

REVIEW



Advances in the structural characterization of complexes of therapeutic antibodies with PD-1 or PD-L1

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ABSTRACT

Antibody-based immune checkpoint blockade (ICB)-based therapeutics have become effective clinical applications for cancers. Applications of monoclonal antibodies (mAbs) to de-activate the PD-1-PD-L1 pathway could effectively reverse the phenotype of depleted activated thymocytes (T cells) to recover their anti-tumoral activities. High-resolution structures of the complexes of the therapeutic monoclonal antibodies with PD-1 or PD-L1 have revealed the key inter-molecular interactions and provided valuable insights into the fundamental mechanisms by which these antibodies inhibit PD-L1-PD-1 binding. Each anti-PD-1 mAb exhibits a unique blockade mechanism, such as interference with large PD-1-PD-L1 contacting interfaces, steric hindrance by overlapping a small area of this site, or binding to an *N*-glycosylated site. In contrast, all therapeutic anti-PD-L1 mAbs bind to a similar area of PD-L1. Here, we summarized advances in the structural characterization of the complexes of commercial mAbs that target PD-1 or PD-L1. In particular, we focus on the unique characteristics of those mAb structures, epitopes, and blockade mechanisms. It is well known that the use of antibodies as anti-tumor drugs has increased recently and both PD-1 and PD-L1 have attracted substantial attention as target for antibodies derived from new technologies. By focusing on structural characterization, this review aims to aid the development of novel antibodies targeting PD-1 or PD-L1 in the future.

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Introduction

The introduction of immune checkpoint blockade (ICB)-based cancer therapeutic approaches has resulted in substantial changes in the clinical treatment paradigm.^{1–3} One of the key molecular mechanisms of ICB therapy involves targeting of the immune suppression PD-1-PD-L1 pathway. PD-1 is localized on the cell surface of activated immune T-cells,⁴ whereas its natural ligand PD-L1 is highly expressed on the surface of tumor cells.^{5–7} Recognition between PD-1 and PD-L1 activates the PD-1/PD-L1 pathway to reduce the activity of the T-cell. Many types of cancer exploit these interactions to escape immune surveillance.^{7–10}

mAbs that bind PD-1 or PD-L1 have been successfully used to suppress the cancer cells' ability to evade immune surveillance.^{11,12} The mAbs bind to PD-1 or PD-L1, block their recognition, and thus re-activate the tumor-specific T cells to kill cancer cells.^{13,14} As of mid-2022, 16 mAbs that target the PD-1-PD-L1 pathway had been approved by the Food and Drug Administration (FDA; USA) or the National Medical Products Administration (NMPA; China) as therapeutics for cancer. Of these, 11 are anti-PD-1 mAbs and 5 are anti PD-L1 antibodies.^{15–29} Aside from envafolimab (KN035), which is a camelid-derived single-domain antibody (i.e., nanobody), all of the other above-mentioned therapeutic antibodies are full-length mAbs. Further details of these antibodies, including their manufacturers and the indications for which they are approved, are listed in Table 1.

Structural characterization of the epitopes on PD-1 or PD-L1 that underpin PD-1-mAb and PD-L1-mAb complexes provides valuable insights into the mechanisms by which these antibodies block the inhibitory signaling pathway of PD-1 and PD-L1.^{48,49} High-resolution structures of the complex of the mAbs with PD-1 or PD-L1 have been obtained to unravel the mechanisms of these antibodies.^{33–50–61} The mechanisms include a mAb's binding plane blocking a large area of the PD-L1-binding site of PD-1,³³ or the mAb generating steric hindrance by overlapping a small area of this site³⁶ or binding to an *N*-glycosylated site.⁴⁰ In contrast, most approved anti-PD-L1 mAbs bind PD-L1 at very similar sites (on the pre- β -sheet), and they all directly compete with PD-1.^{44–62–64} The aforementioned structural findings will aid the future rational design of novel and more effective antibodies.

Herein, we review advances in structural studies of complexes formed between FDA- or NMPA-approved antibodies and PD-1 or PD-L1. We focus on the nature of PD-1-PD-L1 interactions, key contacts that stabilize the interactions between each mAb and PD-1 or PD-L1, and the mechanism by which each mAb blocks PD-1-PD-L1 interactions. An earlier work published in 2019 has reviewed the structure of PD-1-PD-L1 complex and the structures of mAbs complex with PD-1 or PD-L1.⁵⁸ As more antibodies targeting PD-1 or PD-L1 were approved during 2019 to mid-2021, we collected data for all these new approved antibodies and provided a more comprehensive summarization on the complex structures of mAbs with PD-1 or PD-L1.

Table 1. List of the programmed cell death protein 1 (PD-1), programmed cell death ligand 1 (PD-L1) and related structures deposited in the protein data bank

Protein	Ligand/Antibody	Organism	Domain	PDB ID	PD-1 region	Combination of CDR
PD-1	PD-L1	human/human	Ig V extracellular/Ig V	4ZQK ³⁰ , 5IUS ³¹	Front β -sheet	N/A
		mouse/human	Ig V extracellular/Ig V and Ig C extracellular	3BIK ³²		
	pembrolizumab	human	Ig V extracellular/Fv	5B8C ³³	C' D loop	LCDR3, HCDR1, HCDR2 and HCDR3
		human	Ig V extracellular/Fab	5IXE ³⁴ , 5GGS ³⁵		
	nivolumab	human	Ig V extracellular/Fab	5WT9 ³⁶ , 5GGR ³⁷	N-loop; FG and BC loops	HCDR1, HCDR2, HCDR3, LCDR1 and LCDR2
	tislelizumab	human	Ig V extracellular/Fab	7BXA ³⁸ , 7CGW ³⁹	Front β -sheet	LCDR1, HCDR2, HCDR3, LCDR1 and HCDR3
	camrelizumab	human	Ig V extracellular/Fab	7CU5 ⁴⁰	N58 glycosylation; BC, C' D and FG loops	HCDR1, HCDR2, HCDR3, LCDR1 and LCDR3
	toripalimab	human	Ig V extracellular/scFv	6JBT ⁴¹	FG loop	HCDR1, HCDR2, HCDR3, LCDR1 and LCDR3
	cemiplimab	human	Ig V extracellular/Fab	7WWM ³⁷	N58 glycosylation; BC, C' D and FG loops	HCDR1, HCDR2, HCDR3, LCDR1 and LCDR3
	serplulimab	human	Ig V extracellular/scFv	7E9B ⁴²	CD, BC and FG loops	HCDR1, HCDR2, HCDR3, LCDR1, LCDR2 and LCDR3
	sintilimab	human	Ig V extracellular/Fab	N/A	N/A	Structures to be determined
	penpulimab	human	N/A	N/A	N/A	Structures to be determined
	dostarlimab	human	N/A	N/A	N/A	Structures to be determined
	zimberelimab	human	N/A	N/A	N/A	Structures to be determined
	atezolizumab	human	Ig V extracellular/Fab	5XXY ⁴³ , 5X8L ⁴⁴	front β -sheet; CC' and FG loops	HCDR1, HCDR2, HCDR3 and LCDR3
	durvalumab	human	Ig V extracellular/scFv	5XJ4 ⁴⁵	Front β -sheet	HCDR1, HCDR2, HCDR3, LCDR1 and LCDR3
		human	Ig V extracellular/Fab	5X8M ⁴⁴		
PD-L1	avelumab	human	Ig V extracellular/scFv	5GRJ ⁴⁶	C, C', F and G chains and CC' loop	HCDR1, HCDR2, HCDR3, LCDR1 and LCDR3
	envafolimab	human	Ig V extracellular/scFv	5JDS ⁴⁷	Front β -sheet	CDR1 and CDR3
	sugemalimab	human	Ig V extracellular/KN035	N/A	N/A	Structures to be determined

Anti-PD-1/PD-L1 antibodies

Preparation of samples for the structural characterization of PD-1-mAb or PD-L1-mAb complexes

Most of the FDA- and/or the NMPA-approved therapeutic antibodies that specifically bind PD-1 or PD-L1 for cancer therapy are full-length immunoglobulin G (IgG) mAbs (Table 1).^{65,66} The isotypes (IgG1 or IgG4) of each mAb included here are summarized in Table 2. The crystal structures of the complex of PD-1 or PD-L1 with a range of approved antibodies have been reported.^{58–67–71} Because the full-length antibodies are typically too flexible to be crystallized, Fab or scFvs of mAbs have commonly been used to bind with PD-1 or PD-L1 to afford a crystallizable complex. A Fab domain is composed of four folded domains: a variable domain from light chain and heavy chain (V_L and V_H), and a constant domain from light chain and heavy chain (C_L and C_H1). The scFv is a fusion protein of a V_H and a V_L , which is a complete and smallest functional unit.⁷² (Figure 1)

The Fab regions or scFvs of mAbs are usually prepared by one of the three methods. Fab regions are prepared by enzymatic digestion of full-length antibodies. For example, papain degrades IgGs into two Fab regions and one Fc region, and the Fab regions can be further purified for structural studies.¹²¹ Liu and colleagues used this approach to crystallize the complex of Fab region of toripalimab with PD-1, which in turn determines the complex structure.⁴¹

Recombinant co-expression of a mAb's V_H and V_L is an alternative approach in Fab preparation. DNA sequences encoding the V_H and the V_L are constructed in different plasmids, which are then co-transfected into expression hosts that subsequently co-express the V_H and V_L domains. This

generates a random combination of V_H and V_L domains that form a library of V_H - V_L complexes,¹²² which are screened to determine the optimal constructs for structural characterization. This approach has been used to determine the structures of the complexes of PD-1 with pembrolizumab-Fab,³³ nivolumab-Fab,³⁶ tislelizumab-Fab⁷¹ and atezolizumab-Fab.⁴³ Using the third method, scFv recombinant expression plasmids are constructed to contain the genes of an antibody's V_L and V_H domains that are linked by an oligopeptide to form an scFv (Figure 1).¹²³ This method has been used to express and crystallize camrelizumab,⁴⁰ avelumab⁴⁶ and durvalumab.⁴⁵ The second and third methods have obvious advantages in obtaining a Fab region or an scFv from a prokaryotic expression system, i.e., *E. coli* expression systems, and have been the most commonly used methods to obtain Fab regions or scFvs for structural studies.

Structures of PD-1-PD-L1 and PD-1-mAbs complexes

PD-1 and PD-L1 interact and form stable complex. The interface of an mAb and PD-1 or PD-L1 partially or fully overlaps with those of PD-1-PD-L1 complex, resulting in interfacial conflicts and partial steric hindrance that perturb the recognition of PD-L1 with PD-1.^{124,125} Examples of the reported structures are shown in Figure 2.

Structure of PD-1-PD-L1

PD-1 has an extracellular domain comprised by nine β -strands (A, A', B, C, C', D, E, F, G), folding into a β -sandwich Ig topology.^{4,36,126} It also has eight loops connecting the β -strands, and a flexible loop at the *N*-terminus (Figure 3).^{30,34}

Table 2. Details of antibodies.

	Antibody	Manufacturer	Data of Approval	Degree of humanization	Type	Indications
PD-1	pembrolizumab	Merck KGaA	2014	Humanized	IgG4	Melanoma ⁷³ , NSCLC ⁷⁴ , metastatic head and neck squamous-cell carcinoma ⁷⁵ , lung cancer ⁷⁶ and cervical cancer ⁷⁷
	nivolumab	Bristol Myers Squibb	2014	Fully human	IgG4	Melanoma ⁷⁸ , NSCLC ⁷⁹ , metastatic head and neck squamous-cell carcinoma ⁸⁰ and classical Hodgkin's lymphoma ⁸¹
	tislelizumab	BeiGene	2019	Humanized	IgG4	Hodgkin's lymphoma ⁸¹ , urothelial carcinoma ⁸² , NSCLC ⁸³ and hepatocellular carcinoma ⁸⁴
	camrelizumab	Jiangsu Hengrui	2019	Humanized	IgG4	Classical Hodgkin's lymphoma ⁸⁵ , hepatocellular carcinoma ⁸⁶ , esophageal squamous cell carcinoma ⁸⁷ , nasopharyngeal carcinoma ⁸⁸ and non-squamous NSCLC ⁸⁹
	toripalimab	Shanghai Junshi	2018	Humanized	IgG4	Melanoma ⁹⁰ , nasopharyngeal cancer ⁹¹ , urothelial cancer ⁹² and NSCLC ⁹³
	cemiplimab	Sanofi-Regeneron	2018	Humanized	IgG4	Metastatic cutaneous squamous-cell carcinoma (CSCC) ⁹⁴ and locally advanced CSCC ⁹⁵
	serplulimab	Shanghai Fuhong Hanlin Biotechnology	2022	Humanized	IgG4	Unresectable, metastatic and highly microsatellite-unstable solid tumors ⁹⁶
	sintilimab	Innovent	2018	Fully human	IgG4	Squamous-cell lung cancer ⁹⁷ , liver cancer ⁹⁸ , esophageal cancer ⁹⁹ , non-squamous NSCLC ¹⁰⁰ and classical Hodgkin's lymphoma ¹⁰¹
	dostarlimab	GSK Plc	2021	Humanized	IgG4	Adult patients with mismatch repair deficient recurrent or advanced solid tumors ^{97,102}
	penpulimab	Akeso, Inc.	2021	Humanized	IgG1	Relapsed or Refractory Classic Hodgkin's Lymphoma ¹⁰³
PD-L1	zimerelimab	Harbin Gloria Pharmaceuticals	2021	Fully human	IgG4	Relapsed or Refractory Classic Hodgkin's Lymphoma ¹⁰⁴
	atezolizumab	Genentech	2016	Humanized	IgG1	Metastatic urothelial carcinoma ¹⁰⁵ , NSCLC ¹⁰⁶ , renal cell carcinoma ¹⁰⁷ , hepatocellular carcinoma ¹⁰⁸ , triple-negative breast cancer ¹⁰⁹ and colorectal cancer ¹¹⁰
	avelumab	Merck KGaA	2017	Fully human	IgG1	NSCLC ¹¹¹ , advanced renal cell carcinoma ¹¹² and gastric cancer ¹¹³
	durvalumab	AstraZeneca	2017	Fully human	IgG1	NSCLC ¹¹⁴ , head and neck cancer ¹¹⁵ and urothelial carcinoma ¹¹⁶
	envafolimab	Alphamab Oncology	2021	Humanized	Nanobody	Advanced biliary tract cancer and soft tissue sarcoma ^{117–119}
	sugemalimab	Cstone	2021	Fully human	IgG4	NSCLC ¹²⁰

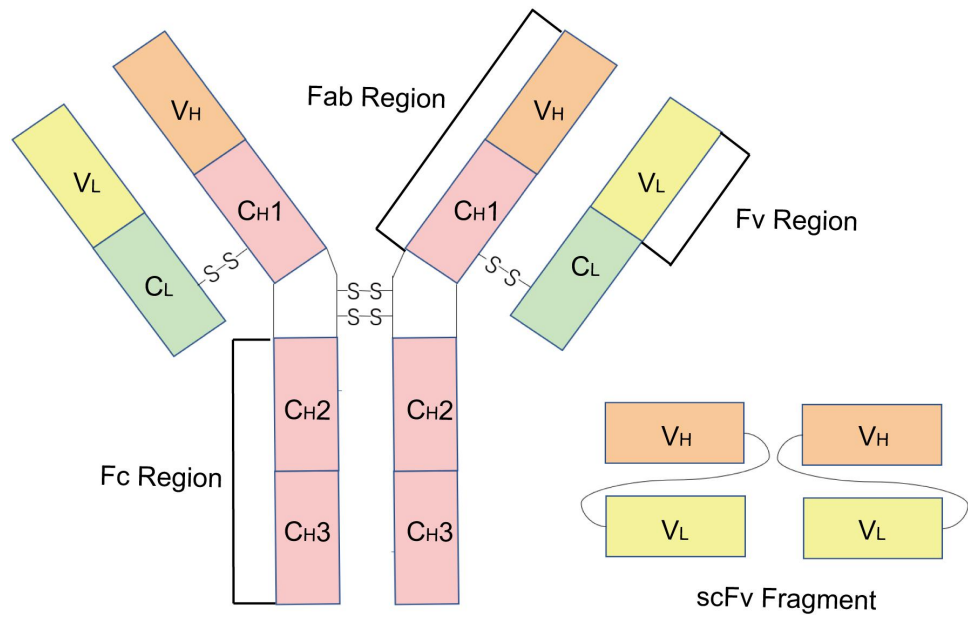


Figure 1. Topology of monoclonal antibody and the scFv fragment. The Fab and scFv domains are indicated.

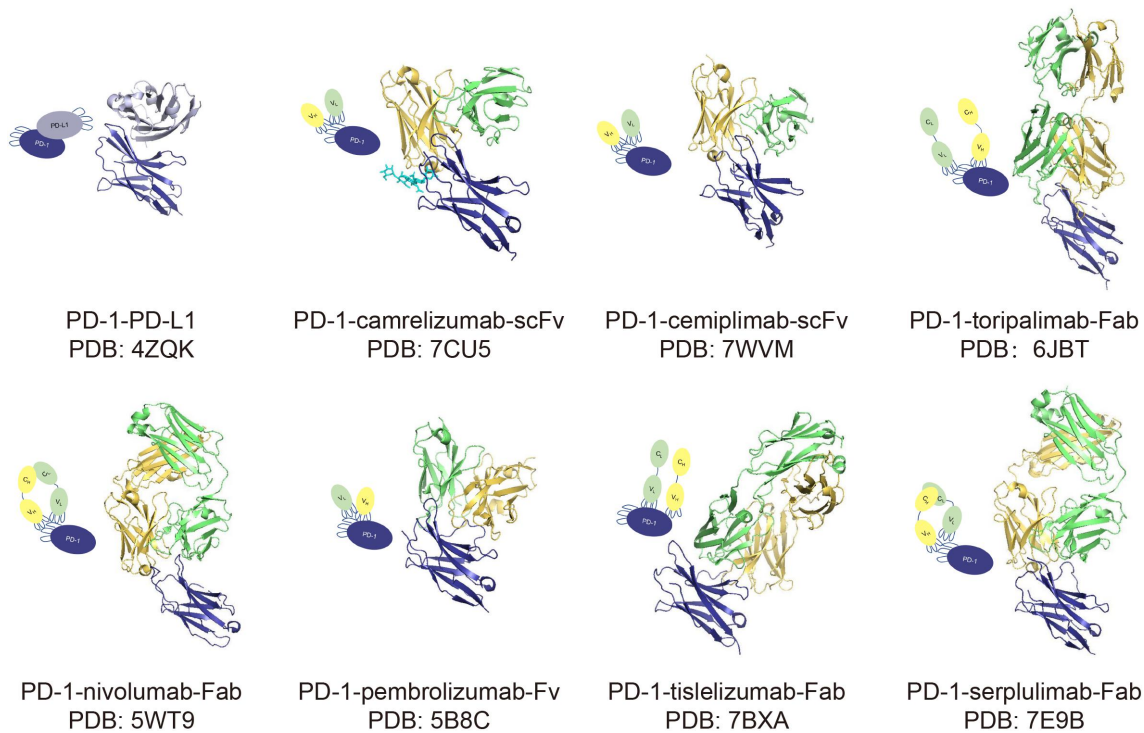


Figure 2. Presentation of structures of PD-1-PD-L1 complex and PD-1-antibody complexes. The complex names and the PDB code of the complexes are given below each structure. PD-1 and PD-L1 are shown in dark blue and light blue, respectively. The Fab or the Fv domains of mAb are shown in yellow and green, respectively.

PD-1 contains multiple *N*-glycosylated sites,³⁶ which affect the binding of certain antibodies.⁴⁰ Similar to PD-1, PD-L1 also has nine β -strands (A, B, C, C', D, E, F, G) and the loops connecting them to fold into an extracellular Ig V domain.^{44,46} The structures of PD-1-PD-L1 complexes have been determined using PD-1 and PD-L1 from different species (Table 2).^{30,32} These structures have revealed that PD-1 and PD-L1 are oriented nearly orthogonal to each other (Figure 3). PD-1 and PD-L1 associate mainly via hydrophobic interactions between residues on the front faces of both proteins

(Figure 3).³⁰ The inter-molecular H-bonds and π - π stacking are other key driving forces that stabilize PD-1-PD-L1 complexes.³⁰ The mAbs mainly compete within this interface to block the PD-1-PD-L1 interactions.

Structure of PD-1-camrelizumab-scFv

Anti-PD-1 camrelizumab was developed by Jiangsu Hengrui Medicine Co., Ltd. It is approved by the NMPA for the treatment of classic Hodgkin's lymphoma, hepatocellular

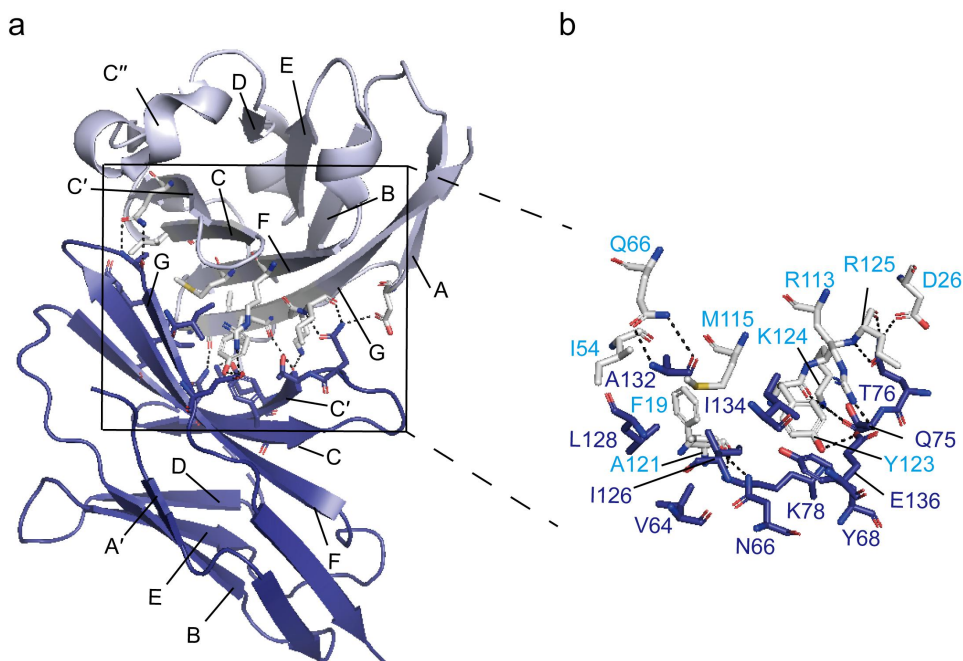


Figure 3. The structure of PD-1-PD-L1. (a) The structure of PD-1-PD-L1 complex (PDB:4ZQK). The PD-1 and the PD-L1 are shown in dark blue and light blue, respectively. The residues that make inter-molecular contacts are highlighted in blue (PD-1) and light gray (PD-L1) sticks. (b) Details of the interacting residues that stabilizing the complex. Residues forming the inter-molecular hydrogen bonds and the hydrophobic contacts are presented as sticks. The inter-molecular H-bonds are connected by dashed lines.

carcinoma, esophageal squamous cell carcinoma, nasopharyngeal carcinoma and non-squamous non-small-cell lung cancer (NSCLC).²¹⁻⁸⁶⁻⁸⁸⁻¹²⁷ Gao and colleagues determined the structure of the PD-1-camrelizumab-scFv complex (PDB code: 7CU5) using a V_L -(glycine)₄-serine- V_H construct of camrelizumab.⁴⁰ All three heavy chain complementarity-determining regions (HCDRs) and two of three light chain (LC) CDRs of camrelizumab form contacts with PD-1 (Figure 4a, b). Three loops of PD-1 constitute several inter-molecular H-bonds that are crucial for the complex stability, such as the H-bonds between the LCDR3 of camrelizumab and the FG loop of PD-1, and between the HCDR2 of camrelizumab and the BC loop of PD-1. The interface between PD-1 and camrelizumab-scFv partially overlaps with that of PD-1 and PD-L1, indicating that camrelizumab prevents the PD-L1-PD-1 interaction.

PD-1 contains several *N*-linked glycosylation sites, which can be modified with a glycan containing two NAGs, two MANs and one FUC. *N*-glycosylation modification on PD-1 has a pronounced effect on camrelizumab binding. The glycosylation of N58 was found to strengthen the camrelizumab-PD-1 binding. The absence of N58-glycosylation, which can be achieved via a N58A single point mutation of PD-1 that eliminates the *N*-glycosylation site, largely reduced its binding affinity with camrelizumab (Figure 4c, d).⁴⁰ The structure of the PD-1-camrelizumab-scFv demonstrated the van der Waals contacts between the glycan chains on N58 and several residues of camrelizumab, such as the S30 of HCDR1 and the G54 and A56 of HCDR2. Moreover, FUC and MAN in the glycan chain form H-bonds with the S31 of HCDR1 and the G53 of HCDR2 (Figure 4). These contacts between the glycan and the PD-1 make significant contributions to the stability of the PD-

1-camrelizumab-scFv complex. Camrelizumab showed some low-affinity binding to other human receptors, leading to side effects in clinical trials, e.g., reactive capillary endothelial proliferation. The low-affinity binding between camrelizumab and other receptors may be correlated with its glycosylation-dependent high-affinity binding to PD-1.

In addition to camrelizumab, a recent publication has shown that glycosylation of PD-1 also affects its binding with cemiplimab, a human IgG4 mAb targeting PD-1 developed by Sanofi and Regeneron.³⁷ The interaction surface of PD-1-cemiplimab-scFv is highly similar to that of PD-1-camrelizumab-scFv. The glycosylation of N58 in PD-1 also promotes the PD-1-cemiplimab-scFv binding.³⁷ The HCDR2 of cemiplimab interacts with N58 glycosylation of PD-1.

Structure of PD-1-toripalimab-Fab

Developed by Shanghai Junshi Biosciences Co., Ltd, toripalimab was approved by the NMPA in 2018. Toripalimab is currently indicated as a treatment for melanoma, nasopharyngeal cancer, urothelial cancer, and NSCLC.⁹¹⁻¹²⁸⁻¹³⁰ The PD-1-toripalimab-Fab structure showed that an unusually long HCDR3 (18 amino acids) at the V_H domain of toripalimab⁴¹ (Figure 5), which is longer than the HCDR3 of any other anti-PD-1 mAb that has been characterized. This long HCDR3 and the LCDR1 and LCDR3 of toripalimab form multiple H-bonds with the residues on the FG loop of PD-1, as key binding factors (Figure 5). In addition, van der Waals interactions between the HCDR1, HCDR2 and LCDR1 of toripalimab and the FG of PD-1 are critical in complex stability.⁵⁷ Like camrelizumab, the interaction sites of the PD-1-toripalimab-Fab partially overlap with that of the PD-1-PD-L1 complex, thus the antibody sterically hinders PD-1-PD-L1 binding.⁴¹ In contrast to camrelizumab,

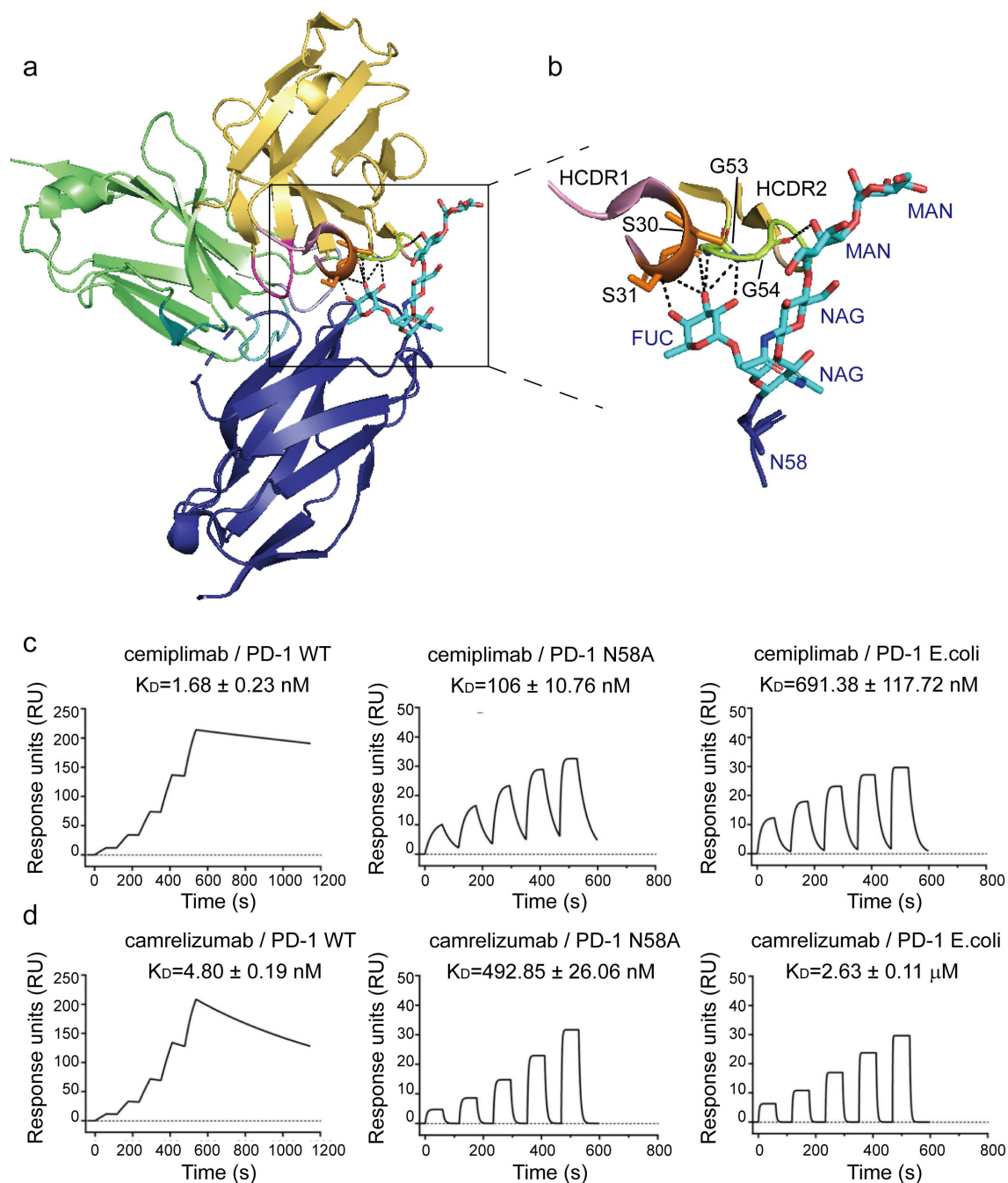


Figure 4. Illustration of the importance of the glycosylation of PD-1 in binding with camrelizumab and cemiplimab³⁷. (a) The structure of PD-1-camrelizumab-scFv (PDB:7CU5)⁴⁰. The PD-1 is colored blue. The camrelizumab are shown in green and yellow orange. The three HCDRs are colored in pink, limon and light magenta. The three LCDRs of light chain are shown in aquamarine, deep teal and light blue. (b) The details of the interacting residues stabilizing the PD-1-camrelizumab-scFv complex. Residues forming the inter-molecular H-bonds are linked with dashed lines and labeled. (c, d) SPR assays of the cemiplimab (c) with various PD-1 mutant proteins and the camrelizumab (d) with various PD-1 mutant proteins. Figure 4(c, d) were reprinted from the *Frontiers in Immunology*.³⁷

toripalimab's binding to PD-1 is not related to glycosylation. It is worth noting that this glycosylation-independent toripalimab-PD-1 binding makes toripalimab less likely to be affected by dysregulated glycosylation modifications of PD-1.

Structure of PD-1-nivolumab-Fab

Nivolumab was developed by Bristol Myers Squibb. First approved by the FDA in 2014, it is now marketed for the treatment of NSCLC, melanoma, advanced kidney cancer,

head and neck squamous cell carcinoma, advanced classical Hodgkin's lymphoma, and bladder cancer.^{60–80,131–138} Two structures of PD-1-nivolumab-Fab have been deposited in the Protein Data Bank (PDB: 5GGR, PDB: 5WT9).^{35,36} The binding interface of the complex comprises the LCDR2, LCDR1 and all three of the HCDRs of nivolumab and the N-terminal, FG and BC loops of PD-1 (Figure 6).

A crucial factor for PD-1-nivolumab-Fab binding was found to be nivolumab's recognition of the N-terminal residues of PD-1. Truncation of N-terminus of PD-1 abolished its binding to

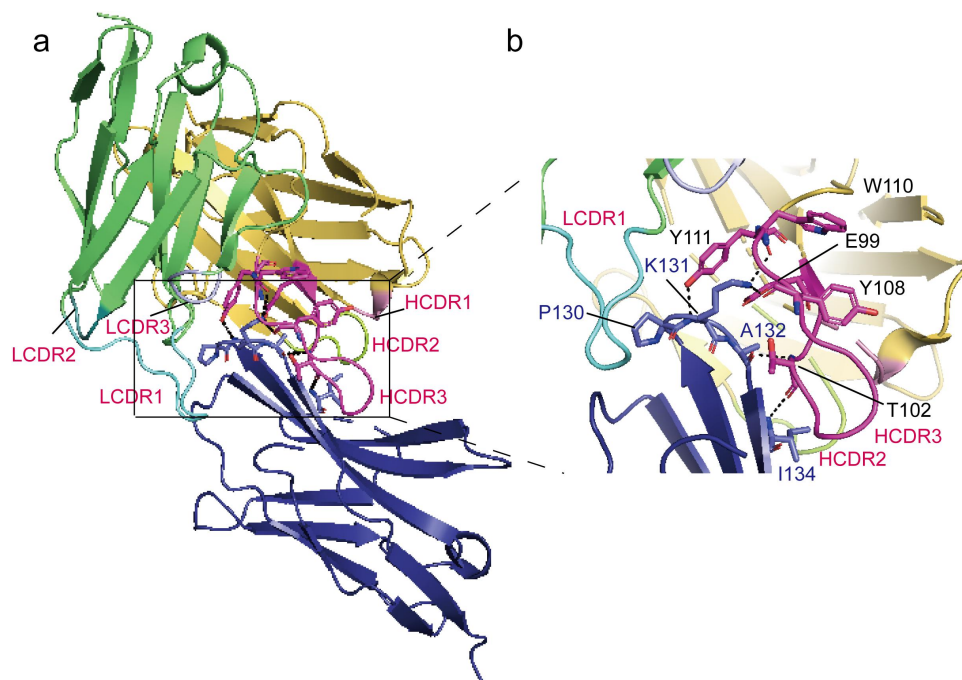


Figure 5. Structure of PD-1-toripalimab-fab (PDB:6JBT). (a). The PD-1 is colored in deep blue. The toripalimab-Fab are shown in green and yellow orange. The three HCDRs are shown in pink, limon, and light magenta. The three LCDRs are shown in aquamarine, deep teal, and light blue. (b) The details of the contacts between PD-1 and toripalimab-Fab. The inter-molecular H-bonds are shown as sticks and labeled.

nivolumab (Figure 6).³⁶ The H-bonds connecting the N-terminus of PD-1 and the HCDR1–2 of nivolumab are the primary driving force to form the complex (Figure 6). The remainder of the key factors of the interactions are between the FG and BC loops of PD-1 and nivolumab. In particular, the FG forms five H-bonds with the HCDR3 and LCDR2, whereas the BC loop has only one inter-molecular H-bond with the HCDR1 of nivolumab.

The nivolumab interferes with PD-1-PD-L1 contact via steric hindrance between its V_L domain and PD-L1, which antagonize PD-1-PD-L1 binding. However, the overlap area of the nivolumab-PD-1 binding region with that of PD-L1-PD-1 is much smaller than the overlap area of camrelizumab or toripalimab on PD-1 with that of PD-L1-PD-1. A common characteristic of these three mAbs is that they all interact with the FG loop of PD-1, which shows the importance of the FG loop in mAb recognition, as suggested by recent research.³⁷ Moreover, the unique ability of nivolumab to bind the N-terminus of PD-1 indicates that the flexible segments of PD-1 are also promising targets for drug design.

Structure of PD-1–pembrolizumab-Fv

Pembrolizumab was approved by the FDA in 2014 and was the first anti-PD-1 mAb to achieve this milestone.³⁴ It was initially used to treat advanced melanoma and thereafter approved for the indications of NSCLC, metastatic head and neck squamous-cell carcinoma, refractory classical Hodgkin's lymphoma and hepatocellular carcinoma.^{74–143} Crystal structures of both full-length pembrolizumab (PDB:5DK3)¹⁴⁴ and PD-1–pembrolizumab-Fv complexes (PDB:5JXE,³⁴ PDB:5B8C³³) have been determined.

The overall topology of the PD-1–pembrolizumab-Fv is unusual in involving more key amino acid residues in binding

with PD-1 than other mAbs (Figure 7). The pembrolizumab-PD-1 binding interface involves 21 amino acid residues of pembrolizumab, whereas camrelizumab, nivolumab, and toripalimab have 10–13 amino acid residues in contacting with PD-1.³³ All of the LCDRs and the HCDRs of pembrolizumab interact with the β -sheet of PD-1, mainly by hydrophilic interactions, i.e., H-bonds and salt bridges. The PD-1–pembrolizumab-binding interface overlaps with most of the binding interface of PD-1–PD-L1 (Figure 7), demonstrating that pembrolizumab sterically prevents the PD-L1–PD-1 contacts.³³ Because of the extensive hydrophilic inter-molecular interactions, the pembrolizumab binds to PD-1 with higher affinity than that PD-L1 binds to PD-1.

Structure of PD-1–tislelizumab-Fab and PD-1–serplulimab-Fab

Developed by Beigene, Ltd., tislelizumab was approved by the NMPA in 2019. It was developed to exhibit less binding to the Fc- γ receptor 1,¹⁴⁵ the interactions of which have negative effect on anti-cancer activity by the anti-PD-1 mAbs.¹⁴⁶ Tislelizumab is used to treat hepatocellular carcinoma, Hodgkin's lymphoma, urothelial carcinoma, and NSCLC.^{81,84,108,147,148} Heo and colleagues solved the PD-1–tislelizumab-Fab structure (PDB:7BXA,³⁸ PDB:7CGW³⁹), which revealed that, unlike other mAbs that recognize the PD-1 loop region, tislelizumab mainly associates with the β -sheet of PD-1, closely mimicking PD-L1–PD-1 binding. In particular, the three LCDRs and the HCDR3 bind PD-1 via several H-bonds and many van der Waals contacts. The binding of tislelizumab to PD-1 sterically hinders the binding of PD-L1 to PD-1.³⁹

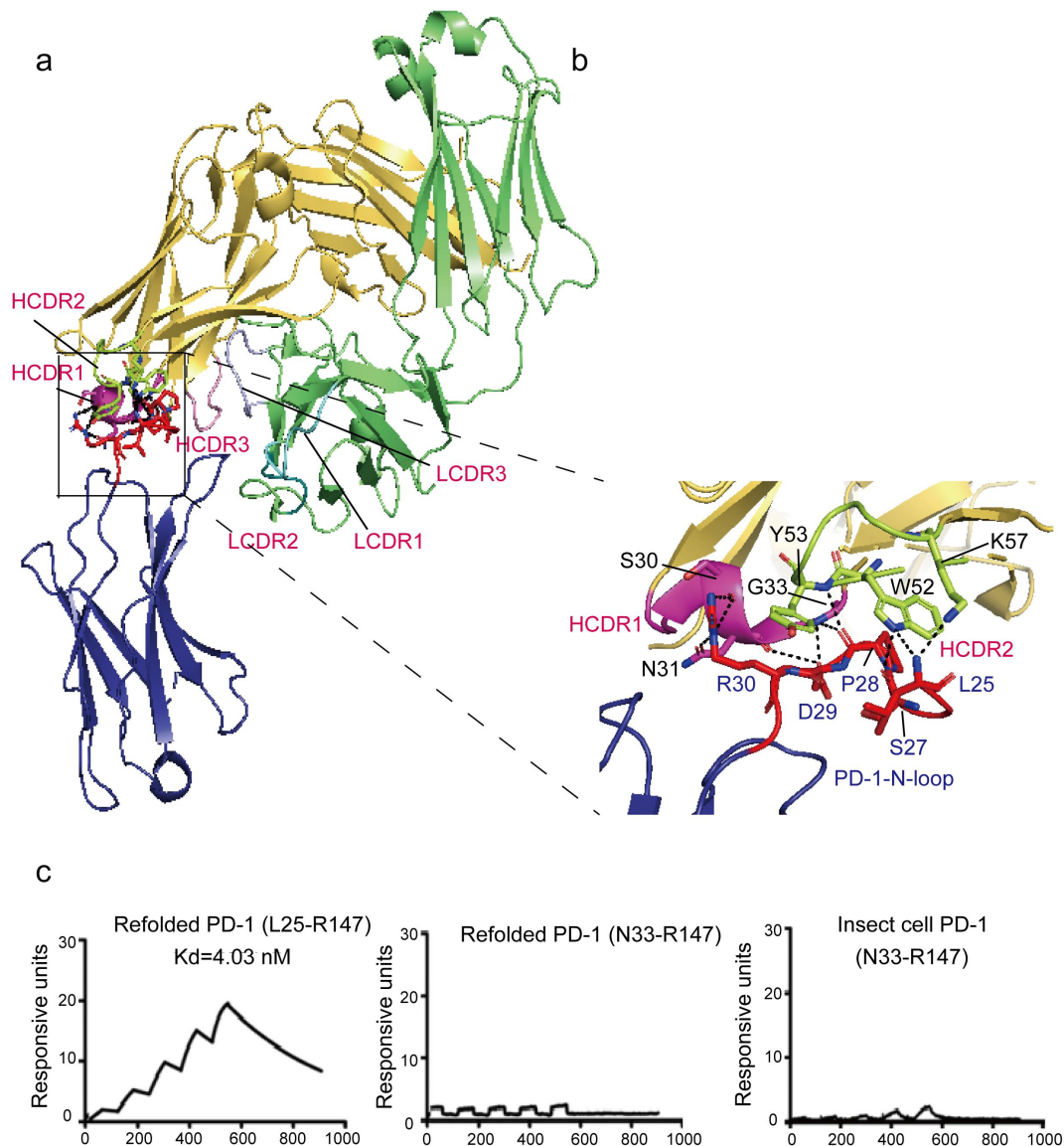


Figure 6. Importance of the N-terminus of PD-1 in stabilization of PD-1-nivolumab-fab complex. (a) The structure of nivolumab-Fab (PDB:5WT9). The nivolumab-Fab is shown in yellow orange, and green. The PD-1 is shown in deep blue. The three HCDRs are colored in light magenta, limon, and pink. The three LCDRs of light chain are shown in aquamarine, deep teal and light blue. (b) Details of PD-1-nivolumab-Fab contacts. (c) SPR results of the nivolumab and different constructs of PD-1. [Figure 6c](#) was reprinted from the reference.³⁶

Serplulimab was developed by Shanghai Fuhong Hanlin Biotechnology Co., Ltd., and was approved by the NMPA in 2022 for the indications of unresectable, metastatic, and highly microsatellite-unstable solid tumors.^{17,96} Issafras and colleagues determined the structure of PD-1-serplulimab-Fab. The three flexible loops of PD-1 and all six CDRs of serplulimab comprise the interface of the complex. The residue Arg86 of PD-1 was found to be vital for complex formation, by forming multiple contacts with residues in the HCDR3 of serplulimab, including salt bridges with Asp104, hydrogen bonds with residue Ser98 and Tyr99, and a π - π interaction with Tyr323. Structural data of the complex demonstrated that many epitopes of PD-1 that are bound by serplulimab are also those contacting with PD-L1. Moreover, it was found that serplulimab binding triggers a structural alteration on the CC' loop of PD-1.

Structures of PD-L1-mAb

As described above, the various anti-PD-1 antibodies exhibit significantly different topologies of binding with PD-1. In contrast, the interface of mAbs with PD-L1 are similar, typically on the β -sheet ([Figure 8](#)).

Structures of PD-L1-full-length mAbs

There are four full-length mAbs targeting PD-L1 on the market: atezolizumab, avelumab, durvalumab and sugemalimab. Atezolizumab was developed by Genentech and approved by the FDA in 2016, both avelumab, developed by Merck KGaA, and durvalumab, developed by AstraZeneca, were approved by FDA in 2017, while sugemalimab, developed by Cstone, was approved in China 2021. The indications of these mAbs are

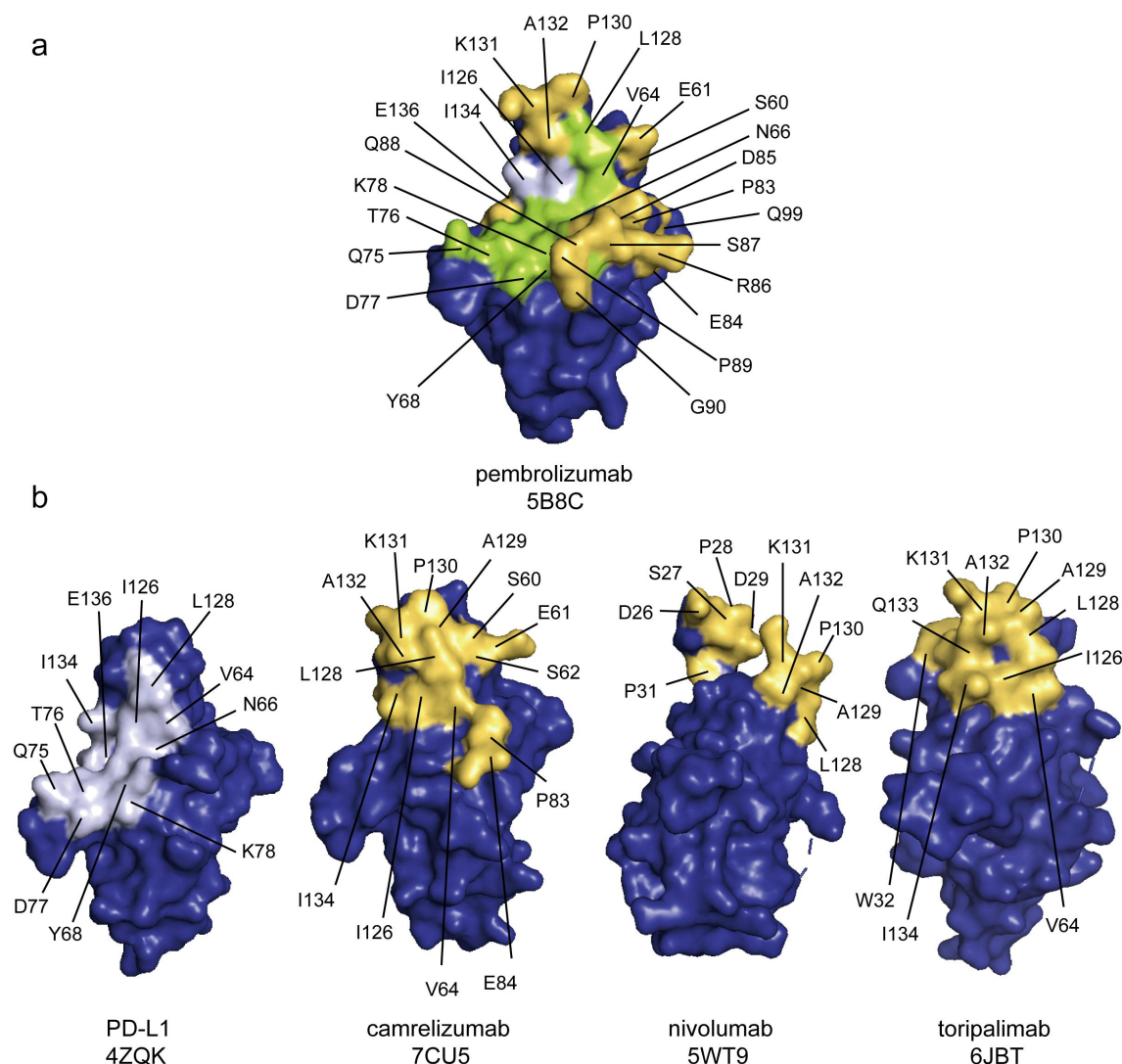


Figure 7. Comparison of PD-1 binding sites to pembrolizumab, PD-L1, camrelizumab, nivolumab and toripalimab. (a) The interaction surface of pembrolizumab on PD-1. The structure of PD-1 is shown in deep blue. The interactions region on PD-1 are shown in yellow-orange (pembrolizumab), light blue (PD-L1) and lime (overlapped surface), respectively. (b) The contact areas to PD-1 of the PD-L1 are shown in light blue, and are yellow for other mAbs, i.e., camrelizumab, nivolumab, and toripalimab. The mAb names and PDB codes for the PD-1-mAb complexes are listed below each structure.

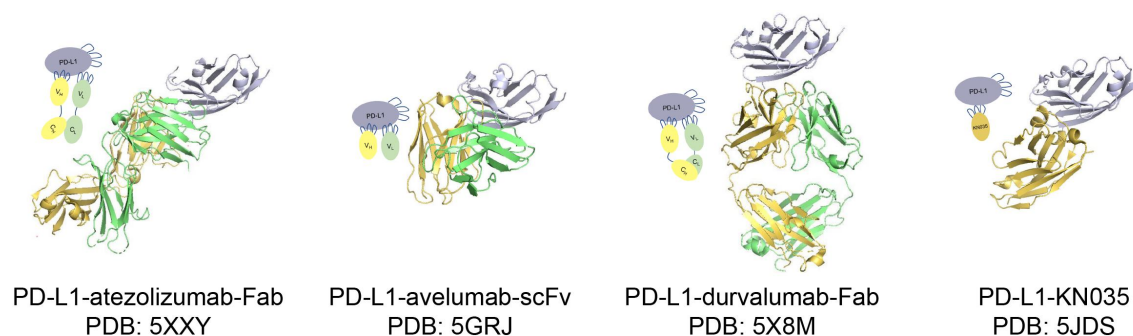


Figure 8. The topology of structures of PD-L1-antibodies. The names of the complexes and the PDB codes of the structures are listed below each structure. The PD-L1 is shown in gray. The mAb are colored in yellow and green. The KN035 is shown in yellow.

listed in Table 2.¹⁰¹⁻¹⁰³⁻¹⁰⁶⁻¹¹⁴⁻¹⁵³ Except sugemalimab, the complex structure of all these anti-PD-L1 mAbs with PD-L1 have been determined.

Zhang and colleagues determined the crystal structures of PD-L1-atezolizumab-Fab complexes (PDB:5XXY⁴³, PDB:5X8L⁴⁴,

which showed that the V_H domain dominates the binding. All three HCDRs are involved in binding with PD-1, while only LCDR3, but not the other two LCDRs, of the V_L form contacts with PD-1. Atezolizumab is associated to the front β -sheet (C chain, C' chain, F chain, and G chain), C'C loop and FG loop of

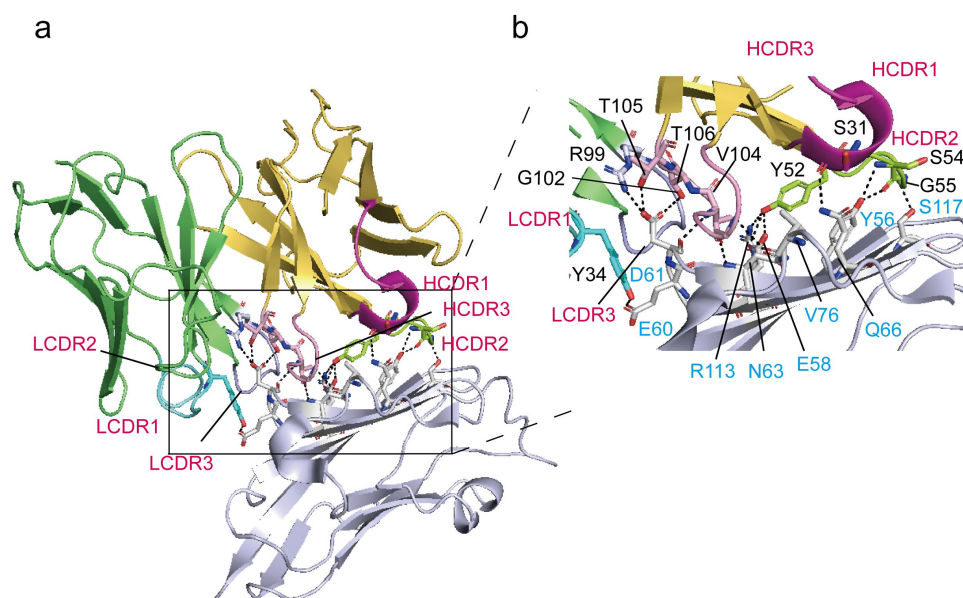


Figure 9. Structure of PD-L1-avelumab-scFv. (a) The scFv fragment of avelumab and PD-L1 is shown in ribbon mode. The avelumab-scFv are colored in green and yellow. The three HCDRs are shown in light magenta, lime and pink. The LCDRs are colored in aquamarine, deep teal and light blue. (b) Key segments that stabilize the PD-L1-avelumab-scFv. Residues forming the inter-molecular H-bonds are linked with dashed black lines.

PD-L1 via its LCDR3 and all three of its HCDRs.⁴³ The atezolizumab-Fab and PD-L1 binding interface consist of 13 hydrogen bonds and approximately 82 other interactions (polar interactions, hydrophobic interactions, and π - π stacking interactions).

The structure of the PD-L1-avelumab-scFv (PDB:5GRJ) was determined by Liu and colleagues.⁴⁶ Similar to atezolizumab, it binds PD-L1 mainly using the V_H domain. All three HCDR loops are involved in binding, whereas the V_L domain

only uses LCDR1 and LCDR3 to form partial contacts. The epitope of PD-L1 comprises parts of the β -sheets and its CC' loop. The CC' loop of PD-L1 makes multiple H-bonds with the HCDR3 and LCDR3 of avelumab, and the latter loops occupy the binding surfaces of the four chains of PD-L1 (Figure 9).

In contrast to the V_H -dominated interactions that are exhibited by atezolizumab and avelumab, Tan and colleagues determined the structure of a PD-L1-durvalumab-scFv

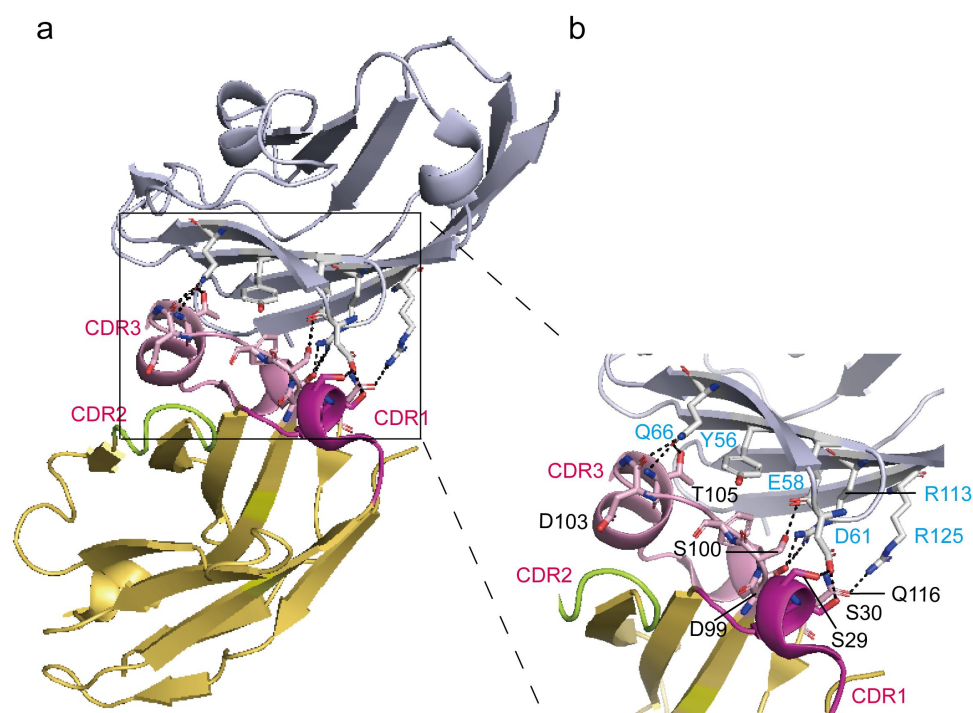


Figure 10. Structure of PD-L1-KN035-scFv. (a) Structure of PD-L1-KN035-scFv complex structure (PDB:5JDS). The scFv fragment of KN035 and PD-L1 are shown in yellow-orange and light blue, respectively. The three CDRs of KN035 are shown in light magenta, lime and pink. (b) Details of the binding interface of PD-L1-KN035-scFv. Residues forming the H-bonds are shown as sticks and labeled.

(PDB:5XJ4)⁴⁵ and identified that the V_L and V_H of durvalumab contribute equally to its binding to PD-L1. In addition, the structure of the PD-L1-durvalumab-Fab has also been reported by Heo and Lee (PDB:5X8M).⁴⁴ The three HCDRs and the LCDR1 and LCDR3 of durvalumab participate in PD-L1 binding; the LCDR1 and HCDR2 contact with the A and F chains of PD-L1, respectively, whereas the HCDR3, HCDR2 and LCDR3 of durvalumab form multiple H-bonds with G chain of PD-L1. The three mAbs- and PD-1-binding surfaces on PD-L1 are highly overlapping, which leads to competing mechanisms between mAbs and PD-1.³⁰

Structure of PD-L1-KN035

Nanobodies are highly stable and easy to produce in high yields through simple bacterial expression systems, making them a promising tool for research and treatment.¹⁵⁴ Envafolelimab (KN035) was developed by Alphamab Oncology and approved by NMPA in 2021 for advanced biliary tract cancer and soft tissue sarcoma. It was the first and currently the only nanobody approved as a cancer therapeutic. Zhou and colleagues of Alphamab Oncology⁴⁷ obtained KN035 by immunizing camels with human PD-L1 and screening a V_H-only nanobody phage library. Subsequently, the structure of KN035-PD-L1 complex was determined. It revealed that KN035 has three CDR loops (CDR1, CDR2 and CDR3),^{155,156} and that CDR1 folds into a short α helix, whereas CDR3 contains a short α helix and a 3_{10} helix. A disulfide bond connects the short α helix of CDR3 to CDR1, and another disulfide bond connects the B and F chains of KN035 (Figure 10).

The interface of KN035 and PD-L1 is composed of the CDR1 and CDR3 of KN035 and the CC'FG chains of PD-L1, mainly through hydrophilic interactions, i.e., H-bonds and salt bridges (Figure 10). KN035 binds PD-L1 on the front β -sheet of PD-L1, in a similar manner as PD-1-PD-L1, which suggested that KN035 blocks the PD-1-PD-L1 binding via a sterically hindering mechanism.

Summary and perspectives

This review summarizes the structures of FDA- and/or NMPA-approved antibodies that recognize PD-1 or PD-L1. The structures of these complexes reveal key driving forces that stabilize interactions and provide insight into the mechanisms of the antibodies to affect the PD-1-PD-L1 recognition. Although the anti-PD-1 mAbs primarily bind PD-1 on its front β -sheet and loops, each mAb exhibits a unique binding pattern that interferes with PD-1-PD-L1 interactions. In contrast, all anti-PD-L1 mAbs recognize nearly identical sites of PD-L1. In addition to the clinically approved antibodies mentioned above, there are other approved anti-PD-1 mAbs (sintilimab, penpulimab, dostarlimab, and zimberelimab) and an anti-PD-L1 mAb (sugemalimab), whose structures and interfaces with PD-1 or PD-L1 have yet to be revealed.

The comparison of the structures of mAbs targeting PD-1 or PD-L1 with the same epitopes revealed that the physicochemical properties of the amino acid residues within

the epitopes determine the type of residues within the CDRs that interact with these epitopes. Specifically, the polar residues within the epitopes tend to recognize polar or charged residues within the CDRs. Interestingly, different mAbs can utilize different CDRs to interact with the same epitope, despite having the same type of amino acid residues to recognize the epitope. Therefore, it is valuable to gather additional structural data to elucidate the correlations between CDR sequences and complex structures. This knowledge can be applied to the design of novel mAbs or the prediction of mAb-antigen structures. Moreover, it can be integrated with mAb discovery methods to draw comprehensive conclusions.

The structural data of mAbs with PD-1 or PD-L1 also facilitated the optimization of antibodies through structure-based mutagenesis. This optimization aimed to improve the affinity of the antibodies with PD-1 or PD-L1, as well as their solubility and stability.^{157–160} For instance, Bullock and colleagues analyzed the structure of nivolumab and identified specific amino acid residues that could influence the solubility of the mAb. This analysis led to the generation of a solubility-enhancing mutant of nivolumab.¹⁶¹ Horita and colleagues³³ used the structural data of the pembrolizumab-PD-1 complex to design cyclopeptide derivatives that bind to PD-1.

All the antibody structures described here do not contain the Fc region. The Fc region should have an impact on the overall structure of the antibody. Scapin and colleagues discovered the unique 120° rotation of the CH₂ domain of pembrolizumab,¹⁴⁴ and mentioned this as one possibility among many conformations. The crystal structure obtained by analytical analysis is the result of an instantaneous state, while the hinge region has great flexibility. The specific effect of Fc regions on the overall structures of complexes between mAbs described above with PD-1 or PD-L1 are still unclear. The Fc region mainly mediates the functional effects of antibodies, such as antibody-dependent cellular cytotoxicity (ADCC) and complement-dependent cytotoxicity (CDC).¹⁶² How the Fc region affects the overall structure of mAbs would reveal new structure–function relationships of anti-PD-1 and anti-PD-L1 mAbs. It is particularly important to IgG1 type anti-PD-L1 mAbs, which act on the surface of tumor cells and take advantage of Fc functions to induce the phagocytosis of target cells.¹⁶³

Another research perspective of clinical anti-PD-1 or anti-PD-L1 mAbs involves the structures in complicated physiological environments (e.g., the blood) or tumor micro-environments.¹⁶⁴ Recently, it has been shown that membrane environments have profound effects on PD-L1 activity.¹⁶⁵ However, all structures described here were obtained in crystals. Thus, structural characterization using integrative structural biology approaches, i.e., NMR spectroscopy and cryogenic electron microscopy, are anticipated to reveal the complex structures of mAbs with PD-1 or PD-L1 in a more native-like conditions, which may shed light on new aspects of the mechanisms of anti-PD-1 or anti-PD-L1 mAbs.

Abbreviations

ADCC	antibody-dependent cellular cytotoxicity
CDC	complement-dependent cytotoxicity
C _H	constant domain from heavy chain;
C _L	constant domain from light chain;
FDA	the Food and Drug Administration;
HCDR	heavy chain complementarity-determining region
ICB	immune checkpoint blockade (ICB);
IgG	immunoglobulin G;
KN035	envafolimab;
LCDR	light chain complementarity-determining region;
mAb	monoclonal antibody;
NMPA	the National Medical Products Administration;
NSCLC	non-squamous non-small-cell lung cancer;
PD-1	programmed cell death 1;
PD-L1	Programmed cell death-ligand 1;
PDB	the Protein Data Bank;
T cells	thymocytes;
V _H	variable domain from heavy chain;
V _L	variable domain from light chain

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