

series of helpful and necessary functions, such as aiding in digestion or preventing growth of pathogenic microbes. Changes to the microbiome composition, such as from the presence or expansion of pathogenic microorganisms or a loss of the diversity of the microflora, may result in a state of dysbiosis. While microbiome dysbiosis has been described in various diseases, safely promoting a 'healthy' microbiome has been difficult, particularly in subjects who may be vulnerable or immunocompromised. Furthermore, microbiomes may vary in healthy individuals, adding to confusion over how a "healthy" microbiome may be defined, let alone promoted or developed. What is needed in the art are compositions and methods for safely treating or ameliorating dysbiosis of the microbiome, as well as disorders or diseases involving inflammation, infection, allergy, or immune dysfunction that may be associated with dysbiosis.

## SUMMARY OF THE INVENTION

[0005] Provided herein are compositions, kits, articles of manufacture, and methods of use thereof, that contain a mixture of one or more prebiotics, *e.g.*, non-digestible carbohydrates such as human milk oligosaccharides, and one or more probiotic strains of bacteria capable of consuming the prebiotics. The provided compositions, kits, and articles of manufacture are particularly useful for treating or preventing diseases or conditions, such as those involving inflammation, immune disorders, allergy, or dysbiosis of the intestinal microbiome. In some aspectsexamples, the provided compositions, kits, and articles of manufacture are useful for treating or preventing graft versus host disease (GVHD).

[0006] Provided herein is a method for treating or preventing a disease, disorder, or condition associated with one or more of dysbiosis of the intestinal microbiome, inflammation, infection, allergy, or immune dysfunction in a subject in need thereof, the method comprising administering to the subject (i) a prebiotic mixture comprising one or more human milk oligosaccharides and (ii) at least one probiotic strain of bacterium capable of consuming the one or more human milk oligosaccharides.

[0007] In particular embodimentexamples, the probiotic strain is capable of internalizing the one or more human milk oligosaccharides. In certain embodimentexamples, the probiotic strain of bacterium comprises a bacterial strain of the genus *Bifidobacterium*. In some embodimentexamples, the probiotic strain of bacterium comprises a strain of *B. longum* subsp. *infantis*, *B. longum* subsp. *longum*, *B. breve*, or *B. bifidum*. In particular embodimentexamples, the probiotic strain of bacterium comprises *B. longum* subsp. *infantis*.

[0007a] In one aspect, the present disclosure refers to use of (i) a prebiotic mixture comprising at least 5, at least 10, at least 25, at least 50, or at least 100 human milk oligosaccharides in the manufacture of a first medicament and use of (ii) a probiotic strain of *B. longum* subsp. *infantis* in the manufacture of a second medicament, wherein the first and second medicaments are to be used in combination therapy to prevent or reduce the incidence or severity of graft versus host disease in a subject in need thereof, wherein the subject has received or will receive an allogenic hematopoietic stem cell transplant.

[0008] Provided herein is a method for treating or preventing a disease, disorder, or condition associated with one or more of dysbiosis of the intestinal microbiome, inflammation, infection, allergy, or immune dysfunction in a subject in need thereof, the method comprising administering to the subject (i) a prebiotic mixture comprising one or more human milk oligosaccharides and (ii) a probiotic strain of *B. longum* subsp. *infantis*. In certain embodimentexamples, the prebiotic mixture comprises at least 5, at least 10, at least 25, at least 50, or at least 100 human milk oligosaccharides.

[0009] Provided herein is a method for treating or preventing a disease, disorder, or condition associated with one or more of dysbiosis of the intestinal microbiome, inflammation, infection, allergy, or immune dysfunction in a subject in need thereof, the method comprising administering to the subject (i) a prebiotic mixture comprising at least 5, at least 10, at least 25, at least 50, or at least 100 human milk oligosaccharides and (ii) a probiotic strain of the genus *Bifidobacterium*.

[0010] In some embodimentexamples, the disease, disorder, or condition comprises one or more of obesity, type II diabetes, a chronic inflammatory disease, an autoimmune disease, an infection, an infectious disease domination, bowel resection, or a condition associated with chronic diarrhea. In particular embodimentexamples, the disease, disorder, or condition comprises one or more of irritable bowel syndrome (IBS), inflammatory bowel disease (IBD), short bowel syndrome (SBS), celiac disease, small intestinal bacterial overgrowth (SIBO), gastroenteritis, leaky gut syndrome, pouchitis, or gastric lymphoma. In certain embodimentexamples, the disease, condition, or disorder is graft versus host disease.

[0011] In some embodimentexamples, the subject has received or will receive an allogenic hematopoietic stem cell transplant. Provided herein is a method of preventing or reducing the incidence or severity of graft versus host disease in a subject in need thereof, wherein the subject has received or will receive an allogenic hematopoietic stem cell transplant, the method comprising administering to the subject (i) a prebiotic mixture comprising at least 5, at least 10, at least 25, at least 50, or at least 100 human milk oligosaccharides and (ii) a probiotic strain of *B. longum* subsp. *infantis*. In particular embodimentexamples, the disease, condition, or disorder is associated with an infection. In certain embodimentexamples, the infection comprises a bacterial infection or gut domination.

[0012] Provided herein is a method for treating or preventing an infection in a subject in need thereof, the method comprising administering to the subject (i) a prebiotic mixture comprising at least 5, at least 10, at least 25, at least 50, or at least 100 human milk

oligosaccharides and (ii) a probiotic strain of *B. longum* subsp. *infantis*, wherein the infection comprises a bacterial infection or gut domination.

[0013] In some [embodimentexamples](#), the bacterial infection or gut domination comprises an infection or gut domination by one or more species, subspecies, or strains of *Aeromonas*, *Bacillus*, *Blautia*, *Bordetella*, *Borrelia*, *Brucella*, *Burkholderia*, *Campylobacter*, *Chlamydia*, *Chlamydophila*, *Citrobacter*, *Clostridium*, *Corynebacterium*, *Coxiella*, *Ehrlichia*, *Enterobacter*, *Enterobacteriaceae*, *Enterococcus*, *Escherichia*, *Faecalicatena*, *Francisella*, *Haemophilus*, *Helicobacter*, *Hungatella*, *Klebsiella*, *Lachnospiraceae*, *Legionella*, *Leptospira*, *Listeria*, *Morganella*, *Mycobacterium*, *Mycoplasma*, *Neisseria*, *Orientia*, *Plesiomonas*, *Proteus*, *Pseudomonas*, *Rickettsia*, *Salmonella*, *Shigella*, *Staphylococcus*, *Streptococcus*, *Treponema*, *Vibrio*, or *Yersinia*, optionally one or more of *Aeromonas hydrophila*, *Bacillus cereus*, *Campylobacter fetus*, *Campylobacter jejuni*, *Clostridium botulinum*, *Clostridium difficile*, *Clostridium perfringens*, enteroaggregative *Escherichia coli*, enterohemorrhagic *Escherichia coli*, enteroinvasive *Escherichia coli*, enteropathogenic *E. coli*, enterotoxigenic *Escherichia coli*, *Escherichia coli* 0157:H7, *Helicobacter pylori*, *Klebsiella pneumonia*, *Listeria monocytogenes*, *Salmonella paratyphi*, *Salmonella typhi*, *Staphylococcus aureus*, *Vibrio cholerae*, *Vibrio parahaemolyticus*, *Vibrio vulnificus*, or *Yersinia enterocolitica*.

[0014] In particular [embodimentexamples](#), the bacterial infection or gut domination comprises an infection or gut domination by one or more of *Anaerostipes caccae*, *Anaerotruncus colihominis*, *Bifidobacterium longum*, *Blautia* spp., *Butyricoccus pullicaecorum*, *Butyrivibrio crossotus*, *Caproiciproducens*, *Citrobacter freundii*, *Citrobacter koseri*, *Clostridium aldenense*, *Clostridium asparagiforme*, *Clostridium bolteae*, *Clostridium citroniae*, *Clostridium clostridioforme*, *Clostridium lavalense*, *Clostridium nexile*, *Clostridium populeti*, *Clostridium symbiosum*, *Coprococcus comes*, *Coprococcus eutactus*, *Dorea formicigenerans*, *Dorea longicatena*, *Enterobacter aerogenes*, *Enterobacter cloacae*, *Enterococcus faecalis*, *Enterococcus faecium*, *Escherichia coli*, *Ethanoligenens*, *Eubacterium hallii*, *Eubacterium rectale*, *Eubacterium ventriosum*, *Eubacterium siraeum*, *Faecalibacterium prausnitzii*, *Faecalicatena*, *Flavonifractor plautii*, *Hungatella*, *Klebsiella oxytoca*, *Klebsiella pneumoniae*, *Lactobacillus acidophilus*, *Morganella morganii*, *Oscillibacter*, *Papillibacter cinnamivorans*, *Papillibacter*, *Proteus mirabilis*, *Pseudoflavonifractor*, *Roseburia hominis*, *Roseburia intestinalis*, *Roseburia inulinivorans*, *Ruminococcus faecis*, *Ruminococcus lactaris*, *Ruminococcus torques*, *Ruminococcus bromii*, *Serratia marcescens*, *Sporobacter*, *Staphylococcus aureus*, *Staphylococcus epidermidis*,

*Streptococcus anginosus*, *Streptococcus australis*, *Streptococcus constellatus*, *Streptococcus cristatus*, *Streptococcus gordonii*, *Streptococcus infantis*, *Streptococcus intermedius*, *Streptococcus mitis*, *Streptococcus mutans*, *Streptococcus oligofermentans*, *Streptococcus oralis*, *Streptococcus parasanguinis*, *Streptococcus peroris*, *Streptococcus pneumoniae*, *Streptococcus pseudopneumoniae*, *Streptococcus salivarius*, *Streptococcus sanguinis*, *Streptococcus sobrinus*, *Streptococcus tigurinus*, *Streptococcus vestibularis*, *Subdoligranulum variable*, *Subdoligranulum*, or *Syntrophococcus sp.*.

[0015] In certain [embodimentexamples](#), the bacterial infection or gut domination comprises an infection or gut domination by drug-resistant bacteria. In some [embodimentexamples](#), the drug-resistant bacteria comprise one or more of antibiotic-resistant bacterium (ARB), Antibiotic-resistant Proteobacteria, Carbapenem-resistant *Enterobacteriaceae* (CRE), Extended Spectrum Beta-Lactamase producing *Enterobacteriaceae* (ESBL-E), fluoroquinolone-resistant *Enterobacteriaceae*, extended spectrum beta-lactam resistant *Enterococci* (ESBL), vancomycin-resistant *Enterococci* (VRE), multi-drug resistant *E. coli*, or multi-drug resistant *Klebsiella*.

[0016] In particular [embodimentexamples](#), the subject has undergone or will undergo an ileal pouch-anal anastomosis (IPAA) surgery, and wherein the disease, condition, or disorder comprises pouchitis. Provided herein is a method of preventing or reducing the incidence or severity of pouchitis in a subject in need thereof, wherein the subject has undergone or will undergo an ileal pouch-anal anastomosis (IPAA) surgery, the method comprising administering to the subject (i) a prebiotic mixture comprising at least 5, at least 10, at least 25, at least 50, or at least 100 human milk oligosaccharides and (ii) a probiotic strain of *B. longum* subsp. *infantis*, wherein the infection comprises a bacterial infection.

[0017] In certain [embodimentexample](#), the prebiotic mixture comprises at least 10 human milk oligosaccharides. In some [embodimentexamples](#), the prebiotic mixture comprises at least 25 human milk oligosaccharides. In particular [embodimentexamples](#), the prebiotic mixture comprises at least 50 human milk oligosaccharides. In certain [embodimentexamples](#), the prebiotic mixture comprises at least 50 human milk oligosaccharides.

[0018] In some [embodimentexamples](#), the prebiotic mixture comprises one or more of 2'-fucosyl-lactose, 3'-fucosyl-lactose, 3'-sialyl-lactose, 6'-sialyl-lactose, Lacto-N-tetraose, lacto-N-difucohexaose I, lactodifucotetraose, Lacto-N-fucopentaose I, sialylacto-N-tetraose c, sialylacto-N-tetraose b, or disialyllacto-N-tetraose. In particular [embodimentexamples](#), the prebiotic mixture comprises 2'-fucosyl-lactose, 3'-fucosyl-lactose, 3'-sialyl-lactose, 6'-sialyl-lactose,

Lacto-N-tetraose, lacto-N-difucohexaose I, lactodifucotetraose, Lacto-N-fucopentaose I, sialylacto-N-tetraose c, sialylacto-N-tetraose b, and disialyllacto-N-tetraose. In certain [embodimentexamples](#), the prebiotic mixture comprises 2'-fucosyl-lactose, 3'-fucosyl-lactose, 3'-sialyl-lactose, 6'-sialyl-lactose, Lacto-N-tetraose, Lacto-N-neo-tetraose, Lacto-N-fucopentaose I, Lacto-N-fucopentaose II, Lacto-N-fucopentaose III, Sialyl-lacto-N-tetraose b, Sialyl-lacto-N-tetraose c, Lacto-N-difuco-hexaose I, Lacto-N-difuco-hexaose II, Lacto-N-hexaose, para-Lacto-N-hexaose, Disialyllacto-N-tetraose, Fucosyl-Lacto-N-hexaose, Difucosyl-Lacto-N-hexaose a, and Difucosyl-Lacto-N-hexaose b.

**[0019]** In some [embodimentexamples](#), the prebiotic mixture is, is derived from, or comprises a concentrated permeate from pooled human milk, wherein the permeate is obtained from the ultrafiltration of human skim milk, wherein the human skim milk is obtained by removing cream from pooled human milk, and wherein the pooled human milk is pooled from the milk of multiple human milk donors.

**[0020]** Provided herein is a method for treating or preventing a disease, disorder, or condition associated with one or more of dysbiosis of the intestinal microbiome, inflammation, infection, allergy, or immune dysfunction in a subject in need thereof, the method comprising administering to the subject (i) a prebiotic mixture comprising at least 5, at least 10, at least 25, at least 50, or at least 100 human milk oligosaccharides, wherein the prebiotic mixture is, is derived from, or comprises a concentrated human milk permeate, wherein the human milk permeate is obtained from the ultrafiltration of human skim milk obtained from pooled human milk, and wherein the pooled human milk is pooled from the milk of multiple human milk donors; and (ii) a probiotic strain of *B. longum* subsp. *infantis*.

**[0021]** In particular [embodimentexamples](#), the pooled human milk is pooled from the milk of at least 25, 50, or 100 human milk donors. In certain [embodimentexamples](#), the prebiotic mixture comprises at least one synthetic human milk oligosaccharide. In some [embodimentexamples](#), the prebiotic mixture and the probiotic strain are administered orally. In particular [embodimentexamples](#), the prebiotic mixture and the probiotic strain are administered on the same day for at least one day. In certain [embodimentexamples](#), the prebiotic mixture and the probiotic strain are administered on the same day for at least three consecutive days. In some [embodimentexamples](#), the prebiotic mixture and the probiotic strain are administered on the same day for at least seven consecutive days.

**[0022]** In particular [embodimentexamples](#), the method comprises at least a first treatment phase and a second treatment phase, wherein the first treatment phase comprises

administering to the subject the prebiotic mixture and the probiotic strain on the same day for at least one day, and wherein the second treatment phase begins after the end of the first treatment phase, optionally immediately after, and comprises administering the prebiotic mixture for at least one day, wherein the probiotic strain is not administered during the second treatment phase.

[0023] In certain [embodimentexamples](#), the second treatment phase comprises administering to the subject the prebiotic mixture each day for at least three consecutive days. In some [embodimentexamples](#), the second treatment phase comprises administering to the subject the prebiotic mixture each day for at least seven consecutive days. In particular [embodimentexamples](#), the first treatment phase is between 3 and 14 days in length and wherein the second treatment phase is between 3 and 14 days in length. In certain [embodimentexamples](#), the first treatment phase is about 7 days in length and wherein the second treatment phase is about 7 days in length. In some [embodimentexamples](#), the probiotic strain is administered in an amount of at least  $5 \times 10^6$  colony forming units (CFU) per day. In particular [embodimentexamples](#), the probiotic strain is administered in an amount of at least  $8 \times 10^7$  colony forming units (CFU) per day. In certain [embodimentexamples](#), the prebiotic mixture is administered in an amount of at least 500 mg of total human milk oligosaccharides per day. In some [embodimentexamples](#), the prebiotic mixture is administered in an amount of between 0.5 g and 25 g, 1 g and 5 g, 2 g and 3 g, 3 g and 6 g, 4 g and 5 g, 5 g and 10 g, 8 g and 10 g, 10 g and 20 g, 15 g and 25 g, 15 g and 20 g, or 17 g and 19 g of total human milk oligosaccharides per day.

[0024] In particular [embodimentexample](#), the prebiotic mixture and/or the probiotic strain is administered with an enteric coating. In certain [embodimentexamples](#), the methods further comprise administering a histamine-2 receptor antagonist, wherein the histamine-2 receptor antagonist is administered at least 1 hour prior to administration of the probiotic strain, optionally wherein the histamine-2 receptor antagonist is famotidine.

[0025] In some [embodimentexamples](#), the subject has not been administered a proton pump inhibitor at least about 6 hours, 12 hours, 24 hours, 36 hours, 48 hours, 60 hours, 2 days, 3 days, 4 days, 5 days, 7 days, 10 days, 2 weeks, 3 weeks, or 4 weeks prior to administration of the probiotic strain. In particular [embodimentexamples](#), the subject has not been administered a benzimidazole derivative for at least about 6 hours, 12 hours, 24 hours, 36 hours, 48 hours, 60 hours, 2 days, 3 days, 4 days, 5 days, 7 days, 10 days, 2 weeks, 3 weeks, or 4 weeks prior to administration of the probiotic strain. In certain [embodimentexamples](#), the subject has not been administered omeprazole for at least about 6 hours, 12 hours, 24 hours, 36 hours, 48 hours,

60 hours, 2 days, 3 days, 4 days, 5 days, 7 days, 10 days, 2 weeks, 3 weeks, or 4 weeks prior to administration of the probiotic strain. In some [embodimentexamples](#), both of the probiotic strain and the prebiotic mixture are free or essentially free of a benzimidazole derivative.

[0026] In particular [embodimentexamples](#), the subject is a human subject at least 6 months, 12 months, 2 years, 5 years, 12 years, or 18 years of age. In certain [embodimentexamples](#), the subject is human subject that is a child, an adolescent, or an adult. In some [embodimentexamples](#), the subject is a human adult.

[0027] Provided herein is a use of a prebiotic mixture and at least one probiotic strain for the treatment or prevention of a disease, disorder, or condition associated with one or more of dysbiosis of the intestinal microbiome, inflammation, infection, allergy, or immune dysfunction in a subject in need thereof, wherein the prebiotic mixture comprises one or more human milk oligosaccharides and the probiotic strain is capable of consuming the one or more human milk oligosaccharides.

[0028] Provided herein is a prebiotic mixture and at least one probiotic strain for use in the manufacture of medicaments for the treatment or prevention of a disease, disorder, or condition associated with one or more of dysbiosis of the intestinal microbiome, inflammation, infection, allergy, or immune dysfunction, wherein the prebiotic mixture comprises one or more human milk oligosaccharides and wherein the probiotic strain is capable of consuming the one or more human milk oligosaccharides.

[0029] ~~Provided herein is~~[In another aspect, the present disclosure refers to](#) a kit comprising at least one probiotic strain of bacterium and a prebiotic mixture comprising one or more human milk oligosaccharides, wherein probiotic strain is capable of internalizing and consuming the one or more human milk oligosaccharides. In particular [embodimentexamples](#), the probiotic strain comprises a strain of Bifidobacterium. In certain [embodimentexamples](#), the probiotic strain comprises a strain of *B. longum* subsp. *infantis*. In some [embodimentexamples](#), the prebiotic mixture comprises at least 2, at least 5, at least 10, at least 25, at least 50, at least 100, at least 125, or at least 150 human milk oligosaccharides. In particular [embodimentexamples](#), the prebiotic mixture comprises at least 25 human milk oligosaccharides. In certain [embodimentexamples](#), the prebiotic mixture is free or essentially free of oligosaccharides that are not human milk oligosaccharides. In some [embodimentexamples](#), the prebiotic mixture is, is obtained from, or comprises a concentrated ultra-filtered permeate from pooled human milk. In particular [embodimentexamples](#), the probiotic strain is incorporated into one or more individual probiotic doses and wherein the prebiotic mixture is incorporated into one or more individual prebiotic doses. In certain [embodimentexamples](#), the one

or more individual probiotic doses each comprise at least  $1 \times 10^3$  colony forming units (CFU). In some [embodimentexamples](#), the one or more individual probiotic doses each comprise at least  $8 \times 10^7$  colony forming units (CFU). In particular [embodimentexamples](#), the one or more prebiotic doses comprises between 500 mg and 25 g of total human milk oligosaccharides. In certain [embodimentexamples](#), the one or more individual prebiotic doses comprises between 0.5 g and 25 g, 1 g and 5 g, 2 g and 3 g, 3 g and 6 g, 4 g and 5 g, 5 g and 10 g, 8 g and 10 g, 10 g and 20 g, 15 g and 25 g, 15 g and 20 g, or 17 g and 19 g of total human milk oligosaccharides per day. In some [embodimentexamples](#), the one or more individual prebiotic doses comprises less than 0.1% lactose. Also provided herein is an article of manufacture, comprising a kit described herein and instructions for use, wherein the instructions for use describing any of the methods provided herein.

**[0030]** Provided herein is a method of treating hyperammonemia, comprising administering to a subject in need thereof a prebiotic mixture comprising oligosaccharides, wherein (i) the mixture is free or essentially free of oligosaccharides that incorporate an N-acetyl glucosamine residue or (ii) the percentage by weight of oligosaccharides comprising nitrogen in the mixture is less than 50%. Also provided herein is a method of decreasing the level or amount of ammonia in a subject in need thereof, comprising administering to the subject a prebiotic mixture comprising oligosaccharides, wherein (i) the mixture is free or essentially free of oligosaccharides that incorporate an N-acetyl glucosamine residue or (ii) the percentage by weight of oligosaccharides comprising nitrogen in the mixture is less than 50%.

**[0031]** In particular [embodimentexample](#), the method further comprises administering a probiotic strain of bacterium capable of consuming each oligosaccharide of the mixture. In certain [embodimentexamples](#), the subject has, is at risk of having, or is suspected of having hyperammonemia, hepatic encephalopathy, or a urea cycle disorder. In some [embodimentexamples](#), the prebiotic mixture comprises one or more human milk oligosaccharides (HMOs). In particular [embodimentexamples](#), all or essentially all of the oligosaccharides of the prebiotic mixture are human milk oligosaccharides (HMOs). In certain [embodimentexamples](#), the prebiotic mixture is free or essentially free of oligosaccharides that incorporate an N-acetyl glucosamine residue. In some [embodimentexamples](#), the prebiotic mixture comprises at least 2, at least 3, at least 4, at least 5, at least 6, or at least 7 oligosaccharides. In particular [embodimentexamples](#), the percentage by weight of oligosaccharides in the prebiotic mixture comprising nitrogen is less than 50%, 40%, 30%, 25%, 20%, 10%, 5%, or 1%. In certain [embodimentexamples](#), the mixture is free or

essentially free of oligosaccharides that comprise nitrogen. In some [embodimentexamples](#), less than 50%, 40%, 30%, 25%, 20%, 10%, 5%, or 1% by weight of the oligosaccharides in the prebiotic mixture are oligosaccharides comprising five or more monosaccharide residues.

**[0032]** In particular [embodimentexamples](#), the prebiotic mixture is free or essentially free of oligosaccharides comprising five or more monosaccharide residues. In certain [embodimentexamples](#), the probiotic strain is a strain of Bifidobacterium, optionally a strain of *B. longum* subsp. *infantis*, *B. longum* subsp. *longum*, *B. breve*, or *B. bifidum*. In some [embodimentexamples](#), the probiotic strain is capable of internalizing the oligosaccharides of the mixture.

**[0033]** Provided herein is a method of treating or preventing hyperammonemia, comprising administering to a subject in need thereof (i) a prebiotic mixture comprising oligosaccharides, wherein all or essentially all of the oligosaccharides of the prebiotic mixture are human milk oligosaccharides that do not incorporate an N-acetyl glucosamine residue, and (ii) a probiotic strain of bacterium, wherein the probiotic strain is a Bifidobacterium capable of internalizing HMOs. Also provided herein is a method of reducing ammonia in a subject in need thereof, comprising administering to a subject (i) a prebiotic mixture of oligosaccharides, wherein all or essentially all of the oligosaccharides of the prebiotic mixture are human milk oligosaccharides (HMOs) that do not incorporate an N-acetyl glucosamine residue, and (ii) a probiotic strain of bacterium, wherein the probiotic bacterium strain is a Bifidobacterium strain capable of internalizing HMOs.

**[0034]** In particular [embodimentexamples](#), the probiotic strain is a strain of *B. longum* subsp. *infantis*. In certain [embodimentexamples](#), the prebiotic mixture comprises one or more of 2'-fucosyllactose, 3-fucosyllactose, difucosyllactose, 3'-sialyllactose, or 6'-sialyllactose. In some [embodimentexamples](#), the prebiotic mixture comprises 2'-fucosyllactose, 3-fucosyllactose, difucosyllactose, 3'-sialyllactose, and 6'-sialyllactose. In particular [embodimentexamples](#), the oligosaccharides of the prebiotic mixture consist or consist essentially of 2'-fucosyllactose, 3-fucosyllactose, difucosyllactose, 3'-sialyllactose, and 6'-sialyllactose. In certain [embodimentexamples](#), the prebiotic mixture comprises one or more of 2'-fucosyllactose, 3-fucosyllactose, or difucosyllactose. In some [embodimentexamples](#), the prebiotic mixture comprises of 2'-fucosyllactose, 3-fucosyllactose, and difucosyllactose. In particular [embodimentexamples](#), the oligosaccharides of the mixture consist or consist essentially of 2'-fucosyllactose, 3-fucosyllactose, and difucosyllactose.

**[0035]** Provided herein is a method of treating or preventing hyperammonemia comprising administering to a subject in need thereof i) a prebiotic mixture comprising 2'-

fucosyllactose, 3-fucosyllactose, difucosyllactose, 3'-sialyllactose, and 6'-sialyllactose and ii) a probiotic strain of *B. longum* subsp. *infantis*. Also provided herein is a method of treating or preventing hyperammonemia comprising administering to a subject in need thereof i) a prebiotic mixture comprising 2'-fucosyllactose, 3-fucosyllactose, and difucosyllactose and ii) a probiotic strain of *B. longum* subsp. *infantis*. In certain [embodimentexamples](#), the subject has, is suspected of having, or is at risk of having hepatic encephalopathy. In some [embodimentexamples](#), the subject has, is suspected of having, or is at risk of having a urea cycle disorder.

**[0036]** Provided herein is a method for treating dysbiosis in a subject in need thereof, wherein the dysbiosis is of the intestinal microbiome, the method comprising administering to the subject (i) a prebiotic mixture comprising one or more human milk oligosaccharides and (ii) at least one probiotic strain of bacterium capable of consuming the human milk oligosaccharides of the mixture.

**[0037]** Also provided herein is a method of preventing or reducing the incidence or severity of graft versus host disease (GVHD) in a subject in need thereof, wherein the subject has received or will receive an allogenic hematopoietic stem cell transplant, the method comprising administering to the subject (i) a prebiotic mixture comprising one or more human milk oligosaccharides and (ii) at least one probiotic strain of bacterium capable of consuming the human milk oligosaccharides of the mixture.

**[0038]** Additionally provided herein is a method of preventing or reducing the incidence or severity of a disease, disorder, or condition associated with inflammation, immune dysfunction, or allergy in a subject in need thereof, the method comprising administering to the subject (i) a prebiotic mixture comprising one or more human milk oligosaccharides and (ii) at least one probiotic strain of bacterium capable of consuming the human milk oligosaccharides of the mixture.

**[0039]** In some [embodimentexamples](#), the subject has, is suspected of having, or is at risk of having obesity, type II diabetes, a chronic inflammatory disease, an autoimmune disease, an infection, an infectious disease domination, bowel resection, and/or a condition associated with chronic diarrhea. In certain [embodimentexamples](#), the subject has, is suspected of having, or is at risk of having one or more of irritable bowel syndrome (IBS), inflammatory bowel disease (IBD), short bowel syndrome (SBS), celiac disease, small intestinal bacterial overgrowth (SIBO), gastroenteritis, leaky gut syndrome, pouchitis, or gastric lymphoma. In particular [embodimentexamples](#), the subject has, is suspected of having, or is at risk of having a disease, disorder, or condition is associated with a bacterial, viral, or parasitic infection or

overgrowth. In some [embodimentexamples](#), the disease, condition, or disorder is associated with infection by drug-resistant bacteria.

[0040] Also provided herein is a method of preventing or reducing the incidence or severity of pouchitis in a subject in need thereof, wherein the subject has undergone or will undergo an ileal pouch-anal anastomosis (IPAA) surgery, the method comprising administering to the subject (i) a prebiotic mixture comprising one or more human milk oligosaccharides and (ii) at least one probiotic strain of bacterium capable of consuming the human milk oligosaccharides of the mixture.

[0041] In certain [embodimentexamples](#), the prebiotic mixture comprises at least 5, at least 10, at least 25, at least 30, at least 50, at least 100, or at least 150 human milk oligosaccharides. In particular [embodimentexamples](#), the prebiotic mixture comprises at least 25 human milk oligosaccharides. In some [embodimentexamples](#), the prebiotic mixture is, is derived from, or comprises a concentrated ultra-filtered permeate from pooled human milk. In certain [embodimentexamples](#), the pooled human milk is pooled from the milk of at least 25, 50, or 100 individual donors.

[0042] In particular [embodimentexamples](#), the at least one probiotic strain of bacterium comprises a bacterial strain of the genus *Bifidobacterium*. In some [embodimentexamples](#), the at least one probiotic strain of bacterium comprises a strain of *B. breve*, *B. bifidum*, *B. longum* subsp. *infantis*, or *B. longum* subsp. *longum*. In certain [embodimentexamples](#), the at least one probiotic strain of bacterium comprises *B. longum* subsp. *infantis*.

[0043] Provided herein is a method of preventing or reducing the incidence or severity of graft versus host disease (GVHD) in a subject in need thereof, wherein the subject has received or will receive an allogeneic hematopoietic stem cell transplant, the method comprising administering to the subject (i) a prebiotic mixture comprising at least 25 human milk oligosaccharides and a probiotic strain of *B. longum* subsp. *infantis*.

[0044] In particular [embodimentexamples](#), the prebiotic mixture and the probiotic strain are administered orally. In some [embodimentexamples](#), the prebiotic mixture and the probiotic strain are administered on the same day for at least three consecutive days. In certain [embodimentexamples](#), the method comprises at least a first treatment phase and a second treatment phase, wherein the first treatment phase comprises administering to the subject the prebiotic mixture and the probiotic strain on the same day for at least three consecutive days, and wherein the second treatment phase begins immediately after the end of the first treatment phase and comprises administering the prebiotic mixture for at least three consecutive days.

[0045] In particular [embodimentexamples](#), the first treatment phase is between 3 and 14 days in length and wherein the second treatment phase is between 3 and 14 days in length. In some [embodimentexamples](#), the first treatment phase is about 7 days in length and wherein the second treatment phase is about 7 days in length. In certain [embodimentexamples](#), the probiotic strain is administered in an amount of at least  $5 \times 10^6$  colony forming units (CFU) per day. In particular [embodimentexamples](#), the probiotic strain is administered in an amount of at least  $8 \times 10^7$  colony forming units (CFU) per day. In some [embodimentexamples](#), the prebiotic mixture is administered in an amount of at least 500 mg of total human milk oligosaccharides per day. In certain [embodimentexamples](#), the prebiotic mixture is administered in an amount of between 1 g and 5 g, 2 g and 3 g, 3 g and 6 g, 4 g and 5 g, 5 g and 10 g, 8 g and 10 g, 10 g and 20 g, 15 g and 20 g, or 17 g and 19 g of total human milk oligosaccharides per day. In particular [embodimentexamples](#), the prebiotic mixture and/or the probiotic strain is administered with an enteric coating. In certain [embodimentexamples](#), the methods further comprise administering a proton pump inhibitor, wherein the proton pump inhibitor is administered at least 1 hour prior to administration of the probiotic strain. In some [embodimentexamples](#), the subject is at least 6 months, 12 months, 2 years, 5 years, 12 years, or 18 years of age. In certain [embodimentexamples](#), the subject is a child, an adolescent, or an adult.

[0046] In some [embodimentexamples](#), the subject has not been administered one or both of a proton pump inhibitor or an antacid for at least about 1 hour, 2 hours, 4 hours, 6 hours, 12 hours, 24 hours, 36 hours, 48 hours, 60 hours, 2 days, 3 days, 4 days, 5 days, 7 days, 10 days, 2 weeks, 3 weeks, or 4 weeks prior to administration of the probiotic strain. In certain [embodimentexamples](#), the subject has not been administered a proton pump inhibitor or an antacid comprising bicarbonate for at least about 1 hour, 2 hours, 4 hours, 6 hours, 12 hours, 24 hours, 36 hours, 48 hours, 60 hours, 2 days, 3 days, 4 days, 5 days, 7 days, 10 days, 2 weeks, 3 weeks, or 4 weeks prior to administration of the probiotic strain. In particular [embodimentexamples](#), the subject has not been administered a proton pump inhibitor comprising a benzimidazole derivative for at least about 1 hour, 2 hours, 4 hours, 6 hours, 12 hours, 24 hours, 36 hours, 48 hours, 60 hours, 2 days, 3 days, 4 days, 5 days, 7 days, 10 days, 2 weeks, 3 weeks, or 4 weeks prior to administration of the probiotic strain. In some [embodimentexamples](#), the subject has not been administered a proton pump inhibitor comprising omeprazole for at least about 1 hour, 2 hours, 4 hours, 6 hours, 12 hours, 24 hours, 36 hours, 48 hours, 60 hours, 2 days, 3 days, 4 days, 5 days, 7 days, 10 days, 2 weeks, 3 weeks, or 4 weeks prior to administration of the probiotic strain. In certain [embodimentexamples](#), at a time when the one or more probiotic is

administered, the subject's stomach has an acidic pH, optionally below 7, between 1 and 7, between 2 and 5, between 1 and 4, or between 1.5 and 3.5. In particular [embodimentexamples](#), both of the one or more probiotics and the prebiotic mixture are free or essentially free of some or all of a proton pump inhibitor and an antacid.

[0047] Provided herein is a kit comprising one or more probiotic strains of bacterium and a prebiotic mixture comprising one or more human milk oligosaccharides, wherein the one or more probiotic strains are capable of internalizing and consuming the one or more human milk oligosaccharides. In certain [embodimentexamples](#), the one or more probiotics comprises a Bifidobacterium probiotic. In particular [embodimentexamples](#), the one or more probiotics comprises one or more of a strain of *B. breve*, *B. bifidum*, *B. longum* subsp. *longum*, or *B. longum* subsp. *infantis*. In some [embodimentexamples](#), the prebiotic mixture comprises at least 2, at least 5, at least 10, at least 25, at least 50, at least 100, at least 125, or at least 150 different human milk oligosaccharides. In certain [embodimentexamples](#), the prebiotic mixture comprises at least 25 human milk oligosaccharides. In particular [embodimentexamples](#), the prebiotic mixture is free or essentially free of oligosaccharides that are not human milk oligosaccharides. In some [embodimentexamples](#), the prebiotic mixture is, is obtained from, or comprises a concentrated ultra-filtered permeate from pooled human milk.

[0048] In certain [embodimentexample](#), the one or more probiotics are incorporated into one or more individual probiotic doses and the prebiotic mixture is incorporated into one or more individual prebiotic doses. In particular [embodimentexamples](#), the one or more individual probiotic doses each comprise at least  $1 \times 10^3$  colony forming units (CFU). In some [embodimentexamples](#), the one or more individual probiotic doses each comprise at least  $8 \times 10^7$  colony forming units (CFU). In certain [embodimentexamples](#), the one or more prebiotic doses comprises between 500 mg and 25 g of total human milk oligosaccharides. In particular [embodimentexamples](#), the one or more individual prebiotic doses comprises between 1 g and 5 g, 2 g and 3 g, 3 g and 6 g, 4 g and 5 g, 5 g and 10 g, 8 g and 10 g, 10 g and 20 g, 15 g and 20 g, or 17 g and 19 g of total human milk oligosaccharides. In some [embodimentexamples](#), the one or more individual prebiotic doses comprises less than 0.1% lactose. In certain [embodimentexamples](#), the kit comprises one or more individual doses of a proton pump inhibitor.

[\[0048a\] In yet another aspect, the present disclosure refers to an article of manufacture, comprising any of the kits disclosed herein and instructions for use, wherein the instructions for use describe any of the uses disclosed herein.](#)

[0049] In certain [embodimentexamples](#), provided herein is an article of manufacture comprising any of the kits provided herein and instructions for use, wherein the instructions for use describes any of the methods described herein.

[0050] Provided herein are compositions, methods, strategies, kits, and articles of manufacture that are useful, inter alia, in the treatment or prevention of graft versus host disease (GVHD) or related conditions and disorders. In some [aspectexamples](#), the provided compositions, methods, kits, or articles of manufacture are or include a prebiotic, such as a mixture of human milk oligosaccharides, and at least one strain of probiotic bacteria. In some [aspectexamples](#), the probiotic bacteria strain is capable of internalizing and metabolizing the human milk oligosaccharides. In particular [embodimentexamples](#), administration of the at least one probiotic strain and oligosaccharides to a subject in need thereof reduces or prevents incidence or severity of GVHD.

[0051] Provided herein is a method of preventing or reducing the incidence or severity of graft versus host disease (GVHD) in a subject in need thereof, wherein the subject has undergone or will undergo an allogenic transplant, the method comprising administering to the subject a mixture of non-digestible carbohydrates and a probiotic strain of bacterium capable of consuming the non-digestible carbohydrates of the mixture.

[0052] In particular [embodimentexamples](#), the mixture comprises at least 10, at least 25, at least 50, at least 100, at least 150, or at least 200 different non-digestible carbohydrates. In certain [embodimentexamples](#), the non-digestible carbohydrates are oligosaccharides. In some [embodimentexamples](#), the non-digestible carbohydrates are human milk oligosaccharides.

[0053] Further provided herein is a method of preventing or reducing the incidence or severity of graft versus host disease (GVHD) in a subject in need thereof, wherein the subject has received or will receive an allogenic transplant, the method comprising administering to the subject a mixture of HMOs and a probiotic strain of bacterium of the genus *Bifidobacterium*.

[0054] In some [embodimentexamples](#), the probiotic strain is a strain of *B. breve*, *B. bifidum*, *B. longum* subsp. *infantis*, or *B. longum* subsp. *longum*. In certain [embodimentexamples](#), the probiotic strain is *B. longum* subsp. *infantis*. In particular [embodimentexamples](#), the mixture comprises at least 10, at least 25, at least 50, at least 100, at least 150, or at least 200 different HMOs.

[0055] In some [embodimentexamples](#), administration of the mixture and the probiotic strain begins at the time of neutrophil engraftment, immediately after completion of an antibiotic regimen taken in association with the transplant. In certain [embodimentexamples](#), the mixture and the probiotic strain are administered orally. In particular [embodimentexamples](#), the mixture and the probiotic strain are administered orally twice a day. In some [embodimentexamples](#), the mixture is

administered at a dose of between or between about 3 g/day and 18 g/day, inclusive. In certain [embodimentexamples](#), the mixture is administered at a dose of or of about 12.5 g/day.

[0056] In particular [embodimentexamples](#), the probiotic strain is administered at a dose of between or between about  $10^4$  units/day and  $10^{12}$  units/day. In some [embodimentexamples](#), the probiotic strain is administered at a dose of or of about  $10^9$  units/day. In certain [embodimentexamples](#), the mixture and the probiotic strain is administered twice daily for the duration of a treatment period. In particular [embodimentexample](#), the treatment period is or lasts between 7 days and 180 days, inclusive. In some [embodimentexamples](#), the treatment period is or lasts for at least 7 days, 14 days, 21 days, 28 days, 30 days, 60 days, 90 days, or 120 days.

[0057] In certain [embodimentexamples](#), one or both of the mixture and the probiotic strain comprises an enteric coating. In particular [embodimentexamples](#), the method further comprises administering a proton pump inhibitor. In some [embodimentexamples](#), administration of the mixture and the probiotic strain treats, ameliorates, or reduces dysbiosis in the subject.

[0058] Also provided herein is a kit comprising a mixture of at least 10, at least 25, at least 50, at least 100, at least 150, or at least 200 different HMOs and a probiotic strain of *B. longum* subsp. *infantis*, wherein the mixture and the probiotic strain is suitable for oral administration.

[0059] In addition, provided herein is a method of preventing or reducing the incidence or severity of pouchitis in a subject in need thereof, wherein the subject has undergone or will undergo an IPAA surgery, the method comprising administering to the subject a mixture of non-digestible carbohydrates and a probiotic strain of bacterium capable of consuming the non-digestible carbohydrates of the mixture.

[0060] In certain [embodimentexample](#), provided herein is a composition or formulation comprising (i) a prebiotic mixture comprising one or more human milk oligosaccharides and (ii) at least one probiotic strain of bacterium capable of consuming the one or more human milk oligosaccharides, for use in treating or preventing a disease, disorder, or condition associated with one or more of inflammation, infection, allergy, immune dysfunction, or dysbiosis of the intestinal microbiome in a subject in need thereof. In particular [embodimentexamples](#), provided herein are formulations for use in accordance with the [invention-disclosure](#) wherein the (i) prebiotic mixture and the (ii) probiotic strain are for administration to the subject simultaneously, separately, or sequentially.

[0061] In some [embodimentexamples](#), provided herein is a composition or formulation comprising (i) a prebiotic mixture comprising one or more human milk oligosaccharides and

(ii) a probiotic strain of *B. longum* subsp. *infantis*, for use in treating or preventing a disease, disorder, or condition associated with one or more of inflammation, infection, allergy, immune dysfunction, or dysbiosis of the intestinal microbiome in a subject in need thereof.

[0062] In various [embodimentexamples](#), provided herein is a composition or formulation comprising (i) a prebiotic mixture comprising at least 5, at least 10, at least 25, at least 50, or at least 100 human milk oligosaccharides and (ii) a probiotic strain of the genus *Bifidobacterium*, for use in treating or preventing a disease, disorder, or condition associated with one or more of inflammation, infection, allergy, immune dysfunction, or dysbiosis of the intestinal microbiome in a subject in need thereof.

[0063] In certain [embodimentexamples](#), provided herein is a composition or formulation comprising (i) a prebiotic mixture comprising at least 5, at least 10, at least 25, at least 50, or at least 100 human milk oligosaccharides and (ii) a probiotic strain of *B. longum* subsp. *infantis*, for use in preventing or reducing the incidence or severity of graft versus host disease in a subject in need thereof, wherein the subject has received or will receive an allogenic hemopoietic stem cell transplant.

[0064] In particular [embodimentexamples](#), provided herein is a composition or formulation comprising (i) a prebiotic mixture comprising at least 5, at least 10, at least 25, at least 50, or at least 100 human milk oligosaccharides and (ii) a probiotic strain of *B. longum* subsp. *infantis*, for use in treating or preventing an infection in a subject in need thereof, wherein the infection comprises a bacterial infection.

[0065] In some [embodimentexamples](#), provided herein is a composition or formulation comprising (i) a prebiotic mixture comprising at least 5, at least 10, at least 25, at least 50, or at least 100 human milk oligosaccharides and (ii) a probiotic strain of *B. longum* subsp. *infantis*, for use in preventing or reducing the incidence or severity of pouchitis in a subject in need thereof, wherein the infection comprises a bacterial infection.

[0066] In particular [embodimentexamples](#), provided herein is a composition or formulation comprising (i) a prebiotic mixture comprising at least 5, at least 10, at least 25, at least 50, or at least 100 human milk oligosaccharides, wherein the prebiotic mixture is, is derived from, or comprises a concentrated human milk permeate, wherein the human milk permeate is obtained from the ultrafiltration of human skim milk obtained from pooled human milk, and wherein the pooled human milk is pooled from the milk of multiple human milk donors; and (ii) a probiotic strain of *B. longum* subsp. *infantis*, for use in treating or preventing a disease,

disorder, or condition associated with one or more of inflammation, infection, allergy, immune dysfunction, or dysbiosis of the intestinal microbiome in a subject in need thereof.

[0067] In some embodimentexamples, provided herein is a composition or formulation comprising a prebiotic mixture and at least one probiotic strain for the treatment or prevention of a disease, disorder, or condition associated with one or more of inflammation, infection, allergy, immune dysfunction, or dysbiosis of the intestinal microbiome in a subject in need thereof, wherein the prebiotic mixture comprises one or more human milk oligosaccharides and the probiotic strain is capable of consuming the one or more human milk oligosaccharides.

[0068] In certain embodimentexamples, provided herein is a composition or formulation comprising a prebiotic mixture and at least one probiotic strain for use in the treatment or prevention of a disease, disorder, or condition associated with one or more of inflammation, infection, allergy, immune dysfunction, or dysbiosis of the intestinal microbiome, wherein the prebiotic mixture comprises one or more human milk oligosaccharides and wherein the probiotic strain is capable of consuming the one or more human milk oligosaccharides.

[0069] In some embodimentexamples, provided herein is a prebiotic mixture comprising oligosaccharides for use treating hyperammonemia, wherein (i) the mixture is free or essentially free of oligosaccharides that incorporate an N-acetyl glucosamine residue or (ii) the percentage by weight of oligosaccharides comprising nitrogen in the mixture is less than 50%.

[0070] In particular embodimentexamples, provided herein is a prebiotic mixture comprising oligosaccharides, wherein (i) the mixture is free or essentially free of oligosaccharides that incorporate an N-acetyl glucosamine residue or (ii) the percentage by weight of oligosaccharides comprising nitrogen in the mixture is less than 50%, for use in decreasing the level or amount of ammonia in a subject in need thereof for the treatment of hyperammonemia.

[0071] In certain embodimentexample, provided herein is a composition or formulation comprising (i) a prebiotic mixture comprising oligosaccharides, wherein all or essentially all of the oligosaccharides of the prebiotic mixture are human milk oligosaccharides that do not incorporate an N-acetyl glucosamine residue, and (ii) a probiotic strain of bacterium, for use in treating or preventing hyperammonemia, wherein the probiotic strain is a Bifidobacterium capable of internalizing HMOs.

[0072] In some [embodimentexamples](#), provided herein is a composition or formulation comprising (i) a prebiotic mixture of oligosaccharides, wherein all or essentially all of the oligosaccharides of the prebiotic mixture are human milk oligosaccharides (HMOs) that do not incorporate an N-acetyl glucosamine residue, and (ii) a probiotic strain of bacterium, for reducing ammonia in a subject in need thereof for the treatment of hyperammonemia, wherein the probiotic bacterium strain is a Bifidobacterium strain capable of internalizing HMOs.

[0073] In certain [embodimentexample](#), provided herein is a composition or formulation comprising i) a prebiotic mixture comprising 2'-fucosyllactose, 3-fucosyllactose, difucosyllactose, 3'-sialyllactose, and 6'-sialyllactose and ii) a probiotic strain of *B. longum* subsp. *infantis*, for use in treating or preventing hyperammonemia.

[0074] In particular [embodimentexamples](#), provided herein is a composition or formulation comprising i) a prebiotic mixture comprising 2'-fucosyllactose, 3-fucosyllactose, and difucosyllactose and ii) a probiotic strain of *B. longum* subsp. *infantis*, for use in treating or preventing hyperammonemia.

## BRIEF DESCRIPTION OF THE DRAWINGS

[0075] **FIG. 1** provides a graph displaying optical density measurements at 600 nm (OD600) collected at various time points in cultures of *B. longum* subsp. *infantis* (BI), stool microbiota (Stool), or both (BI/Stool), incubated in the presence of a mixture of HMOs (HMO). Controls include uninoculated cultures (uninoculated) and/or cultures that were incubated in the absence of a carbon source (No Sugar).

[0076] **FIG. 2** provides a graph displaying results of qPCR targeting *B. longum* subsp. *infantis* at various time points for similar culture conditions as described in FIG. 1. The Limit of detection (LOD) was 502 copies/mL culture.

[0077] **FIGS. 3A and 3B** show graphs displaying anaerobic colony forming unit (CFU) counts following a three-hour incubation of *B. longum* subsp. *infantis* with various acid reducing compounds at concentrations of 1 µg/mL (**FIG. 3A**) or 0.5 µg/mL (**FIG. 3B**) and prior to (Buffer Only T0) or after (Buffer Only T180) a three-hour incubation in buffer alone.

[0078] **FIGS. 4A-4H** show graphs displaying results of OD600 measurements (**FIGS. 4A-4D**) or qPCR targeting *B. longum* subsp. *infantis* following a 24-hour incubation

with various acid reducing compounds at concentrations of 500 µg/mL (FIGS. 4A and 4E), 250 µg/mL (FIGS. 4B and 4F), 125 µg/mL (FIGS. 4C and 4G), 62.5µg/mL (FIGS. 4D and 4H) or with vehicle controls.

**[0079]** **FIG. 5** shows a graph displaying results of qPCR targeting *B. longum* subsp. *infantis* from fecal pellets of individual germ-free mice at various timepoints. Experimental groups of germ free mice included germ free mice that received FMT from the stool of a healthy human adult donor and treatment with HMO (open circles); germ free mice that received FMT from the stool of a healthy human adult donor and treatment with *B. longum* subsp. *infantis* and HMO (solid triangles); germ free mice that received FMT from the stool of a healthy human infant donor and treatment with *B. longum* subsp. *infantis* and HMO (open diamonds); and germ free mice received treatment with *B. longum* subsp. *infantis* and HMO but not FMT (solid squares).

~~**[0080]**—**FIGS. 6A and 6B** show a summary of the experimental design (**FIG. 6A**) and experimental results of qPCR targeting *E. Faecium* (**FIG. 6B**). As shown in **FIG. 6A**, mice were treated with vehicle or ampicillin from days 7 to 1; inoculated with *E. faecium* or vehicle on day 0; administered *B. longum* subsp. *infantis* or vehicle on days 0-7, and administered HMO or vehicle on days 0-13. **FIG. 6B** shows results of qPCR targeting *E. faecium* from stool from individual mice collected daily between days 0 and 14. Results are shown for mice that were inoculated with *E. faecium* but were not administered ampicillin, HMO, or *B. longum* subsp. *infantis* (solid circles); mice that were administered ampicillin and inoculated with *E. faecium* (open squares); mice that were administered ampicillin and *B. longum* subsp. *infantis* and inoculated with *E. faecium* (solid triangles); and mice that were administered ampicillin, HMO, and *B. longum* subsp. *infantis* and inoculated with *E. faecium* (open inverted triangles).~~

## DETAILED DESCRIPTION

~~**[0081]**~~**[0080]** Provided herein are compositions, kits, and articles of manufacture as well as methods of use thereof. In certain embodiments, the provided compositions, kits, and articles of manufacture contain one or more prebiotics, *e.g.*, non-digestible carbohydrates such as human milk oligosaccharides, and at least one probiotic strain, *e.g.*, a Bifidobacterium such as *B. longum* subsp. *infantis*, capable of consuming the one or more prebiotic.

**[0081]** In certain aspects, the provided compositions, kits, and articles are manufacture are particularly useful in the treatment or prevention of diseases or conditions associated with inflammation,

suppositories or retention enemas, using, *e.g.*, conventional suppository bases such as cocoa butter or other glycerides. The compositions may be suspensions, solutions, or emulsions in oily or aqueous vehicles, and may contain suspending, stabilizing and/or dispersing agents.

**[0390]** In some embodiments, disclosed herein are pharmaceutically acceptable compositions continuing one or both of the probiotic strain and prebiotic mixture in single dosage forms. Single dosage forms may be in a liquid or a solid form. Single dosage forms may be administered directly to a subject without modification or may be diluted or reconstituted prior to administration. In certain embodiments, a single dosage form may be administered in bolus form, *e.g.*, single injection, single oral dose, including an oral dose that comprises multiple tablets, capsule, pills, etc. In alternate embodiments, a single dosage form may be administered over a period of time, *e.g.*, by infusion.

**[0391]** Single dosage forms of the pharmaceutical composition containing one or both of the probiotic strain and prebiotic mixture may be prepared by portioning the pharmaceutical composition into smaller aliquots, single dose containers, single dose liquid forms, or single dose solid forms, such as tablets, granulates, nanoparticles, nanocapsules, microcapsules, microtablets, pellets, or powders, which may be enterically coated or uncoated. A single dose in a solid form may be reconstituted by adding liquid, typically sterile water or saline solution, prior to administration to a subject.

**[0001][0391a]** In certain embodiments, the composition can be delivered in a controlled release or sustained release system. In one embodiment, a pump may be used to achieve controlled or sustained release. In another embodiment, polymeric materials can be used to achieve controlled or sustained release of the therapies of the present disclosure (see, *e.g.*, U.S. Pat. No. 5,989,463). Examples of polymers used in sustained release formulations include, but are not limited to, poly((2-hydroxy ethyl methacrylate), poly(methyl methacrylate), poly(acrylic acid), poly(ethylene-co-vinyl acetate), poly(methacrylic acid), polyglycolides (PLG), polyanhydrides, poly(N-vinyl pyrrolidone), poly(vinyl alcohol), poly(ethylene glycol), polylactides (PLA), poly(lactide-co-glycolides) (PLGA), and polyorthoesters. The polymer used in a sustained release formulation may be inert, free of leachable impurities, stable on storage, sterile, and biodegradable. In some embodiments, a controlled or sustained release system can be placed in proximity of the prophylactic or therapeutic target, thus requiring only a fraction of the systemic dose. Any suitable technique known to one of skill in the art may be used.

2° C. and 8° C. and administered within 1 hour, within 3 hours, within 5 hours, within 6 hours, within 12 hours, within 24 hours, within 48 hours, within 72 hours, or within one week after being reconstituted. Cryoprotectants can be included for a lyophilized dosage form, principally 0-10% sucrose (optimally 0.5-1.0%). Other suitable cryoprotectants include trehalose and lactose. Other suitable bulking agents include polydextrose, dextrans (*e.g.*, maltodextrin (*e.g.*, a native maltodextrin or a resistant maltodextrin)), inulin,  $\beta$ -glucan, resistant starches (*e.g.*, resistant maltodextrin), hydrocolloids (*e.g.*, one or more of gum Arabic, pectin, guar gum, alginate, carrageenan, xanthan gum and cellulose gum), corn syrup solids and the like and polysorbate 80.-. Additional surfactants include but are not limited to polysorbate 20 and BRIJ surfactants. The pharmaceutical composition may be prepared as an injectable solution and can further comprise an agent useful as an adjuvant, such as those used to increase absorption or dispersion, *e.g.*, hyaluronidase.

[0395] In some embodiments, the pharmaceutical compositions, *e.g.*, containing one or both of the probiotic strain and prebiotic mixtures are administered with food. In alternate embodiments, the pharmaceutical composition is administered before or after eating food. The pharmaceutical compositions may be administered in combination with one or more dietary modifications, *e.g.*, low-protein diet and amino acid supplementation. The dosage of the pharmaceutical compositions and the frequency of administration may be selected based on the severity of the symptoms and the progression of the disorder. The appropriate therapeutically effective dose and/or frequency of administration can be selected by a treating clinician.

#### **IV. DEFINITIONS**

[0396] Unless defined otherwise, all terms of art, notations and other technical and scientific terms or terminology used herein are intended to have the same meaning as is commonly understood by one of ordinary skill in the art to which the claimed subject matter pertains. In some cases, terms with commonly understood meanings are defined herein for clarity and/or for ready reference, and the inclusion of such definitions herein should not necessarily be construed to represent a substantial difference over what is generally understood in the art.

[0397] As used herein, the singular forms "a," "an," and "the" include plural referents unless the context clearly dictates otherwise. For example, "a" or "an" means "at least one" or

“internalization of an oligosaccharide” refers to the internalization of the intact oligosaccharide.

## **VI. EXAMPLES**

[0414] The following examples are included for illustrative purposes only and are not intended to limit the scope of the invention.

### **EXAMPLE 1: MANUFACTURE OF A CONCENTRATED HMO MIXTURE**

[0415] Human milk from previously screened and approved donors was tested to verify donor identity and then mixed together to generate a pooled bulk of donor milk. In a clean room environment, the pool of donor milk was further tested, including for specific pathogens and bovine proteins. After testing, the pooled donor milk was filtered through a 200 µm filter, heated to a temperature of at least 63°C for 30 minutes, and then cooled to between 22°C and 26°C. The pooled human milk was then transferred to a centrifuge to separate the cream from the skim. The resulting skim milk was processed through an ultra-filtration system with a 10 kDa membrane, and the material that was passed through the filter was collected as the permeate fraction. The permeate was frozen and stored at approximately -20°C. Each permeate lot was tested to confirm quality parameters, including a minimal HMO concentration of approximately 0.2 to 0.4 g/L total HMOs, prior to its release for further processing.

[0416] Multiple qualified lots of the permeate were thawed and pooled. The pH of the pooled permeate was adjusted to a target pH of  $4.5 \pm 0.2$ . The permeate was then heated to approximately 50°C. Lactase enzyme was added to the permeate at a 0.1% w/w concentration and incubated at approximately 50°C for 60 minutes. The permeate and lactase enzyme mixture then was cooled to between 20°C and 30°C and clarified by depth filtration (Filtrox CH113P). The resulting depth filter filtrate was processed through an ultra-filtration skid (Biomax-10K membrane) to remove the lactase. The ultra-filtered permeate was then concentrated by nanofiltration using membranes with an estimated 400 to 500 Dalton molecular weight cut-off (GE G-5 UF). The concentrated HMO composition was then pasteurized and clarified through 0.2 µm sterile filters. This final HMO composition was then filled into containers and stored at  $\leq -20^\circ\text{C}$ . The final concentrations of HMO were targeted to between 84.5 to 105.4 g/L and quantified using high performance anion exchange

**[0454]** Beginning three days after the FMT (day 0), mice were administered doses of  $1 \times 10^8$  colony forming units (CFU) of *B. longum* subsp. *infantis* and 20 mg of total HMO from an HMO mixture (prepared as described in Example 1) twice daily for three days (day 0 to day 2). A group of germ free mice that did not receive an FMT also received treatment with *B. longum* subsp. *infantis* and HMO. A negative control group consisted of mice that received FMT from a healthy human adult donor but received a PBS vehicle in place of *B. longum* subsp. *infantis* and HMO. Fecal pellets were collected from mice in all experimental groups the day of the FMT (day -3) and then daily beginning on day 0 through day 5. DNA was isolated from the fecal pellets, and levels of *B. longum* subsp. *infantis* were assessed by qPCR performed similar to as described in Example 6.

**[0455]** As shown in FIG. 5, *B. longum* subsp. *infantis* was detected in fecal pellets from mice inoculated with *B. longum* subsp. *infantis* but not in stool collected from mice in the negative control group. Consistent with colonization of *B. longum* subsp. *infantis*, this detection persisted to day 5, three days after the final *B. longum* subsp. *infantis* and HMO treatment was administered. Higher levels of *B. longum* subsp. *infantis* were detected in stool from the monocolonized mice (germ free mice that received *B. longum* subsp. *infantis* but not an FMT) and mice that received FMT from healthy infant donors than in mice that received an FMT from healthy adult donors. This trend is consistent with general observations that probiotic strains typically have greater expansion within microbiomes having lower microbial diversity (such as infant or dysbiotic microbiomes) than in healthy adult microbiomes which typically have high microbial diversity.

#### **~~EXAMPLE 10: ADMINISTRATION *B. LONGUM* SUBSP. *INFANTIS* AND HMO TO MICE INOCULATED WITH A STRAIN OF VRE~~**

**~~[0456]~~** ~~Experiments were performed as depicted in FIG. 6A. C57Bl6 mice were treated with ampicillin for 8 days beginning at day -7. At day 0, mice were administered an inoculum of  $3.7 \times 10^7$  CFU *E. Faecium*, a strain of vancomycin resistant *Enterococcus* (VRE) by oral gavage. In addition, mice received either a dose of  $1 \times 10^8$  colony forming units (CFU) of *B. longum* subsp. *infantis* or a PBS vehicle twice daily by oral gavage on days 0-7. Mice also received a 20 mg dose of HMOs prepared as described in Example 1 or a PBS vehicle twice daily by oral gavage on days 0-13. Fecal pellets were removed from cages daily up to day 14 and processed for DNA isolation. DNA was assessed for the presence of *E. faecium* by qPCR similar to as described in Ubeda et al, *J Clin Invest.* 2010;120(12):4332-~~

4341. Control groups included mice inoculated with *E. faecium* that did not receive doses of an antibiotic, *B. longum* subsp. *infantis*, or HMO, as well as groups that received different combinations of antibiotics, HMO, or vehicle, with no *E. faecium* inoculation.

[0457] As shown in FIG. 6B., *E. faecium* could be detected in fecal pellets following inoculation at day 0, consistent with colonization of *E. faecium* within the intestinal microbiome. No *E. faecium* was detected by qPCR in controls that were not inoculated with *E. faecium*. Detection of *E. faecium* persisted to day 14 in fecal pellets from mice that received vehicle-only treatment or *B. longum* subsp. *infantis* treatment without HMO following antibiotic treatment and *E. faecium* inoculation, with highest *E. faecium* copy number observed in fecal pellets collected from the vehicle-only group. Lower *E. Faecium* copy numbers were observed in pellets from mice treated with *B. longum* subsp. *infantis* and HMO, and strikingly, no *E. faecium* could be detected after day 5. These results are consistent with an elimination of *E. faecium* colonization by treatment with *B. longum* subsp. *infantis* and HMO.

## **VII. SEQUENCES**

| SEQ ID NO: | Description                                                                                   |
|------------|-----------------------------------------------------------------------------------------------|
| 1          | <i>B. longum</i> subsp. <i>infantis</i> partial 16S sequence - GenBank: LC071820.1            |
| 2          | <i>B. longum</i> subsp. <i>infantis</i> partial 16S sequence - GenBank: KP326372.1            |
| 3          | <i>B. longum</i> subsp. <i>infantis</i> partial 16S sequence - AB116305.1                     |
| 4          | <i>B. longum</i> subsp. <i>infantis</i> partial 16S sequence - GenBank: X70974.1              |
| 5          | <i>B. longum</i> subsp. <i>infantis</i> partial 16S sequence - GenBank: D86184.1              |
| 6          | <i>B. longum</i> subsp. <i>infantis</i> partial 16S sequence - GenBank: AB507102.1            |
| 7          | <i>B. longum</i> subsp. <i>infantis</i> strain FC13644 16S, partial sequence- MK962475.1      |
| 8          | <i>B. adolescentis</i> 16S sequence - NCBI Reference Sequence: NR_074802.2                    |
| 9          | <i>B. animalis</i> subsp. <i>animalis</i> 16S sequence - NCBI Reference Sequence: NR_119007.1 |
| 10         | <i>B. animalis</i> subsp. <i>lactis</i> partial 16S sequence - GenBank: KU821112              |
| 11         | <i>B. bifidum</i> partial 16S sequence                                                        |
| 12         | <i>B. breve</i> partial 16S sequence - NCBI Reference Sequence: NR_040783.1                   |
| 13         | <i>B. catenulatum</i> partial 16S sequence                                                    |
| 14         | <i>B. longum</i> partial 16S sequence - GenBank: LC306854.1                                   |
| 15         | <i>B. pseudocatanulatum</i> partial 16S sequence                                              |
| 16         | <i>B. pseudolongum</i> partial 16S sequence - GenBank: M58742.1                               |
| 17         | <i>L. acidophilus</i> partial 16S sequence - NCBI Reference Sequence: NR_043182.1             |
| 18         | <i>L. antri</i> partial 16S sequence - NCBI Reference Sequence: NR_027206.1                   |
| 19         | <i>L. brevis</i> partial 16S sequence - NCBI Reference Sequence: NR_044704.2                  |
| 20         | <i>L. casei</i> partial 16S sequence - NR_041893.1                                            |
| 21         | <i>L. coleohominis</i> partial 16S sequence - NCBI Reference Sequence: NR_042436.1            |
| 22         | <i>L. crispatus</i> partial 16S sequence - NCBI Reference Sequence: NR_041800.1               |
| 23         | <i>L. curvatus</i> partial 16S sequence                                                       |
| 24         | <i>L. delbrueckii</i> partial 16S sequence - NCBI Reference Sequence: NR_029106.1             |

|    |                                                                                                 |
|----|-------------------------------------------------------------------------------------------------|
| 25 | <i>L. fermentum</i> partial 16S sequence - NCBI Reference Sequence: NR_104927.1                 |
| 26 | <i>L. gasseri</i> 16S sequence - NCBI Reference Sequence: NR_075051.2                           |
| 27 | <i>L. johnsonii</i> partial 16S sequence - NCBI Reference Sequence: NR_025273.1                 |
| 28 | <i>L. harbinensis</i> partial 16S sequence - NCBI Reference Sequence: NR_041263.1               |
| 29 | <i>L. mucosae</i> partial 16S sequence- NCBI Reference Sequence: NR_024994.1                    |
| 30 | <i>L. pentosus</i> partial 16S sequence                                                         |
| 31 | <i>L. plantarum</i> partial 16S sequence - NCBI Reference Sequence: NR_042254.1                 |
| 32 | <i>L. reuteri</i> partial 16S sequence - NCBI Reference Sequence: NR_025911.1                   |
| 33 | <i>L. rhamnosus</i> partial 16S sequence - NCBI Reference Sequence: NR_043408.1                 |
| 34 | <i>L. sakei</i> partial 16S sequence - NCBI Reference Sequence: NR_042443.1                     |
| 35 | <i>L. salivarius</i> partial 16S sequence - NCBI Reference Sequence: NR_028725.2                |
| 36 | <i>L. paracasei</i> partial 16S sequence - NCBI Reference Sequence: NR_025880.1                 |
| 37 | <i>L. kisonensis</i> partial 16S sequence - NCBI Reference Sequence: NR_041658.1                |
| 38 | <i>L. para-limentarius</i> partial 16S sequence - NCBI Reference Sequence: NR_025045.1          |
| 39 | <i>L. perolens</i> partial 16S sequence - NCBI Reference Sequence: NR_029360.1                  |
| 40 | <i>L. apis</i> partial 16S sequence - NCBI Reference Sequence: NR_125702.1                      |
| 41 | <i>L. ghanensis</i> partial 16S sequence - NCBI Reference Sequence: NR_043896.1                 |
| 42 | <i>L. dextrinicus</i> partial 16S sequence - NCBI Reference Sequence: NR_036861.1               |
| 43 | <i>Lactococcus lactis</i> Partial 16S Sequence - NCBI Reference Sequence: NR_040954.1           |
| 44 | <i>Bacteroides vulgatus</i> partial 16S sequence - NCBI Reference Sequence: NR_074515.1         |
| 45 | <i>Bacteroides fragilis</i> partial 16S sequence - NCBI Reference Sequence: NR_074784.2         |
| 46 | <i>Faecalibacterium prausnitzii</i> partial 16S sequence - NCBI Reference Sequence: NR_121697.2 |
| 47 | <i>E. rectale</i> partial 16S sequence - NCBI Reference Sequence: NR_074634.1                   |
| 48 | <i>S. thermophilus</i> partial 16S sequence - NCBI Reference Sequence: NR_042778.1              |

|    |                                                                                    |
|----|------------------------------------------------------------------------------------|
| 49 | P. parvulus partial 16S sequence - NCBI Reference Sequence: NR_029136.1            |
| 50 | P. lolii partial 16S sequence - NCBI Reference Sequence: NR_041640.1               |
| 51 | P. acidilactici partial 16S sequence - NCBI Reference Sequence: NR_041640.1        |
| 52 | P. argentinicus partial 16S sequence - NCBI Reference Sequence: NR_042623.1        |
| 53 | P. claussenii partial 16S sequence - NCBI Reference Sequence: NR_042232.1          |
| 54 | P. pentosaceus partial 16S sequence - NCBI Reference Sequence: NR_042058.1         |
| 55 | P. stilesii partial 16S sequence - NCBI Reference Sequence: NR_042401.1            |
| 56 | forward primer sequence                                                            |
| 57 | reverse primer sequence                                                            |
| 58 | probe sequence                                                                     |
| 59 | pksS; Polyketide_biosynthesis_cytochrome_P450_PksS - B. longum subsp. infantis     |
| 60 | higB2; Putative_toxin_HigB2 - B. longum subsp. infantis                            |
| 61 | putative Riboflavin biosynthesis gene- B. longum subsp. infantis                   |
| 62 | Endo-B-N-acetylglucosaminadase - B. longum subsp. infantis                         |
| 63 | HTH-type transcriptional activator RhaR - B. longum subsp. infantis                |
| 64 | Putative N-acetylmannosamine-6-phosphate 2-epimerase - B. longum subsp. infantis   |
| 65 | glucokinase - B. longum subsp. infantis                                            |
| 66 | Lactose Transport Protein - B. longum subsp. infantis                              |
| 67 | Sialidase gene - B. longum subsp. infantis                                         |
| 68 | L-fucose mutarotase - B. longum subsp. infantis                                    |
| 69 | Hypothetical gene - B. longum subsp. infantis                                      |
| 70 | ureC: Urease subunit alpha - B. longum subsp. infantis                             |
| 71 | LacG Lactose Transport System - B. longum subsp. infantis                          |
| 72 | putative multiple-sugar transport system permease YteP - B. longum subsp. infantis |
| 73 | por;PolyolNADP_oxidoreductase - B. longum subsp. infantis                          |
| 74 | UxaC: urinate - B. longum subsp. infantis                                          |

|    |                                                                                  |
|----|----------------------------------------------------------------------------------|
| 75 | epsK; putative_membrane_protein_EpsK - B. longum subsp. infantis                 |
| 76 | putative 2-dehydro-3-deoxy-D-pentonate aldolase YjhH - B. longum subsp. infantis |
| 77 | Fucosidase - B. longum subsp. infantis                                           |
| 78 | Hypothetical gene - B. longum subsp. infantis                                    |

## CLAIMS

1. ~~— A method for treating or preventing a disease, disorder, or condition associated with one or more of dysbiosis of the intestinal microbiome, inflammation, infection, allergy, or immune dysfunction in a subject in need thereof, the method comprising administering to the subject (i) a prebiotic mixture comprising one or more human milk oligosaccharides and (ii) at least one probiotic strain of bacterium capable of consuming the one or more human milk oligosaccharides.~~

2. ~~— The method of claim 1, wherein the probiotic strain is capable of internalizing the one or more human milk oligosaccharides.~~

3. ~~— The method of claims 1 or 2, wherein the probiotic strain of bacterium comprises a bacterial strain of the genus Bifidobacterium.~~

4. ~~— The method of any claims 1-3, wherein the probiotic strain of bacterium comprises a strain of *B. longum subsp. infantis*, *B. longum subsp. longum*, *B. breve*, or *B. bifidum*.~~

5. ~~— The method of any claims 1-4, wherein the probiotic strain of bacterium comprises *B. longum subsp. infantis*.~~

6. ~~— A method for treating or preventing a disease, disorder, or condition associated with one or more of dysbiosis of the intestinal microbiome, inflammation, infection, allergy, or immune dysfunction in a subject in need thereof, the method comprising administering to the subject (i) a prebiotic mixture comprising one or more human milk oligosaccharides and (ii) a probiotic strain of *B. longum subsp. infantis*.~~

7. ~~— The method of any of claims 1-6, wherein the prebiotic mixture comprises at least 5, at least 10, at least 25, at least 50, or at least 100 human milk oligosaccharides.~~

8. ~~— A method for treating or preventing a disease, disorder, or condition associated with one or more of dysbiosis of the intestinal microbiome, inflammation,~~

~~infection, allergy, or immune dysfunction in a subject in need thereof, the method comprising administering to the subject (i) a prebiotic mixture comprising at least 5, at least 10, at least 25, at least 50, or at least 100 human milk oligosaccharides and (ii) a probiotic strain of the genus *Bifidobacterium*.~~

~~9. — The method of any of claims 1-8, wherein the disease, disorder, or condition comprises one or more of obesity, type II diabetes, a chronic inflammatory disease, an autoimmune disease, an infection, an infectious disease domination, bowel resection, or a condition associated with chronic diarrhea.~~

~~10. — The method of any of claims 1-8, wherein the disease, disorder, or condition comprises one or more of irritable bowel syndrome (IBS), inflammatory bowel disease (IBD), short bowel syndrome (SBS), celiac disease, small intestinal bacterial overgrowth (SIBO), gastroenteritis, leaky gut syndrome, pouchitis, or gastric lymphoma.~~

~~11. — The method of any of claims 1-8, wherein the disease, condition, or disorder is graft versus host disease.~~

~~12. — The method of claim 11, wherein the subject has received or will receive an allogenic hematopoietic stem cell transplant.~~

~~13. — A method Use of (i) a prebiotic mixture comprising at least 5, at least 10, at least 25, at least 50, or at least 100 human milk oligosaccharides in the manufacture of a first medicament and use of (ii) a probiotic strain of *B. longum* subsp. *infantis* in the manufacture of a second medicament, wherein the first and second medicaments are to be used in combination therapy to preventing or reducing the incidence or severity of graft versus host disease in a subject in need thereof, wherein the subject has received or will receive an allogenic hematopoietic stem cell transplant, the method comprising administering to the subject (i) a prebiotic mixture comprising at least 5, at least 10, at least 25, at least 50, or at least 100 human milk oligosaccharides and (ii) a probiotic strain of *B. longum* subsp. *infantis*.~~

~~14. — The method of any of claims 1-8, wherein the disease, condition, or disorder is associated with an infection.~~

15. — ~~The method of claim 14, wherein the infection comprises a bacterial infection or gut domination.~~

16. — ~~A method for treating or preventing an infection in a subject in need thereof, the method comprising administering to the subject (i) a prebiotic mixture comprising at least 5, at least 10, at least 25, at least 50, or at least 100 human milk oligosaccharides and (ii) a probiotic strain of *B. longum* subsp. *infantis*, wherein the infection comprises a bacterial infection or gut domination.~~

17. — ~~The method of claim 15 or 16, wherein the bacterial infection or gut domination comprises an infection or gut domination by one or more species, subspecies, or strains of *Aeromonas*, *Bacillus*, *Blautia*, *Bordetella*, *Borrelia*, *Brucella*, *Burkholderia*, *Campylobacter*, *Chlamydia*, *Chlamydophila*, *Citrobacter*, *Clostridium*, *Corynebacterium*, *Coxiella*, *Ehrlichia*, *Enterobacter*, *Enterobacteriaceae*, *Enterococcus*, *Escherichia*, *Faecalicatena*, *Francisella*, *Haemophilus*, *Helicobacter*, *Hungatella*, *Klebsiella*, *Lachnospiraceae*, *Legionella*, *Leptospira*, *Listeria*, *Morganella*, *Mycobacterium*, *Mycoplasma*, *Neisseria*, *Orientia*, *Plesiomonas*, *Proteus*, *Pseudomonas*, *Rickettsia*, *Salmonella*, *Shigella*, *Staphylococcus*, *Streptococcus*, *Treponema*, *Vibrio*, or *Yersinia*, optionally one or more of *Aeromonas hydrophila*, *Bacillus cereus*, *Campylobacter fetus*, *Campylobacter jejuni*, *Clostridium botulinum*, *Clostridium difficile*, *Clostridium perfringens*, enteroaggregative *Escherichia coli*, enterohemorrhagic *Escherichia coli*, enteroinvasive *Escherichia coli*, enteropathogenic *E. coli*, enterotoxigenic *Escherichia coli*, *Escherichia coli* 0157:H7, *Helicobacter pylori*, *Klebsiella pneumonia*, *Listeria monocytogenes*, *Salmonella paratyphi*, *Salmonella typhi*, *Staphylococcus aureus*, *Vibrio cholerae*, *Vibrio parahaemolyticus*, *Vibrio vulnificus*, or *Yersinia enterocolitica*.~~

18. — ~~The method of any of claims 15-17, wherein the bacterial infection or gut domination comprises an infection or gut domination by one or more of *Anaerostipes cacciae*, *Anaerotruncus colihominis*, *Bifidobacterium longum*, *Blautia* spp., *Butyrivibrio*, *Butyrivibrio crossotus*, *Caproiciproducens*, *Citrobacter freundii*, *Citrobacter koseri*, *Clostridium aldenense*, *Clostridium asparagiforme*, *Clostridium bolteae*, *Clostridium citroniae*, *Clostridium clostridioforme*, *Clostridium lavalense*, *Clostridium nexile*, *Clostridium populeti*, *Clostridium symbiosum*, *Coprococcus comes*, *Coprococcus eutactus*,~~

~~*Dorea formicigenerans*, *Dorea longicatena*, *Enterobacter aerogenes*, *Enterobacter cloacae*, *Enterococcus faecalis*, *Enterococcus faecium*, *Escherichia coli*, *Ethanoligenens*, *Eubacterium hallii*, *Eubacterium rectale*, *Eubacterium ventriosum*, *Eubacterium siraeum*, *Faecalibacterium prausnitzii*, *Faecalicatena*, *Flavonifractor plautii*, *Hungatella*, *Klebsiella oxytoca*, *Klebsiella pneumoniae*, *Lactobacillus acidophilus*, *Morganella morganii*, *Oscillibacter*, *Papillibacter cinnamivorans*, *Papillibacter*, *Proteus mirabilis*, *Pseudoflavonifractor*, *Roseburia hominis*, *Roseburia intestinalis*, *Roseburia inulinivorans*, *Ruminococcus faecis*, *Ruminococcus lactaris*, *Ruminococcus torques*, *Ruminococcus bromii*, *Serratia marcescens*, *Sporobacter*, *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Streptococcus anginosus*, *Streptococcus australis*, *Streptococcus constellatus*, *Streptococcus cristatus*, *Streptococcus gordonii*, *Streptococcus infantis*, *Streptococcus intermedius*, *Streptococcus mitis*, *Streptococcus mutans*, *Streptococcus oligofermentans*, *Streptococcus oralis*, *Streptococcus parasanguinis*, *Streptococcus peroris*, *Streptococcus pneumoniae*, *Streptococcus pseudopneumoniae*, *Streptococcus salivarius*, *Streptococcus sanguinis*, *Streptococcus sobrinus*, *Streptococcus tigurinus*, *Streptococcus vestibularis*, *Subdoligranulum variable*, *Subdoligranulum*, or *Syntrophococcus* sp.~~

19. — ~~The method of any of claims 15-18, wherein the bacterial infection or gut domination comprises an infection or gut domination by drug-resistant bacteria.~~

20. — ~~The method of any of claims 15-19, wherein the drug-resistant bacteria comprises one or more of antibiotic-resistant bacterium (ARB), Antibiotic-resistant Proteobacteria, Carbapenem-resistant *Enterobacteriaceae* (CRE), Extended Spectrum Beta-Lactamase producing *Enterobacteriaceae* (ESBL-E), fluoroquinolone-resistant *Enterobacteriaceae*, extended-spectrum beta-lactam-resistant *Enterococci* (ESBL), vancomycin-resistant *Enterococci* (VRE), multi-drug-resistant *E. coli*, or multi-drug-resistant *Klebsiella*.~~

21. — ~~The method of any of claims 1-8, wherein the subject has undergone or will undergo an ileal pouch-anal anastomosis (IPAA) surgery, and wherein the disease, condition, or disorder comprises pouchitis.~~

~~22. — A method of preventing or reducing the incidence or severity of pouchitis in a subject in need thereof, wherein the subject has undergone or will undergo an ileal pouch-anal anastomosis (IPAA) surgery, the method comprising administering to the subject (i) a prebiotic mixture comprising at least 5, at least 10, at least 25, at least 50, or at least 100 human milk oligosaccharides and (ii) a probiotic strain of *B. longum* subsp. *infantis*, wherein the infection comprises a bacterial infection.~~

~~232.~~ The ~~method-use~~ of ~~any of~~ claims 1-~~22~~, wherein the prebiotic mixture comprises at least 10 human milk oligosaccharides.

~~243.~~ The ~~method-use~~ of any of claims 1-~~232~~, wherein the prebiotic mixture comprises at least 25 human milk oligosaccharides.

~~254.~~ The ~~method-use~~ of any of claims 1-~~243~~, wherein the prebiotic mixture comprises at least 50 human milk oligosaccharides.

~~265.~~ The ~~method-use~~ of any of claims 1-~~254~~, wherein the prebiotic mixture comprises at least 50 human milk oligosaccharides.

~~276.~~ The ~~method-use~~ of any of claims 1-~~265~~, wherein the prebiotic mixture comprises one or more of 2'-fucosyl-lactose, 3'-fucosyl-lactose, 3'-sialyl-lactose, 6'-sialyl-lactose, Lacto-N-tetraose, lacto-N-difucohexaose I, lactodifucotetraose, Lacto-N-fucopentaose I, sialyl-lacto-N-tetraose c, sialyl-lacto-N-tetraose b, or disialyllacto-N-tetraose.

~~287.~~ The ~~method-use~~ of any of claims 1-~~276~~, wherein the prebiotic mixture comprises 2'-fucosyl-lactose, 3'-fucosyl-lactose, 3'-sialyl-lactose, 6'-sialyl-lactose, Lacto-N-tetraose, lacto-N-difucohexaose I, lactodifucotetraose, Lacto-N-fucopentaose I, sialyl-lacto-N-tetraose c, sialyl-lacto-N-tetraose b, and disialyllacto-N-tetraose.

~~298.~~ The ~~method-use~~ of any of claims 1-~~287~~, wherein the prebiotic mixture comprises 2'-fucosyl-lactose, 3'-fucosyl-lactose, 3'-sialyl-lactose, 6'-sialyl-lactose, Lacto-N-tetraose, Lacto-N-neo-tetraose, Lacto-N-fucopentaose I, Lacto-N-fucopentaose II, Lacto-N-fucopentaose III, Sialyl-lacto-N-tetraose b, Sialyl-lacto-N-tetraose c, Lacto-N-difuco-hexaose

I, Lacto-N-difuco-hexaose II, Lacto-N-hexaose, para-Lacto-N-hexaose, Disialyllacto-N-tetraose, Fucosyl-Lacto-N-hexaose, Difucosyl-Lacto-N-hexaose a, and Difucosyl-Lacto-N-hexaose b.

309. The method-use of any of claims 1-298, wherein the prebiotic mixture is, is derived from, or comprises a concentrated permeate from pooled human milk, wherein the permeate is obtained from the ultrafiltration of human skim milk, wherein the human skim milk is obtained by removing cream from pooled human milk, and wherein the pooled human milk is pooled from the milk of multiple human milk donors.

~~31. — A method for treating or preventing a disease, disorder, or condition associated with one or more of dysbiosis of the intestinal microbiome, inflammation, infection, allergy, or immune dysfunction in a subject in need thereof, the method comprising administering to the subject~~

~~(i) a prebiotic mixture comprising at least 5, at least 10, at least 25, at least 50, or at least 100 human milk oligosaccharides, wherein the prebiotic mixture is, is derived from, or comprises a concentrated human milk permeate, wherein the human milk permeate is obtained from the ultrafiltration of human skim milk obtained from pooled human milk, and wherein the pooled human milk is pooled from the milk of multiple human milk donors; and~~

~~(ii) a probiotic strain of *B. longum* subsp. *infantis*.~~

~~32. — The method of claim 30 or 31, wherein the pooled human milk is pooled from the milk of at least 25, 50, or 100 human milk donors.~~

~~33. — The method of any of claims 1-32, wherein the prebiotic mixture comprises at least one synthetic human milk oligosaccharide.~~

~~34. — The method of any claims 1-33, wherein the prebiotic mixture and the probiotic strain are administered orally.~~

3510. The method-use of any of claims 1-349, wherein the prebiotic mixture and the probiotic strain are to be administered on the same day for at least one day.

~~3611~~. The ~~method-use~~ of any of claims 1-~~3510~~, wherein the prebiotic mixture and the probiotic strain are to be administered on the same day for at least three consecutive days.

~~3712~~. The ~~method-use~~ of any of claims 1-~~3611~~, wherein the prebiotic mixture and the probiotic strain are to be administered on the same day for at least seven consecutive days.

~~3813~~. The ~~method-use~~ of any of claims 1-~~3712~~, wherein the ~~method-use~~ comprises at least a first treatment phase and a second treatment phase, wherein in the first treatment phase the prebiotic mixture and the probiotic strain are ~~comprises administering to be administered~~ to the subject ~~the prebiotic mixture and the probiotic strain~~ on the same day for at least one day, and wherein the second treatment phase begins after the end of the first treatment phase, optionally immediately after, and the prebiotic mixture ~~comprises administering is to be administered~~ the prebiotic mixture for at least one day, wherein the probiotic strain is not to be administered during the second treatment phase.

~~3914~~. The ~~method-use~~ of claim ~~3813~~, wherein in the second treatment phase the prebiotic mixture ~~comprises administering is to be administered~~ to the subject ~~the prebiotic mixture~~ each day for at least three consecutive days.

~~4015~~. The ~~method-use~~ of claim ~~38-13~~ or ~~3914~~, wherein in the second treatment phase the prebiotic mixture ~~comprises administering is to be administered~~ to the subject ~~the prebiotic mixture~~ each day for at least seven consecutive days.

~~4116~~. The ~~method-use~~ of any of claims ~~3813-4015~~, wherein the first treatment phase is between 3 and 14 days in length and wherein the second treatment phase is between 3 and 14 days in length.

~~4217~~. The ~~method-use~~ of any of claims ~~3813-4116~~, wherein the first treatment phase is about 7 days in length and wherein the second treatment phase is about 7 days in length.

~~4318~~. The ~~method-use~~ of any of claims 1-~~4217~~, wherein the probiotic strain is to be administered in an amount of at least  $5 \times 10^6$  colony forming units (CFU) per day.

4419. The ~~method-use~~ of any of claims 1-~~4318~~, wherein the probiotic strain is to be administered in an amount of at least  $8 \times 10^7$  colony forming units (CFU) per day.

4520. The ~~method-use~~ of any of claims 1-~~4419~~, wherein the prebiotic mixture is to be administered in an amount of at least 500 mg of total human milk oligosaccharides per day.

4621. The ~~method-use~~ of any of claims 1-~~4520~~, wherein the prebiotic mixture is to be administered in an amount of between 0.5 g and 25 g, 1 g and 5 g, 2 g and 3 g, 3 g and 6 g, 4 g and 5 g, 5 g and 10 g, 8 g and 10 g, 10 g and 20 g, 15 g and 25 g, 15 g and 20 g, or 17 g and 19 g of total human milk oligosaccharides per day.

4722. The ~~method-use~~ of any of claims 1-~~4621~~, wherein the prebiotic mixture and/or the probiotic strain is to be administered with an enteric coating.

4823. The ~~method-use~~ of any of claims 1-~~4722~~, ~~further comprising administering wherein~~ a histamine-2 receptor antagonist is to be administered, wherein the histamine-2 receptor antagonist is to be administered at least 1 hour prior to administration of the probiotic strain, optionally wherein the histamine-2 receptor antagonist is famotidine.

4924. The ~~method-use~~ of any of claims 1-~~4722~~, wherein the subject has not been administered a proton pump inhibitor at least about 6 hours, 12 hours, 24 hours, 36 hours, 48 hours, 60 hours, 2 days, 3 days, 4 days, 5 days, 7 days, 10 days, 2 weeks, 3 weeks, or 4 weeks prior to administration of the probiotic strain.

5025. The ~~method-use~~ of any of claims 1-~~4722~~, wherein the subject has not been administered a benzimidazole derivative for at least about 6 hours, 12 hours, 24 hours, 36 hours, 48 hours, 60 hours, 2 days, 3 days, 4 days, 5 days, 7 days, 10 days, 2 weeks, 3 weeks, or 4 weeks prior to administration of the probiotic strain.

5126. The ~~method-use~~ of any of claims 1-~~4823~~, wherein the subject has not been administered omeprazole for at least about 6 hours, 12 hours, 24 hours, 36 hours, 48 hours,

60 hours, 2 days, 3 days, 4 days, 5 days, 7 days, 10 days, 2 weeks, 3 weeks, or 4 weeks prior to administration of the probiotic strain.

~~5227.~~ The ~~method-use~~ of any of claims 1-~~5126~~, wherein both of the probiotic strain and the prebiotic mixture are free or essentially free of a benzimidazole derivative.

~~5328.~~ The ~~method-use~~ of any of claims 1-~~5227~~, wherein the subject is a human subject at least 6 months, 12 months, 2 years, 5 years, 12 years, or 18 years of age.

~~5429.~~ The ~~method-use~~ of any of claims 1-~~5328~~, wherein the subject is human subject that is a child, an adolescent, or an adult.

~~5530.~~ The ~~method-use~~ of any of claims 1-~~5429~~, wherein the subject is a human adult.

~~56. — Use of a prebiotic mixture and at least one probiotic strain for the treatment or prevention of a disease, disorder, or condition associated with one or more of dysbiosis of the intestinal microbiome, inflammation, infection, allergy, or immune dysfunction in a subject in need thereof, wherein the prebiotic mixture comprises one or more human milk oligosaccharides and the probiotic strain is capable of consuming the one or more human milk oligosaccharides.~~

~~57. — A prebiotic mixture and at least one probiotic strain for use in the manufacture of medicaments for the treatment or prevention of a disease, disorder, or condition associated with one or more of dysbiosis of the intestinal microbiome, inflammation, infection, allergy, or immune dysfunction, wherein the prebiotic mixture comprises one or more human milk oligosaccharides and wherein the probiotic strain is capable of consuming the one or more human milk oligosaccharides.~~

~~5831.~~ A kit comprising at least one probiotic strain of bacterium and a prebiotic mixture comprising one or more human milk oligosaccharides, wherein probiotic strain is capable of internalizing and consuming the one or more human milk oligosaccharides.

~~5932~~. The kit of claim ~~5831~~, wherein the probiotic strain comprises a strain of Bifidobacterium.

~~6033~~. The kit of claim ~~58-31~~ or claim ~~5932~~, wherein the probiotic strain comprises a strain of *B. longum* subsp. *infantis*, *B. longum* subsp. *longum*, *B. breve*, or *B. bifidum*.

~~6134~~. The kit of any of claims ~~5831-6033~~, wherein the prebiotic mixture comprises at least 2, at least 5, at least 10, at least 25, at least 50, at least 100, at least 125, or at least 150 human milk oligosaccharides.

~~6235~~. The kit of any of claims ~~5831-6134~~, wherein the prebiotic mixture comprises at least 25 human milk oligosaccharides.

~~6336~~. The kit of any of claims ~~5831-6235~~, wherein the prebiotic mixture is free or essentially free of oligosaccharides that are not human milk oligosaccharides.

~~6437~~. The kit of any of claims ~~5831-6336~~, wherein the prebiotic mixture is, is obtained from, or comprises a concentrated ultra-filtered permeate from pooled human milk.

~~6538~~. The kit of any of claims ~~5831-6437~~, wherein the probiotic strain is incorporated into one or more individual probiotic doses and wherein the prebiotic mixture is incorporated into one or more individual prebiotic doses.

~~6639~~. The kit of claim ~~6538~~, wherein the one or more individual probiotic doses each comprise at least  $1 \times 10^3$  colony forming units (CFU).

~~6740~~. The kit of claim ~~65-38~~ or ~~6639~~, wherein the one or more individual probiotic doses each comprise at least  $8 \times 10^7$  colony forming units (CFU).

~~6841~~. The kit of any of claims ~~6538-6740~~, wherein the one or more prebiotic doses comprises between 500 mg and 25 g of total human milk oligosaccharides.

~~6942.~~ The kit of any of claims ~~6538-6841~~, wherein the one or more individual prebiotic doses comprises between 0.5 g and 25 g, 1 g and 5 g, 2 g and 3 g, 3 g and 6 g, 4 g and 5 g, 5 g and 10 g, 8 g and 10 g, 10 g and 20 g, 15 g and 25 g, 15 g and 20 g, or 17 g and 19 g of total human milk oligosaccharides per day.

~~7043.~~ The kit of any of claims ~~6538-6942~~, wherein the one or more individual prebiotic doses comprises less than 0.1% lactose.

~~7144.~~ An article of manufacture, comprising the kit of any of claims ~~5831-7043~~ and instructions for use, wherein the instructions for use describing the ~~method-use~~ of any of claims 1-~~5530~~.

~~72.—A method of treating hyperammonemia, comprising administering to a subject in need thereof a prebiotic mixture comprising oligosaccharides, wherein (i) the mixture is free or essentially free of oligosaccharides that incorporate an N-acetyl-glucosamine residue or (ii) the percentage by weight of oligosaccharides comprising nitrogen in the mixture is less than 50%.~~

~~73.—A method of decreasing the level or amount of ammonia in a subject in need thereof, comprising administering to the subject a prebiotic mixture comprising oligosaccharides, wherein (i) the mixture is free or essentially free of oligosaccharides that incorporate an N-acetyl-glucosamine residue or (ii) the percentage by weight of oligosaccharides comprising nitrogen in the mixture is less than 50%.~~

~~74.—The method of claim 72 or 73, further comprising administering a probiotic strain of bacterium capable of consuming each oligosaccharide of the mixture.~~

~~75.—The method of any of claims 72-74, wherein the subject has, is at risk of having, or is suspected of having hyperammonemia, hepatic encephalopathy, or a urea cycle disorder.~~

~~76.—The method of any of claims 72-75, wherein the prebiotic mixture comprises one or more human milk oligosaccharides (HMOs)~~

~~77. — The method of any of claims 72-76, wherein all or essentially all of the oligosaccharides of the prebiotic mixture are human milk oligosaccharides (HMOs).~~

~~78. — The method of any of claims 72-77, wherein the prebiotic mixture is free or essentially free of oligosaccharides that incorporate an N-acetyl glucosamine residue.~~

~~79. — The method of any of claims 72-78, wherein the prebiotic mixture comprises at least 2, at least 3, at least 4, at least 5, at least 6, or at least 7 oligosaccharides.~~

~~80. — The method of any of claims 72-79, wherein the percentage by weight of oligosaccharides in the prebiotic mixture comprising nitrogen is less than 50%, 40%, 30%, 25%, 20%, 10%, 5%, or 1%.~~

~~81. — The method of any of claims 72-80, wherein the mixture is free or essentially free of oligosaccharides that comprise nitrogen.~~

~~82. — The method of any of claims 72-81, wherein less than 50%, 40%, 30%, 25%, 20%, 10%, 5%, or 1% by weight of the oligosaccharides in the prebiotic mixture are oligosaccharides comprising five or more monosaccharide residues.~~

~~83. — The method of any of claims 72-82, wherein the prebiotic mixture is free or essentially free of oligosaccharides comprising five or more monosaccharide residues.~~

~~84. — The method of any of claims 72-83, wherein the probiotic strain is a strain of Bifidobacterium, optionally a strain of *B. longum* subsp. *infantis*, *B. longum* subsp. *longum*, *B. breve*, or *B. bifidum*.~~

~~85. — The method of any of claims 72-84, wherein the probiotic strain is capable of internalizing the oligosaccharides of the mixture.~~

~~86. — A method of treating or preventing hyperammonemia, comprising administering to a subject in need thereof (i) a prebiotic mixture comprising oligosaccharides, wherein all or essentially all of the oligosaccharides of the prebiotic mixture are human milk~~

oligosaccharides that do not incorporate an N-acetyl-glucosamine residue, and (ii) a probiotic strain of bacterium, wherein the probiotic strain is a *Bifidobacterium* capable of internalizing HMOs.

87. — A method of reducing ammonia in a subject in need thereof, comprising administering to a subject (i) a probiotic mixture of oligosaccharides, wherein all or essentially all of the oligosaccharides of the probiotic mixture are human milk oligosaccharides (HMOs) that do not incorporate an N-acetyl-glucosamine residue, and (ii) a probiotic strain of bacterium, wherein the probiotic bacterium strain is a *Bifidobacterium* strain capable of internalizing HMOs.

88. — The method of any of claims 74-87, wherein the probiotic strain is a strain of *B. longum* subsp. *infantis*.

89. — The method of any of claims 72-88, wherein the probiotic mixture comprises one or more of 2'-fucosyllactose, 3-fucosyllactose, difucosyllactose, 3'-sialyllactose, or 6'-sialyllactose.

90. — The method of any of claims 72-88, wherein the probiotic mixture comprises 2'-fucosyllactose, 3-fucosyllactose, difucosyllactose, 3'-sialyllactose, and 6'-sialyllactose.

91. — The method of any of claims 72-88, wherein the oligosaccharides of the probiotic mixture consist or consist essentially of 2'-fucosyllactose, 3-fucosyllactose, difucosyllactose, 3'-sialyllactose, and 6'-sialyllactose.

92. — The method of any of claims 72-88, wherein the probiotic mixture comprises one or more of 2'-fucosyllactose, 3-fucosyllactose, or difucosyllactose.

93. — The method of any of claims 72-88, wherein the probiotic mixture comprises of 2'-fucosyllactose, 3-fucosyllactose, and difucosyllactose.

94. — ~~The method of any of claims 72-88, wherein the oligosaccharides of the mixture consist or consist essentially of 2' fucosyllactose, 3 fucosyllactose, and difucosyllactose.~~

95. — ~~A method of treating or preventing hyperammonemia comprising administering to a subject in need thereof i) a prebiotic mixture comprising 2' fucosyllactose, 3 fucosyllactose, difucosyllactose, 3' sialyllactose, and 6' sialyllactose and ii) a probiotic strain of *B. longum* subsp. *infantis*.~~

96. — ~~A method of treating or preventing hyperammonemia comprising administering to a subject in need thereof i) a prebiotic mixture comprising 2' fucosyllactose, 3 fucosyllactose, and difucosyllactose and ii) a probiotic strain of *B. longum* subsp. *infantis*~~

97. — ~~The method of any of claims 72-96, wherein the subject has, is suspected of having, or is at risk of having hepatic encephalopathy.~~

98. — ~~The method of any of claims 72-96, wherein the subject has, is suspected of having, or is at risk of having a urea cycle disorder.~~