Treatment with an orally delivered non-replicating, non-colonizing strain of *Veillonella parvula* resolves systemic inflammation

Kritika Ramani, Taylor Cormack, Adam Cartwright, Aula Alami, Shannon Argueta, Divya Raghunathan, Mary Abdou, Fabian B. Romano, Valeria Kravitz, Tyler Rommel, Esra Uckun Kiran, Pallavi Pradeep, Maria Sizova, Holly Ponichtera, Tanmoy Ganguly, Mark Bodmer, and Andrea Itano

Evelo Biosciences, Inc. Cambridge MA

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.

The mucosal immune system comprises of a complex network of intestinal epithelial cells and immune cells. Targeting the mucosal immune axis is of considerable interest for the development of a new modality of oral immunomodulatory therapies.

We report that *Veillonella parvula* (EDP1867), a microbial strain isolated from mucosal tissue of a human donor, rendered non-replicating and non-colonizing, acts through pattern recognition receptor mediated modulation of small intestinal immune responses to induce potent systemic inflammation resolution.

EDP1867 attenuates murine models of Th1 and Th17-inflammation. Orally delivered EDP1867 exerts its effects through direct action on host cells in the gut without exposure beyond the gut. EDP1867 drives broad based resolution of systemic inflammation and can reestablish normal homeostasis.

Orally administered EDP1867 is gut restricted and transits rapidly through the intestine

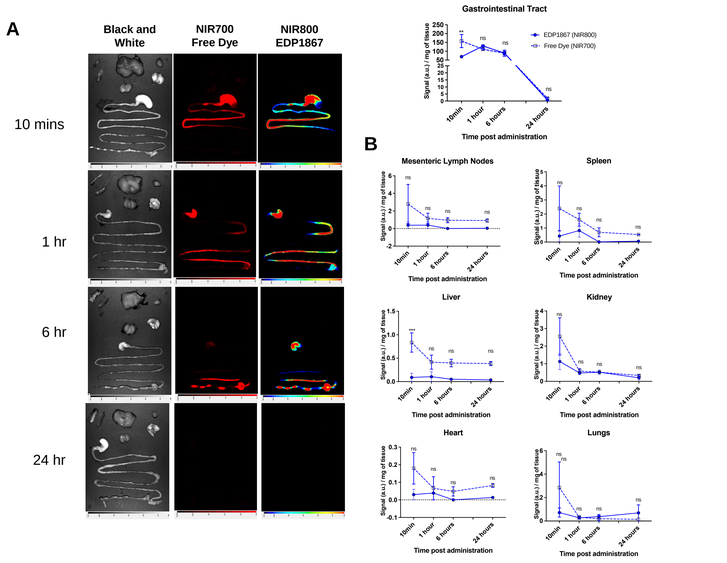

Figure 2: EDP1867 is gut restricted and transits through the intestine within 24 hours
Biodistribution of EDP1867 following a single oral dose of EDP1867 **(A)** Exposure to the small intestine occurs by 10 minutes and complete exposure in 1 hour. By 6 hours, EDP1867 is incorporated into fecal pellets and by 24 hours signal in intestine diminishes. No systemic exposure is observed at any timepoint and >99% of the total signal remains in the gastrointestinal tract. **(B)** Signals for EDP1867 are minimal in mesenteric lymph nodes, spleen, liver, kidney, heart, and lungs. n = 3 mice/timepoint/group. Left column– brightfield images, middle- free dye, right- EDP1867 overlapped with free dye. EDP1867(solid blue line) tracks with baseline levels free dye control (dotted blue line). Data are represented as mean+SEM. **p <0.01, ***p< 0.001, ns > 0.1, as determined by 2-way ANOVA followed by Sidak's multiple comparison test

EDP1867 inhibits cutaneous inflammation in an imiquimod driven model of psoriasis

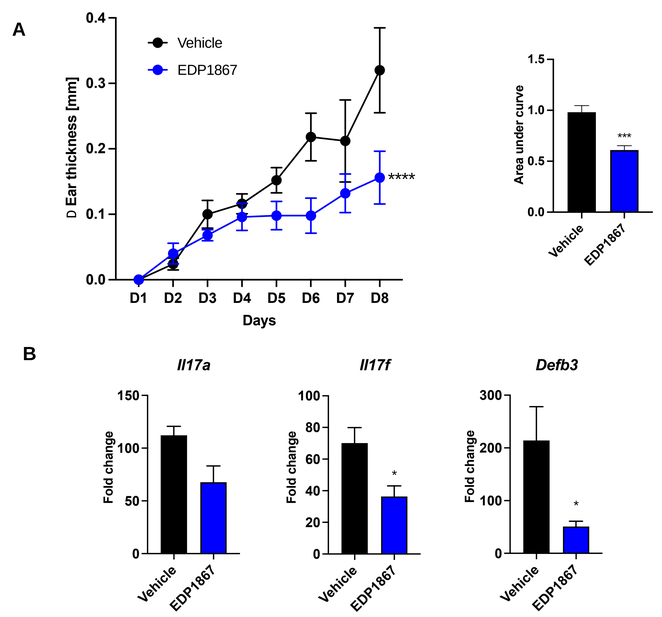

Figure 4: EDP1867 alleviates skin inflammation in imiquimod driven psoriasis
BALB/c mice were topically treated with 20mg 5% imiquimod for 7 days on the ear. Mice were orally dosed daily from day 1 through 7 with vehicle or EDP1867. **(A)** Ear inflammation over the course of 7 days and area under the curve. **(B)** Skin mRNA transcript levels for *Il17a, Il17f* and *Defb3* measured by qPCR. Data are representative from 2 experiments with n = 5/ group. All data show mean+SEM. *p < 0.05, ***p < 0.0005, ****p < 0.0001, as determined by unpaired Student's t-test.

EDP1867 stimulates anti-inflammatory cytokine secretion *in vitro*

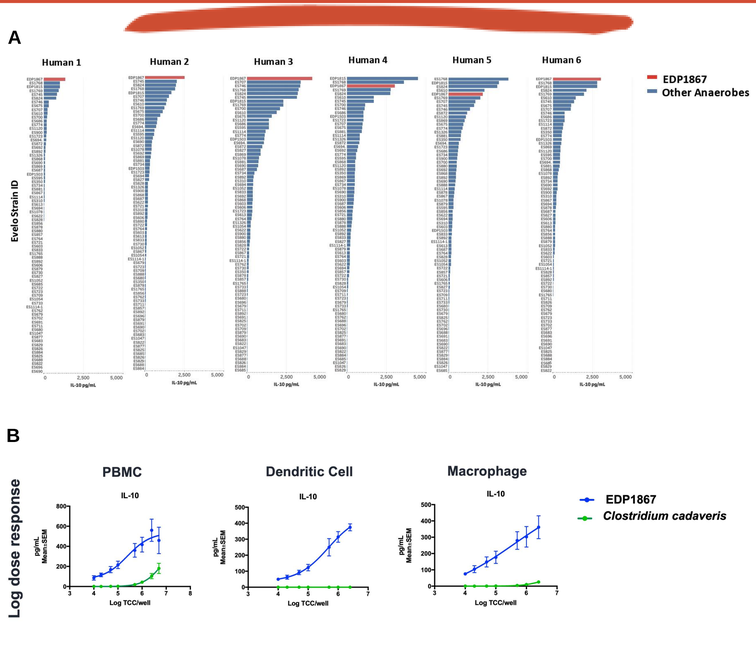

Figure 1: EDP1867 induces IL-10 in primary human cells *in vitro*
(A) Human Macrophages were stimulated with different anaerobic bacterial strains for 24h and flushed with 1% oxygen and supernatants were collected to test for cytokine levels by MSD. Data shown represent collective data from 6 independent human donors. **(B)** Human PBMCs, Dendritic Cells and Macrophages were stimulated with EDP1867 for 24h and flushed with 1% oxygen and supernatants were collected to test for cytokine levels by MSD. Data shown represent collective data from 6 independent human donors.

EDP1867 is dependent on TLR2 and lymphocyte homing to the gut to ameliorate inflammation

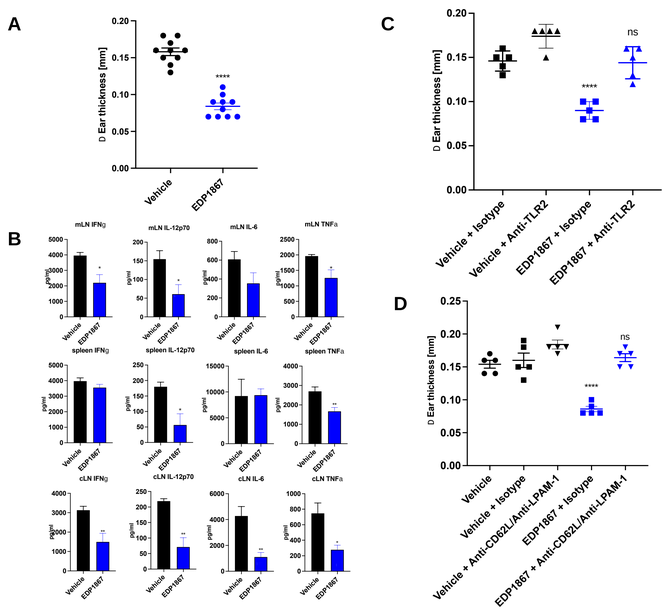

Figure 3: EDP1867 requires multiple pathways for anti-inflammatory effects
C57BL/6 mice were immunized with keyhole limpet hemocyanin (KLH) and CFA and challenged in the ear 9 days later with KLH. Ear inflammation was measured 24 hours after challenge. Mice were dosed daily from day 1 to 8 with EDP1867. Mechanisms of action were interrogated with antibody blockade as indicated. **(A)** Change in ear thickness **(B)** Proinflammatory cytokines from *ex vivo* restimulated mesenteric lymph nodes, spleen and cervical lymph nodes cells (**C)** Change in ear thickness with TLR2 blockade (**D)** Change in ear thickness 24 hours with lymphocyte gut homing blockade. Data are represented as mean+SEM and representative of 2 independent studies. ns = not significant, *p<0.05, **p<0.01, ****p<0.0001 as determined by one-way ANOVA (vs. vehicle)

Conclusions

- EDP1867 induces IL-10 production in primary human cell assays.
- EDP1867 is an orally dosed, gut-restricted therapeutic with no detectable systemic exposure *in vivo.*
- Orally delivered EDP1867 has ability to drive resolution of inflammation and diminished production of proinflammatory cytokines *ex vivo.*
- EDP1867 activity is dependent on TLR2 receptor signaling and migration of lymphocytes through the intestinal lymphoid tissue.
- EDP1867 alleviates skin inflammation in an imiquimod driven psoriasis model and inhibits Th17 cytokines in the skin.

These data support the development of EDP1867 as a therapeutic for Th1- and Th17-driven inflammatory diseases and show that EDP1867 is effective at engaging the mucosal immune axis, acting locally on host cells in the gut to drive systemic inflammation resolution.