Panels A and B show plots of the initial rate of citrate synthase activity (Y-axis, in units of µmol/mL/sec) versus OAA/AcCoA substrate concentration (X-axis, in millimolar units), for wildtype citrate synthase and the A313P and A313V mutants. Panel (A) shows enzymatic parameters for wildtype, A313P mutant, and A313V mutant with variable OAA (0-0.625 mM) and fixed AcCoA (0.3 mM). Panel (B) shows enzymatic parameters for wildtype, A313P mutant, and A313V mutant with fixed OAA (0.5 mM) and variable AcCoA (0-2.5 mM). Three replicates were performed for each condition, with error bars showing ± 1 SD.

Figure 4. Citrate synthase mutants disrupt functional dimerization and destabilize alpha-helix packing

Identified citrate synthase mutants mapped onto the crystal structure of T. thermophilus citrate synthase (PDB 1IOM) show that the mutations are located either at dimerization interfaces or within the interior of hydrophobic packing interfaces.

**Conclusion.** Cellulose metabolism and virulence regulation are intercorrelated in *S. aureus*, as alterations in TCA cycle activity lead to increased persister formation and host macrophage inactivation. Our findings that inactivating citZ mutations are enriched can provide a potential explanation for the mechanisms of persistent bacteremia.

**Disclosures.** All Authors: No reported disclosures

129. Antimicrobial Resistance Genes Were Reduced Following Administration of Investigational Microbiota-Based Live Biotherapeutic RBX2660 to Individuals with Recurrent *Clostridioides difficile* Infection

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**Session:** O-26. New Insights into Microbial Pathogenesis

**Background.** Intestinal colonization by antimicrobial resistant (AMR) pathogens is a known health and infection risk, and is common among individuals with recurrent *Clostridioides difficile* infections (rCDI). Accordingly, therapeutic approaches that decolonize the gut of AMR pathogens could be valuable to patients to reduce risk of associated illnesses. Herein, we assessed gut colonization with AMR bacteria before and after treatment with RBX2660—a microbiota-based investigational live biotherapeutic—in the PUNCH CD3 Phase 3 trial for reducing CDI recurrence.

**Methods.** rCDI participants enrolled in PUNCH CD3 received a blinded single dose of RBX2660 or placebo within 24 to 72 hours after completing antibiotic treatment for the most recent CDI episode. Clinical response was the absence of CDI recurrence at eight weeks after treatment, and participants were asked to submit stool samples prior to RBX2660 or placebo treatment (baseline) and 1, 4 and 8 weeks, and 3 and 6 months after study treatment. Samples were extracted and sequenced using a shallow shotgun method. The presence and number of AMR genes was determined for 175 participant samples and 116 RBX2660 samples using 90% K-mer sequence coverage based on the MEGARes database.

**Results.** Total AMR genes per PUNCH CD3 participant among RBX2660-treated responders at the indicated time points and in the RBX2660 investigational product. The red lines indicate timepoint group medians.

**Conclusion.** In the PUNCH CD3 Phase 3 trial of RBX2660 for rCDI, AMR gene content decreased after RBX2660 treatment and remained low to at least 6 months, consistent with prior RBX2660 trials. This underscores the potential of microbiota-based biotherapeutics for decolonizing AMR bacteria from gut microbiota and thereby reducing AMR infection risks.

**Disclosures.** Heidi Hau, PhD, Rebiotix Inc. (Employee) Dana M. Walsh, PhD, Rebiotix (Employee) Ken Blount, PhD, Rebiotix Inc., a Ferring Company (Employee)

130. Design and Preclinical Characterization of SER-155, an Investigational Cultivated Microbiome Therapeutic to Restore Colonization Resistance and Prevent Infection in Patients Undergoing Hematopoietic Stem Cell Transplantation

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**Session:** O-27. Novel Antimicrobial Agents

**Background.** During allogeneic hematopoietic stem cell transplant (HSCT), the diversity and stability of the GI microbiome is disrupted, increasing the risk of domination by pathogens associated with bacteremia, gVHD, and mortality. SER-155 is an investigational, oral microbiome therapeutic composed of cultivated spores and vegetative bacterial strains rationally designed to reduce the risk of colonization with AMR bacteria and gVHD in HSCT recipients by decolonizing potential pathogens and restoring GI colonization resistance. SER-155 was evaluated in *in vitro* for key pharmacological properties associated with colonization resistance, and in *in vivo* to assess its ability to restore colonization resistance by reducing Enterococcus and Enterobacteriaceae carriage.

**Methods.** The design of SER-155 leveraged genomic data from interventional and observational human datasets to include taxa associated with reduced risk of infection and gVHD in HSCT. Strains of interest were phenotyped, and over 50 candidate consortia containing different combinations of over 150 species were designed and tested *in vitro* and *in vivo*. *In vivo*, candidate compositions were evaluated in mouse models of antibiotic-resistant Enterococcus faecium (VRE) and carbapenem-resistant Klebsiella pneumoniae (CRE) colonization.

**Results.** Oral administration of SER-155 led to a 2-3 Log reduction in VRE and CRE titers compared to untreated mice (Figure 1). In *in vitro*, the carbon source utilization profile of VRE, CRE, and SER-155 strains were assessed using a panel of 85 carbon sources. All 56 carbon sources used by CRE or VRE for anaerobic growth were also utilized by SER-155 strains, supporting a model in which nutrient competition may contribute to reducing CRE and VRE carriage and restoring colonization resistance.
The titers of VRE or CRE were quantified in fecal pellets by plating on selective agar at the indicated time-points. The median A) VRE and B) CRE CFU per gram of feces was calculated for each group and plotted on the line graph (n=6-10 per group). L.O.D., limit of detection. Data were analyzed using the Mann-Whitney t-test and significance was determined as a p-value of p<0.05**, p<0.01***, p<0.001****.

**Conclusion.** SER-155 is an investigational cultivated microbiome therapeutic intended to reduce the risk of infection by engrafting human-commensal bacterial strains in adults undergoing allogeneic HSCT. Preclinical assessments in vitro and in vivo support the ability of SER-155 to reduce VRE and CRE carriage and restore colonization resistance in the gut. A Phase 1b study evaluating SER-155 in allogeneic HSCT patients is being planned.

**Disclosures.** Elizabeth Halvorsen, PhD, Seres Therapeutics (Employee, Shareholder) Marin Vulić, PhD, Seres Therapeutics (Employee) Edward J. O’Brien, PhD, Seres Therapeutics (Employee, Shareholder) Jessica Byrant, PhD, Seres Therapeutics (Employee, Shareholder) Mary-Jane Lombardo, PhD, Seres Therapeutics (Employee, Shareholder) Christopher Ford, PhD, Seres Therapeutics (Employee, Shareholder) Matt Henn, PhD, Seres Therapeutics (Employee, Shareholder)

### 131. AntiViral NL-CVX1 Efficiently Blocks Infection of SARS-CoV-2 Viral Variants of Concern (VOC)

#### Background.

Using a computational approach, NL-CVX1 was developed by Neoleukin Therapeutics, Inc to create a de novo protein that both blocks SARS-CoV-2 infection and is highly resilient to viral escape. In this study we evaluated the efficacy of NL-CVX1 against variants of the original SARS-CoV-2 strain, including important viral variants of concern (VOC) such as B.1.1.7, B.1.351, and P.1.

#### Methods.

The relative binding affinity of NL-CVX1 to the SARS-CoV-2 viral spike protein of VOC was measured using biolayer interferometry (Octet). A competitive ELISA measured the ability of NL-CVX1 to compete with hACE2 for binding to the receptor binding domain (RBD) of the SARS-CoV-2 spike protein from the original strain and VOC. The activity of NL-CVX1 in preventing viral infection was assessed by evaluating the cytopathic effects (CPE) of SARS-CoV-2 in a transmembrane protease, serine 2-expressing Vero E6 cell line (Vero E6/TMPRSS2) and determining the viral load using quantitative real-time reverse transcription polymerase chain reaction in infected cells. A K18hACE2 mouse model of SARS-CoV-2 infection was used to study the dose-response of NL-CVX1 anti-viral activity in vivo.

#### Results.

NL-CVX1 binds the RBD of different VOC of SARS-CoV-2 at low nanomolar concentrations (Fig 1; K<sub>D</sub> 1-5 nM). When competing with hACE2, NL-CVX1 achieved 100% inhibition against hACE2 binding to the RBD of different VOC with IC50 values ranging from 0.7-53 nM (Fig 2). NL-CVX1 neutralized the B.1.1.7 variant as efficiently as the original SARS-CoV-2 strain.

NL-CVX1 prevented clinically significant SARS-CoV-2 infection and protected mice from succumbing to infection. Results from additional in vitro and in vivo experiments to be conducted this summer will be presented.