Structural characterization of the unique pH-responsive anti-TIGIT therapeutic antibody Ociperlimab

TIGIT has attracted tremendous attention in cancer immunotherapy. As an inhibitory immune checkpoint, TIGIT is expressed on most NK and multiple T cell subsets and exerts inhibitory effects on innate and adaptive immunity by binding to its ligands, PVR (CD155) and nectin-2 [1]. Competitive binding of TIGIT and CD226 (an activating receptor) to PVR has been known as a key mechanism of TIGIT-driven immune suppression, and anti-TIGIT blocking mAbs are presumed to reverse the suppression by inhibiting TIGIT binding to PVR, thereby enhancing T cell or NK cell activity (Fig. 1 and Fig. 2(a)). Several clinical trials are currently evaluating the efficacy of anti-TIGIT mAbs in patients with different types of cancer. Ociperlimab from BeiGene and Tiragolumab from Roche block the TIGIT-PVR interaction and are in clinical development. However, the molecular blockade mechanism of these anti-TIGIT mAbs remains elusive.

We determined the crystal structures of the Ociperlimab_Fab/TIGIT complex and the Tiragolumab_Fab/TIGIT complex at resolutions of 2.4 and 2.0 Å with SPring-8 **BL45XU** beamline, respectively [2]. Competitive binding of Ociperlimab_Fab or Tiragolumab_Fab against PVR was revealed by overlaying Ociperlimab_Fab/TIGIT or the Tiragolumab_Fab/TIGIT complex on the PVR/TIGIT complex (Fig. 1). The binding of TIGIT by Ociperlimab_Fab was dominated by the front CC'C'' β-sheets, which have obvious steric clashes with PVR (Fig. 1 and Fig. 2(d)). The overlapping binding surface of Ociperlimab_Fab and PVR on TIGIT is located near the front CC'C'' β-sheets. Similarly, the binding of TIGIT by Tiragolumab_Fab is also mediated by the front CC'C''DF β-sheets. Moreover, the blockade mechanism of Ociperlimab_Fab relies on the steric hindrance of both the VH and VL domains against PVR to abolish its binding to TIGIT, whereas only the VH domain of Tiragolumab_Fab participated in the steric conflict with PVR to prevent its binding. Ociperlimab and Tiragolumab exhibit distinct binding modes toward TIGIT (Fig. 2(c)). We did not observe any obvious conformational change in TIGIT upon Ociperlimab_Fab or Tiragolumab_Fab binding compared with its apo structure (Fig. 2(b)), as both antibodies mainly bind to the β-sheet of TIGIT with rigid secondary structures (Figs. 2(c,d)). However, Ociperlimab and Tiragolumab have distinct epitopes when binding to TIGIT, implying that the antagonistic mechanism of the two antibodies could be different.

Almost all known pH-responsive antibodies sense pH due to histidine residues. pK_a value of the histidine side chain is about 6. Thus, at pH 6.0 or below, the histidine side chain is mostly protonated whereas at physiological pH 7.4 it is deprotonated. As a result, the histidine side chain could change ionization states due to subtle changes in pH, leading to its net charge varying [3]. In some cases, histidine residues in the epitopes could also contribute to the pH-responsive antibodies as demonstrated by this structural study. Our surface plasmon resonance (SPR) data shows that the binding affinity of Ociperlimab towards TIGIT increased approximately 17-fold when the pH decreased from 7.4 to 6.0, whereas Tiragolumab did not show obvious pH-dependent binding to TIGIT (Fig. 3(c)). This observation is further supported by the fluorescence-activated cell sorting experiment which shows the enhanced binding of Ociperlimab to TIGIT when the pH shifts from 7.4 to 6.0 (Fig. 3(d)). In the Ociperlimab_Fab/TIGIT crystal structure, $Asp103_{HCDR3}$ is well positioned with respect to $His76_{TIGIT}$ at a 2.7 Å distance and forms a strong electrostatic interaction, whereas no favorable architecture surrounding $His76_{TIGIT}$ in Tiragolumab_Fab/TIGIT was observed (Figs. 3(a,b)). Consistent with the critical role of $His76_{TIGIT}$ in Ociperlimab binding, a substantial decrease in the binding affinity

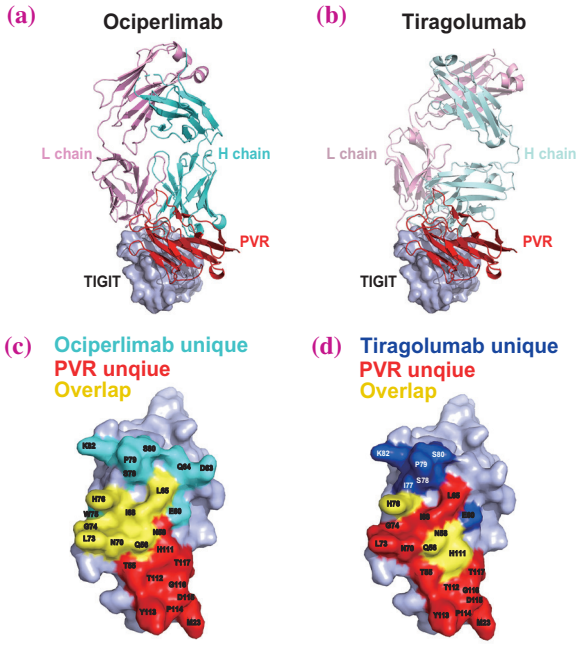


Fig. 1. Competitive binding of Ociperlimab_Fab or Tiragolumab_Fab against PVR for TIGIT. **(a)** Superposition of Ociperlimab_Fab/TIGIT with the PVR/TIGIT (PDB: 3UDW) complex when aligned via TIGIT. **(b)** Superposition of Tiragolumab_Fab/TIGIT with the PVR/TIGIT complex when aligned via TIGIT. **(c)** The binding surface of TIGIT with PVR or Ociperlimab_Fab. **(d)** The binding surface of TIGIT with PVR or Tiragolumab_Fab.

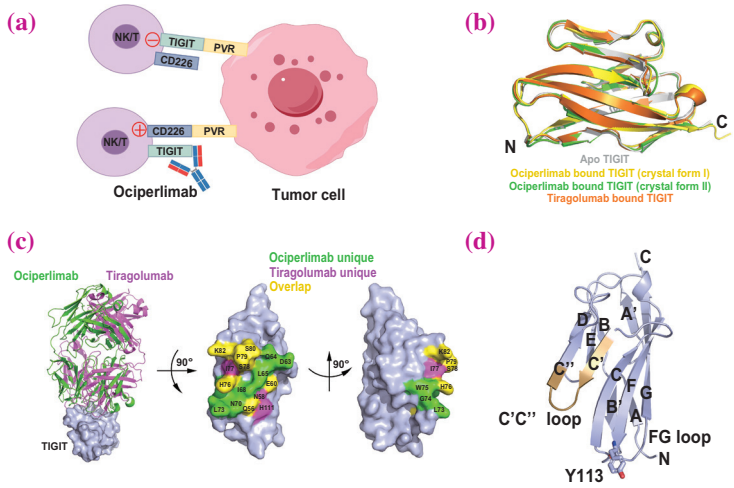
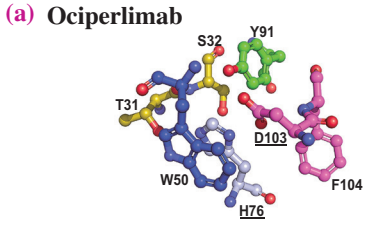
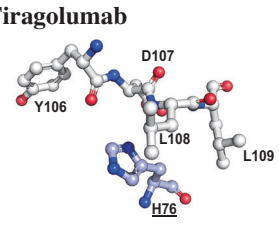


Fig. 2. Mechanisms of action (MOA) of anti-TIGIT therapeutic antibodies. **(a)** A schematic diagram for MOA of anti-TIGIT antibodies. **(b)** Superposition of Fab-bound TIGIT with apo TIGIT (PDB: 3UCR). **(c)** Superposition of Ociperlimab_Fab/TIGIT with the Tiragolumab_Fab/TIGIT complex. **(d)** Structural overview of the TIGIT IgV domain.

of Ociperlimab to $H76A_{TIGIT}$ mutant was observed at pH 6.0, with a nearly 24,303-fold reduction (Fig. 3(c)). Meanwhile, Ociperlimab displayed comparable binding affinities to $H76A_{TIGIT}$ mutant at pH 6.0 and pH 7.4, confirming the critical role of $His76_{TIGIT}$ in mediating this pH-dependent effect for Ociperlimab. These findings clearly demonstrate that His76 of TIGIT is indeed pivotal for the pH-dependent binding characteristics of Ociperlimab. Therefore, Ociperlimab has a stronger binding affinity with TIGIT under acidic pH conditions than physiological conditions, which could be valuable in targeting acidified tumor microenvironment and reducing on-target off-tumor toxicities.

In summary, our structural studies on Ociperlimab and Tiragolumab have revealed the molecular blockade mechanism of these antibodies on TIGIT-driven immune suppression. The Ociperlimab competes with PVR towards TIGIT, which is consistent with previous functional studies by Chen *et al.* [4]. In addition, the pH-responsive property of Ociperlimab, rationalized in our structures, could potentiate the cytotoxicity of immune cells toward cancer cells in the tumor microenvironment. Therefore, the present complex structures should provide useful insights into improving the effectiveness of immunotherapeutic antibody development.





(c)

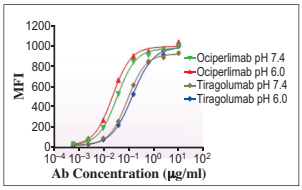
TIGIT binding kinetics with Ociperlimab under different pHs

Analyte	Kon ($M^{-1}s^{-1}$)	Koff (s^{-1})	Ociperlimab KD (M)
TIGIT (WT) pH 7.4	2.4 E+5	1.27 E−4	5.32 E−10
TIGIT (WT) pH 6.0	2.94 E+6	9.28 E−5	3.16 E−11
TIGIT (H76A) pH 7.4	N.A.	N.A.	9.79 E−7
TIGIT (H76A) pH 6.0	N.A.	N.A.	7.68 E−7

TIGIT binding kinetics with Tiragolumab under different pHs

Analyte	Kon ($M^{-1}s^{-1}$)	Koff (s^{-1})	Tiragolumab KD (M)
TIGIT (WT) pH 7.4	1.75 E+5	1.03 E−4	5.88 E−10
TIGIT (WT) pH 6.0	9.05 E+5	4.60 E−4	5.08 E−10
TIGIT (H76A) pH 7.4	6.81 E+5	1.41 E−4	2.06 E−10
TIGIT (H76A) pH 6.0	7.57 E+5	2.42 E−4	3.20 E−10

(d)



Sample code	Ociperlimab pH 7.4	Ociperlimab pH 6.0	Tiragolumab pH 7.4	Tiragolumab pH 6.0
Top, MFI	972.3	990.3	919.6	988
RV of top (%)	100	102	95	102
EC_{50} (μg/ml)	0.03512	0.02298	0.09067	0.1407
RV of EC_{50} (%)	100	153	100	64
Hillslope	1.16	1.177	1.196	1.047
RV of Hillslope (%)	100	101	103	90
R^2	0.9976	0.998	0.9985	0.9975

Fig. 3. Structural basis of the pH-dependent property of Ociperlimab towards TIGIT. **(a)** The surrounding residues of $HIS76_{TIGIT}$ within Ociperlimab. **(b)** The surrounding residues of $HIS76_{TIGIT}$ within Tiragolumab. **(c)** SPR binding kinetics of the interactions of Ociperlimab or Tiragolumab with TIGIT at different pH values. **(d)** Cell binding affinity measurement on Ociperlimab or Tiragolumab toward TIGIT under different pHs.

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