EVO756: A Promising Oral MRGPRX2 Inhibitor Showing Strong Preclinical and Phase 1 Results

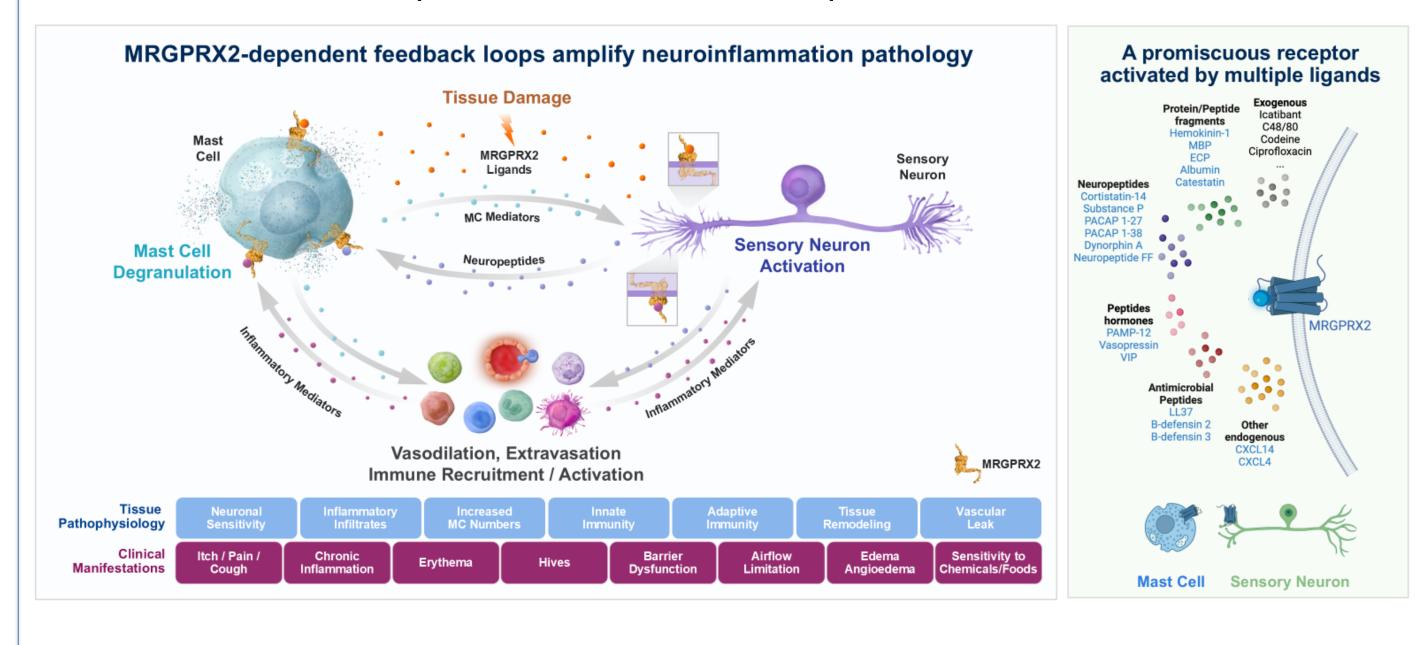


Jamie Harden¹, Sreya Bagchi¹, Stefan Frischbutter^{2,3}, Jorg Scheffel^{2,3}, Han Gao^{2,3}, Nana Shi^{2,3}, Alexandra Pavel¹, Claudia Montlollor-Abalate¹, Janice Drew¹, Polina Bukshpun¹, Kelsey Santisteban¹, Dan Burge¹, Mark Jackson¹, Sarbjit S. Saini⁴, Jeegar Patel¹, Lorena Riol-Blanco¹

¹Evommune, Inc. Palo Alto, CA, USA; ²Institute of Allergology, Charité – Universitätsmedizin Berlin, Corporate Member of Freie Universität Berlin and Humboldt-Universität zu Berlin, Berlin, Germany; ³Fraunhofer Institute for Translational Medicine and Pharmacology ITMP, Immunology and Allergology IA, Berlin, Germany; ⁴Johns Hopkins University, School of Medicine, Division of Allergy and Immunology

Introduction

Mas-Related G-Protein Coupled Receptor X2 (MRGPRX2) is a versatile receptor predominantly found on mast cells and peripheral sensory neurons. It is a key player in FcɛRI-independent mast cell degranulation and drives neurogenic inflammation linked to symptoms such as cough, pain, and itch. MRGPRX2's activation by a wide array of ligands present during chronic inflammation underscores its significant role in tissue pathophysiology. Consequently, targeting MRGPRX2 offers a promising therapeutic strategy for chronic inflammatory diseases like chronic spontaneous urticaria, atopic dermatitis or asthma.



Summary of Key Findings

We introduce EVO756, a novel, oral small molecule inhibitor of MRGPRX2 with robust preclinical and clinical data. EVO756 effectively inhibits MRGPRX2-mediated responses in various models, including transfected CHO cells, LAD2 cells, human skin primary mast cells, and ex vivo skin explants. In a Phase 1, randomized, double-blind, placebo-controlled study, EVO756 demonstrated a well-tolerated safety profile and a pharmacokinetic profile that was proportional to the administered dose. Its pharmacodynamic activity was evaluated through a skin challenge test using icatibant, a known MRGPRX2 ligand. Treatment with EVO756 resulted in statistically significant reductions in icatibant-induced wheal sizes compared to placebo, confirming strong target engagement.

EVO756 broadly inhibits MRGPRX2 activation by all ligands tested

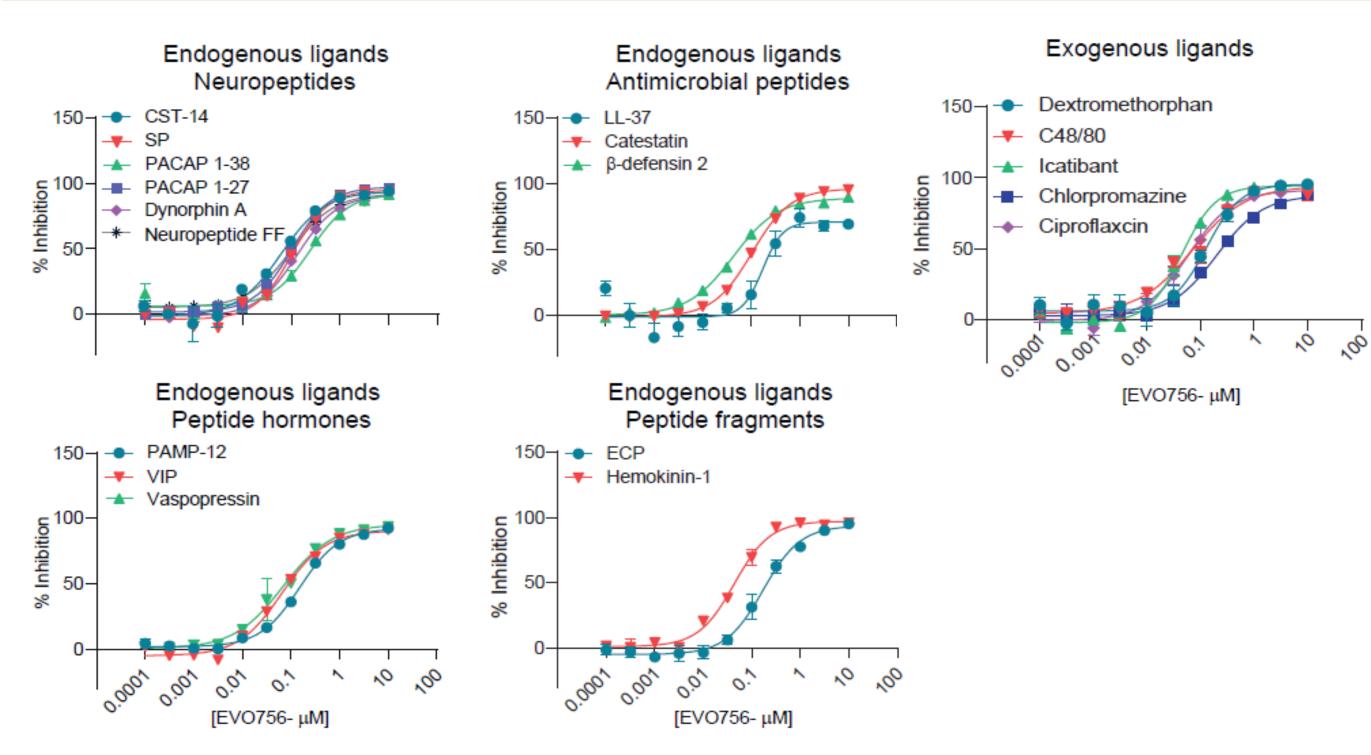


Figure 1. MRGPRX2 ligands and concentration dependent inhibition of MRGPRX2 signaling by EVO756 in CHO-MRGPRX2 transfectant. CHO cells expressing MRGPRX2 were loaded with the FLIPR calcium dye and incubated for 30 min with EVO756 at varying doses. Subsequently, various endogenous and exogenous agonists were added by the FLIPR Penta instrument and calcium flux measured over time. Percent inhibition was calculated based on CHO-MRGPRX2 cells treated with only EVO756 or only agonist.

EVO756 Prevents MC Degranulation In Vitro

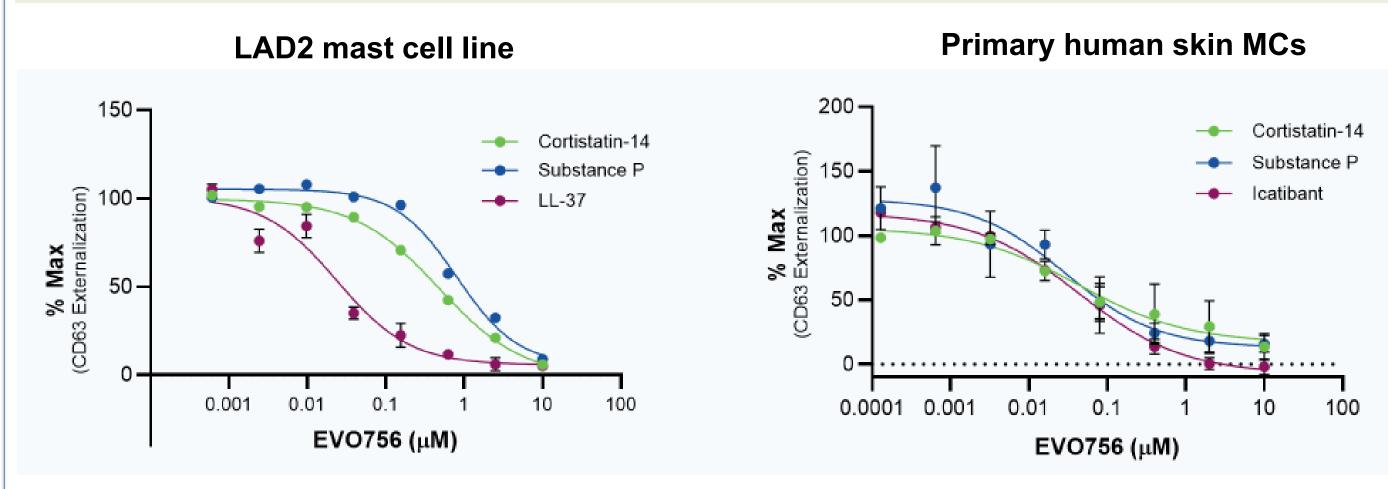


Figure 2. Inhibition of MRGPRX2-mediated mast cell degranulation by EVO756. LAD2 and primary human skin mast cells (phsMCs) were treated with EVO756 at varying concentrations for 5 minutes. Cells were then incubated with MRGPRX2 agonists at greater than EC80 concentrations. For assays assessing CD63 surface expression, cells were incubated for 1 hour with agonists before flow cytometry-based analysis of CD63 expression was performed.

EVO756 Inhibits MRGPRX2-IgE synergistic MC degranulation

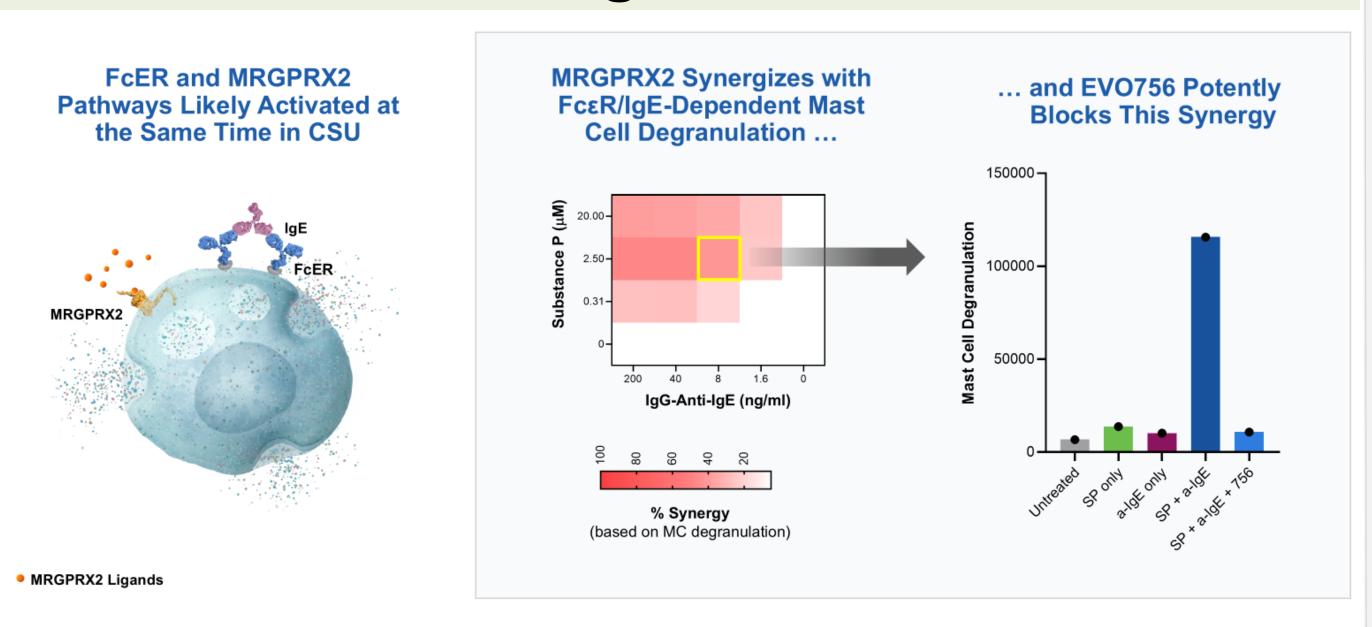
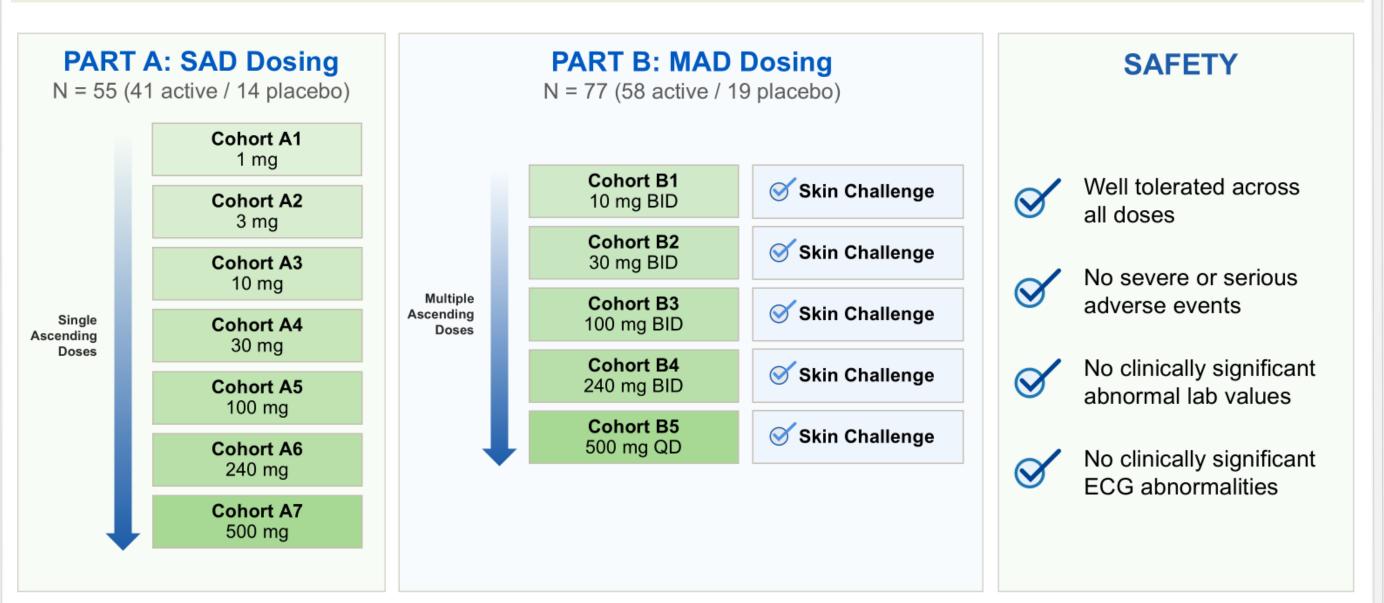


Figure 3. EVO756 blocks MRGPRX2 and FceR1 mediated synergy in primary human skin mast cells. Primary human skin mast cells were treated with different concentrations of SP and/or IgE/anti-IgE in the presence or absence of EVO756 (5 mM). Mast cell degranulation was determined by externalization of CD63 and CD107a. Percent synergy was calculated using the Bliss model of independence.

EVO756: Phase 1 proof of Concept Study Design and Safety



Systemic PK and EVO756 in Skin ISF Post Oral Dosing

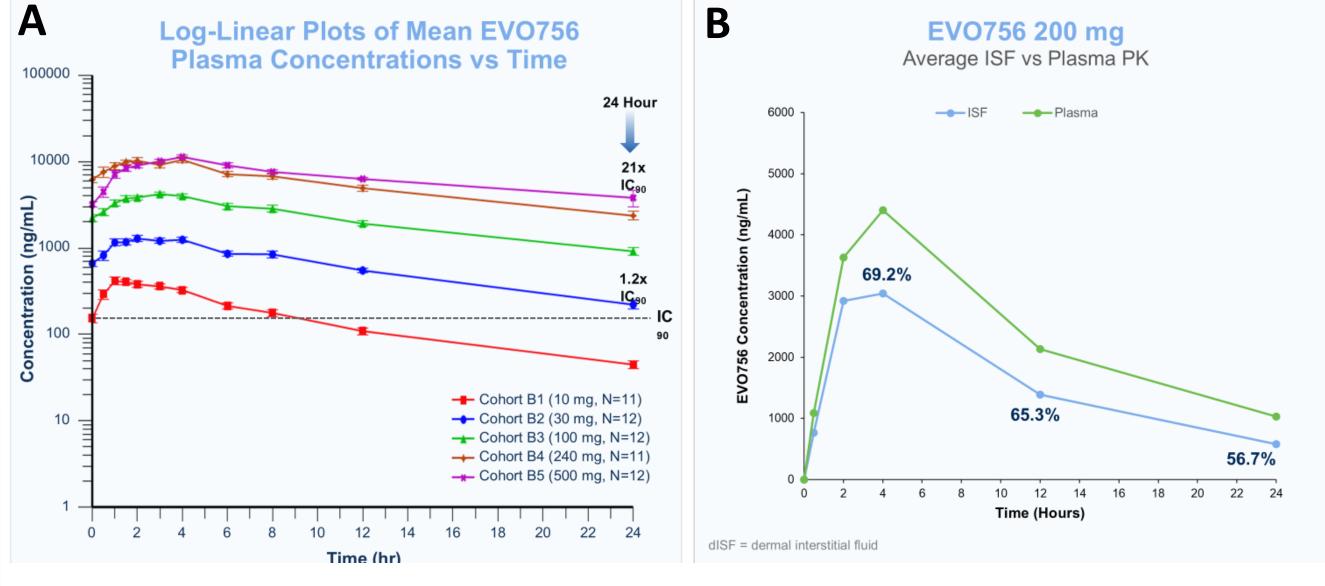


Figure 4. Systemic PK and EVO756 in Skin ISF Post Oral Dosing (A) Plasma concentrations of EVO756 in the multiple ascending dose cohort. EVO756 demonstrates roughly dose-proportional pharmacokinetics. (B) Comparison of systemic plasma levels of EVO756 compared to skin dermal interstitial fluid levels healthy volunteers receiving 200 mg of oral EVO756. Dermal interstitial fluid was collecting using the PELSA device (Ascillion), using silicon hollow microneedles.

Icatibant is a prototypical MRGPRX2 ligand and is inhibited by EVO756

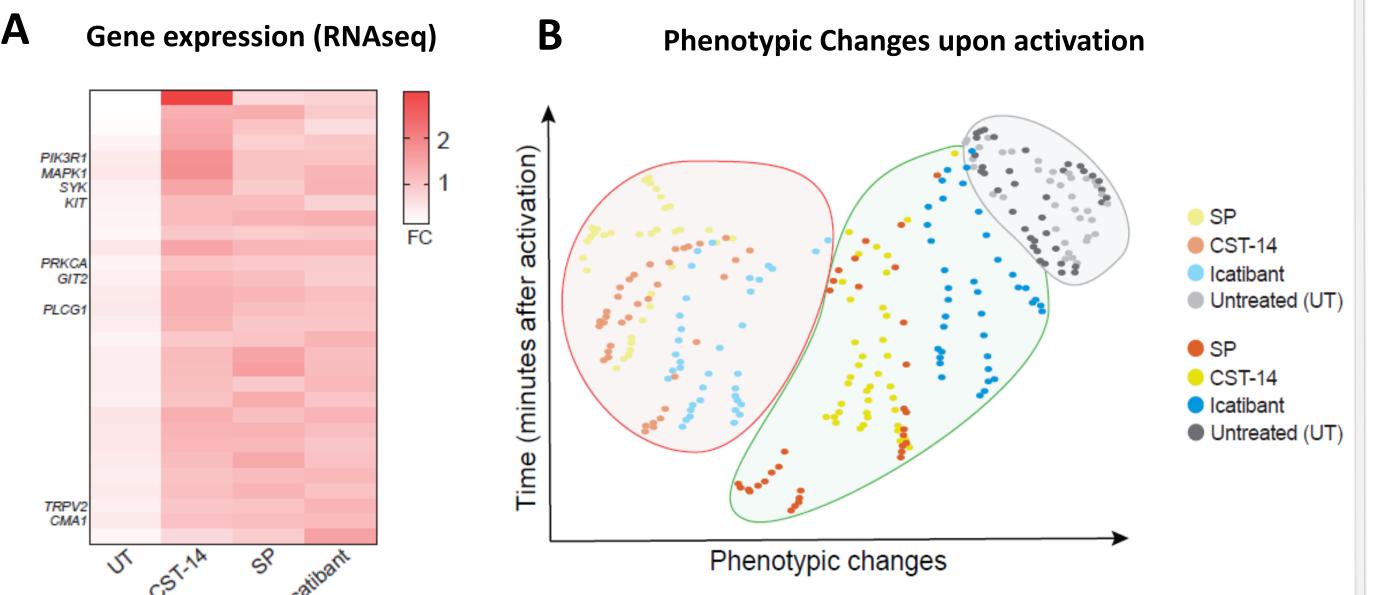


Figure 5. Response of icatibant compared to other MRGPRX2 agonists (A) Common differentially expressed genes in phsMCs stimulated with MRGPRX2 agonists versus untreated cells (B) PCA of morphology of primary human skin mast cells stimulated with MRPGRX2 agonists versus unstimulated control cells

Icatibant is a prototypical MRGPRX2 ligand and is inhibited by EVO756 (cont.)

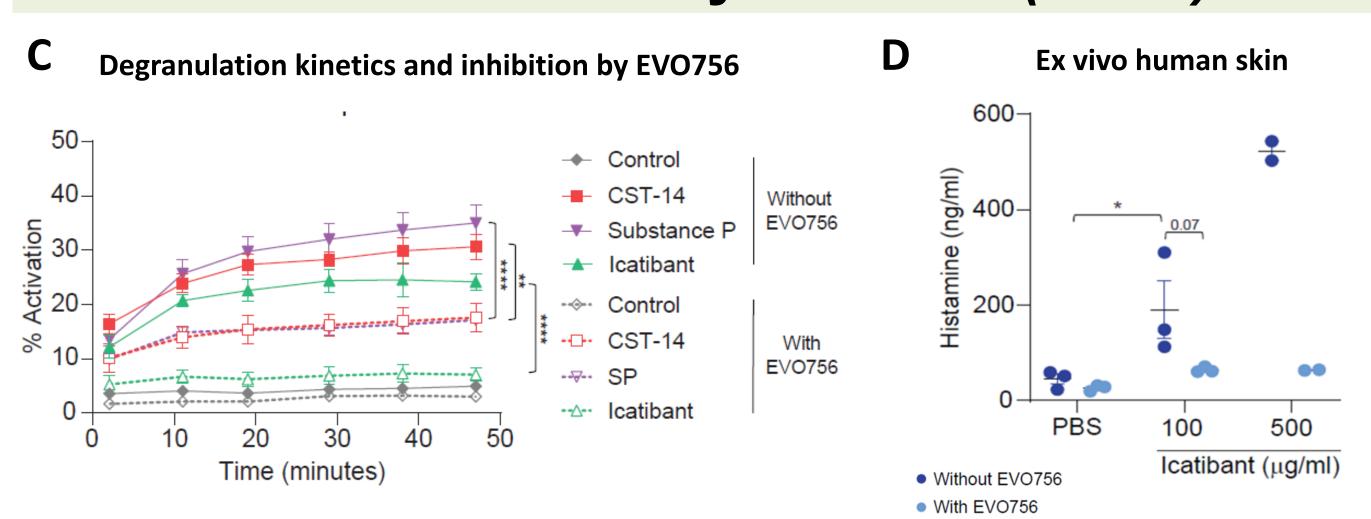
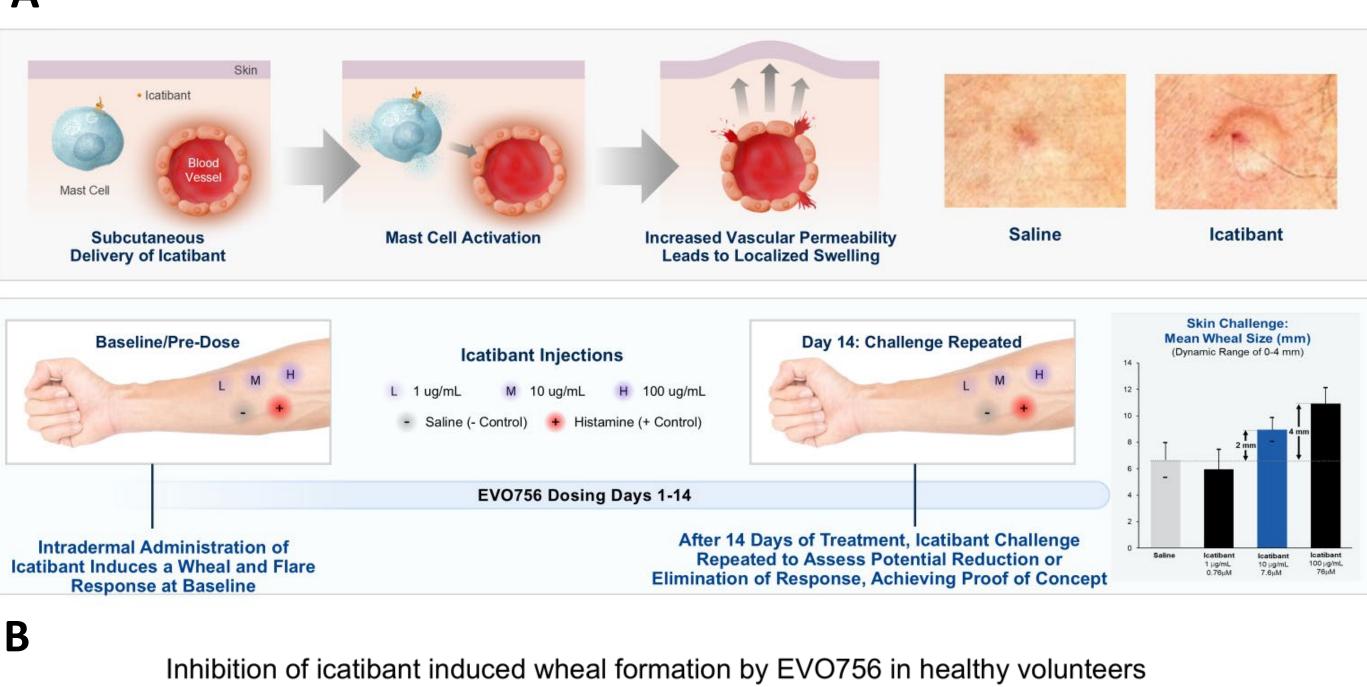


Figure 5 (cont.). Response of icatibant compared to other MRGPRX2 agonists and inhibition by EVO756 is an ex vivo skin assay. (C) Time-course of phsMC degranulation upon stimulation with MRGPRX2 agonists, as determined by the percent of phsMCs with cell-surface CD63 staining (D) Histamine concentrations in dISF collected from ex vivo human skin experiments. EVO756 (EVO) was injected prior to injection at the same site with icatibant. Hollow microneedles (Ascillion) were utilized to extract dISF from the injection sites from which histamine levels were quantified.

Skin Challenge Test: Proof of Concept and Target Engagement for Chronic Urticaria



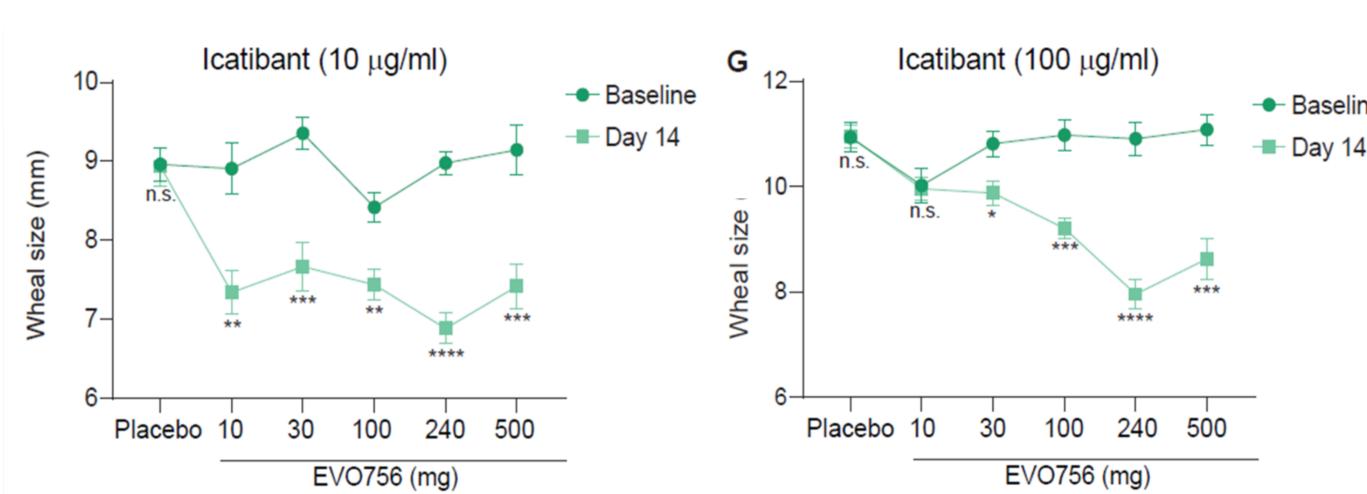


Figure 6. Icatibant skin challenge study design and results (A). Phase 1 healthy volunteer skin challenge study design with oral EVO756. In brief, the wheal response in healthy volunteers was determined at baseline in response to saline, histamine, or 1, 10, and 100ug/mL of an intradermal injection of icatibant. Healthy volunteers were given placebo, 10, 30, 100, 240 mg of EVO756 BID or 500 mg QD for 14 days. After 14 days of oral EVO756, the skin challenge was repeated as performed at baseline, and reduction in wheal size was noted. Average wheal sizes noted in this study shown in lower right (B) Dose-dependent inhibition of icatibant-induced wheal size in healthy volunteers.

Conclusions

- EVO756 is a novel, oral, small molecule inhibitor of MRGPRX2
- EVO756 inhibits MRGPRX2 activation by multiple ligands in several in vitro assays and in synergistic responses with IgE
- EVO756 was evaluated in a Phase 1 Healthy Volunteer Study, which included an icatibant skin challenge component
- EVO756 demonstrated roughly dose-dependent pharmacokinetics and was present in human skin interstitial fluid post oral dosing
- Oral EVO756 inhibited icatibant-induced wheals in a dose-dependent manner

These findings establish EVO756 as a well-tolerated, orally bioavailable inhibitor with the potential to modulate MRGPRX2-driven activation effectively. With promising results from preclinical and Phase 1 studies, EVO756 holds great potential as a treatment for diseases involving mast cells and neuro-inflammation. Further exploration is warranted to unlock its full clinical potential.

Acknowledgements and Disclosures

Some illustrations were created with BioRender.com. JLH, SB, CMA, AP, JL, MJ, JD, PB, KS, DB, JP, and LRB are employees of, and hold stock in, Evommune.