EVO301: an IL18BP-SAFA biologic demonstrates prolonged residence in inflamed skin and efficacy in a preclinical atopic dermatitis model

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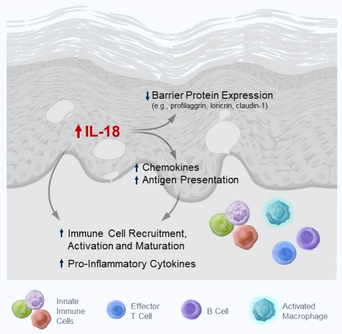
Introduction

IL-18 is a pleotropic cytokine that amplifies inflammation and may contribute to the pathogenesis of multiple chronic inflammatory diseases. IL-18 is robustly expressed in dermal epithelial tissue, and thus is a target of high interest for treating atopic dermatitis, due to several strong scientific rationale listed below:

Key Role for IL-18 Signaling in AD



Strong Scientific Rationale for IL-18BP in AD

- **Human Genetics** — Strong causal link to AD pathogenesis
- **Preclinical Validation** — *In vivo* animal models support pathogenic role
- **Translational Insights** — IL-18 worsens inflammation and impairs barrier integrity
- **Clinical Proof-of-Concept** — IL-18 inhibition improves AD outcomes

EVO301 is a novel biological therapeutic targeting IL-18 mediated inflammation and is currently in a Phase 2 clinical trial in atopic dermatitis (NCT06723405). EVO301 is comprised of the native IL18 binding-protein (IL18BP) and a human serum albumin Fab (SAFA) moiety, which increases the half-life of this therapeutic and may provide a benefit of homing to sites of inflammation.

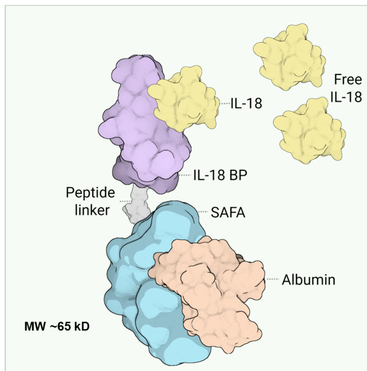
SAFAbody™ Platform Technology

- $T_{½}$ extension: FcRn-mediated recycling of HSA
- Efficient tissue distribution:
  - Smaller size and HSA binding

IL-18 Binding Protein (IL-18BP)

- High binding affinity and specificity
- Functional activity comparable to IL-18 mAb
- Native fully human sequence
  - Low potential for immunogenicity risk and neutralizing antibodies

Footnotes: SAFA - Anti-Serum Albumin Fab-Associated; HSA – Human Serum Albumin SAFABODY™ is a trademark of AprilBio Co., Ltd.



In this study, we evaluated EVO301 PK and preferential homing to oxazolone-inflamed ear skin in human serum albumin (HSA), human neonatal Fc receptor (hFcRn) double transgenic mice. Additionally, we evaluated the ability of EVO301 to improve disease parameters in the chronic oxazolone dermatitis model.

Materials and Methods

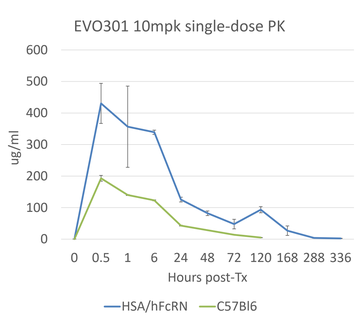
HSA/hFcRn mice and PK study: *HSA/hFcRn mice were purchased from Genoway; these mice have the gene for murine serum albumin replaced with human serum albumin, and the murine FcRn gene replaced with the human FcRn gene. In brief, 8-12 week old mice were given a single intravenous (i.v.) injection of EVO301. Serum samples were collected at various time points post i.v. dosing. An ELISA bioanalysis method for quantification of EVO301 in mouse serum was developed and performed at Charles River Laboratories.*

Fluorescent labeling of EVO301 and FLI in oxazolone-inflamed ear skin: *Fluorescent labeling of EVO301 and the FLI in the oxazolone-induced dermatitis model was performed at Perceptive. EVO301 was fluorescently labeled with CF-750 per manufactures instructions. Male HSA/hFcRN mice (n = 5) (Genoway) were sensitized to oxazolone (1.0% Oxazolone solution (vehicle: ethanol and acetone (1:4)) applied epicutaneously (150 µL) to shaved abdomen 7 days prior to disease induction (D-7). For disease induction (D0), 0.5% Oxazolone solution (vehicle: ethanol and acetone (1:4)) applied topically to the right ear (10 µL), and vehicle solution was applied topically to the left ear, on Days 0, 1, 2, 3, 4, and 5. On Day 3, mice were administered i.v. 10mpk fluorescently labeled EVO301. FLI imaging was performed at 1hr, 4hr, 6hr, 24hr, 48hr, and 72hrs after fluorescently labeled EVO301 injection.*

Cryo-fluorescence tomography (CFT): *CFT was performed at Perceptive. Animals were frozen in n-hexane/dry ice. All samples were stored at -80°C prior to embedding. CFT Image Acquisition: Whole body mice were embedded into one Optimal Cutting Temperature (OCT) compound block. The sample blocks were serially sectioned at 55 µm in a cryomacrotome along with sequential imaging of the block face at each sectioning plane. High resolution images with white light and test article compatible fluorescent channels were acquired using the CFT Xerra system.*

Chronic oxazolone induced dermatitis model: *additional information about this model can be found in the publication by Jang et al., Cytokine 172 (2023) 156413. In brief, SKH-1 mice were sensitized with 100µL 3% oxazolone solution on day 0. From days 7 to 27, mice were challenged with 100µL 0.2 % oxazolone solution thrice weekly. Mice were assigned to eight groups and administered treatment from days 7 to 27. Anti-IL4Rα Ab and anti-IL18 Ab were intraperitoneally administered twice weekly from day 7. EVO301 was intraperitoneally administered every other day from day 7. Skin erythema, scaling, and skin thickness were scored from 0 to 4.*

EVO301 demonstrates longer half-life in HSA/hFcRN mice versus C57Bl/6 mice



	C57BL6	HSA/hFcRN
AUClast (hr*µg/mL)	3,896	17,500
AUCinf (hr*µg/mL)	4,102	17,700
t1/2 (hr)	30.2	47.4
CL (mL/hr/kg)	2.44	0.564
Vss (mL/kg)	81.4	43.2

Figure 1. EVO301 demonstrates longer half-life in HSA/hFcRn vs C57Bl6 mice. A single intravenous (i.v.) dose of 10mpk of EVO301 was administered to HSA/hFcRn mice or C57Bl/6 mice and the systemic PK profile of EVO301 was evaluated through at least 120 hours. Table presents the pharmacokinetic assessment of EVO301 in C57Bl6 mice versus HSA/hFcRn mice

EVO301 preferentially homes to and resides in oxazolone-inflamed ear tissue

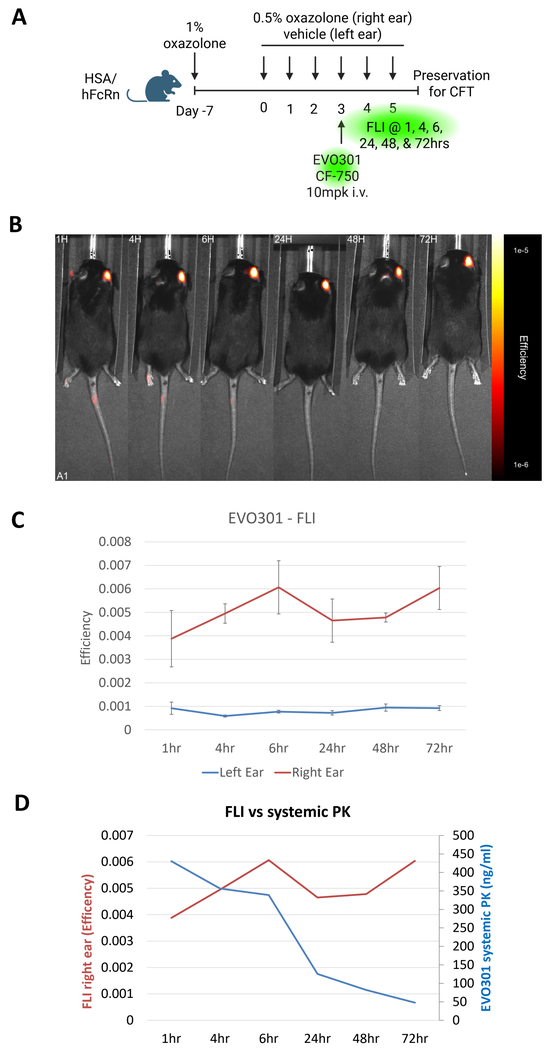


Figure 2. Fluorescence lifetime imaging (FLI) of EVO301 in HSA/hFcRn mice. (A) Schematic of FLI study in oxazolone-induced inflammation in HSA/hFcRn mice. (B) Images of mice at 1, 4, 6, 24, 48, and 72 hrs post 10 mpk i.v. CF-750 labeled EVO301 (C) Quantification of relative fluorescence (Efficiency) in the right and left ear over the duration of the study (D) Comparison of EVO301 FLI results with previous EVO301 systemic PK in HSA/hFcRn mice

Cryo-fluorescence tomography of EVO301

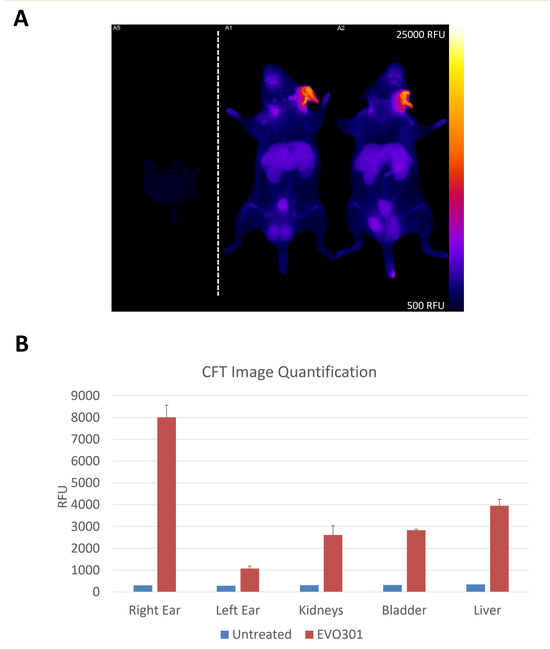


Figure 3. EVO301 demonstrates preferential homing to inflamed tissue compared to internal organs as determined by CFT (A) Images of mice without (left) and with (center and right) fluorescently labeled EVO301 at 10 mpk, 72 hours post dosing (B) Quantification of fluorescence intensity in right (oxazolone inflamed) ear compared to internal tissues

EVO301 reduces disease in the chronic oxazolone-induced dermatitis model in mice

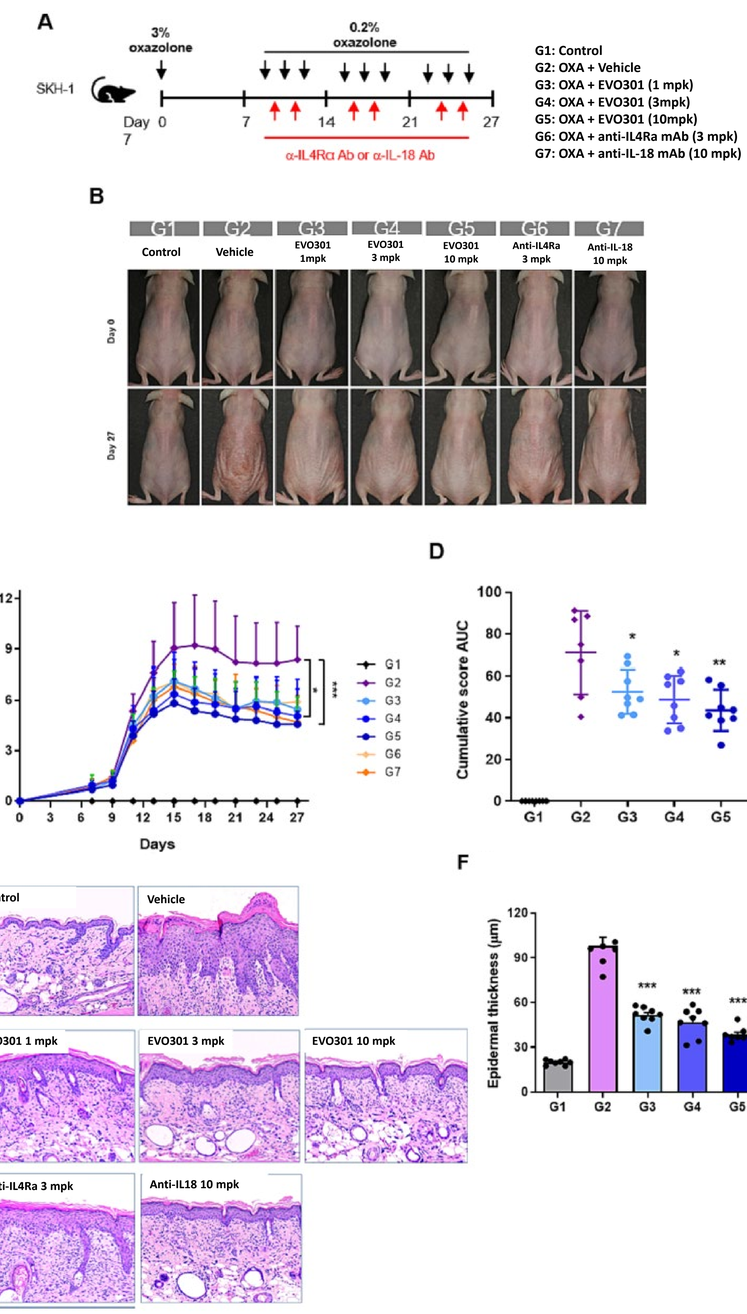


Figure 4. EVO301 demonstrates efficacy in the chronic oxazolone-induced atopic dermatitis model in SKH-1 hairless mice. (A) Experimental design (B) Representative images of mice in each group at Day 0 and Day 27 (C) Cumulative disease score (erythema, scaling, and thickness) of each group (D) AUC of cumulative score. (E) Histopathological features of skin lesions and (F) epidermal thickness of H&E stained skin tissues of each group of mice. This data was also previously published in Jang et al., Cytokine 172 (2023) 156413.

Conclusions

- IL-18 is a proinflammatory cytokine that is proposed to contribute to multiple inflammatory pathways, and is abundantly expressed in epithelial tissues, such as skin.
- EVO301 is comprised of the native IL18 binding-protein (IL18BP) and a human serum albumin Fab (SAFA) moiety. PK studies in C57Bl6 mice versus HSA/hFcRn mice confirmed HSA binding *in vivo,* extending the half-life of EVO301
- In HSA/hFcRn mice, EVO301 preferentially homed to inflamed tissue (oxazolone-induced dermatitis), compared to uninflamed tissue and internal organs
- EVO301 demonstrated efficacy in the chronic oxazolone-induced model of atopic dermatitis in mice
- These data support that EVO301 may be an effective therapeutic for the treatment of chronic inflammatory diseases, such as atopic dermatitis

Acknowledgements and Disclosures

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