EVO756: An Oral MRGPRX2 Blocker Targeting Mast Cell-Neuron Signaling for Chronic Inflammatory Diseases

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Abstract

Chronic inflammatory diseases often involve complex neuro-immune pathways, with Mas-Related G-Protein Coupled Receptor X2 (MRGPRX2) playing a pivotal role. MRGPRX2 is traditionally known for its mast cell-specific expression leading to FcεRI-independent mast cell degranulation. Because MRGPRX2 responds to a wide range of ligands in inflamed tissues, it is a promising target for therapeutics addressing conditions like chronic urticaria and atopic dermatitis.

EVO756, a novel small molecule, has emerged as a promising oral inhibitor of MRGPRX2. Preclinical data demonstrate robust inhibition of MRGPRX2-mediated responses across experimental mast cell models, highlighting its broad activity in targeting this receptor.

MRGPRX2 expression extends beyond mast cells to include subpopulations of primary human sensory neurons implicated in itch, pain and inflammation. MRGPRX2 expression in neurons is functional, as stimulation with the prototypical MRGPRX2 agonist icatibant induces receptor-dependent activation, which is effectively inhibited by EVO756.

Initial human trials support EVO756's potential. A Phase 1 randomized, double-blind study showed it to be well-tolerated with a generally dose-proportional pharmacokinetic profile. Its mechanism of action was confirmed through a skin wheal challenge with icatibant, where significant reductions in wheal size affirmed effective receptor blockade. Taken together, these findings expand the role of MRGPRX2 as a mediator of neuroinflammation and underscore its dual function as a regulator of immune and neuronal responses.

Background

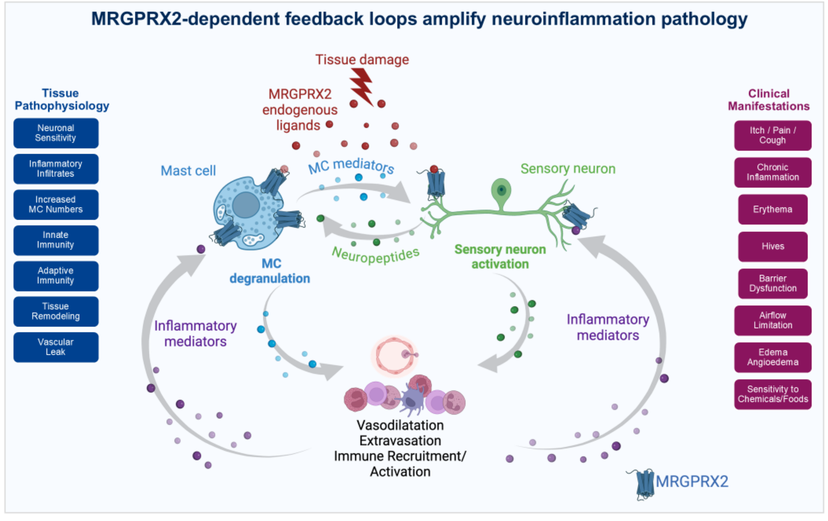


Figure 1. Pivotal Role of MRGPRX2 in Mast Cell Activation and Neuroinflammation

EVO756 broadly inhibits MRGPRX2 activation by all ligands tested

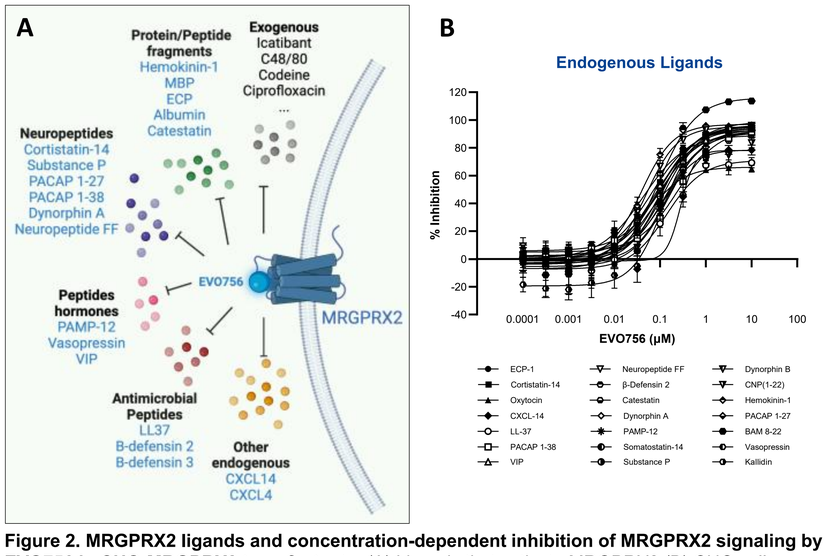


Figure 2. MRGPRX2 ligands and concentration-dependent inhibition of MRGPRX2 signaling by EVO756 in CHO-MRGPRX2 transfectants. (A) Ligands that activate MRGPRX2 (B) CHO cells expressing MRGPRX2 were loaded with the FLIPR calcium dye and incubated for 30 min with EVO756 at varying doses. Subsequently, various endogenous and exogenous (*not shown*) 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 Mast Cell Degranulation *In Vitro*

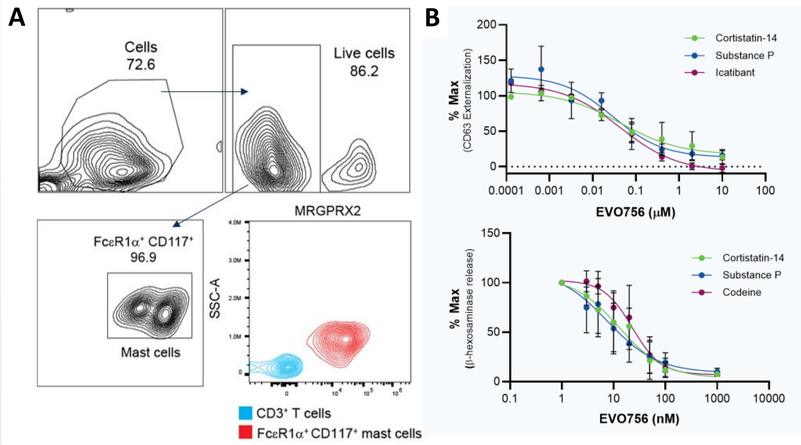


Figure 3. Inhibition of MRGPRX2-mediated mast cell degranulation by EVO756. (A) Gating strategy and MRGPRX2 expression on primary human skin mast cells. (B) Primary human skin mast cells were treated with EVO756 at varying concentrations for 5 minutes. Cells were then incubated with MRGPRX2 agonists 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. For the β-hexosaminidase assay, released versus cellular content of the enzyme was detected in the supernatant and cell pellets after 1 hour of incubation with agonists.

Human sensory neurons express MRGPRX2

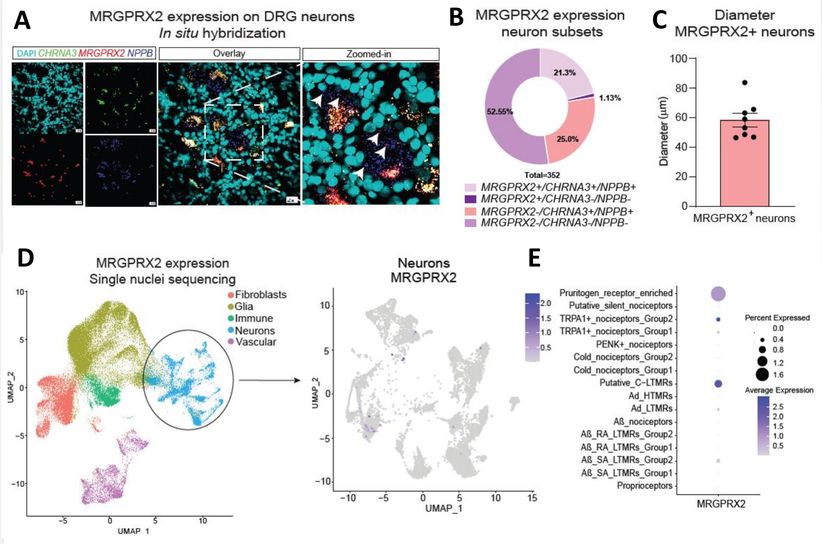


Figure 4. Expression of MRGPRX2 on human sensory neurons (A) *In situ* hybridization and (B) Proportion of MRGPRX2, CHRNA3, and NPPB-expressing human dorsal root ganglia (DRG) neurons (from n=8 organ donors). (C) Diameter of MRGPRX2+ neurons. (D) Left: Unbiased clustering of cell types and Right: Neurons from single nuclei RNA sequencing of human DRG cells from organ donors (n=4). (E) MRGPRX2 expression across human sensory neuron subpopulations.

EVO756 inhibits MRGPRX2 activation of human sensory neurons

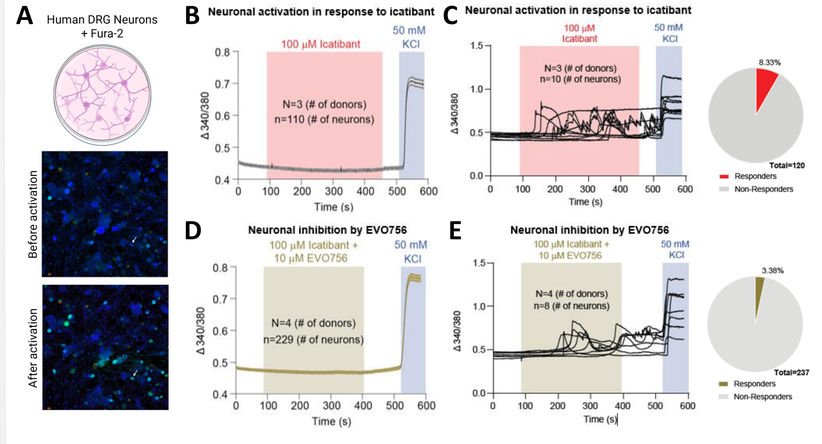
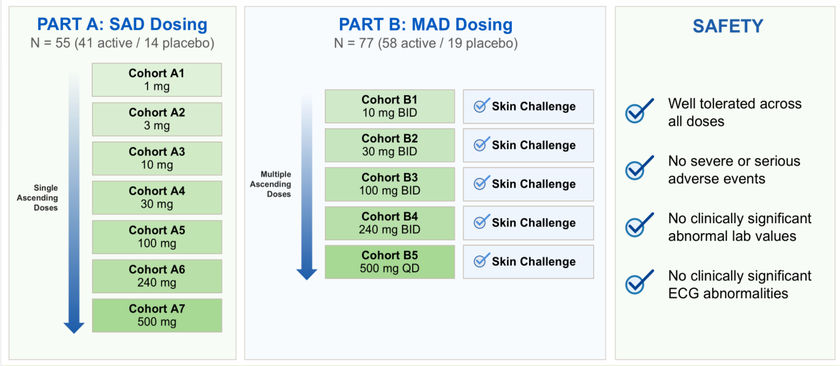


Figure 5. EVO756 inhibits functional MRGPRX2 activation of human sensory neurons. (A) Schematic of calcium imaging: cultured human DRG neurons were pre-loaded with Fura-2 prior to time-lapse fluorescent imaging. (B-E) Calcium imaging traces of human DRG neuron (B,D) non-responders and (C,E) responders after treatment with (B-C) icatibant alone (5 minutes, 100μM) (n=3 organ donors) and (D-E) inhibition in the presence of EVO756 (10μM) (n=4 organ donors) followed by treatment with KCl (50mM).

EVO756 Phase 1 Proof of Concept Study Design and Safety



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

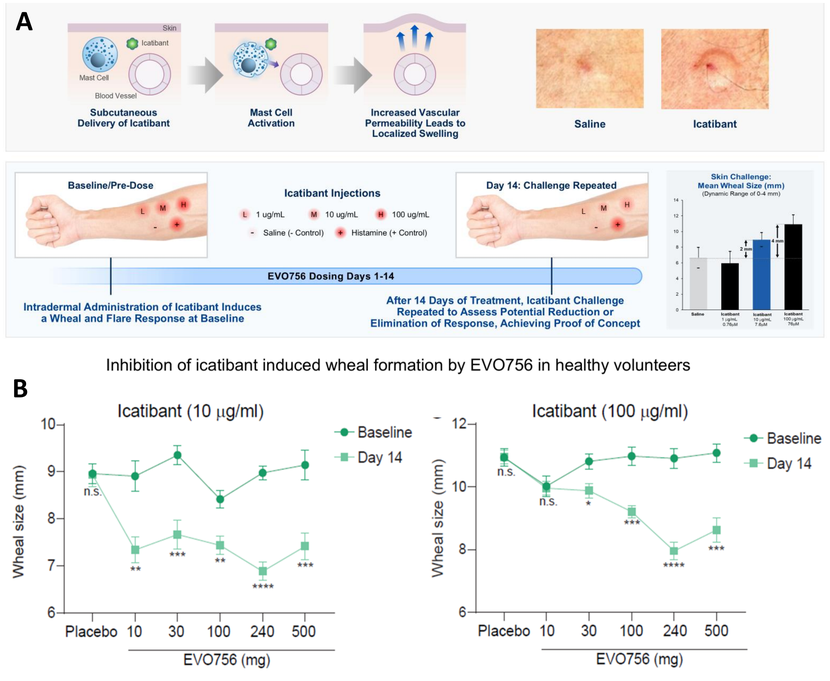


Figure 6. Icatibant skin challenge study design and results (A). Phase 1 healthy volunteer skin challenge study design with oral EVO756. In brief, the wheal response in healthy volunteers was determined at baseline in response to saline, histamine, or 1, 10, and 100ug/mL of an intradermal injection of 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 in mast cells and peripheral sensory neurons involved in itch, pain and inflammation
- EVO756 was evaluated in a Phase 1 Healthy Volunteer Study, which included an icatibant skin challenge component
- EVO756 inhibited icatibant-induced wheals in a dose-dependent manner
- EVO756 is a potential therapeutic for the treatment of diseases caused by overactivation of mast cells and neuroinflammation mediated by MRGPRX2, such as chronic urticarias and atopic dermatitis

Acknowledgements and Disclosures

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