EVO301: a potent, novel IL18 inhibiting biologic demonstrating robust preclinical data for the treatment of atopic dermatitis

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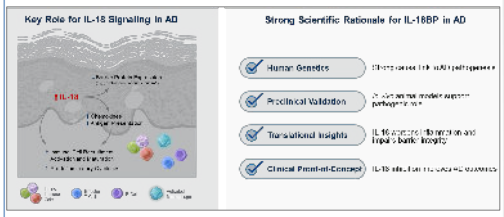
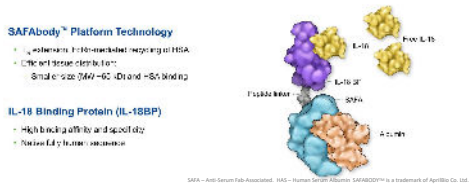
Introduction

Atopic dermatitis (AD) is a common, chronic inflammatory skin condition typically characterized by Th2-driven inflammation. However, AD is highly heterogenous, and not all patients respond to Th2-focused treatments. Therefore, other inflammatory axes may also be involved in pathogenesis. IL18 is a pleotropic cytokine that is robustly expressed in dermal epithelial tissue and amplifies multiple types of inflammation; targeting IL18 may be a novel therapeutic approach to the treatment of a heterogenous indication such as AD.



EVO301 is a novel biological therapeutic targeting IL18, comprised of the native IL18 binding-protein (IL18BP) and a human serum albumin Fab (SAFA) moiety. EVO301 has recently completed a Ph2a trial in AD with positive results.



Here, we present preclinical data supporting the pharmacology of EVO301. In *in vitro* assays, EVO301 demonstrated sub-nanomolar potency at inhibition of IL18, equivalent to competitor therapeutic antibodies and the endogenous IL18BP. In an acute oxazolone induced skin inflammation model, fluorescently labeled EVO301 demonstrated preferential homing to sites of inflammation, while in a chronic oxazolone induced atopic dermatitis model, EVO301 effectively reduced disease parameters. In conclusion, targeting IL18 with therapeutics such as EVO301, may demonstrate efficacy in heterogenous inflammatory conditions such as atopic dermatitis.

EVO301 demonstrates high potency *in vitro*

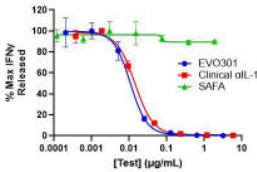
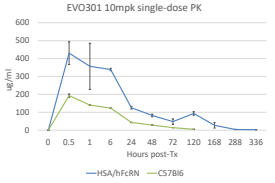


Figure 1. *In vitro* potency of EVO301 versus an anti-IL18 mAb. KG-1 cells were plated in basal media without FCS. Titrations of EVO301, comparator anti-IL18 monoclonal antibody, and SAFA control were combined with human IL18 (8 ng/mL) and placed at 37C for 1hr. KG-1 cells were plated with TNFa (20 ng/mL) and the pre-absorbed Test Article:IL-18 mixtures. After 24hrs, cell supernatant was collected and assayed for IFN-gamma (MSD assay)

EVO301 binds human serum albumin and results in hFcRn mediated half-life extension



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

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

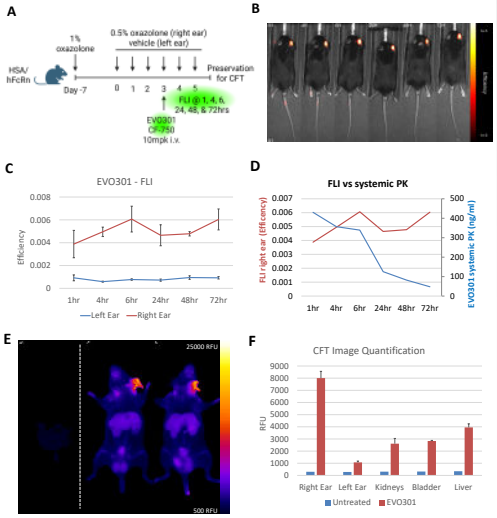


Figure 3. Fluorescence lifetime imaging (FLI) and cryo-fluroescense tomography (CFT) of EVO301 in HSA/hFcRn mice. (A) Schematic of FLI study in oxazolone-induced inflammation in HSA/hFcRn mice. (B) Images of mice at 1, 4, 6, 24, 48, and 72 hrs post 10 mpk i.v. CF-750 labeled EVO301 (C) Quantification 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 (E) Images of mice without (left) and with (center and right) fluorescently labeled EVO301 at 10 mpk, 72 hours post dosing (F) Quantification of fluorescence intensity in right (oxazolone inflamed) ear compared to internal tissues

EVO301 reduces disease in oxazolone-induced atopic dermatitis model in mice

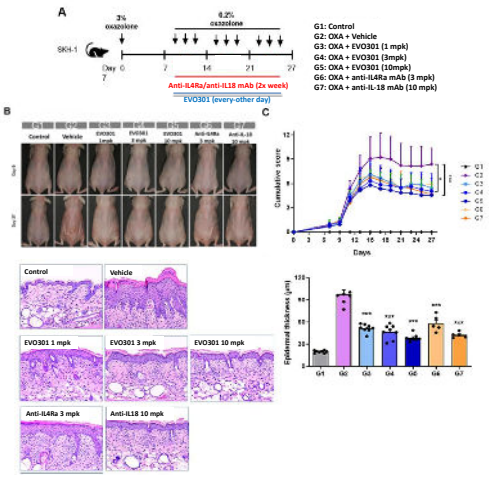


Figure 4. EVO301 demonstrates efficacy in the chronic oxazolone-induced atopic dermatitis model in SKH-1 hairless mice. (A) Experimental design (B) Representative images of mice in each group at Day 0 and 27 (C) Cumulative disease score (erythema, scaling, & thickness). (D) Histopathological features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

EVO301 Phase 2a Clinical Highlights

- Trial met primary efficacy endpoint at week 12
- EVO301 produced rapid, statistically significant EASI reductions at weeks 4, 8 and 12 versus placebo
- 33% placebo-adjusted improvement in EASI at week 12
- 23% of EVO301 patients achieved IGA 0/1 at week 12

Additionally, 23% of patients treated with EVO301 (vs 0% placebo) achieved vIGA-AD 0/1 (percent of patients achieving a score of 0 or 1 on the validated Investigator's Global Assessment for Atopic Dermatitis with ≥ 2-point reduction from baseline) at week 12.

% reduction in EASI at weeks 4, 8, and 12

Visit	EVO301	Placebo	Placebo-adjusted Change	95% CI	p-value
Week 4	-41	-18	-23	(-40, -7)	<0.01
Week 8	-50	-16	-34	(-51, -17)	<0.01
Week 12	-55	-22	-33	(-50, -17)	<0.01

- Pharmacokinetics (PK): Consistent with the Phase 1 healthy volunteer trial; PK and target engagement data continue to support a Q4 week dosing regimen.
- Safety Profile: EVO301 was well tolerated, with no related serious or severe adverse events reported, no treatment related discontinuations due to adverse events and no meaningful differences in events between the active and placebo groups.
- Biomarkers: Robust reduction of both Th2 and non Th2 inflammatory biomarkers in AD including CCL-17 (TARC), CCL-22 and IL-22

https://ir.evommune.com/news-events/press-releases/detail/119/evommune-announces-positive-top-line-data-from-phase-2a-proof-of-concept-trial-of-evo301-in-moderate-to-severe-atopic-dermatitis

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 a high potency inhibitor of IL18 and is comprised of the native IL18 binding-protein (IL18BP) and a human serum albumin Fab (SAFA) moiety, which improves *in vivo* half-life via hFcRn binding
- EVO301 preferentially homed to inflamed tissue (oxazolone-induced dermatitis) and demonstrates efficacy in the chronic oxazolone-induced model of atopic dermatitis in mice
- These data, in addition to recently announced positive Ph2 a data presented above, support that EVO301 may be an effective therapeutic for the treatment of chronic inflammatory diseases, such as atopic dermatitis.

Materials and Methods

In vitro KG-1 assay: *KG-1 cells were purchased from ATCC and cultured in IMDM media supplemented with 20% FCS and 2% Pen/Strep). For the in vitro experiment, KG-1 cells were plated 1.3x10⁶ cells/well in basal media without FCS. Titrations of EVO301, comparator anti-IL18 monoclonal antibody, and SAFA only control were prepared in DPBS+0.3%BSA and then combined with human IL-18 (8 ng/mL) and placed at 37C for 1hr (pre-absorbed Test Article:IL18 mixture). KG-1 cells were plated with TNFa (20 ng/mL) and the pre-absorbed Test Article:IL-18 mixtures. After 24 hrs, cell supernatant was collected and assayed for IFN-gamma (MSD assay).*

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-IL4Ra 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 independently scored from 0 to 4, and summed to generate the cumulative score.*

Acknowledgements and Disclosures

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