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

[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 examples, 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 examples, the probiotic strain is capable of internalizing the one or more human milk oligosaccharides. In certain examples, the probiotic strain of bacterium comprises a bacterial strain of the genus Bifidobacterium. In some examples, the probiotic strain of bacterium comprises a strain of *B. longum subsp. infantis, B. longum subsp. longum*, *B. breve,* or *B. bifidum.* In particular examples, 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 examples, 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 examples, 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 examples, 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 examples, the disease, condition, or disorder is graft versus host disease.

[0011] In some examples, 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 examples, the disease, condition, or disorder is associated with an infection. In certain examples, 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 examples, 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, Klebsiellia pneumonia*, *Lysteria monocytogenes, Salmonella paratyphi, Salmonella typhi, Staphylococcus aureus*, *Vibrio cholerae, Vibrio parahaemolyticus, Vibrio vulnificus*, or *Yersinia enterocolitica*.

[0014] In particular examples, 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., Butyricicoccus 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 variabile, Subdoligranulum, or *Syntrophococcus sp.*.

[0015] In certain examples, the bacterial infection or gut domination comprises an infection or gut domination by drug-resistant bacteria. In some examples, 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 examples, 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 examples, the prebiotic mixture comprises at least 10 human milk oligosaccharides. In some examples, the prebiotic mixture comprises at least 25 human milk oligosaccharides. In particular examples, the prebiotic mixture comprises at least 50 human milk oligosaccharides. In certain examples, the prebiotic mixture comprises at least 50 human milk oligosaccharides.

[0018] In some examples, 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 examples, 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 examples, 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 examples, 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 examples, the pooled human milk is pooled from the milk of at least 25, 50, or 100 human milk donors. In certain examples, the prebiotic mixture comprises at least one synthetic human milk oligosaccharide. In some examples, the prebiotic mixture and the probiotic strain are administered orally. In particular examples, the prebiotic mixture and the probiotic strain are administered on the same day for at least one day. In certain examples, the prebiotic mixture and the probiotic strain are administered on the same day for at least three consecutive days. In some examples, the prebiotic mixture and the probiotic strain are administered on the same day for at least seven consecutive days.

[0022] In particular examples, 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 examples, the second treatment phase comprises administering to the subject the prebiotic mixture each day for at least three consecutive days. In some examples, the second treatment phase comprises administering to the subject the prebiotic mixture each day for at least seven consecutive days. In particular examples, 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 examples, the first treatment phase is about 7 days in length and wherein the second treatment phase is about 7 days in length. In some examples, the probiotic strain is administered in an amount of at least 5×10^6 colony forming units (CFU) per day. In particular examples, the probiotic strain is administered in an amount of at least 8×10^7 colony forming units (CFU) per day. In certain examples, the prebiotic mixture is administered in an amount of at least 500 mg of total human milk oligosaccharides per day. In some examples, 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 examples, the prebiotic mixture and/or the probiotic strain is administered with an enteric coating. In certain examples, 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 examples, 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 examples, 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 examples, 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 examples, both of the probiotic strain and the prebiotic mixture are free or essentially free of a benzimidazole derivative.

[0026] In particular examples, 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 examples, the subject is human subject that is a child, an adolescent, or an adult. In some examples, 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] 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 examples, the probiotic strain comprises a strain of Bifidobacterium. In certain examples, the probiotic strain comprises a strain of *B. longum* subsp. *infantis.* In some examples, 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 examples, the prebiotic mixture comprises at least 25 human milk oligosaccharides. In certain examples, the prebiotic mixture is free or essentially free of oligosaccharides that are not human milk oligosaccharides. In some examples, the prebiotic mixture is, is obtained from, or comprises a concentrated ultra-filtered permeate from pooled human milk. In particular examples, 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 examples, the one

or more individual probiotic doses each comprise at least 1 x 10^3 colony forming units (CFU). In some examples, the one or more individual probiotic doses each comprise at least 8 x 10^7 colony forming units (CFU). In particular examples, the one or more prebiotic doses comprises between 500 mg and 25 g of total human milk oligosaccharides. In certain examples, 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 examples, 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 examples, the method further comprises administering a probiotic strain of bacterium capable of consuming each oligosaccharide of the mixture. In certain examples, the subject has, is at risk of having, or is suspected of having hyperammonemia, hepatic encephalopathy, or a urea cycle disorder. In some examples, the prebiotic mixture comprises one or more human milk oligosaccharides (HMOs). In particular examples, all or essentially all of the oligosaccharides of the prebiotic mixture are human milk oligosaccharides (HMOs). In certain examples, the prebiotic mixture is free or essentially free of oligosaccharides that incorporate an N-acetyl glucosamine residue. In some examples, 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 examples, 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 examples, the mixture is free or

essentially free of oligosaccharides that comprise nitrogen. In some examples, 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 examples, the prebiotic mixture is free or essentially free of oligosaccharides comprising five or more monosaccharide residues. In certain examples, 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 examples, 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 examples, the probiotic strain is a strain of *B. longum* subsp. *infantis*. In certain examples, the prebiotic mixture comprises one or more of 2'-fucosyllactose, 3-fucosyllactose, difucosyllactose, 3'-sialyllactose, or 6'-sialyllactose. In some examples, the prebiotic mixture comprises 2'-fucosyllactose, 3-fucosyllactose, difucosyllactose, 3'-sialyllactose, and 6'-sialyllactose. In particular examples, the oligosaccharides of the prebiotic mixture consist or consist essentially of 2'-fucosyllactose, 3-fucosyllactose, difucosyllactose, 3'-sialyllactose, and 6'-sialyllactose. In certain examples, the prebiotic mixture comprises one or more of 2'-fucosyllactose, 3-fucosyllactose, or difucosyllactose. In some examples, the prebiotic mixture comprises of 2'-fucosyllactose, 3-fucosyllactose, and difucosyllactose. In particular examples, 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 examples, the subject has, is suspected of having, or is at risk of having hepatic encephalopathy. In some examples, 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 examples, 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 examples, 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 examples, 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 examples, 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 examples, 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 examples, the prebiotic mixture comprises at least 25 human milk oligosaccharides. In some examples, the prebiotic mixture is, is derived from, or comprises a concentrated ultra-filtered permeate from pooled human milk. In certain examples, the pooled human milk is pooled from the milk of at least 25, 50, or 100 individual donors.

[0042] In particular examples, the at least one probiotic strain of bacterium comprises a bacterial strain of the genus Bifidobacterium. In some examples, 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 examples, 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 allogenic 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 examples, the prebiotic mixture and the probiotic strain are administered orally. In some examples, the prebiotic mixture and the probiotic strain are administered on the same day for at least three consecutive days. In certain examples, 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 examples, 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 examples, the first treatment phase is about 7 days in length and wherein the second treatment phase is about 7 days in length. In certain examples, the probiotic strain is administered in an amount of at least 5 x 10^6 colony forming units (CFU) per day. In particular examples, the probiotic strain is administered in an amount of at least 8 x 10^7 colony forming units (CFU) per day. In some examples, the prebiotic mixture is administered in an amount of at least 500 mg of total human milk oligosaccharides per day. In certain examples, 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 examples, the prebiotic mixture and/or the probiotic strain is administered with an enteric coating. In certain examples, 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 examples, the subject is at least 6 months, 12 months, 2 years, 5 years, 12 years, or 18 years of age. In certain examples, the subject is a child, an adolescent, or an adult.

[0046] In some examples, 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 examples, 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 examples, 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 examples, 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 examples, 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 examples, 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 examples, the one or more probiotics comprises a Bifidobacterium probiotic. In particular examples, 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 examples, 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 examples, the prebiotic mixture comprises at least 25 human milk oligosaccharides. In particular examples, the prebiotic mixture is free or essentially free of oligosaccharides that are not human milk oligosaccharides. In some examples, the prebiotic mixture is, is obtained from, or comprises a concentrated ultra-filtered permeate from pooled human milk.

[0048] In certain examples, 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 examples, the one or more individual probiotic doses each comprise at least 1 x 10^3 colony forming units (CFU). In some examples, the one or more individual probiotic doses each comprise at least 8 x 10^7 colony forming units (CFU). In certain examples, the one or more prebiotic doses comprises between 500 mg and 25 g of total human milk oligosaccharides. In particular examples, 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 examples, the one or more individual prebiotic doses comprises less than 0.1% lactose. In certain examples, 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 examples, 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 examples, 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 examples, the probiotic bacteria strain is capable of internalizing and metabolizing the human milk oligosaccharides. In particular examples, 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 examples, 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 examples, the non-digestible carbohydrates are oligosaccharides. In some examples, 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 examples, the probiotic strain is a strain of *B. breve, B. bifidum, B. longum* subsp. *infantis, or B. longum* subsp. *longum*. In certain examples, the probiotic strain is *B. longum* subsp. *infantis*. In particular examples, 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 examples, 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 examples, the mixture and the probiotic strain are administered orally. In particular examples, the mixture and the probiotic strain are administered orally twice a day. In some examples, the mixture is

administered at a dose of between or between about 3 g/day and 18 g/day, inclusive. In certain examples, the mixture is administered at a dose of or of about 12.5 g/day.

[0056] In particular examples, the probiotic strain is administered at a dose of between or between about 10^4 units/day and 10^{12} units/day. In some examples, the probiotic strain is administered at a dose of or of about 10^9 units/day. In certain examples, the mixture and the probiotic strain is administered twice daily for the duration of a treatment period. In particular examples, the treatment period is or lasts between 7 days and 180 days, inclusive. In some examples, 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 examples, one or both of the mixture and the probiotic strain comprises an enteric coating. In particular examples, the method further comprises administering a proton pump inhibitor. In some examples, 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 examples, 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 examples, provided herein are formulations for use in accordance with the disclosure wherein the (i) prebiotic mixture and the (ii) probiotic strain are for administration to the subject simultaneously, separately, or sequentially.

[0061] In some examples, 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 examples, 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 examples, 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 examples, 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 examples, 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 examples, 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 examples, 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 examples, 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 examples, 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 examples, 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 examples, 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 examples, 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 examples, 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 examples, 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* sub. sp. *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).

DETAILED DESCRIPTION

[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.

[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, dextrins (*e.g.*, maltodextrin (*e.g.*, a native maltodextrin or a resistant maltodextrin)), inulin, β-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.

V. 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 ± 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 though 0.2 μm sterile filters. This final HMO composition was then filled into containers and stored at ≤ -20°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 x 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.

VII. SEQUENCES

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

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

2. The use of claim 1, wherein the prebiotic mixture comprises at least 10 human milk oligosaccharides.

3. The use of any of claims 1-2, wherein the prebiotic mixture comprises at least 25 human milk oligosaccharides.

4. The use of any of claims 1-3, wherein the prebiotic mixture comprises at least 50 human milk oligosaccharides.

5. The use of any of claims 1-4, wherein the prebiotic mixture comprises at least 50 human milk oligosaccharides.

6. The use of any of claims 1-5, 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, sialylacto-N-tetraose c, sialylacto-N-tetraose b, or disialyllacto-N-tetraose.

7. The use of any of claims 1-6, 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, sialylacto-N-tetraose c, sialylacto-N-tetraose b, and disialyllacto-N-tetraose.

8. The use of any of claims 1-7, 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.

9. The use of any of claims 1-8, 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.

10. The use of any of claims 1-9, wherein the prebiotic mixture and the probiotic strain are to be administered on the same day for at least one day.

11. The use of any of claims 1-10, wherein the prebiotic mixture and the probiotic strain are to be administered on the same day for at least three consecutive days.

12. The use of any of claims 1-11, wherein the prebiotic mixture and the probiotic strain are to be administered on the same day for at least seven consecutive days.

13. The use of any of claims 1-12, wherein the 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 to be administered to the subject 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 is to be administered for at least one day, wherein the probiotic strain is not to be administered during the second treatment phase.

14. The use of claim 13, wherein in the second treatment phase the prebiotic mixture is to be administered to the subject each day for at least three consecutive days.

15. The use of claim 13 or 14, wherein in the second treatment phase the prebiotic mixture is to be administered to the subject each day for at least seven consecutive days.

16. The use of any of claims 13-15, 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.

17. The use of any of claims 13-16, wherein the first treatment phase is about 7 days in length and wherein the second treatment phase is about 7 days in length.

18. The use of any of claims 1-17, wherein the probiotic strain is to be administered in an amount of at least 5×10^6 colony forming units (CFU) per day.

19. The use of any of claims 1-18, wherein the probiotic strain is to be administered in an amount of at least 8×10^7 colony forming units (CFU) per day.

20. The use of any of claims 1-19, wherein the prebiotic mixture is to be administered in an amount of at least 500 mg of total human milk oligosaccharides per day.

21. The use of any of claims 1-20, 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.

22. The use of any of claims 1-21, wherein the prebiotic mixture and/or the probiotic strain is to be administered with an enteric coating.

23. The use of any of claims 1-22, 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.

24. The use of any of claims 1-22, 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.

25. The use of any of claims 1-22, 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.

26. The use of any of claims 1-23, 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.

27. The use of any of claims 1-26, wherein both of the probiotic strain and the prebiotic mixture are free or essentially free of a benzimidazole derivative.

28. The use of any of claims 1-27, wherein the subject is a human subject at least 6 months, 12 months, 2 years, 5 years, 12 years, or 18 years of age.

29. The use of any of claims 1-28, wherein the subject is human subject that is a child, an adolescent, or an adult.

30. The use of any of claims 1-29, wherein the subject is a human adult.

31. 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.

32. The kit of claim 31, wherein the probiotic strain comprises a strain of Bifidobacterium.

33. The kit of claim 31 or claim 32, wherein the probiotic strain comprises a strain of *B. longum* subsp. *infantis, B. longum* subsp. *longum*, *B. breve,* or *B. bifidum.*

34. The kit of any of claims 31-33, 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.

35. The kit of any of claims 31-34, wherein the prebiotic mixture comprises at least 25 human milk oligosaccharides.

36. The kit of any of claims 31-35, wherein the prebiotic mixture is free or essentially free of oligosaccharides that are not human milk oligosaccharides.

37. The kit of any of claims 31-36, wherein the prebiotic mixture is, is obtained from, or comprises a concentrated ultra-filtered permeate from pooled human milk.

38. The kit of any of claims 31-37, 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.

39. The kit of claim 38, wherein the one or more individual probiotic doses each comprise at least 1×10^3 colony forming units (CFU).

40. The kit of claim 38 or 39, wherein the one or more individual probiotic doses each comprise at least 8×10^7 colony forming units (CFU).

41. The kit of any of claims 38-40, wherein the one or more prebiotic doses comprises between 500 mg and 25 g of total human milk oligosaccharides.

42. The kit of any of claims 38-41, 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.

43. The kit of any of claims 38-42, wherein the one or more individual prebiotic doses comprises less than 0.1% lactose.

44. An article of manufacture, comprising the kit of any of claims 31-43 and instructions for use, wherein the instructions for use describe the use of any of claims 1-30.