Orally Delivered Microbial Extracellular Vesicles Modulate Systemic Inflammation Through the Small Intestinal Axis (SINTAX™)

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Introduction

Evelo Biosciences is developing a new class of oral medicines which engage the immune system in the small intestine with anti-inflammatory effects throughout the body.

EDP2939 is an orally-delivered and gut-restricted bacterial EV which potently attenuates inflammation in murine models of Th1 and Th17 inflammation.

The small intestinal axis (SINTAX™) is a network of anatomic and functional connections with the rest of the body. It acts as a sensory system, integrating environmental signals that link gut mucosal immunology with immunological processes throughout the body.

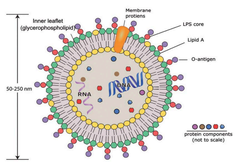
This suggests that SINTAX is a control mechanism for systemic immunity centred in the small intestine. This mechanism has novel features of considerable interest for the development of immunomodulatory therapies. It may be harnessed for orally delivered medicines that are systemically effective without systemic distribution.

We have previously shown clinical proof of the SINTAX mechanism with EDP1815, an orally delivered single strain of commensal bacteria. It has systemic anti-inflammatory effects with a safety profile comparable to placebo. EDP1815 comprises almost entirely non-living bacteria. It exerts its effects through direct action on host cells in the gut with no colonization, alteration of the microbiome, or exposure outside the gut.

Some bacteria produce extracellular vesicles (EVs) that share molecular content with the parent bacterium in a particle that is roughly 1/1000th the volume in a non-replicating form.

We report here the preclinical pharmacological effects, mechanism of action, and biodistribution of EDP2939, an orally administered preparation of EVs derived from a single gram-negative bacterial strain of the family *Prevotellaceae* that was selected from screens of EVs for anti-inflammatory pharmacology.

Extracellular Vesicles (EVs)



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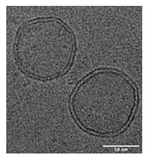

Cryo-EM image of EDP2939

- Extracellular vesicles (EV) are lipoprotein nanoparticles naturally produced by some species of bacteria
- Their macromolecular content is a subset of the parent
- EVs enable bacterial communication and survival during stress, host-immune modulation, material exchange, and cell-cell interactions
- Compared to whole microbes EVs are:
  - ~1/1000th volume of microbes enabling improved target engagement
  - Non-viable
  - Incapable of establishing infection or sepsis

EDP2939 is an effective anti-inflammatory drug requiring multiple pathways for efficacy

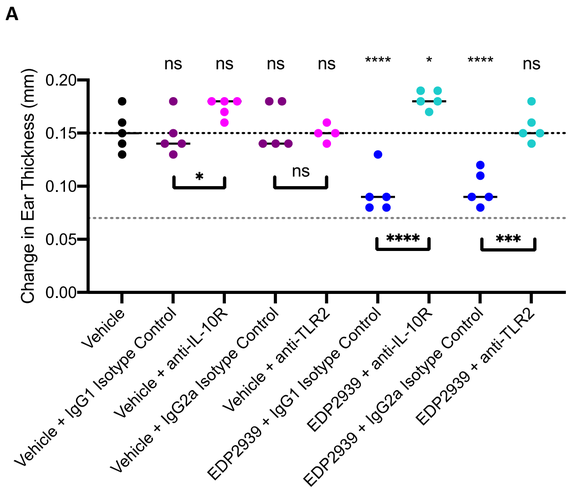

Figure 1: Orally-administered EDP2939 requires multiple pathways for anti-inflammatory effects. Mice undergoing a delayed type hypersensitivity (DTH) reaction against keyhole limpet hemagglutinin (KLH) were dosed with 2E10 particles/dose of EDP2939 by oral gavage on days 5–8. During the study, various mechanisms of action were interrogated by intraperitoneal injection of antibodies as indicated. **A)** Graph shows changes in ear thickness 24 hours after challenge with KLH protein and blockade of TLR2 or IL-10R signaling. **B)** Graph shows changes in ear thickness 24 hours after challenge with KLH protein and inhibition of lymphocyte gut homing. Points indicate individual mice and line shows median change in ear thickness. Data are representative of 2 independent studies. Statistical analyses were performed using a one-way ANOVA (vs. vehicle) or two-tailed unpaired t-test (isotype vs. treated). Ns = not significant, *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001.

Orally administered fluorescently-labelled EDP2939 is gut-restricted

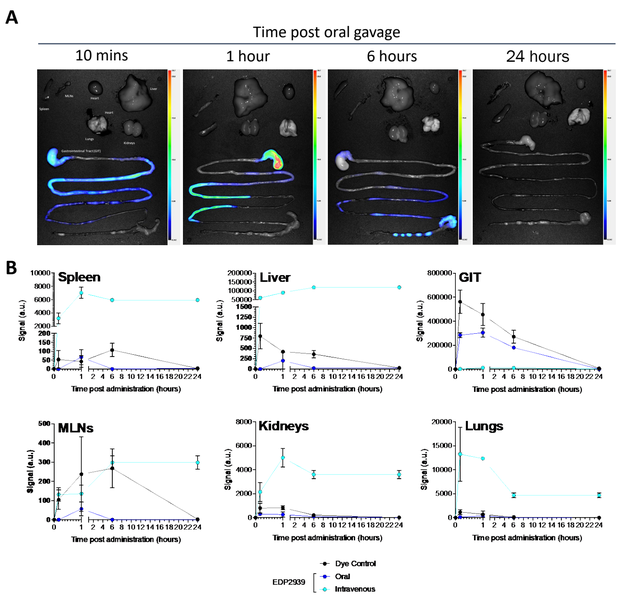

Fig 2. Orally dosed EDP2939 is restricted to the gastrointestinal tract. Mice were injected intravenously or orally dosed with 2E10 EDP2939 particles covalently labeled with IRDye800 or dye-only control. After 10 mins, 1 hour, 6 hours, or 24 hours, fluorescence was measured in the indicated organs using a small animal imaging system (Licor Pearl®). **A)** Representative images showing fluorescence from labelled EDP2939 in various organs at indicated time points post oral gavage. **B)** Graphs showing total signal measured in indicated organs and time points after oral gavage of dye control (*black*) or labelled EDP2939 (*dark blue*) or intravenous injection of labelled EDP2939 (*light blue*). Points show fluorescence intensity mean ± SD. Data are representative of 2 independent experiments.

Conclusions

- Orally-delivered microbial extracellular vesicles enact broad-based resolution of inflammation establishing homeostatic inflammatory status
- Efficacy of EDP2939 requires the stimulation of both the TLR2 receptor and the IL-10 receptor in addition to lymphocyte homing to the intestinal lymphoid tissue
- EDP2939 induces TLR2-dependent release of IL-10
- EVs are an orally-dosed, gut-restricted therapeutic with no apparent safety or tolerability issues in animal models, making for a desirable therapeutic profile

These data support the development of EVs as a new class of immunotherapeutic drugs. They are particularly effective at engaging the small intestinal axis, acting locally on host cells in the gut to activate distal immune responses. EDP2939 is in preclinical development for inflammatory disorders involving both aberrant Th1 and Th17 immune responses.

EDP2939 induces the release of IL-10 through TLR2 stimulation

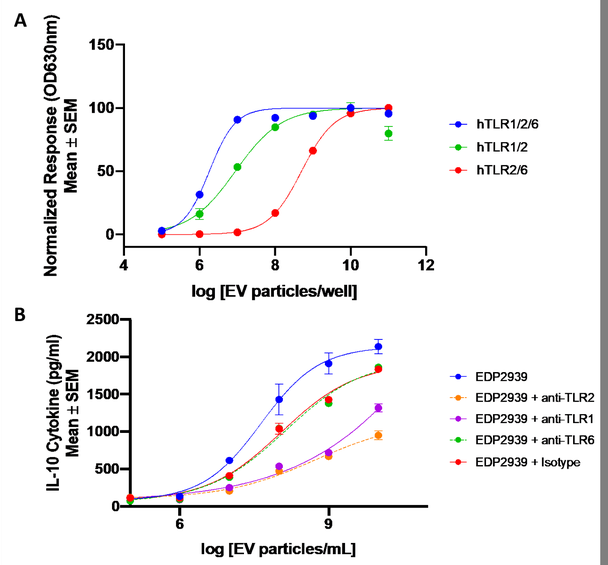

Fig 3. EDP2939 induces release of IL-10 following stimulation of TLR2. A) EDP2939 stimulates both TLR1/2 and TLR2/6 heterodimers, with greater potency towards the TLR1/2 heterodimer. HEK293-SEAP reporter cells (Invivogen) expressing human TLR1, TLR2, and TLR6 combinations were incubated for 24 hours with EDP2939 at the indicated concentrations. Supernatants were collected and analyzed for secreted embryonic alkaline phosphatase (SEAP) production to determine stimulation of TLR2 heterodimers. **B)** EDP2939 stimulated IL-10 release from U937 cells is impaired by antibody-mediated blockade of either TLR1 or TLR2, but not TLR6. PMA-differentiated human monocytic U937 cells were incubated with EDP2939 ± 2.5 µg/mL anti-TLR1, TLR2, TLR6 or isotype control antibody for 24 hours. Supernatants were collected and analyzed for IL-10 response by MSD. Data are representative of 2 independent experiments.

EDP2939 stimulates anti-inflammatory cytokine secretion from human PBMCs

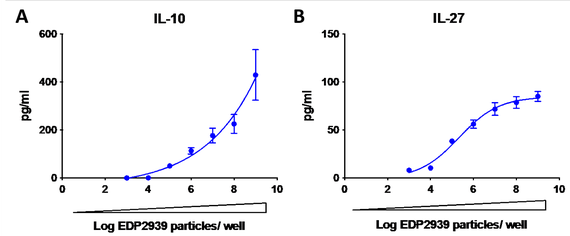

Fig 4. EDP2939 induces IL-10 and IL-27 concentration-dependent production from human PBMCs. PBMCs were isolated from whole blood of six human donors, plated at 100,000 cells per well, rested overnight, and then incubated with varying concentrations of EDP2939 for 24 hours. Supernatants were collected and **A)** IL-10 and **B)** IL-27 concentrations were determined via MSD. Data are representative of six independent human donors.