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

Yingcai Feng1, Yuan Hong1, Hanzi Sun1, Bo Zhang1, HongFu Wu1, Kang Li2, Mike Liu1, Ye Liu*  
Department of 1Discovery Biology and 2Biologics, BeiGene (Beijing) Co., Ltd., Beijing 102206, P.R.China; * Correspondence: ye.liu@beigene.com

Abstract

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

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°C.
- PD-1 mutants were generated by a standard site-directed mutagenesis method.
- Surface plasmon resonance (SPR) analysis was performed at room temperature using a BiAcrore 8K system with CM5 chips.

Results

Figure 1. Overall structure of PD-1/tislelizumab Fab complex. The tislelizumab Fab is shown as a ribbon (VH, green; VL, 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: VH and VL, variable domains of heavy and light chains; CDR, complementarily determining region. The dissociation rate of tislelizumab from PD-1 is much slower than that of pembrolizumab and nivolumab.

Figure 2. Binding kinetics comparison between tislelizumab, 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 grey, green, cyan and magenta, respectively. The BC, CC, CD and FG loops of PD-1 are colored in blue, pink, yellow and orange, respectively.

Figure 4. Epitope mapping results measured by SPR.

Figure 5. Detailed interactions between tislelizumab and its unique epitopes. The PD-1, VH and VL 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