

TPM-1: A Novel Biomaterial for Cancer Immunotherapy Targeting PD-L1

Zixiang Ma¹,

Yichen Zhang^{2*}

1. Department of Life Sciences
Northeast Forestry University
Harbin, China

3061147397@qq.com

2. Department of Clinical Medicine
Wanan Medical College
Wuhu, China

* Corresponding author:

20109010193@stu.wnmc.edu.cn

Abstract:

Immune checkpoint blockade therapy is highly effective for cancer treatment; however, its low response rate limits its clinical application. TPM-1, a novel biomaterial that targets PD-L1, presents a new approach to address this issue. This paper discusses the synthesis, mechanism of action, and drug properties of TPM-1. TPM-1 is a peptide-based self-assembling nanoparticle (TPMI) designed to specifically bind to PD-L1 on the surface of tumor cells, forming a fibrous network in situ. This process promotes the aggregation of PD-L1 and blocks the PD-1/PD-L1 pathway. Compared to traditional inhibitors, TPM-1 offers enhanced targeting and stability, which can help overcome drug resistance and facilitate multi-mechanism synergistic therapy. TPM-1 significantly improves PD-L1 blocking efficiency due to its unique design, demonstrating excellent anti-tumor effects in both in vitro and in vivo experiments. Its self-assembly properties enhance drug retention at tumor sites while minimizing systemic toxicity, providing a promising new strategy for cancer immunotherapy. In the future, the delivery system for TPM-1 can be further optimized, and its potential for combination with other therapies can be explored to promote clinical translation.

Keywords:-Cancer immunotherapy; PD-L1/PD-1 pathway; Self-assembled nanoparticle; Immune checkpoint blockade; Tumor microenvironment component

I. Introduction

Cancer represents a major global health challenge due to its high morbidity and mortality. A critical molecular mechanism underlying tumor immune evasion involves the PD-L1/PD-1 immune checkpoint axis. When PD-L1 expressed on tumor cells engages with PD-1 receptors on CAR-T cells, it initiates an immunosuppressive cascade through re-

cruitment of the protein tyrosine phosphatase SHP-2. This interaction leads to dephosphorylation of key signaling molecules in both the TCR complex and CD28 costimulatory pathway, effectively suppressing downstream PI3K-AKT-mTOR signaling transduction. Such inhibition results in impaired CAR-T cell activation, reduced proliferative capacity, and diminished production of effector cytokines including interferon-gamma (IFN- γ) and interleukin-2

(IL-2), ultimately promoting T cell anergy or apoptosis. Moreover, the inflammatory tumor microenvironment, particularly through IFN- γ secretion, upregulates PD-L1 expression on malignant cells via JAK-STAT signaling, creating a positive feedback loop that reinforces immune suppression and facilitates tumor immune escape [1]. Traditional cancer treatments, including surgery, chemotherapy, and radiotherapy, have various limitations. However, the advent of immune checkpoint blockade (ICB) therapy has introduced new hope for cancer treatment. Among these approaches, immune checkpoint inhibitors (ICIs) targeting PD-1 and PD-L1 have emerged as crucial treatment options for many advanced cancers [2]. Despite their promise, ICIs encounter challenges such as low response rates, toxic side effects, drug resistance, and high costs in clinical applications. For instance, the response rate for anti-PD-1 and anti-PD-L1 monoclonal antibodies can be as low as 25% or less, which constrains their widespread use. Currently, all available PD-L1 immunohistochemical (IHC) assays have been developed based on formalin-fixed tissue specimens. However, obtaining tissue biopsies from patients with advanced cancer is extremely challenging and even unfeasible in clinical practice [3].

In the search for more effective treatment strategies, synthetic peptide drugs have emerged as a promising area of interest. These drugs offer unique advantages, including low production costs and the ease of chemical synthesis. For instance, the GLP-1 peptide has received FDA approval for the treatment of type II diabetes and has demonstrated positive outcomes in clinical applications. Research has indicated that peptides can be modified to enhance their effectiveness, with methods such as glycosylation or PEG conjugation proving to be beneficial. Additionally, small-molecule peptides possess good tissue penetration capabilities, allowing them to reach deeper into tumor tissues [4]. Furthermore, by combining a synthetic targeting sequence with a self-assembled peptide, it is possible to achieve receptor-specific triggering for in situ self-assembly. An example of this is RGD binding to $\alpha_5\beta_1$ to form integrin $\alpha_5\beta_1$, a strategy that enhances the synthesis and efficacy of synthetic peptide drugs [5].

While there are traditional and synthetic drugs targeting PD-L1, these treatments have certain limitations. To address these issues, researchers are exploring new technologies and methods. A novel therapeutic approach involves the development of nanoparticle-transformable peptide monomer 1 (TPM1), engineered using an in situ self-assembly strategy. This synthetic peptide-based therapeutic agent specifically targets PD-L1 on tumor cell membranes, where it undergoes structural transformation into an insoluble fibrillar network. This localized fibrillization effectively sequesters PD-L1, disrupting the PD-1/PD-L1

immune checkpoint interaction and thereby potentiating T cell-mediated antitumor immunity. By physically blocking immune inhibitory signaling, TPM1 enhances the efficacy of cancer immunotherapy while minimizing systemic side effects associated with conventional checkpoint inhibitors. Studies have shown that TPM1 offers several advantages, including efficient targeting, the ability to overcome drug resistance, and multi-mechanism synergy. It has demonstrated significant tumor suppression capabilities and good safety profiles in animal models [6]. This innovation provides a valuable solution for low-response ICB therapies and contributes to advances in cancer immunotherapy.

II. Synthesis and Characterization of TPM-1

A. Synthesis Method

The synthesis of TPM-1 employs an optimized solid-phase peptide synthesis technique, which is a well-established and widely applied preparation method in the field of biomedicine. The entire synthesis process is carried out step by step under strictly controlled reaction conditions. First, Rink amide resin is used as the solid-phase support. A 20% (v/v) piperidine/*N,N*-dimethylformamide (DMF) solution is used to selectively remove the Fmoc protecting group, fully exposing the N-terminal amino group. Subsequently, acetic anhydride is used for acetylation capping. Then, according to the pre-designed amino acid sequence of TPM-1, each functional fragment is sequentially connected through efficient condensation reactions. Among them, the key PD-L1 targeting peptide segment (SGQ-YASYHCWCWRDPGRSGGSK) is first precisely coupled with the β -sheet forming peptide segment (FFVLK). Finally, the photosensitizer chlorin e6 (Ce6) is covalently modified to the ϵ -amino side chain of the terminal lysine (K) through an amide bond. The final product is efficiently cleaved from the resin using a trifluoroacetic acid (TFA)/triethylsilane/water (95%/2.5%/2.5%, V/V/V) mixed solution to obtain a high-purity TPM-1 monomer compound [6].

To systematically study the mechanism of action of each functional fragment, Mao, C.'s research team also carefully designed and synthesized two structural control substances, TPM2 and TPM3. In TPM2, the original FFVLK fragment is replaced with the non- β -sheet forming sequence GGAAK. In TPM3, a sequence-rearranged PD-L1 targeting peptide (SGQGYRWDSYRWHPSACGGSK) is used as a control [6]. This rigorous experimental design provides important molecular tools for the subsequent in-

depth study of the structure-function relationship of TPM-1 and enables accurate evaluation of the specific contributions of each functional domain in the self-assembly process and targeting ability.

B. Structural and Property Characterization

Mao, C.'s research team used a variety of advanced characterization techniques to systematically analyze the structures and physicochemical properties of TPM-1 and its control substances. High-resolution MALDI-TOF MS analysis confirmed the successful synthesis of TPM-1, TPM-2, and TPM-3, as their experimental molecular weights closely matched the theoretical values. ¹H NMR spectroscopic characterization was performed to elucidate the molecular structure. The observed resonance peaks and their relative intensities matched the predicted spectral pattern, confirming the precise atomic arrangement in the synthesized compounds.¹

The TPM-1 molecule possesses distinctive self-assembly properties in aqueous solutions due to its dual structural features—a hydrophilic PD-L1-targeting peptide segment and a hydrophobic Ce6 photosensitizing group coexisting within the same molecular structure. Dynamic light scattering (DLS) analysis confirms that TPM-1 can self-assemble to form a uniform micelle structure with a hydrodynamic diameter of approximately 24 nm and an ideal particle size distribution [7]. Transmission electron microscopy and scanning electron microscopy analyses show that the TPM-1 nanoparticles form a uniform spherical structure. These high-resolution imaging techniques provide a reliable basis for the morphology and size distribution of nanomaterials [8]. The DLS technique detects the fluctuations in the scattered light intensity caused by the Brownian motion of nanoparticles and calculates the particle size based on the Stokes-Einstein equation. This method is particularly suitable for the characterization of particles ranging from a few nanometers to micrometers (such as liposomes and emulsions) and is therefore widely used in the analysis of the particle size and distribution of drug delivery systems [7].

Notably, when TPM-1 interacts with the PD-L1 recombinant protein, a significant structural transformation occurs. DLS detection shows that its particle size sharply increases from the initial 20 nm to 2831 nm, indicating that fibrous supramolecular assembly has occurred in the system [7]. The TEM images intuitively display this dynamic transformation process: the initial spherical nanoparticles gradually elongate and finally form a uniform nanofiber structure with a width of approximately 10 nm. To deeply understand the molecular mechanism of

this structural transformation, the researchers used circular dichroism (CD) for analysis and found that after interacting with the PD-L1 protein, TPM-1 shows a characteristic negative peak at 215 nm and a positive peak at 195 nm. This typical β -sheet characteristic spectrum confirms the rearrangement of the secondary structure. Further analysis by Fourier transform infrared spectroscopy (FTIR) shows that the C=O stretching vibration peak of the amide I band redshifts from 1665 cm^{-1} to 1641 cm^{-1} [7]. This shift is completely consistent with the formation of the β -sheet structure, clarifying the structural transformation mechanism of TPM-1 at the molecular level. The combined use of DLS and TEM provides a comprehensive characterization of the size and morphology of TPM-1 nanoparticles [8].

Mao, C.'s research team also used ultraviolet-visible absorption spectroscopy and fluorescence spectroscopy techniques to quantitatively study the self-assembly behavior of TPM-1 by ingeniously utilizing the aggregation-induced quenching (ACQ) effect of the Ce6 dye molecule. Systematic characterization revealed distinct critical aggregation concentrations (CAC) for TPM-1, TPM-2, and TPM-3, demonstrating their varying self-assembly propensities in aqueous solution,² is greater than 1 and greater than 3. These key parameters provide a quantitative basis for understanding the differences in the self-assembly abilities of molecules with different structures. Comprehensive characterization results fully confirm the unique structural characteristics and controllable assembly properties of TPM-1.

The structural characterization of TPM-1 demonstrates the successful preparation of well-defined nanoparticles via solid-phase peptide synthesis, where the rationally designed molecular architecture - incorporating a PD-L1-targeting motif, β -sheet forming sequence, and Ce6 photosensitizer - confers unique self-organization capabilities in aqueous environments. Multiple characterization techniques confirm that TPM-1 undergoes a significant conformational transformation after binding to PD-L1, reorganizing from spherical nanoparticles (approximately 24 nm) to a fibrous network with a β -sheet structure (width approximately 10 nm). This characteristic is the structural basis for its function realization [9]. Comparative experiments show that the targeting and self-assembly abilities of TPM-1 are significantly superior to those of the control molecules TPM2/TPM3, verifying the rationality of its molecular design and the necessity of the functional modules. [6]

Identify applicable sponsor/s here. (sponsors)

III. The Mechanism of TPM-1

A. Binding and Aggregation of PD-L1

The key function of TPM-1 is to specifically bind to the PD-L1 protein on the tumor cell membrane. After TPM-1 binds to PD-L1, it transforms into a fibrous network in situ. This transformation not only captures the already bound PD-L1 but also promotes the aggregation of unbound PD-L1, efficiently inhibiting the PD-1/PD-L1 pathway.

Computational modeling through molecular dynamics simulations elucidates the molecular recognition mechanism between TPM-1 and PD-L1. The analysis reveals that TPM-1 engages with PD-L1 at binding epitopes analogous to those involved in the native PD-1/PD-L1 interaction. This structural mimicry enables TPM-1 to effectively compete with PD-1 for PD-L1 binding, consequently inhibiting immunosuppressive signaling pathways and potentiating T cell-mediated tumor immunity [9].

This binding and aggregation ability of TPM-1 has been confirmed in various tumor cell line experiments. For example, in the breast cancer cell lines SKBR-3 and 4T1, through protein immunoblotting (western blot) and flow cytometry analysis, it was found that both of these cells highly express the PD-L1 protein. Fluorescence imaging analysis using CLSM demonstrated specific membrane localization of TPM-1 nanoparticles in both SKBR-3 and 4T1 cell lines following 4-hour incubation. The pronounced Ce6-derived red fluorescence signal at the plasma membrane confirmed successful targeting of PD-L1-expressing cells by the designed nanoparticles. Through the combined use of immunofluorescence and electron microscopy (iCLEM) - a technique that combines fluorescently labeled immunostaining and high-resolution electron microscopy imaging to achieve multi-scale observation of the same sample from macro to micro - the experimental results showed that TPM-1 could specifically form a fibrous network structure on the surface of various tumor cell membranes [8]. Through systematic observation using SEM and TEM, it is clearly confirmed that this unique phenomenon in the breast cancer cell lines (SKBR-3 and 4T1). It is worth noting that this TPM-1-mediated PD-L1 aggregation phenomenon has broad-spectrum properties, and similar fibrous network formation was also observed in the liver cancer cell line SKHep-1 and lung cancer cell line LLC, indicating that TPM-1 has efficient binding and aggregation capabilities on the PD-L1 surface of tumor cells from different tissue sources.

B. Regulation of TME

In vivo investigations utilizing 4T1 mammary carcinoma

and LLC pulmonary carcinoma murine models demonstrated significant immunomodulatory effects following TPM-1 administration. Histopathological evaluation through immunohistochemical and immunofluorescence techniques revealed a marked elevation in CD8⁺ cytotoxic T lymphocyte infiltration within treated tumor specimens, concomitant with a substantial reduction in Foxp3⁺ regulatory T cell (Treg) populations. These findings suggest that TPM-1-mediated blockade of the PD-1/PD-L1 axis facilitates enhanced CD8⁺ T cell activation and tumor infiltration while attenuating immunosuppressive Treg activity, thereby promoting immunogenic remodeling of the tumor microenvironment. The experimental evidence supports the conclusion that TPM-1 exhibits high-affinity binding to PD-L1, subsequently inducing in situ fibrilization that effectively interrupts PD-1/PD-L1 signal transduction. This molecular intervention not only potentiates CD8⁺ T cell effector functions but also suppresses Treg-mediated immunosuppression, resulting in comprehensive tumor microenvironment (TME) reprogramming toward an immunologically active state.

IV. Drug Characteristics of TPM-1

A. Pharmacokinetics and Biodistribution in Vivo

After intravenous injection of TPM-1 nanoparticles, Mao, C.'s research team used a systematic research method to comprehensively evaluate the pharmacokinetic characteristics of TPM-1 in mice. During the research process, the researchers used a highly sensitive fluorescence spectrometer to precisely measure the concentration changes of Ce6 in the mouse plasma and thereby calculate the key pharmacokinetic parameters of TPM-1. The experimental data showed that TPM-1 exhibited typical biphasic elimination characteristics, with a distribution half-life ($T_{1/2}(\alpha)$) of 2.54 h and an elimination half-life ($T_{1/2}(\beta)$) of 22.17 h [6]. This result indicates that TPM-1 has excellent pharmacokinetic properties in vivo, maintaining a long systemic circulation time, which provides a guarantee for the sustained exertion of its efficacy. In terms of biodistribution studies, advanced in vivo fluorescence imaging technology was used to systematically observe the mouse model carrying 4T1 tumors. Researchers conducted dynamic monitoring of tumor tissues and major organs at multiple time points after injection (2, 4, 6, 8, 10, 24, 48, 72 and 168 h). The results revealed that TPM-1 was mainly enriched in liver tissues during the initial administration period (2-8 h), with the maximum fluorescence signal intensity reaching at 6 h, and then showing a gradually decreasing trend. This phenomenon may be related

to the biological characteristics of the nanomedicine, that is, it is prone to leakage from the hepatic sinusoidal pores in the early stage of blood circulation and is recognized, taken up and metabolized by the inherent Kupffer cells of the liver. It is worth noting that at the tumor site, TPM-1 exhibits unique accumulation characteristics: the fluorescence signal reaches its peak at 24 h and can maintain a high signal intensity until 168 h (7 days), which is significantly different from TPM2 and TPM3 nanoparticles, which reach their peak fluorescence signal at 24 h but only last until 48 h, and their peak intensity is only about 50% of that of the TPM-1 treatment group. These data fully prove that TPM-1 has more excellent retention ability and targeted accumulation characteristics at the tumor site [6].

B. Efficacy and Safety

Headings, or heads, are organizational devices that guide the read. In the breast cancer and lung cancer mouse models, the researchers conducted a systematic evaluation of the therapeutic effect of TPM-1. The experimental design included multiple control groups: the PBS negative control group, the positive control group of anti-PD-L1 antibody, and three nanoparticle treatment groups of TPM1, TPM2, and TPM3. The efficacy assessment results showed that compared with all the control groups, the TPM-1 treatment group exhibited the most significant tumor growth inhibition effect. Through dynamic monitoring of tumor volume changes and survival period analysis, it was found that the tumor growth curve of the TPM-1 treatment group remained at the lowest level, and the survival time of mice in this group was also significantly prolonged. In addition, the spleen weight of mice in the TPM-1 treatment group was closest to that of the normal group, which suggested that TPM-1 had a relatively small impact on the immune system and could better maintain the normal physiological function and tissue structure of the spleen [6].

Through histopathological analysis, the researchers further verified the anti-tumor mechanism of TPM-1. Tumor tissues were comprehensively analyzed using a combination of hematoxylin-eosin (HE) staining, Ki67 immunohistochemical staining, and TUNEL apoptosis detection. The results revealed that in both 4T1 breast cancer and LLC lung cancer models treated with TPM-1, the tumor tissues exhibited the most severe structural damage, the lowest proportion of Ki67-positive proliferating cells, and the highest number of TUNEL-positive apoptotic cells compared to control groups [6]. These data confirmed from the histological perspective that TPM-1 can effectively induce tumor cell apoptosis and significantly inhibit the proliferation activity of tumor cells.

Safety assessment studies revealed favorable tolerability

profiles for TPM-1 treatment. Longitudinal monitoring of murine body weights demonstrated no significant variations ($p > 0.05$) between TPM-1-treated cohorts and vehicle control groups throughout the experimental period, suggesting absence of treatment-related systemic toxicity. Comprehensive histopathological analysis via hematoxylin-eosin (H&E) staining of vital organs (cardiac, hepatic, splenic, pulmonary, and renal tissues) confirmed preservation of normal cytoarchitecture without evidence of drug-induced pathological alterations. In addition, the systemic blood biochemical test results also confirmed that the various indicators of mice in the TPM-1 treatment group, including liver function markers, were maintained within the normal physiological range [5]. These results fully demonstrated that within the therapeutic dose range, TPM-1 has no obvious toxic effects on the major organs and systems of the body and has high clinical translational potential.

V. Advantages over Traditional PD-L1 Inhibitors

A. Enhanced Targeting and Stability

Although traditional anti-PD-L1 monoclonal antibodies have advantages in terms of target specificity, they still have several limitations in practical clinical applications. The main issues include: limited tissue penetration ability, making it difficult to effectively penetrate deep into tumor tissues; and the tendency to be internalized and degraded by tumor cells, resulting in a relatively short duration of their action in the body.

In contrast, the TPM-1 nanoparticles exhibit multiple technical advantages. The nanoparticle characteristics of TPM-1 were verified for their stability through DLS (particle size distribution PDI < 0.2), which is superior to traditional antibodies (easily internalized and degraded). The short-term detection advantage of DLS supports its efficiency in formulation development [7]. The engineered nanosystem demonstrates precise molecular recognition capabilities for membrane-anchored PD-L1, subsequently initiating a spatially controlled fibrillization process via its intrinsic self-assembly properties to establish an immobilized fibrous matrix at the target site. This structural transformation not only significantly enhances the binding affinity with PD-L1, but more importantly, Can be retained on the surface of tumor cell membranes for a long time. The results of confocal microscopy (CLSM) in cell experiments visually demonstrate this characteristic. After Tested cells were treated with TPM-1, the red fluorescence signal on the cell membrane surface remained strong for

24 hours; while the fluorescence signal in the traditional anti-PD-L1 antibody treatment group significantly weakened after 8 hours, and most of the antibody molecules were internalized and degraded within the cells. These data fully prove that TPM-1 can overcome the limitations of traditional antibodies and achieve long-term residence at the target site, thereby continuously blocking the PD-1/PD-L1 signaling pathway and significantly enhancing the therapeutic effect.

B. Overcoming Resistance Issues

Tumor cells will develop resistance mechanisms through various molecular mechanisms under the pressure of immunotherapy, such as the occurrence of acquired mutations in PD-L1 inhibitor-resistant cases. Genes like STK11, B2M, and SMARCA4 may undergo functional loss mutations, while genes like PIK3CA, SOS1, ERBB2, and BRAF may have activating mutations. These genetic alterations lead to constitutive activation of critical oncogenic pathways, including the MAPK cascade, PI3K-Akt-mTOR axis, and Wnt/ β -catenin signaling network. Such dysregulated signaling enables malignant cells to circumvent immune surveillance mechanisms, ultimately fostering the emergence of treatment-resistant phenotypes [9]. Traditional ICIs often struggle to effectively address the dynamic changes in gene mutations and PD-L1 expression, resulting in limited treatment effects.

TPM-1 demonstrates unique advantages in addressing this clinical challenge. Studies have shown that TPM-1 not only can efficiently bind to the inherent PD-L1 molecules on the surface of tumor cells but also can promptly capture the newly expressed PD-L1 protein induced by cytokines (such as IFN- γ). In mechanism research, researchers first stimulated SKBR-3 and 4T1 cells with IFN- γ to induce the upregulation of PD-L1 expression, and then incubated them with TPM-1 nanoparticles. CLSM observations showed a significant enhancement of the red fluorescence signal on the cell surface, and SEM also confirmed the formation of a denser fiber network structure on the cell membrane surface. These findings reveal the mechanism of TPM-1 at the molecular level: it can dynamically recognize and bind to newly produced PD-L1 molecules, forming a continuous signal blockade, thereby effectively inhibiting the immune escape ability of tumor cells and providing a new idea for solving clinical resistance issues.

C. Multi-mechanism Synergistic Effect

Traditional PD-L1 inhibitors mainly exert their effects by blocking the PD-1/PD-L1 signaling pathway, thereby relieving the inhibition on T cells and restoring their immune activity. [10]. Unlike the single mechanism of

traditional PD-L1 inhibitors, TPM-1, through its unique in situ self-assembly property, can produce multi-target and multi-mechanism synergistic anti-tumor effects. In addition to efficiently blocking the main immune checkpoint pathway of PD-1/PD-L1, TPM-1 may also affect the biological behavior of tumor cells by altering the physical properties of the cell membrane. Mao, C.'s team discovered that SKBR-3 cells treated with TPM-1 would exhibit periodic fluctuations in cell membrane strength, suggesting that the formation of the fiber network may affect the dynamic reorganization of the cytoskeleton through mechanical force transmission. [6]

What is more noteworthy is that TPM-1 can also interact with other important membrane proteins. Experimental evidence indicates that the fibrous network formed by TPM-1 can encapsulate membrane proteins such as progesterone receptor component 1 (PGRMC1). Although the specific biological significance of this interaction remains to be further clarified, this phenomenon suggests that TPM-1 may exert a broader anti-tumor effect by regulating the distribution and function of various membrane proteins. [6] The characteristic of this multi-targeted synergy enables TPM-1 to exhibit a more comprehensive therapeutic profile and greater clinical application potential in tumor treatment.

VI. Conclusions

This study characterizes TPM-1 as an innovative PD-L1-targeting biomaterial with dual functionality. The findings reveal that TPM-1 specifically interacts with membrane-bound PD-L1 on tumor cells, undergoing in situ fibrillar assembly that competitively inhibits PD-1/PD-L1 engagement. This blockade robustly enhances CD8⁺ T cell-mediated tumoricidal activity. Pharmacokinetic analyses demonstrate TPM-1's favorable biodistribution profile, exhibiting prolonged plasma half-life and selective tumor accumulation. Across multiple murine tumor models, TPM-1 administration elicited potent anti-tumor responses while maintaining an excellent safety profile. Compared to traditional PD-L1 inhibitors, TPM-1 has significant advantages: its unique fibrous network structure not only prolongs the retention time in the tumor site but also effectively overcomes tumor resistance issues. At the same time, it synergistically exerts a more comprehensive anti-tumor effect through multiple mechanisms. These findings indicate that TPM-1 has important application prospects in the field of cancer immunotherapy, and its innovative mechanism provides new ideas for addressing the challenges currently faced by immunotherapy. Although further research is still needed to solve the problems of PD-L1 expression heterogeneity

and assembly process, reverse self-assembly refers to the spontaneous assembly of peptide monomers in response to the microenvironment (e.g., pH, enzymes) in cells, and other strategies may become new research and optimization directions in the future. In conclusion, the therapeutic potential of TPM-1 provides a solid foundation for the development of more effective cancer immunotherapy strategies.

Authors Contribution

All the authors contributed equally and their names were listed in alphabetical order.

References

- [1] I. Andreu Saumell, "Exploring the impact of the PD-1/PD-L1 axis on CAR-T cell function," Doctoral dissertation, Universitat de Barcelona, 2024.
- [2] H. Yamaguchi, J.-M. Hsu, W.-H. Yang, and M.-C. Hung, "Mechanisms regulating PD-L1 expression in cancers and associated opportunities for novel small-molecule therapeutics," *Nat. Rev. Clin. Oncol.*, vol. 19, no. 5, pp. 287–305, 2022.
- [3] A. Iaccarino, M. Salatiello, I. Migliatino, C. De Luca, G. Gragnano, M. Russo, C. Bellevicine, U. Malapelle, G. Troncone, and E. Vigliar, "PD-L1 and beyond: Immuno-oncology in cytopathology," *Cytopathology*, vol. 32, no. 6, pp. 596–603, 2021.
- [4] C. Li, N. Zhang, J. Zhou, C. Ding, Y. Jin, X. Cui, ..., and Y. Zhu, "Peptide blocking of PD-1/PD-L1 interaction for cancer immunotherapy," *Cancer Immunol. Res.*, vol. 6, no. 2, pp. 178–188, 2018.
- [5] M.-D. Wang, G.-T. Lv, H.-W. An, N.-Y. Zhang, and H. Wang, "In situ self-assembly of bispecific peptide for cancer immunotherapy," *Angew. Chem. Int. Ed.*, 2022.
- [6] C. Mao, F. Deng, W. Zhu, L. Xie, Y. Wang, G. Li, and J. Shen, "In situ editing of tumour cell membranes induces aggregation and capture of PD-L1 membrane proteins for enhanced cancer immunotherapy," *Nat. Commun.*, vol. 15, no. 1, p. 9723, 2024.
- [7] K. Ashizawa, "Nanosize particle analysis by dynamic light scattering (DLS)," *Yakugaku Zasshi*, vol. 139, no. 2, pp. 237–248, 2019.
- [8] V. Oorschot *et al.*, "TEM, SEM, and STEM-based immuno-CLEM workflows offer complementary advantages," *Sci. Rep.*, vol. 11, p. 899, 2021.
- [9] H. Yamaguchi *et al.*, "Mechanisms regulating PD-L1 expression in cancers and associated opportunities for novel small-molecule therapeutics," *Nat. Rev. Clin. Oncol.*, vol. 19, no. 5, pp. 287–305, 2022.
- [10] H. Cao, T. Wu, X. Zhou, S. Xie, H. Sun, Y. Sun, and Y. Li, "Progress of research on PD-1/PD-L1 in leukemia," *Front. Immunol.*, vol. 14, 1265299, 2023.