Atopic dermatitis disease biomarkers strongly correlate with IL-13 levels, are regulated by IL-13, and are modulated by tralokinumab *in vitro*

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Introduction

- Atopic dermatitis (AD) is a chronic, pruritic skin disease characterized by type 2 immune-mediated inflammation and skin barrier dysfunction[1]
- In a recent large-scale RNA-sequencing-based transcriptomic study of AD, psoriasis, and matched control samples, it was found that the type 2 cytokine interleukin 13 (*IL13*) was the most distinctive marker for AD[2]
  - Increased expression levels of *IL13* were found in both lesional and nonlesional AD skin
  - Expression levels of *IL13* in lesional AD skin correlated with disease severity
- In contrast, expression of the type 2 cytokine *IL4* was detectable in 40% of the AD skin samples and at very low expression levels
- IL-13 has been shown to modulate the expression of inflammatory mediators, such as chemokines, and skin barrier markers related to the pathophysiology of AD[3-8]
- Tralokinumab is a fully human IgG4 monoclonal antibody in Phase 3 development for AD that specifically neutralizes IL-13[9]

Objectives

- To examine the correlation of *IL13* expression with AD disease biomarkers by use of RNA-sequencing data from AD lesional skin samples
- To investigate IL-13-mediated regulation of AD disease biomarkers and their modulation by tralokinumab in primary cultures of human keratinocytes and dermal fibroblasts

Methods

Figure 1. Study design

In silico bioinformatics analysis of RNA-sequencing data[2] to examine the correlation between *IL13* expression and AD disease biomarkers

Validate correlations ***in vitro*** using disease-relevant cell cultures

Human keratinocytes

Human dermal fibroblasts

Can IL-13-driven expression of AD disease biomarkers be reverted by tralokinumab?

Study population

Participants

- Adult patients with a history of AD for at least 3 years
  - The same AD cohort as reported by Tsoi et al from their large-scale transcriptomic study of AD[2]
- Adult volunteers without personal or familial history of allergic atopic and chronic inflammatory diseases

Inclusion criteria

- Dermatologist-confirmed diagnosis of AD (diagnosed on the basis of a skin examination by experienced dermatologists according to standard criteria for AD [American Academy of Dermatology consensus criteria])

Exclusion criteria

- Any other chronic skin disease
- Systemic treatment with immune-efficient medication
- Topical treatment within 1 week prior to material sampling

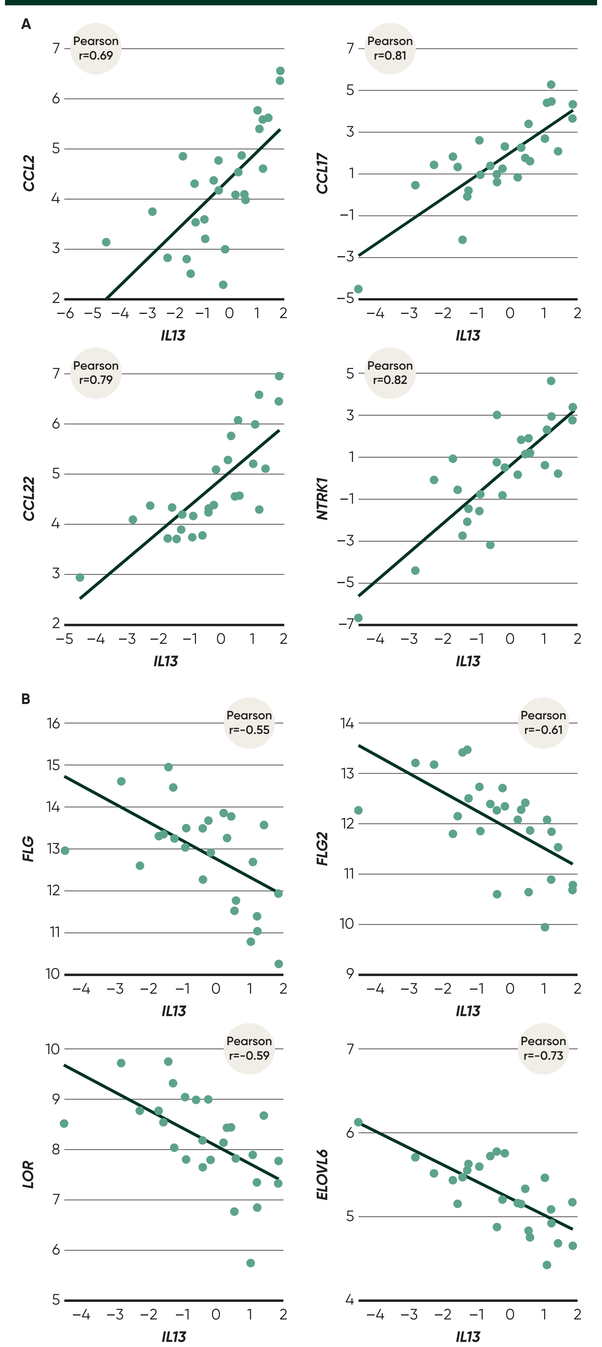
Results

Table 1. Patient samples included in the study

	AD	Controls (healthy)
Number of individuals (male/female)	27 (12/15)	38 (16/22)
Age, years, mean (SD)	34.07 (10.96)	32.63 (11.64)
Objective SCORAD,* mean (SD)	31.11 (10.96)	-
FLG mutation carriers	5	1

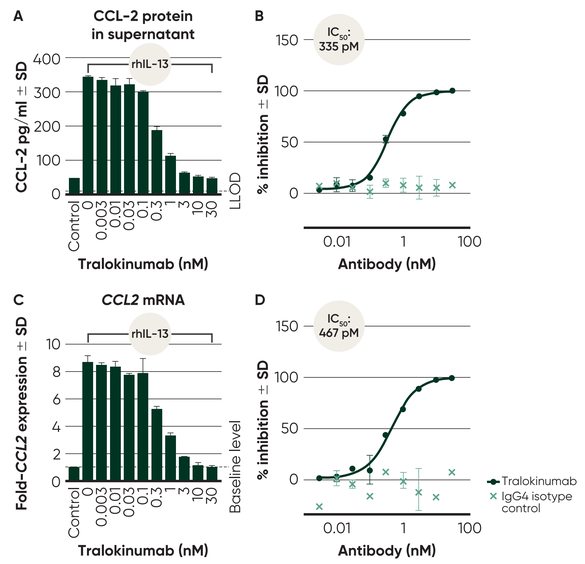
*Objective SCORAD does not include the subjective items daily pruritus and sleeplessness.
FLG, filaggrin; objective SCORAD, objective component of SCORing Atopic Dermatitis; SD, standard deviation.

Figure 2. *IL13* expression strongly correlates with key AD disease biomarkers: A) Positive correlation between *IL13* expression and inflammatory mediators and B) Negative correlation between *IL13* expression and skin barrier markers



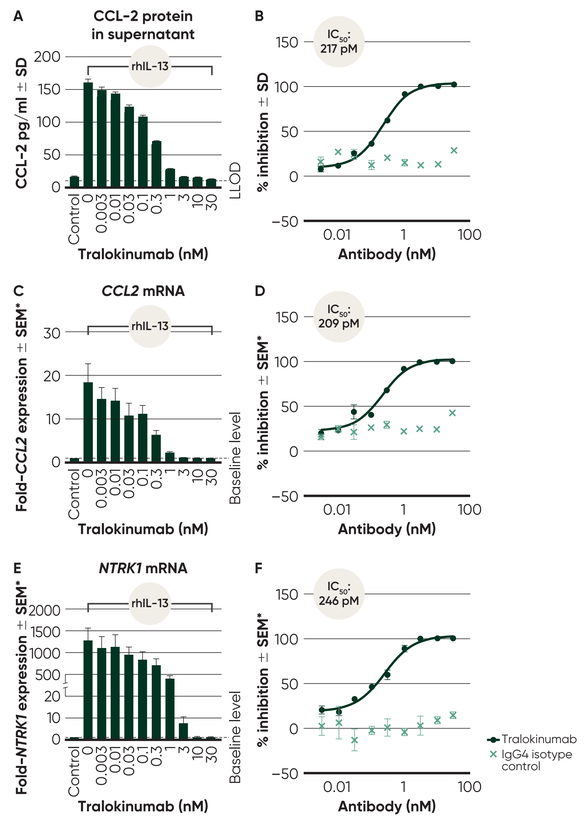
Graph axes represent Log2 (CPM) values.
CCL, C-C motif chemokine ligand; CPM, counts per million; *ELOVL*, elongation of very long chain fatty acids; *IL*, interleukin; *LOR*, loricrin; *NTRK*, neurotrophic tyrosine kinase.

Figure 3. Tralokinumab inhibits IL-13-induced expression of the chemokine CCL-2 in human dermal fibroblasts: A) CCL-2 protein concentration in supernatant; B) Percentage inhibition of IL-13-induced CCL-2 protein secretion; C) *CCL2* mRNA expression; and D) Percentage inhibition of IL-13-induced *CCL2* mRNA expression



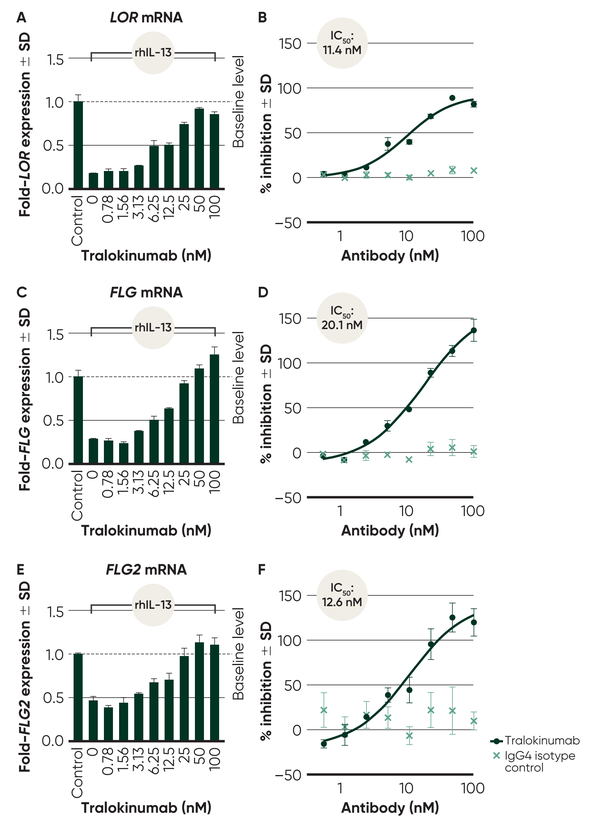
Cells were stimulated with 2 ng/mL (0.16 nM) rhIL-13.
N=1 experiment.
1 nM of antibody=0.15 µg/mL.
Mean change ± SD.
IC_{50}, half maximal inhibitory concentration; LLOD, lower limit of detection; mRNA, messenger RNA; rhIL-13, recombinant human IL-13.

Figure 4. Tralokinumab inhibits IL-13-induced expression of inflammatory mediators in human keratinocytes: A) CCL-2 protein concentration in supernatant; B) Percentage inhibition of IL-13-induced CCL-2 protein secretion; C) *CCL2* mRNA expression; D) Percentage inhibition of IL-13-induced *CCL2* mRNA expression; E) *NTRK1* mRNA expression; and F) Percentage inhibition of IL-13-induced *NTRK1* mRNA expression



*n=3.
Cells were stimulated with 10 ng/mL (0.8 nM) rhIL-13.
N=3 experiments (± SEM) and N=1 on protein measurement.
1 nM of antibody=0.15 µg/mL.
SEM, standard error of the mean.

Figure 5. Tralokinumab restores expression of skin barrier markers decreased by IL-13 in human keratinocytes: A) *LOR* mRNA expression; B) Percentage inhibition of IL-13-induced *LOR* mRNA suppression; C) *FLG* mRNA expression; D) Percentage inhibition of IL-13-induced *FLG* mRNA suppression; E) *FLG2* mRNA expression; and F) Percentage inhibition of IL-13-induced *FLG2* mRNA suppression



Cells were stimulated with 50 ng/mL (4 nM) rhIL-13.
N=1 experiment.
1 nM of antibody=0.15 µg/mL.

Conclusions

- *IL13* expression levels correlate strongly with disease severity and with biomarkers related to the pathophysiology of AD
- The expression of several AD disease biomarkers is regulated by IL-13 and is normalized in a dose-dependent manner by tralokinumab in cultures of human keratinocytes and dermal fibroblasts
- These findings support the rationale for neutralizing excessive levels of IL-13 in AD by utilizing monoclonal antibodies targeting IL-13, such as tralokinumab

References

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Disclosures

- Stephan Weidinger is a speaker, advisory board member, and/or investigator for: AbbVie; Galderma; Incyte; Kymab; La Roche-Posay; LEO Pharma; Lilly; Novartis; Pfizer; and Regeneron and Sanofi-Genzyme
- Maxim A.X. Tollenaere, Thomas Litman, and Hanne Norsgaard are employees of LEO Pharma
- Katharina Drerup has nothing to disclose

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