# EPS-3903 is a Potent and Selective Oral STAT6 Inhibitor That Blocks Th2 Inflammation in an Ovalbumin Asthma Mouse Model

Poster #193

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## BACKGROUND

- Asthma is a complex respiratory disease characterized by airway hyperresponsiveness and inflammation.
- An IL-4/IL-13 signaling-mediated Th2 immune response is the determinant driver of allergic asthma. Dupilumab, an injectable monoclonal antibody that blocks human IL-4Rα, has been approved to treat moderate-to-severe asthma.
- STAT6 is a transcription factor central to Th2 inflammation that mediates IL-4/IL-13 signaling (**Figure 1**). STAT6 activation results in lung inflammation and tissue remodeling via regulation of Th2 cell differentiation, eosinophilic recruitment and epithelial mucus production. STAT6 genetic loss-of-function protects against Th2 inflammation associated asthma in humans[1].
- EPS-3903 is a selective and potent orally bioavailable allosteric small molecule inhibitor of IL-4/IL-13-mediated STAT6 activation with potential to treat a wide range of allergic diseases, including asthma and atopic dermatitis.

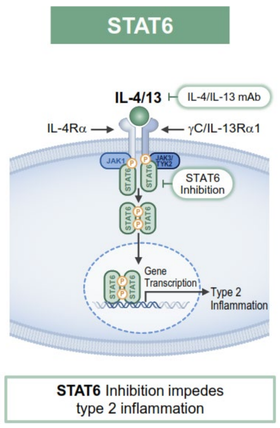


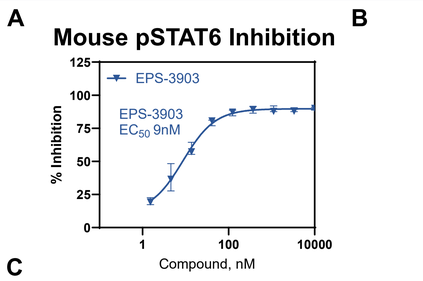

**Figure 1. STAT6 is the key transcription factor mediating IL-4/IL-13-induced Th2 inflammation.**

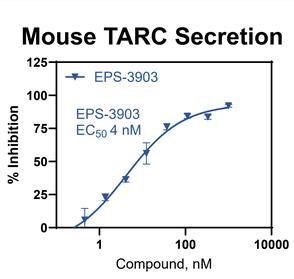
## METHODS

- *In vitro* potency against mouse STAT6 phosphorylation (pSTAT6) and TARC secretion was assessed in primary mouse splenocytes with mouse IL-4 (mIL-4) stimulation. Potency against human STATs was performed in human PBMC with respective cytokines for stimulation. TARC and pSTAT6 levels were measured by MSD.
- *Ex vivo* STAT6 activation was assessed by exogenous mIL-4-stimulated pSTAT6 in whole blood collected from mice that received a single oral dose of EPS-3903 for 24 hours.
- *In vivo* asthma model was established in BALB/cJ mice by intranasal ovalbumin (OVA) sensitization and challenge. EPS-3903 was orally given to mice from day 19 to day 23. Antibodies against mouse IL-4/IL-13 and the isotype IgG control were injected intraperitoneally on day 17 and day 20. Mice were euthanized on day 23, BALF was harvested for leukocyte and eosinophil quantification by flow cytometry. Serum was collected for OVA-specific IgE analysis by ELISA. BALF supernatants and lung tissues were used for Th2 biomarker measurement, such as TARC and eotaxin, by MSD. Goblet cell characterization was identified by PAS staining, and *Muc5ac* gene expression was measured by TaqMan quantitative real-time PCR.

## RESULTS

### EPS-3903 Is a Potent and Selective STAT6 Inhibitor *In Vitro*

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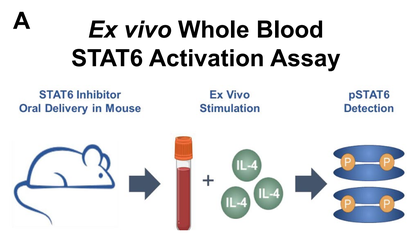
**Selectivity Against Human STAT Family**

| Stimulator | hSTATs | EPS-3903 $EC_{50}$ (nM) |
|---|---|---|
| Type 1 IFN | pSTAT1 | >5000 |
| Type 1 IFN | pSTAT2 | >5000 |
| IL-6 | pSTAT3 | >5000 |
| IL-12 | pSTAT4 | >5000 |
| IL-2 | pSTAT5 | >5000 |
| IL-4 | pSTAT6 | 4 |

**Figure 2. Potency and selectivity of EPS-3903 *in vitro*.** Potency of EPS-3903 against (A) mouse pSTAT6 and (B) TARC secretion in primary mouse splenocytes after mIL-4 stimulation. (C) Potency of EPS-3903 against human STATs in PBMC with respective stimulators. TARC: thymus and activation-regulated chemokine. PBMC: peripheral blood mononuclear cells. $EC_{50}$, half-maximal effective concentration.

## RESULTS

### EPS-3903 Inhibits Whole Blood STAT6 Activity By >90%, 24 Hours After a Single Oral Dose



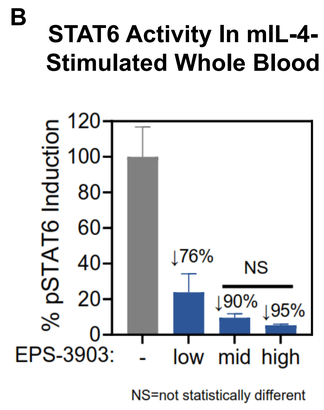
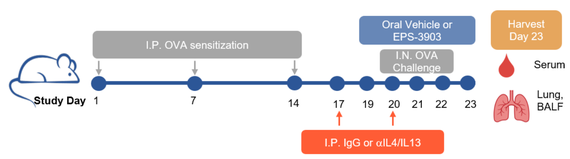




**Figure 3. Whole blood target engagement of EPS-3903.** (A) A schematic of the *ex vivo* STAT6 activation whole blood assay. (B) Whole blood pSTAT6 was measured 24 hours post single oral dose of EPS-3903 at different concentrations in the absence or presence of mIL-4.

### EPS-3903 Blocks OVA-induced Lung STAT6 Activity in an Asthma Mouse Model



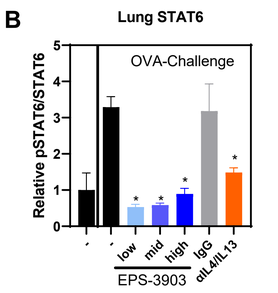




**Figure 4. Lung target engagement of EPS-3903 in an OVA asthma mouse model.** (A) Study design of OVA-induced asthma mouse model. I.P.: intraperitoneal, I.N.: intranasal. αIL4/IL13: mouse IL-4/IL-13 IgG antibodies. (B) Lung pSTAT6 and total STAT6 were measured in lung lysates from OVA-treated mice. Data are presented as the signal intensity ratio between pSTAT6 and total STAT6, normalized to the untreated control group. * p<0.05 vs Corresponding Vehicle.

### EPS-3903 Alleviates Th2 Inflammation With a Comparable Efficacy to Anti-IL4/IL13 Treatment

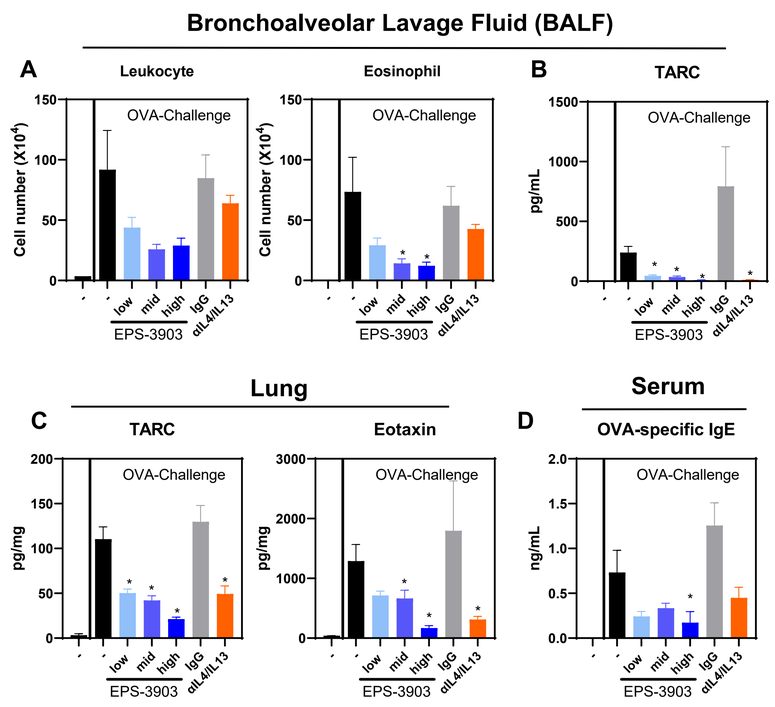



**Figure 5. Effects of EPS-3903 on Lung Th2 inflammation.** (A) Absolute cell numbers of leukocytes and eosinophils in BALF. (B) TARC levels in BALF supernatants. (C) Lung Th2 inflammatory biomarkers, TARC and eotaxin. (D) OVA-specific IgE production in serum. * p<0.05 vs Corresponding Vehicle.

## RESULTS

### EPS-3903 Improves Epithelial Histopathology With a Comparable Efficacy to Anti-IL4/IL13 Treatment

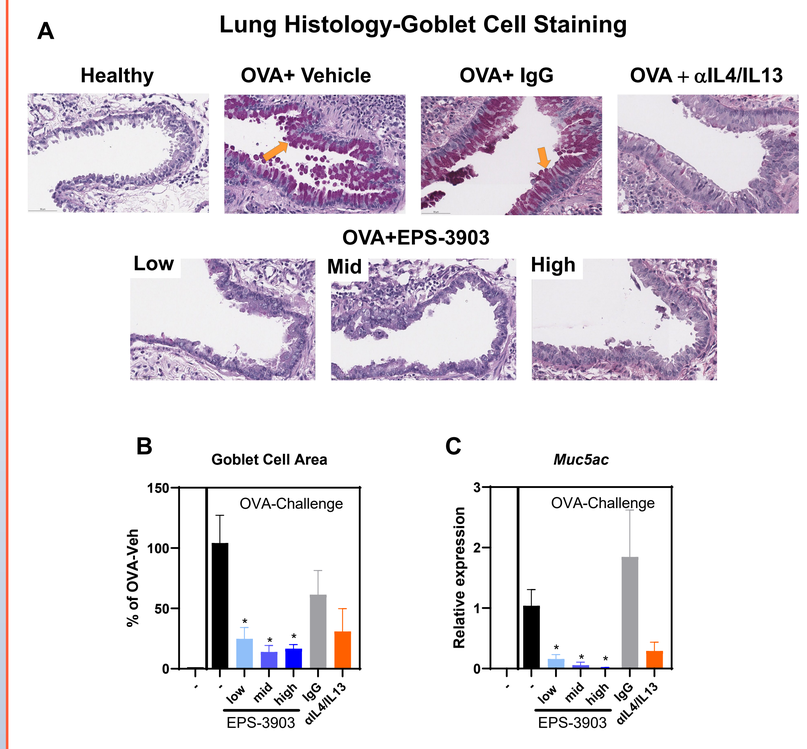



**Figure 6. Lung histology of OVA-induced asthma model treated with EPS-3903.** (A) Representative images of goblet cells by PAS staining in lung. Arrow: goblet cell. Scale bar: 50 μm. (B) Quantification of goblet cells by area in lung epithelium. (C) Goblet cell marker, *Muc5ac*, expression in lung. PAS: periodic acid-schiff . * p<0.05 vs Corresponding Vehicle.

## CONCLUSIONS

- **Potency and Selectivity:** EPS-3903 is an oral small molecule allosteric STAT6 inhibitor with single-digit nanomolar potency and excellent selectivity against other human STATs.
- **Target Engagement:** EPS-3903 inhibits STAT6 activity by >90% for 24 hours in mouse whole blood after a single oral dose and completely inhibits induced STAT6 activity in lung tissue in an OVA-induced asthma mouse model.
- **Preclinical Efficacy:** EPS-3903 alleviates Th2 inflammation and improves lung epithelial histopathology in an OVA asthma mouse model, with comparable efficacy to anti-IL4/IL13 treatment.EPS-3903 may offer an oral therapeutic option for treating allergic diseases including asthma and atopic dermatitis.

## ACKNOWLEDGEMENTS

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## DISCLOSURE

All authors are either current or former employees of Enanta Pharmaceuticals, Inc. and received salary and stock compensation.

## REFERENCE

1. Kristjansdottir *et al*. 2024 “A partial loss-of-unction variant in STAT6 protects against type 2 asthma”, Journal of Allergy and Clinical Immunology, 2024; 155, 228-235.