Oral Serum-Derived Bovine Immunoglobulin (SBI) Administration Leads to Duodenal Gastrointestinal-Associated Lymphoid Tissue CD4+ T-lymphocyte Increases and Improved Small Intestinal Absorption Function in an 8-week Pilot Study in Patients with HIV Enteropathy

David M Asmuth[1,2], Zhong-Min Ma[3,4], Anthony Albanese[2], Sridevi Devaraj[5], Emir Hodzic[3], Juan-Carlos Garcia[1], Thomas H Knight[1], Neil M Flynn[6], Surinder Mann[1], Tammy Yotter[1], Emily Tsuchida[6], Christopher J Miller[3,4].

[1]University of California Davis Medical Center, Sacramento, CA, United States,
[2]Mather Veterans's Administration Hospital, Mather, CA, United States,
[3]University of California at Davis, Davis, CA, United States,
[4]Center for Comparative Medicine, Davis, CA, United States,
[5]Baylor College of Medicine - Texas Children's Hospital, Houston, TX, United States,
[6]Center for AIDS Research, Education and Services (CARES) Clinic, Sacramento, CA, United States

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BACKGROUND

- *HIV Enteropathy* was recognized early in the epidemic as a syndrome of significant gastrointestinal symptoms despite an extensive negative evaluation.[1] It likely represents an imbalance in gut microbiota, or an *HIV Dysbiosis*, that has a spectrum of clinical manifestations.
- In the post-HAART era, as many as 28% of patients report GI symptoms for which an underlying etiology cannot be identified.[2] Unless pressed, patients will minimize these symptoms.
- HIV decimates GALT very early post-acute infection. Despite suppressive ART, repopulation of GALT is blunted and lags far behind the peripheral compartment.
- We have previously shown that pro-inflammatory bacteria are present in higher proportions in HIV infected patients' stools and these correlate with reduced GALT populations of $CD4^+$ lymphocytes.[4] These bacteria may activate pro-fibrogenic pathways and collagen deposition in the small intestinal lamina propria through TLR-4 mediated pathways.

1. Kotler DP, et al. Enteropathy associated with the acquired immunodeficiency syndrome. *Ann Intern Med* 1984,**101**:421-428.
2. Siddiqui U, et al. Prevalence and Impact of Diarrhea on Health-related Quality of Life in HIV-infected Patients in the Era of Highly Active Antiretroviral Therapy. J Clin Gastroenterol 2007;41:484–490)
3. Ellis CL, Ma ZM, Mann SK, Li CS, Wu J, Knight TH, Asmuth DM, *et al.* Molecular Characterization of Stool Microbiota in HIV-Infected Subjects by Panbacterial and Order-Level 16S Ribosomal DNA (rDNA) Quantification and Correlations with Immune Activation. *J.Acquir.Immune.Defic.Syndr.* 2011,**57**:363-370.

Serum-derived bovine immunoglobulin

- SBI is a protein isolate derived from bovine plasma in the process of manufacturing purified BSA. The product contains >50% total immunoglobulins consisting of mainly IgG (>45%) and IgM (>5%).
- Plasma/serum proteins are used extensively in specialty diets in animal husbandry. [1] SBI improves gut health and function and reduces markers of inflammation in porcine and murine models.[2]
- SBI is classified as a medical food with self-affirmed FDA GRAS status. No serious adverse events have been reported in clinical trials in children and adults.[3, 4]

1. Pierce JL, et al. Effects of spray-dried animal plasma and immunoglobulins on performance of early weaned pigs J. Anim. Sci. 2005. 83:2876–2885.
2. Perez-Bosque A, et al. Dietary Plasma Proteins Modulate the Immune Response of Diffuse Gut-Associated Lymphoid Tissue in Rats Challenged with Staphylococcus aureus Enterotoxin J Nutr. 138: 533-537, 2008.
3. Begin F, et al. Effects of bovine serum concentrate, with or without supplemental micronutrients, on the growth, morbidity, and micronutrient status of young children in a low-income, peri-urban Guatemalan community. European J Clin Nutrition. P1-12. 2007.
4. http://www.immunolin.com/

Hypothesis

We hypothesize that oral SBI, which is believed to bind endotoxin and other microbial antigens which induce inflammation in the gut, will

a). Improve GI function and symptoms,

b). Reduce systemic immune activation,

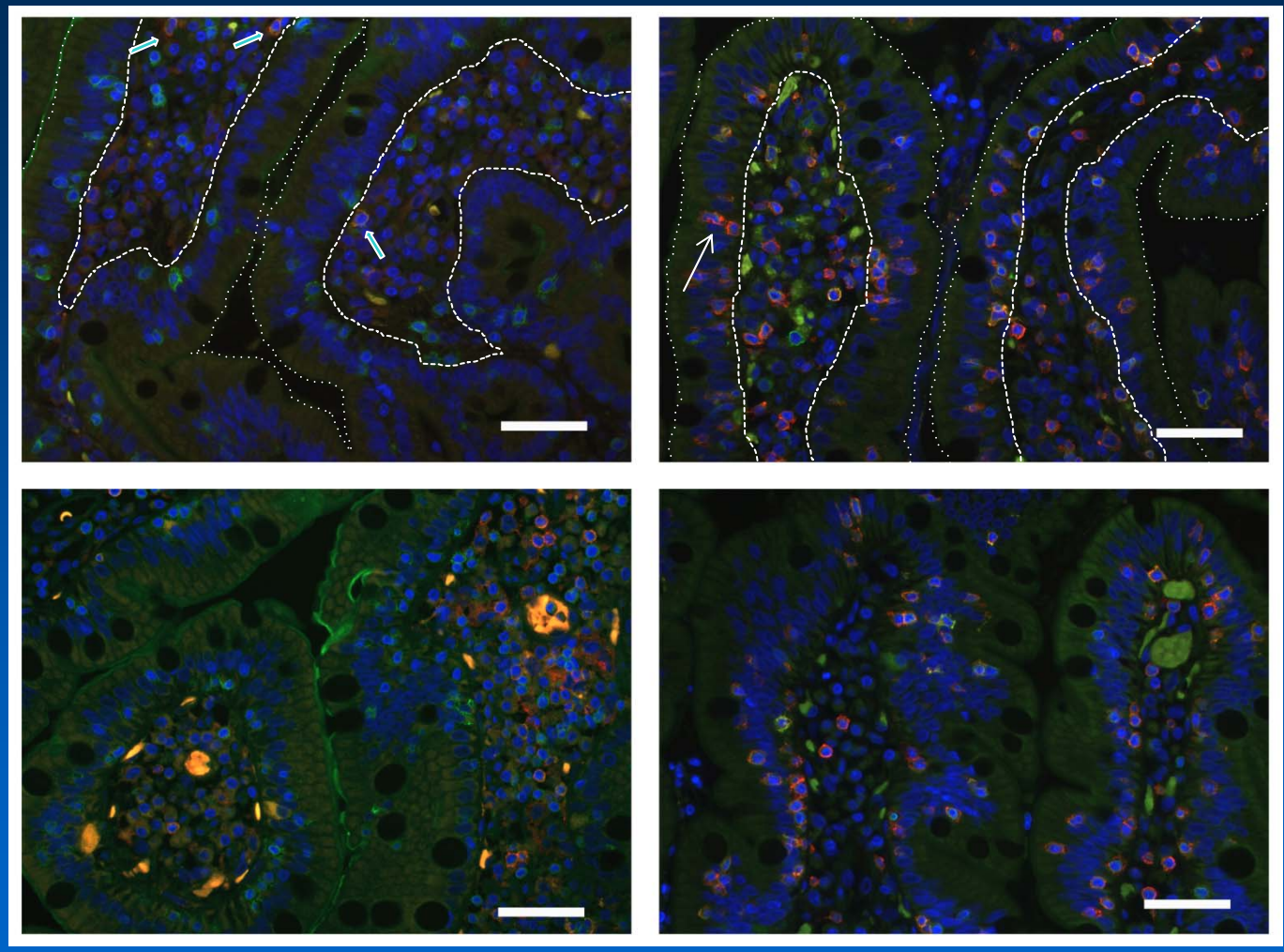
b). Promote immune reconstitution in duodenal GALT, and

c). Increase small intestinal absorptive function.

We targeted patients with HIV enteropathy because we reasoned that these would have the most proinflammatory gut microbiota and might benefit the most from this intervention.

Clinical Trial Design and Methods

- Open-label pilot study of oral SBI 2.5 gms BID for 8 weeks in patients with diagnosis of HIV enteropathy.
- Food diaries and GI symptom questionnaires were assessed at multiple timepoints.
- Paired blood and stool samples were collected before and after the intervention.
- Blood was processed for plasma and serum in endotoxin-free vacutainers or standard tubes (which are not certified pyrogen-free [<5 EU/mL]), for inflammation and bacterial product translocation assays.
- Subjects underwent EGD, and 5-hour urine collection studies following ingestion of a syrup containing lactulose (5 grams), L-rhamnose (1 gram) and D-xylose (500 mg) before and 8-weeks of receiving SBI.
- Biopsy specimens were immediately cryopreserved or processed for collagenase digestion for 9-color flow cytometry or processed for immunohistochemistry.

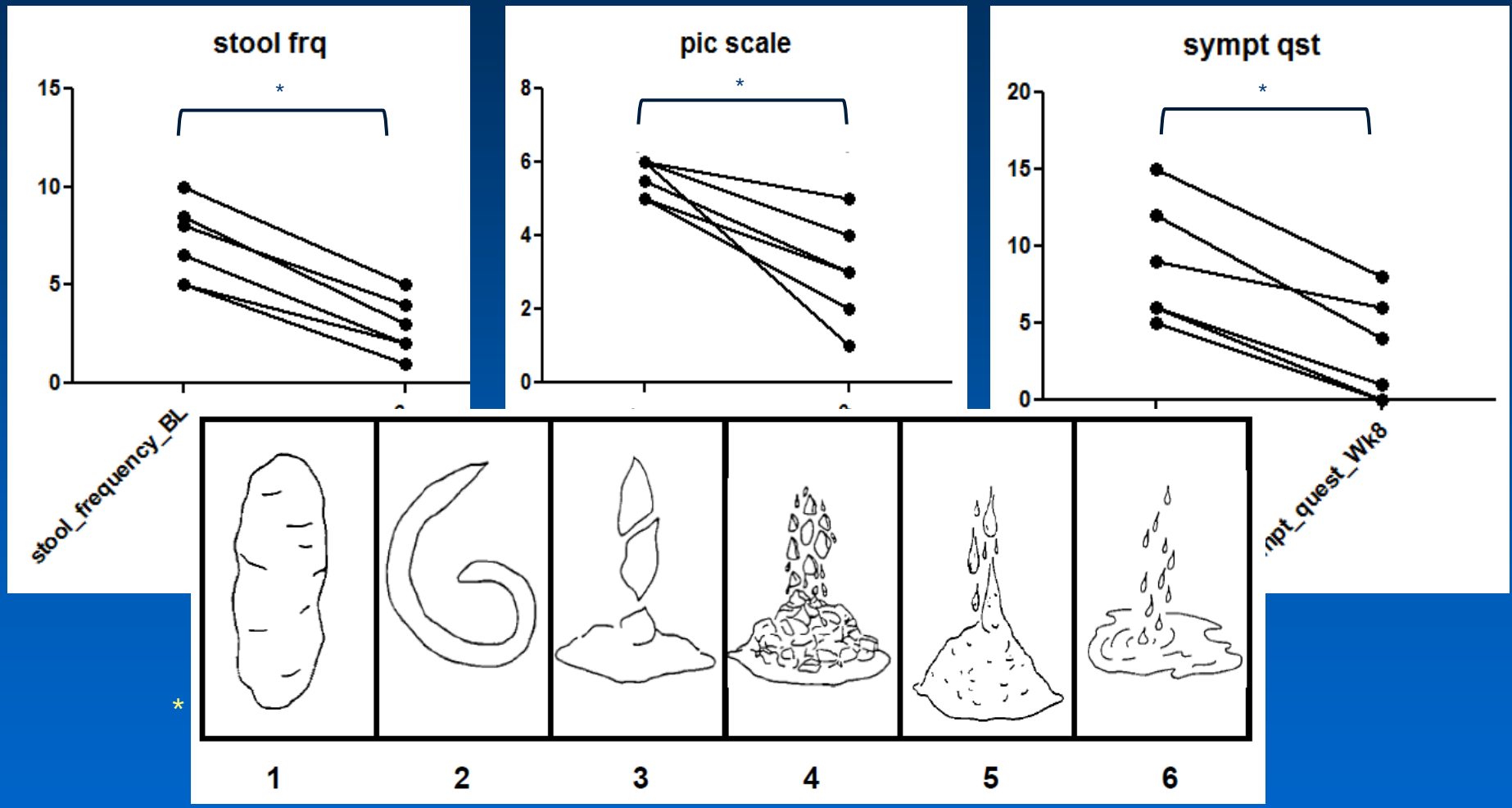


For **IHC** analysis, $CD3^+/CD4^+$ and $CD3^+/CD8^+$ positive cells/mm^2 were **counted by a single observer (Z.-M.M.)** and were quantitated for each of the three zones: 1) lamina propria, 2) intraepithelial regions (above the basement membrane), and 3) total GALT of duodenal mucosa.

Results

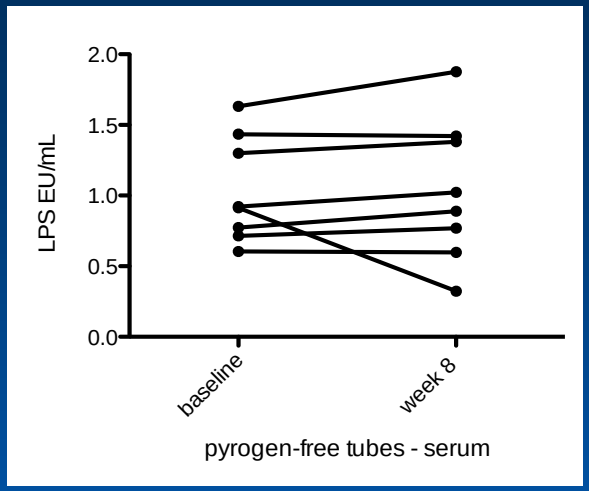
- All 8 pts were male (5 Caucasian, 3 African-American) with a median age of 44.5 years (IQR: 38.8, 47.8) and PB $CD4^+$ count of 372 cells/mL (193, 459). All had been on ART for >1 year with consistently undetectable viral load.
- All tolerated the therapy well with marked symptomatic response which will be described below.
- After 8 weeks peripheral $CD4^+$ counts were unchanged at a median of 339 cells/mL (210, 468). Both $CD4^+$ and $CD8^+$ T-cells with the activated phenotype ($CD38^+$/HLA-DR^+ double positive) remained unchanged over the 8 weeks of the study: CD4 activation – 6.6% (5.1, 9.9) and 6.7% (4.2, 12.9). CD8 activation – 20.1% (17.8, 27.9) and 20.3% (17.8, 29.2).
- All biomarkers for inflammation (CRP, SAA, HU, IFN-γ, IL-10, IL-12p70, IL-6, IL-8, TNF-α, IL-1β) were within the same range as normal controls before and after the intervention.
- By flow cytometry, **Duodenal Tissue $CD4^+$ T-cell percent** remained unchanged at 15.9% (12.0, 26.6) and 15.5% (13.9, 30.2) at wk 0 and 8, respectively.

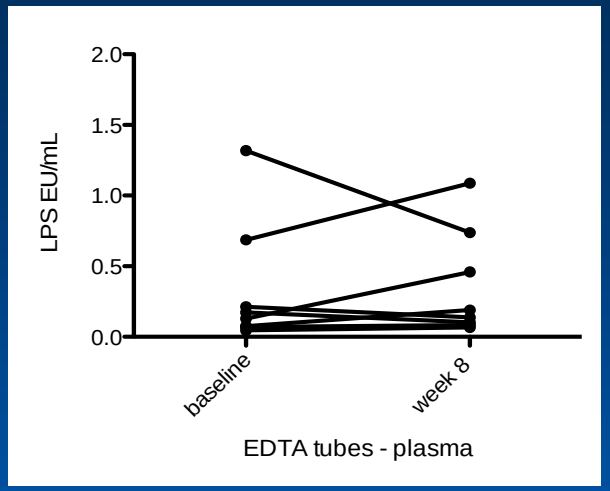
Results: GI Questionnaire

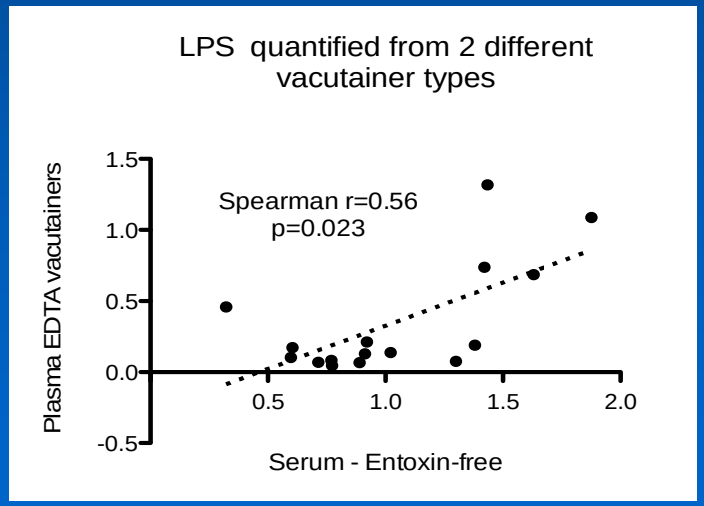


1.Siddiqui U, Bini EJ, Chandarana K, et al. Prevalence and Impact of Diarrhea on Health-related Quality of Life in HIV-infected Patients in the Era of Highly Active Antiretroviral Therapy. J Clin Gastroenterol 2007;41:484–490)

Parameters of Microbial Translocation

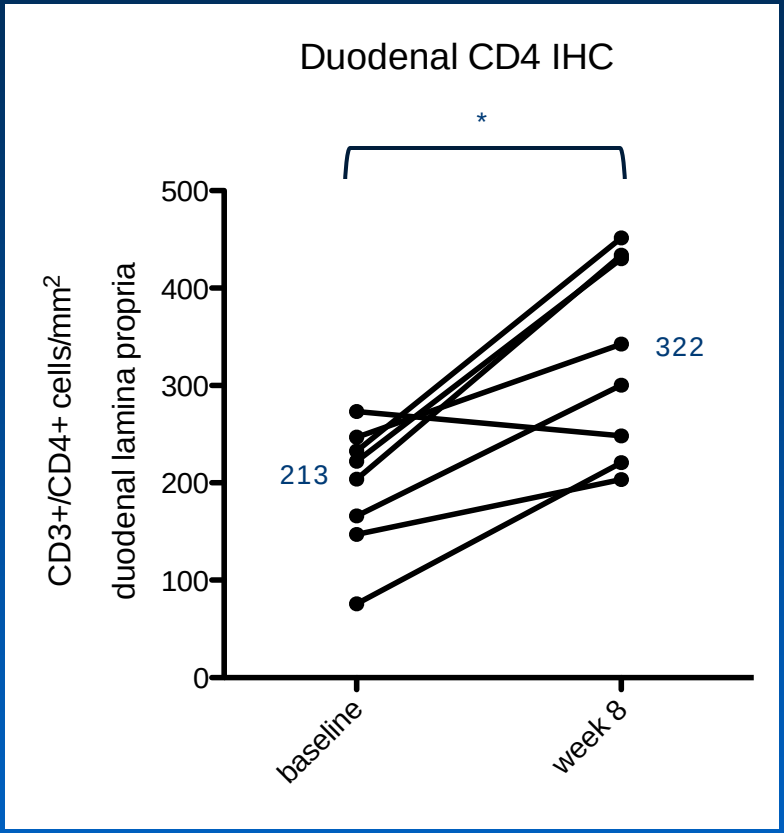


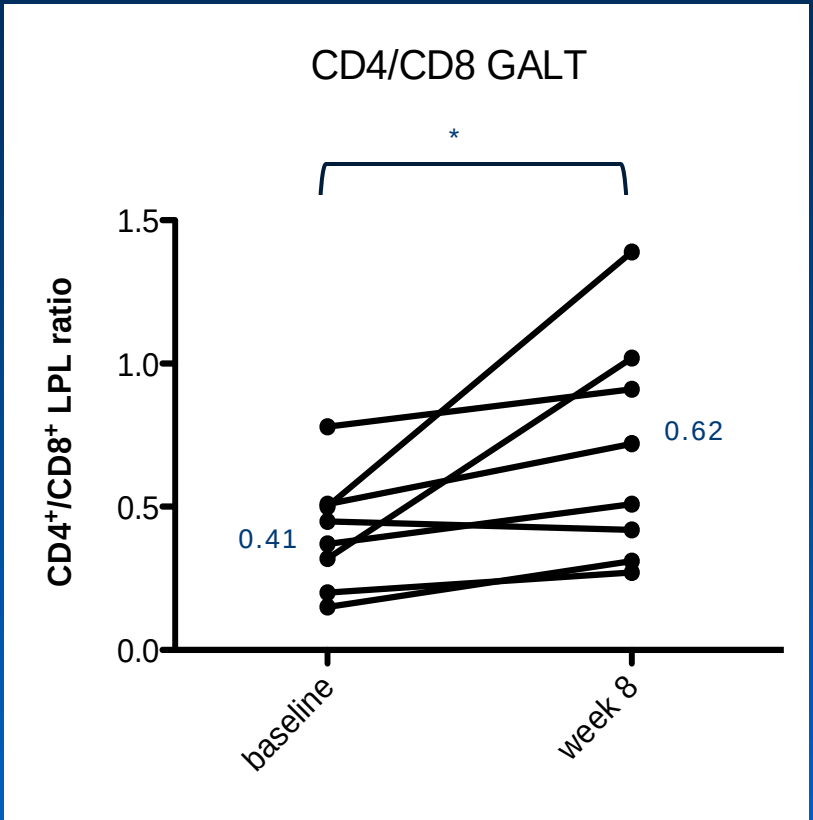




- No change in LPS or sCD14 levels following eight weeks of SBI.

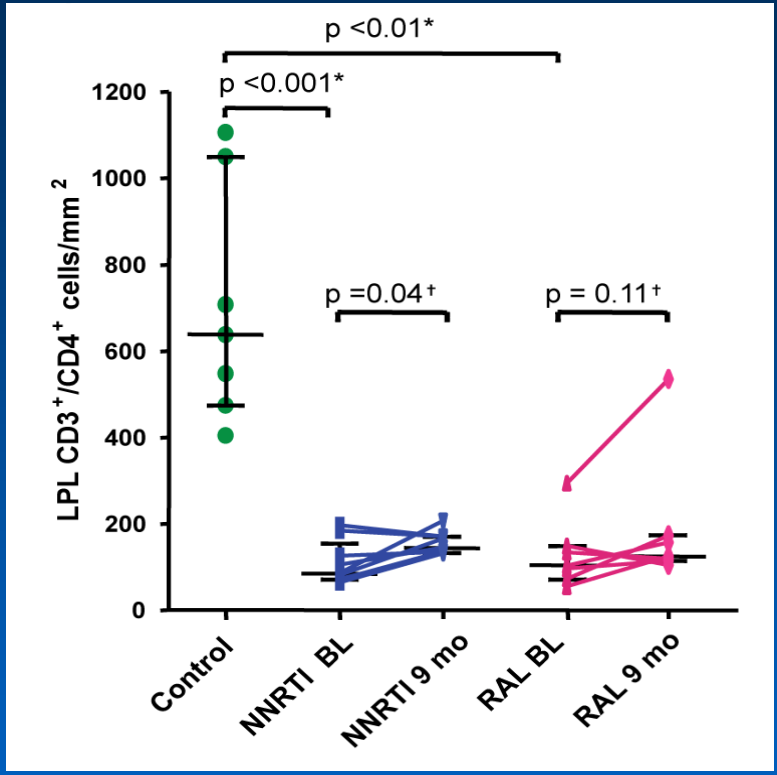
Duodenal GALT Immune Reconstitution with SBI

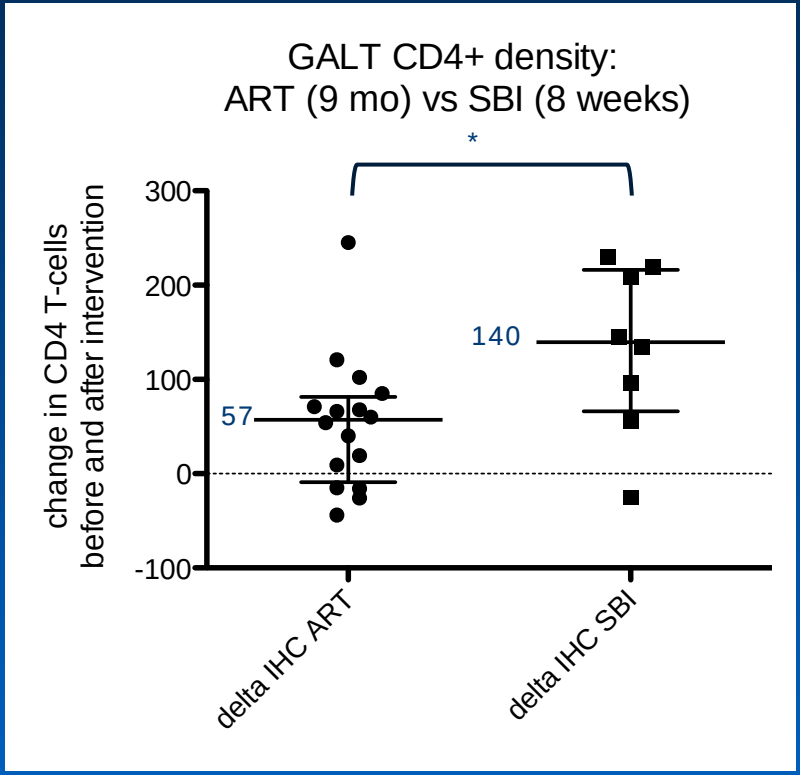




* p=0.016 by Wilcoxon matched-pairs signed rank test

Duodenal GALT Immune Reconstitution ART vs SBI

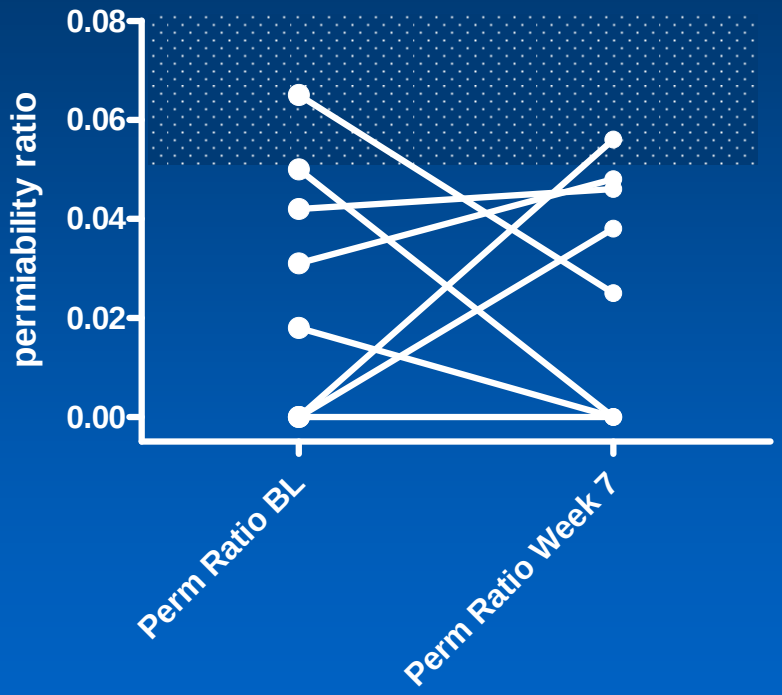


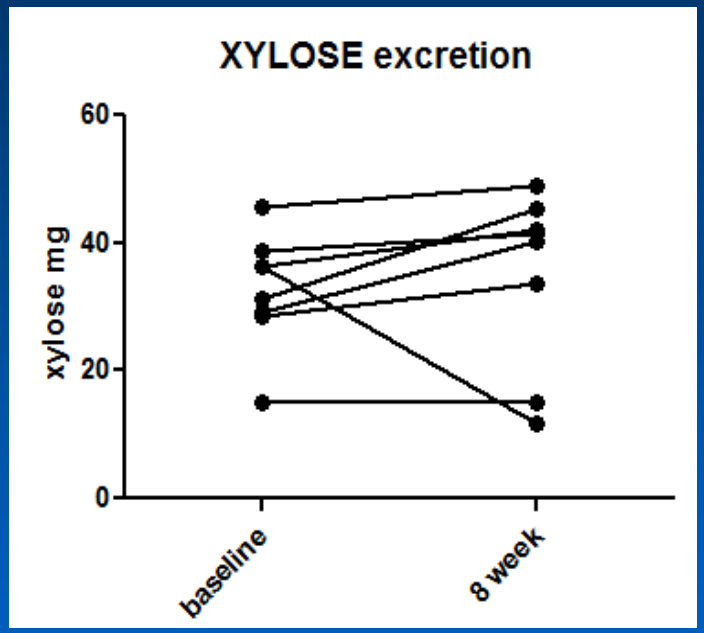


* $p=0.04$ by Mann Whitney test

Asmuth DM, Ma Z-M, Mann S, Knight TH, Yotter T, Albanese A, Melcher GP, Troia-Cancio P, Hayes T, Miller CJ, Pollard RB. Gastrointestinal-Associated Lymphoid Tissue (GALT) Immune Reconstitution in a Randomized Clinical Trial of Raltegravir Versus NNRTI-based Regimens. *AIDS* 2012. ePublished ahead of print

Assay for Gut Permeability Remains in Normal Range, but Proximal Bowel Absorptive Function Improves after SBI





- Xylose absorption and urinary excretion increased in 7/8 subjects over the course of the 8-week intervention. Median 33.8 mg (28.7, 38.2) at baseline and 40.9 mg (19.8, 44.4) at week 7 (p=0.19). HPLC/MS assay performed by Dr. Roy Sherwood of Kings College in London.

Preliminary Conclusions from Pilot Study of SBI in HIV Enteropathy

- SBI was well tolerated and coincided with reduction in HIV enteropathy.
- systemic immune activation as measured by CD8 activation phenotype and inflammatory cytokine plasma levels were unchanged at week 8.
- Lymphocyte repopulation in GALT to near normal levels was observed in 7/8 subjects.
- A direct measure of proximal small bowel function – D-Xylose absorption, suggested improvement in the same 7/8 subjects.
- Leaky gut was not demonstrated employing the gold standard dissaccharide urinary excretion assay nor were changes in LPS or sCD14 levels observed after 8 weeks of intervention
- PBMC, plasma, duodenal tissue, duodenal aspirate, and stool for bacterial DNA extraction and sequencing (hierarchical and functional genes), as well as long term follow-up of 5/8 patients for one year on-going.
- a larger randomized, blinded trial is being designed to confirm these results.

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Catherine A Lozupone
Jesse Strombaugh
Daniel Knight

Jian Wu Laboratory – UCD Hepatology
Xiaoli Chen, MD, PhD
Natalie Torok, MD - UCD Hepatology

Andreas J Baumler, PhD – UCD Med Micro
Ivan Godinez – graduate student

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Iryna Pinchuk, PhD
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Gautam Hebbar

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Donna DeFreitas, MD

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