new challenge to resolve (5, 6). Therefore, understanding the molecular interaction between immunosuppressive cells and T cells in the primary and metastatic tumor microenvironments is of particular interest.

Immunosuppression is triggered by stimuli that emanate from intracellular oncogenic signaling or tumor microenvironments. Over the past several years, investigators have shown that a number of intracellular and extracellular factors, such as IFN $\gamma$ , TGF $\beta$ , MYC, and miR-200 modulate PD-L1 expression via transcriptional and posttranscriptional regulation (for detailed reviews, see refs. 7, 8). Only recently have researchers explored the posttranslational regulations of PD-L1 expression and activity and their effects on immunosuppression. Reported results suggest

that PD-L1-mediated immunosuppression is extensively regulated by protein glycosylation, phosphorylation, and ubiquitination (reviewed below; summarized in Fig. 1 and Table 1). Given that posttranslational modification (PTM) machineries are often therapeutic targets for pharmacologic inhibition of cancer (9), targeting PD-L1 PTMs may be a novel strategy for enhancing antitumor immune responses. This review focuses on recent discoveries regarding PD-L1 PTMs and their potential impact on cancer therapy.

## N-Linked Glycosylation of PD-L1

N-linked glycosylation is a biosynthetic secretory pathway in the endoplasmic reticulum (ER) and Golgi apparatus (10, 11). The N-linked glycosylation process is initiated in the ER by oligosaccharyltransferase, which transfers a 14-sugar core glycan from dolichol to an asparagine residue of an N-X-T/S motif (in which N is asparagine, X is any amino acid except proline, S is serine, and T is threonine) in newly synthesized nascent proteins that have entered the ER lumen (10, 11). The core glycan is then trimmed and further processed in the ER and Golgi apparatus before the glycosylated protein is translocated to the cell membrane (10, 11). Once glycosylation is dysregulated, the protein is transported to the cytosol and rapidly undergoes ER-associated degradation (12). A recent study showed that in the majority of cells in which it is expressed, PD-L1 is highly glycosylated with heterogeneous expression patterns on Western blots (13). Although the 33-kDa form of nonglycosylated PD-L1 can be detected, the majority of PD-L1 is glycosylated with a heterogeneous molecular weight ranging from 45 to 55 kDa on Western blots (13). Inhibitors of N-linked, but not O-linked, glycosylation altered the migratory shift of PD-L1 on SDS-PAGE, suggesting that PD-L1 is primarily N-glycosylated (14). Mass spectrometric analysis demonstrated that the asparagine residues of four N-X-T/S motifs in the PD-L1 extracellular domain—N35, N192, N200, and N219—are highly glycosylated (13). Substitution of these four asparagines with glutamine (4NQ mutant) abolished PD-L1 glycosylation and suppressed the migratory shift of PD-L1 (13).

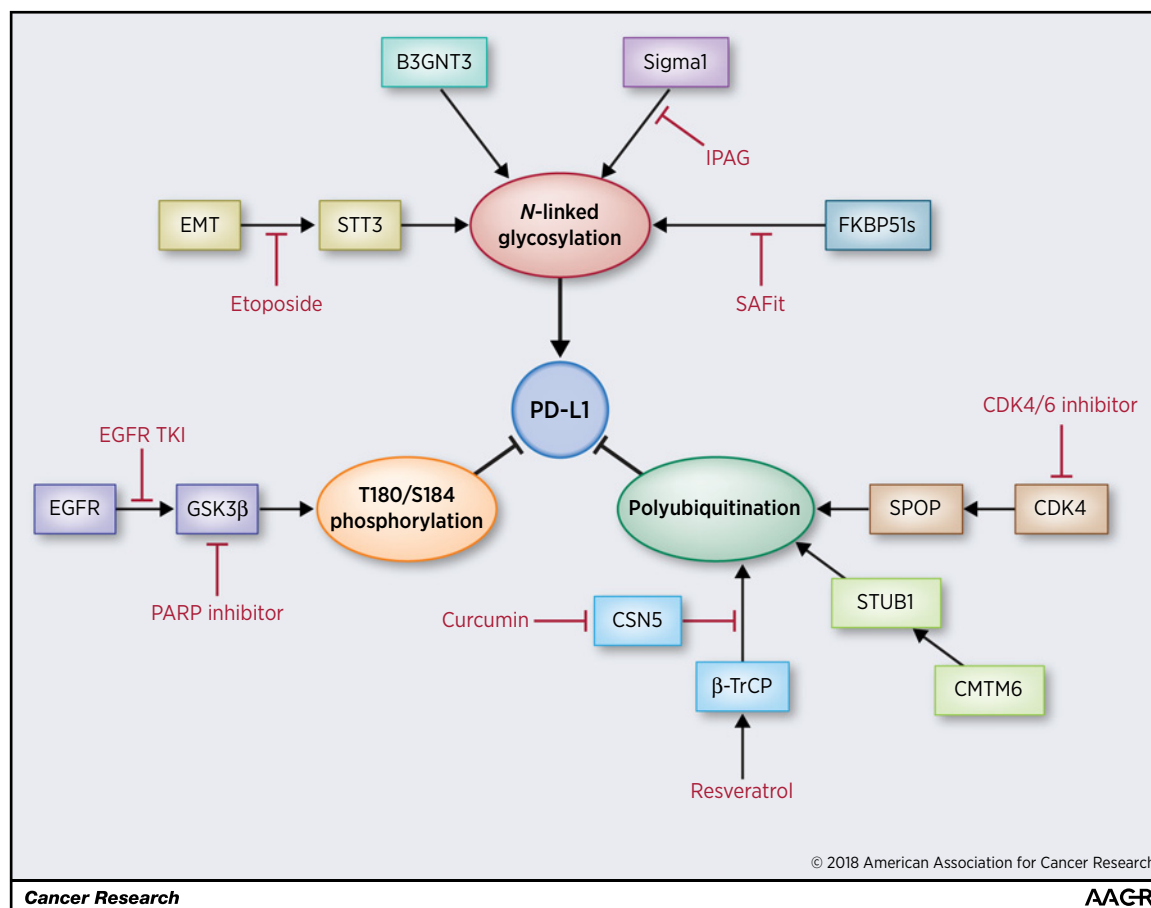
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**doi:** 10.1158/0008-5472.CAN-18-1892

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**Figure 1.**

Regulation of PD-L1 by PTMs. Overview of the molecular (black) and pharmacologic (red) regulations of PD-L1 *N*-linked glycosylation, serine/threonine phosphorylation, and polyubiquitination.

Functionally, *N*-linked glycosylation of PD-L1 is known to be important for PD-L1-mediated immunosuppression in two ways. First, PD-L1 protein is stabilized by its glycosylation. Li and colleagues (13) demonstrated that the protein half-life of glycosylated PD-L1 (gPD-L1) is at least 4-fold longer than that of nonglycosylated PD-L1 in cycloheximide chase assays, suggesting that glycosylation of PD-L1 enhances PD-L1's protein stability. Glycosylation of N192, N200, and N219 but not N35 contributes to PD-L1's protein stability (13). *N*-linked glycosylation of PD-L1 on cancer cells stabilized PD-L1 by preventing its degradation via the 26S proteasome and enhanced its engagement with PD-1 on CD8<sup>+</sup> T cells (13). Moreover, Maher and colleagues (15) and D'Arrigo and colleagues (16) reported that the PD-L1-associated chaperone Sigma1 and cochaperone FKBP51s upregulated PD-L1 expression by facilitating PD-L1 glycosylation and stabilization in the ER (15, 16). Pharmacologic inhibition of Sigma1 (15) or FKBP51s (16) activity reduced PD-L1 expression and activated T cells *in vitro* in prostate cancer, triple-negative breast cancer (TNBC), and glioblastoma models. In addition to its functional effects in the general cell population, Hsu and colleagues (17) recently revealed that PD-L1 glycosylation contributes to PD-L1 enrichment in cancer stem cells. Epithelial-mesenchymal transition-induced high expression of STT3, the catalytically active subunit of oligosaccharyltransferase, in cancer stem cells

increased PD-L1 glycosylation, leading to PD-L1 upregulation in cancer stem cells and cancer immune evasion. Antagonizing this process by etoposide treatment destabilized PD-L1 and sensitized cancer cells to anti-Tim3 therapy in syngeneic mouse models of TNBC and colon cancer (17). These findings suggested that glycosylation is a critical regulator of PD-L1 protein expression and a therapeutic target for PD-L1 blockade.

Second, glycosylation of PD-L1 regulates PD-L1/PD-1 interaction. Li and colleagues (14) found that 4T1 cells expressing wild-type PD-L1 grew much faster than did 4T1 cells expressing the PD-L1 4NQ mutant in immunocompetent BALB/c mice. However, these two cell lines grew similarly after inoculation into SCID mice, suggesting that glycosylation of PD-L1 is important for its immunosuppressive function in mice. Furthermore, mechanical studies of EGF/EGFR-stimulated PD-L1 glycosylation in TNBC revealed that  $\beta$ -1, 3-*N*-acetylglucosaminyltransferase 3 (B3GNT3)-mediated poly-*N*-acetylglucosamine (poly-LacNAc) is required for PD-L1/PD-1 interaction (14). Poly-LacNAc moieties were detected on the glycan structure of both N192 and N200 of PD-L1 (13). B3GNT3-mediated PD-L1 and PD-1 interaction can be blocked by inhibitors of *N*-linked, but not *O*-linked, glycosylation, suggesting that B3GNT3 mediates PD-L1 and PD-1 interaction through *N*-linked glycosylation instead of *O*-linked glycosylation (14). B3GNT3 is highly expressed in basal-like breast

**Table 1.** Targeting PTMs of PD-L1 enhances antitumor immune responses

PD-L1 PTM	Regulator	Therapy	Preclinical tumor model	Reference
N-linked glycosylation	B3GNT3	Anti-gPD-L1-MMAE	TNBC	(14)
	FKBP51s	SAFit	Glioblastoma	(16)
	Sigma1	IPAG	Prostate cancer, TNBC	(15)
	STT3	Etoposide + anti-Tim-3	Colon cancer, TNBC	(17)
Serine/threonine phosphorylation	GSK3 $\beta$	EGFR TKI + anti-PD-1	Colon cancer, TNBC	(13)
		PARP inhibitor + anti-PD-1	TNBC	(23)
Polyubiquitination	$\beta$ -TrCP	Resveratrol	TNBC	(13)
	CSN5	Curcumin + anti-CTLA4	TNBC, colon cancer, melanoma	(25)
	SPOP	CDK4/6 inhibitor + anti-PD-1	Colon cancer	(28)
	STUB1	CMTM6 knockout	Melanoma	(27)
Acetylation	n/a	EGFR TKI	Skin cancer	(22)
Tyrosine phosphorylation	n/a	EGFR TKI	Skin cancer	(22)
Monoubiquitination	n/a	EGFR TKI	Skin cancer	(22)

Abbreviation: n/a, not available.

tumors, and this high expression correlates with poor survival (14), implying that B3GNT3 is a new therapeutic target for TNBC. Indeed, 4T1 cells lacking B3GNT3 expression grew in SCID mice, but not in immunocompetent BALB/c mice. These results suggested that PD-L1 glycosylation is critical for PD-L1-mediated immunosuppression. Anti-gPD-L1 antibodies block PD-L1/PD-1 interaction, and one of these antibodies also induced PD-L1 internalization, resulting in PD-L1 degradation in lysosome, which allowed for the creation of a new antibody-drug conjugate of this anti-gPD-L1 antibody (anti-gPD-L1-MMAE) for the eradication of TNBC cells, even in the surrounding cancer cells with low or no PD-L1 expression (14). This suggests that direct targeting of PD-L1 glycosylation is an effective strategy for enhancing immune checkpoint therapy.

### Serine/Threonine Phosphorylation of PD-L1

Nonglycosylated PD-L1 is a labile protein with a half-life of about 4 hours. Li and colleagues (13) found that nonglycosylated PD-L1 is degraded by the ubiquitin/proteasome system after being phosphorylated by glycogen synthase kinase 3 $\beta$  (GSK3 $\beta$ ). They identified two consensus GSK3 $\beta$  phosphorylation motifs (S/TXXXS/T, in which S is serine, T is threonine, and X is any amino acid) at T180 and S184 of PD-L1's extracellular domain. The phosphorylation of PD-L1 by GSK3 $\beta$  results in the association of PD-L1 with the E3 ligase  $\beta$ -TrCP, leading to degradation of PD-L1 in the cytoplasm. The  $\beta$ -TrCP-binding DSG motif (in which D is aspartate, S is serine, and G is glycine) at S176 catalyzes K48 ubiquitination of PD-L1 and subsequent PD-L1 degradation by the 26S proteasome. Glycosylation of N192, N200, and N219 created a spatial hindrance and disrupted GSK3 $\beta$  and PD-L1 interaction, leading to PD-L1 protein stabilization.

Because EGFR, which is overexpressed in TNBC cells, activates AKT, resulting in GSK3 $\beta$  inhibition, EGFR activation stabilized PD-L1 protein (13). In addition, previous studies demonstrated that EGFR transcriptionally upregulated PD-L1 through multiple pathways (7), including PI3K-AKT-mTOR pathway (18), IL6/JAK/STAT3 pathway (19), and NF $\kappa$ B pathway (20). In line with these findings, Li and colleagues as well as other researchers showed that inhibition of EGF signaling by an EGFR tyrosine kinase inhibitor (TKI) downregulates PD-L1 (13, 21, 22) and enhances the therapeutic efficacy of PD-1 blockade in syngeneic mouse models of TNBC and colon cancer (13). On the other hand, Jiao and colleagues (23) showed that the PARP1 inhibitor

olaparib inactivates GSK3 $\beta$  and in turn upregulates PD-L1 expression in cancer cells, resulting in attenuation of antitumor immune responses. The combination treatment of PD-L1 blockade with olaparib resensitized olaparib-treated cancer cells to T-cell killing and provided better therapeutic efficacy than did single-agent therapy with PD-L1 blockade or olaparib in a syngeneic TNBC mouse model. The combination of PD-L1 blockade with PARP inhibitors is also supported by a hypothesis that the genomic instability induced by PARP inhibition could result in increased neoantigens, leading to activation of antitumor immune responses (24). Via GSK3 $\beta$  phosphorylation-mediated PD-L1 downregulation, investigators in these studies developed two potential mechanism-driven combinations of PD-1/PD-L1 blockade with targeted therapies.

### Polyubiquitination and Degradation of PD-L1

Accumulating evidence demonstrates that PD-L1 is extensively regulated by the ubiquitin/proteasome pathway (13, 25–28), suggesting that targeting of PD-L1 polyubiquitination is an alternative approach to enhancing immune checkpoint therapy. As discussed above,  $\beta$ -TrCP is the E3 ligase responsible for nonglycosylated PD-L1 polyubiquitination and destabilization. Induction of  $\beta$ -TrCP expression by treatment with resveratrol reduced PD-L1 protein expression in TNBC cells (13). In a subsequent study of chronic inflammation-induced cancer cell immune escape, Lim and colleagues (25) further demonstrated that the deubiquitinase CSN5-catalyzed proteolytic removal of polyubiquitination from PD-L1 is critical for tumor infiltrating macrophages to suppress antitumor immune responses. Macrophages secreted proinflammatory cytokine TNF $\alpha$  to activate NF $\kappa$ B and then induce CSN5 expression in cancer cells. Subsequently, CSN5 inhibited the ubiquitination and degradation of PD-L1, thereby enhancing PD-L1/PD-1 interaction to escape T-cell-immune surveillance (25). In this regard, inhibition of CSN5 activity by curcumin reduced chronic inflammation-mediated PD-L1-based immunosuppression and benefited anti-CTLA4 therapy in syngeneic mouse models of TNBC, melanoma, and colon cancer (25).

ER is not the only cellular compartment in which PD-L1 polyubiquitination occurs. Mezzadra and colleagues (26) and Burr and colleagues (27) independently identified CMTM6 as a PD-L1 regulator. Their studies collectively suggested that CMTM6 associated with PD-L1 at the plasma membrane and in recycling endosomes and regulated PD-L1 specifically located in these two

cellular compartments. CMTM6 prolonged the PD-L1 protein half-life by preventing PD-L1 from undergoing polyubiquitination by the E3 ubiquitin ligase STUB1 (26, 27). CMTM6 depletion downregulated PD-L1 expression and enhanced tumor-specific T-cell activity in a syngeneic melanoma mouse model. On the other hand, Zhang and colleagues (28) demonstrated that cullin 3-SPOP is an E3 ubiquitin ligase responsible for fluctuating PD-L1 expression during cell-cycle progression. Cyclin D–cyclin-dependent kinase 4 phosphorylated and stabilized SPOP, leading to polyubiquitination and downregulation of expression of PD-L1 protein in late G<sub>1</sub> and S phases. In syngeneic colon cancer mouse models, treatment with the cyclin-dependent kinase 4/6 inhibitor palbociclib increased PD-L1 protein expression and synergized with anti-PD-1 therapy to elicit enhanced therapeutic efficacy. Taken together, these results suggested that the regulatory mechanisms of PD-L1 polyubiquitination in different cellular compartments or cell-cycle phases can serve as therapeutic targets to improve immunotherapy efficacy in patients with cancer.

## Translating Combination Therapy to the Clinic

The goal of a mechanistic study of PD-L1 is to translate basic research into clinical application. On the basis of the mechanisms underlying the posttranslational regulations of PD-L1 expression and activity, researchers have rationally designed many combinatorial strategies targeting these regulations for cancer treatment and evaluated them in preclinical studies (Table 1). The studies described herein have yielded strong evidence for moving their basic research findings into clinical trials. Several proposed combinations for cancer treatment are being tested in clinical trials, including cyclin-dependent kinase 4/6 inhibitors plus PD-1/PD-L1 blockade therapies (NCT03294694, NCT03147287, NCT03386929, and NCT03292250), PARP inhibitors plus PD-1/PD-L1 blockade therapies (NCT02657889, NCT03307785, and NCT03308942), and EGFR inhibitors plus PD-1/PD-L1 blockade therapies (NCT03082534, NCT02764593, and NCT02039674). Investigators have tested other proposed combinations, such as curcumin plus anti-CTLA4 therapy (25) and etoposide plus anti-Tim-3 therapy (17), to determine their safety and efficacy in preclinical mouse tumor models, suggesting that the success of the combinatorial strategy may provide a scientific base for further analysis in the clinical setting. Thus far, the preclinical data were achieved under specific experimental conditions in certain types of disease models, that is, high EGFR-expressing TNBC mice can benefit from immunotherapy. In this regard, identifying the right patient background is critical for testing in human trials.

## Future Perspectives

In addition to *N*-linked glycosylation, serine/threonine phosphorylation, and polyubiquitination as discussed above, Horita and colleagues (22) reported that PD-L1 is subjected to acetylation, tyrosine phosphorylation, and monoubiquitination upon EGF stimulation. Furthermore, characterization of the functional impacts of these novel PTMs on PD-L1 may extend our understanding of the regulatory network behind PD-L1 expression and provide new approaches to improving immunotherapy efficacy. In addition to PD-L1, the vast majority of immune receptors are glycoproteins. However, researchers have made little progress in understanding the biological significance of glycosylation in immunology. In a systematic study of 22 immune ligand-receptor

pairs, *N*-linked glycosylation was required for coinhibitory ligand/receptor engagement, such as that for PD-L1/PD-1, PD-L1/B7.1, PD-L2/PD-1, and PVR/TIGIT (14). Of note, *N*-linked glycosylation is somewhat less critical for costimulatory ligand/receptor interaction. It remains unclear why glycosylation only seems to be critical for the coinhibitory signaling. It is likely that the glycosylation structure is preferential for engaging with coinhibitory receptors or glycosylation is favorable for activating inhibitory signaling, triggers the ITIM motifs association with phosphatase, and thereby deactivates T cell. However, this result lays the foundation for studying the regulatory effect of glycosylation on signaling of certain types of lymphocyte receptors. Future work should focus on developing preclinical models to unravel the regulatory process stepwise. In addition to immune checkpoint blockade, protein glycosylation is critical for antigen presentation, adaptive T-cell transfer, and cancer vaccine development. A lack of appropriate technology for analyzing protein glycosylation likely has limited our understanding of these bulky and extremely complex carbohydrate structures of proteins. Therefore, developing new technologies to advance investigation of glycosylation and open another avenue for deciphering the impact of glycosylation on cancer immunotherapy is of interest.

As PTM has emerged as an important aspect of PD-L1 regulation, there still are issues and challenges awaiting to be resolved. For example, unglycosylated PD-L1 undergoes fast protein degradation by GSK3 $\beta$  and  $\beta$ -TrCP machinery. However, it remains elusive that how ER-bound PD-L1 can be transported to the cytoplasm and then be degraded by the 26S proteasome. It is likely that ERAD machinery is critical to the PD-L1 stability. Future work may focus on how the PTM of PD-L1 interacts with cytoplasmic degradation machinery, that is, phosphorylation and glycosylation of PD-L1 modulate the ERAD pathway. Although membrane-bound PD-L1 associates with PD-1 for immunosuppression, PD-L1 also expresses in the ER, Golgi, nucleus, and cytoplasm (16, 29). How does PTM of PD-L1 influence its subcellular localization and thus contribute to the oncogenic function of the intracellular PD-L1? With more and more PTM of PD-L1 being identified, harnessing PD-L1 expression in compartment dependent manner may provide new insights for more therapeutic options in the future.

## Disclosure of Potential Conflicts of Interest

M.-C. Hung reports receiving a commercial research grant from STCube Pharmaceuticals, Inc., and has ownership interest (including stocks and patents) in STCube Pharmaceuticals. C.-W. Li has ownership interest (including stocks and patents) in STCube Pharmaceuticals. No potential conflicts of interest were disclosed by the other authors.

## Acknowledgments

We gratefully thank Don Norwood at the Department of Scientific Publications of MD Anderson Cancer Center for editing the manuscript. This work was supported in part by the NIH/NCI P30 CA016672, R01 CA211615, and U01 CA201777 (to M.-C. Hung); a Cancer Prevention & Research Institute of Texas Multi-investigator Research Award (RP160710 to M.-C. Hung); Breast Cancer Research Foundation (BCRF-17-069 to M.-C. Hung); the National Breast Cancer Foundation (to M.-C. Hung); the Patel Memorial Breast Cancer Endowment Fund (to M.-C. Hung); The University of Texas MD Anderson Cancer Center-China Medical University and Hospital Sister Institution Fund (to M.-C. Hung); and the Ministry of Health and Welfare, China Medical University Hospital Cancer Research Center of Excellence (MOHW107-TDU-B-212-114024 and MOHW107-TDU-B-212-112015).

Received June 18, 2018; revised August 1, 2018; accepted September 13, 2018; published first November 2, 2018.

## References

- Pardoll DM. The blockade of immune checkpoints in cancer immunotherapy. *Nat Rev Cancer* 2012;12:252–64.
- Iwai Y, Hamanishi J, Chamoto K, Honjo T. Cancer immunotherapies targeting the PD-1 signaling pathway. *J Biomed Sci* 2017;24:26.
- Yarchoan M, Hopkins A, Jaffee EM. Tumor mutational burden and response rate to PD-1 inhibition. *N Engl J Med* 2017;377:2500–1.
- Pitt JM, Vetizou M, Daillere R, Roberti MP, Yamazaki T, Routy B, et al. Resistance mechanisms to immune-checkpoint blockade in cancer: tumor-intrinsic and -extrinsic factors. *Immunity* 2016;44:1255–69.
- Alexander W. The checkpoint immunotherapy revolution: what started as a trickle has become a flood, despite some daunting adverse effects; new drugs, indications, and combinations continue to emerge. *P T* 2016;41:185–91.
- Li CW, Lim SO, Hsu JL, Hung MC. Rational combination of immunotherapy for triple negative breast cancer treatment. *Chin Clin Oncol* 2017;6:54.
- Sun C, Mezzadra R, Schumacher TN. Regulation and function of the PD-L1 checkpoint. *Immunity* 2018;48:434–52.
- Chen J, Jiang CC, Jin L, Zhang XD. Regulation of PD-L1: a novel role of pro-survival signalling in cancer. *Ann Oncol* 2016;27:409–16.
- Parekh RB, Rohlf C. Post-translational modification of proteins and the discovery of new medicine. *Curr Opin Biotechnol* 1997;8:718–23.
- Breitling J, Aebi M. N-linked protein glycosylation in the endoplasmic reticulum. *Cold Spring Harb Perspect Biol* 2013;5:a013359.
- Schwarz F, Aebi M. Mechanisms and principles of N-linked protein glycosylation. *Curr Opin Struct Biol* 2011;21:576–82.
- Xu C, Ng DT. Glycosylation-directed quality control of protein folding. *Nat Rev Mol Cell Biol* 2015;16:742–52.
- 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.
- 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.
- Maher CM, Thomas JD, Haas DA, Longen CG, Oyer HM, Tong JY, et al. Small-molecule sigma1 modulator induces autophagic degradation of PD-L1. *Mol Cancer Res* 2018;16:243–55.
- D'Arrigo P, Russo M, Rea A, Tufano M, Guadagno E, Del Basso De Caro ML, et al. A regulatory role for the co-chaperone FKBP51s in PD-L1 expression in glioma. *Oncotarget* 2017;8:68291–304.
- Hsu JM, Xia W, Hsu YH, Chan LC, Yu WH, Cha JH, et al. STT3-dependent PD-L1 accumulation on cancer stem cells promotes immune evasion. *Nat Commun* 2018;9:1908.
- Lastwika KJ, Wilson W 3rd, Li QK, Norris J, Xu H, Ghazarian SR, et al. Control of PD-L1 expression by oncogenic activation of the AKT-mTOR pathway in non-small cell lung cancer. *Cancer Res* 2016;76:227–38.
- Zhang N, Zeng Y, Du W, Zhu J, Shen D, Liu Z, et al. The EGFR pathway is involved in the regulation of PD-L1 expression via the IL-6/JAK/STAT3 signaling pathway in EGFR-mutated non-small cell lung cancer. *Int J Oncol* 2016;49:1360–8.
- Lin K, Cheng J, Yang T, Li Y, Zhu B. EGFR-TKI down-regulates PD-L1 in EGFR mutant NSCLC through inhibiting NF-kappaB. *Biochem Biophys Res Commun* 2015;463:95–101.
- Jiang XM, Xu YL, Huang MY, Zhang LL, Su MX, Chen X, et al. Osimertinib (AZD9291) decreases programmed death ligand-1 in EGFR-mutated non-small cell lung cancer cells. *Acta Pharmacol Sin* 2017;38:1512–20.
- Horita H, Law A, Hong S, Middleton K. Identifying regulatory post-translational modifications of PD-L1: a focus on monoubiquitination. *Neoplasia* 2017;19:346–53.
- Jiao S, Xia W, Yamaguchi H, Wei Y, Chen MK, Hsu JM, et al. PARP inhibitor upregulates PD-L1 expression and enhances cancer-associated immunosuppression. *Clin Cancer Res* 2017;23:3711–20.
- Mouw KW, Goldberg MS, Konstantinopoulos PA, D'Andrea AD. DNA damage and repair biomarkers of immunotherapy response. *Cancer Discov* 2017;7:675–93.
- 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.
- Mezzadra R, Sun C, Jae LT, Gomez-Eerland R, de Vries E, Wu W, et al. Identification of CMTM6 and CMTM4 as PD-L1 protein regulators. *Nature* 2017;549:106–10.
- Burr ML, Sparbier CE, Chan YC, Williamson JC, Woods K, Beavis PA, et al. CMTM6 maintains the expression of PD-L1 and regulates anti-tumour immunity. *Nature* 2017;549:101–5.
- Zhang J, Bu X, Wang H, Zhu Y, Geng Y, Nihira NT, et al. Cyclin D-CDK4 kinase destabilizes PD-L1 via cullin 3-SPOP to control cancer immune surveillance. *Nature* 2018;553:91–5.
- Satelli A, Batth IS, Brownlee Z, Rojas C, Meng QH, Kopetz S, et al. Potential role of nuclear PD-L1 expression in cell-surface vimentin positive circulating tumor cells as a prognostic marker in cancer patients. *Sci Rep* 2016;6:28910.