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. 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). 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 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. Structure comparison shows that tislelizumab binds to PD-1 in an orientation different from either pembrolizumab or nivolumab, and the binding surface of PD-1/tislelizumab largely overlaps with PD-1/PD-L1 interface. In addition, tislelizumab shows superior blocking

activity of PD-L1 binding to PD-1 in cellular P3Z assay than pembrolizumab and nivolumab. 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. Mutation on Gln75, Thr76, Asp77 and Arg86 of PD-1 significantly reduces the binding affinity of PD-1 to tislelizumab, but shows relatively little effect to pembrolizumab and nivolumab.

Conclusion:

Both the co-crystal structure and mutagenesis study identified the unique epitopes of tislelizumab that contribute to the extremely slow-off property of tislelizumab after 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.