

Optimized Second-Generation IL-4R α Inhibition: Structural and Molecular Dynamics Properties of Rademikibart Fab-IL-4R α Complex

Yuanjun Shi¹, Minh Ho², Haote Li¹, Raul Collazo³, Christopher Bunick*²

¹*Yale University, Chemistry, New Haven, United States*, ²*Yale University School of Medicine, Dermatology, New Haven, United States*, ³*Connect Biopharma, San Diego, United States*

Introduction: Global Burden of Disease studies showed atopic dermatitis (AD) and asthma are the top two immune-mediated inflammatory diseases at younger age [1]. Dupilumab was the initial first-generation interleukin-4 receptor alpha (IL-4R α) inhibitor for treating both type I and type II IL-4R α -dependent inflammatory disorders. Following recent clinical trials, rademikibart (previously, CBP-201) emerges as an optimized next-generation human monoclonal antibody with higher binding affinity to IL-4R α compared to dupilumab [2]. It demonstrated better effect in inhibiting STAT6 intracellular signaling *in vitro* and provided similar potency inhibiting both IL-4 induced TARC release and IL-4 induced B cell activation [2].

Materials & Methods: X-ray crystallography was used to determine the atomic resolution 3D structure of rademikibart fragment antigen binding (Fab) bound to IL-4R α . This structure was analyzed and compared computationally with the 2.82 Å resolution crystal structure of dupilumab Fab bound to IL-4R α (Protein Data Bank Code 6WGL). Molecular dynamics studies on rademikibart and dupilumab bound to IL-4R α examined the stability of the complexes and effects of amino acid mutations on complex formation.

Results: The x-ray crystal structure of rademikibart Fab bound to IL-4R α was determined at 2.71Å and compared to the complex of dupilumab Fab and IL-4R α . The rotation angle between dupilumab and rademikibart bound to IL-4R α is 59.17°. This rotation enables the epitope of rademikibart, but not dupilumab, on IL-4R α to overlap more closely with the conserved binding interface utilized by IL-4 and IL-13 cytokines. Molecular dynamics simulations of rademikibart Fab and dupilumab Fab complexed with IL-4R α showed the third interface loop (residues 148 to 152 in domain 2) of IL-4R α interacts directly with rademikibart, which is absent in dupilumab/IL-4R α complex. This finding is confirmed by analysis of the hydrogen bond interactions at the interface between the antibodies and IL-4R α , demonstrating superior binding energy for rademikibart. Through single amino acid mutation analysis on rademikibart, we identified residue Y50 on rademikibart as the key residue interacting with IL-4R α 's third interface loop.

Conclusion: Our data provide a molecular and structural rationale for the enhanced IL-4R α inhibition by rademikibart over dupilumab, confirming rademikibart as an optimized second-generation IL-4R α inhibitor.

References:

1. Shin, Y.H., et al., *Global, regional, and national burden of allergic disorders and their risk factors in 204 countries and territories, from 1990 to 2019: A systematic analysis for the Global Burden of Disease Study 2019*. Allergy, 2023. **78**(8): p. 2232- 2254.
2. Zhang, L., et al., *Preclinical immunological characterization of rademikibart (CBP-201), a next-generation human monoclonal antibody targeting IL-4R α , for the treatment of Th2 inflammatory diseases*. Sci Rep, 2023. **13**(1): p. 12411.

Acknowledgement: This study was supported by Connect Biopharma