Cell Insight 3 (2024) 100146

Contents lists available at ScienceDirect

Cell Insight

journal homepage: www.journals.elsevier.com/cell-insight

Original Article

PD-1-mediated inhibition of T cell activation: Mechanisms and strategies for cancer combination immunotherapy

Rui Liu [a,b,c,d,e], Hui-Fang Li [a,b,c,d,e], Shu Li [a,b,c,d,e,*]

[a] Department of Infectious Diseases, Zhongnan Hospital of Wuhan University, Wuhan, 430071, China
[b] Frontier Science Center for Immunology and Metabolism, Wuhan, 430071, China
[c] Medical Research Institute, Wuhan, 430071, China
[d] Research Unit of Innate Immune and Inflammatory Diseases (2019RU063), Chinese Academy of Medical Sciences, Wuhan, 430071, China
[e] Wuhan University, Wuhan, 430071, China

A B S T R A C T

The programmed cell death 1 (PD-1) immune checkpoint of co-inhibitory signaling plays crucial roles in controlling the magnitude and duration of T cell activation to limit tissue damage and maintain self-tolerance. Cancer cells hijack the co-inhibitory pathway and escape immune surveillance by overexpressing the PD-1 ligand PD-L1. Immune checkpoint inhibitors, such as PD-1 blocking antibody have been approved for tumor immunotherapy. However, not all patients can benefit from PD-1 monotherapy. Combination immunotherapy based on PD-1 axis blockade substantially improves clinical anti-tumor efficacy. In this review, we briefly summarize the current progress on the mechanisms of PD-1-mediated inhibition of T cell activation and strategies for cancer combination immunotherapy.

1. Introduction

Cancer represents a substantial global health challenge and ranks among the most pressing health issues worldwide. Conventional therapies, including chemoradiotherapy, are not effective in removing tumors and limited by serious side effects and drug resistance (Rallis et al., 2021; Wang et al., 2017). It is necessary to develop new therapies to surmount the drawbacks. The immune system is crucial in immune surveillance, as immune cells infiltrate into tumor tissues and participate in regulating tumor progression (Boussiotis, 2016; Waldman et al., 2020). An effective immune response can either eliminate malignant cells or inhibit their malignant phenotype (Zhang & Zhang, 2020). However, a variety of mechanisms to evade immune surveillance have been evolved by tumor cells, such as up-regulation of negative regulatory pathways, defective antigen presentation, leading to blocked immune cell effector function and cancellation of anti-tumor immune response (Chen et al., 2018; Schreiber et al., 2011; Vinay et al., 2015).

Immunotherapy is a monumental breakthrough in cancer treatment, which aims to boost natural defenses to eliminate malignant cells (Waldman et al., 2020). In various types of immunotherapy, such as oncolytic viruses, cytokines, adoptive cell transfer and so on, immune checkpoint blockade (ICB) is at the forefront of clinical cancer immunotherapy (Andtbacka et al., 2015; Brahmer et al., 2012; Lin et al., 2022; Rosenberg et al., 1985a; Rosenberg et al., 1985b; Rosenberg & Restifo, 2015; Wang et al., 2022; Yi et al., 2022). James P. Allison and Tasuku Honjo were awarded the Nobel Prize in Physiology or Medicine in 2018 for the discovery of treating cancer by suppressing negative immune regulation (Ishida et al., 1992; Leach et al., 1996). Their research indicates that the immune checkpoints PD-1 and cytotoxic T-lymphocyte-associated protein 4 (CTLA-4) act as brakes in immune system, and immune checkpoint inhibition may significantly reactivate T cells and eliminate cancer cells (Freeman et al., 2000; Hodi et al., 2003; Kwon et al., 1997; Nishimura et al., 1999). Accumulating evidence suggests that targeting blockade of PD-1 axis enhances an effective immune response to against cancer cells and is associated with significant clinical responses in a wide range of cancer patients. (Boussiotis, 2016; Han et al., 2020). In this minireview, we outline the biological function of PD-1 axis, as well as the recent findings on PD-1 signaling antagonizing common cytokine receptor γ chain (γ_c) family cytokine-triggered immune activation, their associations with immunotherapy and potential clinical applications.

2. Overview of PD-1 signaling

PD-1 was first identified in 1992 as a putative mediator of apoptosis (Ishida et al., 1992). Subsequent evidence suggests that PD-1 is a key co-inhibitory receptor in the B7-CD28-CTLA4 family and plays a role in restraining immune system hyperactivation (Boussiotis, 2016). PD-L1 is the ligand for PD-1, which is expressed by APCs stimulated with

* Corresponding author. Department of Infectious Diseases, Zhongnan Hospital of Wuhan University, Wuhan, 430071, China.
E-mail address: shuli@whu.edu.cn (S. Li).

https://doi.org/10.1016/j.cellin.2024.100146
Received 30 November 2023; Received in revised form 12 February 2024; Accepted 20 February 2024
Available online xxxx

pro-inflammatory cytokines, such as interferon-γ (IFN-γ) and can also be constitutively expressed by non-hematopoietic tissues (Dong et al., 1999; Freeman et al., 2000; Strome et al., 2003). The PD-1 axis negatively regulates T cell activation and controls the magnitude and duration of T cell responses to limit tissue damage and maintain self-tolerance under physiological and pathological conditions (Boussiotis, 2016). T cells within peripheral tissues upregulate PD-1 at the mRNA level early after activation. Late after activation in peripheral tissues, PD-1 is further upregulated transcriptionally, leading to greater surface expression of PD-1, which binds to its ligands, thereby promoting T cell exhaustion at sites of infection or when confronted with neoplasms.

PD-1 is a type 1 transmembrane glycoprotein belonging to the immunoglobulin superfamily (Oestreich et al., 2008). There is a single N-terminal immunoglobulin variable region (IgV)-like domain, a transmembrane domain, and a cytoplasmic tail containing C-terminal immunoreceptor tyrosine-based switch motif (ITSM) sequence TEYATI and N-terminal immunoreceptor tyrosine-based inhibition motif (ITIM) sequence VDYGEL (Boussiotis, 2016). The inhibitory function of PD-1 signaling depends on the Y248 of ITSM but not the Y223 of ITIM (Patsoukis et al., 2020). Real-time evens of live-cell imaging during PD-1 ligation reveals that SHP-2 interacts with PD-1, and the ITSM of PD-1 serves as a docking site for SHP-2 (Chemnitz et al., 2004; Sheppard et al., 2004; Yokosuka et al., 2012). The recruitment of SHP-2 to PD-1 Y248 after PD-1 ligation is required for PD-1-mediated inhibitory function.

2.1. *Effects of PD-1 on TCR-mediated signaling*

The mechanism of PD-1 antagonizing TCR signaling has been heavily investigated. Upon PD-1 ligation, the ITSM of PD-1 is phosphorylated by the TCR proximal Src family kinase, subsequently recruiting and activating the allosteric dephosphorylating enzyme SHP-2 within the PD-1 complex (Patsoukis et al., 2020; Yokosuka et al., 2012). The activated SHP-2 mediates dephosphorylation of TCR proximal signaling molecules, including Lck and ZAP-70, leading to the inhibition of downstream events including the PI3K-AKT and Ras-MEK-ERK pathways (Parry et al., 2005; Patsoukis et al., 2012; Sheppard et al., 2004). It has been demonstrated that PD-1 signaling does not induce global signal inhibition, for example, the activation of p38 MAPK is not suppressed by PD-1 signaling (Patsoukis et al., 2012). PD-1 ligation induces expression of certain transcription factors including the AP-1 family member BATF. T cell proliferation and cytokine secretion are impaired when BATF expression is enhanced (Quigley et al., 2010). Unbalanced activation of signaling pathways alters T cell biochemical events, signal transduction, gene transcription, and epigenetic programs, resulting in inhibition of T cell function (Fig. 1). In addition, T cell functions have different sensitivities to PD-1 levels, which is consistent with differential effects of the PD-1 signaling pathway. High levels of PD-1 and ligation lead to the inhibition of MIP1β production, while lower levels are adequate for blocking calcium flux and the production of IFN-γ. Very low levels of PD-1 are sufficient to inhibit the expression of interleukin-2 (IL-2) and tumor necrosis factor-α (TNF-α), as well as to suppress T cell expansion (Wei et al., 2013).

2.2. *Effects of PD-1 on γ_c family cytokine-triggered signaling*

γ_c (also called CD132) is a component of the receptors for IL-2, IL-4, IL-7, IL-9, IL-15 and IL-21 (Leonard et al., 2019a; Rochman et al., 2009). γ_c is constitutively expressed on different immune cells. It has been reported that X-linked severe combined immunodeficiency is associated with mutation of *IL2RG* which encodes γ_c (Leonard et al., 2019a; Noguchi et al., 1993). Cytokines of the γ_c family are considered as pleiotropic regulators in both innate and adaptive immune responses. For example, they promote either survival or death of immune cells depending on the context, and modulate differentiation of precursor immune cells into more terminally differentiated cells (Overwijk et al., 2021; Sakaguchi et al., 2008). Some of the γ_c family cytokines, such as IL-2, IL-15 and IL-21, have been used as immune-enhancing agents due to their functions in regulating T, NK and another immune cell (Leonard et al., 2019b). γ_c family cytokine activates regulatory T cells and effector T cells through three signaling pathways (JAK-STAT, Ras–MEK–ERK pathway, and PI3K-Akt pathways) (Leonard et al., 2019b; Overwijk et al., 2021). Previous studies have shown that PD-1 mediates inhibition of PI3K-AKT pathway and Ras-MEK-ERK pathway (Parry et al., 2005; Patsoukis et al., 2012; Sheppard et al., 2004). PD-1 signal can also antagonize γ_c family cytokine-mediated immune activation by directly targeting γ_c (Liu et al., 2023).

The level of γ_c in $CD8^+$ T cells is negatively correlated with PD-1 activity. PD-1 ligation promotes lysosomal degradation of γ_c. MARCH5 is the E3 ubiquitin ligase of γ_c, which is induced by PD-L1 stimulation. MARCH5-deficiency up-regulates the level of γ_c and blocks PD-1 ligation-induced K27-linked polyubiquitination and lysosomal degradation of γ_c. Mechanistically, the transcription factor BATF induced by PD-L1 ligation binds to the promoter of MARCH5 gene, leading to its transcription. PD-L1-induced up-regulation of *MARCH5* mRNA and down-regulation of γ_c protein level is inhibited in BATF-deficient cells. These studies suggest that PD-1 ligation results in BATF-dependent transcriptional induction of

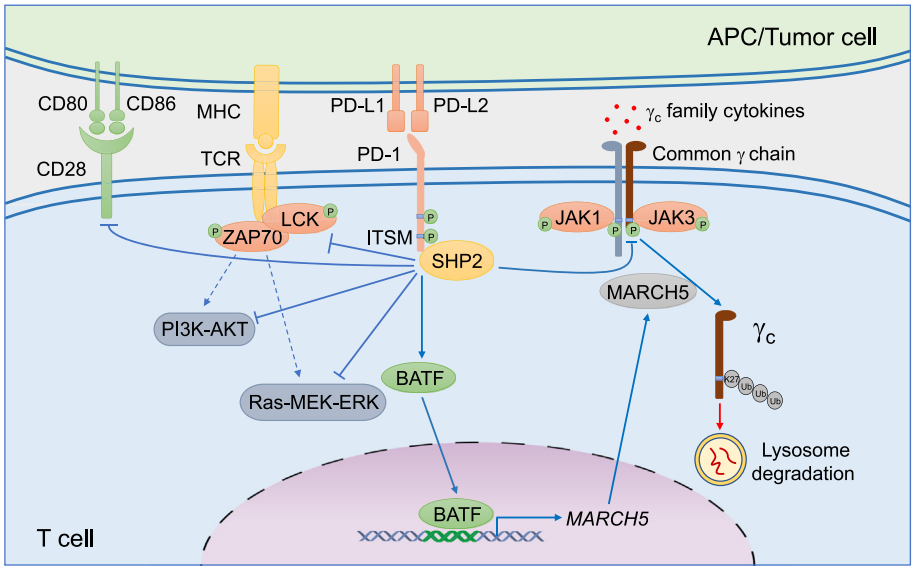

Fig. 1. Effects of PD-1 axis in T cells.
Upon PD-1 engagement, the ITSM of PD-1 is phosphorylated, subsequently recruiting and activating the SHP-2 within the PD-1 complex. The activated SHP-2 mediates dephosphorylation of TCR proximal signaling molecules, including Lck and ZAP-70, T cell costimulatory receptor CD28, and common cytokine receptor γ chain (γ_c). Activation of the PI3K-AKT and Ras-MEK-ERK pathways are inhibited. $CD28^-$and γ_c family cytokine-triggered immune activation is impaired. In contrast, the expression of transcription factor BATF is up-regulated, which subsequently induces transcription of the E3 ubiquitin ligase MARCH5. MARCH5 is recruited to γ_c and mediates its K27-linked polyubiquitination and lysosomal degradation. The imbalanced activation of signaling pathways alters cell-cycle progression, gene transcription and epigenetic programs in T cells, resulting in a net outcome of T cell quiescence and immune suppression.

the E3 ubiquitin ligase MARCH5, leading to K27-linked polyubiquitination and lysosomal degradation of γ_c (Liu et al., 2023).

PD-1-induced inhibition of γ_c-mediated signaling and immune activation is also regulated by SHP-2 (Liu et al., 2023). PD-1 ligation triggers clustering of PD-1 with dynamic TCR microclusters which is necessary for the recruitment of SHP-2 and accumulates at the signaling central supramolecular activation cluster (c-SMAC) (Boussiotis, 2016; Patsoukis et al., 2020; Xu et al., 2020). The activated SHP-2 dephosphorylates TCR proximal signaling molecules. After clustering, PD-1 is partially segregated from TCR, but constitutively interacts with SHP-2, suggesting that PD-1-associated SHP-2 has the potential to mediate dephosphorylation of non TCR-associated molecules (Hui et al., 2017; Xu et al., 2020; Yokosuka et al., 2012). Previous studies have demonstrated that T cell costimulatory receptor CD28 is a target for PD-1-mediated inhibition, which is not a TCR-associated component (Hui et al., 2017). A recent study has also shown that PD-1 ligation-triggered SHP-2 activation induces dephosphorylation of γ_c^{Y357}, resulting in desensitization of γ_c-mediated signaling and immune activation (Liu et al., 2023). SHP-2-deficency impairs PD-1 ligation-induced γ_c^{Y357} dephosphorylation and increases the γ_c family cytokine-induced phosphorylation of γ_c^{Y357}.

These results point to a model on the regulatory mechanisms of γ_c stability and activity by PD-1 signaling (Liu et al., 2023). PD-1 ligation promotes the interaction between SHP-2 and γ_c. The activated SHP-2 dephosphorylates γ_c at Y357, leading to its inactivation and unresponsiveness to γ_c family cytokines. On the other hand, PD-1 signaling triggers the transcription factor BATF, which subsequently induces transcription of the E3 ubiquitin ligase MARCH5. MARCH5 is recruited to γ_c and mediates its K27-linked polyubiquitination at K315 and lysosomal degradation (Fig. 1). PD-1-triggered SHP-2-mediated dephosphorylation of γ_c occurs in minutes, whereas PD-1-triggered induction of MARCH5 and degradation of γ_c is obvious one day after PD-1 ligation. Thus, it has been proposed that PD-1 signal inhibits γ_c family cytokine-triggered immune activation via the two mechanisms in a temporal manner (Liu et al., 2023).

3. PD-1/PD-L1 blockade in cancer

A key mechanism by which tumor cell restricts host immune responses is the upregulation of PD-L1 in tumor microenvironment (TME) and its interaction with PD-1 on tumor-infiltrating $CD8^+$ T cells (Chen et al., 2018; Waldman et al., 2020). PD-L1 can be induced through various mechanisms, such as activation of PI3K-AKT pathways and inflammatory cytokines, of which IFN-γ is the most effective (Akbay et al., 2013; Sun et al., 2018). These cytokines can induce PD-L1 expression not only on tumor cells, but also on dendritic cells (DCs) and macrophages in TME (Zou & Chen, 2008). Tumor-infiltrating T cells that recognize tumor antigens are the producers of IFN-γ, suggesting that the immune system-generated anti-tumor immune response also initiates the immunosuppressive pathway by inducing PD-L1 expression (Saibil & Ohashi, 2020). Since PD-1 is expressed on activated T cells, the inhibition function of PD-1 is exerted after T cells recognize tumor antigens and produce tumor-specific responses, resulting in selective inhibition of cytotoxic T cell function (Waldman et al., 2020). In such contexts, targeted blockade therapy of the PD-1 axis induces the rejuvenation and expansion of tumor-infiltrating $CD8^+$ T cells, thereby promoting effective tumor elimination (Rizvi et al., 2015).

Numerous preclinical efforts have investigated whether blocking the PD-1/PD-L1 pathway can be used for cancer therapy. Overexpression of PD-L1 in cancer cells inhibits the cytotoxic anti-tumor response of $CD8^+$ T cells, whereas tumor rejection is achieved in mice with PD-1 dysfunction (Hirano et al., 2005; Iwai et al., 2002). PD-1 blockade therapy inhibits the growth of myeloma cells transplanted from syngeneic animals, while overexpression of PD-L1 shows the opposite result (Iwai et al., 2002). PD-1 axis-blocking antibody therapy enhances the killing effect of $CD8^+$ T cells on tumor cells (Hirano et al., 2005; Iwai et al., 2002; Strome et al., 2003). In mouse B16 melanoma and CT26 colon carcinoma models, inhibition of the PD-1 axis not only enhances anti--tumor immunity, but also limits hematogenous spread of tumor metastases (Iwai et al., 2005). In addition to the function of the PD-1 axis in cancer therapy, multiple clinical studies have also performed a negative correlation between the expression of PD-1 axis proteins and tumor prognosis (Hamanishi et al., 2007; Thompson et al., 2007).

However, not all cancer patients benefit from PD-1 monotherapy. Clinically, the effective response rate of PD-1 blockade therapy for cancer is only 10%–20% (Sharma et al., 2023; Waldman et al., 2020). The primary reason for this limited response is the low tumor tissue immune scores observed in most patients (Bonaventura et al., 2019). Consequently, there is considerable interest in developing combination therapies aimed at enhancing the overall response rate of cancer patients and eliciting more complete and lasting responses. Immunotherapy, such as CTLA-4 blockade, 4-1BB/OX40 agonists, MITA agonists, and cytokines, which primarily function to boost immunogenicity by stimulating immune cells often induces the expression of PD-1 on T cells (Kennedy & Salama, 2020; Riley et al., 2019). Therefore, combination immunotherapy for cancer based on PD-1 axis blockade has exhibited considerable promise. Examples include dual immune checkpoint blockade, co-stimulatory molecule agonists combined with PD-1 blockade, MITA agonists combined with PD-1 blockade, and cytokines combined with PD-1 blockade (Bommareddy et al., 2018; Yap et al., 2021).

4. Cancer combination therapy with PD-1 blockade and IL-2

PD-1 blockade plus IL-2 is a promising combination, and several clinical trials are underway (Leonard et al., 2019b; Overwijk et al., 2021; Pol et al., 2020). Due to its ability to stimulate proliferation of cytotoxic T lymphocytes and NK cells, IL-2 was used as a cancer therapeutic agent shortly after its discovery (Boyman & Sprent, 2012; Overwijk et al., 2021). However, the therapeutic effects depend on high-dose of IL-2 in certain cancer patients, and systemic toxicity limits its widespread application. (Boyman & Sprent, 2012; Krieg et al., 2010; Marabondo & Kaufman, 2017). Compared with PD-1 blockade or IL-2 monotherapy, PD-1 blockade and IL-2 combination therapy significantly improve anti-tumor efficacy and have been widely used in immunotherapy for various cancers (Overwijk et al., 2021). The combined immunotherapy eliminates the inhibitory brake of PD-1 while providing a stimulatory signal for cytotoxic T lymphocytes by IL-2. Previous studies have indicated that the combination immunotherapy impacts the $CD8^+$ T cell exhaustion program, with bispecific PD1-IL2v and anti-PD-L1 showing potential in overcoming tumor immunity resistance by enhancing stem-like tumor-reactive $CD8^+$ T cells and reprogramming macrophages (Hashimoto et al., 2022; Tichet et al., 2023).

Recent studies have demonstrated that PD-1 signaling down-regulates the level of γ_c, which impairs the cytokines of γ_c family-induced signaling and $CD8^+$ T cell activation in the TME (Liu et al., 2023). PD-1 blockade eliminates the inhibition of γ_c, thereby restoring the responses of $CD8^+$ T cells to the γ_c family cytokines and leading to the synergistic effects of combination immunotherapy of PD-1 blockade and IL-2 (Fig. 2). The studies also show that MARCH5 is a potential target for cancer immunotherapy. MARCH5-deficiency suppresses tumor growth, and promotes the anti-tumor effects of IL-2 as well as its combination with PD-1 blocking antibody in mice (Liu et al., 2023).

Screens of a panel of Food and Drug Administration-approved drugs identifies Pitavastatin Calcium (PC) as an inhibitor of MARCH5 (Liu et al., 2023). PC is a unique lipophilic statin and potent inhibitor of HMG-CoA reductase (Sahebkar et al., 2021). Only PC but not other HMG-CoA reductase inhibitors treatment increases the level of γ_c in tumor-infiltrating lymphocytes and has a synergistic effect with IL-2 on tumor suppression, suggesting that PC promotes antitumor immunity via its inhibition of MARCH5 but not HMG-CoA reductase (Fig. 2). The studies also show that administration of MARCH5 inhibitor leads to improved efficacies of cancer immunotherapy by PD-1 blockade plus IL-2 (Liu et al., 2023).

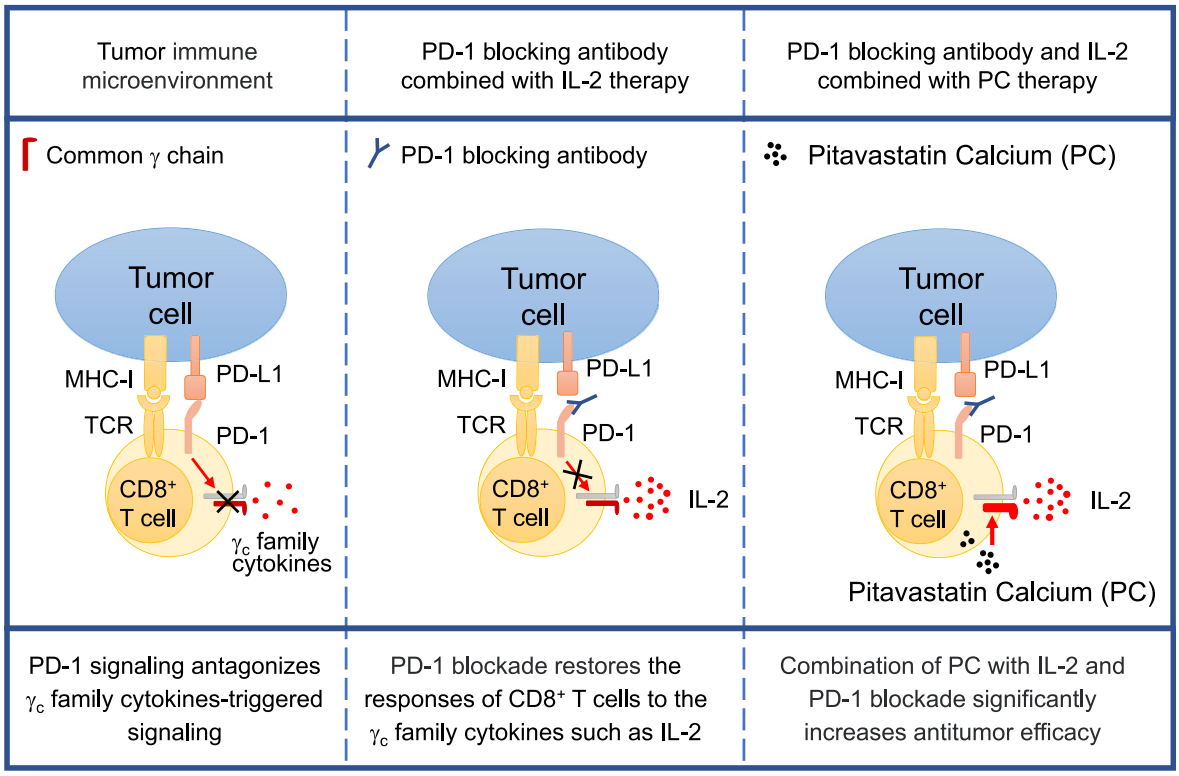

Fig. 2. Mechanisms for the synergistic effects of PD-1 blockade plus IL-2.
In TME, the high activity of PD-1signaling antagonizes γ_c family cytokines-triggered signaling and immune activation (left). PD-1 blockade eliminates the inhibition of γ_c, thereby restoring the responses of $CD8^+$ T cells to the γ_c family cytokines and leading to the synergistic effects of combination immunotherapy of PD-1 blockade and IL-2 (middle). Administration of Pitavastatin Calcium (PC) increases the level of γ_c and leads to improved efficacies of cancer immunotherapy by PD-1 blockade plus IL-2 (right).

5. Conclusion

In conclusion, PD-1 is a key co-inhibitory receptor that regulates the functions of T cells. It plays critical roles in maintaining self-tolerance under physiological and pathological conditions to being used by tumor cells for immune escape. Advancing knowledge of the PD-1 axis-induced signaling effect is helpful to reveal the molecular mechanism of PD-1 associated inhibition in diseases and elucidate the potential factors for the synergistic effects observed in PD-1 blockade-related combination therapy. A deeper understanding of such knowledge will also provide molecular targets and theoretical basis to inform the development of combination therapies targeting PD-1 and its downstream effectors. The future directions of combination immunotherapy should also be based on basic and mechanistic research, so as to develop more effective and less toxic treatment strategies.

Declaration of competing interest

The authors declare no competing interests.

Acknowledgements

This work was supported by grants from the State Key R&D Program of China (2022YFA1304900), the National Natural Science Foundation of China (32070775 and 32300758), the CAMS Innovation Fund for Medical Sciences (2019-I2M-5–071), the Major Scientific and Technological Project of Hubei Province (2022ACA005), the Fundamental Research Funds for the Central Universities (2042022dx0003), and the China Postdoctoral Science Foundation (2022TQ0236, 2023M732694).

References

Akbay, E. A., Koyama, S., Carretero, J., Altabef, A., Tchaicha, J. H., Christensen, C. L., Mikse, O. R., Cherniack, A. D., Beauchamp, E. M., Pugh, T. J., Wilkerson, M. D., Fecci, P. E., Butaney, M., Reibel, J. B., Soucheray, M., Cohoon, T. J., Janne, P. A., Meyerson, M., Hayes, D. N., Shapiro, G. I., et al. (2013). Activation of the PD-1 pathway contributes to immune escape in EGFR-driven lung tumors. *Cancer Discovery, 3*, 1355–1363.

Andtbacka, R. H., Kaufman, H. L., Collichio, F., Amatruda, T., Senzer, N., Chesney, J., Delman, K. A., Spitler, L. E., Puzanov, I., Agarwala, S. S., Milhem, M., Cranmer, L., Curti, B., Lewis, K., Ross, M., Guthrie, T., Linette, G. P., Daniels, G. A., Harrington, K., Middleton, M. R., et al. (2015). Talimogene laherparepvec improves durable response rate in patients with advanced melanoma. *Journal of Clinical Oncology, 33*, 2780–2788.

Bommareddy, P. K., Shettigar, M., & Kaufman, H. L. (2018). Integrating oncolytic viruses in combination cancer immunotherapy. *Nature Reviews Immunology, 18*, 498–513.

Bonaventura, P., Shekarian, T., Alcazer, V., Valladeau-Guilemond, J., Valsesia-Wittmann, S., Amigorena, S., Caux, C., & Depil, S. (2019). Cold tumors: A therapeutic challenge for immunotherapy. *Frontiers in Immunology, 10*, 168.

Boussiotis, V. A. (2016). Molecular and biochemical aspects of the PD-1 checkpoint pathway. *New England Journal of Medicine, 375*, 1767–1778.

Boyman, O., & Sprent, J. (2012). The role of interleukin-2 during homeostasis and activation of the immune system. *Nature Reviews Immunology, 12*, 180–190.

Brahmer, J. R., Tykodi, S. S., Chow, L. Q., Hwu, W. J., Topalian, S. L., Hwu, P., Drake, C. G., Camacho, L. H., Kauh, J., Odunsi, K., Pitot, H. C., Hamid, O., Bhatia, S., Martins, R., Eaton, K., Chen, S., Salay, T. M., Alaparthy, S., Grosso, J. F., Korman, A. J., et al. (2012). Safety and activity of anti-PD-L1 antibody in patients with advanced cancer. *New England Journal of Medicine, 366*, 2455–2465.

Chemnitz, J. M., Parry, R. V., Nichols, K. E., June, C. H., & Riley, J. L. (2004). SHP-1 and SHP-2 associate with immunoreceptor tyrosine-based switch motif of programmed death 1 upon primary human T cell stimulation, but only receptor ligation prevents T cell activation. *The Journal of Immunology, 173*, 945–954.

Chen, G., Huang, A. C., Zhang, W., Zhang, G., Wu, M., Xu, W., Yu, Z., Yang, J., Wang, B., Sun, H., Xia, H., Man, Q., Zhong, W., Antelo, L. F., Wu, B., Xiong, X., Liu, X., Guan, L., Li, T., Liu, S., et al. (2018). Exosomal PD-L1 contributes to immunosuppression and is associated with anti-PD-1 response. *Nature, 560*, 382–386.

Dong, H., Zhu, G., Tamada, K., & Chen, L. (1999). B7-H1, a third member of the B7 family, co-stimulates T-cell proliferation and interleukin-10 secretion. *Nature Medicine, 5*, 1365–1369.

Freeman, G. J., Long, A. J., Iwai, Y., Bourque, K., Chernova, T., Nishimura, H., Fitz, L. J., Malenkovich, N., Okazaki, T., Byrne, M. C., Horton, H. F., Fouser, L., Carter, L., Ling, V., Bowman, M. R., Carreno, B. M., Collins, M., Wood, C. R., & Honjo, T. (2000). Engagement of the PD-1 immunoinhibitory receptor by a novel B7 family member leads to negative regulation of lymphocyte activation. *Journal of Experimental Medicine, 192*, 1027–1034.

Hamanishi, J., Mandai, M., Iwasaki, M., Okazaki, T., Tanaka, Y., Yamaguchi, K., Higuchi, T., Yagi, H., Takakura, K., Minato, N., Honjo, T., & Fujii, S. (2007). Programmed cell death 1 ligand 1 and tumor-infiltrating CD8+ T lymphocytes are prognostic factors of human ovarian cancer. *Proceedings of the National Academy of Sciences of the U S A, 104*, 3360–3365.

Han, Y., Liu, D., & Li, L. (2020). PD-1/PD-L1 pathway: Current researches in cancer. *American Journal of Cancer Research, 10*, 727–742.

Hashimoto, M., Araki, K., Cardenas, M. A., Li, P., Jadhav, R. R., Kissick, H. T., Hudson, W. H., McGuire, D. J., Obeng, R. C., Wieland, A., Lee, J., McManus, D. T., Ross, J. L., Im, S. J., Lee, J., Lin, J. X., Hu, B., West, E. E., Scharer, C. D., Freeman, G. J., et al. (2022). PD-1 combination therapy with IL-2 modifies CD8(+) T cell exhaustion program. *Nature, 610*, 173–181.

Hirano, F., Kaneko, K., Tamura, H., Dong, H., Wang, S., Ichikawa, M., Rietz, C., Flies, D. B., Lau, J. S., Zhu, G., Tamada, K., & Chen, L. (2005). Blockade of B7-H1 and PD-1 by monoclonal antibodies potentiates cancer therapeutic immunity. *Cancer Research, 65*, 1089–1096.

Hodi, F. S., Mihm, M. C., Soiffer, R. J., Haluska, F. G., Butler, M., Seiden, M. V., Davis, T., Henry-Spires, R., MacRae, S., Willman, A., Padera, R., Jaklitsch, M. T., Shankar, S., Chen, T. C., Korman, A., Allison, J. P., & Dranoff, G. (2003). Biologic activity of cytotoxic T lymphocyte-associated antigen 4 antibody blockade in previously vaccinated metastatic melanoma and ovarian carcinoma patients. *Proceedings of the National Academy of Sciences of the U S A, 100*, 4712–4717.

Hui, E., Cheung, J., Zhu, J., Su, X., Taylor, M. J., Wallweber, H. A., Sasmal, D. K., Huang, J., Kim, J. M., Mellman, I., & Vale, R. D. (2017). T cell costimulatory receptor CD28 is a primary target for PD-1-mediated inhibition. *Science, 355*, 1428–1433.

Ishida, Y., Agata, Y., Shibahara, K., & Honjo, T. (1992). Induced expression of PD-1, a novel member of the immunoglobulin gene superfamily, upon programmed cell death. *The EMBO Journal, 11*, 3887–3895.

Iwai, Y., Ishida, M., Tanaka, Y., Okazaki, T., Honjo, T., & Minato, N. (2002). Involvement of PD-L1 on tumor cells in the escape from host immune system and tumor immunotherapy by PD-L1 blockade. *Proceedings of the National Academy of Sciences of the U S A, 99*, 12293–12297.

Iwai, Y., Terawaki, S., & Honjo, T. (2005). PD-1 blockade inhibits hematogenous spread of poorly immunogenic tumor cells by enhanced recruitment of effector T cells. *International Immunology, 17*, 133–144.

Kennedy, L. B., & Salama, A. K. S. (2020). A review of cancer immunotherapy toxicity. *CA: A Cancer Journal for Clinicians, 70*, 86–104.

Krieg, C., Letourneau, S., Pantaleo, G., & Boyman, O. (2010). Improved IL-2 immunotherapy by selective stimulation of IL-2 receptors on lymphocytes and endothelial cells. *Proceedings of the National Academy of Sciences of the U S A, 107*, 11906–11911.

Kwon, E. D., Hurwitz, A. A., Foster, B. A., Madias, C., Feldhaus, A. L., Greenberg, N. M., Burg, M. B., & Allison, J. P. (1997). Manipulation of T cell costimulatory and inhibitory signals for immunotherapy of prostate cancer. *Proceedings of the National Academy of Sciences of the U S A, 94*, 8099–8103.

Leach, D. R., Krummel, M. F., & Allison, J. P. (1996). Enhancement of antitumor immunity by CTLA-4 blockade. *Science, 271*, 1734–1736.

Leonard, W. J., Lin, J. X., & O'Shea, J. J. (2019a). The gamma(c) family of cytokines: Basic biology to therapeutic ramifications. *Immunity, 50*, 832–850.

Leonard, W. J., Lin, J. X., & O'Shea, J. J. (2019b). The gammac family of cytokines: Basic biology to therapeutic ramifications. *Immunity, 50*, 832–850.

Lin, X., Luo, M. L., & Song, E. (2022). Long non-coding RNA and non-coding nucleic acids: Signaling players in the networks of the tumor ecosystem. *Cell Insight, 1*, Article 100004.

Liu, R., Zeng, L.-W., Li, H.-F., Shi, J.-G., Zhong, B., Shu, H.-B., & Li, S. (2023). PD-1 signaling negatively regulates the common cytokine receptor γ chain via MARCH5-mediated ubiquitination and degradation to suppress anti-tumor immunity. *Cell Research, 33*, 923–939.

Marabondo, S., & Kaufman, H. L. (2017). High-dose interleukin-2 (IL-2) for the treatment of melanoma: Safety considerations and future directions. *Expert Opinion on Drug Safety, 16*, 1347–1357.

Nishimura, H., Nose, M., Hiai, H., Minato, N., & Honjo, T. (1999). Development of lupus-like autoimmune diseases by disruption of the PD-1 gene encoding an ITIM motif-carrying immunoreceptor. *Immunity, 11*, 141–151.

Noguchi, M., Yi, H., Rosenblatt, H. M., Filipovich, A. H., Adelstein, S., Modi, W. S., McBride, O. W., & Leonard, W. J. (1993). Interleukin-2 receptor gamma chain mutation results in X-linked severe combined immunodeficiency in humans. *Cell, 73*, 147–157.

Oestreich, K. J., Yoon, H., Ahmed, R., & Boss, J. M. (2008). NFATc1 regulates PD-1 expression upon T cell activation. *The Journal of Immunology, 181*, 4832–4839.

Overwijk, W. W., Tagliaferri, M. A., & Zalevsky, J. (2021). Engineering IL-2 to give new life to T cell immunotherapy. *Annual Review of Medicine, 72*, 281–311.

Parry, R. V., Chemnitz, J. M., Frauwirth, K. A., Lanfranco, A. R., Braunstein, I., Kobayashi, S. V., Linsley, P. S., Thompson, C. B., & Riley, J. L. (2005). CTLA-4 and PD-1 receptors inhibit T-cell activation by distinct mechanisms. *Molecular and Cellular Biology, 25*, 9543–9553.

Patsoukis, N., Brown, J., Petkova, V., Liu, F., Li, L., & Boussiotis, V. A. (2012). Selective effects of PD-1 on Akt and Ras pathways regulate molecular components of the cell cycle and inhibit T cell proliferation. *Science Signaling, 5*, ra46.

Patsoukis, N., Duke-Cohan, J. S., Chaudhri, A., Aksoylar, H. I., Wang, Q., Council, A., Berg, A., Freeman, G. J., & Boussiotis, V. A. (2020). Interaction of SHP-2 SH2 domains with PD-1 ITSM induces PD-1 dimerization and SHP-2 activation. *Communications Biology, 3*, 128.

Pol, J. G., Caudana, P., Paillet, J., Piaggio, E., & Kroemer, G. (2020). Effects of interleukin-2 in immunostimulation and immunosuppression. *Journal of Experimental Medicine, 217*.

Quigley, M., Pereyra, F., Nilsson, B., Porichis, F., Fonseca, C., Eichbaum, Q., Julg, B., Jesneck, J. L., Brosnahan, K., Imam, S., Russell, K., Toth, I., Piechocka-Trocha, A., Dolfi, D., Angelosanto, J., Crawford, A., Shin, H., Kwon, D. S., Zupkosky, J., Francisco, L., et al. (2010). Transcriptional analysis of HIV-specific CD8+ T cells shows that PD-1 inhibits T cell function by upregulating BATF. *Nature Medicine, 16*, 1147–1151.

Rallis, K. S., Lai Yau, T. H., & Sideris, M. (2021). Chemoradiotherapy in cancer treatment: Rationale and clinical applications. *Anticancer Research, 41*, 1–7.

Riley, R. S., June, C. H., Langer, R., & Mitchell, M. J. (2019). Delivery technologies for cancer immunotherapy. *Nature Reviews Drug Discovery, 18*, 175–196.

Rizvi, N. A., Hellmann, M. D., Snyder, A., Kvistborg, P., Makarov, V., Havel, J. J., Lee, W., Yuan, J., Wong, P., Ho, T. S., Miller, M. L., Rekhtman, N., Moreira, A. L., Ibrahim, F., Bruggeman, C., Gasmi, B., Zappasodi, R., Maeda, Y., Sander, C., Garon, E. B., et al. (2015). Cancer immunology. Mutational landscape determines sensitivity to PD-1 blockade in non-small cell lung cancer. *Science, 348*, 124–128.

Rochman, Y., Spolski, R., & Leonard, W. J. (2009). New insights into the regulation of T cells by gamma(c) family cytokines. *Nature Reviews Immunology, 9*, 480–490.

Rosenberg, S. A., Lotze, M. T., Muul, L. M., Leitman, S., Chang, A. E., Ettinghausen, S. E., Matory, Y. L., Skibber, J. M., Shiloni, E., Vetto, J. T., et al. (1985). Observations on the systemic administration of autologous lymphokine-activated killer cells and recombinant interleukin-2 to patients with metastatic cancer. *New England Journal of Medicine, 313*, 1485–1492.

Rosenberg, S. A., Mule, J. J., Spiess, P. J., Reichert, C. M., & Schwarz, S. L. (1985). Regression of established pulmonary metastases and subcutaneous tumor mediated by the systemic administration of high-dose recombinant interleukin 2. *Journal of Experimental Medicine, 161*, 1169–1188.

Rosenberg, S. A., & Restifo, N. P. (2015). Adoptive cell transfer as personalized immunotherapy for human cancer. *Science, 348*, 62–68.

Sahebkar, A., Kiaie, N., Gorabi, A. M., Mannarino, M. R., Bianconi, V., Jamialahmadi, T., Pirro, M., & Banach, M. (2021). A comprehensive review on the lipid and pleiotropic effects of pitavastatin. *Progress in Lipid Research, 84*, Article 101127.

Saibil, S. D., & Ohashi, P. S. (2020). Targeting T cell activation in immuno-oncology. *Current Oncology, 27*, S98–S105.

Sakaguchi, S., Yamaguchi, T., Nomura, T., & Ono, M. (2008). Regulatory T cells and immune tolerance. *Cell, 133*, 775–787.

Schreiber, R. D., Old, L. J., & Smyth, M. J. (2011). Cancer immunoediting: Integrating immunity's roles in cancer suppression and promotion. *Science, 331*, 1565–1570.

Sharma, P., Goswami, S., Raychaudhuri, D., Siddiqui, B. A., Singh, P., Nagarajan, A., Liu, J., Subudhi, S. K., Poon, C., Gant, K. L., Herbrich, S. M., Anandhan, S., Islam, S., Amit, M., Anandappa, G., & Allison, J. P. (2023). Immune checkpoint therapy-current perspectives and future directions. *Cell, 186*, 1652–1669.

Sheppard, K. A., Fitz, L. J., Lee, J. M., Benander, C., George, J. A., Wooters, J., Qiu, Y., Jussif, J. M., Carter, L. L., Wood, C. R., & Chaudhary, D. (2004). PD-1 inhibits T-cell receptor induced phosphorylation of the ZAP70/CD3zeta signalosome and downstream signaling to PKCtheta. *FEBS Letters, 574*, 37–41.

Strome, S. E., Dong, H., Tamura, H., Voss, S. G., Flies, D. B., Tamada, K., Salomao, D., Cheville, J., Hirano, F., Lin, W., Kasperbauer, J. L., Ballman, K. V., & Chen, L. (2003). B7-H1 blockade augments adoptive T-cell immunotherapy for squamous cell carcinoma. *Cancer Research, 63*, 6501–6505.

Sun, C., Mezzadra, R., & Schumacher, T. N. (2018). Regulation and function of the PD-L1 checkpoint. *Immunity, 48*, 434–452.

Thompson, R. H., Dong, H., Lohse, C. M., Leibovich, B. C., Blute, M. L., Cheville, J. C., & Kwon, E. D. (2007). PD-1 is expressed by tumor-infiltrating immune cells and is associated with poor outcome for patients with renal cell carcinoma. *Clinical Cancer Research, 13*, 1757–1761.

Tichet, M., Wullschleger, S., Chryplewicz, A., Fournier, N., Marcone, R., Kauzlaric, A., Homicsko, K., Deak, L. C., Umana, P., Klein, C., & Hanahan, D. (2023). Bispecific PD1-IL2v and anti-PD-L1 break tumor immunity resistance by enhancing stem-like tumor-reactive CD8(+) T cells and reprogramming macrophages. *Immunity, 56*, 162–179 e6.

Vinay, D. S., Ryan, E. P., Pawelec, G., Talib, W. H., Stagg, J., Elkord, E., Lichtor, T., Decker, W. K., Whelan, R. L., Kumara, H., Signori, E., Honoki, K., Georgakilas, A. G., Amin, A., Helferich, W. G., Boosani, C. S., Guha, G., Ciriolo, M. R., Chen, S., Mohammed, S. I., et al. (2015). Immune evasion in cancer: Mechanistic basis and therapeutic strategies. *Seminars in Cancer Biology, 35*(Suppl), S185–S198.

Waldman, A. D., Fritz, J. M., & Lenardo, M. J. (2020). A guide to cancer immunotherapy: from T cell basic science to clinical practice. *Nature Reviews Immunology, 20*, 651–668.

Wang, Z., Kim, J., Zhang, P., Galvan Achi, J. M., Jiang, Y., & Rong, L. (2022). Current therapy and development of therapeutic agents for lung cancer. *Cell Insight, 1*, Article 100015.

Wang, J., Seebacher, N., Shi, H., Kan, Q., & Duan, Z. (2017). Novel strategies to prevent the development of multidrug resistance (MDR) in cancer. *Oncotarget, 8*, 84559–84571.

Wei, F., Zhong, S., Ma, Z., Kong, H., Medvec, A., Ahmed, R., Freeman, G. J., Krogsgaard, M., & Riley, J. L. (2013). Strength of PD-1 signaling differentially affects

T-cell effector functions. *Proceedings of the National Academy of Sciences of the U S A, 110*, E2480–E2489.
Xu, X., Hou, B., Fulzele, A., Masubuchi, T., Zhao, Y., Wu, Z., Hu, Y., Jiang, Y., Ma, Y., Wang, H., Bennett, E. J., Fu, G., & Hui, E. (2020). PD-1 and BTLA regulate T cell signaling differentially and only partially through SHP1 and SHP2. *The Journal of Cell Biology, 219*.
Yap, T. A., Parkes, E. E., Peng, W., Moyers, J. T., Curran, M. A., & Tawbi, H. A. (2021). Development of immunotherapy combination strategies in cancer. *Cancer Discovery, 11*, 1368–1397.
Yi, X. M., Lian, H., & Li, S. (2022). Signaling and functions of interleukin-33 in immune regulation and diseases. *Cell Insight, 1*, Article 100042.
Yokosuka, T., Takamatsu, M., Kobayashi-Imanishi, W., Hashimoto-Tane, A., Azuma, M., & Saito, T. (2012). Programmed cell death 1 forms negative costimulatory microclusters that directly inhibit T cell receptor signaling by recruiting phosphatase SHP2. *Journal of Experimental Medicine, 209*, 1201–1217.
Zhang, Y., & Zhang, Z. (2020). The history and advances in cancer immunotherapy: Understanding the characteristics of tumor-infiltrating immune cells and their therapeutic implications. *Cellular and Molecular Immunology, 17*, 807–821.
Zou, W., & Chen, L. (2008). Inhibitory B7-family molecules in the tumour microenvironment. *Nature Reviews Immunology, 8*, 467–477.