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Structural and mechanistic basis of TSLP neutralization by solrikitug

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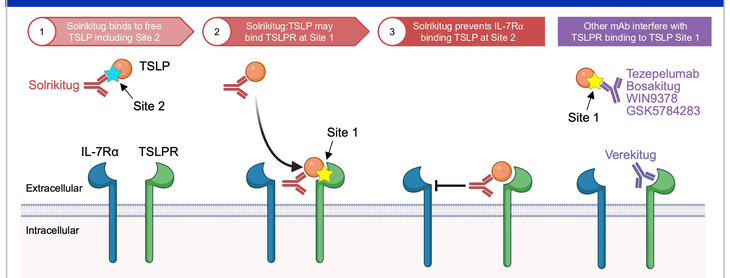
Introduction

- Thymic stromal lymphopoietin (TSLP) is an upstream mediator of type 2 inflammation in respiratory and gastrointestinal inflammatory diseases such as asthma, chronic obstructive pulmonary disease (COPD), and eosinophilic esophagitis (EoE)[1]
- TSLP signals through a heterodimeric receptor comprised of the TSLP receptor (TSLPR) and interleukin-7 receptor alpha (IL-7Rα)[1]
- Solrikitug is a novel, potent, humanized IgG1 monoclonal antibody against TSLP in Phase 2 development for asthma (NCT06496607), COPD (NCT06496620), and EoE (NCT06598462)
- We previously showed that solrikitug uniquely binds to an epitope on TSLP required for IL-7Rα interaction.[2] Consequently, solrikitug potently inhibits TSLP signaling by preventing the assembly of the heterodimeric receptor complex **(Figure 1)**

Figure 1: Solrikitug's distinct mechanism of TSLP inhibition



Objectives

- Elucidate the crystal structure of the solrikitug-TSLP interaction
- Explore how the unique interaction between solrikitug and TSLP could potentially impact TSLP signaling

Methods

Preparation and crystal structure determination of the solrikitug-Fab:TSLP complex

- Human TSLP was expressed in *E. coli*, refolded in vitro, and purified via size-exclusion chromatography (SEC) as previously described.[3] The light chain and heavy chain fragment of solrikitug were co-expressed in HEK293 suspension cells. The solrikitug-Fab fragment was purified via immobilized-metal ion affinity chromatography and SEC.
- Purified solrikitug-Fab was mixed with a 1.5 molar excess of refolded TSLP. A single-domain antibody targeting the human kappa light chain (anti-Kappa VHH) was added to the solrikitug-Fab:TSLP complex as a crystallization chaperone. SEC-elution fractions corresponding to the solrikitug-Fab:TSLP:anti-Kappa-VHH complex were concentrated and subjected to crystallization trials. Crystals were cryocooled and X-ray diffraction data was collected using synchrotron radiation.
- Structural data for TSLPR, IL-7Rα, and tezepelumab interaction with TSLP were previously published.[4]

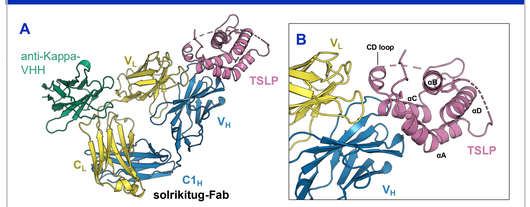
TSLP inhibition assay on HEK-Blue-TSLP reporter cells

- HEK-Blue TSLP reporter cells (InvivoGen) were incubated overnight with pre-mixed TSLP and blocking molecules. The next day, secreted alkaline phosphatase (SEAP) in supernatants was quantified by a calorimetric enzyme assay. Data was fitted with a sigmoidal dose-response model (4PL) to determine the inhibitory concentrations (IC50 and IC90 values). Clinical grade tezepelumab (Myonex) was used in this assay.

Results

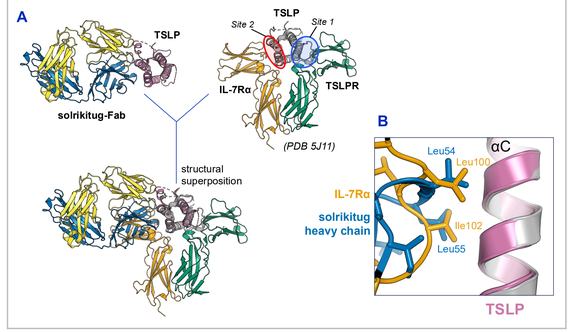
- An anti-kappa VHH was added to the solrikitug-Fab:TSLP complex as a crystallization chaperone, enabling structure determination at 1.85 Å resolution
- Both VH and VL domains of solrikitug contribute extensively to the binding interface, forming an extensive epitope that targets the IL-7Rα interaction site on TSLP (site 2) **(Figure 2A)**
- Notably, the N-terminal segment of the TSLP CD loop becomes ordered upon solrikitug binding and contributes directly to the interaction interface, thereby substantially extending the solrikitug binding epitope beyond site 2 **(Figure 2B)**

Figure 2: Structure of solrikitug-Fab:TSLP complex



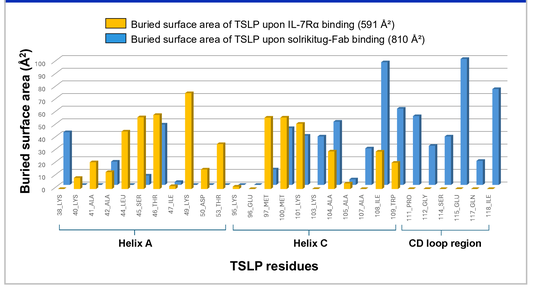
- Superposition of solrikitug-Fab:TSLP complex with the TSLP:TSLPR:IL-7Rα complex (PDB 5J11)[4] demonstrates substantial overlap in the positioning of solrikitug-Fab and IL-7Rα **(Figure 3A)**

Figure 3: Molecular mimicry of solrikitug to interact with αC helix



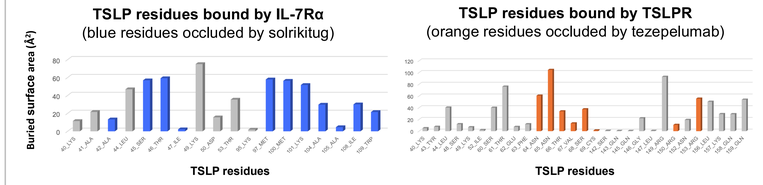
- Solrikitug recapitulates key elements of TSLP recognition by IL-7Rα via site 2, an example of molecular mimicry that sterically prevents binding of IL-7Rα **(Figure 3B)**
- The TSLP residues bound by solrikitug significantly overlap with the residues bound by IL-7Rα
- Solrikitug's binding mode includes hydrophobic interactions with the AC face of TSLP's four helix bundle as well as a substantial extension into the CD loop, collectively burying 810 Å² of solvent-accessible surface area **(Figure 4)**

Figure 4: Solrikitug overlaps IL-7Rα binding site residues on helices A and C with extension into CD loop



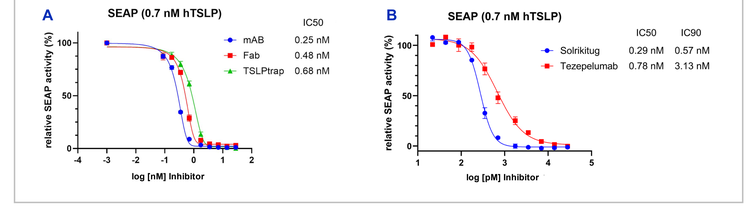
- Solrikitug buries 11 of 18 residues in the IL-7Rα binding site[4], blocking 64.8% (383 Å²) of the IL-7Rα epitope surface area
- Comparatively, tezepelumab only buries 8 of 29 residues from the TSLPR binding site,[4] only blocking 38.5% (317 Å²) of the TSLPR epitope surface area **(Figure 5)**

Figure 5: Solrikitug blocks more of its respective receptor binding site than tezepelumab



- TSLP signaling inhibition was performed using HEK-Blue-TSLP reporter cell line
- Solrikitug is a stronger inhibitor than TSLP-trap, a potent molecule comprised of the extracellular domains of TSLPR and IL-7Rα connected with a long and flexible linker[4] **(Figure 6A)**
- Solrikitug is also more potent than tezepelumab at inhibiting TSLP signaling using this reporter assay **(Figure 6B)**

Figure 6: Solrikitug is highly potent at blocking TSLP signaling



Conclusions

- We have resolved the solrikitug-Fab:TSLP complex crystal structure to 1.85 Å resolution.
- Solrikitug binds TSLP through hydrophobic interactions with its A and C helices as well as strong extension into the CD loop, collectively burying 810 Å² of TSLP's solvent-accessible surface area.
- **Solrikitug's interaction occludes site 2 on TSLP, preventing the binding of IL-7Rα at its canonical binding site.** This interaction demonstrates an impressive example of molecular mimicry, with solrikitug residues recapitulating the orientation of IL-7Rα residues in its interaction with the αC helix.
- **Solrikitug blocks 64.5% of the IL-7Rα binding surface. In contrast, tezepelumab only blocks 38.5% of the TSLPR binding surface**. The greater coverage of the receptor binding site by solrikitug may lead to more complete inhibition of TSLP signaling**.**
- In a TSLP signaling reporter cell assay, solrikitug is a better inhibitor than the highly potent TSLP-trap. **Solrikitug is also more potent than tezepelumab in this reporter assay.**

References **[1]** Ebina-Shibuya and Leonard. Nat Rev Immunol. 2023;23:24-37. **[2]** Komori K, et al. Am J Respir Crit Care Med. 2025;211(Suppl 1):A1393. **[3]** Verstraete K, et al. Protein J. 2009;28:57-65. **[4]** Verstraete K, et al. Nat Commun. 2017;8:14937.

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Disclosure of Interest:
LW is a shareholder and former employee of Uniquity Bio.
HKK is a shareholder and employee of Uniquity Bio.