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

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Introduction

- Inhibiting interleukin-4 receptor alpha (IL-4Rα) with targeted antibodies is a major mechanism for alleviating Type II immune responses in asthma[1,2] and, more recently, COPD[3].
- Rademikibart is an optimized next-generation human monoclonal antibody targeting IL-4Rα. Compared to dupilumab, rademikibart demonstrated better inhibition of STAT6 intracellular signalling, 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)[4].
- In a phase 2b asthma trial, rademikibart demonstrated rapid and statistically significant improvements in lung function; improvements gained during the first few hours/days of treatment were sustained through 24-weeks of rademikibart therapy (See poster #13121).

Objective

- Despite much clinical success with dupilumab in the treatment of asthma and COPD, many patients fail to respond, have incomplete response, suffer waning efficacy over time or experience adverse effects such as eosinophilia[5–9]
- This study examined differences in the atomic-resolution 3D structures of rademikibart and dupilumab, that may potentially lead to an understanding of the differences in efficacy and safety between the two drugs.

Methodology

- X-ray crystallography was used to determine the atomic resolution 3D structure of rademikibart fragment antigen binding (Fab) bound to IL-4Rα. This structure was analyzed and compared computationally with the 2.82 Å resolution crystal structure of dupilumab Fab bound to IL-4Rα (Protein Data Bank Code 6WGL).
- Molecular dynamics studies on rademikibart and dupilumab bound to IL-4Rα 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-4Rα was determined at 2.71Å and compared to the complex of dupilumab Fab and IL-4Rα. The rotation angle between dupilumab and rademikibart bound to IL- 4Rα is 59.17° (Figure 3a) enabling the epitope of rademikibart, but not dupilumab, on IL-4Rα to overlap more closely with the conserved binding interface utilized by IL-4 and IL-13 cytokines.
- Three loops of the IL4Rα at the interface are involved in the binding interactions with antibodies. The major differences between the equilibrated structures of dupilumab-IL4Rα and rademikibart-IL4Rα complexes is at the third interface loop (residue 148 to 152) (Figure 4). The average B factor of the third loop is 133.22 Å² for dupilumab and 53.42 Å² for rademikibart. The third loop of IL4Rα in dupilumab complex has larger B factors and stays further away from the antibody, while all three loops in the rademikibart complex show low B factors and bind closely to the antibody. It indicates that the antibody-antigen binding is more stable for rademikibart than dupilumab.
- Hydrogen (H) bond interactions at the interfaces of both complexes are also analyzed (Figure 3). The rademikibart-IL4Rα complex has more H bonds, each of which can contribute about 2–3 kcal/mol binding energies. The binding surface areas for both complexes are calculated (Figure 4). The buried surface area (BSA) is 779.8 Å² for dupilumab and 893.3 Å² for rademikibart. This evidence (more binding H bonds and a larger BSA) helps explain the stronger binding affinity of the rademikibart than the dupilumab.

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° rotation of rademikibart on IL-4Rα compared to dupilumab, optimizing 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 lung function improvement in asthma trials.
- Our data provide 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.
- The structural differences and distinct epitope binding may explain both the improved lung function and the absence of the eosinophil increase commonly reported with dupilumab (See poster #13132).

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

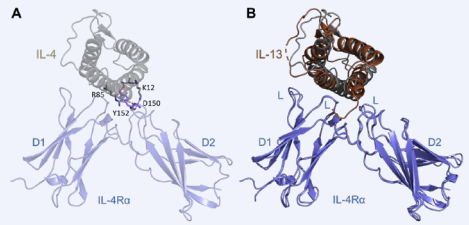

Figure 1. Crystal structure of the IL-4-IL-4R-α complex (PDB: 3bpl) at 2.93 Å resolution. H bond interactions between the L5 loop of IL-4Rα and IL-4 are shown in red dash lines. Residues involved in interactions are shown in sticks (D150 and Y152 on IL-4Rα and R85 and K12 on IL-4) (**A**). Alignment 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

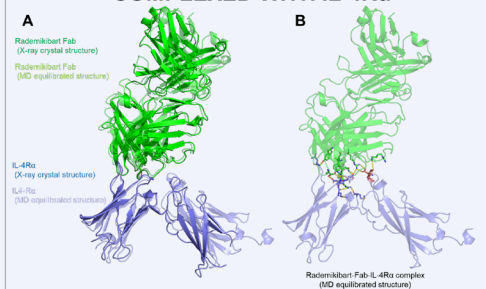

Figure 2. Superposition of rademikibart-Fab crystal structure complexed with IL-4Rα at **2.71 Å** resolution and the equilibrated structure of the same complex derived from MD simulations (**A**). Multiple H bond interactions comprise the binding interface of rademikibart-Fab and IL-4Rα at equilibrium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

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

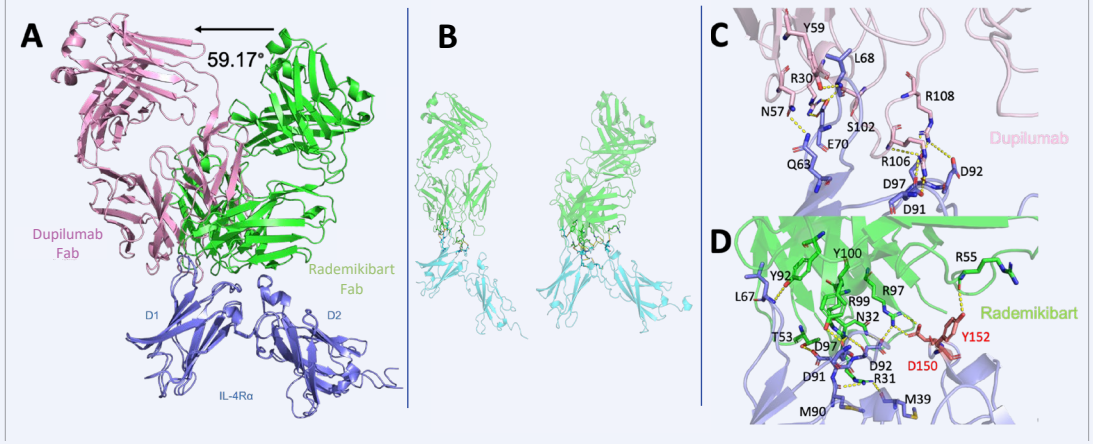

Figure 3. Comparison of the aligned (via IL-4Rα) dupilumab and rademikibart complexes with IL-4Rα. **(A)** There is a 59.17° rotation of rademikibart on IL-4Rα compared to dupilumab. (**B**) H bond interactions at the binding interface of dupilumab and rademikibart Fabs and the IL-4Rα at equilibrium. Note that rademikibart has ~30% more H bonds creating stability with the IL-4Rα. Also note how dupilumab does not engage IL-4Rα at D2. **(C, D)** 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.

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

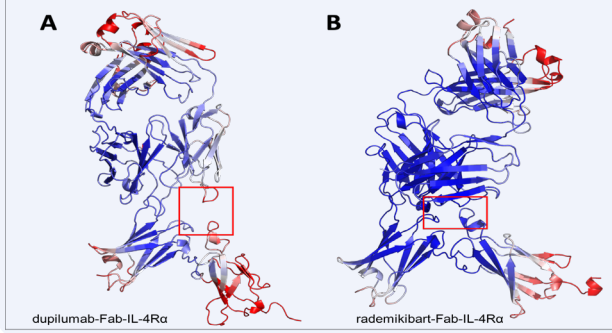

Figure 4. Equilibrated structures of dupilumab-Fab-IL-4Rα (**A**) and rademikibart-Fab-IL-4Rα (**B**) complexes fitted to MD-derived X-ray electron density maps. B factors, which represent motion or elevated protein dynamics when high, are shown in blue-to-red spectrum (low-to-high). Red boxes show the 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.

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