Preclinical profile of STAR-0310, a novel OX40 antagonistic monoclonal antibody

Chunxia Zhao[1*], Julie Macoin[1], Laura Carretero[2], Nikolaos Biris[1]

*Astria Therapeutics, 75 State Street, Suite 1400, Boston, MA 02109 [2]Ichnos Sciences, Route de la Corniche 5A, 1066 Epalinges, Switzerland. *czhao@astriatx.com*

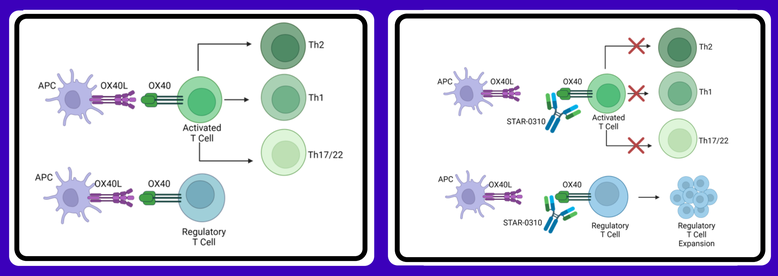
INTRODUCTION

Atopic dermatitis (AD) is a relapsing, inflammatory skin disease with a prevalence of 2–5% in young adults and up to 20% in children. Skin barrier dysfunction, inflammation, and dysbiosis contribute to AD development and chronicity which has a great impact on the patient's quality of life, especially due to pruritus. During the acute phases of AD, there is a notable modulation of Th2 and Th22 immune responses, with variable Th17 involvement; while the chronic phase includes additional Th1 activation.

The co-stimulatory T-cell receptor OX40 is predominantly expressed on effector and regulatory T-cells. Its ligand, OX40L, is expressed on activated antigen-presenting cells, including dendritic cells, endothelial cells, macrophages, and activated B-cells. OX40–OX40L engagement is key to potentiating the expansion of effector T-cells and the prolongation of their survival by suppressing apoptosis, enhancing T-cell effector functions, such as cytokine production, and generating T helper memory cells. OX40 costimulation inhibits regulatory T cell induction via multiple mechanisms (Figure 1A). STAR-0310 targets the OX40 pathway, impacting Th1, Th2 and Th17/22 pathways and preserving the regulatory T cells (Figure 1B).

Figure 1A. Biological function of OX40-OX40L signaling pathway

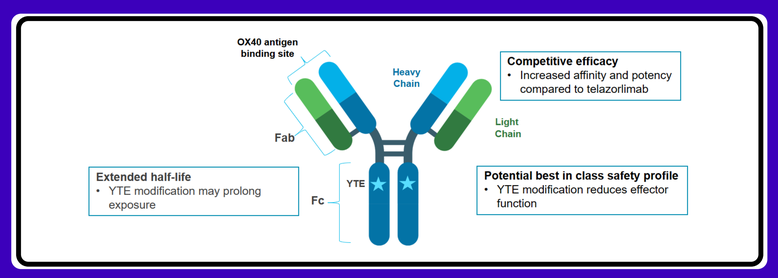
Figure 1B. Proposed mechanism of action for STAR-0310



About STAR-0310

STAR-0310 is a novel, affinity-matured YTE-modified (M252Y/S254T/T256E) monoclonal antibody that inhibits the OX40 receptor. Compared to the parent molecule, telazorlimab (STAR-0305), with previously demonstrated proof of concept in atopic dermatitis, it has increased affinity for OX40 binding and YTE modification in the Fc region. The YTE modification in the Fc region reduces the effector function. Furthermore, YTE modification has the potential for extended half-life in vivo and less frequent dosing (Figure 2). Here, we present the initial preclinical characterization of STAR-0310.

Figure 2: Features of STAR-0310 engineering design



OBJECTIVES

Preclinical characterization of STAR-0310, including affinity to human OX40, potency and effector function.

METHODOLOGY

Surface plasmon resonance (SPR) was used to measure binding affinity of STAR-0310 to human OX40.

STAR-0310 potency was assessed by a cytokine release inhibition assay. Graphs show the cytokine production (IFNγ, TNF-α, IL-5, IL-13) from T cells pre-activated for 24 hours using coated OKT3 and soluble CD28, then incubated with co-coated OKT3 and OX40L in the presence or absence of treatments. All molecules were tested in a dose-response manner starting at 200 nM and diluted by 3. Supernatants were harvested on day 4, then quantified using Meso Scale Discovery assays. Dose-response curves of cytokine secretion and EC50 of cytokine secretion inhibition are shown. Statistical analysis: paired-one way ANOVA followed by Tukey's post-hoc test (ns p>0.05, * 0.05>p>0.01, ** 0.01>p>0.001). Graphs show data obtained from two independent experiments, with a total of eight donors.

STAR-0310 effector function was measured by antibody-dependent cellular cytotoxicity (ADCC) assay. Activated or regulatory T cells were co-cultured with autologous NK in the presence of treatments in a dose-response starting at 20 nM and diluted by 5 or control isotypes at 20 nM. The percentage of ADCC was measured by lactate dehydrogenase release after 4.5 hours of incubation (dose response, maximum ADCC and EC50 of the quantifiable donors). Statistical analysis was performed using paired-one-way ANOVA, followed by Tukey's post-hoc test (ns p>0.05, * 0.05>p>0.01, ** 0.01>p>0.001). Graphs show 6 (activated T cells) or 8 (regulatory T cells) donors, 3 independent experiments.

RESULTS

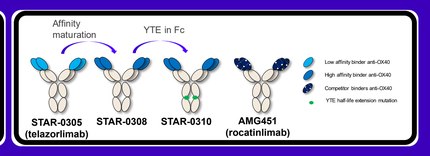
Affinity of STAR-0310 to human OX40

STAR-0310, an affinity-matured, YTE-modified derivative STAR-0305, demonstrates an approximately 8-fold enhancement in binding affinity to human OX40 measured by SPR, Table 1. Comparative analysis reveals that STAR-0310 retains similar binding affinity to human OX40 as STAR-0308, which does not have the YTE modification, suggesting that the YTE modification does not influence the STAR-0310 binding affinity to human OX40.

Table 1. Binding affinity of STAR-0310 to human OX40 measured by SPR

Name	STAR-0305 (telazorlimab*)	STAR-0308	STAR-0310	AMG451 (rocatinlimab*)
Description	parent molecule	affinity maturated	affinity maturated with YTE	comparator
IgG1 backbone modification	n/a	n/a	YTE	afucosylated
Binding affinity K_D (nM)	59 ± 2.0	6.8 ± 2.0	7.2 ± 0.4	3.0 ± 0.4

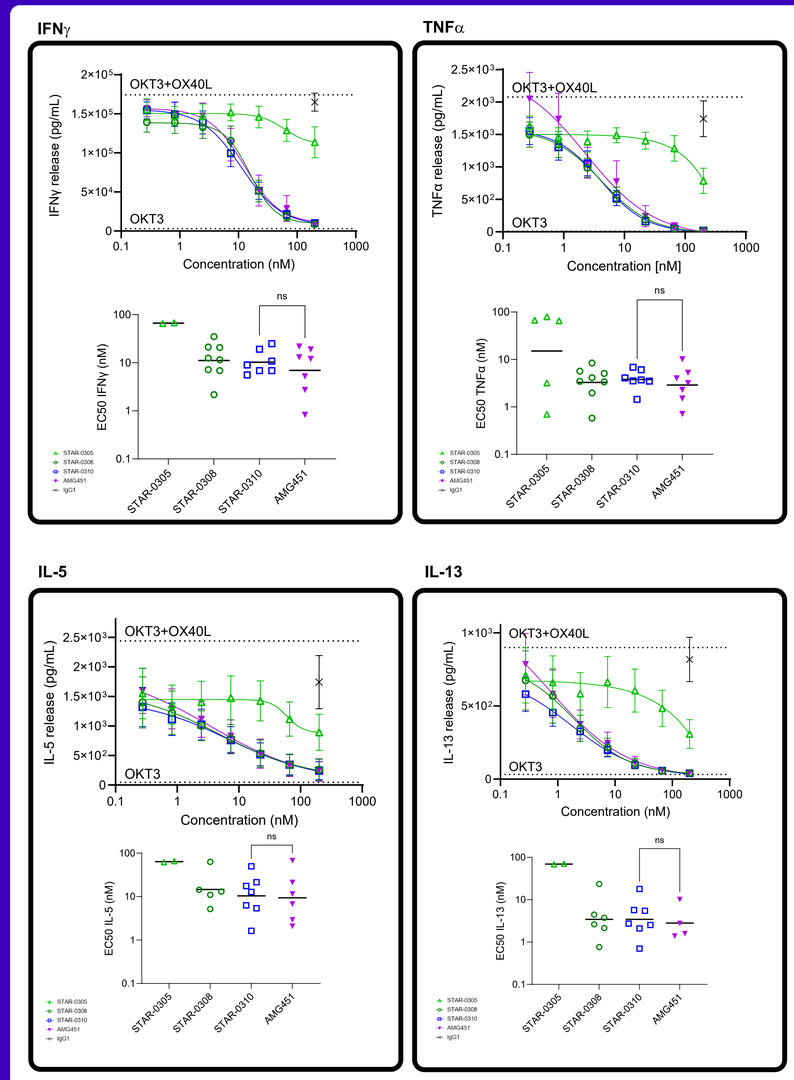
*in-house equivalent of the molecule



STAR-0310 potency

Enhanced affinity to OX40 in STAR-0308 results in a dramatic increase of potency in the cytokine release inhibition compared to STAR-0305. The incorporation of the YTE mutation in STAR-0310 does not affect the potency, showing similar EC50 in cytokine release inhibition for both STAR-0310 and STAR-0308. The potency of STAR-0310 in cytokine release inhibition is comparable to rocatinlimab (AMG451) (Figure 3).

Figure 3. Potency of STAR-0310 assessed in cytokine release inhibition assay

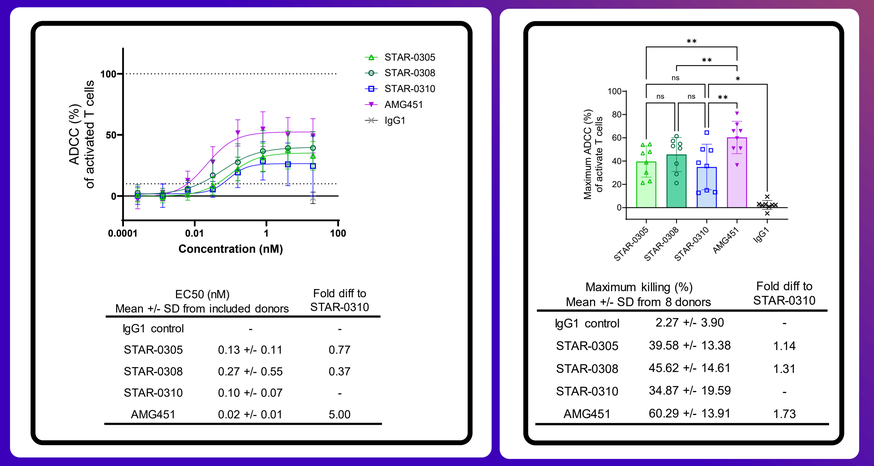


	IFNγ		TNF-α		IL-5		IL-13	
	EC50 (nM) Mean +/- SD	Fold diff to STAR-0305	EC50 (nM) Mean +/- SD	Fold diff to STAR-0305	EC50 (nM) Mean +/- SD	Fold diff to STAR-0305	EC50 (nM) Mean +/- SD	Fold diff to STAR-0305
STAR-0305	66.7 +/- 1.6	-	43.3 +/- 38.1	-	63.6 +/- 2.8	-	69.6 +/- 1.8	-
STAR-0308	14.6 +/- 10.5	4.6	4.1 +/- 2.4	10.6	21.3 +/- 23.5	3.0	6.2 +/- 8.6	11.2
STAR-0310	11.8 +/- 7.3	5.7	4.2 +/- 1.8	10.3	16.4 +/- 16.3	3.9	5.3 +/- 5.9	13.1
AMG451	10.7 +/- 8.0	6.2	3.9 +/- 3.2	11.1	18.6 +/- 24.8	3.4	4.0 +/- 4.2	17.4

STAR-0310 ADCC

STAR-0310 showed less activated T cell depletion compared to rocatinlimab, which demonstrated a 5-fold greater killing capacity; STAR-0310 showed ~75% less maximal killing on activated T cells relative to rocatinlimab (Figure 4).

Figure 4. ADCC on activated T cells

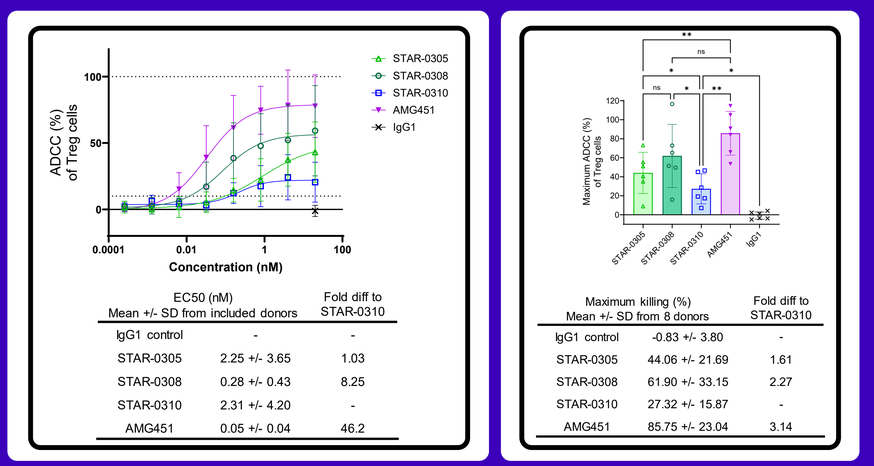


	EC50 (nM) Mean +/- SD from included donors	Fold diff to STAR-0310
IgG1 control	-	-
STAR-0305	0.13 +/- 0.11	0.77
STAR-0308	0.27 +/- 0.55	0.37
STAR-0310	0.10 +/- 0.07	-
AMG451	0.02 +/- 0.01	5.00

	Maximum killing (%) Mean +/- SD from 8 donors	Fold diff to STAR-0310
IgG1 control	2.27 +/- 3.90	-
STAR-0305	39.58 +/- 13.38	1.14
STAR-0308	45.62 +/- 14.61	1.31
STAR-0310	34.87 +/- 19.59	-
AMG451	60.29 +/- 13.91	1.73

Similarly, STAR-0310 exhibits 46-fold less elimination of regulatory T cells than rocatinlimab. Enhanced affinity to OX40 in STAR-0308 results in an 8-fold increase in the cytotoxic activity against regulatory T cells via ADCC compared to STAR-0305. The incorporation of the YTE mutation in STAR-0310 results in statistically lower maximal cytotoxic activity against regulatory T cells, relative to STAR-0305 and STAR-0308 (Figure 5).

Figure 5. ADCC on regulatory T cells



	EC50 (nM) Mean +/- SD from included donors	Fold diff to STAR-0310
IgG1 control	-	-
STAR-0305	2.25 +/- 3.65	1.03
STAR-0308	0.28 +/- 0.43	8.25
STAR-0310	2.31 +/- 4.20	-
AMG451	0.05 +/- 0.04	46.2

	Maximum killing (%) Mean +/- SD from 8 donors	Fold diff to STAR-0310
IgG1 control	-0.83 +/- 3.80	-
STAR-0305	44.06 +/- 21.69	1.61
STAR-0308	61.90 +/- 33.15	2.27
STAR-0310	27.32 +/- 15.87	-
AMG451	85.75 +/- 23.04	3.14

CONCLUSIONS

- STAR-0310 is a high affinity anti-OX40 antibody, demonstrating an ~8 fold increase in binding affinity to human OX40 compared to telazorlimab.
- Enhanced affinity of STAR-0310 results in a significant inhibition of cytokine release, as demonstrated in T cell proliferation assay. STAR-0310 has comparable potency to rocatinlimab.
- There is significantly less ADCC potential with STAR-0310 compared to rocatinlimab which is related to specific Ab engineering, including the YTE modification and lack of afucosylation. Less ADCC in the context of robust potency has the potential to improve safety profile of this OX40 antagonist without impacting potential efficacy. Compared to rocatinlimab, STAR-0310 has 5-fold less depletion of activated T cells, while it shows 46-fold less elimination of regulatory T cells through ADCC.
- Impact of YTE modification on pharmacokinetics of STAR-0310 is under investigation.
- These preclinical data support further development of STAR-0310 for the treatment of moderate-to-severe AD and other immunologic diseases.

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