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(54) Título: COMPOSIÇÕES E MÉTODOS PARA TRATAR CÂNCER COM O USO DE BIFIDOBACTERIUM ANIMALIS SSP. LACTIS

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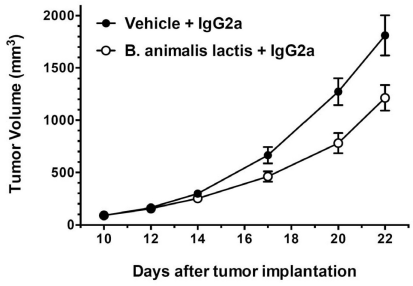
(57) Resumo: A presente invenção refere-se a métodos e composições relacionados a Bifidobacterium animalis ssp. lactis úteis como agentes terapêuticos.



Relatório Descritivo da Patente de Invenção para "**COMPOSIÇÕES E MÉTODOS PARA TRATAR CÂNCER COM O USO DE *BIFIDOBACTERIUM ANIMALIS SSP. LACTIS***".

PEDIDO RELACIONADO

[0001] Este pedido reivindica o benefício de prioridade do Pedido de Patente Provisório US de número de série 62/528.669, depositado em 5 de julho de 2017, que é aqui incorporado pelo presente documento a título de referência em sua totalidade.

SUMÁRIO

[0002] Em certos aspectos, são fornecidos no presente documento métodos e composições relacionados ao tratamento de um câncer em um indivíduo (*por exemplo,* um indivíduo humano) que compreende administrar uma composição bacteriana que compreende *Bifidobacterium animalis ssp. lactis*. Em algumas modalidades, a *Bifidobacterium animalis ssp. lactis* é a Cepa A de *Bifidobacterium animalis ssp. lactis* (Número de Depósito de ATCC PTA-125097). Em algumas modalidades, a *Bifidobacterium animalis ssp. lactis* é uma cepa que compreende pelo menos 99% de identidade de sequência (*por exemplo,* pelo menos 99,5% de identidade de sequência, pelo menos 99,6% de identidade de sequência, pelo menos 99,7% de identidade de sequência, pelo menos 99,8% de identidade de sequência, pelo menos 99,9% de identidade de sequência) com a sequência de nucleotídeos da Cepa A de *Bifidobacterium animalis ssp. lactis* (Tabela 1). Em algumas modalidades, a administração da composição bacteriana induz uma resposta imune contra um tumor no indivíduo. Em algumas modalidades, a administração da composição bacteriana induz infiltração de célula imune de CD3+ no indivíduo. Em algumas modalidades, a administração da composição bacteriana induz regulação ascendente de Classe I de MHC no sítio de tumor. Em algumas modalidades, a administração da composição

bacteriana trata o câncer no indivíduo. Em algumas modalidades, a administração aumenta um microambiente de tumor no indivíduo. Em algumas modalidades, o câncer é um carcinoma colorretal.

[0003] Em certas modalidades, são fornecidos no presente documento métodos de tratar um indivíduo que tem câncer, que compreende administrar ao indivíduo uma composição bacteriana que compreende *Bifidobacterium animalis ssp. lactis* (*por exemplo,* uma bactéria morta, uma bactéria viva e/ou uma bactéria atenuada). Em algumas modalidades, a *Bifidobacterium animalis ssp. lactis* é a Cepa A de *Bifidobacterium animalis ssp. lactis* (Número de Depósito de ATCC PTA-125097). Em algumas modalidades, a *Bifidobacterium animalis ssp. lactis* é uma cepa que compreende pelo menos 99% de identidade de sequência (*por exemplo,* pelo menos 99,5% de identidade de sequência, pelo menos 99,6% de identidade de sequência, pelo menos 99,7% de identidade de sequência, pelo menos 99,8% de identidade de sequência, pelo menos 99,9% de identidade de sequência) com a sequência de nucleotídeos da Cepa A de *Bifidobacterium animalis ssp. lactis*. Em algumas modalidades, pelo menos 50%, 60%, 70%, 80%, 85%, 90%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% ou 99% das bactérias na composição bacteriana são *Bifidobacterium animalis ssp. lactis.* Em algumas modalidades, toda ou substancialmente todas as bactérias na formulação bacteriana são *Bifidobacterium animalis ssp. lactis.* Em algumas modalidades, a formulação bacteriana compreende pelo menos 1×10^5, 5×10^5, 1×10^6, 2×10^6, 3×10^6, 4×10^6, 5×10^6, 6×10^6, 7×10^6, 8×10^6, 9×10^6, 1×10^7, 2×10^7, 3×10^7, 4×10^7, 5×10^7, 6×10^7, 7×10^7, 8×10^7, 9×10^7, 1×10^8, 2×10^8, 3×10^8, 4×10^8, 5×10^8, 6×10^8, 7×10^8, 8×10^8, 9×10^8 ou 1×10^9 unidades de formação de colônia de *Bifidobacterium animalis ssp. lactis.*

[0004] Em certas modalidades, são fornecidas no presente documento

composições bacterianas que compreendem *Bifidobacterium animalis ssp. lactis* (por exemplo, uma bactéria morta, uma bactéria viva e/ou uma bactéria atenuada). Em algumas modalidades, pelo menos 50%, 60%, 70%, 80%, 85%, 90%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% ou 99% das bactérias na composição bacteriana são *Bifidobacterium animalis ssp. lactis.* Em algumas modalidades, a *Bifidobacterium animalis ssp. lactis* é a Cepa A de *Bifidobacterium animalis ssp. lactis* (Número de Depósito de ATCC PTA-125097). Em algumas modalidades, a *Bifidobacterium animalis ssp. lactis* é uma cepa compreendendo pelo menos 99% de identidade de sequência (*por exemplo,* pelo menos 99,5% de identidade de sequência, pelo menos 99,6% de identidade de sequência, pelo menos 99,7% de identidade de sequência, pelo menos 99,8% de identidade de sequência, pelo menos 99,9% de identidade de sequência) com a sequência de nucleotídeos da Cepa A de *Bifidobacterium animalis ssp. lactis*. Em algumas modalidades, todas ou substancialmente todas as bactérias na formulação bacteriana são *Bifidobacterium animalis ssp. lactis.* Em algumas modalidades, a formulação bacteriana compreende pelo menos 1×10^5, 5×10^5, 1×10^6, 2×10^6, 3×10^6, 4×10^6, 5×10^6, 6×10^6, 7×10^6, 8×10^6, 9×10^6, 1×10^7, 2×10^7, 3×10^7, 4×10^7, 5×10^7, 6×10^7, 7×10^7, 8×10^7, 9×10^7, 1×10^8, 2×10^8, 3×10^8, 4×10^8, 5×10^8, 6×10^8, 7×10^8, 8×10^8, 9×10^8 ou 1×10^9 unidades de formação de colônia de *Bifidobacterium animalis ssp. lactis.* Em algumas modalidades, a composição bacteriana compreende pelo menos uma cepa de *Bifidobacterium animalis ssp. lactis* que tem um gene compreendendo uma alteração de base de nucleotídeo como identificado na Tabela 6. Em algumas modalidades, a composição bacteriana compreende pelo menos uma cepa de *Bifidobacterium animalis ssp. lactis* que tem 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, ou 29, genes compreendendo uma alteração

de base de nucleotídeo como identificado na Tabela 6. Em algumas modalidades, a composição bacteriana compreende pelo menos uma cepa de *Bifidobacterium animalis ssp. lactis* que tem genes compreendendo toda a alteração de base de nucleotídeo como identificado na Tabela 6.

[0005] Em algumas modalidades, são fornecidas no presente documento biomassas farmaceuticamente ativas (PhABs) derivadas a partir de e/ou compreendendo *Bifidobacterium animalis ssp. lactis* (por exemplo, Cepa A de *Bifidobacterium animalis ssp. lactis* (Número de Depósito de ATCC PTA-125097)). Em algumas modalidades, as PhABs compreendem células integrais, frações de células, sobrenadante de fermentação, frações de sobrenadante e/ou vesículas extracelulares produzidas a partir de *Bifidobacterium animalis ssp. lactis* descrita no presente documento. Em algumas modalidades, as composições bacterianas fornecidas no presente documento compreendem *PhABs de Bifidobacterium animalis ssp. lactis* fornecidas no presente documento.

[0006] Em algumas modalidades, a composição bacteriana é administrada oralmente, de modo intravenoso, intratumoral ou subcutâneo. Em algumas modalidades, a composição bacteriana é administrada em 2 ou mais (por exemplo, 3 ou mais, 4 ou mais ou 5 ou mais doses). Em algumas modalidades, a administração ao indivíduo das duas ou mais doses é separada por pelo menos 1 hora, 2 horas, 3 horas, 4 horas, 5 horas, 6 horas, 7 horas, 8 horas, 9 horas, 10 horas, 11 horas, 12 horas, 13 horas, 14 horas, 15 horas, 16 horas, 17 horas, 18 horas, 1 dia, 2 dias, 3 dias, 4 dias, 5 dias, 6 dias, 7 dias, 8 dias, 9 dias, 10 dias, 11 dias, 12 dias, 13 dias, 14 dias, 15 dias, 16 dias, 17 dias, 18 dias, 19 dias, 20 dias ou 21 dias. Em algumas modalidades, uma segunda bactéria é administrada como parte de um consórcio ecológico.

[0007] Em algumas modalidades, o método compreende adicionalmente administrar ao indivíduo um antibiótico. Em algumas modalidades, o método compreende adicionalmente administrar ao indivíduo uma ou mais outras terapias de câncer. Em algumas modalidades, a outra terapia de câncer é a remoção cirúrgica de um tumor, a administração de um agente quimioterapêutico, a administração de terapia de radiação, a administração de um antibiótico, a administração de uma imunoterapia contra câncer (por exemplo, um inibidor de ponto de verificação imunológico, um anticorpo específico para câncer, uma vacina para câncer, uma célula apresentadora preparada com antígeno, uma célula T específica para câncer, uma célula T receptora de antígeno quimérico (CAR) específica para câncer, uma proteína de ativação imune, um adjuvante) e/ou a administração de outra bactéria terapêutica.

[0008] Em algumas modalidades, o indivíduo é um mamífero. Em algumas modalidades, o indivíduo é um ser humano. Em algumas modalidades, o indivíduo é um mamífero não humano (por exemplo, um cachorro, um gato, uma vaca, um cavalo, um porco, um jumento, uma cabra, um camelo, um camundongo, um rato, um porquinho da índia, uma ovelha, uma lhama, um macaco, um gorila ou um chimpanzé.

[0009] Em certas modalidades, é fornecido no presente documento um biorreator A compreendendo *Bifidobacterium animalis ssp. Lactis.*

BREVE DESCRIÇÃO DAS FIGURAS

[0010] A **Figura 1** mostra a inibição de crescimento tumoral (em volume) pela administração oral de Cepa A de *Bifidobacterium animalis ssp. lactis* em um modelo de carcinoma colorretal de camundongo.

[0011] A **Figura 2** mostra que em um modelo de carcinoma colorretal de camundongo, a eficácia de Cepa A de *Bifidobacterium animalis ssp. lactis* oralmente administrada é comparável àquela anti-PD-1 administrada

de modo intraperitoneal (i.p.).

[0012] A **Figura 3** mostra que em um modelo de carcinoma colorretal de camundongo, a eficácia de Cepa A de *Bifidobacterium animalis ssp. lactis* oralmente administrada é comparável àquela anti-PD-1 administrada de modo intraperitoneal (i.p.).

[0013] A **Figura 4** mostra que em um modelo de carcinoma colorretal de camundongo, a eficácia de Cepa A de *Bifidobacterium animalis ssp. lactis* de modo intratumoral é comparável àquela anti-PD-1 administrada de modo intraperitoneal (i.p.).

[0014] A **Figura 5** mostra que em um modelo de melanoma de camundongo, tratar camundongos inoculados com tumor com uma combinação de Cepa A de *Bifidobacterium animalis ssp. lactis* e anti-PD-L1 inibe o crescimento tumoral de uma maneira superior tanto à Cepa A de *B. animalis ssp. lactis* quanto anti-PD-L1 sozinha.

[0015] A **Figura 6A** mostra o volume de tumor anterior à infiltração de célula imune de CD3+ e análise de expressão de Classe I de MHC. A **Figura 6A** mostra a inibição de crescimento tumoral (em volume) pela administração oral de Cepa A de *Bifidobacterium animalis ssp. lactis* em um modelo de carcinoma colorretal de camundongo.

[0016] A **Figura 6B** mostra que a infiltração de células imunes de CD3+ foi significativamente aumenta no grupo da Cepa A de Bifidobacterium animalis ssp. lactis em relação ao grupo de veículo.

[0017] A **Figura 6C** mostra que a Cepa A de *Bifidobacterium animalis ssp. lactis* induziu uma regulação ascendente marcante de expressão de Classe I de MHC em relação ao grupo de veículo.

[0018] A **Figura 7** mostra uma visão geral de um estudo de marcação 1/2 aberta de fase da segurança, tolerabilidade e eficácia de uma Cepa A de *Bifidobacterium animalis ssp. lactis* como uma terapia oral em pacientes

com carcinoma colorretal metastático.

[0019] A **Figura 8** mostra que em um modelo de carcinoma colorretal de camundongo, a eficácia de uma combinação tripla de Cepa A de *Bifidobacterium animalis ssp. lactis*, anti-PD-1 e anti-CTLA4 é maior que aquela da combinação de anti-PD-1 e anti-CTLA4.

[0020] A **Figura 9A** e a **Figura 9B** mostram uma comparação de assinaturas in vitro de três cepas de *Bifidobacterium animalis ssp. lactis* (Cepa A, Cepa B e Cepa C) de um ensaio de célula dendrítica de camundongo. A Cepa A exibe um perfil imune com uma indução reduzida de citocinas pró-tumorígenas (IL-10, IL-1b, IL-6, IL-8, TNFα) comparadas com a Cepa B e a Cepa C. Os valores de citocina relatados representam sumário médio geométrico de grupos (n=3) através do ensaio**.**

DESCRIÇÃO DETALHADA

Geral

[0021] Em certos aspectos, são fornecidas no presente documento composições bacterianas compreendendo *Bifidobacterium animalis ssp. lactis* e métodos para tratar câncer em um indivíduo compreendendo administrar ao indivíduo uma composição bacteriana compreendendo *Bifidobacterium animalis ssp. lactis.*

Definições

[0022] "Adjuvante" ou "terapia de adjuvante" se refere amplamente a um agente que afeta uma resposta imunológica ou fisiológica em um paciente ou indivíduo. Por exemplo, um adjuvante pode aumentar a presença de um antígeno ao longo do tempo ou a uma área de interesse como um tumor, ajudar a absorver um antígeno de célula apresentadora de antígeno, ativar macrófagos e linfócitos e suporta a produção de citocinas. Alterando-se uma resposta imune, um adjuvante pode permitir que uma dose menor de um agente de interação imune aumente a eficácia ou segurança de uma

dose particular do agente de interação imune. Por exemplo, um adjuvante pode prevenir a exaustão de célula T e, assim, aumentar a eficácia ou segurança de um agente de interação imune particular.

[0023] "Administração" se refere amplamente a uma via de administração de uma composição a um indivíduo. Os exemplos de vias de administração incluem administração oral, administração retal, administração tópica, inalação (nasal) ou injeção. A administração por injeção inclui administração intravenosa (IV), intramuscular (IM), intratumoral (IT) e subcutânea (SC). As composições farmacêuticas descritas no presente documento podem ser administradas de qualquer forma por qualquer via eficaz, incluindo, mas sem limitações, intratumoral, oral, parenteral, enteral, intravenosa, intraperitoneal, tópica, transdérmica (por exemplo, com o uso de qualquer emplastro padrão), intradérmica, oftálmica, de modo (intra)nasal, local, não oral, como aerossol, inalação, subcutânea, intramuscular, bucal, sublingual, (trans)retal, vaginal, intra-arterial e intratecal, transmucosal (por exemplo, sublingual, lingual, (trans)bucal, (trans)uretral, vaginal (por exemplo, trans- e perivaginal), intravesical, intrapulmonar, intraduodenal, intragástrico e intrabrônquico. Em modalidades preferenciais, as composições farmacêuticas descritas no presente documento são administradas de modo oral, retal, intratumoral, tópico, intravesical, por injeção em ou adjacente a um gânglio de drenagem, intravenoso, por inalação ou aerossol ou subcutâneo.

[0024] Como usado no presente documento, o termo "anticorpo" pode se referir tanto a um anticorpo intacto e um fragmento de ligação de antígeno do mesmo. Os anticorpos intactos são glicoproteínas que incluem pelo menos duas cadeias pesadas (H) e duas cadeias leves (L) interconectadas por ligações de dissulfeto. Cada cadeia pesada inclui uma região variável de cadeia pesada (abreviada no presente documento como V_H) e

uma região constante de cadeia pesada. Cada cadeia leve inclui uma região variável de cadeia leve (abreviada no presente documento como V_L) e uma região constante de cadeia leve. As regiões V_H e V_L podem ser adicionalmente subdivididas em regiões de hipervariabilidade, denominadas regiões determinantes de complementaridade (CDR), intercaladas com regiões que são mais conservadas, denominadas regiões de framework (FR). Cada V_H e V_L é composta por três CDRs e quatro FRs, dispostas a partir da terminação amino para a terminação carbóxi na seguinte ordem: FR1, CDR1, FR2, CDR2, FR3, CDR3, FR4. As regiões variáveis das cadeias pesada e leve contêm um domínio de ligação que interage com um antígeno. O termo "anticorpo" inclui, por exemplo, anticorpos monoclonais, anticorpos policlonais, anticorpos quiméricos, anticorpos humanizados, anticorpos humanos, anticorpos multiespecíficos (por exemplo, anticorpos biespecíficos), anticorpos de cadeia única e fragmentos de anticorpo de ligação de antígeno.

[0025] Os termos "fragmento de ligação de antígeno" e "porção de ligação de antígeno" de um anticorpo, como usado no presente documento, se refere a um ou mais fragmentos de um anticorpo que retém a capacidade de se ligar a um antígeno. Os exemplos de fragmentos de ligação englobados dentro do termo "fragmento de ligação de antígeno" de um anticorpo incluem Fab, Fab', $F(ab')_2$, Fv, scFv, Fv ligado por dissulfeto, Fd, diacorpos, anticorpos de cadeia única, NANOBODIES®, CDRH3 isolada e outros fragmentos de anticorpo que retêm pelo menos uma porção da região variável de um anticorpo intacto. Esses fragmentos de anticorpo podem ser obtidos com o uso de técnicas enzimáticas e/ou de recombinante convencionais e podem ser examinados quanto à ligação de antígeno da mesma maneira que anticorpos intactos.

[0026] "Câncer" se refere amplamente a um crescimento anormal descontrolado das próprias células do hospedeiro, levando à invasão de tecido circundante e potencialmente tecido distal ao sítio inicial do crescimento de célula anormal no hospedeiro. As classes principais incluem carcinomas que são cânceres do tecido epitelial (por exemplo, pele, células escamosas); sarcomas que são cânceres do tecido conectivo (por exemplo, osso, cartilagem, gordura, músculo, vasos sanguíneos, etc.); leucemias que são cânceres de tecido de formação de sangue (por exemplo, tecido da medula óssea); linfomas e mielomas que são cânceres de células imunes; e cânceres do sistema nervoso central que incluem cânceres do cérebro e tecido espinhal. "Câncer (ou cânceres)", "neoplasma (ou neoplasmas)" e "tumor (ou tumores)" são usados no presente documento de modo intercambiável. Como usado no presente documento, "câncer" se refere a todos os tipos de câncer ou neoplasma ou tumores malignos incluindo leucemias, carcinomas e sarcomas, independentemente de serem novos ou recorrentes. Os exemplos específicos de cânceres são: carcinomas, sarcomas, mielomas, leucemias, linfomas e tumores de tipo misturado. Os exemplos não limitantes de cânceres são cânceres novos ou recorrentes do cérebro, melanoma, bexiga, mama, colo do útero, cólon, cabeça e pescoço, rim, pulmão, pulmão de célula não pequena, mesotelioma, ovário, próstata, sarcoma, estômago, útero e meduloblastoma. Os tumores pediátricos e adultos incluem, mas sem limitações, aqueles de bexiga, cérebro, mama, osso, colo do útero, cólon, tecido conectivo, gordura, cabeça e pescoço, rim, fígado, pulmão, mesotélio, melanócitos (melanoma), músculo, ovário, pâncreas, próstata, estômago, intestino pequeno e útero

[0027] O termo "diminuir" ou "esgotar" significa uma alteração, de modo que a diferença seja, dependendo das circunstâncias, pelo menos 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 1/100, 1/1000,

1/10.000, 1/100.000, 1/1.000.000 ou indetectável após o tratamento quando comparado com um estado pré-tratamento.

[0028] O termo "consórcio ecológico" é um grupo de bactérias que trocam metabólitos e corregulam positivamente uma à outra, em contraste com duas bactérias que induzem sinergia de hospedeiro através da ativação de trajetórias de hospedeiro complementares para eficácia aprimorada.

[0029] O termo "epítopo" significa um determinante de proteína capaz de ligação específica a um anticorpo. Os epítopos geralmente consistem em agrupamentos de superfície quimicamente ativa de moléculas como aminoácidos ou cadeias laterais de açúcar. Certos epítopos podem ser definidos por uma sequência particular de aminoácidos aos quais um anticorpo é capaz de se ligar.

[0030] O termo "gene" é usado amplamente para fazer referência a qualquer ácido nucleico associado a uma função biológica. O termo "gene" se aplica a uma sequência genômica específica, assim como a um cDNA ou um mRNA codificado por tal sequência genômica.

[0031] "Identidade" como entre sequência de ácidos nucleicos de duas moléculas de ácido nucleico pode ser determinada como uma porcentagem de identidade com o uso de algoritmos de computador conhecidos como o programa "FASTA", com o uso, por exemplo, dos parâmetros predefinidos como em Pearson *et al.* (1988) Proc. Natl. Acad. Sci. EUA 85:2444 (outros programas incluem o pacote de programa de GCG (Devereux, J., *et al.*, Nucleic Acids Research 12(I):387 (1984)), BLASTP, BLASTN, FASTA Atschul, S. F., *et al.*, J Molec Biol 215:403 (1990); Guide to Huge Computers, Mrtin J. Bishop, ed., Academic Press, San Diego, 1994 e Carillo *et al.* (1988) SIAM J Applied Math 48:1073). Por exemplo, a função

de BLAST do banco de dados de National Center for Biotechnology Information (Centro Nacional para Informações de Biotecnologia) pode ser usado para determinar a identidade. Outros programas disponíveis comercial ou publicamente incluem, o programa DNAStar "MegAlign" (Madison, Wis.) e o programa "Gap" do Grupo de Computador de Genética da Universidade de Wisconsin (University of Wisconsin Genetics Computer Group (UWG)) (Madison Wis.)).

[0032] "Imunoterapia" é o tratamento que usa o sistema imunológico de um indivíduo para tratar câncer e inclui, por exemplo, inibidores de ponto de verificação, vacinas de câncer, citocinas, terapia celular, células T de CAR e terapia de célula dendrítica.

[0033] O termo "aumentar" significa uma alteração, de modo que a diferença seja, dependendo de circunstâncias, pelo menos 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 2 vezes, 4 vezes, 10 vezes, 100 vezes, 10^3 vezes, 10^4 vezes, 10^5 vezes, 10^6 vezes e/ou 10^7 vezes maior após o tratamento em comparação com um estado pré-tratamento. As propriedades que podem ser aumentadas incluem células imunes, células bacterianas, células estromais, células supressoras derivadas de mieloide, fibroblastos, metabólitos e citocinas.

[0034] "Agonistas imunes inatos" ou "imunoadjuvantes" são moléculas pequenas, proteínas ou outros agentes que alvejam especificamente receptores imunes inatos incluindo Receptores Tipo Toll, receptores de NOD, componentes de Trajetória de STING. Por exemplo, LPS é um agonista de TLR-4 que é derivado ou sintetizado de modo bacteriano e alumínio pode ser usado como um adjuvante imunoestimulante. Os imunoadjuvantes são uma classe específica de adjuvante mais amplo ou terapia de adjuvante.

[0035] O termo "isolado" ou "enriquecido" engloba um micróbio, bactérias ou outra entidade ou substância que foi (1) separada de pelo menos

alguns dos componentes aos quais a mesma estava associada quando inicialmente produzida (independentemente de ser na natureza ou em uma configuração experimental) e/ou (2) produzida, preparada, purificada e/ou fabricada pela mão do homem. Os micróbios isolados podem ser separados de pelo menos cerca de 10%, cerca de 20%, cerca de 30%, cerca de 40%, cerca de 50%, cerca de 60%, cerca de 70%, cerca de 80%, cerca de 90% ou mais dos outros componentes com os quais estavam inicialmente associados. Em algumas modalidades, os micróbios isolados são mais que cerca de 80%, cerca de 85%, cerca de 90%, cerca de 91%, cerca de 92%, cerca de 93%, cerca de 94%, cerca de 95%, cerca de 96%, cerca de 97%, cerca de 98%, cerca de 99% ou mais que cerca de 99% puros. Como usado no presente documento, uma substância é "pura" se for substancialmente livre de outros componentes. Os termos "purificar", "purificante" e "purificado" se referem a um micróbio ou outro material que foi separado de pelo menos alguns dos componentes com os quais foi associado quando inicialmente produzido ou gerado (por exemplo, independentemente na natureza ou em uma configuração experimental), ou durante qualquer momento após sua produção inicial. Um micróbio ou uma população microbiana pode ser considerada purificada se for isolada na ou após a produção, como a partir de um material ou ambiente contendo o micróbio ou a população microbiana, e um micróbio purificado ou população microbiana pode conter outros materiais até cerca de 10%, cerca de 20%, cerca de 30%, cerca de 40%, cerca de 50%, cerca de 60%, cerca de 70%, cerca de 80%, cerca de 90%, ou acima de cerca de 90% e ainda ser considerada "isolada". Em algumas modalidades, os micróbios purificados ou população microbiana purificada é mais que cerca de 80%, cerca de 85%, cerca de 90%, cerca de 91%, cerca de 92%, cerca de 93%, cerca de 94%, cerca de 95%, cerca de 96%, cerca de 97%, cerca de 98%, cerca de 99% ou

mais que cerca de 99% pura. No caso de composições microbianas fornecidas no presente documento, o um ou mais tipos microbianos presentes na composição podem ser independentemente purificados de um ou mais outros micróbios produzidos e/ou presente no material ou ambiente contendo o tipo microbiano. As composições microbianas e os componentes microbianos dos mesmos são geralmente purificados a partir de produtos de habitat residual.

[0036] Como usado no presente documento, um gene é "superexpressado" em bactérias se for expressado a um nível superior em bactérias geneticamente modificadas sob pelo menos algumas condições do que é expressado por bactérias de tipo selvagem da mesma espécie sob as mesmas condições. De modo similar, um gene é "subexpressado" em bactérias se for expressado a um nível inferior em bactérias geneticamente modificadas sob pelo menos algumas condições do que é expressado por bactérias de tipo selvagem da mesma espécie sob as mesmas condições.

[0037] Os termos "polinucleotídeo" e "ácido nucleico" são usados de modo intercambiável. Os mesmos se referem a uma forma polimérica de nucleotídeos de qualquer comprimento, deoxirribonucleotídeos ou ribonucleotídeos ou análogos dos mesmos. Os polinucleotídeos podem ter qualquer estrutura tridimensional e podem realizar qualquer função. Os seguintes são exemplos não limitantes de polinucleotídeos: regiões de codificação ou não codificação de um gene ou fragmento de gene, loci (locus) definidos a partir de análise de ligação, éxons, íntrons, RNA mensageiro (mRNA), RNA de transferência, RNA ribossômico, ribozimas, cDNA, polinucleotídeos recombinantes, polinucleotídeos ramificados, plasmídeos, vetores, DNA isolado de qualquer sequência, RNA isolado de qualquer sequência, sondas de ácido nucleico e iniciadores. Um polinucleotídeo pode compreender nucleotídeos modificados, como nucleotídeos metilados e

nucleotídeo análogos. Se estiverem presentes, as modificações na estrutura de nucleotídeo podem ser conferidas antes ou após a montagem do polímero. Um polinucleotídeo pode ser adicionalmente modificado, como por conjugação com um componente de marcação. Em todas as sequências de ácidos nucleicos fornecidas no presente documento, os nucleotídeos de U são intercambiáveis com nucleotídeos de T.

[0038] "Unidades taxonômicas operacionais" e "OTU(s)" se referem a uma folha terminal em uma árvore filogenética e é definida por uma sequência de ácidos nucleicos, por exemplo, o genoma inteiro, ou uma sequência genética específica, e todas as sequências que compartilham identidade de sequência com essa sequência de ácidos nucleicos no nível de espécie. Em algumas modalidades, a sequência genética específica pode ser a sequência de 16S ou uma porção da sequência de 16S. Em outras modalidades, os genomas inteiros de duas entidades são sequenciados e comparados. Em outra modalidade, as regiões seletas como etiquetas de sequência de multilocus (MLST), genes específicos ou conjuntos de genes podem ser geneticamente comparadas. Para 16S, as OTUs que compartilham ≥ 97% de identidade de nucleotídeo média através de toda a 16S ou alguma região variável da 16S são consideradas a mesma OTU. Consultar, por exemplo, Claesson MJ, Wang Q, O'Sullivan O, Greene-Diniz R, Cole JR, Ross RP e O'Toole PW. 2010. A comparação de duas tecnologias de sequenciamento da próxima geração para solucionar a composição microbiota altamente complexa com o uso de regiões de gene de rRNA de 16S variáveis em tandem. Nucleic Acids Res 38: e200. Konstantinidis KT, Ramette A e Tiedje JM. 2006. The bacterial species definition in the genomic era. Philos Trans R Soc Lond B Biol Sci 361: 1929 a 1940. Para genomas completos, as MLSTs, genes específicos, diferentes de 16S ou conjuntos de OTUs de genes que compartilham ≥ 95% de identidade de

nucleotídeo média são consideradas a mesma OTU. Consultar, por exemplo, Achtman M, e Wagner M. 2008. Microbial diversity and the genetic nature of microbial species. Nat. Rev. Microbiol. 6: 431 a 440. Konstantinidis KT, Ramette A e Tiedje JM. 2006. The bacterial species definition in the genomic era. Philos Trans R Soc Lond B Biol Sci 361: 1929 a 1940. As OTUs são frequentemente definidas comparando-se sequências entre organismos. Geralmente, não se considera que as sequências com menos que 95% de identidade de sequência formem parte da mesma OTU. As OTUs também podem ser caracterizadas por qualquer combinação de marcadores de nucleotídeo ou genes, em particular, genes altamente conservados (por exemplo, genes tipo "house-keeping"), ou uma combinação dos mesmos. As Unidades Taxonômicas Operacionais (OTUs) com atribuições taxonômicas produzidas para, por exemplo, gênero, espécie e clade filogenética são fornecidas no presente documento.

[0039] Como usado no presente documento, "ligação específica" se refere à capacidade de um anticorpo para se ligar a um antígeno predeterminado ou a capacidade de um polipeptídeo de se ligar ao seu parceiro de ligação predeterminado. Tipicamente, um anticorpo ou polipeptídeo se liga especificamente ao seu antígeno predeterminado ou parceiro de ligação com uma afinidade correspondente a uma K_D de cerca de 10^{-7} M ou menos, e se liga ao antígeno/parceiro de ligação predeterminado com uma afinidade (como expressado por K_D) que é pelo menos 10 vezes menor, pelo menos 100 vezes menor ou pelo menos 1000 vezes menor que sua afinidade para se ligar a um antígeno/parceiro de ligação não específico e não relacionado (por exemplo, BSA, caseína). Alternativamente, a ligação específica se aplica mais amplamente a um sistema de dois componentes, em que um componente é uma proteína, lipídio ou carboidrato ou combinação dos mesmos e se engata com o segundo componente, que é uma

proteína, lipídio, carboidrato ou combinação dos mesmos de uma forma específica.

[0040] Os termos "indivíduo" ou "paciente" se referem a qualquer animal. Um indivíduo ou um paciente descrito como "precisando do mesmo" se refere a alguém que precisa de um tratamento para uma doença. Os mamíferos (*isto é,* animais mamíferos) incluem seres humanos, animais de laboratório (por exemplo, primatas, ratos, camundongos), gado (por exemplo, vacas, ovelhas, cabras, porcos) e animais domésticos (por exemplo, cachorros, gatos, roedores). Por exemplo, o indivíduo pode ser um mamífero não humano incluindo, mas sem limitações, um cachorro, um gato, uma vaca, um cavalo, um porco, um jumento, uma cabra, um camelo, um camundongo, um rato, um porquinho da índia, uma ovelha, uma lhama, um macaco, um gorila ou um chimpanzé. O indivíduo ou paciente pode estar saudável, ou pode estar sofrendo de um neoplasma em qualquer estágio desenvolvimental, em que qualquer um dos estágios são causados por ou oportunisticamente suportados de um câncer associado ou patógeno causativo, ou pode estar em risco de desenvolver um neoplasma ou transmitir para outros um câncer associado ou patógeno causativo de câncer. Em algumas modalidades, os pacientes têm câncer de pulmão, câncer de bexiga, câncer de próstata, câncer de ovário e/ou melanoma. Os pacientes podem ter tumores que mostram macropinocitose intensificada com a genômica subjacente desse processo incluindo ativação de Ras. Em outras modalidades, os pacientes sofrem de outros cânceres. Em algumas modalidades, o indivíduo foi submetido a uma terapia de câncer.

[0041] "Cepa" se refere a um membro de uma espécie bacteriana com uma assinatura genética, de modo que possa ser diferenciada de membros proximamente relacionados da mesma espécie bacteriana. A assinatura genética pode ser a ausência de todo ou parte de pelo menos um gene, a

ausência de toda ou parte de pelo menos uma região reguladora (por exemplo, um promotor, um terminador, um riboswitch, um sítio de ligação de ribossomo), a ausência ("cura") de pelo menos um plasmídeo nativo, a presença de pelo menos um gene recombinante, a presença de pelo menos um gene mutado, a presença de pelo menos um gene estranho (um gene derivado de outra espécie), a presença de pelo menos uma região reguladora mutada (por exemplo, um promotor, um terminador, um riboswitch, um sítio de ligação de ribossomo), a presença de pelo menos um plasmídeo não nativo, a presença de pelo menos um cassete de resistência ao antibiótico ou uma combinação dos mesmos. As assinaturas genéticas entre cepas diferentes podem ser identificadas por amplificação de PCR, opcionalmente seguida por sequenciamento de DNA da região (ou regiões) genômica de interesse ou do genoma integral. No caso em que uma cepa (comparada com outra da mesma espécie) ganhou ou perdeu resistência ao antibiótico ou ganhou ou perdeu uma capacidade biossintética (como uma cepa auxotrófica), cepas podem ser diferenciadas por seleção ou contrasseleção com o uso de um antibiótico ou nutriente/metabólito, respectivamente.

[0042] Como usado no presente documento, o termo "tratar" uma doença em um indivíduo ou "tratar" um indivíduo que tem ou tem suspeita de ter uma doença se refere a submeter o indivíduo a um tratamento farmacêutico, por exemplo, a administração de um ou mais agentes, de modo que pelo menos um sintoma da doença seja reduzido ou impedido de piorar. Assim, em uma modalidade, "tratar" se refere, entre outros, a retardar a progressão, acelerar a remissão, induzir a remissão, aumentar a remissão, acelerar a recuperação, aumentar a eficácia de ou reduzir a resistência a terapêuticos alternativos, ou uma combinação dos mesmos. Em cer-

tas modalidades, um câncer é tratado se o indivíduo experimentar uma redução no tamanho de tumor, um número reduzido de tumores, uma redução no crescimento tumoral, uma redução na metástase do câncer e/ou um número reduzido de células de câncer totais após o tratamento em relação ao que seria esperado na ausência de tratamento.

Bactérias

[0043] Em certos aspectos, são fornecidas no presente documento composições bacterianas compreendendo *Bifidobacterium animalis ssp. lactis* (por exemplo, uma quantidade eficaz de *Bifidobacterium animalis ssp. lactis*) e métodos para o uso de uma composição bacteriana compreendendo *Bifidobacterium animalis ssp. lactis.* Em algumas modalidades, a *Bifidobacterium animalis ssp. lactis* é a Cepa A de *Bifidobacterium animalis ssp. lactis* (Número de Depósito de ATCC PTA-125097). Em algumas modalidades, a composição bacteriana compreende uma PhAB produzida a partir de ou compreendendo uma *Bifidobacterium animalis ssp. lactis* descrita no presente documento. Em algumas modalidades, a *Bifidobacterium animalis ssp. lactis* é uma cepa que compreende pelo menos 99% de identidade de sequência (*por exemplo,* pelo menos 99,5% de identidade de sequência, pelo menos 99,6% de identidade de sequência, pelo menos 99,7% de identidade de sequência, pelo menos 99,8% de identidade de sequência, pelo menos 99,9% de identidade de sequência) com a sequência de nucleotídeos da Cepa A de *Bifidobacterium animalis ssp. lactis*.

[0044] Mediante os termos do Tratado de Budapeste sobre o Reconhecimento Internacional do Depósito de Micro-organismos para o Propósito de Procedimento de Patente (International Recognition of the Deposit of Microorganisms for the Purpose of Patent Procedure), a Cepa A de *Bifidobacterium animalis ssp. Lactis* foi depositada em 27 de abril de 2018, com a Coleção de Cultura de Tipo Americano (American Type Culture

Collection (ATCC)) de 10801 University Boulevard, Manassas, Va. 20110 a 2209 EUA e foi atribuída ao Número de Acesso de ATCC PTA-125097.

[0045] O requerente representa que a ATCC é um depositário que oferece a permanência do depósito e acessibilidade pronta ao mesmo pelo público se uma patente for concedida. Todas as restrições sobre a disponibilidade ao público do material assim depositado serão irrevogavelmente removidas mediante a concessão de uma patente. O material ficará disponível durante a pendência do pedido de patente a alguém determinado pelo Comissionário a ser intitulado ao mesmo mediante 37 CFR 1.14 e 35 U.S.C. 122. O material depositado será mantido com todos os cuidados necessários para manter o mesmo viável e não contaminado por um período de pelo menos cinco anos após a solicitação mais recente do fornecimento de uma amostra do plasmídeo depositado e, de qualquer forma, por um período de pelo menos trinta (30) anos após a data de depósito ou pela vida executável da patente, o período que for mais longo. O requerente reconhece seu dever de substituir o depósito se o depositário for incapaz de fornecer uma amostra quando solicitado devido à condição do depósito.

[0046] Em algumas modalidades, as bactérias descritas no presente documento são modificadas para aprimorar a colonização e/ou o enxerto no trato gastrointestinal mamífero (por exemplo, metabolismo modificado, como degradação de mucina aprimorada, perfil de competição intensificado, mobilidade aumentada, adesão aumentada às células epiteliais intestinais, quimiotaxia modificada). Em algumas modalidades, as bactérias descritas no presente documento são modificadas para intensificar seu efeito imunomodulador e/ou terapêutico (por exemplo, sozinhas ou em combinação com outro agente terapêutico). Em algumas modalidades, as bactérias descritas no presente documento são modificadas para intensifi-

car a ativação imune (por exemplo, através da produção modificada de polissacarídeos, pili, fimbriae, adesinas, vesículas de membrana externa). Em algumas modalidades, as bactérias descritas no presente documento são modificadas para aprimorar a fabricação bacteriana (por exemplo, tolerância ao oxigênio mais alta, tolerância à condensação de congelamento aprimorada, tempos de geração mais curtos).

[0047] Em certas modalidades, a *Bifidobacterium animalis ssp. lactis* é cultivada em meio de Clostridium Reforçado (meio de RCM) (ATCC 2107 ou meio de outro fabricante), meio de MRS de Lactobacilli (meio de LMRS) e/ou ágar de Bifido. Alternativamente, *Bifidobactrium animalis ssp. lactis* é cultivada com o uso de meio de RCM ATCC 1053 e/ou ágar/caldo de soja de Tripticase com sangue de ovelha desfibrimado ATCC 260 de acordo com as instruções do fabricante.

[0048] A sequência de nucleotídeos da Cepa A de *Bifidobacterium animalis ssp. lactis* é mostrada na Tabela 1.

Tabela 1: Sequência de nucleotídeos da Cepa A de Bifidobacterium animalis ssp. lactis:

Sequência de Nucleotídeos
>1 comprimento=1944302 profundidade=1,00x circular=verdadeiro
CAACGATGCCTCATCCACAGCAGCCAACTCAAAGACATCCTTACACACTAACCTGAGAAGAGCCAACAAA
GGCATCCCATTGCAGCGCATCGCAGGCTTCCACGTCGACGGCGACGAGCAACGACTCGCCATGCTGCATC
TGCGCGGCGAACTGTCGATCGACAGCAATATGAAACGCCTGCTGCTGATTCGTGCGAACGAGCAGCTGGC
AGGGGGCCGTATCACCCGACCGCGTAGTCTCAATGCGGTGCCGCTCATTCCCGTACGTATACAGTCGGGG
TCGGATTTTGCGGATTGGCCTGCCGGTCAGTGCGATTACTGGGCGGAGCGCATAGGCAACTATGTGCTCA
CCCAATCCACCCGCAAATCGATGGAGGGACTCGACGACTTCCAGACGCGACGAGCCCGCATTCTGCGCAG
CCCAAGCTCACGCCAGTTCCCGTTGACGCGCGTGATCGGCGAACTTGAGAAGCTGACGCCGCAGTTCCTG
CAATTGCGGCAGCACCAGATCGTCGAGCTGATCGCACAGGCGTGGAACATCAATCCGGGGGAGCTGGGAT
CCTTGGCAGAGCCGCAGGGGCAGTCCGATCGCACAGGCACATCCGCGCATCGCAGTCGACGCACGTCGAA
GCGTGTGACGGTGGGCGAAGTGGTGCGCGCCGGTCTGCTCACGGTGGGGGACCGATTCGTATGGAACCGC
CCACGCAAGCACGAGATCTGGCGTATCACCGTCACCGAGTCTGGGTTCCGGGGCGAGGACGGCACCGAAT
ATGCGACGCCGACGTCCGCCGCCCGCGCCATCGGAGGTTCCTCGGCGAGCCTGAACGTATGGAAGCGGGA
ATCCGACGGCCGTGCATTGAGCGATATCTGGAAGACCTATCGCACCTCGATGTGATGGTCATGGATGTGA

CGGTCATGCGACGTCAGCCATTCGATGGCGAGCTTCGTGCGGTTCGAGATGTGCGTCTTCGCCAGAATGC
CGCTGATGCGGTTGCGCACGGTGCCCTCGCTCAGGAACAGCTTCGCCGCAATCTCACGATTGTCGAGCTC
CTCGGCGACCAGCTCGACGATGTCATGCTCCCGGTCGGTGAGATGCGCGAATCTGGGATCATCCGGTAAT
GGGGACGCCTTTGGTTCCTCCACATTCCCGGACGCATCGTGCATCGGCAGGTTGCGCAGCACGTCCGCGC
CCAACACCACCTGCCCGGCCATGACCGCCTGCACTGCTGGAATCACCGAAGCCACGTCCTGTTTGATGAG
ATAGCCTTTCGTGCCCAATTCGATGGCACGGCGAATGTACTCCGGGTCGGCGAAAGTGGTGAGCACGAGA
ATGCGCTCGCTGGCTTCAAGGCCGTCGGTGCCCGGCATCTGCACGTCGATGAGCAACACATCCGGTTTCT
GACGTGCATAGGCCACCACGGCGGCGGCCCCGTCATGCGCAGTCCACACGACCTCGGCGGTGCCGGTGGT
CGTGAGGATCGTGCCGATGGACGAGCAGACAATAGGGTCATCGTCAACAATCGCGACCTTCATCGCATAT
TCCTTTCCGCTGTTTTCGGGACGGACATGAACACGCGCCAGCCGCCGTTATGGTAGCCGCAGGTGGCTGT
GCCATCCAATGCGCGCGCCCGTTCCTCGATGTCGGCGATTCCAATGCCCATGCTCGCCACGCGGCCGCGT
TCCGGTACGCCGCCGTTGCCCTGCACGATCAGCTGCCACAACCCCGGCAAATCAATCAGATGCACGTGGG
CCTCAGAAGCTGCGCTATGCCGGATTGTGTTCGTCAACGCTCCGCGAATCACGGCCGTGAATGCATGTGC
CACATGGGAAGGCACGGCATCGATGGCATTGGAGAGGCGAATCTCGACATCAGATTGCTTGGCGGTGGCC
ATGGCATCCTCGACCATCGCCCGAAAATCAGTGCCTTCGTCTTTGAGACCATGCACGGATTTGCGAATCA
TCGTCATCGCCTCGTCGAGGGTATGACCAAGCTGCCCGAATTGATCGGCATGCAGCGTGTCACCGGTGCT
GGTGGCAATCACTTGGTCCGCATTCGTCAGCATGATCGCACGAGTGAGCAGATGTCCCACATTGTCGTGG
ATCTCGCGTGCGATGCGTGTGCGTTCGGTGAGCCGCGCATGCTGCAGGTCCGCCGACCTGGCGGATTCGA
CATCGGCGAGACGAGCGCGCGAACGGCGGATACGTTCCCGTTGGTCGTCTGCCAACGATTGGAAGCACCG
TTGTCTTCGTTGCGCGAGTGCGAATACCCAGCCGCCGAGAACTGCGAGGGAGGTGAGCGCACATAGAATC
CAAAGGGCCATCACGAGCGGCGAGGTGGCTGCGGCTGACAGATCGTGCAATGCCGGACTGTGCTGACCGG
CCACGATTCCGCAGACCAGTGGGGGGACGCAGACCAGCATTTGGAAAGACCTGCCCAACGACTGCACCCG
CAATCCCAGATCGAAGCCCACGATCGGCATCAGCGCGATCCATTGCGGAACAAACAGGCAGACCATGCAG
AACATCATGGCGAGTGCGATCCAGCATCTGAGGGGAGCCAGACGGATCAACGAGCAGAGGATCACGCAGA
ACAGCAGACCAATGACGAAGGTGGTATCGGTGTCGCCGCTCAGCATCACCGAGAACAGCACGCTGACGAC
GAACATCAGCGCATGAGCTCCCACGGAGATGATCATGCCGTTGTCTCTCCCCTCCTATTCTGTTCCATTG
TTCATGACAACAGGGCGAATGTCATGCCGAATGGTGACAAATGTCACCATTCGTACAAAAACGGTGATTC
CTGGCACCGTGTCGGCCGTTGCCTGTTCGGCAATATGGAGGCATGAACACAGCAGAGAACAACGTACAGC
GCGATGCCGCACCAGCGATCGAGGTGCGCGAACTGGTGAAACGATATGCCGACAAGCTCGCGCTCGACTA
TTTGAACATGAACGTGCGCACTGGGGAGATTTACGGATTGCTGGGGCCGAACGGTTCCGGCAAGACCACC
GCGATCAACTGCATTCTCGGGTTGCTCACCTATGATTCGGGCCAAATCCGTGCATTCGGCGGCAAGATGG
GCCCCACCGCCTACGACCTGAAGCGCAGGATCGGCGTGGTGCCTCAGGACGTGGCTGTATTCAACGAGCT
CACCGTGCAGGAGAACATCGAGTACTTCTGCTCGCTGTACGTCGCTGACAAGATGAGACGTGCGCGGCTG
GTGGACGAAGCAATCGAATTCGTGGGACTCGAGGATTACCGCAGATTCCGCCCGCGCAAGCTCTCAGGAG
GTCTGCCGCGCCGATTGAACATTGCATGCGGCATAGTGCACCAACCCGAACCCATCTTCTTCGACGAGCC
GACGGTGGCGGTGGATCCGCAAAGCCGCAACTCGATTCTCGAAGGCATCAAACGGCTCAACGAGCGTGGC
GCGACCGTCGTGTACACGAGCCATTACATGGAGGAGGTCGAGCAGATCTGCGACCGCATCCAGATCATAG
ATCACGGCCGTCAGATCGCCATCGGCACGGCCGACGAGCTCAAGCGCATGATCGAGGCCGGCGAGCGAAT
CACCGTCGAAGCGCCGGCGTTGAGCGCGAATGAGGCCCTGTGCCAACGGGCCGTCTCGATGTTGGGTGAC

TTGGAGGCTGTGCACGAGGTGACGTTCGAGAACGACGAGCTCGTGGTGGTCTGTTCGCAGGGCGAGCACA
ATCTCATCGATGTGCTGCAGGTGCTGCGCTCGCTCGACATCCCCATGGGGCATGTGAGCTCCCGTGCGCC
GACGTTGAACGACGTGTTCCTCGCCCTCACCGGGACCGACCTGCGCGATTGAGCGTGCGGCACATACGAC
GAAGGAGGTGAATACATGTGGAGTACATTCCTCAACACCATGCGCGTGAATCTGCGTGAGAAGAGTTCGG
TGTTCTGGCTCTTCTGCTTCCCGATCATTCTGTCGACGATGTTCATGGGCATGTTCGGCAACATCGGCGA
GGTCTATGAAATACATACGATGAGGTTCGCAGTGGTCGCGGACGCGAACTTCTGCAGGGCGACCAACGCG
CGTCAGATGCTGCATGCGATGCAGCGGAATCCACTGCGGAACGAGGGCTGCGGGAACGCCATGGAAGGCG
CTGCGCCCGATACGGCGTCCGGGCAGGTGGGTTCCGAGCTCAGGAACCTGTTGGAATTGCAGGCCGTGTC
AAACGTCAAGGAGGCCAAGGGCCTCATCAACGCCGACCACGGGGTGCGTGGCTACATCAACGCCACGGAA
GACGGGCTGCTGAACCTGACGATCAGTCGTGCCAGCGTCGCATCCGTCAACGATGACACCGGAAATTCCG
GCTTCAGTGCATCCCTCAACATTCTGCATGGTGCCGTAGGCATGTTCAATAGGCGAGCCATCACCGTGAC
CGAGCTGCTCAACACCGATTCGGGCGTGTTCCGTGATGCCACGTTCACCAAGGAGATGGGCGACACCCCG
ACGTTCACGAAAGTTGTGAGCCCCACCCATTTCAAGCCGACCGAATCCGTCCGATTCTACTATGCGCTGC
TCGCCATGACCGCACTCATGTCGATGACCTTCGCCACCACGACCGTGTGCGCCACGCAGGCGAATCTTTC
GGCGCTTGGTATGAGACGGTCGCTGGCCCCGCTCTCCAAGTTCAACCAGCTCATGGGAGGTTTCCTCGCC
AGCTGGCTATGCGCATCGGTGTCGATGGTGGTTGCGACGCTCTACGTCCATTTCGTGTGCAAGGTCGATT
TCGGCGGCCGTTGGCCCGTGCTCGTCCTGGCGCTGCTGCTCGCGTCCCTTACCGCCAATGCGCTCGGCAC
GCTCATAGGATCGTTGCCGAAGCTCACTTCCGGCGTGAAAATAGGCATCACCACCGCGCTGTCGTGCGTG
CTCTCGATATTCAGTGGACTCTACGGCACCGGCGCAATGAACCTGAGCGACTGGACCCAACGCAACGCCC
CCGGATTCGCCATCGTCAATCCCACCCAGCAGATCACGAACCTGTTCTACGACTTGCTCCACTACGATTC
CTACGCCCCGTCCCTACGCACGTCGGGCATTCTCCTCGCAATGACCGTGCTCGCTCTGACACTGTCCACG
ATCTTCCTCAGGAGGCAACGCTATGAGCACCTTTAGAACATGCTGGCGAGTCCTGTTCGCCCAACGAACC
ATGATCCTCGTCTACATCGTCTACCTGAGCCTCATGATGTTCGGGCTGTCCTGGAGCATCATCCAGAACC
TTTCGAACACCGACAACTCCACCACCTATGAGACGCTGCGACCCGAAGTGGCGGTGGTCGACCGGAATGG
GGGCGTCGGCTCAGGGTTCTCGGATTCGCTGAGGAAATTCCTGTCCAAGGACTGCGACCTCGTCGATGTG
GGCACCACCTCCCAGGACCTGCAGACCGCTGCGGCCTCAAACTATGCCGACCTCATCGTCATCATTCCCG
AACACTATGTCGATGACCTGGCTGCGGCATTCACCTCGGATTCCGCCGTCCCCGAGCTGCAGATGGTCAC
CAGCTTCACCGGAGCCTACGGCTCCCTCGCCAAACTGCAGGTGGAGGATTTCCTGAACCTCACCCGGATG
ACTGCGCTTGCAGATGCAGCGTCGTCGGGTTCCTCCGTTAGCGTCGCCTCATTGAATGCGGCGGCCGGTT
CGGTGATCGCGGATCTTACCGGCAATGCCGACGCCTACCCGCAGATCGCCATCGCCGAGGACCCGGCAGG
GACCGACGACCGGGAGGCGAACGGACGGTACGCGTTCCAGACGATGATGGACATGGGAGCCTATCCGATG
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Composições Bacterianas

[0049] Em certos aspectos, são fornecidas no presente documento composições bacterianas compreendendo *Bifidobacterium animalis ssp. Lactis*. Em algumas modalidades, a *Bifidobacterium animalis ssp. lactis* é a Cepa A de *Bifidobacterium animalis ssp. lactis* (Número de Depósito de ATCC PTA-125097). Em algumas modalidades, a composição bacteriana compreende uma PhAB produzida a partir de ou compreendendo uma *Bifidobacterium animalis ssp. lactis* descrita no presente documento. Em algumas modalidades, a *Bifidobacterium animalis ssp. lactis* é uma cepa compreendendo pelo menos 99% de identidade de sequência (*por exemplo,* pelo menos 99,5% de identidade de sequência, pelo menos 99,6% de identidade de sequência, pelo menos 99,7% de identidade de sequência, pelo menos 99,8% de identidade de sequência, pelo menos 99,9% de identidade de sequência) com a sequência de nucleotídeos da Cepa A de *Bifidobacterium animalis ssp. lactis*. Em algumas modalidades, a formulação bacteriana compreende uma bactéria e/ou uma combinação de bactérias descritas no presente documento e um carreador farmaceuticamente aceitável.

[0050] Em certas modalidades, pelo menos 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% ou 99% das bactérias na composição bacteriana são *Bifidobacterium animalis ssp. lactis.* Em certas modalidades, substancialmente todas as bactérias na composição bacteriana são *Bifidobacterium animalis ssp. lactis.* Em certas modalidades, a composição bacteriana compreende pelo menos 1×10^3 unidades de formação de colônia (CFUs), 1×10^4 unidades de formação de colônia (CFUs), 1×10^5 unidades de formação de colônia (CFUs), 5×10^5 unidades de formação de colônia (CFUs), 1×10^6 unidades de formação de colônia (CFUs), 2×10^6 unidades de formação de colônia

(CFUs), 3 x 10^6 unidades de formação de colônia (CFUs), 4 x 10^6 unidades de formação de colônia (CFUs), 5 x 10^6 unidades de formação de colônia (CFUs), 6 x 10^6 unidades de formação de colônia (CFUs), 7 x 10^6 unidades de formação de colônia (CFUs), 8 x 10^6 unidades de formação de colônia (CFUs), 9 x 10^6 unidades de formação de colônia (CFUs), 1 x 10^7 unidades de formação de colônia (CFUs), 2 x 10^7 unidades de formação de colônia (CFUs), 3 x 10^7 unidades de formação de colônia (CFUs), 4 x 10^7 unidades de formação de colônia (CFUs), 5 x 10^7 unidades de formação de colônia (CFUs), 6 x 10^7 unidades de formação de colônia (CFUs), 7 x 10^7 unidades de formação de colônia (CFUs), 8 x 10^7 unidades de formação de colônia (CFUs), 9 x 10^7 unidades de formação de colônia (CFUs), 1 x 10^8 unidades de formação de colônia (CFUs), 2 x 10^8 unidades de formação de colônia (CFUs), 3 x 10^8 unidades de formação de colônia (CFUs), 4 x 10^8 unidades de formação de colônia (CFUs), 5 x 10^8 unidades de formação de colônia (CFUs), 6 x 10^8 unidades de formação de colônia (CFUs), 7 x 10^8 unidades de formação de colônia (CFUs), 8 x 10^8 unidades de formação de colônia (CFUs), 9 x 10^8 unidades de formação de colônia (CFUs), 1 x 10^9 unidades de formação de colônia (CFUs), 5 x 10^9 unidades de formação de colônia (CFUs), 1 x 10^{10} unidades de formação de colônia (CFUs) 5 x 10^{10} unidades de formação de colônia (CFUs), 1 x 10^{11} unidades de formação de colônia (CFUs) 5 x 10^{11} unidades de formação de colônia (CFUs), 1 x 10^{12} unidades de formação de colônia (CFUs) 5 x 10^{12} unidades de formação de colônia (CFUs), 1 x 10^{13} unidades de formação de colônia (CFUs) de *Bifidobacterium animalis ssp. lactis.*

[0051] Em algumas modalidades, as formulações probióticas contendo *Bifidobacterium animalis ssp. lactis* são fornecidas como formas encapsuladas, revestidas entéricas ou em pó, com doses que variam até 10^{11} cfu

(por exemplo, até 10^{10} cfu). Em algumas modalidades, a composição compreende 5 x 10^{11} cfu de *Bifidobacterium animalis ssp. lactis* e 10% (p/p) de amido de milho em uma cápsula. A cápsula é revestida entérica para liberação duodenal em pH 5,5. Em algumas modalidades, a cápsula é revestida entérica para liberação duodenal em pH 5,5. Em algumas modalidades, a composição compreende um pó de *Bifidobacterium animalis ssp. lactis* seco por congelamento que é considerada a situação "Suposição Qualificada de Segurança" (QPS). Em algumas modalidades, a composição é estável em temperatura congelada ou refrigerada.

[0052] Os métodos para produzir composições microbianas podem incluir três etapas de processamento principais. As etapas são: depósito de organismo, produção de organismo e preservação. Em certas modalidades, uma amostra que contém uma abundância de *Bifidobacterium animalis ssp. lactis* pode ser cultivada evitando-se uma etapa de isolamento.

[0053] Para o depósito, as cepas incluídas na composição microbiana podem ser (1) isoladas diretamente a partir de um espécime ou tomadas a partir de um estoque depositado, (2) opcionalmente cultivadas em um ágar de nutriente ou caldo que suporta o crescimento para gerar biomassa viável e (3) a biomassa opcionalmente preservada em múltiplas alíquotas em armazenamento a longo prazo.

[0054] Em modalidades com o uso de uma etapa de cultivo, o ágar ou caldo pode conter nutrientes que fornecem elementos essenciais e fatores específicos que possibilitam o crescimento. Um exemplo seria um meio composto por 20 g/l de glicose, 10 g/l de extrato de levedura, 10 g/l de peptona de soja, 2 g/l de ácido cítrico, 1,5 g/l de fosfato de sódio monobásico, 100 mg/l de citrato de amônio férrico, 80 mg/l de sulfato de magnésio, 10 mg/l de cloreto de hemina, 2 mg/l de cloreto de cálcio, 1 mg/l de menadiona. Outro exemplo seria um meio composto por 10 g/l de extrato de bife,

10 g/l de peptona, 5 g/l de cloreto de sódio, 5 g/l de dextrose, 3 g/l de extrato de levedura, 3 g/l de acetato de sódio, 1 g/l de amido solúvel e 0,5 g/l de HCl de l-cisteína a pH 6,8. Uma variedade de meios microbiológicos e variações são bem conhecidas na técnica (por exemplo, R.M. Atlas, *Handbook of Microbiological Media* (2010) CRC Press). O meio de cultura pode ser adicionado à cultura no início, pode ser adicionado durante a cultura ou pode ser fluído de modo intermitente/contínuo através da cultura. As cepas na composição bacteriana podem ser cultivadas sozinhas, como um subconjunto da composição microbiana ou como uma coleção inteira compreendendo a composição microbiana. Como um exemplo, uma primeira cepa pode ser cultivada juntamente com uma segunda cepa em uma cultura contínua misturada, a uma taxa de diluição inferior à taxa de crescimento máximo de uma das células para impedir que a cultura seja lavada para fora do cultivo.

[0055] A cultura inoculada é incubada mediante condições favoráveis por um tempo suficiente para construir biomassa. Para as composições microbianas para uso humano, isso é frequentemente à temperatura de 37°C, pH e outros parâmetros com valores similares ao nicho humano normal. O ambiente pode ser controlado ativamente, controlado passivamente (por exemplo, por meio de tampões), ou pode ficar à deriva. Por exemplo, para composições bacterianas anaeróbicas, um ambiente anóxico/de redução pode ser empregado. Isso pode ser alcançado por adição de agentes de redução como cisteína ao caldo e/ou privando o mesmo de oxigênio. Como um exemplo, uma cultura de uma composição bacteriana pode ser cultivada a 37°C, pH 7, no meio acima, pré-reduzida com 1 g/l de HCl de cisteína.

[0056] Quando a cultura tiver gerado biomassa suficiente, a mesma pode ser preservada para depósito. Os organismos podem ser colocados

em um meio químico que protege contra congelamento (adicionando "crioprotetores”), secagem ("lioprotetores”) e/ou choque osmótico ("osmoprotetores”), dispensados em múltiplos recipientes (opcionalmente idênticos) para criar um banco uniforme e, então, tratando a cultura para preservação. Os recipientes são geralmente impermeáveis e têm fechos que asseguram o isolamento do ambiente. O tratamento de criopreservação é alcançado congelando-se um líquido em temperaturas ultrabaixas (por exemplo, a ou abaixo de - 80°C). A preservação seca remove água da cultura por evaporação (no caso de secagem por aspersão ou "secagem fria”) ou por sublimação (por exemplo, para secagem por congelamento, secagem por congelamento por aspersão). A remoção de água aprimora a estabilidade de armazenamento de composição microbiana a longo prazo em temperaturas elevadas acima de condições criogênicas. Se a composição microbiana compreende, por exemplo, espécies de formação de esporo e resulta na produção de esporos, a composição final pode ser purificada por meios adicionais como densidade centrifugação de gradiente e preservada com o uso das técnicas [?]descritas acima[?]. O depósito de composição microbiana pode ser realizado cultivando-se e preservando-se as cepas individualmente, ou misturando-se as cepas em conjunto para criar um banco combinado. Como um exemplo de criopreservação, uma composição microbiana cultura pode ser colhida através de centrifugação para peletizar as células do meio de cultura, o sobrenadante decantado e substituído por caldo de cultura fresco contendo 15% de glicerol. A cultura pode, então, ser aliquotada em criotubos de 1 ml, vedada e colocada em - 80°C para retenção de viabilidade a longo prazo. Esse procedimento alcança a viabilidade aceitável mediante a recuperação do armazenamento congelado.

[0057] A produção microbiana pode ser conduzida com o uso de eta-

pas de cultura similares ao depósito, incluindo o meio composição e condições de cultura descritos acima. Pode ser conduzida em maiores escalas de operação, especialmente para desenvolvimento clínico ou produção comercial. Em escalas maiores, pode haver vários subcultivos da composição microbiana antes do cultivo final. No final do cultivo, a cultura é colhida para possibilitar a formulação adicional em uma forma de dosagem para administração. Isso pode envolver a concentração, remoção de componentes de meio indesejáveis e/ou introdução em um meio químico que preserva a composição microbiana e torna a mesma aceitável para administração por meio da via escolhida. Por exemplo, uma composição microbiana pode ser cultivada a uma concentração de 10^{10} CFU/ml, então, concentrada 20 vezes por microfiltração de fluxo tangencial; o meio gasto pode ser trocado por diafiltração com um meio preservativo que consiste em 2% de gelatina, 100 mM de trealose e 10 mM de tampão de fosfato de sódio. A suspensão pode, então, ser seca por congelamento a um pó e titulada.

[0058] Após a secagem, o pó pode ser mesclado a uma potência adequada, e misturado com outras culturas e/ou uma carga como celulose microcristalina para consistência e facilidade de manuseio, e a composição bacteriana formulada como fornecida no presente documento.

[0059] Em certos aspectos, são fornecidas composições bacterianas para administração em indivíduos. Em algumas modalidades, as composições bacterianas são combinadas com materiais ativos e/ou inativos adicionais para produzir um produto final, que pode ser em unidade de dosagem única ou em um formato de múltiplas doses.

[0060] Em algumas modalidades, a composição compreende pelo menos um carboidrato. Um "carboidrato" se refere a um açúcar ou polímero de açúcares. Os termos "sacarídeo", "polissacarídeo", "carboidrato" e "oligossacarídeo" podem ser usados de modo intercambiável. A maioria dos

carboidratos são aldeídos ou cetonas com muitos grupos hidroxila, geralmente um em cada átomo de carbono da molécula. Os carboidratos geralmente têm a fórmula molecular $C_nH_{2n}O_n$. Um carboidrato pode ser um monossacarídeo, um dissacarídeo, trissacarídeo, oligossacarídeo ou polissacarídeo. O carboidrato mais básico é um monossacarídeo, como glicose, sacarose, galactose, manose, ribose, arabinose, xilose e frutose. Os dissacarídeos são dois monossacarídeos unidos. Os dissacarídeos exemplificativos incluem sacarose, maltose, celobiose e lactose. Tipicamente, um oligossacarídeo inclui entre três e seis unidades de monossacarídeo (por exemplo, rafinose, estaquiose) e polissacarídeos incluem seis ou mais unidades de monossacarídeo. Os polissacarídeos exemplificativos incluem amido, glicogênio e celulose. Os carboidratos podem conter unidades de sacarídeo modificadas como 2'-desoxirribose em que um grupo hidroxila é removido, 2'-fluororribose em que um grupo hidroxila é substituído por um flúor, ou N-acetilglucosamina, uma forma contendo nitrogênio de glicose (por exemplo, 2'-fluororribose, desoxirribose e hexose). Os carboidratos podem existir em muitas formas diferentes, por exemplo, conformadores, formas cíclicas, formas acíclicas, estereoisômeros, tautômeros, anômeros e isômeros.

[0061] Em algumas modalidades, a composição compreende pelo menos um lipídio. Como usado no presente documento, um "lipídio" inclui gorduras, óleos, triglicerídeos, colesterol, fosfolipídeos, ácidos graxos em qualquer forma incluindo ácidos graxos livres. As gorduras, óleos e ácidos graxos podem ser saturados, insaturados (cis ou trans) ou parcialmente insaturados (cis ou trans). Em algumas modalidades, o lipídio compreende pelo menos um ácido graxo selecionado de ácido láurico (12:0), ácido mirístico (14:0), ácido palmítico (16:0), ácido palmitoleico (16:1), ácido margárico (17:0), ácido heptadecenoico (17:1), ácido esteárico (18:0), ácido

oleico (18:1), ácido linoleico (18:2), ácido linolênico (18:3), ácido octadeca-tetraenoico (18:4), ácido araquídico (20:0), ácido eicosenoico (20:1), ácido eicosadienoico (20:2), ácido eicosatetraenoico (20:4), ácido eicosapentae-noico (20:5) (EPA), ácido docosanoico (22:0), ácido docosenoico (22:1), ácido docosapentaenoico (22:5), ácido docosa-hexaenoico (22:6) (DHA) e ácido tetracosanoico (24:0). Em algumas modalidades, a composição com-preende pelo menos um lipídio modificado, por exemplo, um lipídio que foi modificado por cozimento.

[0062] Em algumas modalidades, a composição compreende pelo me-nos um mineral suplementar ou fonte mineral. Os exemplos de minerais incluem, sem limitação: cloreto, sódio, cálcio, ferro, cromo, cobre, iodo, zinco, magnésio, manganês, molibdênio, fósforo, potássio e selênio. As formas adequadas de qualquer um dos minerais supracitados incluem sais minerais solúveis, sais minerais ligeiramente solúveis, sais minerais inso-lúveis, minerais quelados, complexos minerais, minerais não reativos como minerais carbonila e minerais reduzidos e combinações dos mesmos.

[0063] Em algumas modalidades, a composição compreende pelo me-nos uma vitamina suplementar. A pelo menos uma vitamina pode ser vita-minas solúveis em gordura ou solúveis em água. As vitaminas adequadas incluem, mas sem limitações, vitamina C, vitamina A, vitamina E, vitamina B12, vitamina K, riboflavina, niacina, vitamina D, vitamina B6, ácido fólico, piridoxina, tiamina, ácido pantotênico e biotina. As formas adequadas de qualquer um dos supracitados são sais da vitamina, derivados da vitamina, compostos que têm atividade igual ou similar da vitamina e metabólitos da vitamina.

[0064] Em algumas modalidades, a composição compreende um exci-piente. Os exemplos não limitantes de excipientes adequados incluem um

agente de tamponamento, um conservante, um estabilizante, um aglutinante, um agente de compactação, um lubrificante, um intensificador de dispersão, um agente de desintegração, um agente aromatizante, um edulcorante e um agente colorante.

[0065] Em algumas modalidades, o excipiente é um agente de tamponamento. Os exemplos não limitantes de agentes de tamponamento adequados incluem citrato de sódio, carbonato de magnésio, bicarbonato de magnésio, carbonato de cálcio e bicarbonato de cálcio.

[0066] Em algumas modalidades, o excipiente compreende um conservante. Os exemplos não limitantes de conservantes adequados incluem antioxidantes, como alfa-tocoferol e ascorbato e antimicrobianos, como parabenos, clorobutanol e fenol.

[0067] Em algumas modalidades, a composição compreende um aglutinante como um excipiente. Os exemplos não limitantes de aglutinantes adequados incluem amidos, amidos pré-gelatinizados, gelatina, polivinilpirrolidona, celulose, metilcelulose, carboximetilcelulose de sódio, etilcelulose, poliacrilamidas, poliviniloxoazolidona, álcoois polivinílicos, álcool de C_{12}-C_{18} ácido graxo, polietilenoglicol, polióis, sacarídeos, oligossacarídeos e combinações dos mesmos.

[0068] Em algumas modalidades, a composição compreende um lubrificante como um excipiente. Os exemplos não limitantes de lubrificantes adequados incluem estearato de magnésio, estearato de cálcio, estearato de zinco, óleos vegetais hidrogenados, esterotex, monoestearato de polioxietileno, talco, polietilenoglicol, benzoato de sódio, sulfato de laurila de sódio, sulfato de laurila de magnésio e óleo mineral claro.

[0069] Em algumas modalidades, a composição compreende um intensificador de dispersão como um excipiente. Os exemplos não limitantes

de dispersantes adequados incluem amido, ácido algínico, polivinilpirrolidonas, goma guar, caulim, bentonita, celulose de madeira purificada, glicolato de amido de sódio, silicato isoamorfo e celulose microcristalina como tensoativos emulsificantes de HLB alto.

[0070] Em algumas modalidades, a composição compreende um desintegrante como um excipiente. Em algumas modalidades, o desintegrante é um desintegrante não efervescente. Os exemplos não limitantes de desintegrantes não efervescentes adequados incluem amidos como amido de milho, amido de batata, amidos pré-gelatinizados e modificados dos mesmos, edulcorantes, argilas, como bentonita, celulose microcristalina, alginatos, glicolato de amido de sódio, gomas como ágar, guar, alfarroba, caraia, pectina e tragacanto. Em algumas modalidades, o desintegrante é um desintegrante efervescente. Os exemplos não limitantes de desintegrantes efervescentes adequados incluem bicarbonato de sódio em combinação com ácido cítrico e bicarbonato de sódio em combinação com ácido tartárico.

[0071] Em algumas modalidades, a formulação bacteriana compreende um revestimento entérico ou microencapsulamento. Em certas modalidades, o revestimento entérico ou microencapsulamento aprimora o alvejamento a uma região desejada do trato gastrointestinal. Por exemplo, em certas modalidades, a composição bacteriana compreende um revestimento entérico e/ou microcápsulas que dissolvem a um pH associado a uma região particular do trato gastrointestinal. Em algumas modalidades, o revestimento entérico e/ou microcápsulas dissolvem a um pH de cerca de 5,5 a 6,2 para liberação no duodeno, a um valor de pH de cerca de 7,2 a 7,5 para liberação no íleo e/ou a um valor de pH de cerca de 5,6 a 6,2 para liberação no cólon. Os revestimentos entéricos exemplificativos e microcápsulas são descritos, por exemplo, na Publicação de Patente US nº

2016/0022592, que é aqui incorporada a título de referência em sua totalidade.

[0072] Em algumas modalidades, a composição é um produto alimentício (por exemplo, um alimento ou bebida) como um alimento ou bebida para saúde, um alimento ou bebida para recém-nascidos, um alimento ou bebida para mulheres gestantes, atletas, cidadãos idosos ou outro grupo especificado, um alimento funcional, uma bebida, um alimento ou bebida para uso para a saúde especificado, um suplemento dietético, um alimento ou bebida para pacientes ou uma ração para animal. Os exemplos específicos dos alimentos e bebias incluem várias bebidas como sucos, bebidas refrescantes, bebidas de chá, preparações de coquetel, bebidas gelatinosas e bebidas funcionais; bebidas alcoólicas como cervejas; alimentos contendo carboidrato como produtos alimentícios de arroz, macarrões, pães e massas; produtos de pasta como presuntos de peixe, salsichas, produtos de pasta de frutos-do-mar; produtos de embalagens esterilizáveis como caril, alimento temperadas com um molho de amido espesso e sopas chinesas; sopas; produtos de laticínio como leite, bebidas de laticínio, sorvetes, queijos e iogurtes; produtos fermentados como pastas de soja fermentadas, iogurtes, bebidas fermentadas e picles; produtos de feijão; vários produtos de confeitaria, incluindo biscoitos, bolacha e similares, doces, gomas de mascar, gomosas, sobremesas geladas incluindo gelatinas, caramelos cremosos e sobremesas congeladas; alimentos instantâneos como sopas instantâneas e sopas de soja instantâneas; alimentos de micro-ondas; e similares. Adicionalmente, os exemplos também incluem alimentos para saúde e bebidas preparadas nas formas de pós, grânulos, tabletes, cápsulas, líquidos, pastas e gelatinas.

[0073] Em certas modalidades, as bactérias reveladas no presente documento são administradas em conjunto com um pré-biótico ao indivíduo.

Os pré-bióticos são carboidratos que são geralmente digeríveis por um animal hospedeiro e são seletivamente fermentados ou metabolizados por bactérias. Os pré-bióticos podem ser carboidratos de cadeia curta (por exemplo, oligossacarídeos) e/ou açúcares simples (por exemplo, monossacarídeos e dissacarídeos) e/ou mucinas (proteínas pesadamente glicosiladas) que alteram a composição ou o metabolismo de um microbioma no hospedeiro. Os carboidratos de cadeia curta também são chamados de oligossacarídeos e geralmente contêm de 2 ou 3 e até 8, 9, 10, 15 ou mais porções químicas de açúcar. Quando os pré-bióticos são introduzidos a um hospedeiro, os pré-bióticos afetam as bactérias dentro do hospedeiro e não afetam diretamente o hospedeiro. Em certos aspectos, uma composição de pré-biótico pode estimular seletivamente o crescimento e/ou a atividade de um dentre um número limitado de bactérias em um hospedeiro. Os pré-bióticos incluem oligossacarídeos como fruto-oligossacarídeos (FOS) (incluindo inulina), galacto-oligossacarídeos (GOS), trans-galacto-oligossacarídeos, xilo-oligossacarídeos (XOS), quito-oligossacarídeos (COS), oligossacarídeos de soja (por exemplo, estaquiose e rafinose) gentio-oligossacarídeos, isomalto-oligossacarídeos, mano-oligossacarídeos, malto-oligossacarídeos e mananoligossacarídeos. Os oligossacarídeos não são necessariamente componentes únicos e podem ser misturas contendo oligossacarídeos com diferentes graus de oligomerização, por vezes incluindo o dissacarídeo parental e os açúcares monoméricos. Vários tipos de oligossacarídeos são encontrados como componentes naturais em muitos alimentos comuns, incluindo frutas, vegetais, leite e mel. Os exemplos específicos de oligossacarídeos são lactulose, lactossacarose, palatinose, sacarose de glicosila, goma guar, goma arábica, tagalose, amilose, amilopectina, pectina, xilano e ciclodextrinas. Os pré-bióticos também podem ser purificados ou química ou enzimaticamente sintetizados.

Produção de PhABs

[0074] Em certos aspectos, os PhABs descritos no presente documento podem ser preparados com o uso de qualquer método conhecido na técnica.

[0075] Em algumas modalidades, as PhABs descritas no presente documento são preparadas por fracionamento. As células e/ou sobrenadantes bacterianos de células bacterianas cultivadas são fracionados em várias biomassas farmacologicamente ativas (PhABs) e/ou produtos derivados das mesmas. As células e/ou sobrenadantes bacterianos são fracionados com o uso de materiais e métodos conhecidos na técnica (consultar, por exemplo, Sandrini et al. Fractionation by ultracentrifugation of gram negative cytoplasmic and membrane proteins. 2014. Bio-Protocol. 4(21); Scholler et al. Protoplast and cytoplasmic membrane preparations from Streptococcus sanguis and Streptococcus mutans. 1983. J Gen Micro. 129: 3271 a 3279; Thein et al. Efficient subfractionation of gram-negative bacteria for proteomics studies. 2010. Am Chem Society. 9: 6135 a 6147; Hobb et al. Evaluation of procedures for outer membrane isolation from Campylobacter jejuni. 2009. 155(Pt. 3): 979 a 988).

[0076] Adicionalmente, as PhABs obtidas por métodos fornecidos no presente documento podem ser adicionalmente purificadas por cromatografia de coluna com base em tamanho, por cromatografia de afinidade e por ultracentrifugação de gradiente, com o uso de métodos que podem incluir, mas sem limitações, o uso de um gradiente de sacarose ou gradiente de Optiprep. Brevemente, com o uso de um método de gradiente de sacarose, se a precipitação de sulfato de amônio ou ultracentrifugação foram usados para concentrar os sobrenadantes filtrados, os péletes são ressuspensos em 60% de sacarose, 30 mM de Tris, pH 8,0. Se a filtração foi usada para concentrar o sobrenadante filtrado, o concentrado é tampão

trocado em 60% de sacarose, 30 mM de Tris, pH 8,0, com o uso de uma coluna Amicon Ultra. As amostras são aplicadas a um gradiente de sacarose descontínuo de 35 a 60% e centrifugado a 200.000 x g por 3 a 24 horas a 4°C. Brevemente, com o uso de um método de gradiente de Optiprep, se a precipitação de sulfato de amônio ou ultracentrifugação foram usados para concentrar os sobrenadantes filtrados, os péletes são ressuspensos em 35% de Optiprep em PBS. Em algumas modalidades, se a filtração foi usada para concentrar o sobrenadante filtrado, o concentrado é diluído com o uso de 60% de Optiprep a uma concentração final de 35% de Optiprep. As amostras são aplicadas a um gradiente de sacarose descontínuo de 35 a 60% e centrifugadas a 200.000 x g por 3 a 24 horas a 4°C.

[0077] Em algumas modalidades, para confirmar a esterilidade e o isolamento das preparações de PhAB, as PhABs são serialmente diluídas em meio de ágar usado para cultura de rotina das bactérias sendo testadas e incubadas com o uso de condições de rotina. As preparações não estéreis são passadas através de um filtro de 0,22 um para excluir células intactas. Para aumentar adicionalmente a pureza, as PhABs isoladas podem ser DNase ou proteinase tratadas com K.

[0078] Em algumas modalidades, para a preparação de PhABs usadas para injeções *in vivo*, PhABs purificadas são processadas como descrito anteriormente (G. Norheim, et al. *PLoS ONE*. 10(9): e0134353 (2015)). Brevemente, após a centrifugação de gradiente de sacarose, bandas contendo PhABs são ressuspensas a uma concentração final de 50 µg/ml em uma solução contendo 3% de sacarose ou outra solução adequada para injeção *in vivo* conhecida por um elemento versado na técnica. Essa solução também pode conter adjuvante, por exemplo, hidróxido de alumínio a uma concentração de 0 a 0,5% (p/v).

[0079] Em certas modalidades, para produzir amostras compatíveis

com teste adicional (por exemplo, para remover sacarose antes da imaginologia de TEM ou ensaios *in vitro*), as amostras têm tampão trocado para PBS ou 30 mM de Tris, pH 8,0 com o uso de filtração (por exemplo, colunas Amicon Ultra), diálise ou ultracentrifugação (200.000 x g, ≥ 3 horas, 4 ºC) e ressuspensão.

[0080] Em algumas modalidades, a esterilidade das preparações de PhAB pode ser confirmada dispondo-se em placas uma porção das PhABs em meio de ágar usado para cultura padrão das bactérias usadas na geração das PhABs e incubando-se com o uso de condições padrão.

[0081] Em algumas modalidades, PhABs seletas são isoladas e enriquecidas por cromatografia e porções químicas de superfície de ligação em PhABs. Em outras modalidades, PhABs seletas são isoladas e/ou enriquecidas por classificação de célula fluorescente por métodos com o uso de reagentes de afinidade, corantes químicos, proteínas recombinantes ou outros métodos conhecidos por alguém versado na técnica.

Administração

[0082] Em certos aspectos, é fornecido no presente documento um método para entregar uma bactéria e/ou uma composição bacteriana descrita no presente documento a um indivíduo. Em algumas modalidades dos métodos fornecidos no presente documento, as bactérias são administradas em conjunto com a administração de um agente terapêutico de câncer. Em algumas modalidades, as bactérias são coformuladas em uma composição farmacêutica com o agente terapêutico de câncer. Em algumas modalidades, as bactérias são coadministradas com o agente terapêutico de câncer. Em algumas modalidades, o agente terapêutico de câncer é administrado ao indivíduo antes da administração das bactérias (por exemplo, cerca de 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25, 30, 35, 40, 45, 50 ou 55 minutos antes, cerca de 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16,

17, 18, 19, 20, 21, 22 ou 23 horas antes ou cerca de 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13 ou 14 dias antes). Em algumas modalidades, o agente terapêutico de câncer é administrado ao indivíduo após a administração das bactérias (por exemplo, cerca de 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25, 30, 35, 40, 45, 50 ou 55 minutos após, cerca de 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22 ou 23 horas após ou cerca de 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13 ou 14 dias após). Em algumas modalidades, o mesmo modo de entrega é usado para entregar tanto as bactérias quanto o agente terapêutico de câncer. Em algumas modalidades, diferentes modos de entrega são usados para administrar as bactérias e o agente terapêutico de câncer. Por exemplo, em algumas modalidades, as bactérias são administradas oralmente enquanto o agente terapêutico de câncer é administrado por meio de injeção (por exemplo, uma injeção intravenosa, intramuscular e/ou intratumoral).

[0083] Em certas modalidades, as composições farmacêuticas, formas de dosagem e kits descritos no presente documento podem ser administrados em conjunto com qualquer outro tratamento anticâncer convencional, como, por exemplo, terapia de radiação e resseção cirúrgica do tumor. Esses tratamentos podem ser aplicados como necessário e/ou como indicado e podem ocorrer antes, junto com ou após a administração das composições farmacêuticas, formas de dosagem e kits descritos no presente documento.

[0084] O regime de dosagem pode ser qualquer um dentre uma variedade de métodos e quantidades e pode ser determinado por alguém versado na técnica de acordo com fatores clínicos conhecidos. Como é conhecido na técnica medicinal, as dosagens para qualquer um paciente podem depender de muitos fatores, incluindo a espécie do indivíduo, tamanho, área de superfície corporal, idade, sexo, imunocompetência e saúde

geral, o micro-organismo particular a ser administrado, a duração e via de administração, o tipo e estágio da doença, por exemplo, tamanho de tumor e outros compostos como fármacos sendo administrados simultaneamente. Em adição aos fatores acima, tais níveis podem ser afetados pela infectividade do micro-organismo e a natureza do micro-organismo, como pode ser determinado por alguém versado na técnica. Nos presentes métodos, os níveis de dosagem mínimos adequados de micro-organismos podem ser níveis suficientes para que o micro-organismo sobreviva, cresça e se replique em um tumor ou metástase. Os métodos de tratamento descritos no presente documento podem ser adequados para o tratamento de um tumor primário, um tumor secundário ou metástase, assim como para tumores ou cânceres recorrentes. A dose das composições farmacêuticas descritas no presente documento pode ser adequadamente definida ou ajustada de acordo com a forma de dosagem, a via de administração, o grau ou estágio de uma doença-alvo e similares. Por exemplo, a dose eficaz geral dos agentes pode variar entre 0,01 mg/kg de peso corporal/dia e 1000 mg/kg de peso corporal/dia, entre 0,1 mg/kg de peso corporal/dia e 1000 mg/kg de peso corporal/dia, 0,5 mg/kg de peso corporal/dia e 500 mg/kg de peso corporal/dia, 1 mg/kg de peso corporal/dia e 100 mg/kg de peso corporal/dia, ou entre 5 mg/kg de peso corporal/dia e 50 mg/kg de peso corporal/dia. A dose eficaz pode ser 0,01, 0,05, 0,1, 0,5, 1, 2, 3, 5, 10, 20, 30, 40, 50, 60, 70, 80, 90, 100, 200, 500, ou 1000 mg/kg de peso corporal/dia ou mais, porém, a dose não é limitada aos mesmos.

[0085] Em algumas modalidades, a dose administrada a um indivíduo é suficiente para prevenir o câncer, atrasar seu início ou retardar ou interromper sua progressão ou prevenir um relapso de um câncer. Alguém versado na técnica irá reconhecer que a dosagem irá depender de uma vari-

edade de fatores incluindo a força do composto particular empregado, assim como a idade, espécie, condição e peso corporal do indivíduo. O tamanho da dose também será determinado pela via, temporização e frequência de administração, assim como a existência, natureza e extensão de quaisquer efeitos colaterais adversos que possam acompanhar a administração de um composto particular e o efeito fisiológico desejado.

[0086] As doses e regimes de dosagem adequados podem ser determinados por técnicas de busca de faixa convencional conhecidas por aqueles de habilidade comum na técnica. Geralmente, o tratamento é iniciado com dosagens menores, que são menores que a dose ideal do composto. Em seguida, a dosagem é aumentada por pequenos incrementos até que o efeito ideal sob as circunstâncias seja alcançado. Um protocolo de dosagem e tratamento eficaz pode ser determinado por meios de rotina e convencional, a partir, por exemplo, com uma baixa dose em animais de laboratório e, então, aumentar a dosagem enquanto monitora os efeitos e variando sistematicamente o regime de dosagem também. Os estudos de animais são comumente usados para determinar a dose tolerável máxima ("MTD") de agente bioativo por peso em quilograma. Aqueles versados na técnica extrapolam regularmente as doses para eficácia, enquanto evitam a toxicidade, em outra espécie, incluindo seres humanos.

[0087] De acordo com o supracitado, em aplicações terapêuticas, as dosagens dos agentes ativos usados de acordo com a invenção variam dependendo do agente ativo, a idade, peso e condição clínica do paciente recipiente, e a experiência e julgamento do clínico ou técnico administrando a terapia, dentre outros fatores que afetam a dosagem selecionada. Geralmente, a dose deve ser suficiente para resultar em retardamento e preferencialmente regressar o crescimento dos tumores e com máxima preferência, causando regressão completa do câncer.

[0088] As administrações separadas podem incluir qualquer número de duas ou mais administrações (por exemplo, doses), incluindo duas, três, quatro, cinco ou seis administrações. Alguém versado na técnica pode determinar prontamente o número de administrações a realizar, ou a conveniência de realizar uma ou mais administrações adicionais, de acordo com métodos conhecidos na técnica para monitorar métodos terapêuticos e outros métodos de monitoramento fornecidos no presente documento. Em algumas modalidades, as doses podem ser separadas por pelo menos 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29 ou 30 dias ou 1, 2, 3 ou 4 semanas. Consequentemente, os métodos fornecidos no presente documento incluem métodos para fornecer ao indivíduo uma ou mais administrações de uma bactéria, em que o número de administrações pode ser determinado através do monitoramento do indivíduo e, com base nos resultados do monitoramento, determinar a possibilidade de fornecer ou não uma ou mais administrações adicionais. A decisão quanto à possibilidade de fornecer ou não uma ou mais administrações adicionais pode ser baseada em uma variedade de resultados de monitoramento, incluindo, mas sem limitações, indicação de crescimento tumoral ou inibição de crescimento tumoral, aparecimento de novas metástases ou inibição de metástase, o título de anticorpo antibactéria do indivíduo, o título de anticorpo antitumor do indivíduo, a saúde geral do indivíduo e/ou o peso do indivíduo.

[0089] O período de tempo entre administrações pode ser qualquer um dentre uma variedade de períodos de tempo. O período de tempo entre administrações pode ser uma função de qualquer um dentre uma variedade de fatores, incluindo etapas de monitoramento, como descrito em relação ao número de administrações, ao período de tempo para que um indivíduo monte uma resposta imune e/ou o período de tempo para que um

indivíduo limpe as bactérias do tecido normal. Em um exemplo, o período de tempo pode ser uma função do período de tempo para que um indivíduo monte uma resposta imune; por exemplo, o período de tempo pode ser mais que o período de tempo para que um indivíduo monte uma resposta imune, como mais que cerca de uma semana, mais que cerca de dez dias, mais que cerca de duas semanas ou mais que cerca de um mês; em outro exemplo, o período de tempo pode ser menor que o período de tempo para que um indivíduo monte uma resposta imune, como menor que cerca de uma semana, menor que cerca de dez dias, menor que cerca de duas semanas, ou menos que cerca de um mês. Em outro exemplo, o período de tempo pode ser uma função do período de tempo para que um indivíduo elimine as bactérias do tecido normal; por exemplo, o período de tempo pode ser mais que o período de tempo para que um indivíduo elimine as bactérias do tecido normal, como mais que cerca de um dia, mais que cerca de dois dias, mais que cerca de três dias, mais que cerca de cinco dias ou mais que cerca de uma semana.

[0090] Em algumas modalidades, a entrega de um agente terapêutico de câncer em combinação com as bactérias descritas no presente documento reduz os efeitos adversos e/ou aprimora a eficácia do agente terapêutico de câncer.

[0091] A dose eficaz de um agente terapêutico de câncer descrito no presente documento é a quantidade do agente terapêutico que é eficaz para alcançar a resposta terapêutica desejada para um paciente, composição e modo particular de administração, com a menor toxicidade para o paciente. O nível de dosagem eficaz pode ser identificado com o uso dos métodos descritos no presente documento e irá depender de uma variedade de fatores farmacocinéticos incluindo a atividade das composições

particulares administradas, a via de administração, o tempo de administração, a taxa de excreção do composto particular sendo empregado, a duração do tratamento, outros fármacos, compostos e/ou materiais usados em combinação com as composições particulares empregadas, a idade, o sexo, peso, condição, saúde geral e histórico médico anterior do paciente sendo tratado e fatores similares bem conhecidos na técnica medicinal. Em geral, uma dose eficaz de uma terapia de câncer será a quantidade do agente terapêutico que é a dose mais baixa eficaz para produzir um efeito terapêutico. Tal dose eficaz geralmente irá depender dos fatores descritos acima.

[0092] A toxicidade de uma terapia de câncer é o nível de efeitos adversos experimentados pelo indivíduo durante e após o tratamento. Os eventos adversos associados à terapia de câncer toxicidade incluem, mas sem limitações, dor abdominal, indigestão ácida, refluxo ácido, reações alérgicas, alopécia, anafilaxia, anemia, ansiedade, falta de apetite, artralgias, astenia, ataxia, azotemia, perda de equilíbrio, dor óssea, hemorragia, coágulos sanguíneos, baixa pressão sanguínea, pressão sanguínea elevada, dificuldade para respirar, bronquite, hematomas, contagem baixa de glóbulos brancos, contagem baixa de hemácias, contagem baixa de plaquetas, cardiotoxicidade, cistite, cistite hemorrágica, arritmias, doença de válvula cardíaca, cardiomiopatia, doença da artéria coronária, catarata, neurotoxicidade central, disfunção cognitiva, confusão, conjuntivite, constipação, tosse, cãibra, cistite, trombose de veia profunda, desidratação, depressão, diarreia, tontura, boca seca, pele seca, dispepsia, dispneia, edema, desequilíbrio de eletrólito, esofagite, fadiga, perda de fertilidade, febre, flatulência, ruborização, refluxo gástrico, doença de refluxo fastroesofageal, dor genital, granulocitopenia, ginecomastia, glaucoma, perda de

cabelo, síndrome de mão-pé, dor de cabeça, perda de audição, insuficiência cardíaca, palpitações cardíacas, azia, hematoma, cistite hemorrágica, hepatotoxicidade, hiperamilasemia, hipercalcemia, hipercloremia, hiperglicemia, hipercalemia, hiperlipasemia, hipermagnesemia, hipernatremia, hiperfosfatemia, hiperpigmentação, hipertrigliceridemia, hiperuricemia, hipoalbuminemia, hipocalcemia, hipocloremia, hipoglicemia, hipocalemia, hipomagnesemia, hiponatremia, hipofosfatemia, impotência, infecção, reações de sítio de injeção, insônia, deficiência de ferro, coceira, dor de articulação, insuficiência renal, leucopenia, disfunção do fígado, perda de memória, menopausa, dores na boca, mucosite, dor muscular, mialgias, mielossupressão, miocardite, febre neutropênica, náusea, nefrotoxicidade, neutropenia, sangramento nasal, dormência, ototoxicidade, dor, palmar-plantar eritrodisestesia, pancitopenia, pericardite, neuropatia periférica, faringite, fotofobia, fotossensibilidade, pneumonia, pneumonite, proteinuria, êmbolo pulmonar, fibrose pulmonar, toxicidade pulmonar, coceira, batida cardíaca rápida, hemorragia retal, inquietação, rinite, convulsões, falta de ar, sinusite, trombocitopenia, zumbido, infecção do trato urinário, hemorragia vaginal, secura vaginal, vertigem, retenção de água, fraqueza, perda de peso, ganho de peso e xerostomia. Em geral, a toxicidade é aceitável se os benefícios para o indivíduo alcançados através da terapia superarem os eventos adversos experimentados pelo indivíduo devido à terapia.

[0093] Em algumas modalidades, a administração da composição bacteriana trata o câncer. Em algumas modalidades, a composição bacteriana induz uma resposta imune antitumoral no indivíduo.

Agentes Terapêuticos

[0094] Em certos aspectos, os métodos fornecidos no presente documento incluem a administração a um indivíduo de uma bactéria e/ou uma composição bacteriana descrita no presente documento (por exemplo,

uma composição bacteriana contendo *Bifidobacterium animalis ssp. Lactis*) sozinha ou em combinação com outro agente terapêutico de câncer. Ou outro agente terapêutico de câncer pode incluir, por exemplo, resseção cirúrgica, radioterapia ou um agente terapêutico de câncer. Em algumas modalidades, a composição bacteriana e a outra terapia de câncer podem ser administradas ao indivíduo em qualquer ordem. Em algumas modalidades, a composição bacteriana e a outra terapia de câncer são administradas de modo conjunto.

[0095] Em algumas modalidades, a bactéria é administrada ao indivíduo antes que o agente terapêutico de câncer seja administrado (por exemplo, pelo menos 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23 ou horas antes ou pelo menos 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29 ou 30 dias antes). Em algumas modalidades, a bactéria é administrada ao indivíduo após o agente terapêutico de câncer ser administrado (por exemplo, pelo menos 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23 ou 24 horas após ou pelo menos 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29 ou 30 dias após). Em algumas modalidades, a bactéria e o agente terapêutico de câncer são administrados ao indivíduo simultaneamente ou quase simultaneamente (por exemplo, administrações ocorrem em até uma hora uma da outra). Em algumas modalidades, o indivíduo recebe um antibiótico antes que a bactéria seja administrada ao indivíduo (por exemplo, pelo menos 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23 ou 24 horas antes ou pelo menos 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29 ou 30 dias antes). Em algumas modalidades, o indivíduo recebe um antibiótico após a bactéria ser administrada ao indivíduo (por exemplo, pelo menos 1, 2, 3,

4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23 ou 24 horas antes ou pelo menos 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29 ou 30 dias após). Em algumas modalidades, a bactéria e o antibiótico são administrados ao indivíduo simultaneamente ou quase simultaneamente (por exemplo, administrações ocorrem em até uma hora uma da outra).

[0096] Em certas modalidades, o indivíduo pode ser submetido à cirurgia. Os tipos de cirurgia incluem, mas sem limitações, cirurgia preventiva, diagnóstica ou testes, curativa e paleativa. A cirurgia curativa é um tratamento de câncer que pode ser usado em conjunto com outras terapias, como o tratamento da presente invenção, quimioterapia, radioterapia, terapia hormonal, terapia genética, imunoterapia e/ou terapias alternativas.

[0097] A cirurgia curativa inclui resseção, em que todo ou parte do tecido canceroso é fisicamente removido, excisado e/ou destruído. A ressecção de tumor se refere à remoção física de pelo menos parte de um tumor. Em adição à resseção de tumor, o tratamento por cirurgia inclui cirurgia a laser, criocirugia, eletrocirurgia e cirurgia controlada microscopicamente (cirurgia de Mohs). Mediante a excisão de parte ou de todas as células, tecido ou tumor canceroso, uma cavidade pode ser formada no corpo.

[0098] Em certas modalidades, o indivíduo pode ser submetido à terapia de radiação. A terapia de radiação inclui a administração ou aplicação de agentes radioterapêuticos e fatores incluindo, mas sem limitações. Além de bandejas, irradiação de UV, micro-ondas, emissões eletrônicas e radioisótopos. O sítio de tumor localizado pode ser irradiado, incluindo por uma ou mais das formas descritas acima de radiações. Todos esses fatores podem afetar uma ampla faixa de DNA prejudicado, nos precursores de DNA, a replicação e reparo de DNA, e a montagem e manutenção de cromossomos.

[0099] As faixas de dosagem para raios X faixa de doses diárias de 50 a 200 roentgens por períodos de tempo prolongados (3 a 4 semanas), a doses únicas de 2.000 a 6.000 roentgens. As faixas de dosagem para radioisótopos variam amplamente e depende da meia vida do isótopo, da força e tipo de radiação emitida, e a absorção das células neoplásticas.

[00100] Em certos aspectos, os métodos fornecidos no presente documento compreendem adicionalmente administrar outro agente terapêutico de câncer ao indivíduo.

[00101] Em algumas modalidades, o agente terapêutico de câncer é um agente quimioterapêutico. Os exemplos de tais agentes quimioterapêuticos incluem, mas sem limitações, agentes de alquilação como tiotepa e ciclosfosfamida; sulfonatos de alquila como bussulfano, improssulfano e piposssulfano; aziridinas como benzodopa, carboquona, meturedopa e uredopa; etileniminas e metilamelaminas incluindo altretamina, trietilenomelamina, trietilenofosforamida, trieti-ilenotiofosforamida e trimetilolomelamina; acetogeninas (especialmente bulatacina e bulatacinona); a camptotecina (incluindo o análogo sintético topotecano); briostatina; calistatina; CC-1065 (incluindo sua adozelesina, carzelesina e análogos sintéticos de bizelesina); criptoficinas (particularmente criptoficina 1 e criptoficina 8); dolastatina; duocarmicina (incluindo os análogos sintéticos, KW-2189 e CB1-TM1); eleuterrobina; pancratistatina; uma sarcodictiína; espongistatina; mostardas de nitrogênio como clorambucila, clornafazina, colofosfamida, estramustina, ifosfamida, mecloretamina, cloridrato de óxido de mecloretamina, melfalano, novembiquina, fenesterina, prednimustina, trofosfamida, mostarda de uracila; nitrosureia como carmustina, clorozotocina, fotemustina, lomustina, nimustina e ranimnustina; antibióticos como os antibióticos de enediína (por exemplo, caliqueamicina, especialmente caliqueamicina

gamal e caliqueamicina omegal1; dinemicina, incluindo dinemicina A; bisfosfonatos, como clodronato; uma esperamicina; assim como cromóforo de neocarzinostatina e cromóforos de antibiótico de enediína de cromoproteína relacionados, aclacinomosinas, actinomicina, autrarnicina, azasserina, bleomicinas, cactinomicina, carabicina, caminomicina, carzinofilina, cromomicinais, dactinomicina, daunorrubicina, detorrubicina, 6-diazo-5-oxo-l-norleucina, doxorrubicina (incluindo morfolino-doxorrubicina, cianomorfolino-doxorrubicina, 2-pirrolino-doxorrubicina e deoxidoxorrubicina), epirrubicina, esorrubicina, idarrubicina, marcelomicina, mitomicinas como mitomicina C, ácido micofenólico, nogalamicina, olivomicinas, peplomicina, potfiromicina, puromicina, quelamicina, rodorrubicina, estreptonigrina, estreptozocina, tubercidina, ubenimex, zinostatina, zorrubicina; antimetabólitos como metotrexato e 5-fluorouracila (5-FU); análogos de ácido fólico como denopterina, metotrexato, pteropterina, trimetrexato; análogos de purina como fludarabina, 6-mercaptopurina, tiamiprina, tioguanina; análogos de pirimidina como ancitabina, azacitidina, 6-azauridina, canfora, citarabina, dideoxiuridina, doxifluridina, enocitabina, floxuridina; androgênios como calusterona, propionato de dromostanolona, epitiostanol, mepitiostano, testolactona; anti-adrenais como aminoglutetimida, mitotano, trilostano; reabastecedor de ácido fólico como ácido frolínico; aceglatona; glicosídeo de aldofosfamida; ácido aminolevulínico; eniluracila; amsacrina; bestrabucila; bisantreno; edatraxato; defofamina; demecolcina; diaziquona; elformitino; acetato de eliptinio; uma epotilona; etoglucide; nitrato de gálio; hidroxiureia; lentinana; lonidainina; maitansinoides como maitansina e ansamitocinas; mitoguazona; mitoxantrona; mopidanmol; nitraerina; pentostatina; fenamete; pirarubicina; losoxantrona; ácido podofilínico; 2-etil-hidrazida; procarbazina; complexo de polissacarídeo de PSK); razoxana; rizoxina; sizo-

furano; espirogermânio; ácido tenuazónico; triaziquona; 2,2',2''-triclorotrietilamina; tricotecenos (especialmente toxina de T-2, verracurina A, roridina A e anguidina); uretanos; vindesina; dacarbazina; manomustina; mitobronitol; mitolactol; pipobromano; gacitosina; arabinosídeo ("Ara-C"); ciclofosfamida; tiotepa; taxoides, *por exemplo,* paclitaxel e doxetaxel; clorambucila; gemcitabina; 6-tioguanina; mercaptopurina; metotrexato; complexos de coordenação de platina como cisplatina, oxaliplatina e carboplatina; vinblastina; platina; etoposídeo (VP-16); ifosfamida; mitoxantrona; vincristina; vinorelbina; novantrona; teniposídeo; edatrexato; daunomicina; aminopterina; xeloda; ibandronato; irinotecano (*por exemplo,* CPT-11); inibidor de topoisomerase RFS 2000; difluorometilomitina (DMFO); retinoides como ácido retinoico; capecitabina; e sais, ácidos ou derivados farmaceuticamente aceitáveis de qualquer um dos supracitados.

[00102] Em algumas modalidades, o agente terapêutico de câncer é um agente de imunoterapia contra câncer. A imunoterapia se refere a um tratamento que usa um sistema imunológico de um indivíduo para tratar o câncer, *por exemplo,* inibidores de ponto de verificação, vacinas de câncer, citocinas, terapia celular, células T de CAR e terapia de célula dendrítica. Os exemplos não limitantes de imunoterapias são inibidores de ponto de verificação incluem Nivolumab (BMS, anti-PD-1), Pembrolizumab (Merck, anti-PD-1), Ipilimumab (BMS, anti-CTLA-4), MEDI4736 (AstraZeneca, anti-PD-L1) e MPDL3280A (Roche, anti-PD-L1). Outras imunoterapias podem ser vacinas contra tumor, como Gardail, Cervarix, BCG, sipulencel-T, Gp100:209-217, AGS-003, DCVax-l, Algenpantucel-l, Tergenpantucel-l, TG4010, ProstAtak, Prostvac-V/R-TRICOM, Rindopepimul, acetato de peptídeo E75, IMA901, POL-103A, Belagenpumatucel-l, GSK1572932A, MDX-1279, GV1001 e Tecemotide. A imunoterapia pode ser administrada por meio de injeção (*por exemplo,* intravenosa, intratumoral, subcutânea

ou em gânglios linfáticos), mas também pode ser administrada oralmente, de modo tópico ou por meio de aerossol. As imunoterapias podem compreender adjuvantes como citocinas. .

[00103] Em algumas modalidades, o agente de imunoterapia é um inibidor de ponto de verificação imunológico. A inibição de ponto de verificação imune se refere amplamente a inibir os pontos de verificação que as células de câncer podem produzir para prevenir ou regular de modo descendente uma resposta imune. Os exemplos de proteína de ponto de verificação imunológico incluem, mas sem limitações, CTLA4, PD-1, PD-L1, PD-L2, A2AR, B7-H3, B7-H4, BTLA, KIR, LAG3, TIM-3 ou VISTA. Os inibidores de ponto de verificação imunológico podem ser anticorpos ou fragmentos de ligação de antígeno dos mesmos que se ligam a e inibem uma proteína de ponto de verificação imunológico. Os exemplos de inibidores de ponto de verificação imunológico incluem, mas sem limitações, nivolumab, pembrolizumab, pidilizumab, AMP-224, AMP-514, STI-A1110, TSR-042, RG-7446, BMS-936559, MEDI-4736, MSB-0020718C, AUR-012 e STI-A1010. Em algumas modalidades, a imunoterapia contra câncer inclui administrar um inibidor de ponto de verificação imunológico adicional (por exemplo, administra 2, 3, 4 ou 5, inibidores de ponto de verificação imunológico) ao indivíduo

[00104] Em certas modalidades, os inibidores de ponto de verificação imunológico podem ser uma molécula de ácido nucleico inibidora (por exemplo, uma molécula de siRNA, uma shRNA molécula ou uma molécula de RNA antissenso) que inibe a expressão de uma proteína de ponto de verificação imunológico que inibe a expressão de uma proteína de ponto de verificação imunológico.

[00105] Em algumas modalidades, o inibidor de ponto de verificação imunológico é uma molécula de siRNA. Tais moléculas de siRNA devem

incluir uma região de homologia suficiente à região-alvo, e ter comprimento suficiente em termos de nucleotídeos, de modo que a molécula de siRNA regule de modo descendente o RNA-alvo (por exemplo, RNA de uma proteína de ponto de verificação imunológico). O termo "ribonucleotídeo" ou "nucleotídeo" pode, no caso de um RNA modificado ou substituto de nucleotídeo, ainda, se referir a um nucleotídeo modificado, ou porção química de substituição em uma ou mais posições. Não é necessário que haja complementaridade perfeita entre a molécula de siRNA e o alvo, porém, a correspondência deve ser suficiente para possibilitar que a molécula de siRNA silencie a sequência específica direta, como por clivagem de RNAi do RNA-alvo. Em algumas modalidades, o filamento de senso precisa ser apenas suficientemente complementar ao filamento antissenso para manter o caráter geral de filamento duplo da molécula.

[00106] Além disso, uma molécula de siRNA pode ser modificada ou incluir substitutos de nucleosídeo. As regiões de filamento único de uma molécula de siRNA podem ser modificadas ou incluir substitutos de nucleosídeo, *por exemplo,* a região ou regiões não emparelhadas de uma estrutura tipo grampo, *por exemplo,* uma região que liga duas regiões complementares, pode ter modificações ou substitutos de nucleosídeo. A modificação para estabilizar uma ou mais terminações 3′ ou 5′ de uma molécula de siRNA, *por exemplo,* contra exonucleases, ou para favorecer que o agente de siRNA de antissenso entre em RISC também é útil. As modificações podem incluir C3 (ou C6, C7, C12) ligantes de amino, ligantes de tiol, ligantes de carboxila, espaçadores não nucleotídicos (C3, C6, C9, C12, abásico, trietileno glicol, hexaetileno glicol), reagentes de biotina ou fluoresceína especiais que são fornecidos como fosforamiditas e que têm outro grupo hidroxila protegido por DMT, que permite múltiplos acoplamentos durante a síntese de RNA.

[00107] Cada filamento de uma molécula de siRNA pode ter igual ou menos que 35, 30, 25, 24, 23, 22, 21 ou 20 nucleotídeos de comprimento. Em algumas modalidades, o filamento tem pelo menos 19 nucleotídeos de comprimento. Por exemplo, cada filamento pode ter entre 21 e 25 nucleotídeos de comprimento. Em algumas modalidades, agentes de siRNA têm uma região de dúplex de 17, 18, 19, 29, 21, 22, 23, 24, ou 25 pares de nucleotídeos, e uma ou mais saliências, como uma ou duas saliências de 3′, de 2 a 3 nucleotídeos.

[00108] Em algumas modalidades, o inibidor de ponto de verificação imunológico é uma molécula de shRNA. Um "RNA tipo grampo pequeno" ou "RNA tipo grampo curto" ou "shRNA" inclui uma sequência de RNA curta que realiza uma curva de grampo justa que pode ser usada para silenciar a expressão de gene por meio de interferência de RNA. As shRNAs fornecidas no presente documento podem ser quimicamente sintetizadas ou transcritas a partir de um cassete transcricional em um plasmídeo de DNA. A estrutura tipo grampo de shRNA é clivada pelo maquinário celular em siRNA, que é, então, ligada ao complexo de silenciamento induzido por RNA (RISC).

[00109] Em algumas modalidades, shRNAs têm cerca de 15 a 60, 15 a 50 ou 15 a 40 (dúplex) nucleotídeos de comprimento, cerca de 15 a 30, 15 a 25 ou 19 a 25 (dúplex) nucleotídeos de comprimento ou têm cerca de 20 a 24, 21 a 22 ou 21 a 23 (dúplex) nucleotídeos de comprimento (*por exemplo,* cada sequência complementar do shRNA de filamento duplo tem 15 a 60, 15 a 50, 15 a 40, 15 a 30, 15 a 25 ou 19 a 25 nucleotídeos de comprimento, ou cerca de 20 a 24, 21 a 22 ou 21 a 23 nucleotídeos de comprimento, e o shRNA de filamento duplo tem cerca de 15 a 60, 15 a 50, 15 a 40, 15 a 30, 15 a 25 ou 19 a 25 pares de base in comprimento, ou cerca

de 18 a 22, 19 a 20 ou 19 a 21 pares de base de comprimento). Os dúplexes de shRNA podem compreender saliências de 3' de cerca de 1 a cerca de 4 nucleotídeos ou cerca de 2 a cerca de 3 nucleotídeos no filamento antissenso e/ou terminações 5'-fosfato no filamento de senso. Em algumas modalidades, a shRNA compreende um filamento de senso e/ou sequência de filamento antissenso de cerca de 15 a cerca de 60 nucleotídeos de comprimento (*por exemplo,* cerca de 15 a 60, 15 a 55, 15 a 50, 15 a 45, 15 a 40, 15 a 35, 15 a 30 ou 15 a 25 nucleotídeos de comprimento), ou de cerca de 19 a cerca de 40 nucleotídeos de comprimento (*por exemplo,* cerca de 19 a 40, 19 a 35, 19 a 30 ou 19 a 25 nucleotídeos de comprimento), ou de cerca de 19 a cerca de 23 nucleotídeos de comprimento (*por exemplo,* 19, 20, 21, 22 ou 23 nucleotídeos de comprimento).

[00110] Os exemplos não limitantes de shRNA incluem uma molécula de polinucleotídeo de filamento duplo montada a partir de uma molécula de filamento único, em que as regiões de senso e antissenso são ligadas por um ligante à base de ácido nucleico ou sem base em ácido nucleico; e uma molécula de polinucleotídeo de filamento duplo com uma estrutura secundária tipo grampo que tem regiões de senso e antissenso de autocomplementaridade. Em algumas modalidades, os filamentos de senso e antissenso da shRNA são ligados por uma estrutura de laço compreendendo de cerca de 1 a cerca de 25 nucleotídeos, de cerca de 2 a cerca de 20 nucleotídeos, de cerca de 4 a cerca de 15 nucleotídeos, de cerca de 5 a cerca de 12 nucleotídeos ou 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, ou mais nucleotídeos.

[00111] As modalidades adicionais relacionadas às shRNAs, assim como métodos para projetar e sintetizar tais shRNAs, são descritos na publicação de patente de número U.S. 2011/0071208, cuja revelação está

incorporada no presente documento a título de referência em sua totalidade para todos os propósitos.

[00112] Em algumas modalidades, o inibidor de ponto de verificação imunológico consiste em compostos de oligonucleotídeo de antissenso que inibem a expressão de uma proteína de ponto de verificação imunológico. Em certas modalidades, o grau de complementaridade entre a sequência-alvo e a sequência de alvejamento de antissenso é suficiente para formar um dúplex estável. A região de complementaridade dos oligonucleotídeos antissenso com a sequência de RNA-alvo pode ser tão curta quanto 8 a 11 bases, porém, pode ter 12 a 15 bases ou mais, por exemplo, 10 a 40 bases, 12 a 30 bases, 12 a 25 bases, 15 a 25 bases, 12 a 20 bases ou 15 a 20 bases, incluindo todos os números inteiros entre essas faixas. Um oligonucleotídeo de antissenso de cerca de 14 a 15 bases é geralmente longo o suficiente para ter uma sequência complementar exclusiva.

[00113] Em certas modalidades, os oligonucleotídeos antissenso podem ser 100% complementares à sequência-alvo, ou podem incluir incompatibilidades, *por exemplo,* para aprimorar o alvejamento seletivo de alelo contendo a mutação associada à doença, desde que um heterodúplex formado entre o oligonucleotídeo e a sequência-alvo seja suficientemente estável para suportar a ação de nucleases celulares e outros modos de degradação que possam ocorrer *in vivo*. Portanto, certos oligonucleotídeos podem ter cerca de ou pelo menos cerca de 70% de complementaridade de sequência, *por exemplo,* 70%, 71%, 72%, 73%, 74%, 75%, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% ou 100% de complementaridade de sequência entre o oligonucleotídeo e a sequência-alvo. As cadeias principais de oligonucleotídeo que são menos suscetíveis à cliva-

gem por nucleases são discutidas no presente documento. As incompatibilidades, se presentes, são tipicamente menos desestabilizantes em direção às regiões de extremidade do dúplex híbrido do que no meio. O número de incompatibilidades permitidas irá depender do comprimento do oligonucleotídeo, da porcentagem de pares de base de G:C no dúplex e da posição da incompatibilidade (ou incompatibilidades) no dúplex, de acordo com princípios bem entendidos de estabilidade de dúplex.

[00114] A molécula de ácido nucleico inibidora pode ser preparada, por exemplo, por síntese química, transcrição in vitro, ou digestão de dsRNA longos por Rnase III ou Dicer. Esses podem ser introduzidos em células por transfecção, eletroporação ou outros métodos conhecidos na técnica. Consultar Hannon, GJ, 2002, RNA Interference, Nature 418: 244 a 251; Bernstein E et al., 2002, The rest is silence. RNA 7: 1509 a 1521; Hutvagner G et al., RNAi: Nature abhors a double-strand. Curr. Opin. Genetics & Development 12: 225 a 232; Brummelkamp, 2002, A system for stable expression of short interfering RNAs in mammalian cells. Science 296: 550 a 553; Lee NS, Dohjima T, Bauer G, Li H, Li M-J, Ehsani A, Salvaterra P e Rossi J. (2002). Expression of small interfering RNAs targeted against HIV-1 rev transcripts in human cells. Nature Biotechnol. 20:500 a 505; Miyagishi M e Taira K. (2002). U6-promoter-driven siRNAs with four uridine 3' overhangs efficiently suppress targeted gene expression in mammalian cells. Nature Biotechnol. 20:497 a 500; Paddison PJ, Caudy AA, Bernstein E, Hannon GJ e Conklin DS. (2002). Short hairpin RNAs (shRNAs) induce sequence-specific silencing in mammalian cells. Genes & Dev. 16:948 a 958; Paul CP, Good PD, Winer I e Engelke DR. (2002). Effective expression of small interfering RNA in human cells. Nature Biotechnol. 20:505 a 508; Sui G, Soohoo C, Affar E-B, Gay F, Shi Y, Forrester WC de Shi Y. (2002). A DNA vector-based RNAi technology to suppress gene expression in mammalian

cells. Proc. Natl. Acad. Sci. EUA 99(6):5515 a 5520; Yu J-Y, DeRuiter SL e Turner DL. (2002). RNA interference by expression of short-interfering RNAs and hairpin RNAs in mammalian cells. Proc. Natl. Acad. Sci. EUA 99(9):6047 a 6052.

[00115] Nos presentes métodos, a molécula de ácido nucleico inibidora pode ser administrada ao indivíduo, por exemplo, como ácido nucleico puro, em combinação com um reagente de entrega e/ou como um ácido nucleico compreendendo sequências que expressam uma molécula de ácido nucleico de interferência. Em algumas modalidades, o ácido nucleico compreendendo sequências que expressam as moléculas de ácido nucleico de interferência são entregues dentro dos vetores, *por exemplo,* plasmídeo, vetores virais e bacterianos. Qualquer método de entrega de ácido nucleico conhecido na técnica pode ser usado nos métodos descritos no presente documento. Os reagentes de entrega adequados incluem, mas sem limitações, *por exemplo,* o reagente lipofílico Mirus Transit TKO; lipofectina; lipofectamina; celfectina; policátions (*por exemplo,* polilisina), atelocolágeno, nanoplexos e lipossomos. O uso de atelocolágeno como um veículo de entrega para moléculas de ácido nucleico é descrito em Minakuchi *et al.* Nucleic Acids Res., 32(13):e109 (2004); Hanai *et al.* Ann NY Acad Sci., 1082:9 a 17 (2006); e Kawata *et al.* Mol Cancer Ther., 7(9):2904-12 (2008); cada um dos quais é incorporado no presente documento em sua totalidade. Os sistemas de entrega de ácido nucleico de interferência exemplificativos são fornecidos nas Patentes nºs U.S. 8.283.461, 8.313.772, 8.501.930. 8.426.554, 8.268.798 e 8.324,366, cada uma das quais está aqui incorporada a título de referência em sua totalidade.

[00116] Em algumas modalidades, o agente de imunoterapia é um anticorpo ou fragmento de ligação de antígeno do mesmo que, por exemplo,

se liga a um antígeno associado a câncer. Os exemplos de antígenos associados a câncer incluem, mas sem limitações, adipofilina, AIM-2, ALDH1A1, alfa-actinina-4, alfa-fetoproteína ("AFP"), ARTC1, B-RAF, BAGE-1, BCLX (L), proteína de fusão de BCR-ABL b3a2, beta-catenina, BING-4, CA-125, CALCA, antígeno carcinoembriônico ("CEA"), CASP-5, CASP-8, CD274, CD45, Cdc27, CDK12, CDK4, CDKN2A, CEA, CLPP, COA-1, CPSF, CSNK1A1, CTAG1, CTAG2, ciclina D1, Ciclina-A1, proteína tipo dek-can, DKK1, EFTUD2, fator de alongamento 2, ENAH (hMena), Ep-CAM, EpCAM, EphA3, antígeno de tumor epitelial ("ETA"), proteína de fusão de ETV6-AML1, EZH2, FGF5, FLT3-ITD, FN1, G250/MN/CAIX, GAGE-1,2,8, GAGE-3,4,5,6,7, GAS7, glipicano-3, GnTV, gp100/Pmel17, GPNMB, HAUS3, Hepsina, HER-2/neu, HERV-K-MEL, HLA-A11, HLA-A2, HLA-DOB, hsp70-2, IDO1, IGF2B3, IL13Ralfa2, esterase de carboxila intestinal, K-ras, Kalicreína 4, KIF20A, KK-LC-1, KKLC1, KM-HN-1, KMHN1 também conhecido como CCDC110, LAGE-1, proteína de fusão de LDLR-fucosil-transferaseAS, Lengsin, M-CSF, MAGE-A1, MAGE-A10, MAGE-A12, MAGE-A2, MAGE-A3, MAGE-A4, MAGE-A6, MAGE-A9, MAGE-C1, MAGE-C2, enzima málica, mamaglobina-A, MART2, MATN, MC1R, MCSP, mdm-2, ME1, Melano-A/MART-1, Meloe, Midkine, MMP-2, MMP-7, MUC1, MUC5AC, mucina, MUM-1, MUM-2, MUM-3, Miosina, Classe de miosina, N-raw, NA88-A, neo-PAP, NFYC, NY-BR-1, NY-ESO-1/LAGE-2, OA1, OGT, OS-9, polipeptídeo de P, p53, PAP, PAX5, PBF, proteína de fusão de pml-RARalfa, mucina epitelial polimórfica ("PEM"), PPP1R3B, PRAME, PRDX5, PSA, PSMA, PTPRK, RAB38/NY-MEL-1, RAGE-1, RBAF600, RGS5, RhoC, RNF43, RU2AS, SAGE, secernina 1, SIRT2, SNRPD1, SOX10, Sp17, SPA17, SSX-2, SSX-4, STEAP1, survivina, SYT-SSX1 ou proteína de fusão de SSX2, TAG-1, TAG-2, Telomerase, TGF-betaRII, TPBG, TRAG-3, isomerase de Triosefosfato, TRP-1/gp75, TRP-2,

TRP2-INT2, tirosinase, tirosinase ("TYR"), VEGF, WT1, XAGE-1b/GAGED2a. Em algumas modalidades, o antígeno é um neo-antígeno.

[00117] Em algumas modalidades, o agente de imunoterapia é uma vacina para câncer e/ou um componente de uma vacina para câncer (*por exemplo,* uma proteína e/ou peptídeo antigênico). A vacina para câncer pode ser uma vacina de proteína, uma vacina de ácido nucleico ou uma combinação das mesmas. Por exemplo, em algumas modalidades, a vacina para câncer compreende um polipeptídeo compreendendo um epítopo de um antígeno associado a câncer. Em algumas modalidades, a vacina para câncer compreende um ácido nucleico (*por exemplo,* DNA ou RNA, como mRNA) que codifica um epítopo de um antígeno associado a câncer. Em algumas modalidades, o ácido nucleico é um vetor (por exemplo, um vetor bacteriano, vetor viral). Os exemplos de vetores bacterianos incluem, mas sem limitações, Mycobacterium bovis (BCG), Salmonella Typhimurium ssp., Salmonella Typhi ssp., Clostridium sp. spores, Escherichia coli Nissle 1917, Escherichia coli K-12/LLO, Listeria monocitogenes e Shigella flexneri. Os exemplos de vetores virais incluem, mas sem limitações, vaccinia, adenovírus, vírus de RNA e avipox com deficiência de replicação, fowlpox com deficiência de replicação, canarypox com deficiência de replicação, MVA com deficiência de replicação e adenovírus com deficiência de replicação.

[00118] Em algumas modalidades, a imunoterapia contra câncer compreende administração de uma célula apresentadora de antígeno (APC) preparada com um antígeno específico para câncer. Em algumas modalidades, o APC é uma célula dendrítica, um macrófago ou uma célula B.

[00119] Os exemplos de antígenos associados a câncer incluem, mas sem limitações, adipofilina, AIM-2, ALDH1A1, alfa-actinina-4, alfa-fetoproteína ("AFP"), ARTC1, B-RAF, BAGE-1, BCLX (l), proteína de fusão de

BCR-ABL b3a2, beta-catenina, BING-4, CA-125, CALCA, antígeno carcinoembriônico ("CEA"), CASP-5, CASP-8, CD274, CD45, Cdc27, CDK12, CDK4, CDKN2A, CEA, CLPP, COA-1, CPSF, CSNK1A1, CTAG1, CTAG2, ciclina D1, Ciclina-A1, proteína tipo dek-can, DKK1, EFTUD2, fator de alongamento 2, ENAH (hMena), Ep-CAM, EpCAM, EphA3, antígeno de tumor epitelial ("ETA"), proteína de fusão de ETV6-AML1, EZH2, FGF5, FLT3-ITD, FN1, G250/MN/CAIX, GAGE-1,2,8, GAGE-3,4,5,6,7, GAS7, glipicano-3, GnTV, gp100/Pmel17, GPNMB, HAUS3, Hepsina, HER-2/neu, HERV-K-MEL, HLA-A11, HLA-A2, HLA-DOB, hsp70-2, IDO1, IGF2B3, IL13Ralfa2, esterase de carboxila intestinal, K-ras, Kalicreína 4, KIF20A, KK-LC-1, KKLC1, KM-HN-1, KMHN1 também conhecido como CCDC110, LAGE-1, proteína de fusão de LDLR-fucosiltransferaseAS, Lengsin, M-CSF, MAGE-A1, MAGE-A10, MAGE-A12, MAGE-A2, MAGE-A3, MAGE-A4, MAGE-A6, MAGE-A9, MAGE-C1, MAGE-C2, enzima málica, mamaglobina-A, MART2, MATN, MC1R, MCSP, mdm-2, ME1, Melano-A/MART-1, Meloe, Midkine, MMP-2, MMP-7, MUC1, MUC5AC, mucina, MUM-1, MUM-2, MUM-3, Miosina, Classe de miosina, N-raw, NA88-A, neo-PAP, NFYC, NY-BR-1, NY-ESO-1/LAGE-2, OA1, OGT, OS-9, polipeptídeo de P, p53, PAP, PAX5, PBF, proteína de fusão de pml-RARalfa, mucina epitelial polimórfica ("PEM"), PPP1R3B, PRAME, PRDX5, PSA, PSMA, PTPRK, RAB38/NY-MEL-1, RAGE-1, RBAF600, RGS5, RhoC, RNF43, RU2AS, SAGE, secernina 1, SIRT2, SNRPD1, SOX10, Sp17, SPA17, SSX-2, SSX-4, STEAP1, survivina, SYT-SSX1 ou proteína de fusão de SSX2, TAG-1, TAG-2, Telomerase, TGF-betaRII, TPBG, TRAG-3, isomerase de Triosefosfato, TRP-1/gp75, TRP-2, TRP2-INT2, tirosinase, tirosinase ("TYR"), VEGF, WT1, XAGE-1b/GAGED2a. Em algumas modalidades, o antígeno é um neo-antígeno.

[00120] Em algumas modalidades, a imunoterapia contra câncer compreende a administração de um receptor de antígeno quimérico específico

para câncer (CAR). Em algumas modalidades, o CAR é administrado na superfície de uma célula T. Em algumas modalidades, a CAR se liga especificamente a um antígeno associado a câncer.

[00121] Em algumas modalidades, o imunoterapia contra câncer compreende a administração de uma célula T específica para câncer ao indivíduo. Em algumas modalidades, a célula T é uma célula T de $CD4^+$. Em algumas modalidades, a célula T de $CD4^+$ é uma célula T de T_H1, uma célula T de T_H2 ou uma célula T de T_H17. Em algumas modalidades, a célula T expressa um receptor de célula T específico para um antígeno associado a câncer.

[00122] Em algumas modalidades, a vacina para câncer é administrada com um adjuvante. Os exemplos de adjuvantes incluem, mas sem limitações, uma proteína imunomoduladora, Adjuvante 65, α-GalCer, fosfato de alumínio, hidróxido de alumínio, fosfato de cálcio, Peptídeo β-Glucano, CpG DNA, GPI-0100, lipídio A, lipopolissacarídeo, Lipovant, Montanide, N-acetil-muramil-l-alanil-D-isoglutamina, Pam3CSK4, quil A e dimicolato de trealose.

[00123] Em algumas modalidades, o agente de imunoterapia é uma proteína imunomoduladora para o indivíduo. Em algumas modalidades, a proteína imunomoduladora é uma citocina. Os exemplos de proteínas imunomoduladoras incluem, mas sem limitações, quimioatrativo de linfócito B ("BLC"), quimiocina de motivo de C-C 11 ("Eotaxina-1"), proteína quimiotática de Eosinofila 2 ("Eotaxina-2"), fator estimulante de colônia de granulótico ("G-CSF"), fator estimulante de colônia de macrófago de granulótico ("GM-CSF"), 1-309, Molécula de Adesão Intercelular 1 ("ICAM-1"), Interferón gama ("IFN-gama"), Interlucina-1 alfa ("IL-1 alfa"), Interlucina-1 beta ("IL-1 beta"), antagonista de receptor de Interleucina 1 ("IL-1 ra"), Interleucina-2 ("IL-2"), Interleucina-4 ("IL-4"), Interleucina-5 ("IL-5"), Interleucina-6

("IL-6"), receptor solúvel de Interleucina-6 ("IL-6 sR"), Interleucina-7 ("IL-7"), Interleucina-8 ("IL-8"), Interleucina- 10 ("IL-10"), Interleucina- 11 ("IL-11"), Subunidade beta de Interleucina- 12 ("IL-12 p40" ou "IL-12 p70"), Interleucina-13 ("IL-13"), Interleucina-15 ("IL-15"), Interleucina-16 ("IL-16"), Interleucina-17 ("IL-17"), Ligante 2 de quimiocina (motivo de C-C) ("MCP-1"), fator estimulante de colônia de macrófago ("M-CSF"), Monocina induzida por interferón de gama ("MIG"), Ligante 2 de quimiocina (motivo de C-C) ("MIP-1 alfa"), Ligante 4 de quimiocina (Motivo de C-C) ("MIP-1 beta"), Proteína inflamatória de macrófago- 1 -delta ("MIP-1 delta"), Subunidade de fator de crescimento derivada de plaqueta B ("PDGF-BB"), Ligante 5 de Quimiocina (Motivo de C-C), Célula T normal regulada em ativação, expressada e secretada ("RANTES"), inibidor de metalopeptidase de TIMP 1 ("TIMP-1"), Inibidor de metalopeptidase de TIMP 2 ("TIMP-2"), fator de necrose de tumor, linfotoxina-alfa ("TNF alfa"), fator de necrose de tumor, linfotoxina-beta ("TNF beta"), recepto de TNF tipo 1 ("sTNFRI"), sTNFRIIAR, fator neurotrófico derivado do cérebro ("BDNF"), fator de crescimento de fibroblasto básico ("bFGF"), proteína morfogenética óssea 4 ("BMP-4"), proteína morfogenética óssea 5 ("BMP-5"), Proteína morfogenética óssea 7 ("BMP-7"), fator de crescimento de nervo ("b-NGF"), fator de crescimento epidérmico ("EGF"), receptor de fator de crescimento epidérmico ("EGFR"), Fator de crescimento endotelial vascular derivado da glândula endócrina ("EG-VEGF"), Fator de crescimento de fibroblasto 4 ("FGF-4"), Fator de crescimento de ceratinócito ("FGF-7"), Fator de diferenciação de crescimento 15 ("GDF-15"), fator neurotrófico derivado de célula Glial ("GDNF"), Hormônio de crescimento, Fator de crescimento tipo EGF de ligação tipo grampo ("HB-EGF"), fator de crescimento de hepatócito ("HGF"), Proteína de ligação de fator de crescimento tipo insulina 1 ("IGFBP-1"), Proteína de ligação de fator de crescimento tipo insulina 2 ("IGFBP-2"), Proteína de

ligação de fator de crescimento tipo insulina 3 (" IGFBP-3"), Proteína de ligação de fator de crescimento tipo insulina 4 ("IGFBP-4"), Proteína de ligação de fator de crescimento tipo insulina 6 ("IGFBP-6"), Fator de crescimento tipo insulina 1 ("IGF-1"), Insulina, Fator estimulante de colônia de macrófago ("M-CSF R"), receptor de fator de crescimento de nervo ("NGF R"), Neurotrofina-3 ("NT-3"), Neutrofina-4 ("NT-4"), Fator inibidor de osteoclastogênese ("Osteoprotegerina"), Receptores de fator de crescimento derivados de plaqueta ("PDGF-AA"), Biossíntese de fosfatidilinositol-glicano ("PIGF"), Skp, Culina, complexo contendo F-box ("SCF"), Receptor de fator de célula-tronco ("SCF R"), Fator de crescimento de transformação alfa ("TGFalfa"), Fator de crescimento de transformação beta-1 ("TGF beta 1"), Fator de crescimento de transformação beta-3 ("TGF beta 3"), Fator de crescimento endotelial vascular ("VEGF"), receptor de fator de crescimento endotelial vascular 2 ("VEGFR2"), Receptor de fator de crescimento endotelial vascular 3 ("VEGFR3"), VEGF-D 6Ccina, Receptor de cinase de Tirosina-proteína UFO ("Axl"), Betacelulina ("BTC"), Quimiocina epitelial associada à Mucosae ("CCL28"), Ligante 27 de quimiocina (Motivo de C-C) ("CTACK"), Ligante 16 de quimiocina (motivo de C-X-C) ("CXCL16"), quimiocina de motivo de C-X-C 5 ("ENA-78"), Ligante 26 de quimiocina (Motivo de C-C) ("Eotaxina-3"), Proteína quimiotática de granulócito 2 ("GCP-2"), GRO, Ligante 14 de quimiocina (Motivo de C-C) ("HCC-l"), Ligante 16 de quimiocina (Motivo de C-C) ("HCC-4"), Interleucina-9 ("IL-9"), Interleucina-17 F ("IL-17F"), Proteína de ligação à Interleucina-18 ("IL-18 BPa"), Interleucina-28 A ("IL-28A"), Interleucina 29 ("IL-29"), Interleucina 31 ("IL-31"), Quimiocina de motivo de C-X-C 10 ("IP-10"), Receptor de quimiocina CXCR3 ("I-TAC"), Fator inibidor de leucemia ("LIF"), Light, Ligante de quimiocina (motivo de C) ("Linfotactina"), Proteína quimioatrativa de monócito 2 ("MCP-2"), Proteína quimioatrativa de monócito 3 ("MCP-3"), Proteína

quimioatrativa de monócito 4 ("MCP-4"), Quimiocina derivada de macrófago ("MDC"), Fator inibidor de migração de macrófago ("MIF"), Ligante 20 de quimiocina (Motivo de C-C) ("MIP-3 alfa"), Quimiocina de motivo de C-C 19 ("MIP-3 beta"), Ligante 23 de quimiocina (Motivo de C-C) ("MPIF-1"), Cadeia alfa de proteína estimulante de macrófago ("MSPalfa"), Proteína de monagem de nucleossomo 1-tipo 4 ("NAP-2"), Fosfoproteína secretada 1 ("Osteopontina"), Citocina pulmonar e regulada para ativação ("PARC"), Fator de plaqueta 4 ("PF4"), Fator derivado de célula de estroma- 1 alfa ("SDF-1 alfa"), Ligante 17 de quimiocina (Motivo de C-C) ("TARC"), Quimiocina expressada em Timo ("TECK"), Linfopoietina estromal tímica ("TSLP 4- IBB"), antígeno de CD 166 ("ALCAM"), Agrupamento de Diferenciação 80 ("B7-1"), Membro da superfamília de receptor de fator de necrose de tumor 17 ("BCMA"), Agrupamento de Diferenciação 14 ("CD14"), Agrupamento de Diferenciação 30 ("CD30"), Agrupamento de Diferenciação 40 ("CD40 Ligand"), Molécula de adesão celular relacionada ao antígeno carcinoembriônico 1 (glocoproteína biliar) ("CEACAM-1"), Receptor de Morte 6 ("DR6"), Desoxitimidina cinase ("Dtk"), glicoproteína de membrana tipo 1 ("Endoglina"), Receptor Tirosina-proteína cinase erbB-3 ("ErbB3"), molécula de adesão de leucócito endotelial 1 ("E-Seletina"), Antígeno de apoptose 1 ("Fas"), cinase de Tirosina tipo Fms 3 ("Flt-3L"), Membro da superfamília de receptor de fator de necrose de tumor 1 ("GITR"), Membro da superfamília de receptor de fator de necrose de tumor 14 ("HVEM"), molécula de adesão intercelular 3 ("ICAM-3"), IL-1 R4, IL-1 RI, IL-10 Rbeta, IL-17R, IL-2Rgama, IL-21R, proteína membranar de lisosomo 2 ("LIMPII"), lipocalina associada à gelatinase de neutrófilo ("Lipocalina-2"), CD62L ("L-Seletina"), Endotélio linfático ("LYVE-1"), sequência A relacionada à polipeptídeo de classe I de MHC ("MICA"), sequência B relacionada à polipep-

tídeo de classe I de MHC ("MICB"), NRGl-betal, Receptor de fator de crescimento derivado de plaqueta tipo Beta ("PDGF Rbeta"), Molécula de adesão de célula endotelial de plaqueta ("PECAM-1"), RAGE, Receptor celular do vírus de hepatite A 1 ("TIM-1"), Membro da superfamília de receptor de fator de necrose de tumor IOC ("TRAIL R3"), domínio de ligação de transglutaminase de proteína Trapina ("Trapina-2"), receptor de urocinase ("uPAR"), Proteína de adesão de célula vascular 1 ("VCAM-1"), XEDARActivina A, proteína relacionada a Agouti ("AgRP"), Ribonuclease 5 ("Angiogenina"), Angiopoietina 1, Angiostatina, Cateprina S, CD40, Proteína de família Crítica IB ("Cripto-1"), DAN, proteína relacaionada a Dickkopf 1 ("DKK-1"), E-Caderina, molécula de adesão de célula epitelial ("EpCAM"), Ligante de Fas (FasL ou CD95L), Fcg RIIB/C, FoUistatina, Galectina-7, molécula de adesão intercelular 2 ("ICAM-2"), IL-13 RI, IL-13R2, IL-17B, IL-2 Ra, IL-2 Rb, IL-23, LAP, molécula de adesão de célula neuronal ("NrCAM"), Inibidor de ativador de plasminogênio- 1 ("PAI-1"), receptores de fator de crescimento derivado de plaqueta ("PDGF-AB"), Resistina, fator derivado de célula estromal 1 ("SDF-1 beta"), sgpl30, proteína relacionada à frizzled secretada 2 ("ShhN"), lectinas tipo imunoglobulina de ligação de ácido siálico ("Siglec-5"), ST2, Fator de crescimento de transformação-beta 2 ("TGF beta 2"), Tie-2, Trombopoietina ("TPO"), Membro da superfamília de receptor de fator de necrose de tumor 10D ("TRAIL R4"), receptor de acionamento expressado em células mieloides 1 ("TREM-1"), Fator de crescimento endotelial vascular C ("VEGF-C"), VEGFRIAdiponectina, Adipsina ("AND"), Alfa-fetoproteina ("AFP"), Angiopoietina-tipo 4 ("ANGPTL4"), Beta-2-microglobulina ("B2M"), molécula de adesão de célula basal ("BCAM"), antígeno de carboidrato 125 ("CA125"), Antígeno de câncer 15-3 ("CA15-3"), antígeno carcinoembriônico ("CEA"), proteína receptora de cAMP ("CRP"), Receptor de fator de crescimento epidérmico humano 2

("ErbB2"), Folistatina, hormônio de estímulo de folículo ("FSH"), ligante 1 de quimiocina (motivo C-X-C) ("GRO alfa"), gonadotropino coriônica humana ("beta HCG"), receptor de fator de crescimento tipo insulina 1 ("IGF-1 sR"), IL-1 sRII, IL-3, IL-18 Rb, IL-21, Leptina, metaloproteinase-1 de matriz ("MMP-1"), metaloproteinase-2 de matriz ("MMP-2"), metaloproteinase-3 de matriz ("MMP-3"), metaloproteinase-8 de matriz ("MMP-8"), metaloproteinase-9 de matriz ("MMP-9"), metaloproteinase-10 de matriz ("MMP-10"), metaloproteinase-13 de matriz ("MMP-13"), Molécula de adesão de célula neural ("NCAM-1"), Entactina ("Nidogênio-1"), enolase específica de neurônio ("NSE"), Oncostatina M ("OSM"), Procalcitonina, Prolactina, antígeno específico de próstata ("PSA"), Lectina tipo Ig de ligação ao ácido Siálico 9 ("Siglec-9"), ADAM 17 endopeptidase ("TACE"), Tiroglobulina, inibidor de Metaloproteinase 4 ("TIMP-4"), TSH2B4, Disintegrina e proteína 9 contendo domínio de metaloproteinase ("ADAM-9"), Angiopoietina 2, Membro de superfamília de ligante de fator de necrose de tumor 13/membro de família B de fosfoproteina nuclear rica em leucina ácida 32 ("APRIL"), Proteína morfogenética óssea 2 ("BMP-2"), Proteína morfogenética óssea 9 ("BMP-9"), Componente de complemento 5a ("C5a"), Catepsina L, CD200, CD97, Quemerina, Membro da superfamília de receptor de fator de necrose de tumor 6B ("DcR3"), proteína de ligação de ácido graxo 2 ("FABP2"), proteína de ativação de fibroblasto, alfa ("FAP"), Fator de crescimento de fibroblasto 19 ("FGF-19"), Galectina-3, receptor de fator de crescimento de hepatócito ("HGF R"), IFN-gamalfa/beta R2, Fator de crescimento tipo insulina 2 ("IGF-2"), receptor de fator de crescimento tipo insulina 2 ("IGF-2 R"), receptor de Interleucina-1 6 ("IL-1R6"), Interleucina 24 ("IL-24"), Interleucina 33 ("IL-33", Kallikrein 14, Asparaginil endopeptidase ("Legumaina"), Receptor de lipoproteína de baixa densidade oxidada 1

("LOX-1"), lectina de ligação de manose ("MBL"), Neprilisina ("NEP"), homologo de Notch 1, associado à translocação (Drosofila) ("Notch-1"), Nefroblastoma superexpressado ("NOV"), Osteoactivina, proteína de morte celular programada 1 ("PD-1"), N-acetilmuramoil-L-alanina amidase ("PGRP-5"), Serpina A4, proteína relacionada à frizzled secretada 3 ("sFRP-3"), Trombomodulina, receptor tipo Toll 2 ("TLR2"), Membro da superfamília de receptor de fator de necrose de tumor 10A ("TRAIL RI"), Transferrina ("TRF"), WIF-lACE-2, Albumina, AMICA, Angiopoietina 4, Fator de ativação de célula B ("BAFF"), antígeno de carboidrato 19-9 ("CA19-9"), CD 163 , Clusterina, CRT AM, Ligante 14 de quimiocina (motivo C-X-C) ("CXCL14"), Cistatina C, Decorina ("DCN"), proteína relacionada a Dickkopf 3 ("Dkk-3"), proteína tipo delta 1 ("DLL1"), Fetuina A, fator de crescimento de ligação de heparina 1 ("aFGF"), receptor de folato alfa ("FOLR1"), Furina, proteína de classificação associada a GPCR 1 ("GASP-1"), proteína de classificação associada a GPCR 2 ("GASP-2"), Receptor de fator estimulante de colônia de granulócito ("GCSF R"), hepsina de serina protease ("HAI-2"), Receptor de Interleucina-17B ("IL-17B R"), Interleucina 27 ("IL-27"), gene de ativação de linfócito 3 ("LAG-3"), Apolipoproteína A-V ("LDL R"), Pepsinogênio I, proteína de ligação a Retinol 4 ("RBP4"), SOST, Proteoglicano de sulfato de heparano ("Sindecano-1"), Membro da superfamília de receptor de fator de necrose de tumor 13B ("TACI"), Inibidor de trajetória de fator de tecido ("TFPI"), TSP-1, Superfamília de receptor de fator de necrose de tumor, membro 10b ("TRAIL R2"), TRANCE, Troponina I, Ativador de Plaminogênio de Urocinase ("uPA"), Caderina 5, caderina tipo 2 ou VE-caderina (endotelial vascular) também conhecida como CD144 ("VE-Caderina"), proteína de sinalização induzível por WNTI 1 ("WISP-1") e Ativador de Receptor de Fator Nuclear κ B ("RANK").

[00124] Em algumas modalidades, o agente terapêutico de câncer é

uma porção química radioativa que compreende um radionuclídeo. Os radionuclídeos exemplificativos incluem, mas sem limitações Cr-51, Cs-131, Ce-134, Se-75, Ru-97, I-125, Eu-149, Os-189m, Sb-119, I-123, Ho-161, Sb-117, Ce-139, In-111, Rh-103m, Ga-67, Tl-201, Pd-103, Au-195, Hg-197, Sr-87m, Pt-191, P-33, Er-169, Ru-103, Yb-169, Au-199, Sn-121, Tm-167, Yb-175, In-113m, Sn-113, Lu-177, Rh-105, Sn-117m, Cu-67, Sc-47, Pt-195m, Ce-141, I-131, Tb-161, As-77, Pt-197, Sm-153, Gd-159, Tm-173, Pr-143, Au-198, Tm-170, Re-186, Ag-111, Pd-109, Ga-73, Dy-165, Pm-149, Sn-123, Sr-89, Ho-166, P-32, Re-188, Pr-142, Ir-194, In-114m/In-114 e Y-90.

[00125] Em algumas modalidades, o agente terapêutico de câncer é um inibidor de angiogênese para o indivíduo. Os exemplos de tais inibidores de angiogênese incluem, mas sem limitações Bevacizumabe (Avastin®), Ziv-aflibercepte (Zaltrap®), Sorafenibe (Nexavar®), Sunitinibe (Sutent®), Pazopanibe (Votrient®), Regorafenibe (Stivarga®) e Cabozantinibe (Cometriq™).

[00126] Em algumas modalidades, o agente terapêutico de câncer é um antibiótico. Por exemplo, se a presença de bactérias associadas ao câncer e/ou um perfil de microbioma associado ao câncer for detectado de acordo com os métodos fornecidos no presente documento, antibióticos podem ser administrados para eliminar as bactérias associadas ao câncer do indivíduo. "Antibióticos" se referem amplamente a compostos capazes de inibir ou prevenir uma infecção bacteriana. Os antibióticos podem ser classificados de diversas formas, incluindo seu uso para infecções específicas, seu mecanismo de ação, sua biodisponibilidade ou seu espectro de micróbio-alvo (*por exemplo,* bactérias Gram-negativas vs. Gram-positivas, bactérias aeróbicas vs. anaeróbicas, etc.) e esses podem ser usados para matar bactérias específicas em áreas específicas do hospedeiro ("nichos") (Leekha, et al 2011. General Principles of Antimicrobial Therapy. Mayo Clin

Proc. 86(2): 156 a 167). Em certas modalidades, antibióticos podem ser usados para alvejar seletivamente bactérias de um nicho específico. Em algumas modalidades, os antibióticos conhecidos para tratar uma infecção particular que inclui um nicho de câncer podem ser usados para alvejar micróbios associados ao câncer, incluindo bactérias associadas ao câncer em tal nicho. Em outras modalidades, os antibióticos são administrados após o tratamento bacteriano. Em algumas modalidades, os antibióticos são administrados após o tratamento bacteriano para remover o enxerto.

[00127] Em alguns aspectos, os antibióticos podem ser selecionados com base em suas propriedades bactericidas e bacteriostáticas. Os antibióticos bactericidas incluem mecanismos de ação que perturbam a parede celular (*por exemplo,* β-lactamas), a membrana celular (*por exemplo,* daptomicina), ou DNA bacteriano (*por exemplo,* fluoroquinolonas). Os agentes bacteriostáticos inibem a replicação bacteriana e incluem sulfonamidas, tetraciclinas e macrolídeos e atuam inibindo a síntese de proteína. Ademais, embora alguns fármacos podem ser bactericidas em certos organismos e bacteriostáticos em outros, conhecer o organismo-alvo permite que o versado na técnica selecione um antibiótico com as propriedades adequadas. Em certas condições de tratamento, os antibióticos bacteriostáticos inibem a atividade de antibióticos bactericidas. Assim, em certas modalidades, os antibióticos bactericidas e bacteriostáticos não são combinados.

[00128] Os antibióticos incluem, mas sem limitações aminoglicosídeos, ansamicinas, carbacefemas, carbapenemas, cefalosporinas, glicopeptídeos, lincosamidas, lipopeptídeos, macrólidos, monobactamas, nitrofuranos, oxazolidononas, penicilinas, antibióticos de polipeptídeo, quinolonas, fluoroquinolona, sulfonamidas, tetraciclinas e compostos antimicobacterianos e combinações dos mesmos.

[00129] Os aminoglicosídeos incluem, mas sem limitações Amicacina,

Gentamicina, Canamicina, Neomicina, Netilmicina, Tobramicina, Paromomicina e Espectinomicina. Os aminoglicosídeos são eficazes, *por exemplo,* contra bactérias Gram-negativas, como Escherichia coli, Klebsiella, Pseudomonas aeruginosa e Francisella tularensis, e contra certas bactérias aeróbicas, porém, menos eficazes contra anaeróbios obrigatórios/facultativos. Acredita-se que os aminoglicosídeos se liguem à subunidade ribossômica 30S ou 50S bacteriana, inibindo, assim, a síntese de proteína bacteriana.

[00130] A ansamicinas incluem, mas sem limitações, Geldanamicina, Herbimicina, Rifamicina e Estreptovaricina. Acredita-se que a Geldanamicina e Herbimicina inibem ou alteram a função da Proteína de Choque Térmico 90.

[00131] As Carbacefemas incluem, mas sem limitações, Loracarbef. Acredita-se que as Carbacefemas inibem a síntese de parede celular bacteriana.

[00132] As Carbapenemas incluem, mas sem limitações, Ertapenem, Doripenem, Imipenem/Cilastatina e Meropenem. As Carbapenemas são bactericidas tanto para bactérias Gram-positivas quanto Gram-negativas como antibióticos de amplo espectro. Acredita-se que as Carbapenemas inibem a síntese de parede celular bacteriana.

[00133] As Cefalosporinas incluem, mas sem limitações, Cefadroxila, Cefazolina, Cefalotina, Cefalotina, Cefalexina, Cefaclor, Cefamandol, Cefoxitina, Cefprozila, Cefuroxime, Cefixime, Cefdinir, Cefditoreno, Cefoperazona, Cefotaxime, Cefpodoxime, Ceftazidime, Ceftibuteno, Ceftizoxime, Ceftriaxona, Cefepime, Ceftaroline fosamila e Ceftobiprol. As Cefalosporinas selecionadas são eficazes, *por exemplo,* contra bactérias Gram-negativas e contra bactérias Gram-positivas, incluindo *Pseudomonas*, certas Cefalosporinas são eficazes contra *Staphilococcus aureus* (MRSA) resiliente a meticilina. Acredita-se que as Cefalosporinas inibem a síntese de

parede celular bacteriana através da perturbação da síntese da camada de peptidoglicano de paredes celulares bacterianas.

[00134] Os Glicopeptídeos incluem, mas sem limitações, Teicoplanina, Vancomicina e Telavancina. Os Glicopeptídeos são eficazes, *por exemplo,* contra bactérias Gram-positivas aeróbicas e anaeróbicas incluindo MRSA e *Clostridium difficile*. Acredita-se que os Glicopeptídeos inibem a síntese de parede celular bacteriana através da perturbação da síntese da camada de peptidoglicano de paredes celulares bacterianas.

[00135] As Lincosamidas incluem, mas sem limitações, Clindamicina e Lincomicina. As Lincosamidas são eficazes, *por exemplo,* contra bactérias anaeróbicas, assim como Staphilococcus e Streptococcus. Acredita-se que as Lincosamidas se liguem à subunidade ribossômica 50S bacteriana, inibindo, assim, a síntese de proteína bacteriana.

[00136] Os Lipopeptídeos incluem, mas sem limitações, Daptomicina. Os Lipopeptídeos são eficazes, *por exemplo,* contra bactérias Gram-positivas. Acredita-se que os Lipopeptídeos se liguem à membrana bacteriana e causem despolarização rápida.

[00137] Os Macrolídeos incluem, mas sem limitações, Azitromicina, Claritromicina, Diritromicina, Eritromicina, Roxitromicina, Troleandomicina, Telitromicina e Espiramicina. Os Macrolídeos são eficazes, *por exemplo,* contra Streptococcus e Mycoplasma. Acredita-se que os Macrolídeos se liguem à subunidade ribossômica 50S ou bacteriana, inibindo, assim, a síntese de proteína bacteriana.

[00138] Os Monobactames incluem, mas sem limitações, Aztreonam. Os Monobactames são eficazes, *por exemplo,* contra bactérias Gram-negativas. Acredita-se que os Monobactames inibem a síntese de parede celular bacteriana através da perturbação da síntese da camada de peptidoglicano de paredes celulares bacterianas.

[00139] Os Nitrofuranos incluem, mas sem limitações, Furazolidona e Nitrofurantoina.

[00140] As Oxazolidononas incluem, mas sem limitações, Linezolide, Posizolide, Radezolide e Torezolide. Acredita-se que as Oxazolidononas sejam inibidores de síntese de proteína.

[00141] As Penicilinas incluem, mas sem limitações, Amoxicilina, Ampicilina, Azlocilina, Carbenicilina, Cloxacilina, Dicloxacilina, Flucloxacilina, Mezlocilina, Meticilina, Nafcilina, Oxacilina, Penicilina G, Penicilina V, Piperacilina, Temocilina e Ticarcilina. As Penicilinas são eficazes, *por exemplo,* contra bactérias Gram-positivas, anaeróbios facultativos, *por exemplo,* Streptococcus, Borrelia e Treponema. Acredita-se que as Penicilinas inibem a síntese de parede celular bacteriana através da perturbação da síntese da camada de peptidoglicano de paredes celulares bacterianas.

[00142] As combinações de penicilina incluem, mas sem limitações, Amoxicilina/clavulanato, Ampicilina/sulbactame, Piperacilina/tazobactame e Ticarcilina/clavulanato.

[00143] Os antibióticos de polipeptídeo incluem, mas sem limitações, Bacitracina, Colistina e polimixina B e E. Os antibióticos de polipeptídeo são eficazes, *por exemplo,* contra bactérias Gram-negativas. Acredita-se que certos antibióticos de polipeptídeo inibem pirofosfato de isoprenila envolvido na síntese da camada de peptidoglicano de paredes celulares bacterianas, enquanto outros desestabilizam a membrana externa bacteriana deslocando-se contraíons bacterianos.

[00144] As Quinolonas e Fluoroquinolona incluem, mas sem limitações, Ciprofloxacina, Enoxacina, Gatifloxacina, Gemifloxacina, Levofloxacina, Lomefloxacina, Moxifloxacina, Ácido nalidíxico, Norfloxacina, Ofloxacina, Trovafloxacina, Grepafloxacina, Esparfloxacina e Temafloxacina. As Quinolonas/Fluoroquinolona são eficazes, *por exemplo,* contra *Streptococcus*

e *Neisseria*. Acredita-se que as Quinolonas/Fluoroquinolona inibem a girase ou topoisomerase IV de DNA bacteriano, inibindo, assim, a replicação e transcrição de DNA.

[00145] As Sulfonamidas incluem, mas sem limitações, Mafenida, Sulfacetamida, Sulfadiazina, sulfadiazina de prata, Sulfadimetoxina, Sulfametizol, Sulfametoxazol, Sulfanilimide, Sulfassalazina, Sulfisoxazol, Trimetoprim-Sulfametoxazol (Cotrimoxazol) e Sulfonamidocrisoidina. Acredita-se que as Sulfonamidas inibem a síntese de folato através de inibição competitiva de sintetase de di-hidropteroato, inibindo, assim, a síntese de ácido nucleico.

[00146] As Tetraciclinas incluem, mas sem limitações, Demeclociclina, Doxiciclina, Minociclina, Oxitetraciclina e Tetraciclina. As Tetraciclinas são eficazes, *por exemplo,* contra bactérias Gram-negativas. Acredita-se que as Tetraciclinas se liguem à subunidade ribossômica 30S bacteriana, inibindo, assim, a síntese de proteína bacteriana.

[00147] Os compostos antimicobacterianos incluem, mas sem limitações, Clofazimina, Dapsona, Capreomicina, Ciclosserina, Etambutol, Etionamida, Isoniazide, Pirazinamida, Rifampicina, Rifabutina, Rifapentina e Estreptomicina.

[00148] Os antibióticos adequados também incluem arsfenamina, cloramfenicol, fosfomicina, ácido fusídico, metronidazol, mupirocina, platensimicina, quinupristina/dalfopristina, tigeciclina, tinidazol, amoxicilina/clavulanato de trimetoprime, ampicilina/sulbactame, anfomicina ristocetina, azitromicina, bacitracina, buforina II, carbomicina, cecropina PI, claritromicina, eritromicinas, furazolidona, ácido fusídico, fusidato de Na, gramicidina, imipeneme, indolicidina, josamicina, magainana II, metronidazol, nitroimidazóis, micamicina, mutacina B-Ny266, mutacina B-JHI 140, mutacina J-T8,

nisina, nisina A, novobiocina, oleandomicina, ostreogricina, piperacilina/tazobactame, pristinamicina, ramoplanina, ranalexina, reuterina, rifaximina, rosamicina, rosaramicina, spectinomicina, espiramicina, estafilomicina, estreptogramina, estreptogramina A, sinergistina, taurolidina, teicoplanina, telitromicina, ticarcilina/ácido clavulânico, triacetiloleandomicina, tilosina, tirocidina, tirotricina, vancomicina, vemamicina e virginiamicina.

[00149] Em algumas modalidades, a terapia de câncer compreende administrar bactérias terapêuticas e/ou a combinação terapêutica de bactérias ao indivíduo, de modo que um microbioma saudável possa ser reconstituído no indivíduo. Em algumas modalidades, as bactérias terapêuticas são bactérias não associadas ao câncer. Em algumas modalidades, as bactérias terapêuticas consistem em bactérias probióticas.

Câncer

[00150] Em algumas modalidades, os métodos e composições descritos no presente documento se referem ao tratamento de câncer. Os exemplos de cânceres que podem ser tratados por métodos descritos no presente documento incluem, mas sem limitações, malignidade hematológica, leucemia não linfocítica aguda, leucemia linfocítica crônica, leucemia granulócita aguda, leucemia granulócita crônica, leucemia pró-mielocítica aguda, leucemia de célula T adulta, leucemia aleucêmica, uma leucemia leucocitêmica, leucemia basofílica, leucemia de blastócitos, leucemia bovina, leucemia mielocítica crônica, leucemia cutânea, leucemia embrionária, leucemia eosinofílica, leucemia de Gross, leucemia de célula de Rieder, leucemia de Schilling, leucemia de célula-tronco, leucemia subleucêmica, leucemia de célula não diferenciada, leucemia de célula pilosa, leucemia hemoblástica, leucemia hemocitoblástica, leucemia histiocítica, leucemia de célula-tronco, leucemia monocítica aguda, leucemia leucopênica, leucemia linfática, leucemia linfoblástica, leucemia linfocítica, leucemia linfogênica,

leucemia linfoide, leucemia de célula de linfossarcoma, leucemia de mastócito, leucemia megacariocítica, leucemia micromieloblástica, leucemia monocítica, leucemia mieloblástica, leucemia mielocítica, leucemia granulocítica mieloide, leucemia mielomonocítica, leucemia de Naegeli, leucemia de célula plasmática, leucemia plasmacítica, leucemia pró-mielocítica, carcinoma acinar, carcinoma acinoso, carcinoma adenocístico, carcinoma cístico adenoide, carcinoma adenomatoso, carcinoma do córtex adrenal, carcinoma alveolar, carcinoma de célula alveolar, carcinoma de célula basal, carcinoma basocelular, carcinoma basaloide, carcinoma de célula basoescamosa, carcinoma bronquioalveolar, carcinoma bronquiolar, carcinoma broncogênico, carcinoma cerebriforme, carcinoma colangiocelular, carcinoma coriônico, carcinoma coloide, comedocarcinoma, carcinoma do corpo, carcinoma cribriforme, carcinoma en cuirasse, carcinoma cutâneo, carcinoma cilíndrico, carcinoma de célula cilíndrica, carcinoma do duto, carcinoma duro, carcinoma embrionário, carcinoma encefaloide, carcinoma epienoide, carcinomas epiteliais adenoides, carcinoma exofítico, carcinoma ex úlcera, carcinoma fibroso, carcinoma gelatiniforme, carcinoma gelatinoso, carcinoma de célula gigante, carcinoma de células em anel de sinete, carcinoma simples, carcinoma de célula pequena, carcinoma solanoide, carcinoma de célula esferoide, carcinoma de célula fusiforme, carcinoma espongioso, carcinoma escamoso, carcinoma de célula escamosa, carcinoma de corda, carcinoma telangiectásico, telangiectódios de carcinoma, carcinoma de célula transicional, carcinoma tuberosum, carcinoma tuberoso, carcinoma verrucoso, carcinoma viloso, carcinoma gigantocelular, carcinoma glandular, carcinoma de célula granulosa, carcinoma da matriz capilar, carcinoma hematoide, carcinoma hepatocelular, carcinoma de célula de Hurthle, carcinoma hialina, carcinoma hipernefroide, carcinoma

embrionário infantil, carcinoma in situ, carcinoma intraepidérmico, carcinoma intraepitelial, carcinoma de Krompecher, carcinoma de célula de Kulchitzky, carcinoma de célula grande, carcinoma lenticular, carcinoma lenticular, carcinoma lipomatoso, carcinoma linfoepitelial, carcinoma medullar, carcinoma medular, carcinoma melanótico, carcinoma mole, carcinoma mucinoso, carcinoma muciparum, carcinoma mucocelular, carcinoma mucoepidermoide, carcinoma mucoso, carcinoma muscoso, carcinoma mixomatoso, carcinoma nasofaríngeo, carcinoma de células tipo grão de aveia, carcinoma ossificante, carcinoma osteoide, carcinoma papilar, carcinoma periportal, carcinoma pré-invasivo, carcinoma de célula espinhosa, carcinoma pultáceo, carcinoma de células renais dos rins, carcinoma de células de reserva, carcinoma sarcomatoso, carcinoma schneideriano, carcinoma cirrótico, carcinoma escrotal, condrossarcoma, fibrossarcoma, linfossarcoma, melanossarcoma, mixossarcoma, osteossarcoma, sarcoma endometrial, sarcoma estromal, sarcoma de Ewing, sarcoma da fáscia, sarcoma fibroblástico, sarcoma de célula gigante, sarcoma de Abemethy, sarcoma adiposo, lipossarcoma, sarcoma da parte mole alveolar, sarcoma ameloblástico, sarcoma botrioide, sarcoma cloroma, coriocarcinoma, sarcoma embrionário, sarcoma de tumor de Wilms, sarcoma granulócito, sarcoma de Hodgkin, sarcoma hemorrágico pigmentado múltiplo idiopático, sarcoma imunoblástico de células B, linfoma, sarcoma imunoblástico de células T, sarcoma de Jensen, sarcoma de Kaposi, sarcoma de célula de Kupffer, angiossarcoma, leucossarcoma, sarcoma mesenquimoma maligno, sarcoma parosteal, sarcoma reticulócito, rabdossarcoma, sarcoma serocístico, sarcoma sinovial, sarcoma telangiectáltico, doença de Hodgkin, linfoma não Hodgkin, mieloma múltiplo, neuroblastoma, câncer de bexiga, câncer de mama, câncer de ovário, câncer de pulmão, câncer colorretal,

rabdomiossarcoma, trombocitose primária, macroglobulinemia primária, tumores de pulmão de células pequenas, tumores cerebrais primários, câncer de estômago, câncer de cólon, insulanoma pancreático maligno, carcinoide maligno, lesões de pele pré-malignas, câncer testicular, linfomas, câncer da tireoide, neuroblastoma, câncer esofágico, câncer do trato genitourinário, hipercalcemia maligna, câncer cervical, câncer endometrial, câncer adrenal cortical, melanoma de Harding-Passey, melanoma juvenil, melanoma lentigo maligno, melanoma maligno, melanoma acral-lentiginoso, melanoma amelanótico, melanoma juvenil benigno, melanoma de Cloudman, melanoma S91, melanoma nodular, melanoma subungular e melanoma de difusão superficial.

[00151] Em algumas modalidades, os métodos e composições fornecidos no presente documento se referem ao tratamento de uma leucemia. O termo "leucemia" significa doenças malignas amplamente progressivas dos órgãos/sistemas hematopoiéticos e é geralmente caracterizada por uma proliferação e desenvolvimento distorcidos de leucócitos e seus precursores no sangue e medula óssea. Os exemplos não limitantes de doenças de leucemia incluem, leucemia não linfocítica aguda, leucemia linfocítica crônica, leucemia granulocítica aguda, leucemia granulocítica crônica, leucemia promielocítica aguda, leucemia de célula T adulta, leucemia aleucêmica, uma leucemia leucocitêmica, leucemia basofílica, leucemia de célula de blasto, leucemia bovina, leucemia mielocítica crônica, leucemia cutânea, leucemia embrionária, leucemia eosinofílica, leucemia de Gross, leucemia de célula de Rieder, leucemia de Schilling, leucemia de célula-tronco, leucemia subleucêmica, leucemia de célula não diferenciada, leucemia de célula capilar, leucemia hemoblástica, leucemia hemocitoblástica, leucemia histiocítica, leucemia de célula-tronco, leucemia monocítica aguda, leucemia leucopênica, leucemia linfática, leucemia linfoblástica,

leucemia linfocítica, leucemia linfogenosa, leucemia linfoide, leucemia de célula de linfossarcoma, leucemia de célula de masto, leucemia megacariocítica, leucemia micromieloblástica, leucemia monocítica, leucemia mieloblástica, leucemia mielocítica, leucemia granulocítica mieloide, leucemia mielomonocítica, leucemia de Naegeli, leucemia de célula plasmática, leucemia plasmática e leucemia pró-mielocítica.

[00152] Em algumas modalidades, os métodos e composições fornecidos no presente documento se referem ao tratamento de um carcinoma. O termo "carcinoma" se refere a um crescimento maligno constituído por células epiteliais que tendem a infiltrar os tecidos circundantes e/ou resistir sinais de morte celular fisiológicos e não fisiológicos e gera as metástases. Os tipos exemplificativos não limitantes de carcinomas incluem, carcinoma acinar, carcinoma acinoso, carcinoma adenocístico, carcinoma cístico adenoide, carcinoma adenomatoso, carcinoma do córtex adrenal, carcinoma alveolar, carcinoma de célula alveolar, carcinoma de célula basal, carcinoma basocelular, carcinoma basaloide, carcinoma de célula basoescamosa, carcinoma bronquioalveolar, carcinoma bronquiolar, carcinoma broncogênico, carcinoma cerebriforme, carcinoma colangiocelular, carcinoma coriônico, carcinoma coloide, comedocarcinoma, carcinoma do corpo, carcinoma cribriforme, carcinoma en cuirasse, carcinoma cutâneo, carcinoma cilíndrico, carcinoma de célula cilíndrica, carcinoma do duto, carcinoma duro, carcinoma embrionário, carcinoma encefaloide, carcinoma epienoide, carcinoma epiteliais adenoides, carcinoma exofítico, carcinoma ex úlcera, carcinoma fibroso, carcinoma gelatiniforme, carcinoma gelatinoso, carcinoma de célula gigante, carcinoma de células em anel de sinete, carcinoma simples, carcinoma de célula pequena, carcinoma solanoide, carcinoma de célula esferoide, carcinoma de célula fusiforme, carcinoma espongioso, carcinoma escamoso, carcinoma de célula escamosa,

carcinoma de corda, carcinoma telangiectásico, telangiectódios de carcinoma, carcinoma de célula transicional, carcinoma tuberosum, carcinoma tuberoso, carcinoma verrucoso, carcinoma viloso, carcinoma gigantocelular, carcinoma glandular, carcinoma de célula granulosa, carcinoma da matriz capilar, carcinoma hematoide, carcinoma hepatocelular, carcinoma de célula de Hurthle, carcinoma hialina, carcinoma hipernefroide, carcinoma embrionário infantil, carcinoma in situ, carcinoma intraepidérmico, carcinoma intraepitelial, carcinoma de Krompecher, carcinoma de célula de Kulchitzky, carcinoma de célula grande, carcinoma lenticular, carcinoma lenticulare, carcinoma lipomatoso, carcinoma linfoepitelial, carcinoma medullare, carcinoma medular, carcinoma melanótico, carcinoma mole, carcinoma mucinoso, carcinoma muciparum, carcinoma mucocelular, carcinoma mucoepidermoide, carcinoma mucosum, mucous carcinoma, carcinoma mixomatoso, carcinoma nasofaríngeo, carcinoma de células tipo grão de aveia, carcinoma ossificante, carcinoma osteoide, carcinoma papilar, carcinoma periportal, carcinoma pré-invasivo, carcinoma de célula espinhosa, carcinoma pultáceo, carcinoma de células renais dos rins, carcinoma de células de reserva, carcinoma sarcomatoso, carcinoma schneideriano, carcinoma cirrótico e carcinoma escrotal.

[00153] Em algumas modalidades, os métodos e composições fornecidos no presente documento se referem ao tratamento de um sarcoma. O termo "sarcoma" geralmente se refere a um tumor que é constituído por uma substância como o tecido conectivo embrionário e é geralmente composto por células embaladas próximas embutidas em uma substâncias fibrilar, heterogênea ou homogênea. Os sarcomas incluem, mas sem limitações, condrossarcoma, fibrossarcoma, linfossarcoma, melanossarcoma, mixossarcoma, osteossarcoma, sarcoma endometrial, sarcoma estromal,

sarcoma de Ewing, sarcoma da fáscia, sarcoma fibroblástico, sarcoma de célula gigante, sarcoma de Abemethy, sarcoma adiposo, lipossarcoma, sarcoma da parte mole alveolar, sarcoma ameloblástico, sarcoma botrioide, sarcoma cloroma, coriocarcinoma, sarcoma embrionário, sarcoma de tumor de Wilms, sarcoma granulócito, sarcoma de Hodgkin, sarcoma hemorrágico múltiplo idiopático, sarcoma imunoblástico de células B, linfoma, sarcoma imunoblástico de células T, sarcoma de Jensen, sarcoma de Kaposi, sarcoma de célula de Kupffer, angiossarcoma, leucossarcoma, sarcoma mesenquimoma maligno, sarcoma parosteal, sarcoma reticulócito, sarcoma de Rous, sarcoma serocístico, sarcoma sinovial e sarcoma telangiectáltico.

[00154] As neoplasias exemplificativas adicionais que podem ser tratadas com o uso dos métodos e composições descritos no presente documento incluem doença de Hodgkin, linfoma não Hodgkin, mieloma múltiplo, neuroblastoma, câncer de mama, câncer de ovário, câncer de pulmão, rabdomiossarcoma, trombocitose primária, macroglobulinemia primária, tumores de pulmão de células pequenas, tumores cerebrais primários, câncer de estômago, câncer de cólon, insulanoma pancreático maligno, carcinoide maligno, lesões de pele pré-malignas, câncer testicular, linfomas, câncer da tireoide, neuroblastoma, câncer esofágico, câncer do trato genitourinário, hipercalcemia maligna, câncer cervical, câncer endometrial e câncer adrenal cortical.

[00155] Em algumas modalidades, o câncer tratado é um melanoma. O termo "melanoma" significa um tumor que surge a partir do sistema melanocítico da pele e outros órgãos. Os exemplos não limitantes de melanomas são melanoma de Harding-Passey, melanoma juvenil, melanoma lentigo maligna, melanoma maligno, melanoma acral-lentiginoso, melanoma amelanótico, melanoma juvenil benigno, melanoma de Cloudman, mela-

noma S91, melanoma nodular, melanoma subungal e melanoma de difusão superficial.

[00156] As categorias de tumores que podem ser tratadas com o uso de métodos e composições descritos no presente documento incluem distúrbios linfoproliferativos, câncer de mama, câncer de ovário, câncer de próstata, câncer de colo de útero, câncer endometrial, câncer ósseo, câncer do fígado, câncer do estômago, câncer do cólon, câncer colorretal, câncer pancreático, câncer da tiroide, cabeça e pescoço câncer, câncer do sistema nervoso central, câncer do sistema nervoso periférico, câncer de pele, câncer do rim, assim como metástases de todos os supracitados. Os tipos particulares de tumores incluem carcinoma hepatocelular, hepatoma, hepatoblastoma, rabdomiossarcoma, carcinoma esofageal, carcinoma de tiroide, ganglioblastoma, fibrossarcoma, mixossarcoma, lipossarcoma, condrossarcoma, sarcoma osteogênico, cordoma, angiossarcoma, endoteliossarcoma, tumor de Ewing, leimiossarcoma, rabdoterliossarcoma, carcinoma dutal invasivo, adenocarcinoma papilar, melanoma, carcinoma de célula escamosa pulmonar, carcinoma de célula basal, adenocarcinoma (bem diferenciada, moderadamente diferenciada, insatisfatoriamente diferenciada ou não diferenciada), carcinoma bronquioalveolar, carcinoma de célula renal, hipernefroma, adenocarcinoma hipernefroide, carcinoma de duto biliar, coriocarcinoma, seminoma, carcinoma embrionário, tumor de Wilms, tumor testicular, carcinoma pulmonar incluindo carcinoma pulmonar de célula pequena, de célula não pequena e de célula grande, carcinoma de bexiga, glioma, astrocioma, meduloblastoma, craniofaringioma, ependimoma, pinealoma, retinoblastoma, neuroblastoma, carcinoma de cólon, carcinoma retal, malignâncias hematopoiéticas incluindo todos os tipos de leucemia e linfoma incluindo: leucemia mielogenosa aguda, leucemia mielocítica aguda, leucemia linfocítica aguda, leucemia mielogenosa crônica,

leucemia linfocítica crônica, leucemia de célula de masto, leucemia megacariocíticao, linfoma mieloide, linfoma de Hodgkin, linfoma não Hodgkin.

[00157] Os cânceres tratados em certas modalidades também incluem lesões pré-cancerígenas, *por exemplo,* ceratose actínica (ceratose solar), molas (nervos displásticos), quelite actínica (lábio de agricultor), chifres cutâneos, esôfago de Barrett, gastrite atrófica, disceratose congenita, disfagia sideropênica, líquen plano, fibrose submucosa oral, elastose actínica (solar) e displasia cervical.

[00158] Os cânceres tratados em algumas modalidades incluem tumores não cancerígenos ou benignos, *por exemplo,* de origem endodérmica, ectodérmica ou mesenquimal, incluindo, mas sem limitações colangioma, pólipo colônico, adenoma, papiloma, cistadenoma, adenoma celular do fígado, mola hidatidiforme, adenoma tubular renal, papiloma de célula escamosa, pólipo gástrico, hemangioma, osteoma, condroma, lipoma, fibroma, linfangioma, leiomioma, rabdomioma, astrocitoma, nevo, meningioma e ganglioneuroma.

EXEMPLOS

Exemplo 1: *Produção de Bifidobacterium animalis ssp. lactis*

[00159] Meios enriquecidos são usados para cultivar e preparar *Bifidobacterium animalis ssp. lactis* para uso in vitro e in vivo. Por exemplo, os meios podem conter açúcar, extratos de levedura, peptonas, elementos vestigiais e vitaminas. Alternativamente, os meios podem ser preparados e *Bifidobacterium animalis ssp. lactis* cultivada como mostrado por Saarela *et al., J. Applied Microbiology*. 2005. 99: 1330 a 1339, que é aqui incorporado a título de referência. A influência do tempo de fermentação, crioprotetor e neutralização do concentrado celular sobre a sobrevivência à secagem por congelamento, estabilidade de armazenamento e exposição a ácido e bile de células de *Bifidobacterium animalis ssp. lactis* produzidas

sem ingredientes à base de leite,

[00160] Em grande escala, o meio é esterilizado. A esterilização pode ser por processamento de Temperatura Ultra-Alta (UHT). O processamento de UHT é realizado a uma temperatura muito alta por períodos de tempo curtos. A faixa de UHT pode ser de 135 a 180°C. Por exemplo, o meio pode ser esterilizado entre 10 a 30 segundos a 135°C.

[00161] O inóculo pode ser preparado em frascos ou em biorreatores menores e o crescimento é monitorado. Por exemplo, o tamanho de inóculo pode ser entre aproximadamente 0,5 e 3% do volume de biorreator total. Dependendo da aplicação e da necessidade de material, o volume de biorreator pode ser pelo menos 2 l, 10 l, 80 l, 100 l, 250 l, 1.000 l, 2.500 l, 5.000 l, 10.000 l.

[00162] Antes da inoculação, o biorreator é preparado e pronto com meio em pH, temperatura e concentração de oxigênio desejados. Por exemplo, o pH pode ser definido entre 4,5 e 8,0. Durante a fermentação, o pH pode ser controlado através do uso de hidróxido de sódio, hidróxido de potássio ou hidróxido de amônio. A temperatura pode ser controlada a partir de 25°C a 45°C, por exemplo, a 37°C. Condições anaeróbicas são criadas através da redução do nível de oxigênio no caldo de cultura a partir de cerca de 8 mg/l a 0 mg/l. Por exemplo, nitrogênio ou misturas de gás (N2, CO2, e H2) podem ser usadas para estabelecer condições anaeróbicas. Alternativamente, nenhum gás é usado e as condições anaeróbicas são estabelecidas por células que consomem o oxigênio restante do meio. Dependendo da cepa e do tamanho de inóculo, o tempo de fermentação de biorreator pode variar. Por exemplo, o tempo de fermentação pode variar de aproximadamente 5 horas a 48 horas.

[00163] A colheita pode ser realizada por centrifugação contínua. O produto será ressuspenso com vários excipientes a uma concentração final

desejada. Os excipientes podem ser adicionados para crioproteção ou para proteção durante a liofilização. Os excipientes podem incluir, mas sem limitações, sacarose, trealose ou lactose, e essas podem ser alternativamente misturadas com tampão e antioxidantes. Antes da liofilização, gotículas de péletes de célula misturadas com excipientes são submersas em nitrogênio líquido.

[00164] A liofilização de material, incluindo bactérias vivas, começa com secagem primária. Durante a fase de secagem primária, o gelo é removido. Aqui, um vácuo é gerado e uma quantidade adequada de calor é suprida ao material para que o gelo seja submetido à sublimação. Durante a fase de secagem secundária, as moléculas de água ligadas ao produto são removidas. Aqui, a temperatura é elevada acima da fase de secagem primária para romper quaisquer interações físico-químicas que tenham se formado entre as moléculas de água e o material de produto. A pressão também pode ser reduzida adicionalmente para intensificar a dessorção durante esse estágio. Após o processo de secagem por congelamento ser concluído, a câmara pode ser preenchida com um gás inerte, como nitrogênio. O produto pode ser vedado dentro da secadora por congelamento sob condições secas, prevenindo a exposição à água e contaminantes atmosféricos.

Exemplo 2: *Bifidobacterium animalis ssp. lactis* administrada oralmente inibe o crescimento tumoral de carcinoma colorretal

[00165] Camundongos tipo Balb/c fêmeas com 6 a 8 semanas de idade foram obtidos junto à Taconic (Germantown, NY). 100.000 células de tumor colorretal de CT-26 (ATCC CRL-2638) foram ressuspensas em PBS estéril e inoculadas na presença de 50% de Matrigel. As células de tumor de CT-26 foram injetadas de modo subcutâneo em um flanco traseiro de cada camundongo. Quando os volumes de tumor alcançaram uma média de 100

mm^3 (aproximadamente 10 a 12 dias após a inoculação de célula de tumor), os animais foram distribuídos nos seguintes grupos: 1) Veículo + anticorpo de controle de isótipo IgG2a; e 3) *Cepa A de Bifidobacterium animalis ssp. lactis* (número de acesso ao ATCC PTA-125097) + IgG2a. As bactérias *Bifidobacterium animalis ssp. lactis* ($1x10^9$) foram administradas por gavagem oral (p.o.) diariamente, começando no dia 1 até a conclusão do estudo. O grupo de *Bifidobacterium animalis ssp. lactis* + IgG2a mostrou inibição significativa de crescimento tumoral em comparação com o grupo de veículo + IgG2a (Consultar a **Figura 1**).

Exemplo 3: *Bifidobacterium animalis ssp. lactis* administrada oralmente inibe o crescimento tumoral de carcinoma colorretal em comparação com a inibição vista com tratamento de anti-PD-1

[00166] As células de tumor de CT-26 foram injetadas de modo subcutâneo em um flanco traseiro de cada camundongo Balb/c conforme supracitado. Quando os volumes de tumor alcançaram uma média de 100 mm^3 (aproximadamente 10 a 12 dias após a inoculação de célula de tumor), os animais foram distribuídos nos seguintes grupos: 1) Veículo + PBS; 2) Veículo + anticorpo de controle de isótipo IgG2a; e 3) Veículo + anti-PD-1; 4) *Cepa A de Bifidobacterium animalis ssp. lactis* (número de acesso ao ATCC PTA-125097) + IgG2a; e 5) *Cepa A de Bifidobacterium animalis ssp. lactis* + anti-PD-1. Os anticorpos foram administrados de modo intraperitoneal (i.p.) a 100 ug/camundongo (volume final de 100 ul) a cada quatro dias, começando no dia 1, e bactérias de *Bifidobacterium animalis ssp. lactis* ($1x10^9$) foram administradas por gavagem oral (p.o.) diariamente, começando no dia 1 até a conclusão do estudo. O grupo de *Bifidobacterium animalis ssp. lactis* + IgG2a mostrou inibição de crescimento tumoral comparável com aquela vista no grupo de veículo + anti-PD-1 (Consultar **Figura 2**).

<u>Exemplo 4</u>: Tratamento de carcinoma colorretal com o uso de diferentes cepas de *Bifidobacterium animalis ssp. lactis*

Como descrito nos exemplos 2 e 3, as células de tumor de CT-26 foram injetadas de modo subcutâneo em um flanco traseiro de camundongos e animais foram atribuídos aos grupos recebendo os seguintes tratamentos: 1) Veículo; 2) *Cepa A de Bifidobacterium animalis ssp. lactis* (número de acesso ao ATCC PTA-125097)*;* 3) Cepa B de *B. animalis lactis;* 4) Cepa C *B. animalis ssp. lactis*; e 5) anti-PD-1. $1x10^9$ células bacterianas foram administradas diariamente (p.o.), iniciando no dia 1 de atribuição de grupo de animais, e os tumores medidos como descrito acima. Os anticorpos anti-PD-1 foram administrados de modo intraperitoneal (i.p.) a 100 ug/camundongo (volume final de 100 ul) a cada quatro dias, começando no dia 1. Como visto na Tabela 2, a Cepa A mostrou inibição significativa de crescimento tumoral em comparação com os outros grupos de tratamento de *B. animalis lactis* (**Tabela 2** e **Figura 3**).

Tabela 2. Volume de tumor de carcinoma colorretal (mm^3) no Dia 22 (12º dia de dosagem)

	Anti PD-1	**Veículo**	**Cepa C**	**Cepa B**	**Cepa A**
Vol. de tumor mediano (mm^3)	1096,97	1775,89	1294,94	1819,68	1788,83
Vol. de tumor médio (mm^3)	1029,97	1757,75	1231,34	1753,12	1704,33
SEM (mm^3)	193,37	154,75	190,00	89,48	119,03

<u>Exemplo 5</u>: *Bifidobacterium animalis ssp. lactis* administrada de modo intratumoral inibe o crescimento tumoral de carcinoma colorretal

[00167] Como descrito nos exemplos 2 e 3, as células de tumor de CT-26 foram injetadas de modo subcutâneo em um flanco traseiro de camundongos e animais foram atribuídos aos grupos recebendo os seguintes tra-

tamentos: 1) Veículo; 2) Cepa A de *Bifidobacterium animalis ssp. lactis* (número de acesso ao ATCC PTA-125097)*;* e 3) anti-PD-1. $2x10^9$ células bacterianas foram administradas de modo intratumoral (IT) no dia 1 de atribuição de grupo de animais, e os tumores medidos como descrito acima. Os camundongos receberam uma segunda dose no dia 4. Os anticorpos anti-PD-1 foram administrados de modo intraperitoneal (i.p.) a 100 ug/camundongo (volume final de 100 ul) a cada quatro dias, começando no dia 1. O grupo de Cepa A de *Bifidobacterium animalis ssp. lactis* mostrou inibição significativa de crescimento tumoral em comparação com o grupo de controle (Consultar **Figura 4**).

Exemplo 6: *Bifidobacterium animalis ssp. lactis* em um modelo de melanoma de camundongo

[00168] A *B. animalis ssp. lactis* é testada quanto à eficácia em um modelo de melanoma de camundongo. Por exemplo, camundongos tipo C57B1/6 fêmeas de 6 a 8 semanas de idade são obtidas junto à Taconic (Germantown, NY). Entre $1x10^4$ e $1x10^6$ de células de tumor de B16-F10 (ATCC CRL-6475) podem ser usadas para inocular vários grupos de camundongos. As células de tumor de B16-F10 podem ser ressuspensas em PBS estéril contendo 50% de Matrigel e inoculadas em um volume final de 100 ul em um flanco traseiro (o primeiro flanco) de cada camundongo. O tratamento com *Bifidobacterium animalis ssp. lactis* é iniciado em algum momento após a inoculação de célula de tumor em doses variadas e a intervalos definidos. Por exemplo, alguns camundongos recebem entre 1 a $5x10^9$ CFU (volume final de 100 µl) por dose. As vias possíveis de administração incluem gavagem oral (p.o.), injeção intravenosa, injeção intratumoral (IT) ou injeção peritumoral, ou subtumoral ou subcutânea. Para avaliar os efeitos antitumorais sistêmicos de tratamento de *B. animalis lactis*, camundongos adicionais podem ser inoculados com células de tumor no

flanco contralateral (não tratado, segundo) antes do tratamento IT, peritumoral ou subtumoral com *B. animalis lactis* no primeiro flanco.

[00169] Alguns camundongos podem receber *Bifidobacterium animalis ssp. lactis* (p.o.) no dia 1 (o dia seguinte à injeção de célula de tumor). Outros camundongos podem receber sete (7) doses consecutivas de *Bifidobacterium animalis ssp. lactis* (uma dose por dia nos dias 14 a 21). Outros camundongos recebem dosagem diariamente ou, alternativamente, alguns camundongos recebem dosagem em dias intercalados. Alternativamente, os camundongos são randomizados em vários grupos de tratamento em um ponto no tempo definido (*por exemplo,* no dia 13) ou quando os tumores alcançam um certo tamanho (*por exemplo,* 100 mm^3) e o tratamento é, então, iniciado consequentemente. Por exemplo, quando os volumes de tumor alcançam uma média de 100 mm^3 (aproximadamente 10 a 12 dias após a inoculação de célula de tumor), os animais são distribuídos em grupos e tratados com veículo ou *Bifidobacterium animalis ssp. lactis* (p.o. ou IT). Alguns grupos adicionais de camundongos podem ser tratados com um agente terapêutico de câncer adicional ou anticorpo de controle adequado. Um exemplo de um agente terapêutico de câncer que pode ser administrado é um inibidor de um ponto de verificação imune, por exemplo, anti-PD-1, anti-PD-L1 ou outro tratamento que bloqueie a ligação de um ponto de verificação imune ao seu ligante (ou ligantes). Os inibidores de ponto de verificação anti-PD-1 e anti-PD-L1 podem ser formulados em PBS e administrados de modo intraperitoneal (i.p.) em doses eficazes. Por exemplo, os camundongos recebem 100 ug de anti-PD-1 (i.p.) a cada quatro dias, começando no dia 1, e continuando pela duração do estudo.

[00170] Além disso, alguns camundongos são tratados com antibióticos antes do tratamento. Por exemplo, vancomicina (0,5 g/l), ampicilina (1,0 g/l), gentamicina (1,0 g/l) e anfotericina B (0,2 g/l) são adicionados à água

para beber e o tratamento de antibiótico é interrompido no momento do tratamento ou alguns dias antes do tratamento. Alguns camundongos são inoculados com células de tumor sem receber tratamento anterior com antibióticos. Além disso, em alguns grupos, as células de B16-f10 podem ser cultivadas com 100 U/ml de penicilina e 10 mg/ml de estreptomicina antes da inoculação.

[00171] Por exemplo, as células de B16-F10 foram expandidas com o uso de técnica de cultura de tecido padrão, mantidas em meio de DMEM suplementado com 10% de soro bovino fetal (FBS), 100 U/ml de penicilina e 10 mg/ml de estreptomicina em um incubador a 37°C com 5% de CO_2. No dia de implantação de célula de tumor, as células de B16-F10 foram ressuspensas em HBSS gelado a $1x10^6$ células de tumor/ml e cada camundongo foi injetado com 100 ul de células de tumor ressuspensas. O crescimento de tumor foi medido três vezes por semana com o uso de calibradores digitais de acordo com métodos conhecidos na técnica. Quando o tamanho de tumor médio alcançou aproximadamente 100 mm^3, os camundongos foram aleatoriamente separados em grupos de tratamento e o tratamento se iniciou de acordo com a Tabela 3 abaixo.

Tabela 3. Tratamentos

Grupo, N=20	Tratamento nº 1	Tratamento nº 2
1	Veículo (PO, 100 µl de vol., QD)	PBS (IP, 100 µl, Q4D)
2	Veículo (PO, 100 µl de vol., QD)	anti-PD-L1 (IP, 200 µ g/100 µl de vol, Q4D)
3	Cepa A de B. animalis ssp. lactis (PO, $6,5x10^9$ CFU em 100 µl de vol., QD)	PBS (IP, 100 µl, Q4D)
4	Cepa A de B. animalis ssp. lactis (PO, $6,5x10^9$ CFU em 100 µl de vol., QD)	anti-PD-L1 (IP, 200 µ g/100 µl de vol, Q4D)

[00172] Os animais receberam o Tratamento nº 1 (Veículo ou Cepa A

de B. animalis ssp. lactis) por meio de gavagem oral todos os dias. Os camundongos receberam o Tratamento nº 2 (PBS ou anti-PD-L1 intraperitoneal a cada 4 dias até a finalização do estudo. Tratar camundongos inoculados com tumor com uma combinação de Cepa A de *Bifidobacterium animalis ssp. lactis* e anti-PD-L1 inibe o crescimento tumoral de uma maneira superior tanto à Cepa A de *B. animalis ssp. lactis* quanto anti-PD-L1 sozinha **(Figura 5)**.

Exemplo 7: A infiltração de células de CD3+ é significativamente aumentada em tumores de CT26 em camundongos oralmente gavados com Cepa A de B. animalis lactis.

[00173] Em alguns estudos, em vários pontos no tempo, os camundongos são sacrificados e os tumores, gânglios linfáticos ou outros tecidos podem ser removidos para análise citométrica de fluxo *ex vivo* com o uso de métodos conhecidos na técnica. Por exemplo, os tumores são dissociados com o uso de um coquetel de enzima de dissociação de tumor de Miltenyi de acordo com as instruções do fabricante. Os pesos de tumor são registrados e os tumores são cortados, então, colocados em tubos de 15 ml contendo o coquetel de enzima e colocados em gelo. As amostras são, então, colocadas em um agitador suave a 37°C por 45 minutos e arrefecidas bruscamente com até 15 ml de RPMI completo. Cada suspensão celular é passada através de um filtro de 70 µm para um tubo falcon de 50 ml e centrifugada a 1.000 rpm por 10 minutos. As células são ressuspensas em tampão de FACS e lavadas para remover detritos restantes. Se for necessário, as amostras são passadas novamente através de um segundo filtro de 70 µm para um novo tubo. As células são manchadas para análise por citometria de fluxo com o uso de técnicas conhecidas na técnica. Os anticorpos de manchamento podem incluir anti-CD11c (células dendríticas), anti-CD80, anti-CD86, anti-CD40, anti-MHCII, anti-CD8a, anti-CD4 e

anti-CD103. Outros marcadores que podem ser analisados incluem marcador de célula pan-imune CD45, os marcadores de célula T (CD3, CD4, CD8, CD25, Foxp3, T-bet, Gata3, Rorγt, Granzima B, CD69, PD-1, CTLA-4) e marcadores de macrófago/mieloide (CD11b, MHCII, CD206, CD40, CSF1R, PD-L1, Gr-1). Além da imunofenotipagem, as citocinas são analisadas incluindo, mas sem limitações, IP-10, TNFa, IL-17, IL-13, IL-12p70, IL12p40, IL-10, IL-6, IL-5, IL-4, IL-2, IL-1b, IFNy, GM-CSF, G-CSF, M-CSF, MIG, IP10, MIP1b, RANTES e MCP-1, com o uso de técnicas conhecidas na área. A análise de citocina pode ser analisada em células imunes obtidas a partir de gânglios linfáticos ou outro tecido, seções de tumor, células de tumor dissociadas, e/ou em células imunes infiltradas em tumor de CD45+ purificadas obtidas ex vivo. Os níveis de citocina podem ser averiguados com o uso de várias técnicas de manchamento, qPCR, ou outras técnicas conhecidas nas técnicas. A imuno-histoquímica também pode ser realizada em seções de tumor para medir as células T, macrófagos, células dendríticas e molécula de ponto de verificação ou outra expressão de proteína com o uso de técnicas conhecidas na técnica.

[00174] Os camundongos foram inoculados com células de tumor de CT26, divididas em grupos como descrito acima, e oralmente gavadas com Cepa A de *B. animalis ssp. lactis* $8{,}3x10^{9}$ CFU/camundongo diariamente. Outros camundongos receberam veículo como um controle negativo. Os camundongos desse estudo foram sacrificados no dia 10 e os tumores colhidos para análise. Com o uso de técnicas conhecidas descritas acima, as seções de tumor foram manchadas com anticorpos anti-CD3 (marcador de célula T) para avaliar as células de CD3+ médias por seção de tumor e a expressão de gene de tumor foi analisada por qPCR com o uso de um ensaio de TaqMan conforme a seguir: O RNA de tumor foi isolado com o Minikit RNeasy da Qiagen (nº de catálogo 74104), com o uso do protocolo

do fabricante. O cDNA foi isolado com o uso do Kit de Síntese de cDNA da BioRad iScript™ de acordo com o protocolo do fabricante (nº de catálogo 1708891). A reação de qPCR foi examinada com o uso de SsoAdvanced™ Universal Probes Supermix 1725281, usando o protocolo do fabricante no instrumento BioRad CFX384. As sondas específicas de camundongo de TaqMan foram da Invitrogen, MHC I (b2m) Mm00437762_m1 e β-actina Mm01205647_g1 para normalizar a expressão de gene. O valor de Ct de delta para cada tumor foi calculado para MHC I com o uso de β-actina como o gene de referência. A expressão para MHC I foi calculada com o uso da fórmula 2^-delta Ct para cada tumor de camundongo e esse valor foi dividido por seu respectivo volume final de tumor no final do estudo.

[00175] Os volumes de tumor foram tomados antes da análise de expressão de infiltrado de célula imune de CD3+ e Classe I de MHC. Como descrito na **Figura 1**, o volume de tumor foi notavelmente reduzido no dia 9 no grupo *B. animalis ssp. lactis* (**Figura 6A**). A infiltração de células imunes de CD3+ foi significativamente aumentada no grupo de *Cepa A de Bifidobacterium animalis ssp. lactis* em relação ao veículo **(Figura 6B)**, e a Cepa A de *B. animalis ssp. lactis* induziu uma regulação ascendente marcante de expressão de Classe I de MHC **(Figura 6C).**

[00176] Em vez de serem sacrificados, alguns camundongos puderam ser testados novamente com injeção de célula de tumor no contralateral flanco contralateral (ou outra área) para determinar o impacto da resposta de memória do sistema imunológico sobre o crescimento tumoral.

Exemplo 8: *Bifidobacterium animalis ssp. lactis* em um modelo de câncer de pulmão de camundongo

[00177] A *Bifidobacterium animalis ssp. lactis* é testada quanto à sua eficácia no modelo de câncer de pulmão de camundongo, sozinha ou em

combinação com outras terapias de câncer, incluindo inibidor (ou inibidores) de ponto de verificação. Os camundongos são divididos em grupos recebendo *Bifidobacterium animalis ssp. lactis* com ou sem inibidor de ponto de verificação tratamento. Como descrito no Exemplo 6, *Bifidobacterium animalis ssp. lactis* é administrada em doses variadas em intervalos definidos. Por exemplo, alguns camundongos recebem *Bifidobacterium animalis ssp. lactis* (p.o.) no dia seguinte à injeção de célula de tumor (dia 1). Alguns camundongos recebem sete (7) doses consecutivas de *Bifidobacterium animalis ssp. lactis* (uma dose por dia nos dias 14 a 21). Outros camundongos recebem dosagem diariamente ou, alternativamente, alguns camundongos recebem dosagem em dias intercalados. Alternativamente, os camundongos são randomizados em vários grupos de tratamento em um ponto no tempo definido (*por exemplo,* no dia 13) ou quando os tumores alcançam um certo tamanho (*por exemplo,* 100 mm^3) e o tratamento é, então, iniciado consequentemente.

[00178] $1x10^6$ células de LLC1, ou um número adequado de células de câncer de pulmão a partir de outra linhagem celular de câncer de pulmão, são injetadas no flanco traseiro de camundongos singênicos. Os tumores dos vários grupos de tratamento são medidos com calibradores em intervalos regulares. Como descrito no Exemplo 6, alguns camundongos são sacrificados para análise de tumor ex vivo com o uso de citometria de fluxo. Outros camundongos puderam ser testados novamente com injeção de célula de tumor no contralateral flanco contralateral para determinar o impacto da resposta de memória do sistema imunológico sobre o crescimento tumoral.

<u>Exemplo 9</u>: *Bifidobacterium animalis ssp. lactis* em um modelo de câncer de mama de camundongo

[00179] A *Bifidobacterium animalis ssp. lactis* é testada quanto à sua

eficácia no modelo de câncer de mama de camundongo, sozinha ou em combinação com outras terapias de câncer, incluindo inibidor (ou inibidores) de ponto de verificação. Os camundongos são divididos em grupos recebendo *Bifidobacterium animalis ssp. lactis* com ou sem inibidor de ponto de verificação tratamento. Como descrito no Exemplo 6, *Bifidobacterium animalis ssp. lactis* é administrada em doses variadas em intervalos definidos. Por exemplo, alguns camundongos recebem *Bifidobacterium animalis ssp. lactis* (p.o.) no dia seguinte à injeção de célula de tumor (dia 1). Alguns camundongos recebem sete (7) doses consecutivas de *Bifidobacterium animalis ssp. lactis* (uma dose por dia nos dias 14 a 21). Outros camundongos recebem dosagem diariamente ou, alternativamente, alguns camundongos recebem dosagem em dias intercalados. Alternativamente, os camundongos são randomizados em vários grupos de tratamento em um ponto no tempo definido (*por exemplo,* no dia 13) ou quando os tumores alcançam um certo tamanho (*por exemplo,* 100 mm^3) e o tratamento é, então, iniciado consequentemente.

[00180] As células de carcinoma de mama de camundongo 4T1 são obtidas junto ao ATCC e $1x10^6$ células em PBS a 50 ul são injetadas de modo subcutâneo em um ou ambos os membros traseiros de camundongos fêmeas tipo Balb/c (como descrito por Wang *et al.* 2003, Systemic dissemination of viral vectors during intratumoral injection. Molecular Cancer Therapeutics; 2(11)). Alternativamente, as células de carcinoma de mama de camundongo EMT6 são obtidas a partir do ATCC e $1x10^6$ células em PBS a 50µl são injetadas de modo subcutâneo em um ou ambos os membros traseiros de camundongos fêmeas tipo Balb/c com 6 a 8 semanas de idade (como descrito por Guo *et al.* 2014, Combinatorial Photothermal e Immuno Cancer Therapi Using Chitosan-Coated Hollow Copper Sulfide Nanoparti-

cles. ASC Nano.; 8(6): 5670 a 5681). Além disso, outras linhagens celulares de mama de camundongo disponíveis podem ser usadas.

[00181] Os tumores dos vários grupos de tratamento são medidos com calibradores em intervalos regulares. Como descrito no Exemplo 6, *Bifidobacterium animalis ssp. lactis* é administrada em doses variadas em intervalos definidos. Por exemplo, alguns camundongos são sacrificados para análise de tumor ex vivo com o uso de citometria de fluxo. Outros camundongos puderam ser testados novamente com injeção de célula de tumor no contralateral flanco contralateral para determinar o impacto da resposta de memória do sistema imunológico sobre o crescimento tumoral.

[00182] Alternativamente, as células de 4T1 podem ser usadas em um modelo murino ortotópico de câncer de mama como descrito por Tao *et al.* (Tao *et al.* 2008. O modelo de 4T1 pode ser imageado para o estudo de câncer de mama de estágio tardio. 8: 288). Os camundongos são sacrificados para a análise de tumor ex vivo. Os tumores são analisados por citometria de fluxo e imuno-histoquímica.

Exemplo 10: *Bifidobacterium animalis ssp. lactis* em um modelo de câncer pancreático de camundongo

[00183] A *Bifidobacterium animalis ssp. lactis* é testada quanto à sua eficácia no modelo de câncer pancreático de camundongo, sozinha ou em combinação com outras terapias de câncer, incluindo inibidor (ou inibidores) de ponto de verificação. Os camundongos são divididos em grupos recebendo *Bifidobacterium animalis ssp. lactis* com ou sem inibidor de ponto de verificação tratamento. Como descrito no Exemplo 6, alguns camundongos recebem *Bifidobacterium animalis ssp. lactis* (p.o.) no dia seguinte à injeção de célula de tumor (dia 1). Alguns camundongos recebem sete (7) doses consecutivas de *Bifidobacterium animalis ssp. lactis* (uma dose por dia nos dias 14 a 21). Outros camundongos recebem dosagem

diariamente ou, alternativamente, alguns camundongos recebem dosagem em dias intercalados. Alternativamente, os camundongos são randomizados em vários grupos de tratamento em um ponto no tempo definido (*por exemplo,* no dia 13) ou quando os tumores alcançam um certo tamanho (*por exemplo,* 100 mm^3) e o tratamento é, então, iniciado consequentemente.

[00184] As células de Panc02 são mantidas em DMEM, suplementadas com 10% de soro de bezerro fetal e 1% de penicilina/estreptomicina, e incubadas a 37°C a 5% de CO2. Camundongos tipo C57Bl/6 fêmeas de 8 a 10 semanas de idade são obtidos junto à Charles River, Inc. ou outro comerciante certificado. Os camundongos tipo C57Bl/6 fêmeas são injetados de modo subcutâneo no flanco traseiro direito com $1x10^6$ células de Panc02. Esse protocolo é baseado nos modelos de tumor de Panc02 padronizados (Maletzki *et al*. 2008. A regressão de câncer pâncreático por injeção intratumoral de streptococcus piogenes vivo em um modelo de camundongo singênico. Gut. 57:483 a 491). Os tumores dos vários grupos de tratamento são medidos com calibradores em intervalos regulares. Como descrito no Exemplo 6, alguns camundongos são sacrificados para a análise de tumor ex vivo com o uso de citometria de fluxo, enquanto outros camundongos são testados novamente para determinar o impacto da resposta de memória no crescimento tumoral.

[00185] Alternativamente, as linhagens celulares de Panc02, 6606PDA ou Capan-1 podem ser usadas em um modelo murino ortotópico de câncer pancreático como descrito por Partecke *et al.* (Partecke *et al.* 2011. Um modelo murino ortotópico singênico de adenocarcinoma pancreático no camundongo tipo C57/Bl6 com o uso das linhagens celulares de Panc02 e 6606PDA. Eur. Surg. Res. 47(2):98 a 107) ou Chai *et al.* (Chai *et al.* 2013. Bioluminescent orthotopic model of pancreatic cancer progression. J. Vis.

Exp. 76: 50395). Os camundongos são sacrificados para a análise de tumor ex vivo. Os tumores são analisados por citometria de fluxo e imunohistoquímica.

<u>Exemplo 11</u>: *Bifidobacterium animalis ssp. lactis* em um modelo de camundongo de carcinoma hepatocelular

[00186] A *Bifidobacterium animalis ssp. lactis* é testada quanto à sua eficácia no modelo de carcinoma hepatocelular de camundongo, sozinha ou em combinação com outras terapias de câncer, incluindo inibidor (ou inibidores) de ponto de verificação. Os camundongos são divididos em grupos recebendo *Bifidobacterium animalis ssp. lactis* com ou sem inibidor de ponto de verificação tratamento. Como descrito no Exemplo 6, *Bifidobacterium animalis ssp. lactis* é administrada em doses variadas em intervalos definidos. Por exemplo, alguns camundongos recebem *Bifidobacterium animalis ssp. lactis* (p.o.) no dia seguinte à injeção de célula de tumor (dia 1). Alguns camundongos recebem sete (7) doses consecutivas de *Bifidobacterium animalis ssp. lactis* (uma dose por dia nos dias 14 a 21). Outros camundongos recebem dosagem diariamente ou, alternativamente, alguns camundongos recebem dosagem em dias intercalados. Alternativamente, os camundongos são randomizados em vários grupos de tratamento em um ponto no tempo definido (*por exemplo,* no dia 13) ou quando os tumores alcançam um certo tamanho (*por exemplo,* 100 mm^3) e o tratamento é, então, iniciado consequentemente.

[00187] O carcinoma hepatocelular é induzido em camundongos por inoculação subcutânea de $1x10^6$ células de Hepa129 (obtidas a partir de NCI ou outra fonte), ou um número adequado de células de outra linhagem celular de carcinoma hepatocelular (como descrito por Gonzalez-Carmona *et al*. 2008. As células dendríticas que expressam o ligante CD40 induzem a regressão de carcinoma hepatocelular através da ativação de imunidade

inata ou adquirida in vivo. Hepatology. 48(1):157 a 168). As células de tumor são inoculadas em um ou ambos os flancos. Os tumores dos vários grupos de tratamento são medidos com calibradores em intervalos regulares. Como descrito no Exemplo 6, alguns camundongos são sacrificados para a análise de tumor ex vivo com o uso de citometria de fluxo, enquanto outros camundongos são testados novamente para determinar o impacto da resposta de memória no crescimento tumoral.

Exemplo 12: *Bifidobacterium animalis ssp. lactis* em um modelo de linfoma de camundongo

[00188] A *Bifidobacterium animalis ssp. lactis* é testada quanto à sua eficácia no modelo de linfoma de camundongo, sozinha ou em combinação com outras terapias de câncer, incluindo inibidor (ou inibidores) de ponto de verificação. Os camundongos são divididos em grupos recebendo *Bifidobacterium animalis ssp. lactis* com ou sem inibidor de ponto de verificação tratamento. Como descrito no Exemplo 6, *Bifidobacterium animalis ssp. lactis* é administrada em doses variadas em intervalos definidos. Por exemplo, alguns camundongos recebem *Bifidobacterium animalis ssp. lactis* (p.o.) no dia seguinte à injeção de célula de tumor (dia 1). Alguns camundongos recebem sete (7) doses consecutivas de *Bifidobacterium animalis ssp. lactis* (uma dose por dia nos dias 14 a 21). Outros camundongos recebem dosagem diariamente ou, alternativamente, alguns camundongos recebem dosagem em dias intercalados. Alternativamente, os camundongos são randomizados em vários grupos de tratamento em um ponto no tempo definido (*por exemplo,* no dia 13) ou quando os tumores alcançam um certo tamanho (*por exemplo,* 100 mm^3) e o tratamento é, então, iniciado consequentemente.

[00189] Uma linhagem celular de linfoma é o linfoma A20, embora outras linhagens celulares de linfoma possam ser usadas com camundongos

singênicos. As células A20 de linfoma são obtidas a partir de ATCC e $5x10^6$ células em PBS a 50 ul são injetadas de modo subcutâneo em um ou ambos os membros traseiros dos camundongos fêmeas tipo Balb/c (como descrito por Houot *et al.* 2009. A modulação de célula T combinada com CpG intratumoral cura o linfoma em um modelo de camundongo sem a necessidade de quimioterapia. Blood. 113(15): 3546 a 3552). Os tumores dos vários grupos de tratamento são medidos com calibradores em intervalos regulares. Como descrito no Exemplo 6, alguns camundongos são sacrificados para a análise de tumor ex vivo com o uso de citometria de fluxo, enquanto outros camundongos são testados novamente para determinar o impacto da resposta de memória no crescimento tumoral.

Exemplo 13: *Bifidobacterium animalis ssp. lactis* em um modelo de câncer de próstata de camundongo

[00190] A *Bifidobacterium animalis ssp. lactis* é testada quanto à sua eficácia no modelo de câncer de próstata de camundongo, sozinha ou em combinação com outras terapias de câncer, incluindo inibidor (ou inibidores) de ponto de verificação. Os camundongos são divididos em grupos recebendo *Bifidobacterium animalis ssp. lactis* com ou sem inibidor de ponto de verificação tratamento. Como descrito no Exemplo 6, *Bifidobacterium animalis ssp. lactis* é administrada em doses variadas em intervalos definidos. Por exemplo, alguns camundongos recebem *Bifidobacterium animalis ssp. lactis* (p.o.) no dia seguinte à injeção de célula de tumor (dia 1). Alguns camundongos recebem sete (7) doses consecutivas de *Bifidobacterium animalis ssp. lactis* (uma dose por dia nos dias 14 a 21). Outros camundongos recebem dosagem diariamente ou, alternativamente, alguns camundongos recebem dosagem em dias intercalados. Alternativamente, os camundongos são randomizados em vários grupos de tratamento em um ponto no tempo definido (*por exemplo,* no dia 13) ou quando os tumores

alcançam um certo tamanho (*por exemplo,* 100 mm^3) e o tratamento é, então, iniciado consequentemente.

[00191] As células de câncer de próstata de camundongo ($1x10^5$ RM-1 células ou um número adequado de células de outra linhagem celular de câncer de próstata) são injetadas em camundongos singênicos. Os tumores dos vários grupos de tratamento são medidos com calibradores em intervalos regulares. Como descrito no Exemplo 6, alguns camundongos são sacrificados para a análise de tumor ex vivo com o uso de citometria de fluxo, enquanto outros camundongos são testados novamente para determinar o impacto da resposta de memória no crescimento tumoral.

Exemplo 14: *Bifidobacterium animalis ssp. lactis* em um modelo de plasmacitoma de camundongo

[00192] A *Bifidobacterium animalis ssp. lactis* é testada quanto à sua eficácia no modelo de plasmacitoma de camundongo, sozinha ou em combinação com outras terapias de câncer, incluindo inibidor (ou inibidores) de ponto de verificação. Os camundongos são divididos em grupos recebendo *Bifidobacterium animalis ssp. lactis* com ou sem inibidor de ponto de verificação tratamento. Como descrito no Exemplo 6, *Bifidobacterium animalis ssp. lactis* é administrada em doses variadas em intervalos definidos. Por exemplo, alguns camundongos recebem *Bifidobacterium animalis ssp. lactis* (p.o.) no dia seguinte à injeção de célula de tumor (dia 1). Alguns camundongos recebem sete (7) doses consecutivas de *Bifidobacterium animalis ssp. lactis* (uma dose por dia nos dias 14 a 21). Outros camundongos recebem dosagem diariamente ou, alternativamente, alguns camundongos recebem dosagem em dias intercalados. Alternativamente, os camundongos são randomizados em vários grupos de tratamento em um ponto no tempo definido (*por exemplo,* no dia 13) ou quando os tumores alcançam um certo tamanho (*por exemplo,* 100 mm^3) e o tratamento é, então, iniciado

consequentemente.

Modelo de plasmacitoma induzido por óleo mineral

[00193] Para examinar a eficácia de *Bifidobacterium animalis ssp. lactis* em um modelo de plasmacitoma ou mieloma múltiplo, os camundongos são injetados intraperitoneal três vezes com 500 ul de 2,6,10,12-tetrametil-pentadecano ("óleo pristano") em vários pontos no tempo entre 0 e 60 dias, como descrito por Potter *et al.* 1983. A plasmacitomagenese peritoneal em camundongos: comparação de diferentes regimes de dose de pristano. J. Natl. Cancer Inst. 71(2):391-5 (consultar, ainda, Lattanzio *et al*. 1997. Desenvolvimento Defeituoso de Plasmacitomas Induzidos por Óleo de Pristano em camundongos tipo BALB/C deficientes de Interleucina-6. Am. J. Pathology: 151(3):689696). A progressão da doença é medida pelo grau de inchaço abdominal e células imunes e partículas na ascite. O fluido de ascite é analisado quanto ao fenótipo de célula imune por citometria de fluxo como descrito no Exemplo 2.

Modelo de plasmacitoma induzido por linhagem celular

[00194] Para examinar a eficácia de *Bifidobacterium animalis ssp. lactis* em um modelo de plasmacitoma ou mieloma múltiplo, células de MOPC-104E ou células de plasmacitoma de J558 (TIB-6 ATCC) são injetadas de modo subcutâneo em um ou mais flancos traseiros de camundongos tipo Balb/c ($5x10^6$ células), com base no modelo descrito por Bhoopalam *et al.* 1980. O efeito de dextrano-S (alfa, 1-3 dextrano) no crescimento de plasmacitomas MOPC-104E e J558. J. Immunol. 125(4):1454-8 (consultar, ainda, Wang *et al.* 2015. IL-10 intensifica a rejeição ao tumor mediada por CTL através da inibição de células T de CD4+ altamente supressoras e da promoção de persistência de CTL em um modelo murino de plasmacitoma. OncoImmunology. 4(7): e1014232-1-9). Os camundongos são divididos em grupos recebendo *Bifidobacterium animalis ssp. lactis* por gavagem

oral e com ou sem inibidor de ponto de verificação tratamento. Os tumores dos vários grupos de tratamento são medidos com calibradores em intervalos regulares. Como descrito no Exemplo 6, alguns camundongos são sacrificados para a análise de tumor ex vivo com o uso de citometria de fluxo, enquanto outros camundongos são testados novamente para determinar o impacto da resposta de memória no crescimento tumoral.

Exemplo 15: *Bifidobacterium animalis ssp. lactis* em um modelo de camundongo de mieloma de camundongo

[00195] A *Bifidobacterium animalis ssp. lactis* é testada quanto à sua eficácia no modelo de mieloma de camundongo SCID, sozinha ou em combinação com outras terapias de câncer, incluindo inibidor (ou inibidores) de ponto de verificação. Os camundongos são divididos em grupos recebendo *Bifidobacterium animalis ssp. lactis* com ou sem inibidor de ponto de verificação tratamento. Como descrito no Exemplo 6, *Bifidobacterium animalis ssp. lactis* é administrada em doses variadas em intervalos definidos. Por exemplo, alguns camundongos recebem *Bifidobacterium animalis ssp. lactis* (p.o.) no dia seguinte à injeção de célula de tumor (dia 1). Alguns camundongos recebem sete (7) doses consecutivas de *Bifidobacterium animalis ssp. lactis* (uma dose por dia nos dias 14 a 21). Outros camundongos recebem dosagem diariamente ou, alternativamente, alguns camundongos recebem dosagem em dias intercalados. Alternativamente, os camundongos são randomizados em vários grupos de tratamento em um ponto no tempo definido (*por exemplo,* no dia 13) ou quando os tumores alcançam um certo tamanho (*por exemplo,* 100 mm^3) e o tratamento é, então, iniciado consequentemente.

[00196] Para examinar a eficácia de *Bifidobacterium animalis ssp. lactis* com o uso de uma leucemia de célula plasmática humana, $1x10^7$ linhagens celulares de mieloma humano, células de ARH77 (ARH77-ATCC CRL-

1621, ou um número adequado de células de outra linhagem celular de mieloma como KPMM2) são usadas. As células de mieloma são injetadas de modo subcutâneo em um ou ambos os flancos traseiros de camundongos de SCID (Consultar Caers *et al*. 2004. Of mice and men: disease models of multiple myeloma. Drug Discovery Today: Disease Models. 1(4): 373-380. Os tumores dos vários grupos de tratamento são medidos com calibradores em intervalos regulares. Como descrito no Exemplo 6, alguns camundongos são sacrificados para a análise de tumor ex vivo com o uso de citometria de fluxo, enquanto outros camundongos são testados novamente para determinar o impacto da resposta de memória no crescimento tumoral.

Exemplo 16: *Bifidobacterium animalis ssp. lactis* em um modelo de carcinoma de célula renal de camundongo

[00197] A *Bifidobacterium animalis ssp. lactis* é testada quanto à sua eficácia no modelo de carcinoma de célula renal de camundongo, sozinha ou em combinação com outras terapias de câncer, incluindo inibidor (ou inibidores) de ponto de verificação. Os camundongos são divididos em grupos recebendo *Bifidobacterium animalis ssp. lactis* com ou sem inibidor de ponto de verificação tratamento. Como descrito no Exemplo 6, *Bifidobacterium animalis ssp. lactis* é administrada em doses variadas em intervalos definidos. Por exemplo, alguns camundongos recebem *Bifidobacterium animalis ssp. lactis* (p.o.) no dia seguinte à injeção de célula de tumor (dia 1). Alguns camundongos recebem sete (7) doses consecutivas de *Bifidobacterium animalis ssp. lactis* (uma dose por dia nos dias 14 a 21). Outros camundongos recebem dosagem diariamente ou, alternativamente, alguns camundongos recebem dosagem em dias intercalados. Alternativamente, os camundongos são randomizados em vários grupos de tratamento em um ponto no tempo definido (*por exemplo,* no dia 13) ou quando os tumores

alcançam um certo tamanho (*por exemplo,* 100 mm^3) e o tratamento é, então, iniciado consequentemente.

[00198] Para examinar a eficácia de *Bifidobacterium animalis ssp. lactis* em um modelo de camundongo de carcinoma de célula renal, células de Renca (ATCC CRL-2947) ou outras células de carcinoma de célula renal são injetadas de modo subcutâneo em um ou ambos os flancos de camundongos singênicos tipo Balb/c de 7 a 8 semanas de idade ($5x10^6$ in 0,1 ml de PBS). Os tumores dos vários grupos de tratamento são medidos com calibradores em intervalos regulares. Como descrito no Exemplo 6, alguns camundongos são sacrificados para a análise de tumor ex vivo com o uso de citometria de fluxo, enquanto outros camundongos são testados novamente para determinar o impacto da resposta de memória no crescimento tumoral.

<u>Exemplo 17</u>: *Bifidobacterium animalis ssp. lactis* em um modelo de câncer de bexiga de camundongo

[00199] A *Bifidobacterium animalis ssp. lactis* é testada quanto à sua eficácia no modelo de câncer de bexiga de camundongo, sozinha ou em combinação com outras terapias de câncer, incluindo inibidor (ou inibidores) de ponto de verificação. Os camundongos são divididos em grupos recebendo *Bifidobacterium animalis ssp. lactis* com ou sem inibidor de ponto de verificação tratamento. Como descrito no Exemplo 6, *Bifidobacterium animalis ssp. lactis* é administrada em doses variadas em intervalos definidos. Por exemplo, alguns camundongos recebem *Bifidobacterium animalis ssp. lactis* (p.o.) no dia seguinte à injeção de célula de tumor (dia 1). Alguns camundongos recebem sete (7) doses consecutivas de *Bifidobacterium animalis ssp. lactis* (uma dose por dia nos dias 14 a 21). Outros camundongos recebem dosagem diariamente ou, alternativamente, alguns camundongos recebem dosagem em dias intercalados. Alternativamente,

os camundongos são randomizados em vários grupos de tratamento em um ponto no tempo definido (*por exemplo,* no dia 13) ou quando os tumores alcançam um certo tamanho (*por exemplo,* 100 mm^3) e o tratamento é, então, iniciado consequentemente.

[00200] No dia da inoculação, as células de MBT-2 (ou outra linhagem celular de câncer de bexiga) são colhidas e ressuspensas em 1:1 de mistura de PBS/Matrigel. $2x10^5$ células de MBT-2 são suspensas em 100 ul de mistura e injetadas de modo subcutâneo em um ou ambos os flancos traseiros de camundongos singênicos. Os tumores são medidos com calibradores em intervalos regulares.

[00201] Como descrito no Exemplo 6, alguns camundongos são sacrificados para a análise de tumor ex vivo com o uso de citometria de fluxo, enquanto outros camundongos são testados novamente para determinar o impacto da resposta de memória no crescimento tumoral.

Exemplo 18: A eficácia de uma cepa de *Bifidobacterium animalis ssp. lactis* é comparada com outras cepas de *Bifidobacterium* quanto à eficácia em modelos de câncer

[00202] Com o uso dos métodos descritos acima, várias *Bifidobacterium animalis ssp. lactis* e outras cepas de *Bifidobacterium* são testadas e comparadas quanto à eficácia em vários modelos de câncer. Tais modelos podem incluir, mas sem limitações, melanoma, câncer de pulmão, câncer de mama, câncer do cólon, câncer colorretal, câncer pancreático, câncer hepatocelular, linfoma, câncer de próstata, plasmacitoma, um modelo de SCID de mieloma, carcinoma de célula renal e/ou câncer de bexiga.

Exemplo 19: Um estudo aberto sobre a segurança, tolerabilidade e eficácia de um agente terapêutico oral de *Cepa A de Bifidobacterium animalis ssp. lactis* em pacientes com carcinoma colorretal metastático

[00203] Um estudo clínico aberto de múltiplos centros com escalações

de dose e expansões de dose para avaliar a segurança preliminar, a tolerabilidade e a eficácia da *Cepa A de Bifidobacterium animalis ssp. lactis* é realizado. O estudo segue em três partes: Parte A: Escalação de dose em carcinoma colorretal metastático (CRC) estável em microssatélite (MSS) e indicações alternativas, Parte B: O CRC MSS e as indicações alternativas e Parte C: CRC instável em microssatélite (MSI). A escalação de dose ocorre em um projeto de 3+3 predefinido. Na parte A, de 9 a 18 pacientes são inscritos. As partes B e C são registradas simultaneamente. Na Parte B, até 15 e não menos que 10 pacientes são inscritos. Na Parte C, não menos que 5 e até 10 pacientes são inscritos. Com base em estudos in vivo de camundongos que portam aloenxerto, uma dose eficaz humana estimada exemplificativa é de $2x10^{11}$ organismos por dia, entregue por cápsula entérica. Uma visão geral esquemática do estudo é representada na Figura 7.

[00204] Os objetivos primários do estudo incluem a determinação da dose máxima tolerada dentro da faixa de doses testada para a *Cepa A de Bifidobacterium animalis ssp. lactis* em pacientes com malignâncias avançadas; a determinação da fase recomendada de 2 doses para a *Cepa A de Bifidobacterium animalis ssp. lactis* em CRC; a avaliação da segurança e tolerabilidade, incluindo toxicidades de limitação de dose da Cepa de *Bifidobacterium animalis ssp. lactis*; e a demonstração de atividade antitumor da *Cepa A de Bifidobacterium animalis ssp. lactis* em pacientes com CRC (*MSI e MSS*). A atividade antitumor será avaliada monitorando-se alterações em células de tumor circulantes, subconjuntos de célula imune no sangue e tumor, e viabilidade de tumor; taxa de resposta objetiva e duração de resposta; sobrevivência livre de progressão; sobrevivência geral; taxa de benefício clínico e duração de taxa de benefício clínico; e taxa de con-

trole de doença e duração de taxa de controle de doença. O objetivo secundário é determinar a distribuição e eliminação humana da *Cepa A de Bifidobacterium animalis ssp. lactis*. Os objetivos exploratórios consistem em avaliar as correlações de resposta de *Cepa A de Bifidobacterium animalis ssp. lactis* com marcadores moleculares relevantes a cada tipo de tumor e identificar possíveis mecanismos de resistência à *Cepa A de Bifidobacterium animalis ssp. lactis*.

Critérios de Inclusão e Exclusão:

[00205] Os critérios de inclusão para todas as partes do estudo incluem os seguintes:

1. Consentimento informado por escrito obtido antes de quaisquer procedimentos de examinação e de acordo com orientações federais, locais e institucionais.

2. Idade ≥ 18 anos.

3. Função hepática adequada:

a. Bilirrubina total ≤ 2 vezes o limita superior ao normal (ULN) (exceto pacientes com síndrome de Gilbert [hiperbilirubinemia indireta hereditária] que devem ter uma bilirrubina total de ≤ 3 vezes o ULN),

b. aspartato aminotransferase (AST) e alanina aminotransferase (ALT) ≤ 2,5 vezes o ULN (exceto pacientes com envolvimento de fígado reconhecido de seu tumor que devem ter seu AST e ALT ≤ 5,0 vezes o ULN).

4. Função renal adequada: folga de creatinina estimada de ≥ 30 ml/min, calculada com o uso da fórmula de Cockcroft e Gault (140 - Idade) • Massa (kg)/(72 • creatinina mg/dL); multiplica por 0,85 se for do sexo feminino.

5. Todos os pacientes na parte B + C devem estar dispostos a realizar biópsias novas no início da terapia e após 4 semanas de terapia.

6. Contracepção: Pacientes do sexo feminino com potencial para gestação devem concordar em usar métodos duplos de contracepção (incluindo um método altamente eficaz e um método eficaz de contracepção) e realizarem um teste de gravidez soro negativo na Examinação, e pacientes do sexo masculino devem usar um método de barreira eficaz de contracepção se forem sexualmente ativos com alguém do sexo feminino com potencial para gestação. Tanto para pacientes do sexo feminino quanto masculino, os métodos eficazes de contracepção devem ser usados ao longo do estudo e por 3 meses após a última dose.

[00206] Para as porções do estudo que testam câncer colorretal remissivo/refratário, os critérios de inclusão também incluem o seguinte:

7. Documentação histológica ou citológica de adenocarcinoma do cólon ou reto.

8. Situação de MSI/MSS reconhecida.

9. Doença mensurável por RECIST v1.1.

10. Doença metastática não adequada para cirurgia de intenção curativa inicialmente.

11. Evidência documentada de doença progressiva de acordo com RECIST v1.1.

12. Tratamento anterior (com conclusão de um curso de terapia, ou à progressão ou intolerabilidade da doença) com cada um dos seguintes:

a. Quimioterapias à base de luoropirimidina, oxaliplatina, irinotecano (por exemplo, FOLFOX e FOLFIRI)

b. Se KRAS tipo selvagem, uma terapia anti-EGFR,

c. Regorafenibe ou TAS 102 (Parte A exigido, partes B+C opcional)

d. A radiação e a cirurgia não são consideradas como regimes

anticâncer anteriores

13. Os pacientes não devem ser dependentes de transfusão.

14. Função hematopoiética adequada: ANC ≥ 1.000/mm3, hemoglobina (Hb) ≥ 9,0 g/dL e contagem de plaquetas ≥ 100.000/mm3.

15. A situação de desempenho do Eastern Cooperative Oncology Grupo (ECOG) de ≤ 1.

16. Expectativa de vida de ≥ 3 meses.

[00207] As seguintes categorias de paciente são excluídas do estudo:

1. Pacientes do sexo feminino que são gestantes ou lactantes.
2. Grande cirurgia até 4 semanas antes de C1D1.
3. Função cardíaca prejudicada ou doenças cardíacas clinicamente significativas, incluindo qualquer uma das seguintes:

a. Angina instável ou infarto do miocárdio agudo ≤ 3 meses antes do C1D1;

b. Doença do coração clinicamente significativa (por exemplo, insuficiência cardíaca congestiva sintomática [por exemplo, > Classe 2 de NYHA]; arritmia não controlada ou hipertensão; histórico de hipertensão lábil ou cumprimento insatisfatório com um regime anti-hipertensivo).

4. Infecção sistêmica grave ativa não controlada que exige antibióticos parenterais, antivirais ou antifúngicos em uma semana antes de C1D1.
5. Qualquer tratamento de antibiótico em andamento que não tenha sido descontinuado pelo menos 3 dias antes da iniciação da terapia
6. Os pacientes com metástase no cérebro sintomática confirmada não são adequados para inscrição. Os pacientes com metástases de cérebro tratadas, estáveis, assintomáticas são elegíveis para entrada no estudo.

7. Os pacientes com um histórico confirmado de vírus de imunodeficiência humana (HIV); o teste de HIV não é exigido como parte deste estudo.

8. Infecção de hepatite A, B ou C ativa confirmada; ou se souber que é positivo para RNA de HCV ou HBsAg (antígeno de superfície de HBV).

9. Malignâncias anteriores:

a. Os pacientes com carcinoma basal ou de célula escamosa adequadamente ressecado da pele, ou carcinoma adequadamente ressecado in situ (por exemplo, colo do útero) podem se inscrever independentemente do momento de diagnóstico.

b. As malignâncias anteriores que podem interferir com a interpretação do estudo. Câncer tratado com intenção curativa < 5 anos antes não serão permitidos exceto se aprovados pelo Patrocinador. O câncer tratado com intenção curativa > 5 anos antes e sem evidência de recorrência será permitido.

10. Os pacientes com malignância ativa do sistema nervoso central (CNS). Os pacientes que tiveram apenas quimioterapia intratecal ou intravenosa profilática contra doença de CNS doença são elegíveis.

11. Os pacientes com doença de trato gastrointestinal (ou vômito ou diarreia não controlados) que poderiam interferir com a absorção de EVP001.

12. Afecções psiquiátricas ou médicas sérias que, na opinião do Investigador, poderiam interferir com o tratamento, a conformidade ou a capacidade para fornecer consentimento.

13. Os pacientes não dispostos a cumprirem o protocolo incluindo biópsias e coletas de amostra exigidas para medir a doença.

14. Radioterapia até semanas antes da examinação. Os pacientes devem ter se recuperado de toxicidades clinicamente significativas.

[00208] O uso de qualquer medição/terapia concomitante, incluindo medicações sem prescrição médica (OTC) consideradas necessárias para o cuidado do paciente é permitido durante o estudo. Medicações necessárias para tratar AEs, gerenciar sintomas de câncer, doenças concorrentes estáveis e agentes de cuidados de suporte (por exemplo, transfusões de produto de sangue), medicações para dor, antieméticos e antidiarreia são permitidos. A terapia concorrente com os fatores de crescimento é permitida. O uso de quaisquer agentes imunossupressores deve ser discutido entre o Investigador e o M Monitor Médico em uma base de caso para caso. Qualquer procedimento de diagnóstico, terapêutico ou cirúrgico realizado durante o período de estudo deve ser registrado, incluindo as datas, descrição do procedimento (ou procedimentos), e quaisquer constatações clínicas, se for aplicável. Todos os antibióticos são contraindicados.

[00209] Os contraceptivos hormonais são permitidos em mulheres com potencial para gestação. Os contraceptivos hormonais incluem qualquer agente contraceptivo comercializado que inclui um estrogênio e/ou um agente pré-gestacional.

[00210] Os agentes anticâncer investigacionais ou comerciais diferentes da *Cepa A de Bifidobacterium animalis ssp. lactis* não são permitidos durante o estudo. A iniciação de qualquer tratamento antitumor específico de não protocolo é considerada uma indicação de remissão/progressão de doença e deve ser registrado adequadamente nos formulários de relatório de caso eletrônicos.

[00211] A terapia de radiação paliativa às lesões não alvejadas é permitida, porém, o tratamento de estudo é retido por ≥ 1 dia antes do início da terapia de radiação paliativa e ≥ 1 dia após cada dose de terapia de

radiação paliativa. O tratamento com a *Cepa A de Bifidobacterium animalis ssp. lactis* não é descontinuado somente devido à radiação paliativa.

[00212] As medidas de apoio para cuidados médicos ideais devem ser fornecidas aos pacientes durante a participação nesse estudo. Essas devem ser baseadas em orientações institucionais e/ou na Rede Nacional de Câncer Compreensiva (NCCN).

Estudo de escalação de dose

[00213] Todos os pacientes recebem doses da *Cepa A de Bifidobacterium animalis ssp. lactis* durante o período de tratamento de 7 dias, ou receberam uma toxicidade de limitação de dose (DLT) dentro do período de tratamento para ser considerada avaliável para decisões de escalação de dose. A *Cepa A de Bifidobacterium animalis ssp. lactis* é administrada oralmente como tabletes ou cápsulas revestidas entéricas. As decisões de escalação de dose ocorrem quando o coorte de pacientes satisfez esses critérios.

[00214] Uma DLT é definida como um evento adverso (AE) ou valor de laboratório anormal que ocorre dentro dos primeiros 7 dias de tratamento com a *Cepa A de Bifidobacterium animalis ssp. lactis*, exceto para aqueles que são claramente e incontestavelmente devido à doença subjacente, progressão de doença ou causas estranhas e satisfaz qualquer um dos critérios incluídos na Tabela 4. Os Critérios de Terminologia Comuns do Instituto Nacional de Câncer para Eventos Adversos (National Cancer Institute Common Terminology Criteria for Adverse Events (NCI CTCAE)) (Versão 4.03) são usados para todas as pontuações. Além disso, > 4 de doses perdidas de *Cepa A de Bifidobacterium animalis ssp. lactis* serão consideradas uma DLT. As decisões de escalação de dose ocorrem quando o coorte de pacientes satisfez esses critérios.

Tabela 4: Critérios para definir DLT

Toxicidade	**Qualquer um dos seguintes critérios (com base em CTCAE [Versão 4.03]):**
Não hematológico	Grau ≥ 3 náusea/vômito, enquanto toma medicações de apoio ideais.
	Qualquer outro Grau ≥ 3 toxicidade não hematológica exceto a alopécia ou anomalias de eletrólito corrigíveis com terapia de apoio.
Hematológico	Neutropenia de grau 4 que dura mais que 5 dias.
	Neutropenia febril (contagem de neutrófilo absoluta [ANC] < 1 x 10^9/l, febre > 38,5°C.).
	Trombocitopenia de grau 4 ou trombocitopenia de grau 3 com hemorragia ou qualquer necessidade de transfusão de plaqueta.
O CTCAE, Versão 4.03, será usado para pontuar todas as AEs e anomalias de laboratório. Os pacientes podem receber cuidados de apoio de acordo com orientações institucionais.	

[00215] Para implementar as decisões de escalação de dose, as informações de toxicidade disponíveis (isto é, todas as AEs e todas as anomalias de laboratório, independente de avaliação de DLT) são avaliadas pelos Investigadores de inscrição e os Patrocinadores médicos monitoram em uma reunião ou teleconferência de decisão de dose. As decisões são baseadas em uma avaliação de todos os dados relevantes disponíveis a partir de todos os coortes de dose avaliados no estudo em andamento incluindo informações de segurança, DLTs, todos os NCI CTCAE, dados de toxicidade de Versão 4.03 toxicidade durante o Ciclo 1 de pacientes avaliáveis. A administração de fármaco no seguinte coorte de dose superior não pode prosseguir até que o Investigador receba confirmação escrita do Patrocinador indicando que os resultados do coorte de dose anterior foram avali-

ados e que é permissível prosseguir para o próximo coorte de dose superior.

[00216] A Tabela 5 abaixo descreve a dose inicial e os níveis de dose que podem ser avaliados durante o estudo para todas as partes do estudo.

Tabela 5: Níveis de Escalação de Dose de *Cepa A de Bifidobacterium animalis ssp. lactis*

Coorte	**Níveis de Dose** *dosagem QD; ciclos de 3 semanas*	**Número de Pacientes**
1	$2x10^8$ organismos	3+3
2	$1 x10^9$ organismos	3+3
3	$2x10^9$ organismos	3+3
4 (opcional)	$4x10^9$ organismos	3

[00217] Uma escalação de dose de 3+3 predefinida é conduzida conforme a seguir:

- Se 0 de 3 pacientes experimentarem uma DLT, escalar para o próximo coorte de dose superior.
- Se 1 de 3 pacientes experimentar uma DLT, tal coorte será expandido para 6 pacientes. Se 1 de 6 pacientes experimentar uma DLT, escalar para o próximo coorte de dose superior;
- Se ≥ 2 de 3 ou ≥ 2 de 6 pacientes experimentarem uma DLT, a dose máxima tolerada (MTD) foi excedida.
- Se a MTD for excedida, a inscrição de pacientes adicionais será a um nível de dose inferior. Se uma dose inicial não for aprovada na avaliação de DLT, a desescalação de dose irá prosseguir com o uso de regras de 3+3 predefinidas

[00218] As escalações de dose intrapaciente são permitidas para todos os coortes após o nível de dose pretendido ter se mostrado seguro (isto é,

todos os pacientes tratados no nível de dose pretendido concluírem avaliações de DLT e ≤ 1 paciente experimentar uma DLT).

[00219] Para pacientes de escalação de dose após 7 dias e pacientes de expansão de dose, se um evento que satisfaz a definição de uma DLT, mas sem necessariamente ocorrer dentro dos primeiros 21 dias, for observado em > 33% dos pacientes a qualquer momento, ou se > 33% de pacientes tratados removerem o consentimento devido à toxicidade, a inscrição será realizada e uma reunião com todos os Investigadores e o Patrocinador irá ocorrer para revisar os eventos e discutir sua significância clínica. Com base nessa revisão, o Patrocinador pode escolher reduzir a dose para os pacientes inscritos e retomar a inscrição do coorte de expansão nessa dose inferior, ou a inscrição no coorte de expansão pode ser interrompida.

[00220] A segurança e a tolerabilidade são avaliadas por meio de DLTs (apenas coortes de escalonamento de dose), relatórios de AE, examinações físicas, eletrocardiogramas e avaliações de segurança de laboratório.

[00221] A atividade antitumor é avaliada pelo Investigador de acordo com critérios de resposta específicos de doença e descritos em termos de taxa de resposta objetiva, duração de resposta, sobrevivência livre de progressão, taxa de benefício clínico, sobrevivência geral e taxa de controle de doença.

[00222] Na Parte A: Os subconjuntos de WBC de sangue periférico e citocinas são analisados na linha de base e 7 dias. As citocinas são adicionalmente analisadas em 24 e 48 horas.

[00223] Nas Partes B e C: Os subconjuntos de WBC de sangue periférico e citocinas são analisados na linha de base e 7 e 21 dias. A linha de base e as biópsias de tumor do dia 28 são analisadas quanto à infiltração de subconjunto imune, assim como marcadores de angiogênese, proliferação e morte.

[00224] Os pacientes continuam recebendo a *Cepa A de Bifidobacterium animalis ssp. lactis* até que o paciente tenha farmacodinâmica confirmada, remova o consentimento, não tenha realizado o acompanhamento, experimente toxicidade intolerável que prejudique o tratamento adicionalmente com *Cepa A de Bifidobacterium animalis ssp. lactis*, ou o tratamento seja descontinuado pelo paciente, Investigador ou Patrocinador. Os pacientes que têm progressão de doença objetiva, mas têm evidência de benefício clínico geral podem, mediante a solicitação do médico responsável, continuar o tratamento com *Cepa A de Bifidobacterium animalis ssp. lactis* após a discussão com o Monitor Médico.

Exemplo 20: *Bifidobacterium animalis ssp. lactis* administrada de modo intratumoral inibe o crescimento tumoral de carcinoma colorretal

[00225] Como descrito nos exemplos 2 e 3, as células de tumor de CT-26 foram injetadas de modo subcutâneo em um flanco traseiro de camundongos e animais foram atribuídos aos grupos recebendo os seguintes tratamentos: 1) Veículo de Sacarose; 2) anti-PD-1 e anti-CTLA4; e 3) *Cepa A de Bifidobacterium animalis ssp. lactis*, anti-PD-1 e anti-CTLA4. $2x10^9$ células bacterianas foram administradas por gavagem oral (p.o.) todos os dias por 21 dias. Os anticorpos anti-PD-1 foram administrados de modo intraperitoneal (i.p.) a 200 ug/camundongo (volume final de 100 ul) a cada quatro dias, começando no dia 1, seis vezes no total. Os anticorpos anti-CTLA4 foram administrados de modo intraperitoneal (i.p.) a 100 ug/camundongo (volume final de 100 ul) a cada quatro dias, começando no dia 1, seis vezes no total. Os volumes de tumor foram medidos como descrito acima. O grupo de combinação tripla de *Cepa A de Bifidobacterium animalis ssp. lactis,* anti-PD-1 e anti-CTLA4 mostrou inibição significativa de crescimento tumoral em comparação com o grupo de controle e o grupo anti-PD-1 e anti-CTLA4 (Consultar **Figura 8**).

<u>Exemplo 21</u>: Análise genômica de Cepa A de *Bifidobacterium animalis ssp. Lactis*

[00226] Ao longo da duração de experimentos de modelo de CT26 como descrito acima, o volume de tumor variou quando algumas cepas de Bifidobacterium animalis ssp. Lactis foram administradas. Os micróbios de realização negativa foram determinados através de uma comparação de três vias de volumes de tumor na terminação de grupos tratados com micróbios, anti-PD1 e veículo. As distribuições de volumes de tumor na terminação do grupo anti-PD1 vs. o grupo de veículo foram usadas como um marco para classificar o desempenho negativo de micróbios. Se as distribuições foram mais proximamente sobrepostas ao veículo que o grupo tratado com anti-PD1, a cepa foi considerada um micróbio com desempenho negativo.

[00227] Por exemplo, foi constatado que o volume de tumor é superior para grupos tratados com duas espécies de Bifidobacterium animalis lactis, Cepa B e Cepa C, em comparação com o grupo tratado com a Cepa A.

[00228] Consistente com essas constatações, uma comparação de assinaturas in vitro das três cepas de um ensaio de célula dendrítica de camundongo mostra que a Cepa A exibe um perfil imune com uma indução reduzida de citocinas pró-tumorígenas em comparação com a Cepa B e a Cepa C (consultar as **Figuras 9A** e **9B**). Foi constatado que várias dessas citocinas são associadas ao prognóstico mais insatisfatório no soro de pacientes de CRC (Mager et al., (2016) Cytokine-Induced Modulation of Colorectal Cancer. Frontiers in Oncology. 6. 96. 10.3389).

[00229] Para elucidar as diferenças entre as cepas, Cepas A, B e C, foram sequenciadas a ~500x com o uso de Illumina MiSeq. O genoma completo e circular da Cepa A (Tabela 1) foi usado como a referência para sinalizar polimorfismos de nucleotídeo único (SNPs) entre as cepas. As

regiões de codificação foram previstas, e as SNPs que ocorreram nas regiões de codificação foram registradas, juntamente com a natureza da alteração de codificação (sinônimos, troca de sentido, frameshift, etc.) Usando agrupamentos estabelecidos através de resultados no modelo de tumor de CT26, SNPs que estavam presentes em cepas negativas (Cepas B e C) quando comparados com a Cepa A de referência foram identificados, e estruturas exclusivas às cepas positivas (por exemplo, Cepa A) foram identificadas.

[00230] Quando comparada com cepas não eficazes altamente homólogas, a Cepa A contém polimorfismos de nucleotídeo único (SNPs) e ou eventos de inserção/deleção nos seguintes genes listados nas Tabelas 6 e 7.

Tabela 6: SNPs e/ou inserção/deleções na Cepa A em comparação com cepas não eficazes

gene	produto	Tipo de Variante	Posição no Gene	Cepa A Alteração de Base	Cepa A Alteração de Aminoácido
glcU	proteína de absorção de glicose putativa GlcU	Troca de sentido	301	C	Arg
glcU	proteína de absorção de glicose putativa GlcU	Troca de sentido	814	C	Pro
relA	Sintase de (p)ppGpp /hidrolase RelA funcional	Troca de sentido	251	G	Gly
relA	Sintase de (p)ppGpp /hidrolase RelA funcional	Troca de sentido	2053	G	Val
metF	5,10-metilenotetra-hidrofolato redutase	Troca de sentido	527	T	Phe
nnr	enzima de reparo de NAD(P)H-hidrato bifuncional	Troca de sentido	574	C	Pro
ykoE_1	proteína de HMP putativa/tiamina permease YkoE	Troca de sentido	533	C	Ala
tcrY	Cinase de histidina de sensor putativo TcrY	Troca de sentido	300	G	Gln
tcrY	Cinase de histidina de sensor putativo TcrY	Troca de sentido	49	C	Pro
dac	D-alanil-D-alanina carboxipeptidase	mudança de quadro	199	DELEÇÃO	His
proteína hipotética	proteína hipotética	Troca de sentido	739	A	Ile
proteína hipotética	proteína hipotética	mudança de quadro	990	DELEÇÃO	His
rdgB	dITP/XTP pirofosfatase	Troca de sentido	547	A	Ser
proteína hipotética	proteína hipotética	Troca de sentido	290	C	Ala
glnB	proteína reguladora de nitrogênio P-II	Troca de sentido	161	A	Asp
accC	Carboxilase de biotina	mudança de quadro	826	DELEÇÃO	-
trxB_2	Redutase de tioredoxina	Troca de sentido	926	T	Val
afsK	Serina/treonina-proteína cinase AfsK	Troca de sentido	686	G	Gly

gene	produto	Tipo de Variante	Posição no Gene	Cepa A Alteração de Base	Cepa A Alteração de Aminoácido
nhaK	Sódio, potássio, lítio e rubídio/H(+) antiporte	Troca de sentido	989	T	Val
proteína hipotética	proteína hipotética	inserção de infraestrutura	45-77	INSERÇÃO (GGCGCTCGGCAC-CCTGGCGATTGGCGCGG-CAAC)	-
oxc_1	Oxalil-CoA decarboxilase	mudança de quadro	183-184	DELEÇÃO	-
wbgU	UDP-N-acetilglucosamina 4-epimerase	Troca de sentido	7	G	Val
wcaJ	UDP-glicose:undecaprenil-fosfato glicose-1-fosfato transferase	Troca de sentido	292	G	Gly
proteína hipotética	proteína hipotética	deleção de infraestrutura	126-128	DELEÇÃO	-
yciV	5'-3' exorribonuclease	Troca de sentido	100	T	Ser
aspA	Aspartato amônia-liase	Troca de sentido	1355	C	Ala
tcrY	Cinase de histidina de sensor putativo TcrY	Troca de sentido	368	T	Phe
tcrY	Cinase de histidina de sensor putativo TcrY	Troca de sentido	278	C	Pro
nagC_2	repressor de N-acetilglucosamina	Troca de sentido	457	G	Ala

Tabela 7: Genes com variantes sinônimos

gene	**produto**	**Posição no Gene**	**Cepa A Alteração de Base**	**Cepa A Alteração de Aminoácido**
proteína hipotética	proteína hipotética	753	T	Leu
hemN	proteína tipo coproporfirinogênio-III independente de oxigênio	912	A	Gly
proteína hipotética	proteína hipotética	951	G	Pro
proteína hipotética	proteína hipotética	1722	T	Pro
cytR_2	repressor transcricional tipo HTH CytR	372	C	Ile
egtC	sulfóxido de gama-glutamil-hercinilcisteína hidrolase	135	C	Ile
proteína hipotética	proteína hipotética	30	G	Arg

Incorporação por Referência

[00231] Todas os pedidos de patente de publicações mencionados no presente documento são aqui incorporados a título de referência em sua totalidade como se cada publicação ou pedido de patente individual fosse específica e individualmente indicado para ser incorporado a título de referência. No caso de conflito, o presente pedido, incluindo quaisquer definições no presente documento, irá controlar.

Equivalentes

[00232] Aqueles versados na técnica irão reconhecer, ou serão capazes de averiguar com o uso de não mais que experimentação de rotina, muitos equivalentes às modalidades específicas da invenção descrita no presente documento. Tais equivalentes se destinam a serem englobados pelas seguintes reivindicações.

REIVINDICAÇÕES

1. Método para tratar câncer em um indivíduo, caracterizado pelo fato de que compreende administrar ao indivíduo uma composição bacteriana que compreende *Bifidobacterium animalis ssp. lactis*.

2. Método, de acordo com a reivindicação 1, caracterizado pelo fato de que a *Bifidobacterium animalis ssp. lactis* é uma cepa que compreende pelo menos 99% de identidade de sequência com a sequência de nucleotídeos da Cepa A de *Bifidobacterium animalis ssp. lactis* (Número de Depósito no ATCC PTA-125097).

3. Método, de acordo com a reivindicação 1, caracterizado pelo fato de que a *Bifidobacterium animalis ssp. lactis* é uma cepa que compreende pelo menos 99,9% de identidade de sequência com a sequência de nucleotídeos da Cepa A de *Bifidobacterium animalis ssp. lactis* (Número de Depósito no ATCC PTA-125097).

4. Método, de acordo com a reivindicação 1, caracterizado pelo fato de que a *Bifidobacterium animalis ssp. lactis* é a Cepa A de *Bifidobacterium animalis ssp. lactis* (Número de Depósito no ATCC PTA-125097).

5. Método, de acordo com qualquer uma das reivindicações 1 a 4, caracterizado pelo fato de que o câncer é selecionado a partir do grupo que consiste em malignidade hematológica, leucemia não linfocítica aguda, leucemia linfocítica crônica, leucemia granulócita aguda, leucemia granulócita crônica, leucemia pró-mielocítica aguda, leucemia de célula T adulta, leucemia aleucêmica, uma leucemia leucocitêmica, leucemia basofílica, leucemia de blastócitos, leucemia bovina, leucemia mielocítica crônica, leucemia cutânea, leucemia embrionária, leucemia eosinofílica, leucemia de Gross, leucemia de célula de Rieder, leucemia de Schilling, leucemia de célula-tronco, leucemia subleucêmica, leucemia de célula não diferenciada, leucemia de célula pilosa, leucemia hemoblástica, leucemia hemocitoblástica, leucemia histiocítica, leucemia

de célula-tronco, leucemia monocítica aguda, leucemia leucopênica, leucemia linfática, leucemia linfoblástica, leucemia linfocítica, leucemia linfogênica, leucemia linfoide, leucemia de célula de linfossarcoma, leucemia de mastócito, leucemia megacariocítica, leucemia micromieloblástica, leucemia monocítica, leucemia mieloblástica, leucemia mielocítica, leucemia granulocítica mieloide, leucemia mielomonocítica, leucemia de Naegeli, leucemia de célula plasmática, leucemia plasmacítica, leucemia pró-mielocítica, carcinoma acinar, carcinoma acinoso, carcinoma adenocístico, carcinoma cístico adenoide, carcinoma adenomatoso, carcinoma do córtex adrenal, carcinoma alveolar, carcinoma de célula alveolar, carcinoma de célula basal, carcinoma basocelular, carcinoma basaloide, carcinoma de célula basoescamosa, carcinoma bronquioalveolar, carcinoma bronquiolar, carcinoma broncogênico, carcinoma cerebriforme, carcinoma colangiocelular, carcinoma coriônico, carcinoma coloide, comedocarcinoma, carcinoma do corpo, carcinoma cribriforme, carcinoma en cuirasse, carcinoma cutâneo, carcinoma cilíndrico, carcinoma de célula cilíndrica, carcinoma do duto, carcinoma duro, carcinoma embrionário, carcinoma encefaloide, carcinoma epienoide, carcinomas epiteliais adenoides, carcinoma exofítico, carcinoma ex úlcera, carcinoma fibroso, carcinoma gelatiniforme, carcinoma gelatinoso, carcinoma de célula gigante, carcinoma de células em anel de sinete, carcinoma simples, carcinoma de célula pequena, carcinoma solanoide, carcinoma de célula esferoide, carcinoma de célula fusiforme, carcinoma espongioso, carcinoma escamoso, carcinoma de célula escamosa, carcinoma de corda, carcinoma telangiectásico, telangiectódios de carcinoma, carcinoma de célula transicional, carcinoma tuberosum, carcinoma tuberoso, carcinoma verrucoso, carcinoma viloso, carcinoma gigantocelular, carcinoma glandular, carcinoma de célula granulosa, carcinoma da matriz capilar, carcinoma hematoide, carcinoma he-

patocelular, carcinoma de célula de Hurthle, carcinoma hialina, carcinoma hipernefroide, carcinoma embrionário infantil, carcinoma in situ, carcinoma intraepidérmico, carcinoma intraepitelial, carcinoma de Krompecher, carcinoma de célula de Kulchitzky, carcinoma de célula grande, carcinoma lenticular, carcinoma lenticular, carcinoma lipomatoso, carcinoma linfoepitelial, carcinoma medullar, carcinoma medular, carcinoma melanótico, carcinoma mole, carcinoma mucinoso, carcinoma muciparum, carcinoma mucocelular, carcinoma mucoepidermoide, carcinoma mucoso, carcinoma mucoso, carcinoma mixomatoso, carcinoma nasofaríngeo, carcinoma de células tipo grão de aveia, carcinoma ossificante, carcinoma osteoide, carcinoma papilar, carcinoma periportal, carcinoma pré-invasivo, carcinoma de célula espinhosa, carcinoma pultáceo, carcinoma de células renais dos rins, carcinoma de células de reserva, carcinoma sarcomatoso, carcinoma schneideriano, carcinoma cirrótico, carcinoma escrotal, condrossarcoma, fibrossarcoma, linfossarcoma, melanossarcoma, mixossarcoma, osteossarcoma, sarcoma endometrial, sarcoma estromal, sarcoma de Ewing, sarcoma da fáscia, sarcoma fibroblástico, sarcoma de célula gigante, sarcoma de Abemethy, sarcoma adiposo, lipossarcoma, sarcoma da parte mole alveolar, sarcoma ameloblástico, sarcoma botrioide, sarcoma cloroma, coriocarcinoma, sarcoma embrionário, sarcoma de tumor de Wilms, sarcoma granulócito, sarcoma de Hodgkin, sarcoma hemorrágico pigmentado múltiplo idiopático, sarcoma imunoblástico de células B, linfoma, sarcoma imunoblástico de células T, sarcoma de Jensen, sarcoma de Kaposi, sarcoma de célula de Kupffer, angiossarcoma, leucossarcoma, sarcoma mesenquimoma maligno, sarcoma parosteal, sarcoma reticulócito, sarcoma de Rous, sarcoma serocístico, sarcoma sinovial, sarcoma telangiectáltico, doença de Hodgkin, linfoma não Hodgkin, mieloma múltiplo, neuroblastoma, câncer de bexiga, câncer de

mama, câncer de ovário, câncer de pulmão, rabdomiossarcoma, trombocitose primária, macroglobulinemia primária, tumores de pulmão de células pequenas, tumores cerebrais primários, câncer de estômago, câncer de cólon, insulanoma pancreático maligno, carcinoide maligno, lesões de pele pré-malignas, câncer testicular, linfomas, câncer da tireoide, neuroblastoma, câncer esofágico, câncer do trato genitourinário, hipercalcemia maligna, câncer cervical, câncer endometrial, câncer adrenal cortical, melanoma de Harding-Passey, melanoma juvenil, melanoma lentigo maligno, melanoma maligno, melanoma acral-lentiginoso, melanoma amelanótico, melanoma juvenil benigno, melanoma de Cloudman, melanoma S91, melanoma nodular, melanoma subungular e melanoma de difusão superficial.

6. Método, de acordo com qualquer uma das reivindicações 1 a 4, caracterizado pelo fato de que o câncer é câncer de próstata, câncer de pulmão, câncer de cólon, câncer colorretal, melanoma, câncer de mama, câncer pancreático, carcinoma hepatocelular ou linfoma.

7. Método, de acordo com qualquer uma das reivindicações 1 a 6, caracterizado pelo fato de que a composição bacteriana é administrada por via oral, por via retal, por via intravenosa, por via intratumoral ou por via subcutânea.

8. Método, de acordo com qualquer uma das reivindicações 1 a 7, caracterizado pelo fato de que pelo menos 50% das bactérias na composição bacteriana são *Bifidobacterium animalis ssp. lactis*.

9. Método, de acordo com qualquer uma das reivindicações 1 a 7, caracterizado pelo fato de que pelo menos 90% das bactérias na composição bacteriana são *Bifidobacterium animalis ssp. lactis*.

10. Método, de acordo com qualquer uma das reivindicações 1 a 7, caracterizado pelo fato de que substancialmente todas as bactérias na composição bacteriana são *Bifidobacterium animalis ssp. lactis*.

11. Método, de acordo com qualquer uma das reivindicações

1 a 10, caracterizado pelo fato de que a composição bacteriana compreende pelo menos 1 x 10^6 unidades de formação de colônia (CFUs) de *Bifidobacterium animalis ssp. lactis*.

12. Método, de acordo com a reivindicação 11, caracterizado pelo fato de que a composição bacteriana compreende pelo menos 1 x 10^7 CFUs de *Bifidobacterium animalis ssp. lactis*.

13. Método, de acordo com a reivindicação 1, caracterizado pelo fato de que a composição bacteriana compreende pelo menos 1 x 10^8 CFUs de *Bifidobacterium animalis ssp. lactis*.

14. Método, de acordo com qualquer uma das reivindicações 1 a 13, caracterizado pelo fato de que a composição bacteriana é administrada em duas ou mais doses.

15. Método, de acordo com a reivindicação 14, caracterizado pelo fato de que a administração ao indivíduo das duas ou mais doses é separada por pelo menos 1 dia.

16. Método, de acordo com a reivindicação 15, caracterizado pelo fato de que a administração das duas ou mais doses é separada por pelo menos 1 semana.

17. Método, de acordo com qualquer uma das reivindicações 1 a 16, caracterizado pelo fato de que a composição bacteriana compreende bactérias vivas.

18. Método, de acordo com qualquer uma das reivindicações 1 a 16, caracterizado pelo fato de que a composição bacteriana compreende bactérias atenuadas.

19. Método, de acordo com qualquer uma das reivindicações 1 a 19, caracterizado pelo fato de que a composição bacteriana compreende bactérias mortas.

20. Método, de acordo com qualquer uma das reivindicações 1 a 19, caracterizado pelo fato de que a administração da composição bacteriana trata o câncer.

21. Método, de acordo com qualquer uma das reivindicações 1 a 20, caracterizado pelo fato de que administração da composição bacteriana induz uma resposta imunológica anticâncer.

22. Método, de acordo com qualquer uma das reivindicações 1 a 21, caracterizado pelo fato de que o método compreende, ainda, administrar ao indivíduo uma segunda terapia contra câncer.

23. Método, de acordo com a reivindicação 22, caracterizado pelo fato de que a segunda terapia contra câncer compreende a administração de um agente quimioterápico ao indivíduo.

24. Método, de acordo com a reivindicação 23, caracterizado pelo fato de que o agente quimioterápico é selecionado dentre o grupo que consiste em tiotepa, ciclofosfamida, bussulfano, improssulfano, pipossulfano, benzodopa, carboquona, meturedopa, uredopa, altretamina, trietilenomelamina, trietilenofosforamida, trietiilenotiofosforamida, trimetilolomelamina, bulatacina, bulatacinona, camptotecina, topotecano, briostatina, calistatina, CC-1065, criptoficina 1, criptoficina 8, dolastatina, duocarmicina, eleuterobina, pancratistatina, sarcodictiina, espongistatina, clorambucila, clornafazina, colofosfamida, estramustina, ifosfamida, mecloretamina, cloridrato de óxido de mecloretamina, melfalan, novembiquina, fenesterina, prednimustina, trofosfamida, mostarda de uracila, carmustina, clorozotocina, fotemustina, lomustina, nimustina, ranimnustina, caliqueamicina, dinemicina, clodronato, esperamicina; cromóforo de neocarzinostatina, aclacinomisinas, actinomicina, autrarnicina, azaserina, bleomicinas, cactinomicina, carabicina, caminomicina, carzinofilina, cromomicinais, dactinomicina, daunorrubicina, detorrubicina, 6-diazo-5-oxo-L-norleucina, doxorrubicina, epirrubicina, esorrubicina, idarrubicina, marcelomicina, mitomicina, mitomicina C, ácido micofenólico, nogalamicina, olivomicina, peplomicina, potfiromicina, puromicina, quelamicina, rodorrubicina, estreptonigrina, estreptozocina, tubercidina, ubenimex, zinostatina, zorrubicina, metotrexato, 5-

fluorouracila (5-FU), denopterina, metotrexato, pteropterina, trimetrexato, fludarabina, 6-mercaptopurina, tiamiprina, tioguanina, ancitabina, azacitidina, 6-azauridina, carmofur, citarabina, dideoxiuridina, doxifluridina, enocitabina, floxuridina, calusterona, propionato de dromostanolona, epitiostanol, mepitiostano, testolactona, aminoglutetimida, mitotano, trilostano, ácido frolínico, aceglatona, aldofosfamida glicosídeo, ácido aminolevulínico, eniluracila, amsacrina, bestrabucila, bisantreno, edatraxato, defofamina, demecolcina, diaziquona, elformitina, acetato de eliptínio, epotilona, etoglucid, nitrato de gálio, hidroxiureia, lentinano, lonidainina, maitansina, ansamitocinas, mitoguazona, mitoxantrona, mopidanmol, nitraerina, pentostatina, fenamet, pirarrubicina, losoxantrona, ácido podofilínico, 2-etil-hidrazida, procarbazina, complexo de polissacarídeo PSK, razoxana, rizoxina, sizofurano, espirogermânio, ácido tenuazônico, triaziquona; 2,2',2''-triclorotrietilamina, tricoteceno, toxina T-2, verracurina A, roridina A, anguidina, uretano, vindesina, dacarbazina, manomustina, mitobronitol, mitolactol, pipobromano, gacitosina, arabinosídeo, ciclofosfamida, tiotepa, paclitaxel, doxetaxel, clorambucil, gencitabina, 6-tioguanina, mercaptopurina, metotrexato, cisplatina, oxaliplatina, carboplatina, vinblastina, platina, etoposídeo, ifosfamida, mitoxantrona, vincristina, vinorelbina, novantrona, teniposídeo, edatrexato, daunomicina, aminopterina, xeloda, ibandronato, irinotecano, RFS 2000, difluorometilomitina, ácido retinoico e capecitabina.

25. Método, de acordo com qualquer uma das reivindicações 22 a 24, caracterizado pelo fato de que a segunda terapia contra câncer compreende imunoterapia contra câncer.

26. Método, de acordo com a reivindicação 25, caracterizado pelo fato de que a imunoterapia contra câncer compreende administrar um inibidor de ponto de verificação imunológico ao indivíduo.

27. Método, de acordo com a reivindicação 26, caracterizado pelo fato de que a imunoterapia contra câncer compreende administrar

um inibidor de ponto de verificação imunológico adicional ao indivíduo.

28. Método, de acordo com a reivindicação 26 ou 27, caracterizado pelo fato de que o inibidor de ponto de verificação imunológico é um anticorpo ou fragmento de ligação a antígeno do mesmo que se liga especificamente a uma proteína de ponto de verificação imunológico.

29. Método, de acordo com a reivindicação 28, caracterizado pelo fato de que o inibidor de ponto de verificação imunológico é uma molécula de siRNA, uma molécula de shRNA ou uma molécula de RNA antissenso que inibe a expressão de uma proteína de ponto de verificação imunológico.

30. Método, de acordo com a reivindicação 28 ou 29, caracterizado pelo fato de que a proteína de ponto de verificação imunológico é CTLA4, PD-1, PD-L1, PD-L2, A2AR, B7-H3, B7-H4, BTLA, KIR, LAG3, TIM-3 ou VISTA.

31. Método, de acordo com a reivindicação 26 ou 27, caracterizado pelo fato de que o inibidor de ponto de verificação imunológico é atezolizumabe, avelumabe, durvalumabe, ipilimumabe, nivolumabe, pembrolizumabe, pidilizumabe, AMP-224, AMP-514, BGB-A317, STI-A1110, TSR-042, RG-7446, BMS-936559, MEDI-4736, MSB-0020718C, AUR-012 ou STI-A1010.

32. Método, de acordo com qualquer uma das reivindicações 25 a 31, caracterizado pelo fato de que a imunoterapia contra câncer compreende a administração de um anticorpo específico para câncer ou um fragmento de ligação a antígeno do mesmo ao indivíduo.

33. Método, de acordo com a reivindicação 32, caracterizado pelo fato de que o anticorpo específico para câncer ou fragmento de ligação a antígeno do mesmo se liga a um antígeno associado a câncer.

34. Método, de acordo com a reivindicação 33, caracterizado pelo fato de que o antígeno associado a câncer é selecionado dentre o

grupo que consiste em adipofilina, AIM-2, ALDH1A1, alfa-actinina-4, alfa-fetoproteína ("AFP"), ARTC1, B-RAF, BAGE-1, BCLX (L), proteína de fusão de BCR-ABL b3a2, beta-catenina, BING-4, CA-125, CALCA, antígeno carcinoembrionário ("CEA"), CASP-5, CASP-8, CD274, CD45, Cdc27, CDK12, CDK4, CDKN2A, CEA, CLPP, COA-1, CPSF, CSNK1A1, CTAG1, CTAG2, ciclina D1, Ciclina-A1, proteína de fusão dek-can, DKK1, EFTUD2, Fator de alongamento 2, ENAH (hMena), Ep-CAM, EpCAM, EphA3, antígeno de tumor epitelial ("ETA"), proteína de fusão ETV6-AML1, EZH2, FGF5, FLT3-ITD, FN1, G250/MN/CAIX, GAGE-1,2,8, GAGE-3,4,5,6,7, GAS7, glipicano-3, GnTV, gp100/Pmel17, GPNMB, HAUS3, Hepsina, HER-2/neu, HERV-K-MEL, HLA-A11, HLA-A2, HLA-DOB, hsp70-2, IDO1, IGF2B3, IL13Ralfa2, carboxil esterase intestinal, K-ras, Calicreína 4, KIF20A, KK-LC-1, KKLC1, KM-HN-1, KMHN1 também conhecido como CCDC110, LAGE-1, proteína de fusão LDLR-fucosiltransferaseAS, Lengsina, M-CSF, MAGE-A1, MAGE-A10, MAGE-A12, MAGE-A2, MAGE-A3, MAGE-A4, MAGE-A6, MAGE-A9, MAGE-C1, MAGE-C2, enzima málica, mamaglobina-A, MART2, MATN, MC1R, MCSP, mdm-2, ME1, Melan-A/MART-1, Meloe, Midkina, MMP-2, MMP-7, MUC1, MUC5AC, mucina, MUM-1, MUM-2, MUM-3, Miosina, Miosina classe I, N-raw, NA88-A, neo-PAP, NFYC, NY-BR-1, NY-ESO-1/LAGE-2, OA1, OGT, OS-9, polipeptídeo P, p53, PAP, PAX5, PBF, proteína de fusão pml-RARalfa, mucina epitelial polimórfica ("PEM"), PPP1R3B, PRAME, PRDX5, PSA, PSMA, PTPRK, RAB38/NY-MEL-1, RAGE-1, RBAF600, RGS5, RhoC, RNF43, RU2AS, SAGE, secernina 1, SIRT2, SNRPD1, SOX10, Sp17, SPA17, SSX-2, SSX-4, STEAP1, survivina, proteína de fusão SYT-SSX1 ou SSX2, TAG-1, TAG-2, Telomerase, TGF-betaRII, TPBG, TRAG-3, Triosefosfato isomerase, TRP-1/gp75, TRP-2, TRP2-INT2, tirosinase, tirosinase ("TYR"), VEGF, WT1 e XAGE-1b/GAGED2a.

35. Método, de acordo com a reivindicação 33, caracterizado

pelo fato de que o antígeno associado a câncer é um neo-antígeno.

36. Método, de acordo com qualquer uma das reivindicações 25 a 35, caracterizado pelo fato de que a imunoterapia contra câncer compreende a administração de uma vacina contra câncer ao indivíduo.

37. Método, de acordo com a reivindicação 36, caracterizado pelo fato de que a vacina contra câncer compreende um polipeptídeo que compreende um epítopo de um antígeno associado a câncer.

38. Método, de acordo com a reivindicação 37, caracterizado pelo fato de que o antígeno associado a câncer é selecionado dentre o grupo que consiste em adipofilina, AIM-2, ALDH1A1, alfa-actinina-4, alfa-fetoproteína ("AFP"), ARTC1, B-RAF, BAGE-1, BCLX (L), proteína de fusão de BCR-ABL b3a2, beta-catenina, BING-4, CA-125, CALCA, antígeno carcinoembrionário ("CEA"), CASP-5, CASP-8, CD274, CD45, Cdc27, CDK12, CDK4, CDKN2A, CEA, CLPP, COA-1, CPSF, CSNK1A1, CTAG1, CTAG2, ciclina D1, Ciclina-A1, proteína de fusão dek-can, DKK1, EFTUD2, Fator de alongamento 2, ENAH (hMena), Ep-CAM, EpCAM, EphA3, antígeno de tumor epitelial ("ETA"), proteína de fusão ETV6-AML1, EZH2, FGF5, FLT3-ITD, FN1, G250/MN/CAIX, GAGE-1,2,8, GAGE-3,4,5,6,7, GAS7, glipicano-3, GnTV, gp100/Pmel17, GPNMB, HAUS3, Hepsina, HER-2/neu, HERV-K-MEL, HLA-A11, HLA-A2, HLA-DOB, hsp70-2, IDO1, IGF2B3, IL13Ralfa2, carboxil esterase intestinal, K-ras, Calicreína 4, KIF20A, KK-LC-1, KKLC1, KM-HN-1, KMHN1 também conhecido como CCDC110, LAGE-1, proteína de fusão LDLR-fucosiltransferaseAS, Lengsina, M-CSF, MAGE-A1, MAGE-A10, MAGE-A12, MAGE-A2, MAGE-A3, MAGE-A4, MAGE-A6, MAGE-A9, MAGE-C1, MAGE-C2, enzima málica, mamaglobina-A, MART2, MATN, MC1R, MCSP, mdm-2, ME1, Melan-A/MART-1, Meloe, Midkina, MMP-2, MMP-7, MUC1, MUC5AC, mucina, MUM-1, MUM-2, MUM-3, Miosina, Miosina classe I, N-raw, NA88-A, neo-PAP, NFYC, NY-BR-1, NY-ESO-1/LAGE-2, OA1, OGT, OS-9, polipeptídeo P, p53,

PAP, PAX5, PBF, proteína de fusão pml-RARalfa, mucina epitelial polimórfica ("PEM"), PPP1R3B, PRAME, PRDX5, PSA, PSMA, PTPRK, RAB38/NY-MEL-1, RAGE-1, RBAF600, RGS5, RhoC, RNF43, RU2AS, SAGE, secernina 1, SIRT2, SNRPD1, SOX10, Sp17, SPA17, SSX-2, SSX-4, STEAP1, survivina, proteína de fusão SYT-SSX1 ou SSX2, TAG-1, TAG-2, Telomerase, TGF-betaRII, TPBG, TRAG-3, Triosefosfato isomerase, TRP-1/gp75, TRP-2, TRP2-INT2, tirosinase, tirosinase ("TYR"), VEGF, WT1 e XAGE-1b/GAGED2a.

39. Método, de acordo com a reivindicação 37, caracterizado pelo fato de que o antígeno associado a câncer é um neo-antígeno.

40. Método, de acordo com qualquer uma das reivindicações 37 a 39, caracterizado pelo fato de que o polipeptídeo é uma proteína de fusão.

41. Método, de acordo com a reivindicação 36, caracterizado pelo fato de que a vacina contra câncer compreende um ácido nucleico que codifica um epítopo de um antígeno associado a câncer.

42. Método, de acordo com a reivindicação 41, caracterizado pelo fato de que o antígeno associado a câncer é selecionado dentre o grupo que consiste em adipofilina, AIM-2, ALDH1A1, alfa-actinina-4, alfa-fetoproteína ("AFP"), ARTC1, B-RAF, BAGE-1, BCLX (L), proteína de fusão de BCR-ABL b3a2, beta-catenina, BING-4, CA-125, CALCA, antígeno carcinoembrionário ("CEA"), CASP-5, CASP-8, CD274, CD45, Cdc27, CDK12, CDK4, CDKN2A, CEA, CLPP, COA-1, CPSF, CSNK1A1, CTAG1, CTAG2, ciclina D1, Ciclina-A1, proteína de fusão dek-can, DKK1, EFTUD2, Fator de alongamento 2, ENAH (hMena), Ep-CAM, EpCAM, EphA3, antígeno de tumor epitelial ("ETA"), proteína de fusão ETV6-AML1, EZH2, FGF5, FLT3-ITD, FN1, G250/MN/CAIX, GAGE-1,2,8, GAGE-3,4,5,6,7, GAS7, glipicano-3, GnTV, gp100/Pmel17, GPNMB, HAUS3, Hepsina, HER-2/neu, HERV-K-MEL,

HLA-A11, HLA-A2, HLA-DOB, hsp70-2, IDO1, IGF2B3, IL13Ralfa2, carboxil esterase intestinal, K-ras, Calicreína 4, KIF20A, KK-LC-1, KKLC1, KM-HN-1, KMHN1 também conhecido como CCDC110, LAGE-1, proteína de fusão LDLR-fucosiltransferaseAS, Lengsina, M-CSF, MAGE-A1, MAGE-A10, MAGE-A12, MAGE-A2, MAGE-A3, MAGE-A4, MAGE-A6, MAGE-A9, MAGE-C1, MAGE-C2, enzima málica, mamaglobina-A, MART2, MATN, MC1R, MCSP, mdm-2, ME1, Melan-A/MART-1, Meloe, Midkina, MMP-2, MMP-7, MUC1, MUC5AC, mucina, MUM-1, MUM-2, MUM-3, Miosina, Miosina classe I, N-raw, NA88-A, neo-PAP, NFYC, NY-BR-1, NY-ESO-1/LAGE-2, OA1, OGT, OS-9, polipeptídeo P, p53, PAP, PAX5, PBF, proteína de fusão pml-RARalfa, mucina epitelial polimórfica ("PEM"), PPP1R3B, PRAME, PRDX5, PSA, PSMA, PTPRK, RAB38/NY-MEL-1, RAGE-1, RBAF600, RGS5, RhoC, RNF43, RU2AS, SAGE, secernina 1, SIRT2, SNRPD1, SOX10, Sp17, SPA17, SSX-2, SSX-4, STEAP1, survivina, proteína de fusão SYT-SSX1 ou SSX2, TAG-1, TAG-2, Telomerase, TGF-betaRII, TPBG, TRAG-3, Triosefosfato isomerase, TRP-1/gp75, TRP-2, TRP2-INT2, tirosinase, tirosinase ("TYR"), VEGF, WT1 e XAGE-1b/GAGED2a.

43. Método, de acordo com a reivindicação 41, caracterizado pelo fato de que o antígeno associado a câncer é um neo-antígeno.

44. Método, de acordo com qualquer uma das reivindicações 41 a 43, caracterizado pelo fato de que o ácido nucleico é DNA.

45. Método, de acordo com qualquer uma das reivindicações 41 a 43, caracterizado pelo fato de que o ácido nucleico é RNA.

46. Método, de acordo com a reivindicação 45, caracterizado pelo fato de que o RNA é mRNA.

47. Método, de acordo com qualquer uma das reivindicações 41 a 46, caracterizado pelo fato de que o ácido nucleico está em um vetor.

48. Método, de acordo com a reivindicação 47, caracterizado

pelo fato de que o vetor é um vetor bacteriano.

49. Método, de acordo com a reivindicação 48, caracterizado pelo fato de que o vetor bacteriano é selecionado dentre o grupo que consiste em Mycobacterium bovis (BCG), Salmonella Typhimurium ssp., Salmonella Typhi ssp., esporos de Clostridium sp., Escherichia coli Nissle 1917, Escherichia coli K-12/LLO, Listeria monocytogenes e Shigella flexneri.

50. Método, de acordo com a reivindicação 47, caracterizado pelo fato de que o vetor é um vetor viral.

51. Método, de acordo com a reivindicação 50, caracterizado pelo fato de que o vetor viral é selecionado dentre o grupo que consiste em vaccinia, adenovírus, vírus de RNA e vírus avipox defectivo em replicação, vírus da bouba aviária defectivo em replicação, vírus da varíola de canário defectivo em replicação, MVA defectivo em replicação e adenovírus defectivo em replicação.

52. Método, de acordo com qualquer uma das reivindicações 25 a 51, caracterizado pelo fato de que a imunoterapia contra câncer compreende a administração de uma célula apresentadora de antígeno (APC) preparada com antígeno específico para câncer.

53. Método, de acordo com a reivindicação 51, caracterizado pelo fato de que a APC é uma célula dendrítica, um macrófago ou uma célula B.

54. Método, de acordo com a reivindicação 52 ou 53, caracterizado pelo fato de que o antígeno associado a câncer é selecionado dentre o grupo que consiste em adipofilina, AIM-2, ALDH1A1, alfa-actinina-4, alfa-fetoproteína ("AFP"), ARTC1, B-RAF, BAGE-1, BCLX (L), proteína de fusão de BCR-ABL b3a2, beta-catenina, BING-4, CA-125, CALCA, antígeno carcinoembrionário ("CEA"), CASP-5, CASP-8, CD274, CD45, Cdc27, CDK12, CDK4, CDKN2A, CEA, CLPP, COA-1, CPSF, CSNK1A1, CTAG1, CTAG2, ciclina D1, Ciclina-A1, proteína de

fusão dek-can, DKK1, EFTUD2, Fator de alongamento 2, ENAH (hMena), Ep-CAM, EpCAM, EphA3, antígeno de tumor epitelial ("ETA"), proteína de fusão ETV6-AML1, EZH2, FGF5, FLT3-ITD, FN1, G250/MN/CAIX, GAGE-1,2,8, GAGE-3,4,5,6,7, GAS7, glipicano-3, GnTV, gp100/Pmel17, GPNMB, HAUS3, Hepsina, HER-2/neu, HERV-K-MEL, HLA-A11, HLA-A2, HLA-DOB, hsp70-2, IDO1, IGF2B3, IL13Ralfa2, carboxil esterase intestinal, K-ras, Calicreína 4, KIF20A, KK-LC-1, KKLC1, KM-HN-1, KMHN1 também conhecido como CCDC110, LAGE-1, proteína de fusão LDLR-fucosiltransferaseAS, Lengsina, M-CSF, MAGE-A1, MAGE-A10, MAGE-A12, MAGE-A2, MAGE-A3, MAGE-A4, MAGE-A6, MAGE-A9, MAGE-C1, MAGE-C2, enzima málica, mamaglobina-A, MART2, MATN, MC1R, MCSP, mdm-2, ME1, Melan-A/MART-1, Meloe, Midkina, MMP-2, MMP-7, MUC1, MUC5AC, mucina, MUM-1, MUM-2, MUM-3, Miosina, Miosina classe I, N-raw, NA88-A, neo-PAP, NFYC, NY-BR-1, NY-ESO-1/LAGE-2, OA1, OGT, OS-9, polipeptídeo P, p53, PAP, PAX5, PBF, proteína de fusão pml-RARalfa, mucina epitelial polimórfica ("PEM"), PPP1R3B, PRAME, PRDX5, PSA, PSMA, PTPRK, RAB38/NY-MEL-1, RAGE-1, RBAF600, RGS5, RhoC, RNF43, RU2AS, SAGE, secernina 1, SIRT2, SNRPD1, SOX10, Sp17, SPA17, SSX-2, SSX-4, STEAP1, survivina, proteína de fusão SYT-SSX1 ou SSX2, TAG-1, TAG-2, Telomerase, TGF-betaRII, TPBG, TRAG-3, Triosefosfato isomerase, TRP-1/gp75, TRP-2, TRP2-INT2, tirosinase, tirosinase ("TYR"), VEGF, WT1 e XAGE-1b/GAGED2a.

55. Método, de acordo com a reivindicação 52 ou 53, caracterizado pelo fato de que o antígeno associado a câncer é um neo-antígeno.

56. Método, de acordo com qualquer uma das reivindicações 25 a 55, caracterizado pelo fato de que a imunoterapia contra câncer compreende a administração de um receptor de antígeno quimérico específico para câncer (CAR).

57. Método, de acordo com a reivindicação 56, caracterizado pelo fato de que o CAR é administrado na superfície de uma célula T.

58. Método, de acordo com a reivindicação 56 ou 57, caracterizado pelo fato de que o CAR se liga especificamente a um antígeno associado a câncer.

59. Método, de acordo com a reivindicação 58, caracterizado pelo fato de que o antígeno associado a câncer é selecionado dentre o grupo que consiste em adipofilina, AIM-2, ALDH1A1, alfa-actinina-4, alfa-fetoproteína ("AFP"), ARTC1, B-RAF, BAGE-1, BCLX (L), proteína de fusão de BCR-ABL b3a2, beta-catenina, BING-4, CA-125, CALCA, antígeno carcinoembrionário ("CEA"), CASP-5, CASP-8, CD274, CD45, Cdc27, CDK12, CDK4, CDKN2A, CEA, CLPP, COA-1, CPSF, CSNK1A1, CTAG1, CTAG2, ciclina D1, Ciclina-A1, proteína de fusão dek-can, DKK1, EFTUD2, Fator de alongamento 2, ENAH (hMena), Ep-CAM, EpCAM, EphA3, antígeno de tumor epitelial ("ETA"), proteína de fusão ETV6-AML1, EZH2, FGF5, FLT3-ITD, FN1, G250/MN/CAIX, GAGE-1,2,8, GAGE-3,4,5,6,7, GAS7, glipicano-3, GnTV, gp100/Pmel17, GPNMB, HAUS3, Hepsina, HER-2/neu, HERV-K-MEL, HLA-A11, HLA-A2, HLA-DOB, hsp70-2, IDO1, IGF2B3, IL13Ralfa2, carboxil esterase intestinal, K-ras, Calicreína 4, KIF20A, KK-LC-1, KKLC1, KM-HN-1, KMHN1 também conhecido como CCDC110, LAGE-1, proteína de fusão LDLR-fucosiltransferaseAS, Lengsina, M-CSF, MAGE-A1, MAGE-A10, MAGE-A12, MAGE-A2, MAGE-A3, MAGE-A4, MAGE-A6, MAGE-A9, MAGE-C1, MAGE-C2, enzima málica, mamaglobina-A, MART2, MATN, MC1R, MCSP, mdm-2, ME1, Melan-A/MART-1, Meloe, Midkina, MMP-2, MMP-7, MUC1, MUC5AC, mucina, MUM-1, MUM-2, MUM-3, Miosina, Miosina classe I, N-raw, NA88-A, neo-PAP, NFYC, NY-BR-1, NY-ESO-1/LAGE-2, OA1, OGT, OS-9, polipeptídeo P, p53, PAP, PAX5, PBF, proteína de fusão pml-RARalfa, mucina epitelial polimórfica ("PEM"), PPP1R3B, PRAME, PRDX5, PSA, PSMA, PTPRK,

RAB38/NY-MEL-1, RAGE-1, RBAF600, RGS5, RhoC, RNF43, RU2AS, SAGE, secernina 1, SIRT2, SNRPD1, SOX10, Sp17, SPA17, SSX-2, SSX-4, STEAP1, survivina, proteína de fusão SYT-SSX1 ou SSX2, TAG-1, TAG-2, Telomerase, TGF-betaRII, TPBG, TRAG-3, Triosefosfato isomerase, TRP-1/gp75, TRP-2, TRP2-INT2, tirosinase, tirosinase ("TYR"), VEGF, WT1 e XAGE-1b/GAGED2a.

60. Método, de acordo com a reivindicação 58, caracterizado pelo fato de que o antígeno associado a câncer é um neo-antígeno.

61. Método, de acordo com qualquer uma das reivindicações 25 a 60, caracterizado pelo fato de que a imunoterapia contra câncer compreende a administração de uma célula T específica para câncer ao indivíduo.

62. Método, de acordo com a reivindicação 61, caracterizado pelo fato de que a célula T é uma célula T CD4+.

63. Método, de acordo com a reivindicação 62, caracterizado pelo fato de que a célula T CD4+ T é uma célula T T_H1, uma célula T T_H2 ou uma célula T T_H17.

64. Método, de acordo com qualquer uma das reivindicações 61 a 63, caracterizado pelo fato de que a célula T expressa um receptor de célula T específico para um antígeno associado a câncer.

65. Método, de acordo com a reivindicação 64, caracterizado pelo fato de que o antígeno associado a câncer é selecionado dentre o grupo que consiste em adipofilina, AIM-2, ALDH1A1, alfa-actinina-4, alfa-fetoproteína ("AFP"), ARTC1, B-RAF, BAGE-1, BCLX (L), proteína de fusão de BCR-ABL b3a2, beta-catenina, BING-4, CA-125, CALCA, antígeno carcinoembrionário ("CEA"), CASP-5, CASP-8, CD274, CD45, Cdc27, CDK12, CDK4, CDKN2A, CEA, CLPP, COA-1, CPSF, CSNK1A1, CTAG1, CTAG2, ciclina D1, Ciclina-A1, proteína de fusão dek-can, DKK1, EFTUD2, Fator de alongamento 2, ENAH (hMena), Ep-CAM, EpCAM, EphA3, antígeno de tumor epitelial ("ETA"), proteína de

fusão ETV6-AML1, EZH2, FGF5, FLT3-ITD, FN1, G250/MN/CAIX, GAGE-1,2,8, GAGE-3,4,5,6,7, GAS7, glipicano-3, GnTV, gp100/Pmel17, GPNMB, HAUS3, Hepsina, HER-2/neu, HERV-K-MEL, HLA-A11, HLA-A2, HLA-DOB, hsp70-2, IDO1, IGF2B3, IL13Ralfa2, carboxil esterase intestinal, K-ras, Calicreína 4, KIF20A, KK-LC-1, KKLC1, KM-HN-1, KMHN1 também conhecido como CCDC110, LAGE-1, proteína de fusão LDLR-fucosiltransferaseAS, Lengsina, M-CSF, MAGE-A1, MAGE-A10, MAGE-A12, MAGE-A2, MAGE-A3, MAGE-A4, MAGE-A6, MAGE-A9, MAGE-C1, MAGE-C2, enzima málica, mamaglobina-A, MART2, MATN, MC1R, MCSP, mdm-2, ME1, Melan-A/MART-1, Meloe, Midkina, MMP-2, MMP-7, MUC1, MUC5AC, mucina, MUM-1, MUM-2, MUM-3, Miosina, Miosina classe I, N-raw, NA88-A, neo-PAP, NFYC, NY-BR-1, NY-ESO-1/LAGE-2, OA1, OGT, OS-9, polipeptídeo P, p53, PAP, PAX5, PBF, proteína de fusão pml-RARalfa, mucina epitelial polimórfica ("PEM"), PPP1R3B, PRAME, PRDX5, PSA, PSMA, PTPRK, RAB38/NY-MEL-1, RAGE-1, RBAF600, RGS5, RhoC, RNF43, RU2AS, SAGE, secernina 1, SIRT2, SNRPD1, SOX10, Sp17, SPA17, SSX-2, SSX-4, STEAP1, survivina, proteína de fusão SYT-SSX1 ou SSX2, TAG-1, TAG-2, Telomerase, TGF-betaRII, TPBG, TRAG-3, Triosefosfato isomerase, TRP-1/gp75, TRP-2, TRP2-INT2, tirosinase, tirosinase ("TYR"), VEGF, WT1 e XAGE-1b/GAGED2a.

66. Método, de acordo com qualquer uma das reivindicações 25 a 65, caracterizado pelo fato de que a imunoterapia contra câncer compreende a administração de uma proteína imunomoduladora ao indivíduo.

67. Método, de acordo com a reivindicação 66, caracterizado pelo fato de que a proteína imunomoduladora é uma citocina.

68. Método, de acordo com a reivindicação 67, caracterizado pelo fato de que a proteína imunomoduladora é selecionada dentre o grupo que consiste em quimioatraente de linfócito B ("BLC"), quimiocina

de motivo C-C 11 ("Eotaxina-1"), Proteína quimiotática de eosinófilo 2 ("Eotaxina-2"), Fator estimulante de colônia de granulócitos ("G-CSF"), Fator estimulante de colônia de granulócitos e macrófagos ("GM-CSF"), 1-309, Molécula de Adesão Intercelular 1 ("ICAM-1"), Interferon gama ("IFN-gama"), Interleucina-1 alfa ("IL-1 alfa"), Interleucina-1 beta ("IL-1 beta"), antagonista de receptor de Interleucina 1 ("IL-1 ra"), Interleucina-2 ("IL-2"), Interleucina-4 ("IL-4"), Interleucina-5 ("IL-5"), Interleucina-6 ("IL-6"), receptor solúvel de Interleucina-6 ("IL-6 sR"), Interleucina-7 ("IL-7"), Interleucina-8 ("IL-8"), Interleucina-10 ("IL-10"), Interleucina-11 ("IL-11"), Subunidade beta de Interleucina-12 ("IL-12 p40" ou "IL-12 p70"), Interleucina-13 ("IL-13"), Interleucina-15 ("IL-15"), Interleucina-16 ("IL-16"), Interleucina-17 ("IL-17"), Ligante 2 de quimiocina (motivo C-C) ("MCP-1"), Fator estimulante de colônia de macrófagos ("M-CSF"), Monocina induzida por interferon gama ("MIG"), Ligante 2 de quimiocina (motivo C-C) ("MIP-1 alpha"), Ligante 4 de quimiocina (motivo C-C) ("MIP-1 beta"), proteína-1-delta inflamatória de macrófago ("MIP-1 delta"), subunidade B de fator de crescimento derivado de plaquetas ("PDGF-BB"), Ligante 5 de quimiocina (motivo C-C) 5, Regulado em Ativação, Expresso e Secretado em células T normais ("RANTES"), inibidor de TIMP metalopeptidase 1 ("TIMP-1"), inibidor de TIMP metalopeptidase 2 ("TIMP-2"), Fator de necrose tumoral, linfotoxina-alfa ("TNF alfa"), Fator de necrose tumoral, linfotoxina-beta ("TNF beta"), Receptor de TNF solúvel tipo 1 ("sTNFRI"), sTNFRIIAR, Fator neutrotrófico derivado do cérebro ("BDNF"), Fator de crescimento de fibroblasto básico ("bFGF"), Proteína morfogenética óssea 4 ("BMP-4"), Proteína morfogenética óssea 5 ("BMP-5"), Proteína morfogenética óssea 7 ("BMP-7"), Fator de crescimento de nervo ("b-NGF"), Fator de crescimento epidérmico ("EGF"), Receptor de fator de crescimento epidérmico ("EGFR"), Fator de crescimento endotelial vascular derivado de glândula endó-

crina ("EG-VEGF"), Fator de crescimento de fibroblasto 4 ("FGF-4"), Fator de crescimento de queratinócito ("FGF-7"), Fator de diferenciação de crescimento 15 ("GDF-15"), Fator neurotrófico derivado de célula glial ("GDNF"), Hormônio de crescimento, Fator de crescimento semelhante a EGF de ligação a heparina ("HB-EGF"), Fator de crescimento hepatócito ("HGF"), Proteína de ligação a fator de crescimento semelhante a insulina 1 ("IGFBP-1"), Proteína de ligação a fator de crescimento semelhante a insulina 2 ("IGFBP-2"), Proteína de ligação a fator de crescimento semelhante a insulina 3 ("IGFBP-3"), Proteína de ligação a fator de crescimento semelhante a insulina 4 ("IGFBP-4"), Proteína de ligação a fator de crescimento semelhante a insulina 6 ("IGFBP-6"), Fator de crescimento semelhante a insulina 1 ("IGF-1"), Insulina, Fator estimulante de colônia de macrófagos ("M-CSF R"), Receptor de fator de crescimento de nervos ("NGF R"), Neurotrofina-3 ("NT-3"), Neurotrofina-4 ("NT-4"), Fator inibidor de osteoclastogênese ("Osteoprotegerina"), Receptores de fator de crescimento derivado de plaqueta ("PDGF-AA"), Biossíntese de fosfatidilinositol-glicano ("PIGF"), Skp, Culina, Complexo contendo F-box ("SCF"), Receptor de fator de célula-tronco ("SCF R"), Fator de crescimento transformante alfa ("TGFalfa"), Fator de crescimento transformante beta-1 ("TGF beta 1"), Fator de crescimento transformante beta-3 ("TGF beta 3"), Fator de crescimento endotelial vascular ("VEGF"), Receptor de fator de crescimento endotelial vascular 2 ("VEGFR2"), Receptor de fator de crescimento endotelial vascular 3 ("VEGFR3"), VEGF-D 6Ckina, Receptor de proteína tirosina quinase UFO ("Axl"), Betacelulina ("BTC"), Quimiocina epitelial associada a mucosas ("CCL28"), Ligante de quimiocina 27 (motivo C-C) ("CTACK"), Ligante de quimiocina 16 (motivo C-X-C) ("CXCL16"), Quimiocina 5 de motivo C-X-C ("ENA-78"), Ligante de quimiocina 26 (motivo C-C) ("Eotaxina-3"), Proteína quimiotática de granulócito 2 ("GCP-2"), GRO, Ligante de quimiocina 14 (motivo C-C) ("HCC-l"), Ligante de quimiocina

16 (motivo C-C) ("HCC-4"), Interleucina-9 ("IL-9"), Interleucina-17 F ("IL-17F"), Proteína de ligação à interleucina 18 ("IL-18 BPa"), Interleucina-28 A ("IL-28A"), Interleucina 29 ("IL-29"), Interleucina 31 ("IL-31"), Quimiocina 10 de motivo C-X-C ("IP-10"), Receptor de quimiocina CXCR3 ("I-TAC"), Fator inibidor de leucemia ("LIF"), Leve, Ligante de quimiocina (motivo C) ("Linfotactina"), Proteína quimioatraente de monócito 2 ("MCP-2"), Proteína quimioatraente de monócito 3 ("MCP-3"), Proteína quimioatraente de monócito 4 ("MCP-4"), Quimiocina derivada de macrófago ("MDC"), Fator inibidor de migração de macrófago ("MIF"), Ligante de quimiocina 20 (motivo C-C) ("MIP-3 alfa"), motivo de quimiocina 19 C-C ("MIP-3 beta"), Ligante de quimiocina 23 (motivo C-C) ("MPIF-1"), Cadeia alfa de proteína estimulante de macrófago ("MSPalfa"), Proteína de montagem de nucleossomo 4 semelhante a 1 ("NAP-2"), Fosfoproteína secretada 1 ("Osteopontina"), Citocina pulmonar e com regulação de ativação ("PARC"), Fator de plaqueta 4 ("PF4"), Fator-1 alfa derivado de célula do estroma ("SDF-1 alfa"), Ligante de quimiocina 17 (motivo C-C) ("TARC"), Quimiocina expressa no timo ("TECK"), Linfopoietina estromal tímica ("TSLP 4- IBB"), antígeno CD 166 ("ALCAM"), Agrupamento de Diferenciação 80 ("B7-1"), Membro da superfamília do receptor de fator de necrose tumoral 17 ("BCMA"), Agrupamento de Diferenciação 14 ("CD14"), Agrupamento de Diferenciação 30 ("CD30"), Agrupamento de Diferenciação 40 ("Ligante CD40"), Molécula de adesão celular relacionada a antígeno carcinoembrionário 1 (glicoproteína biliar) ("CEACAM-1"), Receptor de Morte 6 ("DR6"), Desoxitimidina quinase ("Dtk"), Glicoproteína de membrana tipo 1 ("Endoglina"), Proteína tirosina quinase receptora erbB-3 ("ErbB3"), Molécula de adesão de leucócito endotelial 1 ("E-Selectina"), Antígeno de apoptose 1 ("Fas"), Tirosina quinase semelhante a Fms 3 ("Flt-3L"), Membro da superfamília do receptor de fator de necrose tumoral 1 ("GITR"), Membro da superfamília do receptor de fator de necrose tumoral 14

("HVEM"), Molécula de adesão intercelular 3 ("ICAM-3"), IL-1 R4, IL-1 RI, IL-10 Rbeta, IL-17R, IL-2Rgama, IL-21R, Proteína de membrana de lisossomo 2 ("LIMPII"), Lipocalina associada a gelatinase de neutrófilo ("Lipocalina-2"), CD62L ("L-Selectina"), Endotélio linfático ("LYVE-1"), Sequência A relacionada a polipeptídeo de MHC classe I ("MICA"), Sequência B relacionada a polipeptídeo de MHC classe I ("MICB"), NRGl-betal, Receptor de fator de crescimento derivado de plaqueta do tipo beta ("PDGF Rbeta"), Molécula de adesão de célula endotelial de plaqueta ("PECAM-1"), RAGE, Receptor 1 celular do vírus da hepatite A ("TIM-1"), Membro da superfamília do receptor de fator de necrose tumoral IOC ("TRAIL R3"), Domínio de ligação a transglutaminase de proteína trapina ("Trapina-2"), Receptor de uroquinase ("uPAR"), Proteína de adesão celular vascular 1 ("VCAM-1"), XEDARActivina A, Proteína relacionada a Agouti ("AgRP"), Ribonuclease 5 ("Angiogenina"), Angiopoietina 1, Angiostatina, Cateprina S, CD40, Proteína da família críptica IB ("Cripto-1"), DAN, Proteína relacionada a Dickkopf 1 ("DKK-1"), E-Caderina, Molécula de adesão de célula epitelial ("EpCAM"), Ligante Fas (FasL ou CD95L), Fcg RIIB/C, FoUistatina, Galectina-7, Molécula de adesão intercelular 2 ("ICAM-2"), IL-13 Rl, IL-13R2, IL-17B, IL-2 Ra, IL-2 Rb, IL-23, LAP, Molécula de adesão de célula neuronal ("NrCAM"), Inibidor de ativador de plasminogênio 1 ("PAI-1"), Receptores de fator de crescimento derivado de plaqueta ("PDGF-AB"), Resistina, Fator derivado de célula estromal 1 ("SDF-1 beta"), sgpl30, Proteína secretada relacionada a frizzled 2 ("ShhN"), lecitinas do tipo imunoglobulina de ligação a ácido siálico ("Siglec-5"), ST2, Fator de crescimento transformante beta 2 ("TGF beta 2"), Tie-2, Trombopoietina ("TPO"), Membro da superfamília do receptor de fator de necrose tumoral 10D ("TRAIL R4"), Receptor de desencadeamento expresso em células mieloides 1 ("TREM-1"), Fator de crescimento endotelial vascular C ("VEGF-C"),

VEGFRIAdiponectina, Adipsina ("AND"), Alfa-fetoproteína ("AFP"), Angiopoietina semelhante a 4 ("ANGPTL4"), Microglobulina Beta-2 ("B2M"), Molécula de adesão de célula basal ("BCAM"), Antígeno de carboidrato 125 ("CA125"), Antígeno de Câncer 15-3 ("CA15-3"), Antígeno carcinoembrionário ("CEA"), proteína de receptor de cAMP ("CRP"), Receptor de Fator de Crescimento Epidérmico Humano 2 ("ErbB2"), Folistatina, Hormônio estimulante de folículo ("FSH"), Ligante de quimiocina 1 (motivo C-X-C) 1 ("GRO alfa"), gonadotropina coriônica humana ("beta HCG"), Receptor de fator de crescimento semelhante a insulina 1 ("IGF-1 sR"), IL-1 sRII, IL-3, IL-18 Rb, IL-21, Leptina, metaloproteinase de matriz 1 ("MMP-1"), metaloproteinase de matriz 2 ("MMP-2"), metaloproteinase de matriz 3 ("MMP-3"), metaloproteinase de matriz 8 ("MMP-8"), metaloproteinase de matriz 9 ("MMP-9"), metaloproteinase de matriz 10 ("MMP-10"), metaloproteinase de matriz 13 ("MMP-13"), Molécula de adesão de célula neural ("NCAM-1"), Entactina ("Nidogênio 1"), enolase específica de neurônio ("NSE"), Oncostatina M ("OSM"), Procalcitonina, Prolactina, Antígeno específico para próstata ("PSA"), Lecitina semelhante a Ig de ligação a ácido siálico 9 ("Siglec-9"), ADAM 17 endopeptidase ("TACE"), Tiroglobulina, Inibidor de metaloproteinase 4 ("TIMP-4"), TSH2B4, Proteína contendo domínio de desintegrina e metaloproteinase 9 ("ADAM-9"), Angiopoietina 2, Membro da superfamília de ligante de fator de necrose tumoral 13/ membro da família de fosfoproteína 32 nuclear rica em leucina ácida B ("APRIL"), Proteína morfogenética óssea 2 ("BMP-2"), Proteína morfogenética óssea 9 ("BMP-9"), Componente de complemento 5a ("C5a"), Catepsina L, CD200, CD97, Chemerina, Membro da superfamília do receptor de fator de necrose tumoral 6B ("DcR3"), Proteína de ligação a ácido graxo 2 ("FABP2"), Proteína de ativação de fibroblasto, alfa ("FAP"), Fator de crescimento de fibroblasto 19 ("FGF-19"), Galectina-3, Receptor do fator de crescimento de hepatócitos ("HGF R"), IFN-gamalfa/beta R2, Fator

de crescimento semelhante a insulina 2 ("IGF-2"), Receptor de Fator de crescimento semelhante a insulina 2 ("IGF-2 R"), Receptor 6 de Interleucina-1 ("IL-1R6"), Interleucina 24 ("IL-24"), Interleucina 33 ("IL-33", Calicreína 14, Asparaginil endopeptidase ("Legumaina"), Receptor de lipoproteína de baixa densidade oxidada 1 ("LOX-1"), Lectina de ligação a manose ("MBL"), Neprilisina ("NEP"), homólogo de Notch 1, associado a translocação (Drosofila) ("Notch-1"), Nefroblastoma superexpresso ("NOV"), Osteoactivina, Proteína de morte celular programada 1 ("PD-1"), N-acetilmuramoíl-L-alanina amidase ("PGRP-5"), Serpina A4, Proteína secretada relacionada a frizzled 3 ("sFRP-3"), Trombomodulina, receptor semelhante a Toll 2 ("TLR2"), Membro da superfamília do receptor de fator de necrose tumoral 10A ("TRAIL Rl"), Transferrina ("TRF"), WIF-lACE-2, Albumina, AMICA, Angiopoietina 4, Fator de ativação de célula B ("BAFF"), Antígeno de carboidrato 19-9 ("CA19-9"), CD 163 , Clusterina, CRT AM, Ligante de quimiocina 14 (motivo C-X-C) ("CXCL14"), Cistatina C, Decorina ("DCN"), proteína relacionada a Dickkopf 3 ("Dkk-3"), Proteína 1 semelhante a Delta ("DLL1"), Fetuína A, Fator de crescimento de ligação a heparina 1 ("aFGF"), Receptor de folato alfa ("FOLR1"), Furina, Proteína de seleção associada a GPCR 1 ("GASP-1"), Proteína de seleção associada a GPCR 2 ("GASP-2"), Receptor de fator estimulante de colônia de granulócitos ("GCSF R"), Serina protease hepsina ("HAI-2"), Receptor de Interleucina-17B ("IL-17B R"), Interleucina 27 ("IL-27"), gene de ativação de linfócitos 3 ("LAG-3"), Apolipoproteína A-V ("LDL R"), Pepsinogênio I, Proteína de ligação a retinol 4 ("RBP4"), SOST, Heparano sulfato proteoglicano ("Syndecan-1"), Membro da superfamília do receptor de fator de necrose tumoral 13B ("TACI"), Inibidor de trajetória de fator de tecido ("TFPI"), TSP-1, Superfamília do receptor de fator de necrose tumoral, membro 10b ("TRAIL R2"), TRANCE, Troponina I, Ativador de Uroquinase Plasminogênio ("uPA"), Caderina 5, tipo 2 ou VE-caderina (endotelial vascular)

também conhecida como CD144 ("VE- Caderina"), Proteína de trajetória de sinalização induzível por WNTl 1 ("WISP-1") e Ativador de Receptor de Fator Nuclear κ B ("RANK").

69. Método, de acordo com qualquer uma das reivindicações 25 a 68, caracterizado pelo fato de que a imunoterapia contra câncer compreende administrar um adjuvante ao indivíduo.

70. Método, de acordo com a reivindicação 69, caracterizado pelo fato de que o adjuvante é selecionado dentre o grupo que consiste em uma proteína imunomoduladora, Adjuvante 65, α-GalCer, fosfato de alumínio, hidróxido de alumínio, fosfato de cálcio, Peptídeo β-Glucano, CpG DNA, GPI-0100, lipídio A, lipopolissacarídeo, Lipovant, Montanide, N-acetil-muramil-L-alanil-D-isoglutamina, Pam3CSK4, quil A e dimicolato de trealose.

71. Método, de acordo com qualquer uma das reivindicações 22 a 70, caracterizado pelo fato de que a segunda terapia contra câncer compreende administrar um inibidor de angiogênese ao indivíduo.

72. Método, de acordo com a reivindicação 71, caracterizado pelo fato de que o inibidor de angiogênese é selecionado dentre o grupo que consiste em Bevacizumabe (Avastin®), Ziv-aflibercepte (Zaltrap®), Sorafenibe (Nexavar®), Sunitinibe (Sutent®), Pazopanibe (Votrient®), Regorafenibe (Stivarga®) e Cabozantinibe (Cometriq™).

73. Método, de acordo com qualquer uma das reivindicações 22 a 72, caracterizado pelo fato de que a segunda terapia contra câncer compreende radioterapia.

74. Método, de acordo com qualquer uma das reivindicações 22 a 73, caracterizado pelo fato de que a terapia contra câncer compreende administrar um antibiótico ao indivíduo.

75. Método, de acordo com a reivindicação 74, caracterizado pelo fato de que o antibiótico é selecionado dentre o grupo que consiste

em aminoglicosídeos, ansamicinas, carbacefemas, carbapenemas, cefalosporinas, glicopeptídeos, lincosamidas, lipopeptídeos, macrólidos, monobactamas, nitrofuranos, oxazolidononas, penicilinas, antibióticos de polipeptídeo, quinolonas, fluoroquinolona, sulfonamidas, tetraciclinas, compostos antimicobacterianos e combinações dos mesmos.

76. Método, de acordo com qualquer uma das reivindicações 22 a 75, caracterizado pelo fato de que a terapia contra câncer compreende administrar ao indivíduo uma segunda bactéria terapêutica.

77. Método, de acordo com qualquer uma das reivindicações 1 a 76, caracterizado pelo fato de que o método compreende, ainda, administrar um prebiótico ao indivíduo.

78. Método, de acordo com a reivindicação 77, caracterizado pelo fato de que o prebiótico é um fruto-oligossacarídeo, um galacto-oligossacarídeo, um trans-galacto-oligossacarídeo, um xilo-oligossacarídeo, um quito-oligossacarídeo, um oligossacarídeo de soja, um gentio-oligossacarídeo, um isomalto-oligossacarídeo, um mano-oligossacarídeo, um malto-oligossacarídeo, um mananoligossacarídeo, lactulose, lactosacarose, palatinose, glicosil sacarose, goma guar, goma arábica, tagalose, amilose, amilopectina, pectina, xilano ou uma ciclodextrina.

79. Método para aumentar um ambiente tumoral contendo uma célula imune em um indivíduo, caracterizado pelo fato de que compreende administrar uma composição bacteriana que compreende *Bifidobacterium animalis ssp. lactis* ao indivíduo.

80. Método, de acordo com a reivindicação 79, caracterizado pelo fato de que a célula imune é uma célula apresentadora de antígeno, uma célula dendrítica, uma célula T, uma célula B, uma célula NK, uma célula NKT, um macrófago ou um neutrófilo.

81. Método, de acordo com a reivindicação 79 ou 80, caracterizado pelo fato de que a composição é administrada por via oral.

82. Método para aumentar um ambiente tumoral, caracterizado pelo fato de que compreende um biomarcador de seleção de paciente em um indivíduo que compreende administrar uma composição bacteriana que compreende *Bifidobacterium animalis ssp. lactis* ao indivíduo.

83. Método, de acordo com a reivindicação 82, caracterizado pelo fato de que o biomarcador é PD-L1 e a terapia é um inibidor de ponto de verificação.

84. Método para aumentar um ambiente tumoral, caracterizado pelo fato de que compreende um biomarcador associado à atividade de célula imune em um indivíduo que compreende administrar uma composição bacteriana que compreende *Bifidobacterium animalis ssp. lactis* ao indivíduo.

85. Método, de acordo com a reivindicação 84, caracterizado pelo fato de que o biomarcador é CD86, CD80 ou CD206.

86. Método, de acordo com a reivindicação 84 ou 85, caracterizado pelo fato de que a composição é administrada por via oral.

87. Método, de acordo com qualquer uma das reivindicações 1 a 86, caracterizado pelo fato de que o indivíduo é um ser humano.

88. Método, de acordo com qualquer uma das reivindicações 1 a 86, caracterizado pelo fato de que o indivíduo é um mamífero não humano.

89. Método, de acordo com a reivindicação 88, caracterizado pelo fato de que o mamífero é selecionado dentre o grupo que consiste em um cão, um gato, uma vaca, um cavalo, um porco, um jumento, uma cabra, um camelo, um camundongo, um rato, um porquinho da índia, uma ovelha, uma lhama, um macaco, um gorila ou um chimpanzé.

90. Método, de acordo com qualquer uma das reivindicações 1 a 89, caracterizado pelo fato de que uma segunda bactéria é administrada como parte de um consórcio ecológico.

91. Composição bacteriana, caracterizada pelo fato de que compreende *Bifidobacterium animalis ssp. Lactis*.

92. Composição bacteriana, de acordo com a reivindicação 91, caracterizada pelo fato de que compreende, ainda, um veículo farmaceuticamente aceitável.

93. Composição bacteriana, caracterizada pelo fato de que compreende uma biomassa farmaceuticamente ativa (PhABs) derivada de *Bifidobacterium animalis ssp. lactis*.

94. Composição bacteriana, caracterizada pelo fato de que compreende uma biomassa farmaceuticamente ativa (PhABs) isolada de *Bifidobacterium animalis ssp. lactis*.

95. Composição bacteriana, de acordo com qualquer uma das reivindicações 91 a 94, caracterizada pelo fato de que a *Bifidobacterium animalis ssp. lactis* é uma cepa que compreende pelo menos 99% de identidade de sequência com a sequência de nucleotídeos da Cepa A de *Bifidobacterium animalis ssp. lactis* (Número de Depósito no ATCC PTA-125097).

96. Composição bacteriana, de acordo com qualquer uma das reivindicações 91 a 94, caracterizada pelo fato de que a *Bifidobacterium animalis ssp. lactis* é uma cepa que compreende pelo menos 99,9% de identidade de sequência com a sequência de nucleotídeos da Cepa A de *Bifidobacterium animalis ssp. lactis* (Número de Depósito no ATCC PTA-125097).

97. Composição bacteriana, de acordo com qualquer uma das reivindicações 91 a 94, caracterizada pelo fato de que a *Bifidobacterium animalis ssp. lactis* é a Cepa A de *Bifidobacterium animalis ssp. lactis* (Número de Depósito no ATCC PTA-125097).

98. Composição bacteriana, de acordo com qualquer uma das reivindicações 91 a 94, caracterizada pelo fato de que a composição

compreende uma quantidade eficaz de Cepa A de *Bifidobacterium animalis ssp. lactis* (Número de Depósito no ATCC PTA-125097).

99. Composição bacteriana, de acordo com qualquer uma das reivindicações 91 a 98, caracterizada pelo fato de que a composição bacteriana é formulada para administração oral, retal, intravenosa, intratumoral ou subcutânea.

100. Composição bacteriana, de acordo com qualquer uma das reivindicações 91 a 99, caracterizada pelo fato de que pelo menos 50% das bactérias na composição bacteriana são *Bifidobacterium animalis ssp. lactis*.

101. Composição bacteriana, de acordo com qualquer uma das reivindicações 91 a 99, caracterizada pelo fato de que pelo menos 90% das bactérias na composição bacteriana são *Bifidobacterium animalis ssp. lactis*.

102. Composição bacteriana, de acordo com qualquer uma das reivindicações 91 a 101, caracterizada pelo fato de que substancialmente todas as bactérias na composição bacteriana são *Bifidobacterium animalis ssp. lactis*.

103. Composição bacteriana, de acordo com qualquer uma das reivindicações 91 a 103, caracterizada pelo fato de que a composição bacteriana compreende pelo menos 1 x 10^6 unidades de formação de colônia (CFUs) de *Bifidobacterium animalis ssp. lactis*.

104. Composição bacteriana, de acordo com a reivindicação 103, caracterizada pelo fato de que a composição bacteriana compreende pelo menos 1 x 10^7 CFUs de *Bifidobacterium animalis ssp. lactis*.

105. Composição bacteriana, de acordo com a reivindicação 103, caracterizada pelo fato de que a composição bacteriana compreende pelo menos 1 x 10^8 CFUs de *Bifidobacterium animalis ssp. lactis*.

106. Composição bacteriana, de acordo com qualquer uma

das reivindicações 91 a 105, caracterizada pelo fato de que a composição bacteriana compreende bactérias vivas.

107. Composição bacteriana, de acordo com qualquer uma das reivindicações 91, 92, 93, 94, 95, 96, 97, 98, 99, 100, 101, 103, 104 ou 105, caracterizada pelo fato de que a composição bacteriana compreende bactérias atenuadas.

108. Composição bacteriana, de acordo com qualquer uma das reivindicações 91, 92, 93, 94, 95, 96, 97, 98, 99, 100, 101, 103, 104 ou 105, caracterizada pelo fato de que a composição bacteriana compreende bactérias mortas.

109. Composição bacteriana, de acordo com qualquer uma das reivindicações 91 a 108, caracterizada pelo fato de que a administração da composição bacteriana trata o câncer.

110. Composição bacteriana, de acordo com qualquer uma das reivindicações 91 a 109, caracterizada pelo fato de que administração da composição bacteriana induz uma resposta imunológica anticâncer.

111. Composição bacteriana, de acordo com qualquer uma das reivindicações 91 a 109, caracterizado pelo fato de que a administração da composição bacteriana induz infiltração de células imunes CD3+.

112. Composição bacteriana, de acordo com qualquer uma das reivindicações 91 a 109, caracterizada pelo fato de que a administração da composição bacteriana induz a regulação crescente de MHC Classe I no sítio do tumor.

113. Composição bacteriana, de acordo com qualquer uma das reivindicações 91 a 112, caracterizada pelo fato de que a *Bifidobacterium animalis ssp. lactis* é formulada com um revestimento entérico ou microencapsulação.

114. Biorreator, caracterizado pelo fato de que compreende

Bifidobacterium animalis ssp. lactis.

115. Biorreator, de acordo com a reivindicação 114, caracterizado pelo fato de que a *Bifidobacterium animalis ssp. lactis* é uma cepa que compreende pelo menos 99% de identidade de sequência com a sequência de nucleotídeos da Cepa A de *Bifidobacterium animalis ssp. lactis* (Número de Depósito no ATCC PTA-125097).

116. Biorreator, de acordo com a reivindicação 114, caracterizado pelo fato de que a *Bifidobacterium animalis ssp. lactis* é uma cepa que compreende pelo menos 99,9% de identidade de sequência com a sequência de nucleotídeos da Cepa A de *Bifidobacterium animalis ssp. lactis* (Número de Depósito no ATCC PTA-125097).

117. Biorreator, de acordo com a reivindicação 114, caracterizado pelo fato de que a *Bifidobacterium animalis ssp. lactis* é a Cepa A de *Bifidobacterium animalis ssp. lactis* (Número de Depósito no ATCC PTA-125097).

118. Composição bacteriana, caracterizada pelo fato de que compreende pelo menos uma cepa de *Bifidobacterium animalis ssp. lactis* que tem um gene que compreende uma alteração de base de nucleotídeo, conforme identificado na Tabela 6.

119. Composição bacteriana, de acordo com a reivindicação 118, caracterizada pelo fato de que compreende pelo menos uma cepa de *Bifidobacterium animalis ssp. lactis* que tem dois genes que compreendem uma alteração de base de nucleotídeo, conforme identificado na Tabela 6.

120. Composição bacteriana, de acordo com a reivindicação 118, caracterizada pelo fato de que compreende pelo menos uma cepa de *Bifidobacterium animalis ssp. lactis* que tem três genes que compreendem uma alteração de base de nucleotídeo, conforme identificado na Tabela 6.

121. Composição bacteriana, de acordo com a reivindicação

118, caracterizada pelo fato de que compreende pelo menos uma cepa de *Bifidobacterium animalis ssp. lactis* que tem quatro genes que compreendem uma alteração de base de nucleotídeo, conforme identificado na Tabela 6.

122. Composição bacteriana, de acordo com a reivindicação 118, caracterizada pelo fato de que compreende pelo menos uma cepa de *Bifidobacterium animalis ssp. lactis* que tem cinco genes que compreendem uma alteração de base de nucleotídeo, conforme identificado na Tabela 6.

123. Composição bacteriana, de acordo com a reivindicação 118, caracterizada pelo fato de que compreende pelo menos uma cepa de *Bifidobacterium animalis ssp. lactis* que tem seis genes que compreendem uma alteração de base de nucleotídeo, conforme identificado na Tabela 6.

124. Composição bacteriana, de acordo com a reivindicação 118, caracterizada pelo fato de que compreende pelo menos uma cepa de *Bifidobacterium animalis ssp. lactis* que tem sete genes que compreendem uma alteração de base de nucleotídeo, conforme identificado na Tabela 6.

125. Composição bacteriana, de acordo com a reivindicação 118, caracterizada pelo fato de que compreende pelo menos uma cepa de *Bifidobacterium animalis ssp. lactis* que tem oito genes que compreendem uma alteração de base de nucleotídeo, conforme identificado na Tabela 6.

126. Composição bacteriana, de acordo com a reivindicação 118, caracterizada pelo fato de que compreende pelo menos uma cepa de *Bifidobacterium animalis ssp. lactis* que tem nove genes que compreendem uma alteração de base de nucleotídeo, conforme identificado na Tabela 6.

127. Composição bacteriana, de acordo com a reivindicação

118, caracterizada pelo fato de que compreende pelo menos uma cepa de *Bifidobacterium animalis ssp. lactis* que tem dez genes que compreendem uma alteração de base de nucleotídeo, conforme identificado na Tabela 6.

128. Composição bacteriana, de acordo com a reivindicação 118, caracterizada pelo fato de que compreende pelo menos uma cepa de *Bifidobacterium animalis ssp. lactis* que tem onze genes que compreendem uma alteração de base de nucleotídeo, conforme identificado na Tabela 6.

129. Composição bacteriana, de acordo com a reivindicação 118, caracterizada pelo fato de que compreende pelo menos uma cepa de *Bifidobacterium animalis ssp. lactis* que tem doze genes que compreendem uma alteração de base de nucleotídeo, conforme identificado na Tabela 6.

130. Composição bacteriana, de acordo com a reivindicação 118, caracterizada pelo fato de que compreende pelo menos uma cepa de *Bifidobacterium animalis ssp. lactis* que tem treze genes que compreendem uma alteração de base de nucleotídeo, conforme identificado na Tabela 6.

131. Composição bacteriana, de acordo com a reivindicação 118, caracterizada pelo fato de que compreende pelo menos uma cepa de *Bifidobacterium animalis ssp. lactis* que tem quatorze genes que compreendem uma alteração de base de nucleotídeo, conforme identificado na Tabela 6.

132. Composição bacteriana, de acordo com a reivindicação 118, caracterizada pelo fato de que compreende pelo menos uma cepa de *Bifidobacterium animalis ssp. lactis* que tem quinze genes que compreendem uma alteração de base de nucleotídeo, conforme identificado na Tabela 6.

133. Composição bacteriana, de acordo com a reivindicação

118, caracterizada pelo fato de que compreende pelo menos uma cepa de *Bifidobacterium animalis ssp. lactis* que tem genes que compreendem todas as alterações de base de nucleotídeo, conforme identificado na Tabela 6.

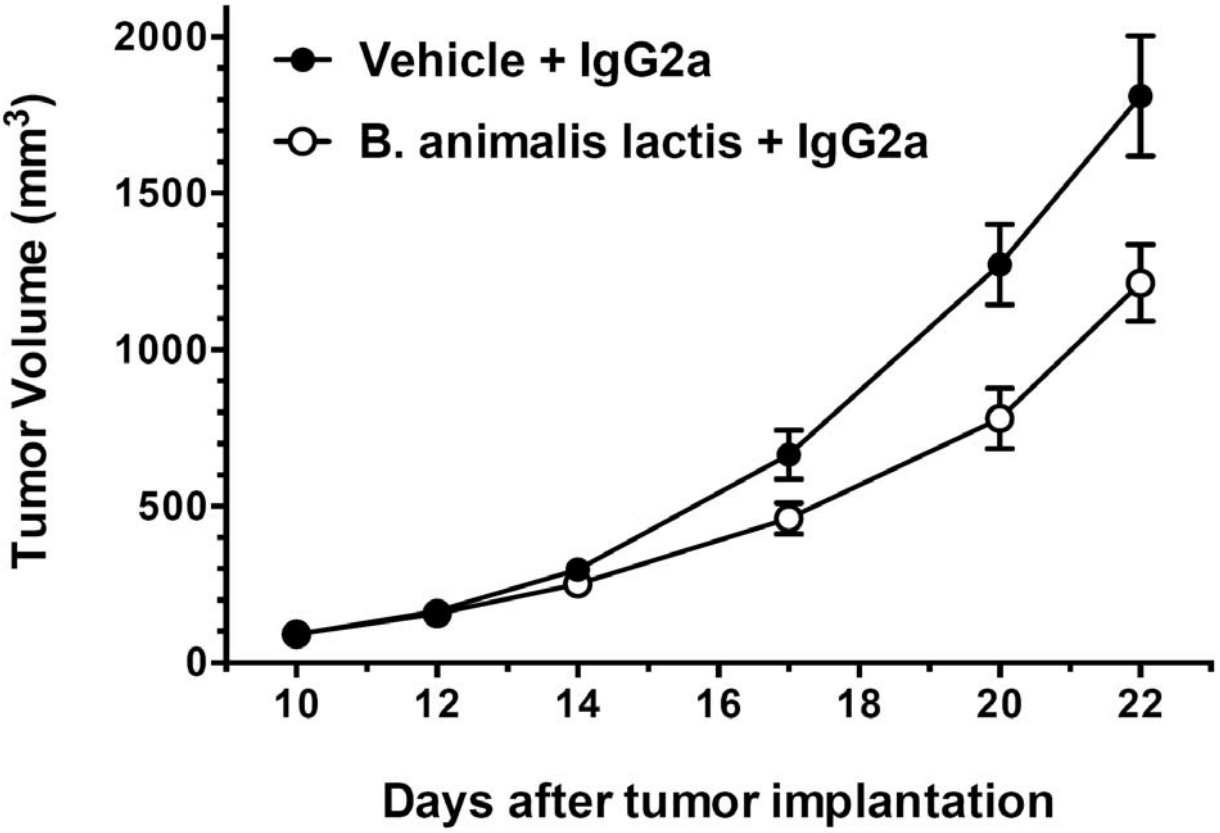
Vehicle + IgG2a
B. animalis lactis + IgG2a
Tumor Volume (mm3)
2000
1500
1000
500
0
10
12
14
16
18
20
22
Days after tumor implantation

FIG. 1

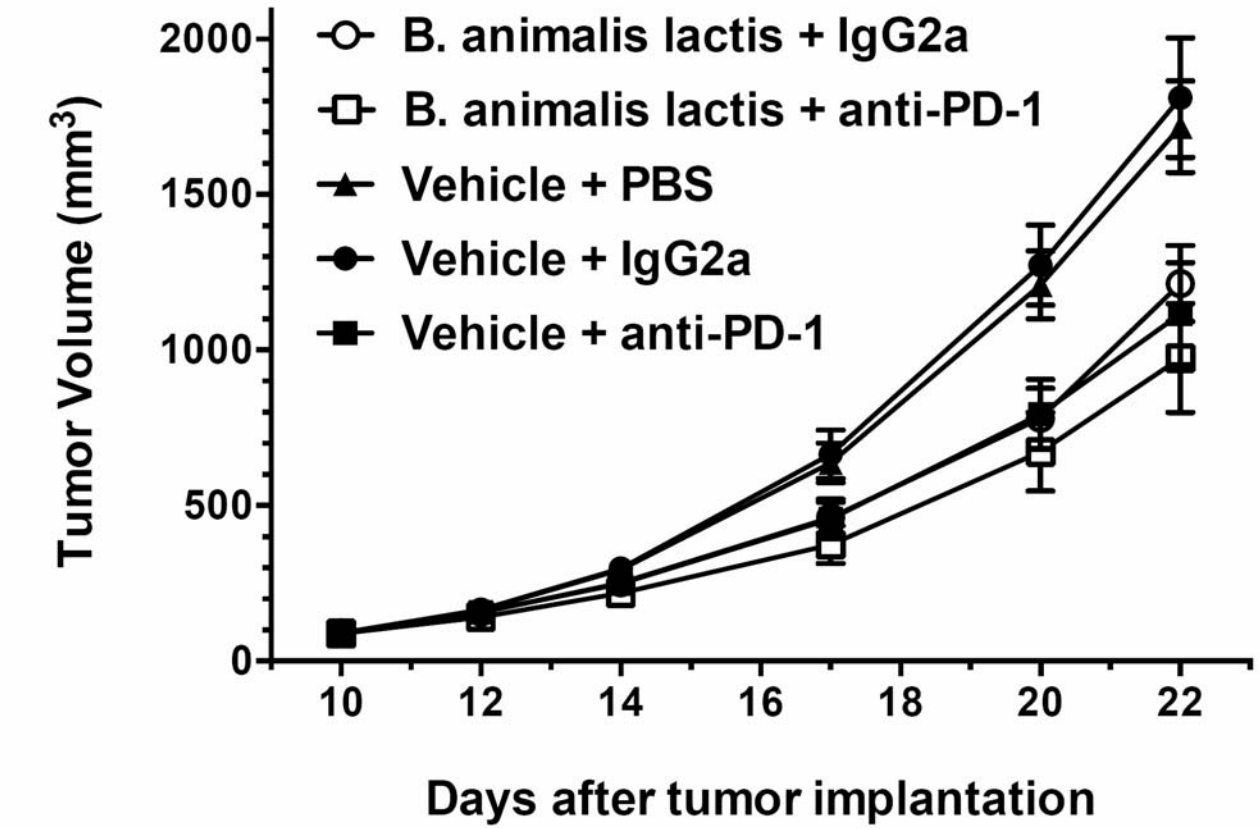
B. animalis lactis + IgG2a
B. animalis lactis + anti-PD-1
Vehicle + PBS
Vehicle + IgG2a
Vehicle + anti-PD-1
Tumor Volume (mm3)
2000
1500
1000
500
0
10
12
14
16
18
20
22
Days after tumor implantation

FIG. 2

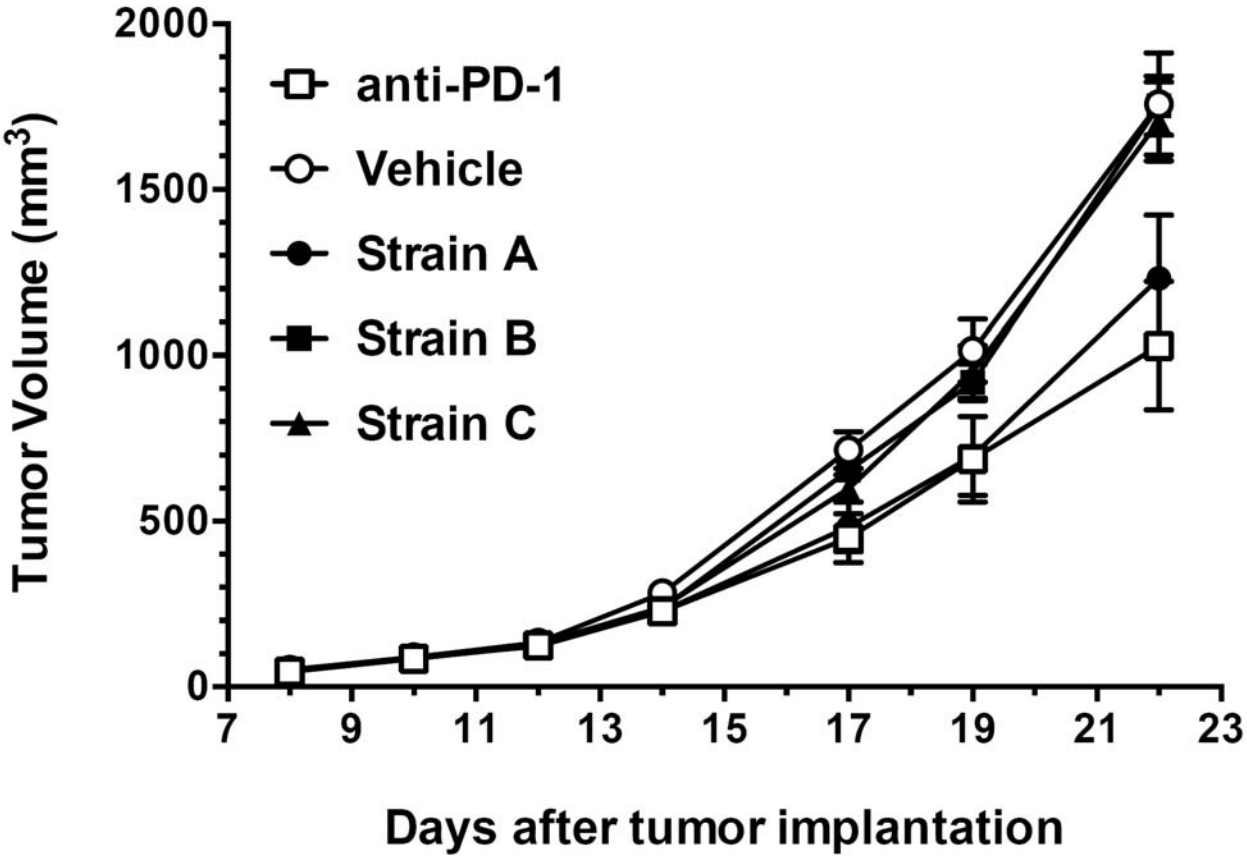
2000
1500
1000
500
0
Tumor Volume (mm3)
anti-PD-1
Vehicle
Strain A
Strain B
Strain C
7
9
11
13
15
17
19
21
23
Days after tumor implantation

FIG. 3

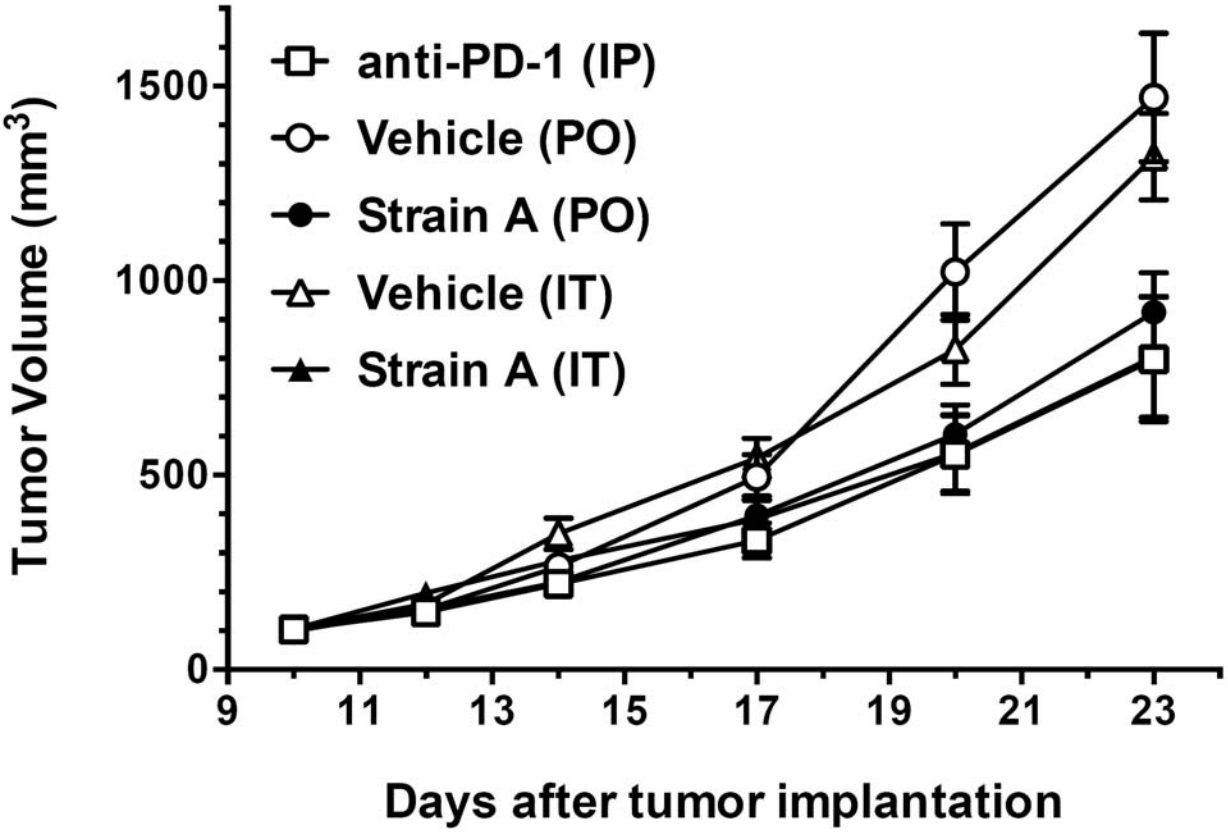
anti-PD-1 (IP)
Vehicle (PO)
Strain A (PO)
Vehicle (IT)
Strain A (IT)
Tumor Volume (mm3)
1500
1000
500
0
9
11
13
15
17
19
21
23
Days after tumor implantation

FIG. 4

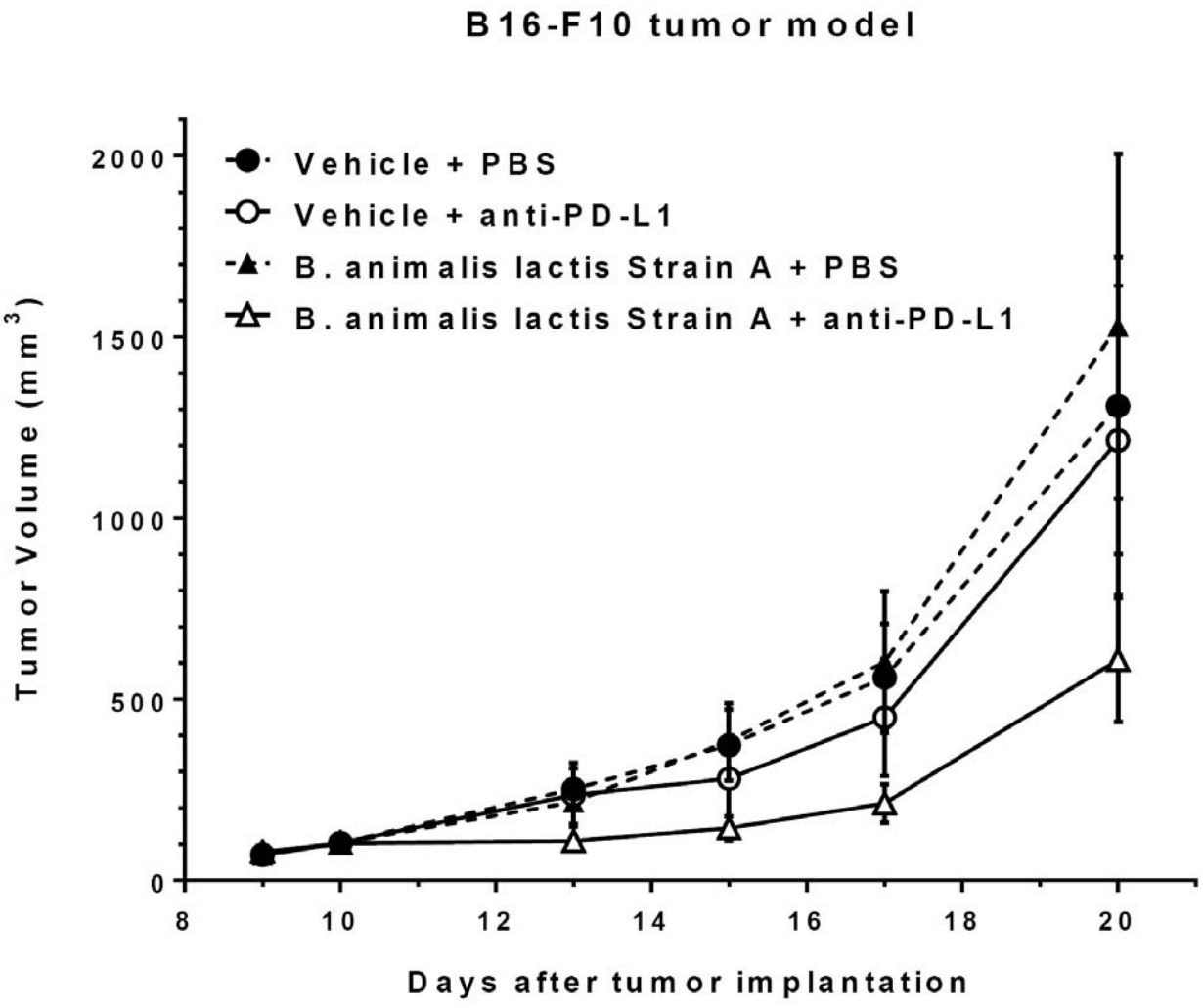
B16-F10 tumor model
Vehicle + PBS
Vehicle + anti-PD-L1
B. animalis lactis Strain A + PBS
B. animalis lactis Strain A + anti-PD-L1
Tumor Volume (mm3)
2000
1500
1000
500
0
8
10
12
14
16
18
20
Days after tumor implantation

FIG. 5

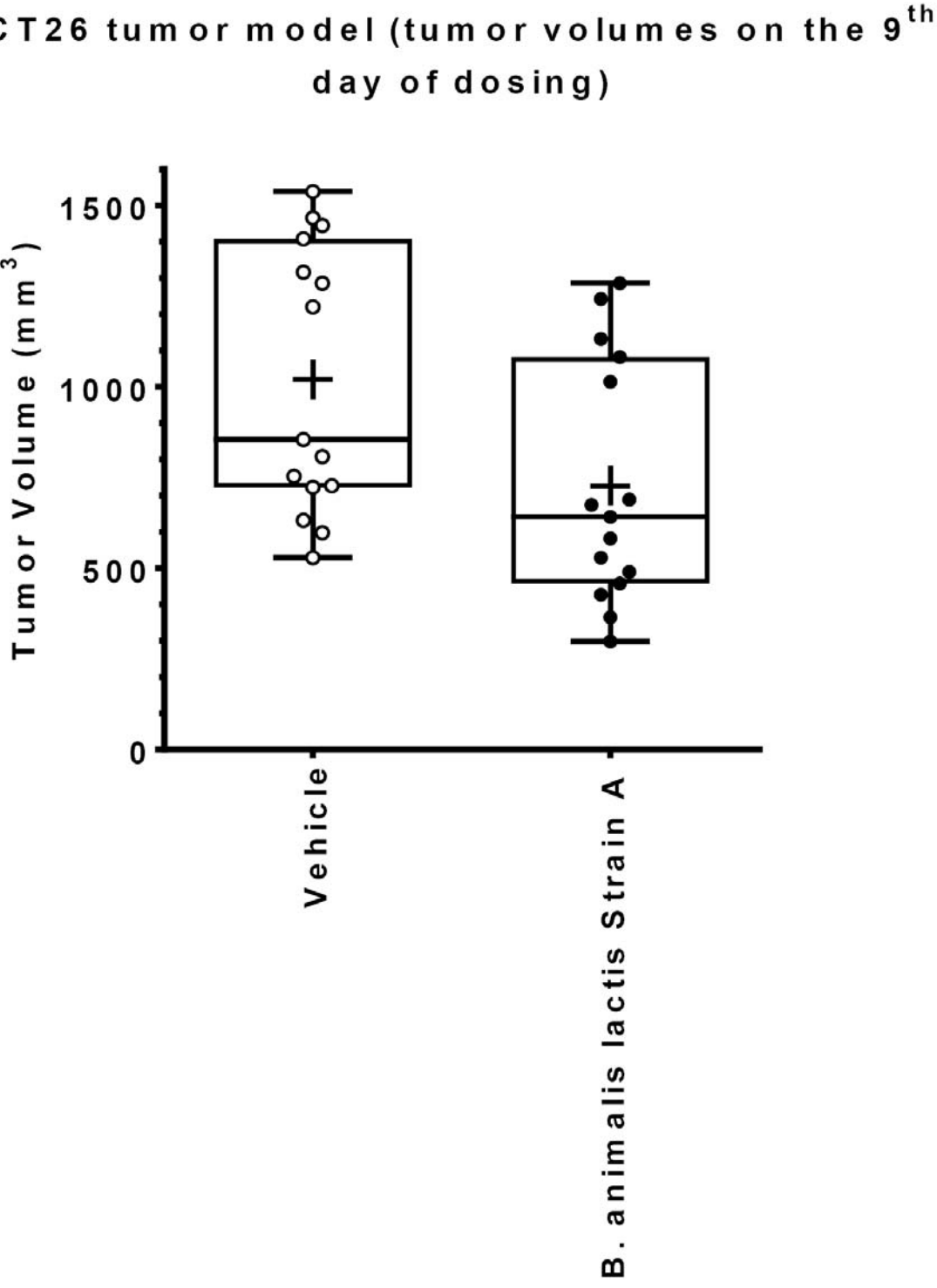
CT26 tumor model (tumor volumes on the 9th day of dosing)
Tumor Volume (mm3)
1500
1000
500
0
Vehicle
B. animalis lactis Strain A

FIG. 6A

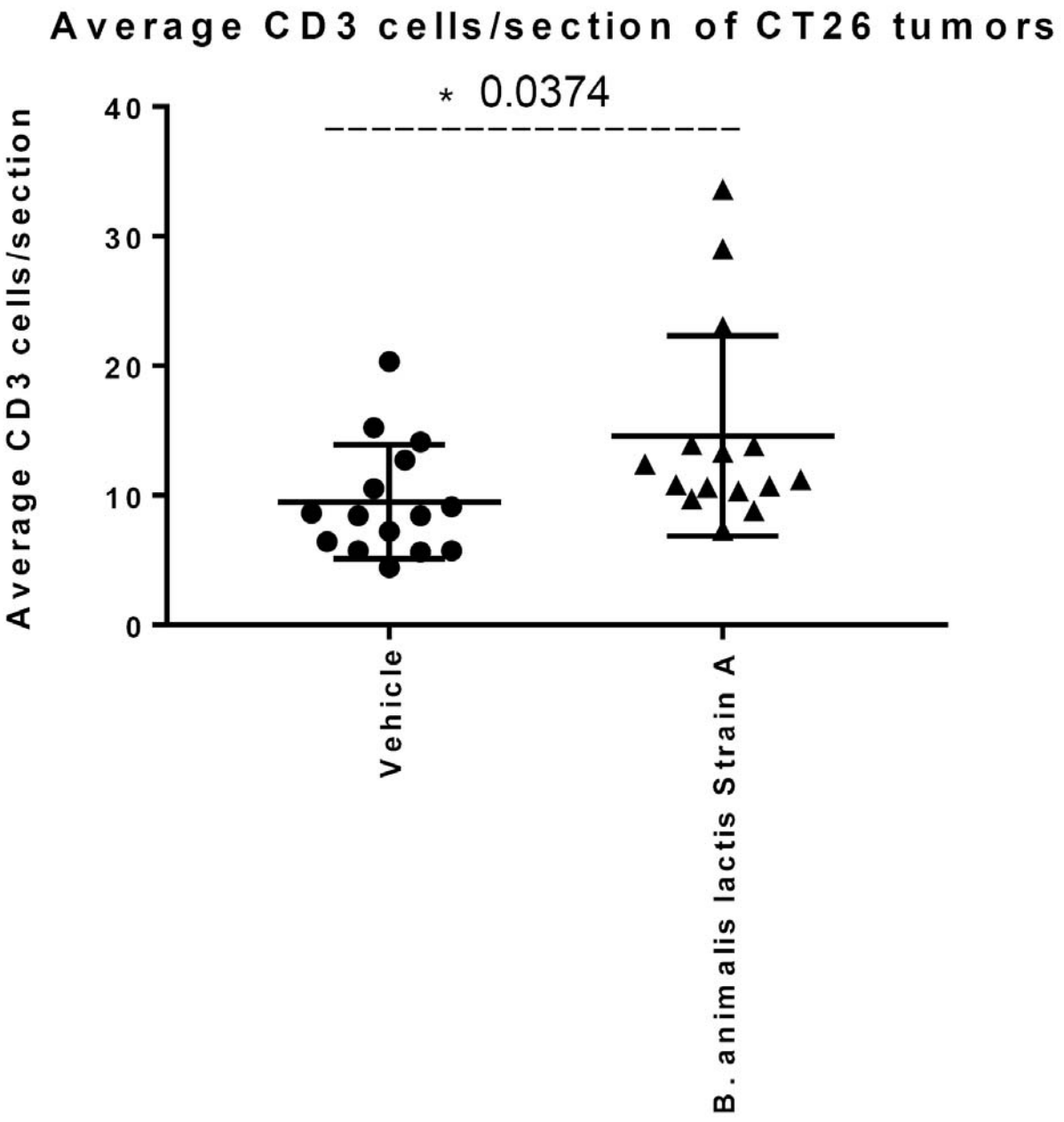
Average CD3 cells/section of CT26 tumors
* 0.0374
Average CD3 cells/section
40
30
20
10
0
Vehicle
B. animalis lactis Strain A

FIG. 6B

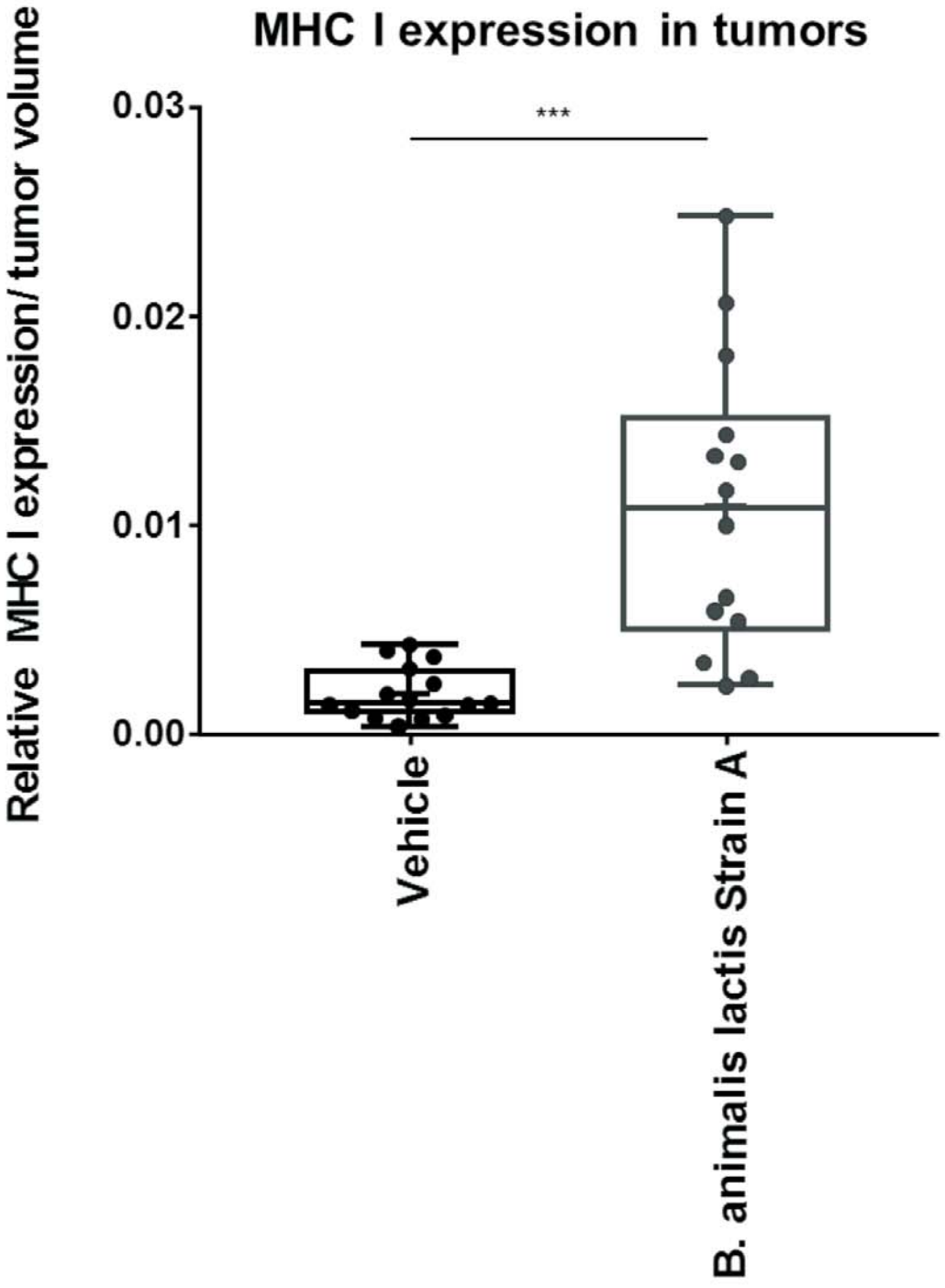
MHC I expression in tumors
Relative MHC I expression/ tumor volume
0.03
0.02
0.01
0.00
***
Vehicle
B. animalis lactis Strain A

FIG. 6C

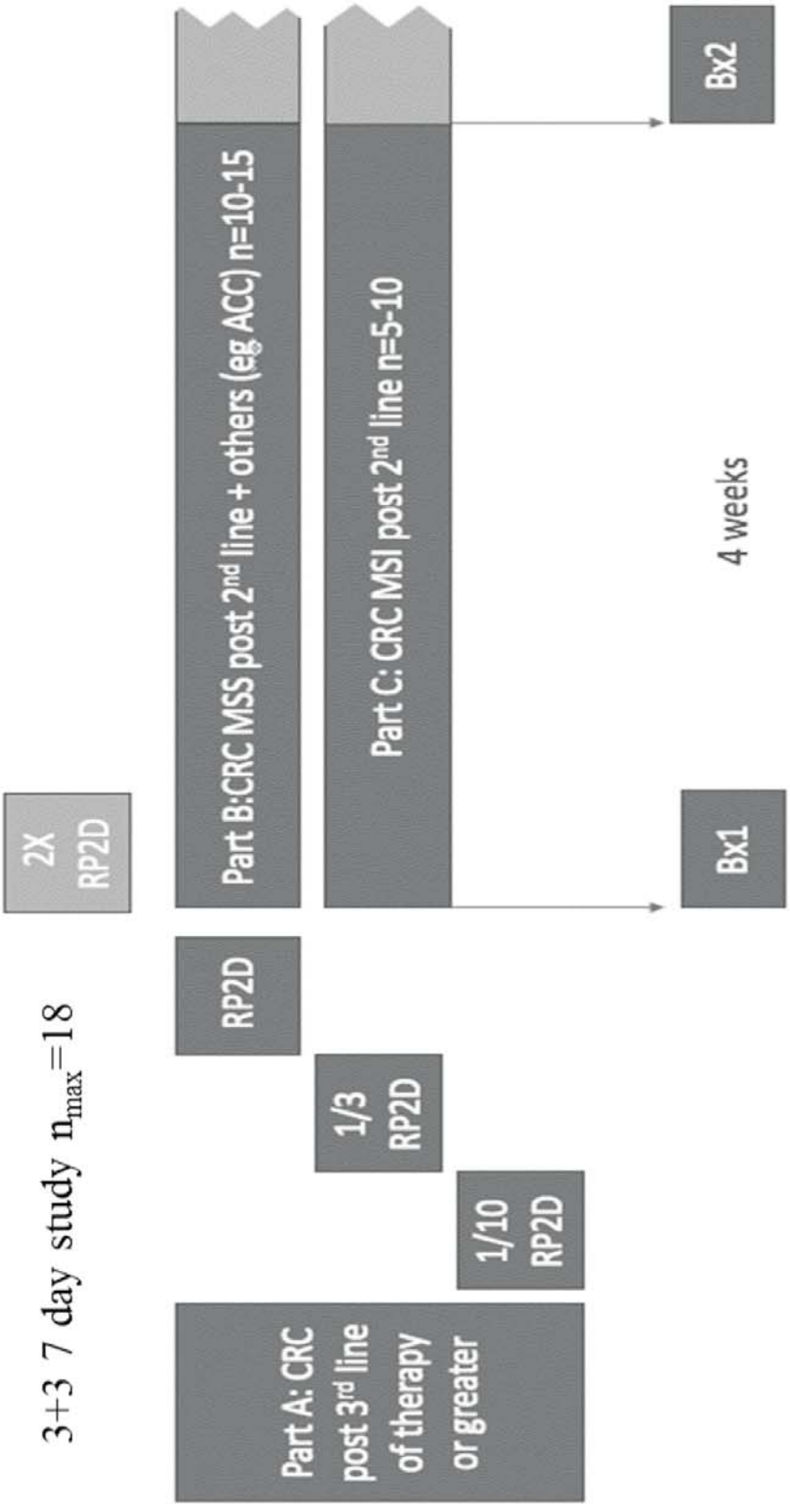
3+3 7 day study n_{max}=18
Part A: CRC post 3rd line of therapy or greater
1/10 RP2D
1/3 RP2D
RP2D
2X RP2D
Part B:CRC MSS post 2nd line + others (eg ACC) n=10-15
Part C: CRC MSI post 2nd line n=5-10
Bx1
4 weeks
Bx2

FIG. 7

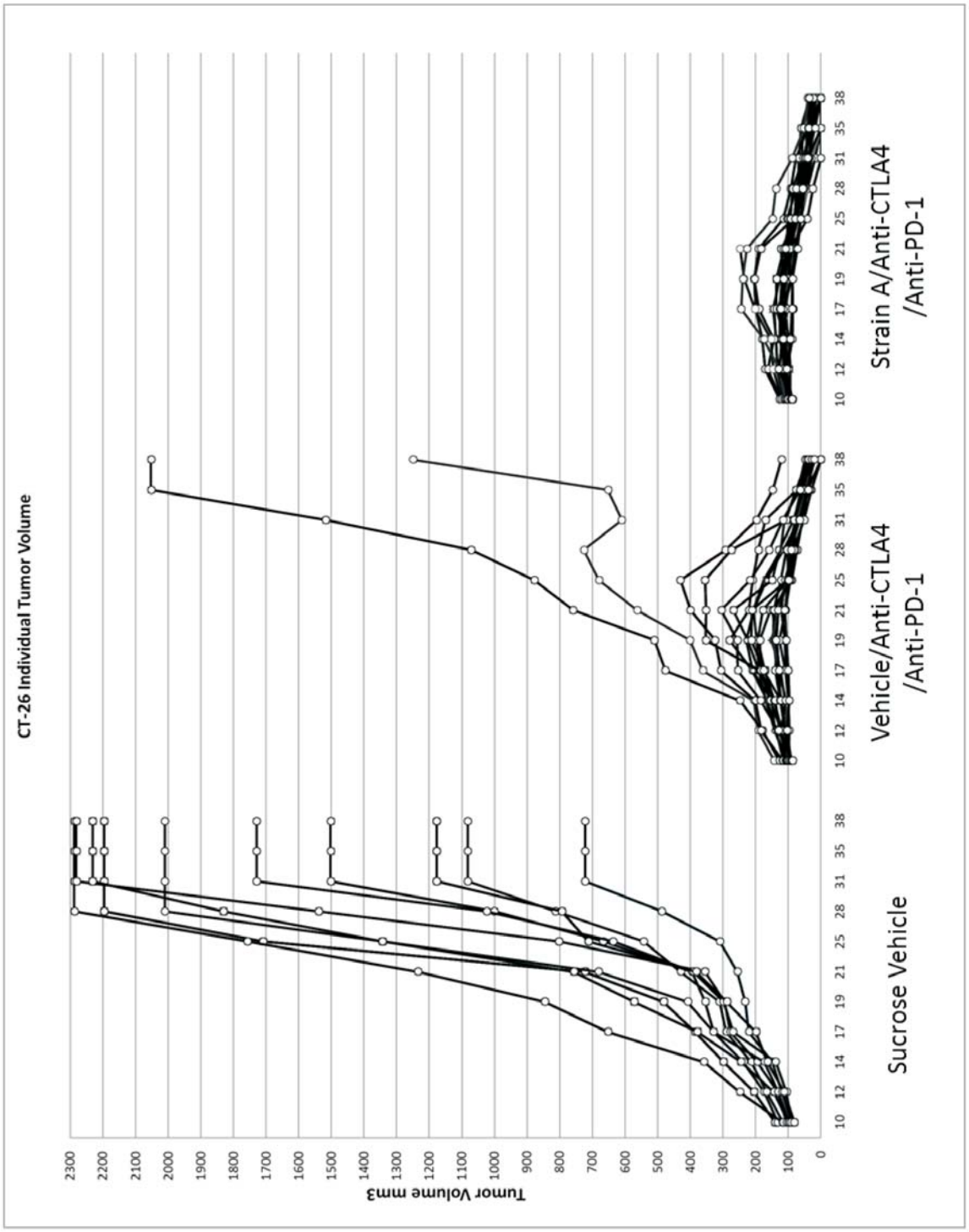
CT-26 Individual Tumor Volume
Tumor Volume mm3
0
100
200
300
400
500
600
700
800
900
1000
1100
1200
1300
1400
1500
1600
1700
1800
1900
2000
2100
2200
2300
10 12 14 17 19 21 25 28 31 35 38
Sucrose Vehicle
Vehicle/Anti-CTLA4
/Anti-PD-1
Strain A/Anti-CTLA4
/Anti-PD-1

FIG. 8

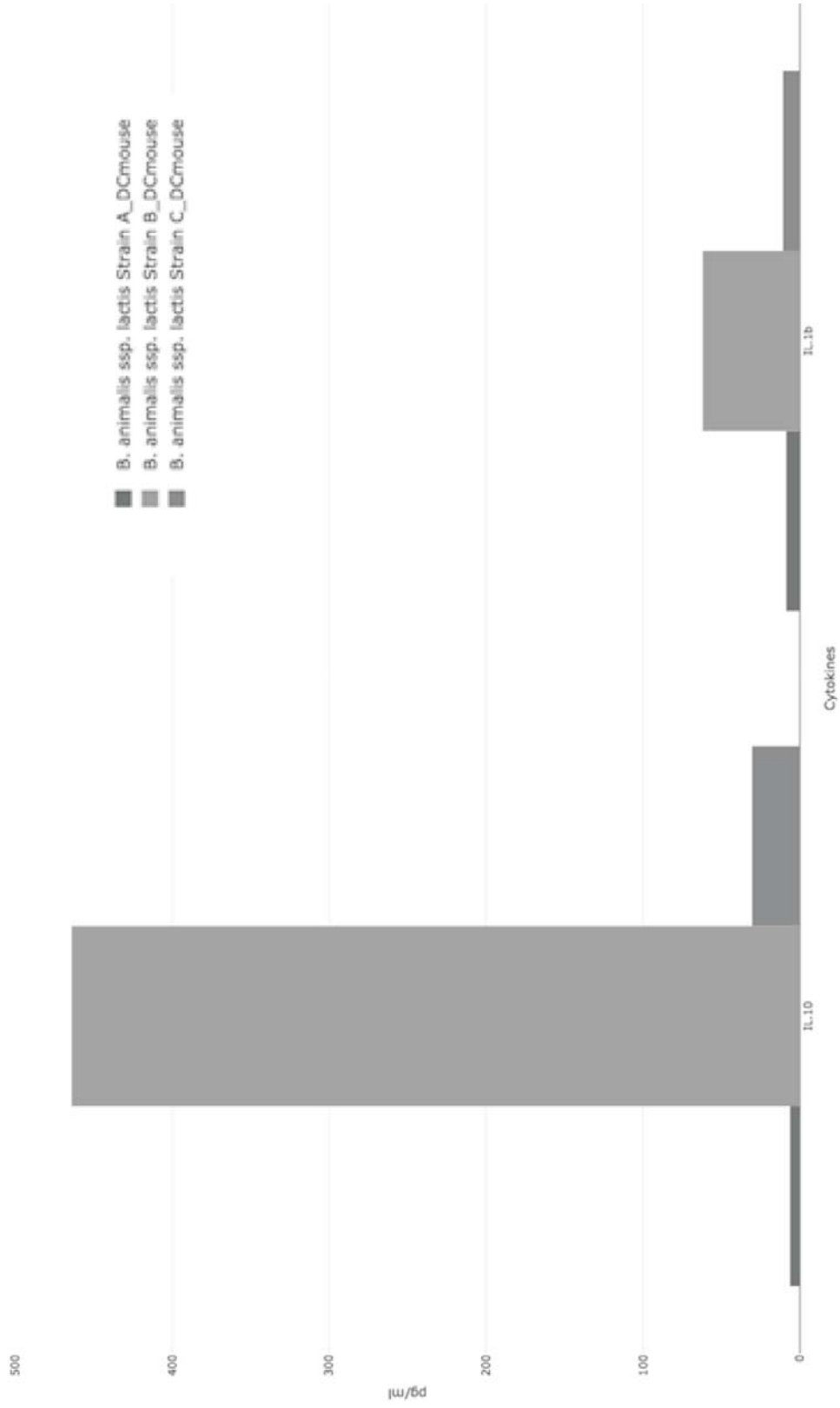
B. animalis ssp. lactis Strain A_DCmouse
B. animalis ssp. lactis Strain B_DCmouse
B. animalis ssp. lactis Strain C_DCmouse
pg/ml
500
400
300
200
100
0
IL.10
IL.1b
Cytokines

FIG. 9A

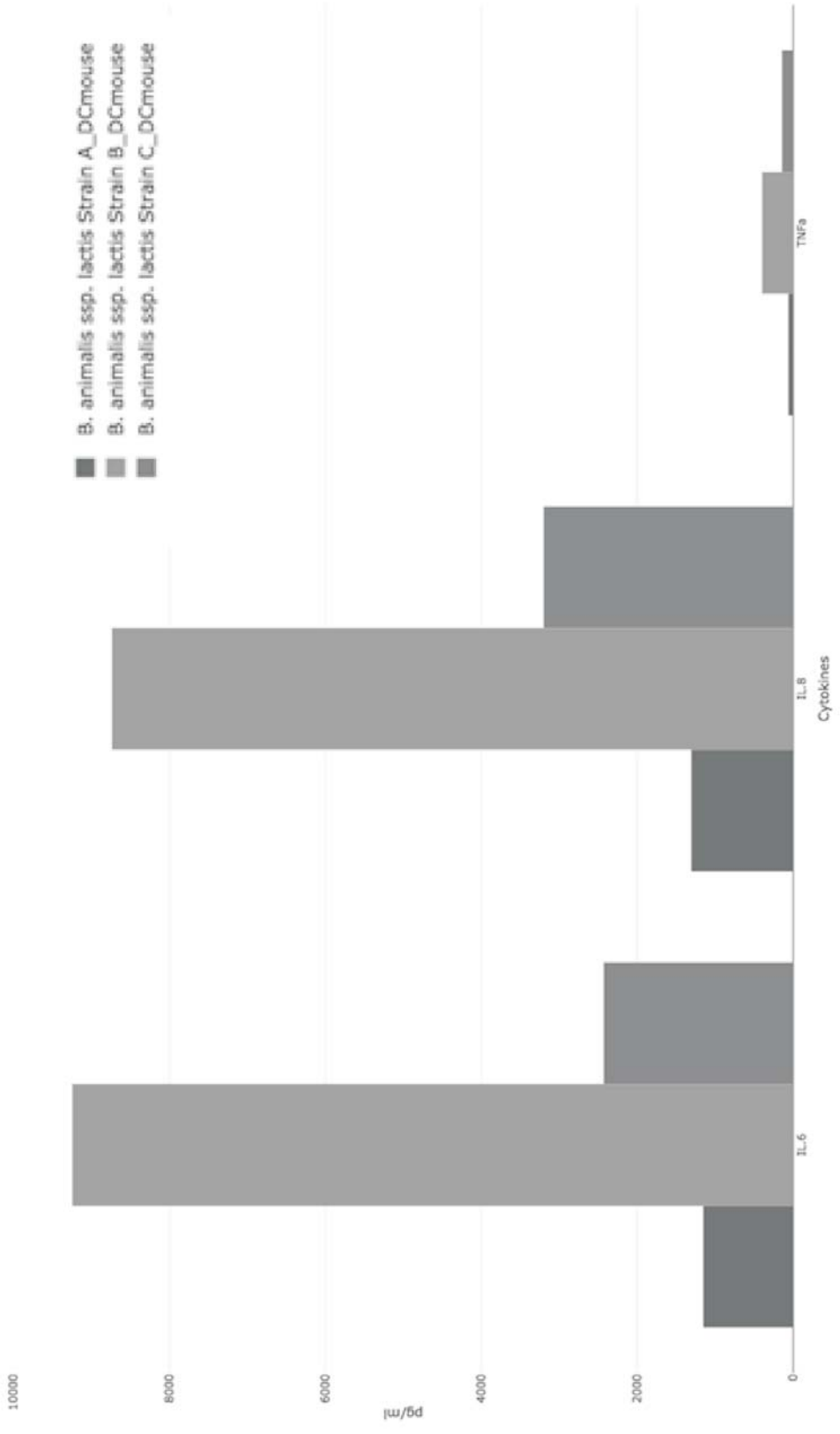
B. animalis ssp. lactis Strain A_DCmouse
B. animalis ssp. lactis Strain B_DCmouse
B. animalis ssp. lactis Strain C_DCmouse
pg/ml
0
2000
4000
6000
8000
10000
IL-6
IL-8
TNFa
Cytokines

FIG. 9B

RESUMO

Patente de Invenção: **"COMPOSIÇÕES E MÉTODOS PARA TRATAR CÂNCER COM O USO DE *BIFIDOBACTERIUM ANIMALIS SSP. LACTIS".***

A presente invenção refere-se a métodos e composições relacionados a *Bifidobacterium animalis ssp. lactis* úteis como agentes terapêuticos.

Este anexo apresenta o código de controle da listagem de sequências biológicas.

Código de Controle

Campo 1



Campo 2



Outras Informações:

- Nome do Arquivo: LISTAGEM DE SEQUÊNCIA EMENDAS EMENDAS-
- Data de Geração do Código: 03/02/2020
- Hora de Geração do Código: 13:19:39
- Código de Controle:
  - Campo 1: A3E25988EC159347
  - Campo 2: A9162F85706FC891