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

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INTRODUCTION

Inhibiting interleukin-4 receptor alpha (IL-4Rα) with targeted antibodies is major mechanism for alleviating Type II immune responses underlying atopic dermatitis (AD) and asthma [1-5]. Dupilumab is a first-generation IL-4Rα antibody inhibitor that binds to IL-4Rα to block its interaction with IL-4 and IL-13/IL-13Rα1 [1]. Despite much clinical success with dupilumab in the treatment of AD and asthma, many patients fail to respond, experience incomplete response, suffer waning efficacy over time or experience adverse effects such as conjunctivitis and arthralgias [6]. This raises the question, "What is it about dupilumab's molecular function that limits its clinical efficacy and potentially contributes to adverse events?" To answer this question, we performed a detailed molecular analysis of dupilumab compared to a new, second-generation IL-4Rα blocker, rademikibart.

Rademikibart (formally, CBP-201) is an optimized next-generation human monoclonal antibody targeting IL-4Rα. Compared to dupilumab, rademikibart demonstrated better inhibition of STAT6 intracellular signaling, provided similar potency in prohibiting both IL-4-induced TARC release and IL-4-induced B cell activation and has more than 2-fold higher binding affinity to IL-4Rα compared to dupilumab (20.7 pM vs 45.8 pM, respectively) [7]. Phase 3 clinical trials demonstrated that ~48% of dupilumab-treated patients achieve EASI-75 at Week 16 [8], in contrast to ~63% for rademikibart [9].

Here, we determined the 2.71Å x-ray crystal structure of rademikibart Fab bound to IL-4Rα and compared it to the complex of dupilumab Fab and IL-4Rα. Molecular dynamics simulations of rademikibart Fab and dupilumab Fab bound to IL-4Rα demonstrated the third interface loop (loop L5, residues 148 to 152) of IL-4Rα interacts directly with rademikibart, which is absent in the dupilumab/IL-4Rα complex. This finding is confirmed by analysis of the hydrogen bond interactions at the interface between the antibodies and IL-4Rα, demonstrating superior binding energy for rademikibart. Our data provides a molecular and structural rationale for the enhanced IL-4Rα inhibition by rademikibart over dupilumab [10-11], which corresponds to rademikibart's optimized epitope on IL-4Rα that overlaps more closely with the natural IL-4 epitope.

MOLECULAR DYNAMICS TRAJECTORIES

Rademikibart forms a more stable complex with IL-4Rα

SCAN ME

Dupilumab/IL-4Rα

SCAN ME

Rademikibart/IL-4Rα

IL-4 AND IL-13 BIND IL-4Rα USING A CONSERVED MECHANISM

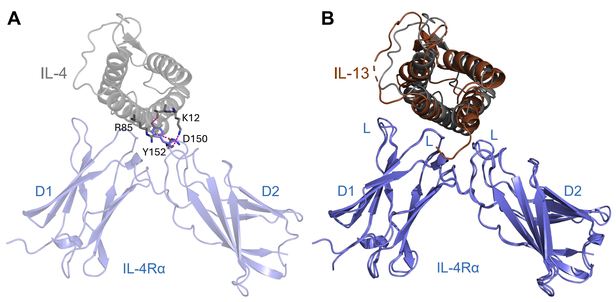

Figure 1. Crystal structure of the IL-4-IL-4R-α complex (PDB: 3bpl) at 2.93 Å resolution. H bond interactions between the L5 loop of IL-4Rα and IL-4 are shown in red dash lines. Residues involved in interactions are shown in sticks (D150 and Y152 on IL-4Rα and R85 and K12 on IL-4) (**A**). Alignment of IL-13-IL-4Rα complex and IL-4-IL-4Rα complex (**B**). Importantly, both IL-4 and IL-13 engage three major loops (L) on domains 1 (D1) and 2 (D2) of IL-4Rα.

STRUCTURE OF RADEMIKIBART Fab COMPLEXED WITH IL-4Rα

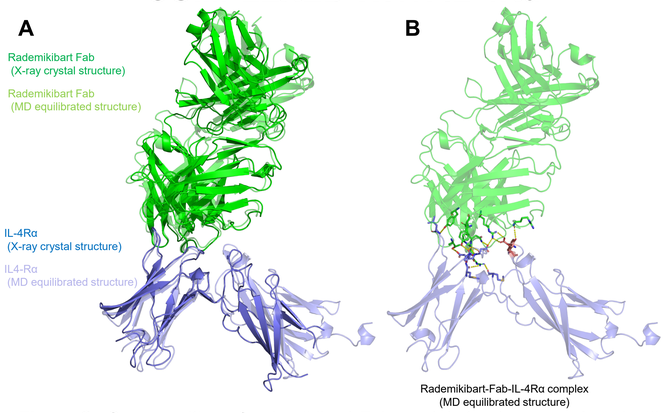

Figure 2. Superposition of rademikibart-Fab crystal structure complexed with IL-4Rα at **2.71 Å** resolution and the equilibrated structure of the same complex derived from MD simulations (**A**). Multiple H bond interactions comprise the binding interface of rademikibart-Fab and IL-4Rα at equilibrium (**B**).

CRYSTAL STRUCTURE B FACTOR ANALYSIS SHOWS RADEMIKIBART FORMS A MORE STABLE COMPLEX THAN DUPILUMAB

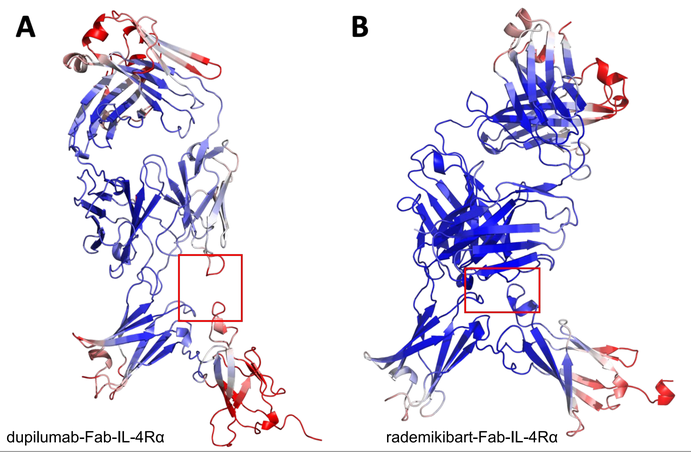

Figure 4. Equilibrated structures of dupilumab-Fab-IL-4Rα (**A**) and rademikibart-Fab-IL-4Rα (**B**) complexes fitted to MD-derived X-ray electron density maps. B factors, which represent motion or elevated protein dynamics when high, are shown in blue-to-red spectrum (low-to-high). Red boxes show the major area of B factor difference at the binding interface of the two complexes. The average B factor of the L5 loop is **133.22 Å²** for dupilumab complex and **53.42 Å²** for rademikibart complex. In addition, the L5 loop is positioned farther away from dupilumab than from rademikibart. This data demonstrates that antibody-antigen binding is more stable for rademikibart over dupilumab.

RADEMIKIBART BINDING INTERFACE WITH IL-4Rα CONTAINS MORE HYDROGEN BONDS THAN THE DUPILUMAB-IL-4Rα COMPLEX

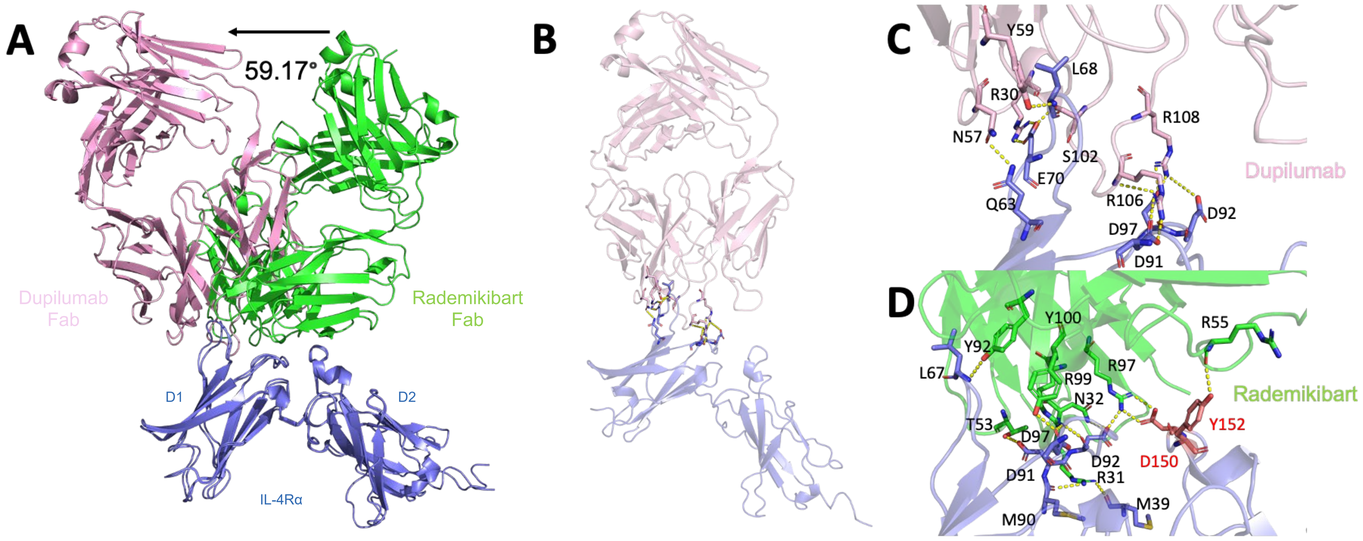

Figure 3. Comparison of the aligned (via IL-4Rα) dupilumab and rademikibart complexes with IL-4Rα. There is a 59.17° rotation of rademikibart on IL-4Rα compared to dupilumab (**A**). H bond interactions at the binding interface of dupilumab Fab and IL-4Rα at equilibrium (**B**). Note how dupilumab does not engage IL-4Rα D2. Close-up views of dupilumab and rademikibart complexes with IL-4Rα showing that the interacting residues at L5 loop of IL-4Rα (D150 and Y152, highlighted in red) are unique to rademikibart and provide enhanced antibody-antigen stability (**C**, **D**).

RADEMIKIBART'S EFFICACY AND CLINICAL RESPONSE IS MAINTAINED AT HIGH LEVELS THROUGH WEEK 52

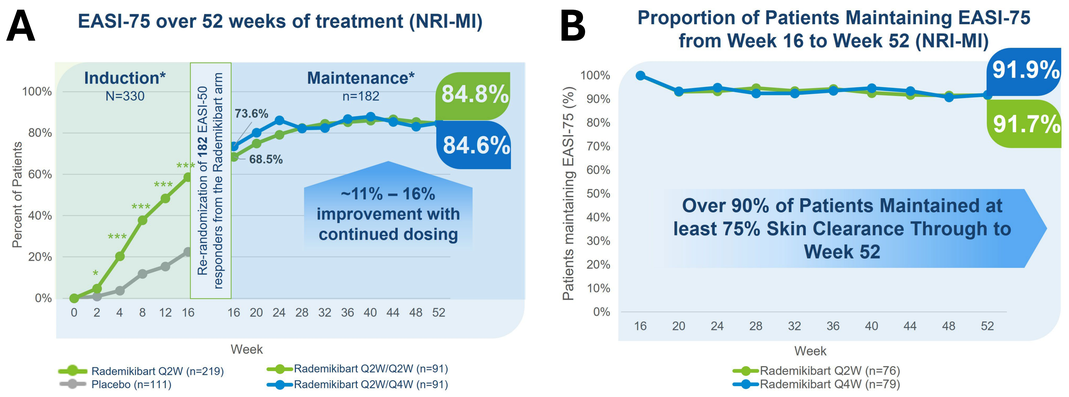

Figure 5. ~85% of patients achieved EASI-75 with rademikibart treatment at 52 weeks. Over 90% of those achieving this level of clearance (EASI-75) at week 16 were able to maintain their response through 52 weeks with either Q2W or Q4W dosing frequency.

*EASI, Eczema Area and Severity Index. EASI-50, EASI-75, and EASI-90 (i.e. at least 50%, 75%, and 90% decreases from baseline). IGA 0/1, Investigator Global Assessment (Clear/Almost Clear assessment). NRI-MI, Non-responder imputation for rescue medications and multiple imputation for remaining missing data.
Full Expanded Patient Population (N=330)

CONCLUSIONS

- IL-4 and IL-13 use a conserved structural mechanism to bind IL-4Rα, which spans both domains 1 and 2 of IL-4Rα.
- Dupilumab binds to IL-4Rα through only domain 1, the consequence being it incompletely engages the natural IL-4/IL-13 epitope on IL-4Rα.
- Molecular dynamics studies revealed that the binding interface between dupilumab Fab and IL-4Rα is highly mobile and weaker than that of rademikibart.
- The x-ray crystal structure of rademikibart Fab-IL-4Rα complex revealed an ~60 degree rotation of rademikibart on IL-4Rα compared to dupilumab, optimizing its interference with the natural IL-4/IL-13 epitope.
- Molecular dynamics studies showed rademikibart forms a very strong and stable interaction with IL-4Rα, confirmed structurally by lower B-factors (less motion) and more hydrogen bonds (stronger binding) than dupilumab.
- Rademikibart is a next-generation, optimized antibody inhibitor of IL-4Rα with 2-fold stronger binding affinity than dupilumab and clinically significant and durable EASI-75 responses over 52 weeks of treatment.

ACKNOWLEDGEMENTS

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DISCLOSURES

CGB has served as investigator or consultant for AbbVie, Apogee, Connect BioPharma, Eli Lilly, Incyte, LEO Pharma, Pfizer, and Sanofi-Regeneron. RC is an employee of Connect Biopharma. Other authors report no conflicts.

REFERENCES

1. Le Floc'h, A., et al., Dual blockade of IL-4 and IL-13 with dupilumab, an IL-4Ralpha antibody, is required to broadly inhibit type 2 inflammation. Allergy, 2020. 75(5): p. 1188-1204.
2. Ricciardolo, F.L.M., F. Bertolini, and V. Carriero, The Role of Dupilumab in Severe Asthma. Biomedicines, 2021. 9(9).
3. Scioscia, G., et al., Switching Biological Therapies in Severe Asthma. Int J Mol Sci, 2023. 24(11).
4. Collaborators, G.I., Global, regional, and national incidence of six major immune-mediated inflammatory diseases: findings from the global burden of disease study 2019. EClinicalMedicine, 2023. 64: p. 102193.
5. 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.
6. Bieber, T., Interleukin-13: Targeting an underestimated cytokine in atopic dermatitis. Allergy, 2020. **75**(1): p. 54-62.
7. Wang, J., et al., Rademikibart (CBP-201), a next-generation monoclonal antibody targeting human IL-4Ralpha: Two phase I randomized trials, in healthy individuals and patients with atopic dermatitis. Clin Transl Sci, 2023. **16**(12): p. 2614-2627.
8. D. Thaçi et al., Journal of Dermatological Science 94 (2019) 266–275.
9. Zhang et al., CBP 1 -201, a Next-Generation IL-4Rα Antibody, Achieved All Primary and Secondary Efficacy Endpoints in the Treatment of Adults with Moderate-to-Severe Atopic Dermatitis (AD): A Randomized, Double-blind, Pivotal Trial in China (CBP-201-CN002), AAD oral presentation, New Orleans 2023.
10. LaPorte, S.L., et al., Molecular and structural basis of cytokine receptor pleiotropy in the interleukin-4/13 system. Cell, 2008. 132(2): p. 259-72.
11. Zhang, L., et al., *Preclinical immunological characterization of rademikibart (CBP-201), a next-generation human monoclonal antibody targeting IL-4Ralpha, for the treatment of Th2 inflammatory diseases.* Sci Rep, 2023. **13**(1): p. 12411.