



Development and In Vitro Characterization of a Novel Anti-IL-6/Anti-PD-L1 Bispecific Antibody



Lu Yang¹, A. Aili¹, Baoshan Cao¹

¹Department of Medical Oncology and Radiation Sickness, Peking University Third Hospital, Beijing, China

INTRODUCTION

Although immune checkpoint inhibitors (ICIs) have revolutionized the therapeutic landscape for non-small cell lung cancer (NSCLC), the emergence of resistance remains a critical clinical hurdle.

Interleukin-6 (IL-6) has been identified as a pivotal mediator of the immunosuppressive tumor microenvironment (TME) and a primary driver of ICI resistance. Preclinical investigations have indicated that dual blockade of IL-6 and PD-L1 exerts synergistic anti-tumor activity and promotes CD8⁺ T cell infiltration. However, conventional combinatorial strategies are often constrained by disparate pharmacokinetics and cumulative toxicities. To address these limitations, we developed a novel bispecific antibody (BsAb) simultaneously targeting IL-6 and PD-L1.

METHODS

We designed and constructed a novel anti-IL-6/anti-PD-L1 BsAb. Recombinant expression was performed in a mammalian system, followed by purification via affinity chromatography. Structural integrity and purity were assessed using SDS-PAGE and size-exclusion high-performance liquid chromatography (SEC-HPLC). Target binding potency (EC₅₀) was quantified by enzyme-linked immunosorbent assay (ELISA), while binding kinetics (KD, kon, koff) were determined via bio-layer interferometry (BLI).

RESULTS

SEC-HPLC analysis confirmed a high degree of purity (97.54%) with a distinct monodisperse peak. ELISA demonstrated simultaneous and potent binding to both recombinant IL-6 and PD-L1, with EC₅₀ values of 0.223 nM and 0.275 nM, respectively. Kinetic characterization via BLI revealed high-affinity binding, with KD values of 2.84×10^{-10} M for IL-6 and 5.17×10^{-9} M for PD-L1. Notably, the anti-IL-6 arm of the BsAb exhibited enhanced affinity relative to its monoclonal counterpart (KD = 1.01×10^{-9} M), suggesting potential intramolecular synergy.

RESULTS

Figure 1. A. Schematic structure of the bispecific anti-IL-6/anti-PD-L1 bispecific antibody (BsAb). The antibody comprises an IL-6-targeting IgG1 antibody and a PD-L1-targeting VHH nanobody, with the anti-PD-L1 VHH nanobody linked to the C-terminus of the anti-IL-6 IgG1 heavy chain via a flexible peptide linker.

B. Physicochemical characterization of the anti-IL-6/anti-PD-L1 BsAb.

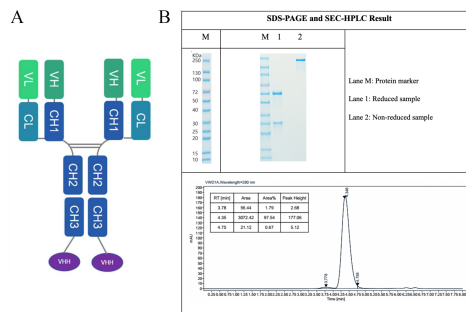
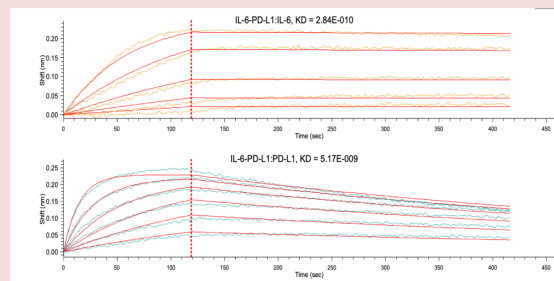


Figure 2. Bio-layer interferometry (BLI) kinetic analysis of the anti-IL-6/anti-PD-L1 BsAb binding to IL-6 or PD-L1. The BsAb exhibited a KD of 2.84×10^{-10} M for IL-6 and 5.17×10^{-9} M for PD-L1. Notably, compared with the corresponding parental monoclonal antibodies (anti-IL-6 mAb KD = 1.01×10^{-9} M; anti-PD-L1 mAb KD = 3.73×10^{-10} M), the anti-IL-6 arm of the bispecific antibody displayed enhanced binding affinity (lower KD), suggesting a potential intramolecular synergistic effect.

Sample	Fixed	Conc. (nM)	koff (1/s)	kon (1/M·s)	KD (M)	Response	Full R ²
Anti IL-6	IL-6	3.13–50	4.99E-04	4.97E+05	1.01E-09	0.0204–0.0984	0.9463
IL-6-PD-L1	IL-6	6.25–100	4.78E-05	1.68E+05	2.84E-10	0.0085–0.2215	0.9912
Anti PD-L1	PD-L1	3.13–100	1.43E-04	3.83E+05	3.73E-10	0.0951–0.5591	0.9987
IL-6-PD-L1	PD-L1	6.25–200	1.77E-03	3.43E+05	5.17E-09	0.0482–0.244	0.9875

Table note. Conc., analyte concentration range; KD, equilibrium dissociation constant (Molar); kon and koff, association and dissociation rate constants; Response, sensor response range; Full R², goodness of fit.

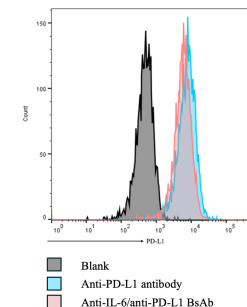
Figure 3. BLI kinetic analysis of the anti-IL-6/anti-PD-L1 BsAb binding to IL-6 (top, KD = 2.84×10^{-10} M) and PD-L1 (bottom, KD = 5.17×10^{-9} M). Individual traces show binding/dissociation at different analyte concentrations; the red dashed line marks the association-to-dissociation switch. Calculated equilibrium dissociation constants (KD) indicate high-affinity binding to IL-6 and nanomolar binding to PD-L1.



RESULTS

Figure 4.

Flow cytometric analysis of binding of the anti-IL-6/anti-PD-L1 BsAb to PD-L1-high human H292 cells. Flow cytometry showed that both the anti-PD-L1 monoclonal antibody and the anti-IL-6/anti-PD-L1 BsAb shifted the PD-L1 signal to the right compared with the blank control, indicating PD-L1 binding on cells. The bispecific antibody showed a slightly lower/left-shifted signal relative to the anti-PD-L1 monoclonal antibody, suggesting comparable cell-surface binding signal under this assay condition.



CONCLUSION

We successfully engineered a structurally stable, high-purity anti-IL-6/anti-PD-L1 bispecific antibody with robust dual-targeting capabilities. These in vitro findings demonstrate that the BsAb maintains or exceeds the binding potency of the parental monoclonal antibodies, establishing a robust foundation for further evaluation of its in vivo efficacy and potential to overcome immunotherapy resistance.

FUNDING

This work was supported by the National Natural Science Foundation of China (NSFC) [Grant No. 82202992], Youth Science Fund Project (Category C) and Young Backbone Talent Fund of Peking University Third Hospital (BYSYQY202604).

ACKNOWLEDGEMENTS

The authors declare that they have no competing interests.

Email: yanglu_0508@126.com