### INTRODUCTION

Atopic dermatitis is a common, chronic inflammatory skin disease which has a significant impact on the patient’s quality of life, especially due to pruritus. Atopic dermatitis affects 2-5% in young adults and up to 25% in children. During the acute phases of AD, there is a notable increase in Th2 and Th22 immune responses, with variable Th17 involvement; while the chronic phase includes additional Th1 activation.

The co-stimulatory T-cell receptor CD40 is primarily expressed on activated effector and regulatory T-cells. Its ligand, CD40L, is present on activated antigen-presenting cells, including dendritic cells, endothelial cells, macrophages and activated B-cells. Engagement of CD40 with CD40L, is crucial for the proliferation of effector T-cells and enhancement of cytokine production. Upon binding to the CD40 pathway, modulates Th1, Th2, and Th17 pathways, while preserving the regulatory T cells (Figure 1B).

### 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 (pIFN-γ, pTNF-α, pIL-17a) from T cells pre-activated for 24 hours using OX40L and soluble OKT3, then incubated with concentrated OX40L and OKT3 to assess presence or absence of treatments. Analysis was performed 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 (MSCD) assays. Three response surfaces of cytokine production and MSCD of cytokine release inhibition are shown. Statistical analysis: paired one-way ANOVA followed by Tukey’s post-hoc test (ns p>0.05, * p<0.05, ** p<0.01).

Graphs show data obtained from two independent experiments, with a total of eight donors.

### RESULTS

#### AFFINITY OF STAR-0310 TO HUMAN OX40

**STAR-0310, an affinity-matured, YTE-modified derivative of telazorlimab (STAR-0305), exhibits an approximately 8-fold increase in binding affinity to OX40 as measured by SPR**.

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

<table>
<thead>
<tr>
<th>Name</th>
<th>pIC50 of STAR-0305</th>
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<th>pIC50 of STAR-0310</th>
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<tbody>
<tr>
<td>OKT3</td>
<td>4.4</td>
<td>4.4</td>
<td>4.1</td>
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<tr>
<td>OKT3+OX40L</td>
<td>3.7</td>
<td>3.7</td>
<td>3.3</td>
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<tr>
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<td>3.5</td>
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<tr>
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<td>3.4</td>
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<td>2.9</td>
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<tr>
<td>AMG451</td>
<td>3.2</td>
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**STAR-0310 POTENCY (Figure 3)**

Enhanced affinity of STAR-0310 to OX40 results in a significant 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 to inhibit cytokine release compared to STAR-0310. The potency of STAR-0310 in cytokine release inhibition is comparable to rocatinlimab.

**Table 2. Potency of STAR-0310 assessed in cytokine release inhibition assay**

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**STAR-0310 ADCC (Figure 4, Figure 5)**

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. Similarly, STAR-0310 exhibits 46-fold less elimination of regulatory T cells than rocatinlimab. Enhanced affinity to OX40 in STAR-0310 results in an approximately 8-fold increase in ADCC 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.

**STAR-0310 HAS PROLONGED t1/2 IN CYMOMOLGUS MONKEYS**

Pharmacokinetic (PK) analysis of STAR-0310 in cynomolgus monkeys showed an estimated mean half-life of 26 days (Figure 6).

### 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 profiles of this OX40 antagonistic antibody, impacting patient efficacy.** Compared to rocatinlimab, STAR-0310 has 50-fold less depletion of activated T cells, while it shows 46-fold less elimination of regulatory T cells through ADCC.
- **Star-0310 is a long mean half-life of 26 days in cynomolgus monkeys, potentially attributed to the YTE modification.**
- **These preclinical data support further development of STAR-0310 for the treatment of moderate-to-severe AD and other immunologic diseases.**

### ACKNOWLEDGEMENTS

Authors acknowledge Valerie Keller for her assistance in creating Figures 1 and 2; Jingang Liu for her expert help with statistical analysis and creating Figures 3 and 4; Eliza Krueger and Matt Dough for image acquisition and manuscript editing; and Luna Zhang for her contribution to data analysis. Authors acknowledge Isabella Miller for medical writing and design of the poster.

### REFERENCES


### SUPPORTING INFORMATION

- These preclinical data support further development of STAR-0310 for the treatment of moderate-to-severe AD and other immunologic diseases.

### SCAN QR FOR DIGITAL COPY OF THIS POSTER.

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

**Figure 2.** Features of STAR-0310 engineering design

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

**Figure 4.** ADCC on activated T cells

**Figure 5.** ADCC on regulatory T cells

**Figure 6.** STAR-0310 concentration-time profile

**Figure 7.** Single dose in vivo pharmacometric data from cynomolgus monkeys

**Figure 8.** Pharmacokinetic data analysis using non-compartmental methods.