Mice Clinical Trials

PATHOGENIC E. COLI STRAINS
The clinically isolated enterotoxigenic Escherichia coli strain, H10407 (serotype O78:H11) was used to represent the major serotypes isolated worldwide from major microbial imbalances. This ETEC strain H10407 was originally isolated in Bangladesh from a patient with severe, cholera-like diarrheal illness. Marcia Wolfe provided the H10407 isolate used in this study. It was derived from good manufacturing practice (GMP) lots of H10407 produced at Walter Reed Army Institute of Research. This strain is fully virulent in human volunteer clinical challenge studies.

PHAGE PURIFICATION AND SOURCE
Phage isolation and purification was performed by Deerland Enzymes.

EXPERIMENTAL ANIMALS

INTESTINAL INFECTION OF MICE WITH ETEC H10407
Mice were infected orally with ETEC strain H10407 as previously described by Allen et al.: Briefly, strain H10407 was grown to mid-logarithmic phase in Luria broth, pH 7.4, and resuspended in sterile PBS such that the final concentration of bacteria was approximately 5x10⁹ colony forming units (CFU) per dose plus 2.5x10⁷ CFU per dose of Bifidobacterium longum in a final volume of 300:1. This amount was then administered by gavage to 12 ETEC-naïve ICR mice that had been pretreated with streptomycin to eliminate native flora and cimetidine to reduce stomach acidity prior to challenge. This procedure was repeated with the addition of 1x10⁶ plaque forming units (PFU) per dose of the E.coli phage cocktail PreforPro®. Fecal matter was taken 2 times at 6 and 24 hours after inoculation and mice were subsequently sacrificed at 24 hours. The ileum and large intestine were harvested and plated for E.coli counts, B. longum counts and phage counts.

RESULTS
Comparing to the control, the phage cocktail resulted in the following results; E.coli decreased in the ileum -10 fold (50170 to 3135 with PreforPro), the large intestine -100 fold (11180 to 49 with PreforPro) and in the fecal matter (10525 to 67). The B. longum counts increased -100 fold in the ileum (40423 to 73), -100 fold in the large intestine (1001 to 12) and -40 fold in the 24 hour fecal sample (18050 to 505). Phage counts went up in the ileum from 897 with B. longum to 51150 with E.coli and B.longum. Then in the large intestines phage counts went up from 695 to 91500. In the 24 hour fecal count, the phage counts increased from 582 to 87000. Mice with Ecoli and with E.coli and B.longum were constipated and the ileum, cecal valve and large intestine were swollen, red and leaking when compared to the control mice with no inoculation. The mice that were infected with E.coli and PreforPro exhibited normal bowel movements and experienced no change in color or size of the various compartments of the intestine when compared with control mice.

DISCUSSION
An oral phage cocktail decreased intestinal pathogenic Ecoli populations from 10-1000 fold while simultaneously increasing probiotic populations by 10-100 fold. The increase in probiotic counts reflects the decrease in competition and release of nutrients from the pathogenic E.coli bacteria. These in vivo results strongly support the application of phage as a prebiotic to regulate and enhance the intestinal microflora, providing support for good bacteria, helping promote and maintain a healthy digestive tract.

References