Abstract #4048

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

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Introduction

Programmed cell death protein 1 (PD-1) is an immune checkpoint receptor expressed by activated T, B, and NK cells, which interacts with its ligands PD-L1/L2 to inhibit T-cell proliferation and effector functions such as tumor cell killing and cytokine production [1]. Two anti-PD-1 antibodies approved by the FDA, pembrolizumab and nivolumab, have shown efficacy in many cancer types; nevertheless there are some indications where limited efficacy is observed [2]. Tislelizumab (BGB-A317), 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) [3]. Additionally, tislelizumab is being studied in global pivotal trials in a number of malignancies, including non-small cell lung cancer, hepatocellular carcinoma, and esophageal squamous cell carcinoma [4]. Here we report the co-crystal structure of the PD-1 extracellular domain with the Fab of tislelizumab. By structure-guided mutagenesis and biacore studies, we observed that tislelizumab is differentiated structurally from pembrolizumab and nivolumab by its unique binding epitopes as well as binding kinetics.

Methods

- The ectodomain of the PD-1 protein and tislelizumab Fab were expressed in 293F cells, and were purified by protein A and Ni affinity column, respectively.
- The co-crystals of PD-1/tislelizumab Fab were cultured by vapour-diffusion sitting-drop method at 20℃.
- PD-1 mutants were generated by a standard site-directed mutagenesis method.
- Surface plasmon resonance (SPR) analysis was performed at room temperature using a BIAcore 8K system with CM5 chips.

Results

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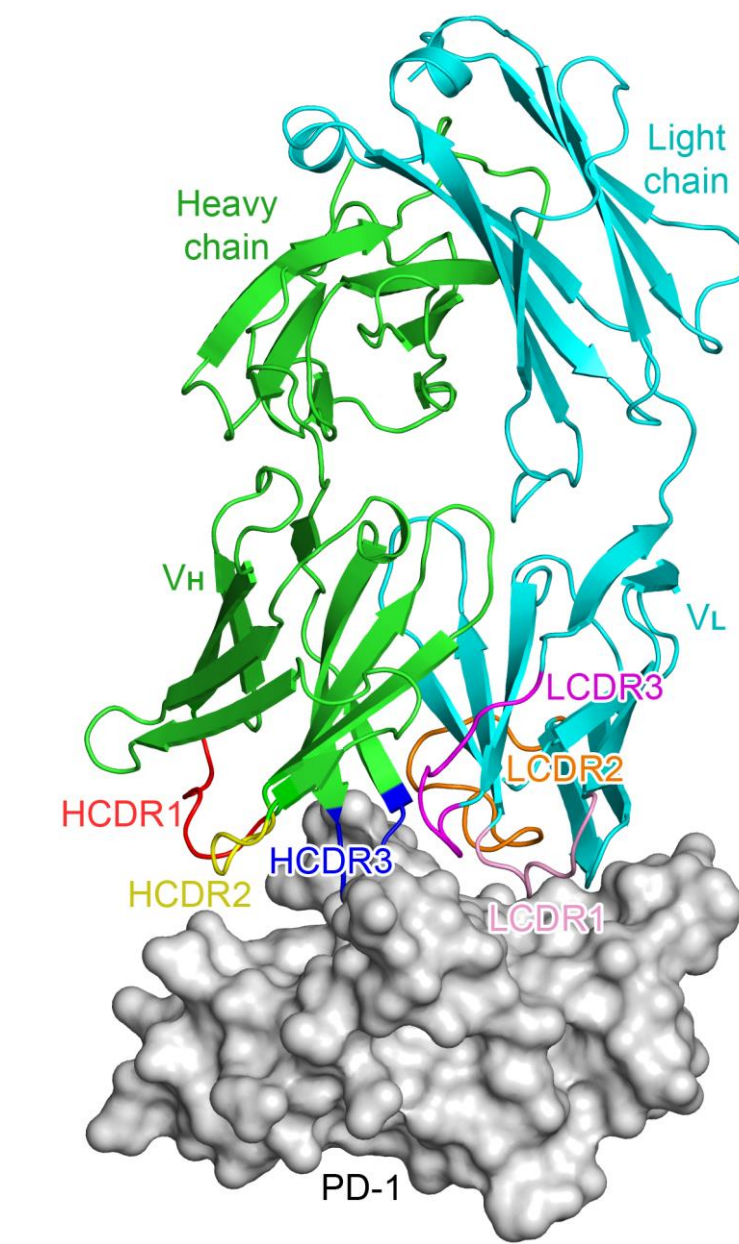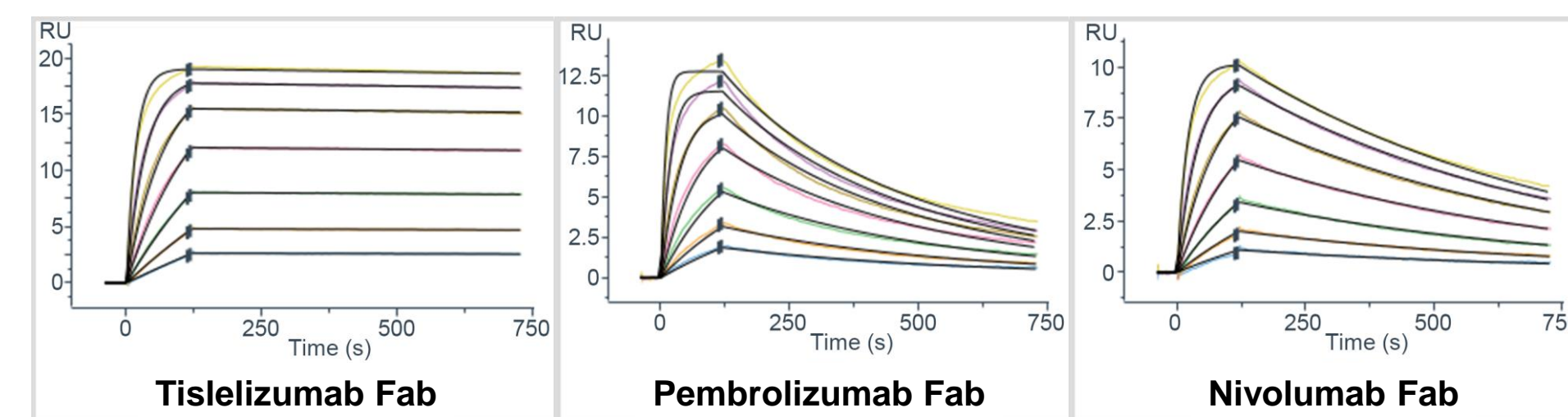

Figure 1. Overall structure of PD-1/tislelizumab Fab complex. The tislelizumab Fab is shown as a ribbon (V_H, green; V_L, cyan), and PD-1 is shown as a surface representation (gray). The HCDR1, HCDR2, HCDR3, LCDR1, LCDR2 and LCDR3 are colored in red, yellow, blue, pink, orange and magenta, respectively.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

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 2. Binding kinetics comparison between tislelizumab, pembrolizumab and nivolumab

Results

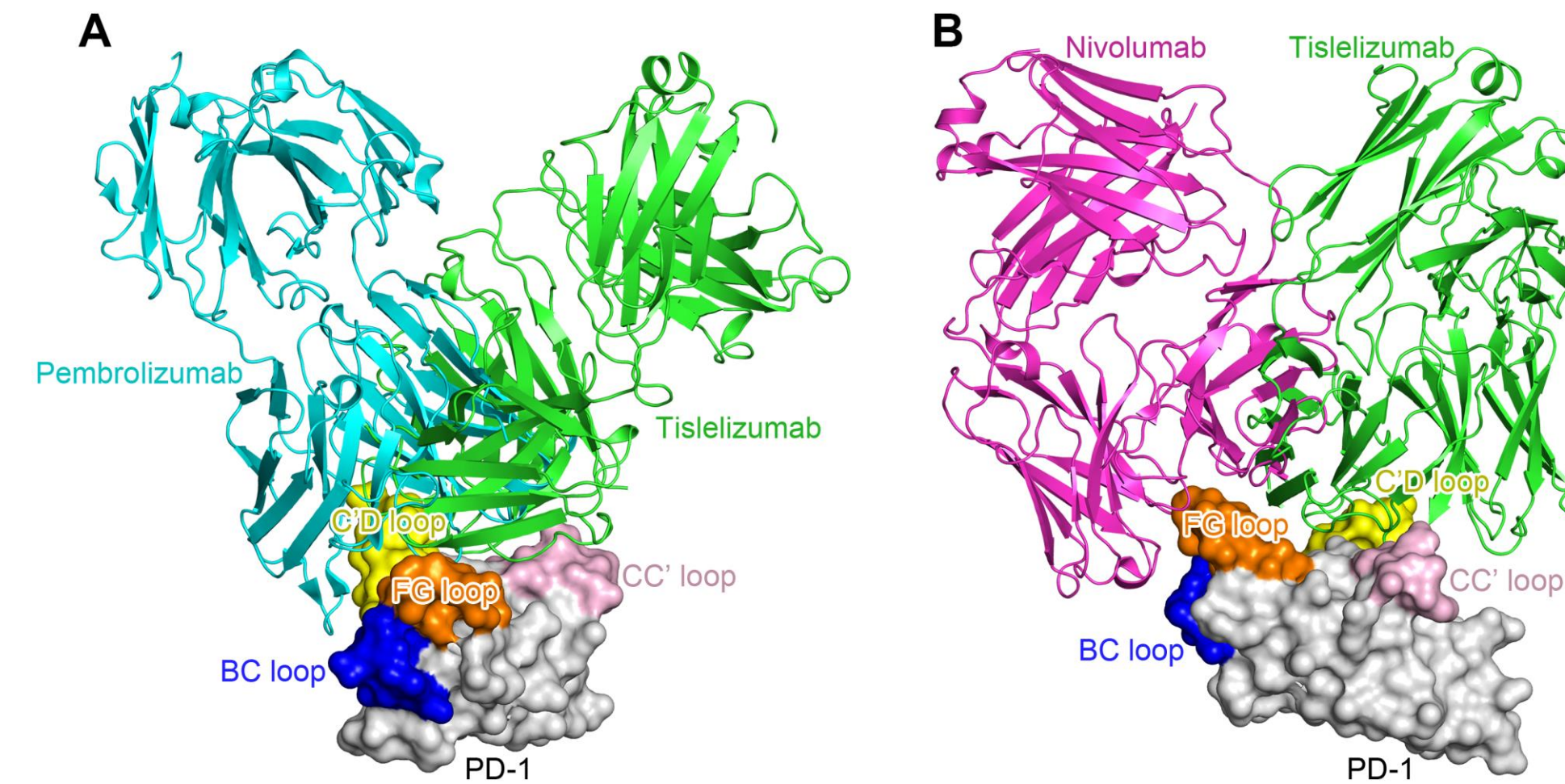### Tislelizumab binds to PD-1 in an orientation different from pembrolizumab and nivolumab

Figure 3. 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) [5, 6]. 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
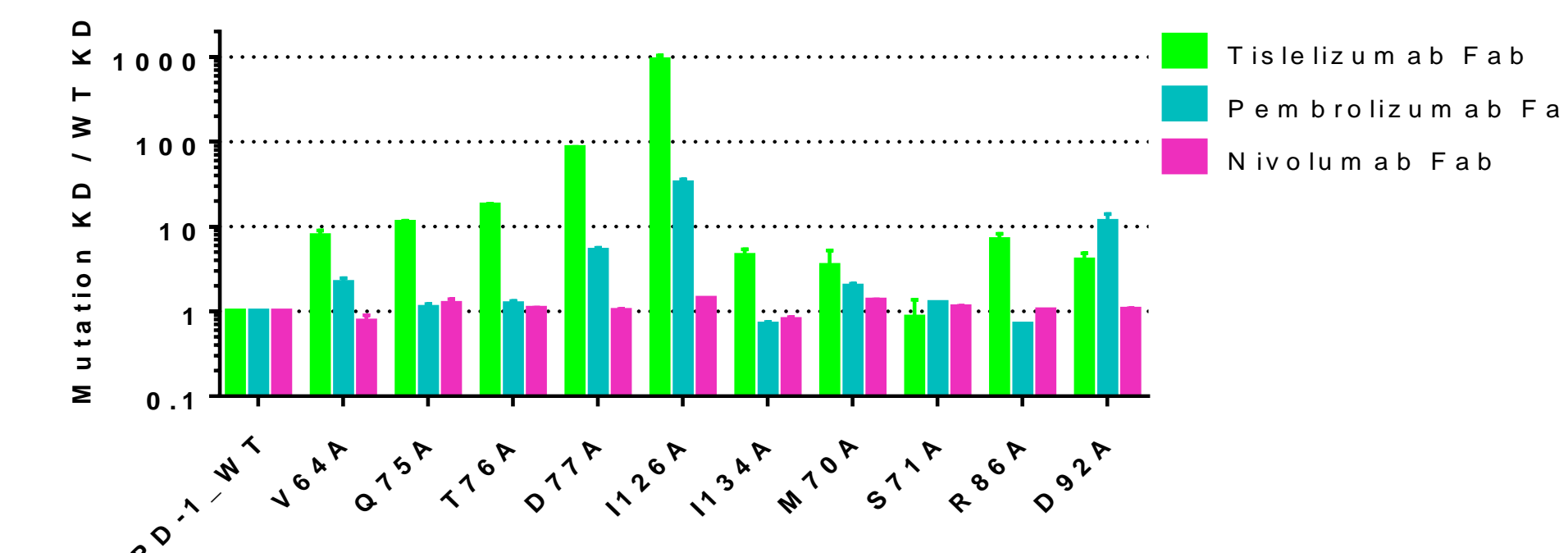

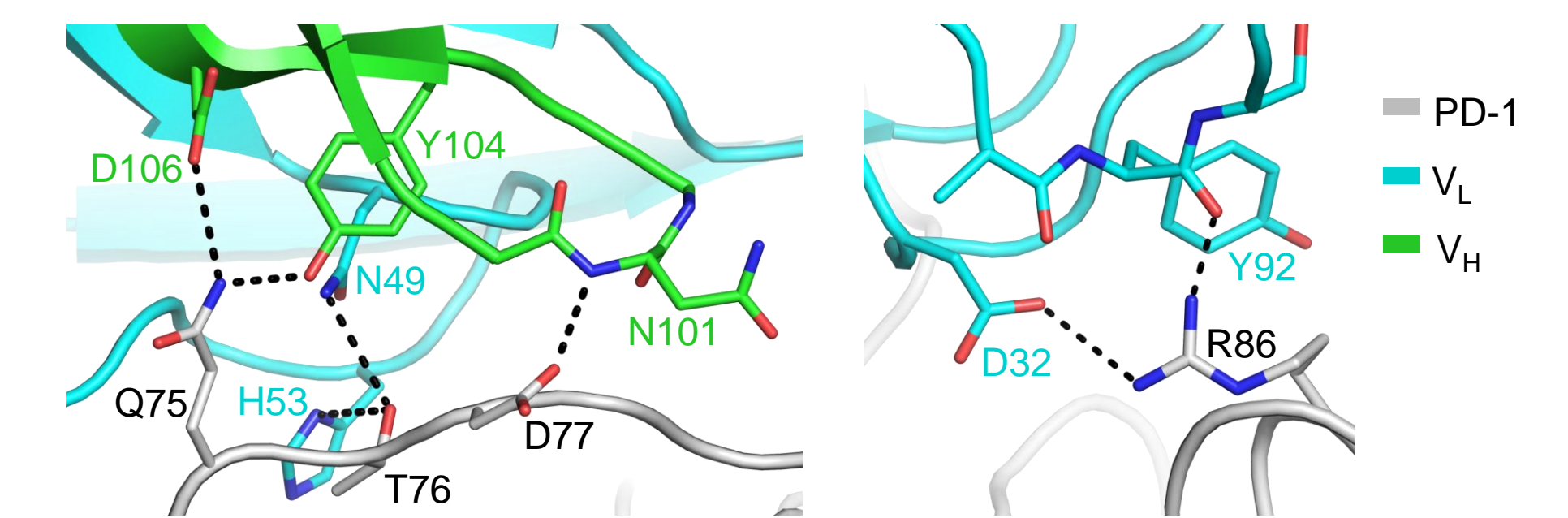**Figure 4.** Epitope mapping results measured by SPR

Results

Figure 5. 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.

Conclusion

- Tislelizumab shows higher affinity to PD-1 and an approximately 100-fold and 50-fold slower off-rate than pembrolizumab and nivolumab, respectively.
- Tislelizumab has a distinctive binding orientation to PD-1 compared to pembrolizumab and nivolumab.
- Gln75, Thr76, Asp77 and Arg86 of PD-1 have been identified as unique binding epitopes of tislelizumab.
- Tislelizumab is differentiated from pembrolizumab and nivolumab by its unique binding epitopes as well as binding kinetics.

References

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