Design and Evaluation of SER-262: A Fermentation-Derived Microbiome Therapeutic for the Prevention of Recurrence in Patients with Primary *Clostridium difficile* Infection

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Background

Clostridium difficile infection (CDI) is the leading hospital-acquired infection in the US and Europe, with an estimated 29,000 US deaths/year[1].

The leading risk factor for primary and recurrent CDI is exposure to antibiotics, which create ecologic gaps within the healthy microbiome[2]. Yet, the current paradox is to treat this antibiotic-associated disease with more antibiotics, which exacerbate the underlying dysbiosis[3].

Commensal microbes directly outcompete pathogens through mechanisms such as competition for nutrients, such as carbohydrates or bile acid conversion, which inhibits *C. difficile* germination[4,5]. These observations suggest that functional restoration of the microbiome may offer therapeutic benefit.

Seres' first product **SER-109** is composed of Firmicutes in spore form, fractionated from the stool of rigorously screened healthy donors.

In an open-label Phase 1b/2 study of 30 patients with multiply recurrent CDI, **SER-109** rapidly diversified the gut microbiome and 87% of subjects met the primary endpoint of no recurrence of CDI up to 8 weeks following dosing.

The **SER-109** manufacturing process, which removes unwanted bacteria, viruses, fungi and parasites, reduces the risk of pathogen transmission to a level that cannot be achieved through donor screening alone[7].

Here we present details related to the research and development of **SER-262**, a product derived entirely from fermented bacteria.

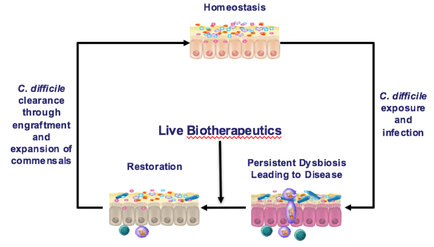
SER-262 is a rationally-designed composition of commensal bacteria, delivered orally in spore form and manufactured using anaerobic fermentation, for the prevention of recurrence in patients with primary CDI.

Background References

[1]*Lessa et al., NEJM 2015, adapted from Lawley TD PLoS Pathog 2012*
[2]*Bagdasarian JAMA 2015*
[3]*Gerding D Nat Rev Gastro Hep 2016*
[4]*Ng KM Nature 2013;*
[5]*Theriot C Annu Rev Microbiol 2015*
[6]*Khanna S, J Infect Dis 2016*
[7]*McKenzie ASM 2016 Poster 450*

Model of Microbiome Disruption and Restoration

Ecobiotic® therapeutics restore the colonization resistance of a healthy microbiome and break the antibiotic-induced cycle of dysbiosis and recurrence.



A phylogenetically diverse therapeutic composition will maximize the likelihood of providing the functional characteristics necessary to promote colonization resistance across a broad range of recipients.

SER-262 is a Rationally-Designed, Fermentation-Derived Microbiome Therapeutic

SER-262 contains bacteria from clades that are prevalent in healthy individuals and engraft well in SER-109 treated subjects

Table 1: Prevalence of SER-262 Clades in the HMP Gut Microbiome Dataset and in SERES-001 Dose and Endpoint Datasets

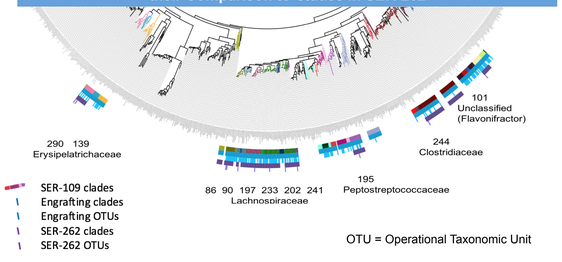
Seres Clade Designation	Clade Prevalence HMP*	Clade Prevalence SER-109 Doses	Clade Prevalence Engrafting at Any Timepoint in Subjects Treated in SERES-001
clade_86	94%	100%	100%
clade_90	91%	100%	100%
clade_197	97%	100%	100%
clade_233	99%	100%	100%
clade_241	42%	95%	63%
clade_195	32%	90%	90%
clade_290	15%	86%	50%
clade_101	98%	76%	100%
clade_244	14%	76%	83%
clade_139	37%	38%	80%
clade_202	4%	14%	16%

SER-262 is manufactured using well-controlled fermentation and purification processes to create a product that substantially reduces the risk of transmission of potential pathogens

	Biological Sourced	Fermented
Composition	• Complex mixture of microbes fractionated from human material from extensively screened healthy donors	• Complex mixture of microbes designed for function; sourced from stock cultures that are obtained by isolation and purification from extensively screened healthy human donors
Manufacturing Process	• Microbes grow in natural *in vivo* environment in human matrix • Fractionated, clarified, and purified through a defined process	• Maintained in a genomically and physiologically characterized strain library by passage in controlled environment • Grown & purified under controlled conditions with supporting analytics

SER-262 captures a broad phylogenetic breadth of the bacterial diversity present in SER-109 with reduced taxonomic complexity

Figure 1: Distribution of 30 Spore Forming Clades Found in SER-109 and their Comparison to Clades in SER-262



OTU = Operational Taxonomic Unit

SER-262 in a Mouse Model of CDI

- The mouse prophylactic model of CDI was first published by Chen, et al. (Chen 2008; **Figure 2**)
- Days -14 to -6: nine- to ten-week-old female C57BL/6 mice receive an antibiotic cocktail in their drinking water consisting of 1% glucose, kanamycin (0.5 mg/mL), gentamicin (0.044 mg/mL), colistin (1062.5 U/mL), metronidazole (0.269 mg/mL), ciprofloxacin (0.156 mg/mL), ampicillin (0.1 mg/mL) and vancomycin (0.056 mg/mL)
- Day -3: animals received a dose of 10 mg/kg clindamycin by oral gavage
- Day -1: positive control and test articles treatments are administered
- Day 0: mice were challenged by administration of approximately 4.5 $\log_{10}$ spores of *C. difficile* or sterile PBS (for the naïve control) via oral gavage
- Infection and its consequences were evaluated by daily assessment of mortality, weight loss and clinical signs (scoring of lethargy, grooming, wet tail/abdomen and hypothermia).

Figure 2: Schematic of Mouse Model of CDI

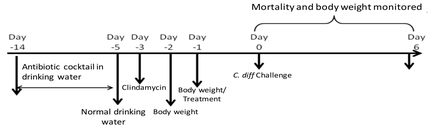
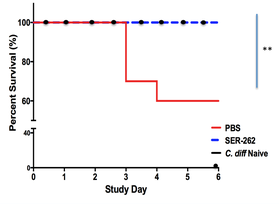
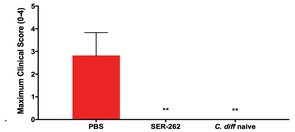


Figure 3: SER-262 is Effective at Preventing CDI in a Mouse Model

SER-262 prevents CDI-Related Mortality



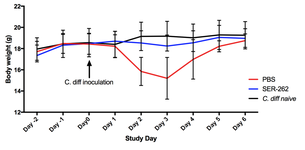
SER-262 prevents CDI-Related Clinical Symptoms



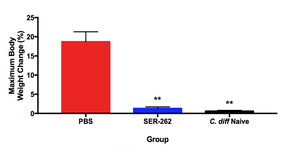
** p≤0.001 using a Fisher's exact test

SER-262 prevents CDI-Related Body Weight Loss

Body Weight Change over Time



Maximum Body Weight Change (%)



** p≤0.001 using a Fisher's exact test

SER-262 Bacteria Compete with *C. difficile* for Carbon Sources and Facilitate Conversion of Primary to Secondary Bile Acids that Inhibit Spore Germination

- SER-262 strains can utilize 26 of the 29 (90%) C-sources used by *C. difficile*, comprising 19 of the 19 (100%) carbohydrates and carboxylic acids tested
- The majority (8 out of 12) of SER-262 strains convert primary into secondary bile acids

Table 2: SER-262 Strain Carbon Source- and Bile Acid Conversion-Characterization

Strain Designation	Sugars, sugar alcohols, glucosides															Carboxylic acids			Amino acids										Bile Acid Enzymatic Activity
	fru	glu	man	rha	rib	xyl	cel	scr	tre	mel	pec	mtl	sor	NAG	gln	sal	for	suc	pyr	ala	asp	Leu	ileu	met	orn	phe	ser	thr	val	
C. difficile																														-
1																														-
2																														+
3																														+
4																														+
5																														+
6																														+
7																														-
8																														+
9																														+
10																														+
11																														+
12																														+

Comparison of carbon source utilization profile of *C. difficile* and SER-262 strains. Full cells (in red) indicate utilization of the listed C-sources. Of the 59 carbon sources tested, only 29 used by *C. difficile* are presented. Highlighted in yellow are C-sources not utilized by any SER-262 strains. (**fru**-fructose, **glu**-glucose, **man**-mannose, **rha**-rhamnose, **rib**-ribose, **xyl**-xylose, **cel**-cellobiose, **scr**-sucrose, **tre**-trehalose, **mel**-melezitose, **pec**-pectin, **mtl**-mannitol, **sor**-sorbitol, **NAG**-N-acetylglucosamine, **gln**-glucosamine, **sal**-salicin, **for**-formate, **suc**-succinate, **pyr**-pyruvate, **ala**-alanine, **asp**-asparagine, **Ile**-isoleucine, **leu**-leucine, **met**-methionine, **orn**-ornithine, **ser**-serine, **thr**-threonine, **val**-valine). + BSH activity, - No BSH activity.

Conclusions

- SER-262 is a rationally-designed, fermentation-derived composition of spores that was based on the prevalence of clades in healthy individuals and observed rates of SER-109 engraftment in our Phase 1b/2 study.
- Strains comprising SER-262 utilize 90% of overall carbon-sources, 100% specifically of the carbohydrates and carboxylic acid used by *C. difficile*, supporting carbon source competition as a mechanism to prevent CDI.
- SER-262 strains can convert primary to secondary bile acids. Secondary bile acids prevent *C. difficile* germination or growth.
- SER-262 is efficacious at preventing CDI in the mouse model of disease
  - Complete prevention of mortality
  - 100% protection against body weight loss and clinical symptoms
- The SER-262 fermentation-based approach enables manufacturing for global requirements by eliminating the need for donors and providing a scalable, standardized product that mitigates patient risk.

First-in-human clinical trial of SER-262 for prevention of CDI recurrence in adults with primary Infection following completion of standard of care antibiotics is expected in mid-2016