Unveiling the Underlying Mechanism of Differentiation for STAR-0310, an Anti-OX40 Antibody for Atopic Dermatitis (AD)

NIKOLAOS BIRIS[1*], CHUNXIA ZHAO[1], MICHELE GUNSIOR[1], JINGPING GE[1], JAMES FULLER[2], RAFIF DAGHER[1]

[1]ASTRIA THERAPEUTICS, 22 BOSTON WHARF ROAD, BOSTON, MA 02210
[2]HELIX BIOSTRUCTURES, 5225 EXPLORATION DRIVE, INDIANAPOLIS, IN 46241

*PRESENTING AUTHOR

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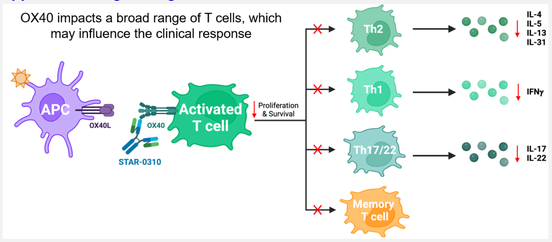
INTRODUCTION

Atopic Dermatitis (AD) is a chronic inflammatory skin disorder with significant unmet medical need. T-cell inflammation is a key component of AD pathogenesis driven by antigen stimulation and co-stimulatory receptors. The co-stimulatory receptor OX40, expressed on activated T cells, including Th1, Th2, and Th17 subtypes, plays a pivotal role in sustaining T cell-mediated inflammation by promoting survival, proliferation, and cytokine production (**Fig. 1**). The interaction between OX40 and its ligand OX40L, is a key immunological checkpoint that facilitates the activation of downstream signaling cascades, including the nuclear factor kappa B (NFκB) pathway, essential for T cell longevity and effector function.

Figure 1. Atopic dermatitis is driven by a range of T cell responses. However, approved biologics target Th2.



STAR-0310 is a novel, potent, and selective long-acting investigational monoclonal antibody antagonist targeting OX40 engineered to fully inhibit OX40 signaling, without inducing receptor activation, disrupting the inflammatory feedback loop central to AD pathogenesis. Here, we will examine the Cryogenic Electron Microscopy (Cryo-EM) structures of STAR-0310 to understand the structural basis of this potential therapy in AD.

METHODS

Structures of therapeutic antibodies in complex with OX40

- STAR-0310, in-house rocatinlimab and IMG-007 analogues Fab/OX40 complex structures were obtained by Cryo-EM.
- The complexes were initially screened to evaluate the best conditions for imaging, followed by vitrification and ThermoFisher Krios G3i electron microscope imaging.
- Image data were analyzed to obtain a high-resolution structure of the complexes.

Agonism assessment

- Agonist activity of STAR-0310, in-house rocatinlimab and IMG-007 analogues was evaluated using a Jurkat-OX40-NFκB-Luc reporter assay, which measures NFκB pathway activation via luciferase expression in OX40-expressing Jurkat cells.

Surface Plasmon Resonance (SPR) -Based OX40/OX40L Complex Disruption Assay

- Anti-Avi tag antibody was immobilized on a CM5 sensor chip to capture biotinylated human OX40L trimer. Human OX40-Fc was then injected to form the complex, followed by injection of the test antibodies. Disruption was monitored over a 150-second dissociation phase.

Summary

1. The high resolution Cryo-EM structure of the STAR-0310 Fab/OX40 complex uncovered an allosteric inhibition mechanism of the OX40/OX40L interaction.
2. STAR-0310 showed a higher rate of dissociation of preformed complexes of OX40/OX40L in a SPR-based complex disruption assay as compared to rocatinlimab and IMG-007.
3. STAR-0310 was shown to have a distinct Fc orientation and antagonistic activity without inducing agonistic activity, which together could potentially lead to achieving more comprehensive inhibition of T cell responses.

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STAR-0310 PREVENTS THE BINDING OF OX40 TO THE OX40L TRIMER INHIBITING T CELL SURVIVAL AND PROLIFERATION

The extracellular domain of OX40 consists of 4 Cysteine Rich Domains (CRD1-CRD4). OX40L binds OX40 through CRD1 and CRD2 (**Fig.2a**). STAR-0310 binds to OX40 CRD2 and diametrically opposite from the OX40/OX40L binding site (**Fig.2b**).

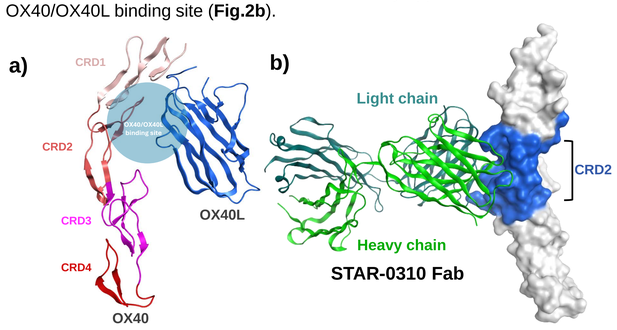


Figure 2. a) OX40/OX40L interaction (PDB ID: 2HEV), b) STAR-0310 binds away from OX40L site in the OX40-CRD2

OX40L, expressed as a trimer predominantly on professional antigen-presenting cells (APCs), engages OX40 on activated T cells to induce OX40 trimerization (**Fig. 3a**), a critical step that triggers downstream signaling, including NF-κB activation, promoting T cell survival, proliferation and cytokine production.
STAR-0310 binds to OX40 in a manner that sterically obstructs the formation of the hexameric OX40/OX40L signaling complex, due to significant steric hindrance between its Fab region and the native trimeric conformation of OX40L (**Fig. 3b**).

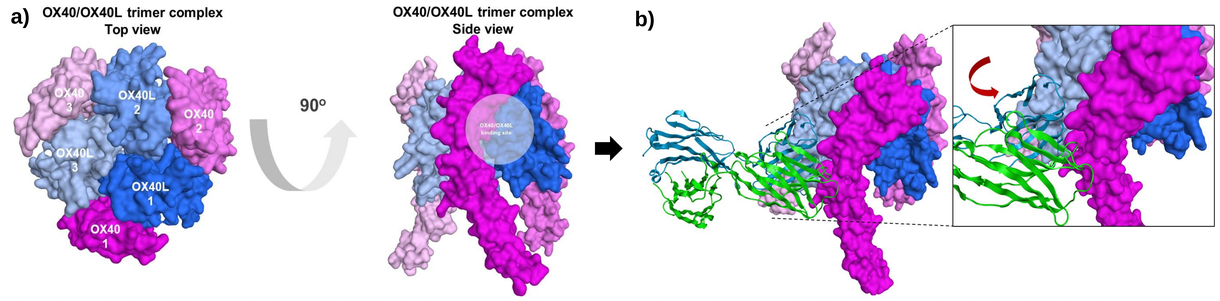
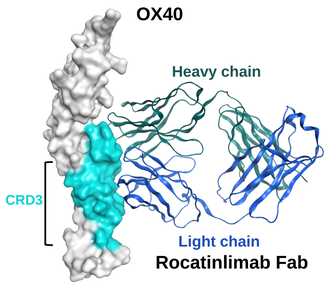


Figure 3. Mechanism of OX40 activation and inhibition by STAR-0310. a) OX40L, expressed as a trimer on APCs, induces OX40 trimerization initiating downstream T cell signaling. b) STAR-0310 sterically inhibits the assembly of the hexameric OX40/OX40L complex.

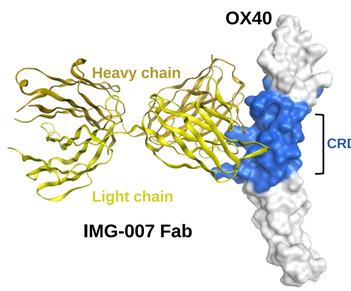
ROCATINLIMAB STERICALLY BLOCKS THE OX40L INTERACTION SITE



Unlike STAR-0310, rocatinlimab targets the OX40 CRD3 domain, positioning itself just below the OX40L binding site in a manner that sterically blocks OX40L from engaging with OX40 (**Fig.4**).

Figure 4. Structure of rocatinlimab Fab in complex with OX40

IMG-007 ENGAGES OX40 THROUGH A DIFFERENT BINDING MODE COMPARED TO STAR-0310



Although IMG-007 binds to an epitope that overlaps with STAR-0310 (**Fig.5**), its Fab domains are oriented very differently, a structural divergence that may reduce its downstream signaling inhibition (**Fig. 7**).

Figure 5. Structure of IMG-007 Fab in complex with OX40

STAR-0310 EXHIBITS A HIGHER RATE OF DISSOCIATION OF PREFORMED OX40/OX40L COMPLEXES

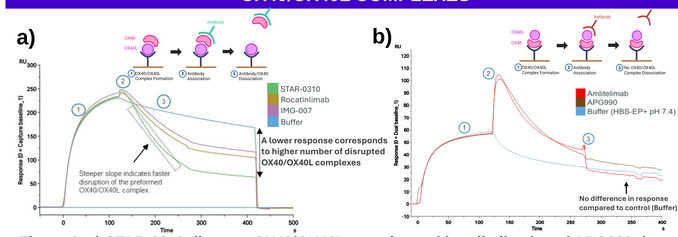
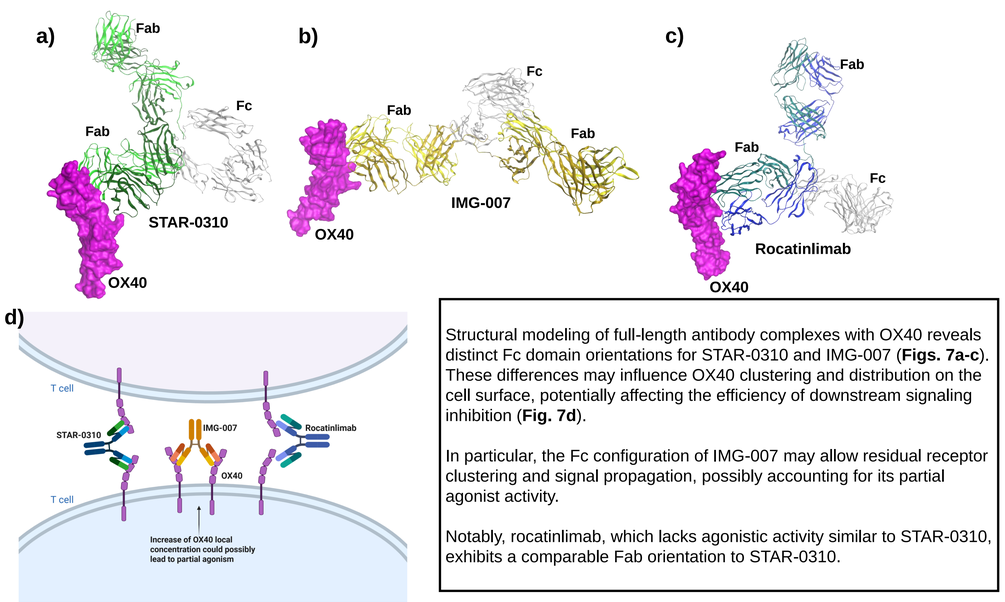


Figure 6. a) STAR-0310 disrupts OX40/OX40L complexes, b) amlitelimab and APG990 do not disrupt the pre-formed OX40/OX40L complexes

STAR-0310 not only inhibits OX40/OX40L binding but also disrupts pre-formed OX40/OX40L complexes with approximately twice the rate and efficiency of rocatinlimab and IMG-007 (**Fig. 6a**), an effect not seen with OX40L-targeting antibodies like amlitelimab or APG990 (**Fig.6b**).

DISTINCT ORIENTATIONS OF STAR-0310 AND IMG-007 COULD INFLUENCE OX40 SIGNALING



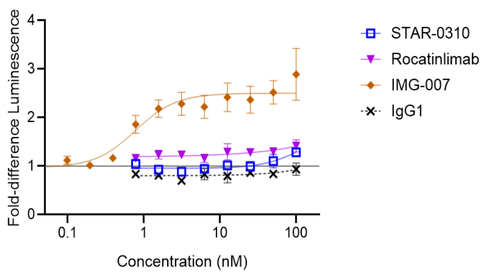
Structural modeling of full-length antibody complexes with OX40 reveals distinct Fc domain orientations for STAR-0310 and IMG-007 (**Figs. 7a-c**). These differences may influence OX40 clustering and distribution on the cell surface, potentially affecting the efficiency of downstream signaling inhibition (**Fig. 7d**).

In particular, the Fc configuration of IMG-007 may allow residual receptor clustering and signal propagation, possibly accounting for its partial agonist activity.

Notably, rocatinlimab, which lacks agonistic activity similar to STAR-0310, exhibits a comparable Fab orientation to STAR-0310.

Figure 7. Structural models of full-length complexes with OX40 of a)STAR-0310, b) IMG-007, c) rocatinlimab depicting the different Fc orientations, d) schematic demonstrating the potential mechanism of inhibition of STAR-0310, IMG-007 and rocatinlimab

STAR-0310: A FULL ANTAGONIST THAT AVOIDS UNWANTED T CELL ACTIVATION



STAR-0310 does not induce agonistic activity on Jurkat-NFκB-OX40 cells, whereas IMG-007 showed a 3-fold higher luminescence than the untreated control (**Fig. 8**).

STAR-0310 functions as a pure OX40 antagonist, effectively inhibiting receptor signaling without inducing activation, which could potentially lead to achieving more comprehensive inhibition of T cell responses than partial agonists such as IMG-007.

Figure 8. STAR-0310 does not induce agonism of OX40 receptor

Conclusions:

1. STAR-0310 is a high-potency, novel OX40 antagonist that binds the CRD2 domain of OX40, as revealed by Cryo-EM. This unique mechanism disrupts receptor trimerization and activation, which may support favorable clinical differentiation.
2. STAR-0310 demonstrates enhanced antagonism by effectively disrupting pre-formed OX40/OX40L complexes with greater potency and efficiency relative to other OX40 and OX40L comparators, highlighting its unique potential to inhibit this aspect of OX40-mediated signaling in inflammatory settings such as atopic dermatitis.
3. Based on a Jurkat-OX40-NFκB-Luc reporter assay, STAR-0310 is a pure OX40 antagonist that blocks receptor signaling without inducing activation, possibly resulting in deeper inhibition of T cell responses compared to IMG-007.
4. Combined with the previously reported lower antibody-dependent cellular cytotoxicity (ADCC) activity and robust cytokine inhibition, the data presented here support STAR-0310 as a potential best-in-class OX40 antagonist, currently being evaluated in an ongoing Phase 1 clinical trial (NCT 06782477).