Evaluating the expression of OX40 and OX40 ligand in lesional skin versus non-lesional skin in volunteers with atopic dermatitis and in healthy control skin

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Introduction/Background: Growing evidence suggests that blocking the OX40 ligand (OX40L)/OX40 costimulatory pathway could have therapeutic effects in inflammatory diseases such as atopic dermatitis (AD). Amlitelimab (SAR445229; KY1005) is a potential first-in-class, fully human, non-depleting anti-OX40L monoclonal antibody that blocks OX40L-OX40 interactions. We report OX40L and OX40 expression from skin biopsies collected in two studies: an amlitelimab Phase 2a (Ph2a) study in patients with moderate-to-severe AD, and a small-scale study of patients with mild AD and healthy volunteers.

Objectives: Assess OX40L and OX40 expression in AD skin (lesional and non-lesional) and healthy control skin.

Methods: As part of a Ph2a randomized, double-blind, placebo-controlled study (NCT03754309) of amlitelimab in moderate-to-severe AD, 174 baseline-paired lesional/non-lesional skin biopsies were collected from all patients (n=87). In a separate small-scale study, 24 skin biopsies were collected, including healthy control skin from healthy volunteers (n=6) and paired lesional/non-lesional skin from patients with mild AD (n=9).

Each biopsy underwent immunohistochemical (IHC; formalin-fixed paraffin-embedded) analyses using various antibodies (OX40L: rabbit IgG antibody from Cell Signaling Technology for Ph2a
biopsies, proprietary mouse IgG2a KY1005 for small-scale study biopsies; OX40: clone Ber-ACT35 from BioLegend for all biopsies). A trained pathologist assessed IHC staining in the dermis and/or epidermis using semi-quantitative scoring (0 [no stained cells] to 10 [all cells stained]) or quantitative methods (cells/mm², Tissue Studio 4.0, and HALO link image analysis software). Wilcoxon matched-pairs signed-rank test was used to assess IHC staining of Ph2a biopsies.

Ph2a biopsies also underwent transcriptomic analyses (stored in RNALater) for OX40L (TNFSF4) and OX40 (TNFRSF4) on sequenced RNA using a linear mixed model with Benjamini-Hochberg adjustment.

**Results:** In baseline Ph2a biopsies, IHC analysis revealed elevated OX40L and OX40 staining in lesional versus non-lesional AD skin ($P<0.0001$ for both markers). For OX40L, the median score was 3 (lesional) versus 1 (non-lesional) and the Wilcoxon matched-pairs signed-rank test found $P<0.0001$. For OX40+ cells, the median expression was 179.8 cells/mm² in lesional AD skin versus 54.45 cells/mm² in non-lesional AD skin ($P<0.0001$ using the Wilcoxon matched-pairs signed-rank test). These findings were confirmed by RNA sequencing analysis, with TNFSF4 (OX40L) and TNFRSF4 (OX40) differentially expressed in lesional and non-lesional AD skin ($P<0.01$ and $P<0.0001$, respectively). A larger scale study is ongoing that may further support this finding.

In the small-scale study including patients with mild AD and healthy volunteers, there was a trend for an increase of mean OX40L+ and OX40+ cells/mm² in lesional skin versus non-lesional skin and healthy control skin in the dermis and epidermis (OX40L+ dermal lesional, non-lesional, healthy, respectively: 17.6, 11.2, 11.5; OX40L+ epidermal: 5.8, 2.1, 1.9; OX40+ dermal: 5.7, 2.1, 0.4; OX40+ epidermal: 10.9, 9.0, 1.8); the increase was significant for lesional versus healthy control skin for dermal OX40+ cells/mm² ($P<0.001$).
**Conclusions:** We confirm with IHC and RNA sequencing that OX40L and OX40 expression is elevated in skin lesions of patients with moderate-to-severe AD. We also demonstrate a trend to elevated OX40L and elevated OX40 in mild AD skin versus healthy volunteers. A potential explanation for the lower levels of OX40L and OX40 in patients with mild AD in this study may be their lower disease severity versus Ph2a study patients. These findings support the OX40L/OX40 pathway as a relevant target in T cell immune dysregulation; its blockade may represent a novel treatment approach for moderate-to-severe AD.

**Keywords:** Monoclonal antibody, anti-OX40L, Phase 2a, biologic therapy, biomarkers

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