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# Apparent deficiencies in the phase III data for Seres Therapeutics, SER-109: a closer inspection of the data prior to a decision by the FDA expert committee.

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**Abstract**

The purpose of our research was to determine if Seres- 109, a microbiome therapeutic treatment created by Seres Therapeutics Inc., produced a significant therapeutic effect in its Phase III clinical trial to be granted approval by the FDA for the treatment of recurrent *C. Difficile* infection (CDI). When studying the results of both, the phase II and phase III clinical trials, we observed that a disproportionately greater number of women were enrolled in the treatment arm while an equal number of men and women were enrolled in the placebo arm. We performed a literature search to determine if there were any differences in the intestinal microbiota environment between men and women and if repopulating the dysbiotic microbiome would result in a better clinical response (less CDI recurrence) in women. We found that repopulation of the dysbiotic microbiome resulted in greater levels of secondary bile acids in female (when compared with male) mammalian animal models. We also found that secondary bile acids inhibited intestinal *C.Difficile* germination and outgrowth through recently discovered molecular mechanisms. These findings suggest that the unblinded data from the Phase III trial also be analyzed using gender stratification to discount the possibility of a gender skewed efficacy increase in the treatment arm and a (gender skewed) futility in the placebo arm.

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**Introduction**

Seres Therapeutics Inc. is among a few companies trying to deliver on the significant potential of the intestinal microbiome. On August 10, 2020, it made available to the public, phase III results of its microbiome consortia drug; SER-109; in recurrent *Clostridium. Difficile* infection (CDI). Utilizing orally administered spores of a consortia of *Firmicutes* sp. bacteria, the ECOSPOR phase III study met its primary endpoint; a significant difference in the CDI recurrence rate between SER-109 and placebo (p=0.001) at 8 weeks post dosing. There was also no significant difference in adverse effects (AEs') between drug and placebo. Based on these ostensibly positive results, the market drove up Seres Therapeutics (ticker: MCRB) stock from $2 to $25 within a span of hours.

We have examined this phase III data as a part of our Project Lead the Way (PLTW), Biomedical Innovation (BI) curriculum. Our objective was to determine if the data were robust enough to warrant FDA approval. Upon closer inspection of the data, we found that the ratio of female to male patients who were administered SER-109 was more than twice that of the cohort who were administered placebo (2.2 versus 1.0 respectively). We investigated the implications - if any - of this skewed ratio.

CDI is the most common cause of healthcare associated infection in US hospitals (1). The first-line treatment for CDI are antibiotics, such as vancomycin and fidaxomicin. Despite the immediate success of most antibiotics, approximately 20% of patients with prior infection of CDI, experience recurrence within 3 weeks of antibiotic discontinuation (2).

Antibiotics not only kill active *C. difficile* bacteria, but also healthy gut microbes such as *Firmicutes sp.*and *Bacteroidetes sp.*, leading to dysbiosis. Additionally, the antibiotics are unable to kill the dormant spores of *C. difficile* inside the gut, which are hence better able to grow in an environment that lacks colonization resistance due to dysbiosis (5). Spore forming *Firmicutes sp.,* one of the dominant healthy phyla in the gastrointestinal microbiome, converts primary bile acids; secreted from the liver into the intestine; into secondary bile acids. Recent studies showed that secondary bile acids inhibit *C. difficile* spore germination and growth *in vitro*, as well as *ex vivo* (3). CDI is associated with a lack of gut microbial diversity and microbe derived secondary bile acids, which inhibit *C. Difficile* germination and growth (27).

Seres Therapeutics, a late clinical stage biotechnology company, developed an investigational, oral medication to combat *C. Difficile* recurrence in adults. The drug is a consortia of purified bacterial spores from *Firmicute sp.* derived from the stool of healthy donors (4). It is designed to be equivalent to a fecal microbiota transplantation (FMT) that increases gut microbial diversity for adults who experience recurrence of CDI after antibiotics course completion. The drug is intended to repopulate the gut with microbes that defend against toxigenic *C. Difficile* colonization and outgrowth. The repopulated microbiome regains the ability to convert primary bile acids into secondary bile acids, which in turn creates an environment that

prevents CDI recurrence in adults (27). The oral drug, SER-109, led to a statistically significant reduction of CDI recurrence compared to a placebo, with minimal adverse effects. The FDA granted the drug Breakthrough Therapy designation and Orphan Drug designation (4).

As mentioned earlier, as a part of our PLTW Biomedical Innovations curriculum, we examined the data in the Phase III trial in order to determine if the drug warranted FDA approval. During our research, we noticed that the study had more females than males in the SER-109 arm (68.9% and 31.1% respectively) compared to those in the placebo arm (51.1% and 48.9% respectively) in the trial (4). We hypothesized that this gender disparity could influence the Phase III trial results due to the intestinal microbiome differences between males and females. In CONV-R mice, the *Firmicutes/Bacteroidetes* ratio and total *Firmicutes* levels were higher in females, whereas the total *Bacteroides* levels were higher in males. Furthermore, independent of the influence of microbiota, females had greater total and primary bile acid levels; however, in the presence of microbiota, females had higher levels of secondary bile acids compared to males (23). Assuming that these gender differences in the absolute concentrations of bile acids were translatable into differential *C. Difficile* inhibition, the activity of SER-109 could be gender specific. We hypothesize that this gender-skewed ratio could influence the statistically significant results (30.2% absolute reduction rate compared to the placebo) that were seen in the Phase III trial of SER-109 (4).

**Methods**

We performed a literature search to see if there was any difference between males and females with respect to intestinal microbiota, *C. Difficile* colonization or resistance. We asked if repopulating the dysbiotic microbiome in women would elicit a different metabolic or physiological response when compared to the response generated by men. If we did come across such a differential response, we would then ask if the response mechanism had been researched and elucidated.

**Results**

The literature indicated that a greater expression, replacement (5) and/or detection of intestinal secondary bile acids is associated with a inhibition of vegetative outgrowth of *C. Difficile* (6). This is accompanied by the restoration of the bacterial taxonomic composition to pre-dysbiotic levels (7). Secondary bile acids inhibit *C. Difficile* germination in vitro (8) in a dose dependent manner (9) , while primary bile acids promote it (10), although these effects are strain dependent (11). A decreased ratio of secondary to primary bile acids stimulates colony formation of *C. Difficile* spores (12). Recent research demonstrated that secondary bile acids bind to TcdB - the toxin secreted by *C. Difficile* - thereby inducing a major conformational change in the structure of TcdB, which prevents receptor binding and uptake into cells (13). Taken together, the data demonstrate a

strong inverse correlation; with causative undertones; between the ratio of the secondary to primary bile acids and CDI recurrence.

The metabolism of bile acids; i.e. the conversion of primary to secondary bile acids; by the microbiota involves enzymatic deconjugation by bile salt hydrolases (BSH) and dehydroxylation by 7α-dehydroxylation (7αDH) enzymes. The literature is replete with reports of Lactobacillus strains preventing CDI (14). In addition, BSH expressing Lactobacillus strains have been shown to modify primary bile acids *in vitro* thus decreasing *C. Difficile* germination (15) . Since the SER-109 phase III data had no eligibility requirements concerning dietary intake during the trial (16), it could not be determined whether there was a significant difference in dietary intake (including probiotic ingestion) between the drug and placebo arms, or when stratified by gender. Therefore, the possibility of *C. Difficile* germination, or vegetative outgrowth, being modulated by diet could not be discounted (17) and must be regarded as a confounding factor.

The female gender is an independent predictor of community acquired CDI (18). It is known that sex-specific differences exist in the gut microbiome (19) that can further be amplified by hormone levels (20). In addition, women; as primary caregivers; are more likely to come into contact with infants; who have high *C .Difficile* colonization, a known risk factor (21). Women are also more likely to seek medical care (22), leading to increased exposure to antibiotics. It therefore appears that women, even though metabolically less susceptible to CDI (due to an increased secondary to primary bile acid ratio (23, 24) are at a greater occupational and voluntary exposure and risk of CDI, thereby explaining this paradox. Females would be expected to respond better to dysbiosis corrective and gut microbiota repopulating medications such as SER-109 because of the greater resulting colon concentrations of secondary bile acids with greater consequent reduction of *C. Difficile* colonization and outgrowth.

A literature search revealed that in the presence of a repopulated *Firmicutes* microbiome - such as would be achieved with SER-109 administration - the levels of microbiome-produced secondary bile acids could be upto 2.5 times greater in females than in males (23). While this in-itself is not conclusive evidence for a greater effectiveness in females, the minimum inhibitory concentration (MIC) provided greater insight. A colon deoxycholic acid (DCA) - a secondary fatty acid - concentration of 1.24 ± 0.24 mM was obtained after Fecal microbiota transplantation (FMT) procedure on 12 CDI patients (83% female) (25). The minimum inhibitory concentration (MIC) of DCA against two strains of *C. Difficile* was 1.00 ± 0.00 mM (26). Hence, as a first approximation, it could be assumed that a 2.5X lesser DCA concentration in males would translate to 0.6 mM and (assuming a linear relationship); to 0.99 mM and 1.12 mM in a population of 50% and 68% females respectively. It can be seen that the gender enrollment ratio favors efficacy in the treatment arm and futility in the placebo arm of the Phase III trial. Furthermore, since secondary bile acids have been shown to decrease *C.Difficile* spore germination and outgrowth in the small intestine, it may be that SER-109 exhibits significantly greater bactericidal activity against *C.Difficile* in females,

than in males. This phenomenon could have led to a greater therapeutic effect in this gender skewed population than would otherwise have been observed, had there been no gender bias between subjects dosed with SER-109 and placebo.

Although our data derives primarily from animal models, we conjecture that the mechanism of action across mammalian species is unlikely to change, hence giving pause to the overtly exuberant interpretation that the phase III clinical study results have thus far elicited. We also note that a similar gender biased pattern also existed in a phase II study with SER-109 (27). The results of this phase II study did not meet statistical significance for efficacy presumably because the eligibility criteria were met either with PCR or toxin testing. The former method cannot distinguish colonization from infection, hence the authors hypothesized that recurrence in the treatment arm may have been overestimated. It is also interesting to note that a 67% female enrollment in the phase II trial with a 2:1 randomization between drug and placebo arms can be accomplished in 31 ways as shown in Table 1.

Table 1: Different possible combinations of males and females for the Phase II study of a total of 89 enrolled subjects with a 67% female enrollment and a 2:1 randomization ratio between the drug and placebo arms. Not all combinations are shown.

| Treatment arm (n=59) | | Placebo arm (n=30) | |
|---|---|---|---|
| # males | # females | # males | # females |
| 1 | 58 | 30 | 0 |
| 2 | 57 | 29 | 1 |
| 3 | 56 | 28 | 2 |
| Pattern continues for combinations in between | | | |
| 10 | 49 | 21 | 9 |
| 11 | 48 | 20 | 10 |
| 12 | 47 | 19 | 11 |
| Pattern continues for combinations in between | | | |
| 17 | 42 | 14 | 16 |
| 18 | 41 | 13 | 17 |
| 19 | 40 | 12 | 18 |
| 20 | 39 | 11 | 19 |
| 21 | 38 | 10 | 20 |
| Pattern continues for combinations in between | | | |
| 29 | 30 | 2 | 28 |
| 30 | 29 | 1 | 29 |
| 31 | 28 | 0 | 30 |

The green region of the table is where the ratio of females to males in the placebo arm is <1, with this ratio becoming > 1 in the orange shaded part of the table. If, as hypothesized, females respond more favorably to SER-109, then statistical significance is progressively harder to reach as one goes down the table due to the placebo arm starting to show decreased CDI recurrence. The published results from the phase II study did not explicitly present enrollment by gender. The study did show that non-recurrence was associated with increased secondary bile acid concentrations.

**Conclusion**

Data from *In vitro* studies and murine mammalian models suggested that females are likely to respond more favorably than males, to a repopulation of their dysbiotic microbiome with regard to inhibition of *C. Difficile*. Since the phase III clinical trial for SER-109 had a significantly greater number of female subjects in the treatment arm than in the placebo arm, this raises the question whether the efficacy of the drug was overestimated due to this skewed gender distribution.

We realize that we may not have access to all the data that the company has submitted to the FDA, and perhaps, there is an explanation for this phenomenon in the submitted data. If not, we would need to see a reclassification/recalculation of the submitted data - such as perhaps results from sex-balanced ratios for the treatment and placebo arms or from sex categorized stratified randomization in the SER-109 arm - to discount this possibility before we can judge SER-109 on its scientific and clinical merit. At the time of publishing of this manuscript, the FDA review of SER-109 had not taken place. It remains to be seen whether the expert committee reaches similar conclusions and asks for more data and clarification before approval.

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