The molecular binding mechanism of tislelizumab, an investigational anti-PD-1 antibody, is differentiated from pembrolizumab and nivolumab

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Background

Programmed cell death protein 1 (PD-1) is an immune checkpoint receptor expressed by activated T, B, and NK cells, which interacts with its ligand PD-L1/L2 to inhibit T-cell proliferation and effector functions[1]. Tislelizumab, an investigational anti-PD-1 antibody, has demonstrated significant clinical activity (85.7% ORR, including 61.4% CR) in relapsed/refractory classical Hodgkin's lymphoma (R/R cHL)[2]. The purpose of this study is to investigate the molecular binding mechanism of tislelizumab in comparison to pembrolizumab and nivolumab, the two FDA-approved anti-PD-1 antibodies.

Methods

The x-ray co-crystal structure of PD-1/tislelizumab Fab was solved to study the molecular binding mechanism. Structure-guided mutagenesis of PD-1 and surface plasmon resonance (SPR) were performed to compare the binding of the three anti-PD-1 antibodies to mutant and wild type PD-1. Cellular P3Z assay was used to quantify the PD-L1 blocking activity of the anti-PD-1 antibodies.

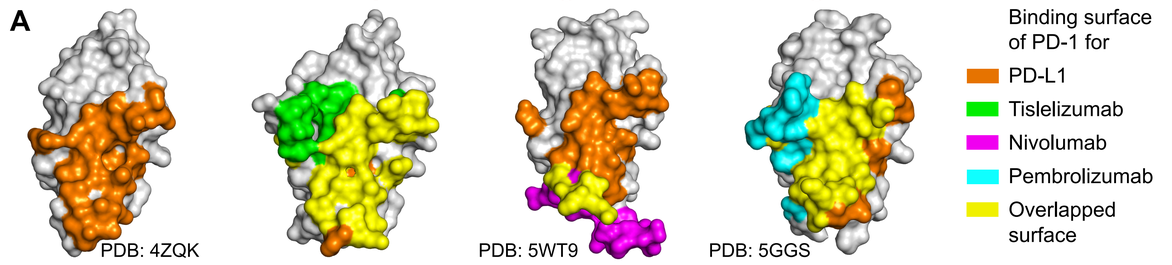
Results

The co-crystal structure of PD-1 and the Fab of tislelizumab was solved at 2.9 Å resolution (Fig. 1). Structure comparison shows that tislelizumab binds to PD-1 in an orientation different from either pembrolizumab or nivolumab (Fig. 2), and the binding surface of PD-1/tislelizumab largely overlaps with PD-1/PD-L1 interface (Fig. 3A). In addition, tislelizumab is superior to pembrolizumab and nivolumab in blocking PD-L1 binding to PD-1 in cellular P3Z assay (Fig. 3B). The dissociation rate (K_d) of tislelizumab from PD-1 is about 100-fold and 50-fold slower than that of pembrolizumab and nivolumab, respectively (Fig. 4). Mutation on Gln75, Thr76, Asp77 and Arg86 of PD-1 significantly reduces the binding affinity of PD-1 to tislelizumab, but shows little effect on binding to pembrolizumab and nivolumab (Fig. 5).

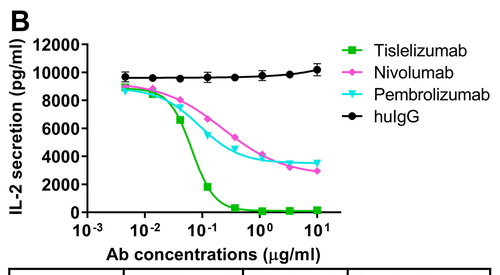
Conclusion

Both the co-crystal structure and mutagenesis study identified the unique epitopes of tislelizumab that contribute to the exceptionally slow off-rate of tislelizumab's binding to PD-1. In conclusion, we observed that tislelizumab is differentiated from pembrolizumab and nivolumab by its unique binding epitopes, binding kinetics and PD-L1 blocking activity.

Tislelizumab/PD-1 binding surface largely overlaps with PD-1/PD-L1 binding surface and leads to complete blocking of PD-L1 binding



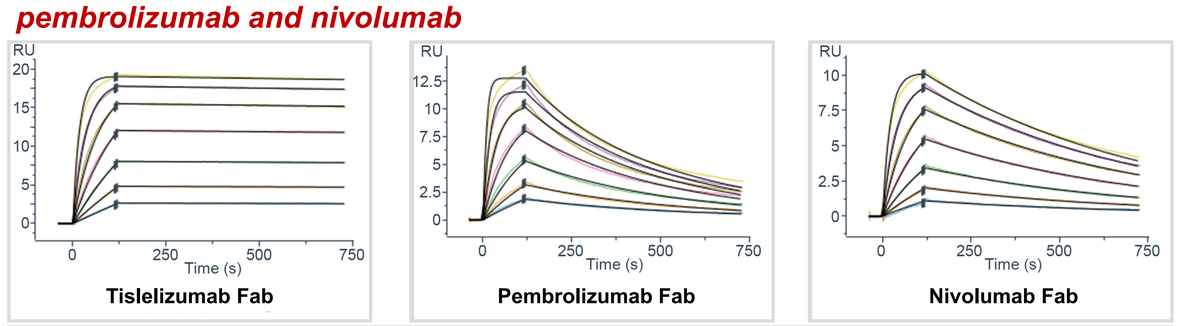
	PD-L1	Tislelizumab	Nivolumab	Pembrolizumab
Binding surface on PD-1 (Å^2)	778.2	1112.2	869.7	1120.4
Surface overlapped with that for PD-L1 (%)	-	82	44	66



	Tislelizumab	Nivolumab	Pembrolizumab
IC50 (μg/ml)	0.066	0.23	0.089

Figure 3. A. Binding surface comparison between PD-L1 and anti-PD-1 antibodies[3-5]. The surface of PD-1 molecule colored in orange, green, magenta or cyan are the binding surface for PD-L1, tislelizumab, pembrolizumab or nivolumab, respectively. The surface colored in yellow is overlapped area with PD-L1 binding surface. **B**. Comparison of tislelizumab functional activities with pembrolizumab and nivolumab by P3Z assay.

The dissociation rate of tislelizumab from PD-1 is much slower than that of pembrolizumab and nivolumab



Sample	Ka (1/Ms)	Kd (1/s)	KD (M)
Tislelizumab Fab	5.75E+05	3.43E-05	5.97E-11
Pembrolizumab Fab	1.29E+06	3.01E-03	2.32E-09
Nivolumab Fab	5.20E+05	1.66E-03	3.20E-09

Figure 4. Binding kinetics comparison between tislelizumab, pembrolizumab and nivolumab

Tislelizumab utilizes all three CDRs of V_L and CDR2 and CDR3 of V_H to interact with PD-1 to form extensive interactions

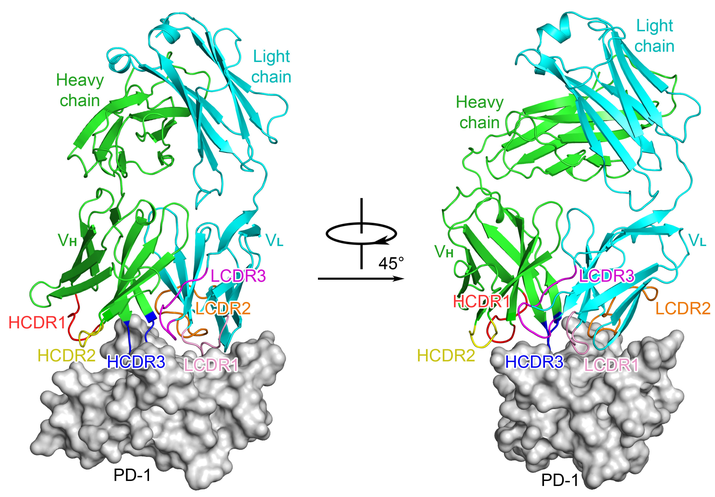


Figure 1. Overall structure of PD-1/tislelizumab Fab complex. The tislelizumab Fab is shown as a ribbon (V_H, green; V_L, cyan), and PD-1 is shown as a surface representation (gray). The HCDR1, HCDR2, HCDR3, LCDR1, LCDR2 and LCDR3 are colored in red, yellow, blue, pink, orange and magenta, respectively. Abbreviations: V_H and V_L, variable domains of heavy and light chains; CDR, complementarity determining region.

Tislelizumab binds to PD-1 in an orientation different from pembrolizumab and nivolumab

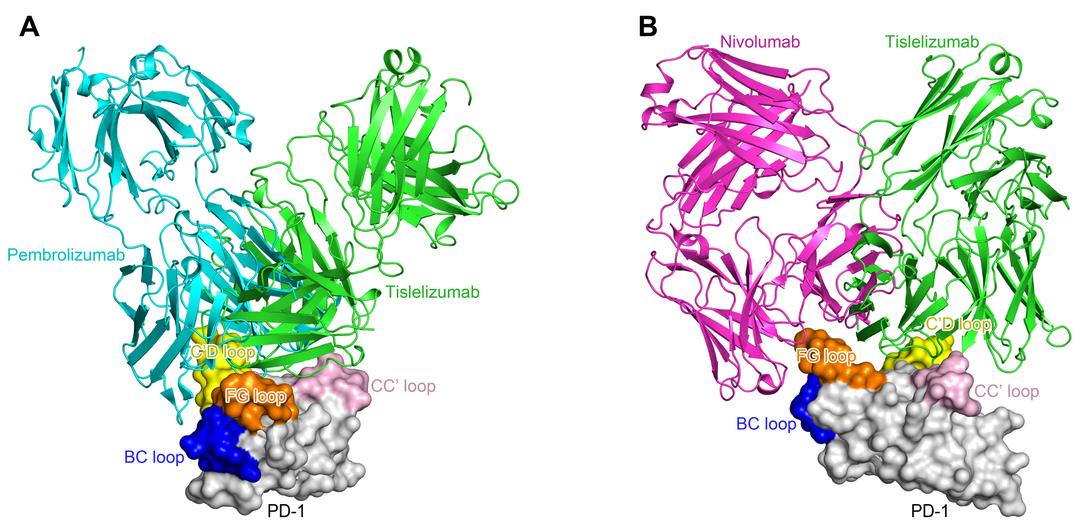


Figure 2. Distinct binding orientation compared with pembrolizumab and nivolumab. Superposition of PD-1/ tislelizumab Fab complex with that of pembrolizumab (**A**, PDB: 5GGS) and nivolumab (**B**, PDB: 5WT9)[3, 4]. PD-1, tislelizumab, pembrolizumab and nivolumab are colored in gray, green, cyan and magenta, respectively. The BC, CC', C'D and FG loops of PD-1 are colored in blue, pink, yellow and orange, respectively.

Unique epitopes of tislelizumab, Q75, T76, D77 and R86, are identified by a structure guided mutagenesis study and SPR

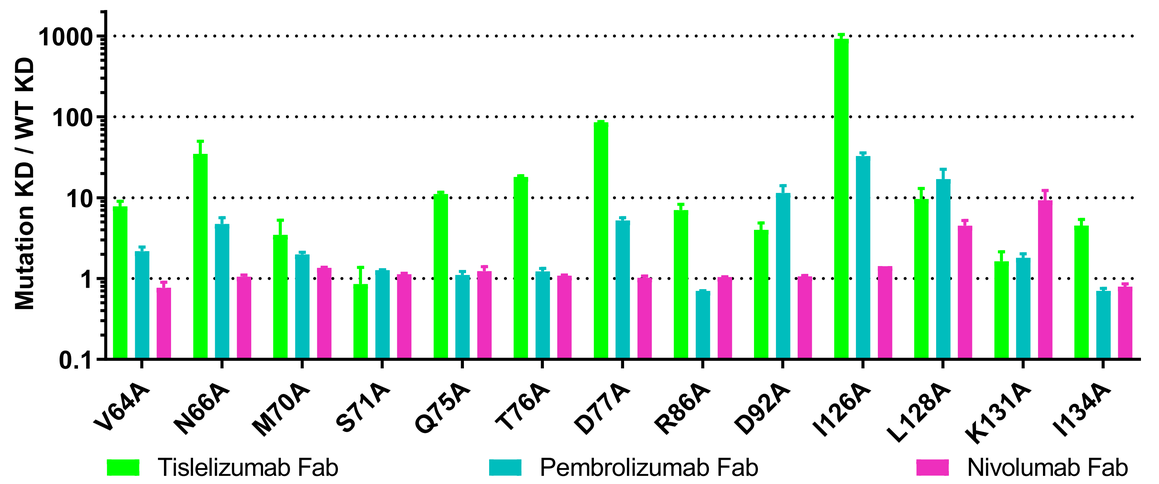


Figure 5. Epitope mapping results measured by SPR

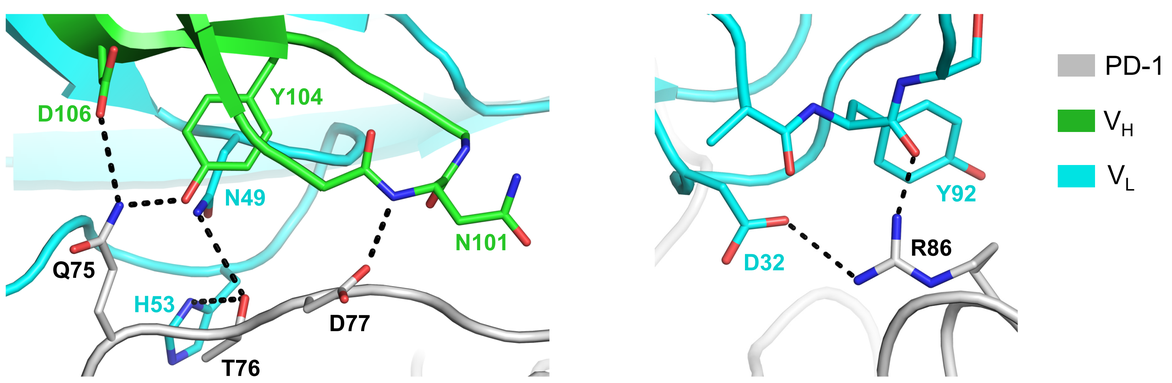


Figure 6. Detailed interactions between tislelizumab and its unique epitopes. The PD-1, V_L and V_H of tislelizumab are colored in grey, cyan and green, respectively. Hydrogen bonds and a salt bridge are indicated with black dashed lines.

References

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