COMPOSICIONES Y MÉTODOS DE TRATAMIENTO DE CÁNCER UTILIZANDO *BIFIDOBACTERIUM ANIMALIS SSP. LACTIS*

SOLICITUD RELACIONADA

Esta solicitud reivindica el beneficio de la prioridad del número de serie de la Solicitud Provisional de Patente de los Estados Unidos 62/528,669, presentada el 5 de julio de 2017, que se incorpora en el presente documento como referencia en su totalidad.

SUMARIO

En ciertos aspectos, en el presente documentose proporcionan métodos y composiciones relacionadas con el tratamiento de un cáncer en un sujeto (por ejemplo, un sujeto humano) que comprende administrar una composición bacteriana que comprende *Bifidobacterium animalis ssp. lactis*. En algunas realizaciones, *Bifidobacterium animalis ssp. lactis* es la cepa A de *Bifidobacterium animalis ssp. lactis* (número de depósito ATCC PTA-125097). En algunas realizaciones, *Bifidobacterium animalis ssp. lactis* es una cepa que comprende al menos 99 % de identidad de secuencia, al menos 99,6 % de identidad de secuencia, al menos 99,6 % de identidad de secuencia, al menos 99,7 % de identidad de secuencia, al menos 99,8 % de identidad de secuencia, al menos 99,9 % de identidad de secuencia. la secuencia de nucleótidos de la cepa A de *Bifidobacterium animalis ssp. lactis* (Tabla 1). En algunas realizaciones, la administración de la composición bacteriana induce una respuesta inmune contra un tumor en el sujeto. En algunas realizaciones, la administración de la composición bacteriana induce la infiltración de células inmunitarias CD3+ en el sujeto. En algunas realizaciones, la administración de la composición bacteriana induce una

regulación al alza del MHC de Clase I en el sitio del tumor. En algunas realizaciones, la administración de la composición bacteriana trata el cáncer en el sujeto. En algunas realizaciones, la administración aumenta un microambiente tumoral en el sujeto. En algunas realizaciones, el cáncer es un carcinoma colorrectal.

En ciertas realizaciones, se proporcionan en la presente los métodos para tratar a un sujeto que tiene cáncer, que comprende administrar al sujeto una composición bacteriana que comprende *Bifidobacterium animalis ssp. lactis* (por ejemplo, una bacteria muerta, una bacteria viva y/o una bacteria atenuada). En algunas realizaciones, *Bifidobacterium animalis ssp. lactis* es la cepa A de *Bifidobacterium animalis ssp. lactis* (número de depósito ATCC PTA-125097). En algunas realizaciones, *Bifidobacterium animalis ssp. lactis* es una cepa que comprende al menos 99 % de identidad de secuencia, al menos 99,6 % de identidad de secuencia, al menos 99,6 % de identidad de secuencia, al menos 99,7 % de identidad de secuencia, al menos 99,8 % de identidad de secuencia, al menos 99,9 % de identidad de secuencia. la secuencia de nucleótidos de la cepa A de *Bifidobacterium animalis ssp. lactis*. En algunas realizaciones, al menos 50 %, 60 %, 70 %, 80 %, 85 %, 90 %, 90 %, 91 %, 92 %, 93 %, 94 %, 95 %, 96 %, 97 %, 98 % o 99 % de las bacterias en la composición bacteriana son *Bifidobacterium animalis ssp. lactis*. En algunas realizaciones, todas o sustancialmente todas las bacterias en la formulación bacteriana son *Bifidobacterium animalis ssp. lactis*. En algunas realizaciones, la formulación bacteriana comprende al menos 1 x 10^5, 5 x 10^5, 1 x 10^6, 2 x 10^6, 3 x 10^6, 4 x 10^6, 5 x 10^6, 6 x 10^6, 7 x 10^6, 8 x 10^6, 9 x 10^6, 1 x10^7, 2 x10^7, 3 x10^7, 4 x10^7, 5 x10^7, 6 x10^7, 7

$x10^7$, 8 $x10^7$, 9 $x10^7$, 1 $x10^8$, 2 $x10^8$, 3 $x10^8$, 4 $x10^8$, 5 $x10^8$, 6 $x10^8$, 7 $x10^8$, 8 $x10^8$, 9 $x10^8$ o 1 $x10^9$ unidades formadoras de colonias de *Bifidobacterium animalis ssp. lactis.*

En ciertas realizaciones, se proporcionan en el presente documento composiciones bacterianas que comprenden *Bifidobacterium animalis ssp. lactis* (por ejemplo, una bacteria muerta, una bacteria viva y/o una bacteria atenuada). En algunas realizaciones, al menos 50 %, 60 %, 70 %, 80 %, 85 %, 90 %, 90 %, 91 %, 92 %, 93 %, 94 %, 95 %, 96 %, 97 %, 98 % o el 99 % de las bacterias en la composición bacteriana son *Bifidobacterium animalis ssp. lactis*. En algunas realizaciones, *Bifidobacterium animalis ssp. lactis* es la cepa A de *Bifidobacterium animalis ssp. lactis* (número de depósito ATCC PTA-125097). En algunas realizaciones, *Bifidobacterium animalis ssp. lactis* es una cepa que comprende al menos 99 % de identidad de secuencia, al menos 99,6 % de identidad de secuencia, al menos 99,6 % de identidad de secuencia, al menos 99,7 % de identidad de secuencia, al menos 99,8 % de identidad de secuencia, al menos 99,9 % de identidad de secuencia. la secuencia de nucleótidos de la cepa A de *Bifidobacterium animalis ssp. lactis*. En algunas realizaciones, todas o sustancialmente todas las bacterias en la formulación bacteriana son *Bifidobacterium animalis ssp. lactis*. En algunas realizaciones, la formulación bacteriana comprende al menos 1 x 10^5, 5 x 10^5, 1 x 10^6, 2 x 10^6, 3 x 10^6, 4 x 10^6, 5 x 10^6, 6 x 10^6, 7 x 10^6, 8 x 10^6, 9 x 10^6, 1 $x10^7$, 2 $x10^7$, 3 $x10^7$, 4 $x10^7$, 5 $x10^7$, 6 $x10^7$, 7 $x10^7$, 8 $x10^7$, 9 $x10^7$, 1 $x10^8$, 2 $x10^8$, 3 $x10^8$, 4 $x10^8$, 5 $x10^8$, 6 $x10^8$, 7 $x10^8$, 8 $x10^8$, 9 $x10^8$ o 1 $x10^9$ unidades formadoras de colonias de *Bifidobacterium animalis ssp. lactis*. En algunas realizaciones, la composición bacteriana comprende al menos

una cepa de *Bifidobacterium animalis ssp. lactis* que tiene un gen que comprende un cambio de base de nucleótidos como se identifica en la Tabla 6. En algunas realizaciones, la composición bacteriana comprende al menos una cepa de *Bifidobacterium animalis ssp. lactis* con 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 o 29, genes que comprenden un cambio en la base de nucleótidos como se identifica en la Tabla 6. En algunas realizaciones, la composición bacteriana comprende al menos una cepa de *Bifidobacterium animalis ssp. lactis* que tiene genes que comprenden todos los cambios de bases de nucleótidos identificados en la Tabla 6.

En algunas realizaciones, en el presente documentose proporcionan biomasas farmacéuticamente activas (PhAB) derivadas de y/o que comprenden *Bifidobacterium animalis ssp. lactis* (por ejemplo, cepa *Bifidobacterium animalis ssp. lactis* A (número de depósito ATCC PTA-125097)). En algunas realizaciones, los PhAB comprenden células enteras, fracciones de células, sobrenadante de la fermentación, fracciones de sobrenadante y/o vesículas extracelulares preparadas a partir de *Bifidobacterium animalis ssp. lactis* descrito en este documento. En algunas realizaciones, las composiciones bacterianas proporcionadas en el presente documentocomprenden *Bifidobacterium animalis ssp. lactis* PhAB proporcionados en este documento.

En algunas realizaciones, la composición bacteriana se administra por vía oral, intravenosa, intratumoral o subcutánea. En algunas realizaciones, la composición bacteriana se administra en 2 o más (p. Ej., 3 o más, 4 o más o 5 o más dosis). En algunas realizaciones, la administración al sujeto de las dos o más dosis se separa

por al 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 día, 2 días, 3 días, 4 días, 5 días, 6 días, 7 días, 8 días, 9 días, 10 días, 11 días, 12 días, 13 días, 14 días, 15 días, 16 días, 17 días, 18 días, 19 días, 20 días o 21 días. En algunas realizaciones, se administra una segunda bacteria como parte de un consorcio ecológico.

En algunas realizaciones, el método comprende además administrar al sujeto un antibiótico. En algunas realizaciones, el método comprende además administrar al sujeto una o más terapias contra el cáncer diferentes. En algunas realizaciones, la otra terapia contra el cáncer es la extirpación quirúrgica de un tumor, la administración de un agente quimioterapéutico, la administración de radioterapia, la administración de un antibiótico, la administración de una inmunoterapia contra el cáncer (por ejemplo, un inhibidor del punto de control inmune, anticuerpo específico contra el cáncer, una vacuna contra el cáncer, una célula presentadora de antígeno cebada, una célula T específica de cáncer, una célula T de receptor de antígeno quimérico (CAR) específico de cáncer, una proteína activadora de inmunidad, un adyuvante), y/o la administración de otra bacteria terapéutica.

En algunas realizaciones, el sujeto es un mamífero. En algunas realizaciones, el sujeto es un ser humano. En algunas realizaciones, el sujeto es un mamífero no humano (por ejemplo, un perro, un gato, una vaca, un caballo, un cerdo, un burro, una cabra, un camello, un ratón, una rata, un conejillo de Indias, en ovejas, una llama, un mono, un gorila o un chimpancé).

En ciertas realizaciones, se proporciona en el

presente documento un biorreactor que comprende *Bifidobacterium animalis ssp. lactis*.

BREVE DESCRIPCIÓN DE LAS FIGURAS

La **Figura 1** muestra la inhibición del crecimiento tumoral (en volumen) mediante la administración oral de la cepa A de *Bifidobacterium animalis ssp. lactis* en un modelo de carcinoma colorrectal de ratón.

La **Figura 2** muestra que en un modelo de carcinoma colorrectal de ratón, la eficacia de la cepa A de *Bifidobacterium animalis ssp lactis* administrada por vía oral es comparable a la del anti-PD-1 administrado intraperitonealmente (i.p.).

La **Figura 3** muestra que en un modelo de carcinoma colorrectal de ratón, la eficacia de la cepa A de *Bifidobacterium animalis ssp lactis* administrada por vía oral es comparable a la del anti-PD-1 administrado intraperitonealmente (i.p.).

La **Figura 4** muestra que en un modelo de carcinoma colorrectal de ratón, la eficacia de la cepa A de *Bifidobacterium animalis ssp lactis* administrada por vía oral es comparable a la del anti-PD-1 administrado intraperitonealmente (i.p.).

La **Figura 5** muestra que en un modelo de melanoma de ratón, se tratan ratones inoculados con un tumor con una combinación de la cepa A de *Bifidobacterium animalis ssp lactis* y anti-PD-L1 inhibe el crecimiento tumoral de una manera superior a la cepa A de *Bifidobacterium animalis ssp lactis* o anti-PD-L1 de forma individual.

La **Figura 6A** muestra el volumen tumoral antes del infiltrado de células inmunitarias CD3+ y el análisis de expresión de MHC de Clase I. La Figura 6A muestra la inhibición del crecimiento tumoral (en volumen) mediante la

administración oral de la cepa A de *Bifidobacterium animalis ssp. lactis* en un modelo de carcinoma colorrectal de ratón.

La **Figura 6B** muestra que la infiltración de células inmunitarias CD3+ se incrementó significativamente en el grupo de la cepa A de *Bifidobacterium animalis ssp. lactis* respecto a grupo de vehículo.

La **Figura 6C** muestra que la cepa A de *Bifidobacterium animalis ssp. lactis* indujo una sorprendente regulación de la expresión del MHC de clase I en relación con el grupo de vehículo.

La **Figura 7** muestra una visión general de un estudio abierto de fase 1/2 de la seguridad, tolerabilidad y eficacia de la cepa A de *Bifidobacterium animalis ssp. lactis* como un medicamento oral en pacientes con carcinoma colorrectal metastásico.

La **Figura 8** muestra que en un modelo de carcinoma colorrectal de ratón, la eficacia de una combinación triple de la cepa A de *Bifidobacterium animalis ssp. lactis*, anti-PD-1 y anti-CTLA4 es mayor que la de la combinación de anti-PD-1 y anti-CTLA4.

La **Figura 9A** y la **Figura 9B** muestran una comparación de firmas *in vitro* de tres cepas de *Bifidobacterium animalis ssp. lactis* (cepa A, cepa B y cepa C) de un ensayo de células dendríticas de ratón. La cepa A presenta un perfil inmune con una disminución de la inducción de citocinas protumorigénicas (IL-10, IL-1b, IL-6, IL-8, TNFα) en comparación con la cepa B y la cepa C. Los valores de las citocinas indicados representan el resumen de la media geométrica de grupos (n = 3) en todo el ensayo.

DESCRIPCIÓN DETALLADA

Aspectos generales

En ciertos aspectos, se proporcionan en el presente documento composiciones bacterianas que comprenden *Bifidobacterium animalis ssp. lactis* y métodos para tratar el cáncer en un sujeto que comprenden administrar al sujeto una composición bacteriana que comprende *Bifidobacterium animalis ssp. lactis*.

Definiciones

"Adyuvante" o "terapia adyuvante" se refiere en general a un agente que afecta a una respuesta inmunológica o fisiológica en un paciente o sujeto. Por ejemplo, un adyuvante puede aumentar la presencia de un antígeno a lo largo del tiempo o en un área de interés, como un tumor, ayudar a absorber un antígeno celular presentador de antígeno, activar macrófagos y linfocitos y apoyar la producción de citocinas. Al cambiar una respuesta inmune, un adyuvante podría permitir una dosis más pequeña de un agente que interactúa con el sistema inmunológico para aumentar la efectividad o la seguridad de una dosis particular del agente que interacciona con el sistema inmunológico. Por ejemplo, un adyuvante puede evitar el agotamiento de las células T y así aumentar la efectividad o la seguridad de un agente de interacción inmune particular.

"Administración" se refiere ampliamente a una ruta de administración de una composición a un sujeto. Los ejemplos de vías de administración incluyen administración oral, administración rectal, administración tópica, inhalación (nasal) o inyección. La administración por inyección incluye la administración intravenosa (IV), intramuscular (IM), intratumoral (IT) y subcutánea (SC). Las composiciones farmacéuticas descritas en el presente documentopueden administrarse en cualquier forma mediante

cualquier ruta eficaz, que incluye, pero no se limita a, intratumoral, oral, parenteral, enteral, intravenosa, intraperitoneal, tópica, transdérmica (por ejemplo, usando cualquier parche estándar), intradérmica, oftálmica, (intra) nasal, local, no oral, como aerosol, inhalación, subcutánea, intramuscular, bucal, sublingual, (trans) rectal, vaginal, intraarterial e intratecal, transmucosa (p. ej., sublingual, lingual, (trans) bucal, (trans) uretral, vaginal (p. ej., trans y perivaginal), intravesical, intrapulmonar, intraduodenal, intragástrico e intrabronquial. En realizaciones preferidas, las composiciones farmacéuticas descritas en el presente documentose administran por vía oral, rectal, intratumoral, tópica, intravesical, mediante inyección. dentro o adyacente a un ganglio linfático que drena, por vía intravenosa, por inhalación o aerosol, o por vía subcutánea.

Como se usa en este documento, el término "anticuerpo" puede referirse tanto a un anticuerpo intacto como a un fragmento de unión a antígeno del mismo. Los anticuerpos intactos son glicoproteínas que incluyen al menos dos cadenas pesadas (H) y dos cadenas ligeras (L) interconectadas por enlaces disulfuro. Cada cadena pesada incluye una región variable de cadena pesada (abreviada en el presente documentocomo VH) y una región constante de cadena pesada. Cada cadena ligera incluye una región variable de cadena ligera (abreviada en el presente documentocomo VL) y una región constante de cadena ligera. Las regiones VH y VL pueden subdividirse adicionalmente en regiones de hipervariabilidad, denominadas regiones determinantes de complementariedad (CDR), intercaladas con regiones que están más conservadas, denominadas regiones

marco (FR). Cada VH y VL está compuesto por tres CDR y cuatro FR, dispuestas desde el extremo amino al extremo carboxi en el siguiente orden: FR1, CDR1, FR2, CDR2, FR3, CDR3, FR4. Las regiones variables de las cadenas pesada y ligera contienen un dominio de unión que interactúa con un antígeno. El término "anticuerpo" incluye, por ejemplo, anticuerpos monoclonales, anticuerpos policlonales, anticuerpos quiméricos, anticuerpos humanizados, anticuerpos humanos, anticuerpos multiespecíficos (por ejemplo, anticuerpos biespecíficos), anticuerpos monocatenarios y fragmentos de anticuerpos que se unen a antígenos.

Los términos "fragmento de unión a antígeno" y "porción de unión a antígeno" de un anticuerpo, como se usan en el presente documento, se refieren a uno o más fragmentos de un anticuerpo que retienen la capacidad de unirse a un antígeno. Los ejemplos de fragmentos de unión abarcados dentro del término "fragmento de unión a antígeno" de un anticuerpo incluyen Fab, Fab ', F (ab') 2, Fv, scFv, Fv unido por disulfuro, Fd, diacuerpos, anticuerpos de cadena única, NANOBODIES®, CDRH3 aislado, y otros fragmentos de anticuerpo que retienen al menos una porción de la región variable de un anticuerpo intacto. Estos fragmentos de anticuerpo pueden obtenerse usando técnicas recombinantes y/o enzimáticas convencionales y pueden cribarse para la unión al antígeno de la misma manera que los anticuerpos intactos.

"Cáncer" se refiere en general a un crecimiento anormal e incontrolado de las propias células del huésped que conduce a la invasión del tejido circundante y potencialmente al tejido distal al sitio inicial de crecimiento celular anormal en el huésped. Las clases

principales incluyen carcinomas que son cánceres del tejido epitelial (por ejemplo, piel, células escamosas); sarcomas que son cánceres del tejido conjuntivo (por ejemplo, hueso, cartílago, grasa, músculo, vasos sanguíneos, etc.); leucemias que son cánceres de tejido formador de sangre (por ejemplo, tejido de médula ósea); linfomas y mielomas que son cánceres de células inmunes; y cánceres del sistema nervioso central que incluyen cánceres del cerebro y del tejido espinal. "Cáncer (es)", "neoplasma (s)" y "tumor (es)" se usan en el presente documento de forma intercambiable. Como se usa en el presente documento, "cáncer" se refiere a todos los tipos de cáncer o neoplasia o tumores malignos que incluyen leucemias, carcinomas y sarcomas, ya sean nuevos o recurrentes. Los ejemplos específicos de cánceres son: carcinomas, sarcomas, mielomas, leucemias, linfomas y tumores de tipo mixto. Los ejemplos no limitantes de cánceres son cánceres nuevos o recurrentes del cerebro, melanoma, vejiga, mama, cuello uterino, colon, cabeza y cuello, riñón, pulmón, pulmón de células no pequeñas, mesotelioma, ovario, próstata, sarcoma, estómago y útero y meduloblastoma. Los tumores pediátricos y adultos incluyen, entre otros, los de vejiga, cerebro, mama, hueso, cuello uterino, colon, tejido conjuntivo, grasa, cabeza y cuello, riñón, hígado, pulmón, mesotelio, melanocitos (melanoma), músculo, ovario, páncreas, próstata, estómago, intestino delgado y útero

El término "disminución" o "reducción" significa un cambio, de modo que la diferencia es, según las circunstancias, al menos 10 %, 20 %, 30 %, 40 %, 50 %, 60 %, 70 %, 80 %, 90 %, 1/100, 1/1000, 1/10,000, 1/100.000, 1/1,000,000 o indetectable después del tratamiento en comparación con un estado previo al tratamiento.

El término "consorcio ecológico" es un grupo de bacterias que intercambian metabolitos y se co-regulan positivamente entre sí, en contraste con dos bacterias que inducen la sinergia del hospedador a través de la activación de rutas del hospedador complementarias para mejorar la eficacia.

El término "epítope" significa un determinante de proteína capaz de unirse específicamente a un anticuerpo. Los epítopos generalmente consisten en agrupaciones superficiales químicamente activas de moléculas tales como aminoácidos o cadenas laterales de azúcar. Ciertos epítopos se pueden definir mediante una secuencia particular de aminoácidos a la que un anticuerpo es capaz de unirse.

El término "gen" se usa ampliamente para referirse a cualquier ácido nucleico asociado con una función biológica. El término "gen" se aplica a una secuencia genómica específica, así como a un ADNc o un ARNm codificado por esa secuencia genómica.

La "identidad" entre las secuencias de ácido nucleico de dos moléculas de ácido nucleico se puede determinar como un porcentaje de identidad usando algoritmos informáticos conocidos tales como el programa "FASTA", usando, por ejemplo, los parámetros por defecto como en Pearson et al. (1988) Proc. Natl. Acad. Sci. Sci. USA 85: 2444 (otros programas incluyen el paquete de programa 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, and Carillo *et al.* (1988) SIAM J Applied Math 48:1073). Por ejemplo, la función BLAST de la base de datos de información del Centro Nacional de Biotecnología se puede usar para determinar la

identidad. Otros programas comercial o públicamente disponibles incluyen, el programa "MegAlign" de DNAStar (Madison, Wis.) Y el programa "Gap" de la Genetics Computer Group (UWG) de la Universidad de Wisconsin (Madison Wis.)).

"Inmunoterapia" es un tratamiento que usa el sistema inmunológico de un sujeto para tratar el cáncer e incluye, por ejemplo, inhibidores del punto de control, vacunas contra el cáncer, citocinas, terapia celular, células CAR-T y terapia con células dendríticas.

El término "aumento" significa un cambio, tal que la diferencia es, según las circunstancias, al menos 10 %, 20 %, 30 %, 40 %, 50 %, 60 %, 70 %, 80 %, 90 %, 2- doble, 4 veces, 10 veces, 100 veces, 10 ^ 3 veces, 10 ^ 4 veces, 10 ^ 5 veces, 10 ^ 6 veces, y/o 10 ^ 7 veces mayor después del tratamiento en comparación con un pre estado de tratamiento. Las propiedades que pueden aumentar incluyen las células inmunitarias, las células bacterianas, las células del estroma, las células supresoras derivadas de mieloides, los fibroblastos, los metabolitos y las citocinas.

Los "agonistas inmune innato" o "inmunoadyuvantes" son pequeñas moléculas, proteínas u otros agentes que se dirigen específicamente a los receptores inmune innato, incluidos los receptores Toll-Like, los receptores NOD, los componentes STING Pathway. Por ejemplo, LPS es un agonista de TLR-4 que se deriva o sintetiza bacterianamente y el aluminio se puede usar como un adyuvante de estimulación inmune. inmuno-adyuvantes son una clase específica de adyuvante más amplio o terapia adyuvante.

El término "aislado" o "enriquecido" abarca un microbio, bacteria u otra entidad o sustancia que ha sido (1) separada de al menos algunos de los componentes con los

que estaba asociada cuando se produjo inicialmente (ya sea en la naturaleza o en un entorno experimental)), y/o (2) producido, preparado, purificado y/o fabricado por la mano del hombre. Los microbios aislados pueden separarse de al menos aproximadamente 10 %, aproximadamente 20 %, aproximadamente 30 %, aproximadamente 40 %, aproximadamente 50 %, aproximadamente 60 %, aproximadamente 70 %, aproximadamente 80 %, aproximadamente 90 % o más de los otros componentes con el que fueron inicialmente asociados. En algunas realizaciones, los microbios aislados son más de aproximadamente 80 %, aproximadamente 85 %, aproximadamente 90 %, aproximadamente 91 %, aproximadamente 92 %, aproximadamente 93 %, aproximadamente 94 %, aproximadamente 95 %, aproximadamente 96 %, aproximadamente 97 %, aproximadamente 98 %, aproximadamente 99 %, o más de aproximadamente 99 % de pureza. Como se usa en el presente documento, una sustancia es "pura" si está sustancialmente libre de otros componentes. Los términos "purificar", "purificar" y "purificar" se refieren a un microbio u otro material que se ha separado de al menos algunos de los componentes con los que se asociaba cuando se producía o se generaba inicialmente (por ejemplo, en la naturaleza o en un ajuste experimental), o durante cualquier tiempo después de su producción inicial. Un microbio o una población microbiana puede considerarse purificada si se aísla en o después de la producción, como de un material o entorno que contiene el microbio o la población microbiana, y una población microbiana o microbiana purificada puede contener otros materiales de hasta aproximadamente 10 %, aproximadamente un 20 %, aproximadamente un 30 %, aproximadamente un 40 %, aproximadamente un 50 %, aproximadamente un 60 %, aproximadamente un 70 %,

aproximadamente un 80 %, aproximadamente un 90 % y aún se considera "aislado". En algunas realizaciones, se purifica microbios o población microbiana son más de aproximadamente 80 %, aproximadamente 85 %, aproximadamente 90 %, aproximadamente 91 %, aproximadamente 92 %, aproximadamente 93 %, aproximadamente 94 %, aproximadamente 95 %, aproximadamente 96 %, aproximadamente 97 %, aproximadamente 98 %, aproximadamente 99 %, o más de aproximadamente 99 % de pureza. En el caso de composiciones microbianas proporcionadas en la presente memoria, uno o más tipos microbianos presentes en la composición pueden purificarse independientemente de uno o más de otros microbios producidos y/o presentes en el material o entorno que contiene el tipo microbiano. Las composiciones microbianas y sus componentes microbianos generalmente se purifican a partir de productos de hábitat residuales.

Como se usa en este documento, un gen se "sobreexpresa" en una bacteria si se expresa a un nivel superior en una bacteria modificada genéticamente en al menos algunas condiciones que se expresa por una bacteria de tipo salvaje de la misma especie en las mismas condiciones. De forma similar, un gen está "subexpresionado" en una bacteria si se expresa en un nivel inferior en una bacteria modificada genéticamente en al menos algunas condiciones que las expresadas por una bacteria de tipo salvaje de la misma especie en las mismas condiciones.

Los términos "polinucleótido" y "ácido nucleico" se usan indistintamente. Se refieren a una forma polimérica de nucleótidos de cualquier longitud, ya sean desoxirribonucleótidos o ribonucleótidos, o análogos de los mismos. Los polinucleótidos pueden tener cualquier

estructura tridimensional y pueden realizar cualquier función. Los siguientes son ejemplos no limitantes de polinucleótidos: regiones codificantes o no codificantes de un gen o fragmento de gen, loci (locus) definido a partir del análisis de ligamiento, exones, intrones, ARN mensajero (ARNm), ARN de transferencia, ARN ribosómico, ribozimas, ADNc, polinucleótidos recombinantes, polinucleótidos ramificados, plásmidos, vectores, ADN aislado de cualquier secuencia, ARN aislado de cualquier secuencia, sondas de ácidos nucleicos y cebadores. Un polinucleótido puede comprender nucleótidos modificados, tales como nucleótidos metilados y análogos de nucleótidos. Si está presente, pueden impartirse modificaciones a la estructura de nucleótidos antes o después del ensamblaje del polímero. Un polinucleótido puede modificarse adicionalmente, tal como mediante conjugación con un componente de marcaje. En todas las secuencias de ácido nucleico proporcionadas en el presente documento, los nucleótidos U son intercambiables con nucleótidos T.

"Unidades taxonómicas operativas" y "OTU (s)" se refieren a una hoja terminal en un árbol filogenético y se definen por una secuencia de ácido nucleico, por ejemplo, el genoma completo o una secuencia genética específica, y todas las secuencias que comparten identidad de secuencia esta secuencia de ácido nucleico a nivel de especie. En algunas realizaciones, la secuencia genética específica puede ser la secuencia 16S o una porción de la secuencia 16S. En otras realizaciones, los genomas completos de dos entidades se secuencian y se comparan. En otra realización, se pueden comparar genéticamente regiones seleccionadas tales como etiquetas de secuencia multilocus (MLST), genes específicos o conjuntos de genes. Para 16S, las OTU que

comparten ≥ 97 % de identidad de nucleótidos promedio en todo el 16S o alguna región variable del 16S se consideran la misma OTU. Ver p. Claesson MJ, Wang Q, O'Sullivan O, Greene-Diniz R, Cole JR, Ross RP y O'Toole PW. 2010. Comparación de dos tecnologías de secuenciación de próxima generación para resolver la composición de microbiotas altamente complejas utilizando regiones de genes rRNA 16S en tándem variable. Nucleic Acids Res 38: e200. Konstantinidis KT, Ramette A y Tiedje JM. 2006. La definición de especie bacteriana en la era genómica. Philos Trans R Soc Lond B Biol Sci 361: 1929-1940. Para genomas completos, MLSTs, genes específicos, distintos de 16S, o conjuntos de genes OTUs que comparten ≥ 95 % de identidad de nucleótidos promedio se consideran la misma OTU. Véase, por ejemplo, Achtman M y Wagner M. 2008. Diversidad microbiana y la naturaleza genética de las especies microbianas. Nat. Rev. Microbiol. 6: 431-440. Konstantinidis KT, Ramette A y Tiedje JM. 2006. La definición de especie bacteriana en la era genómica. Philos Trans R Soc Lond B Biol Sci 361: 1929-1940. Las OTU se definen con frecuencia al comparar secuencias entre organismos. En general, las secuencias con una identidad de secuencia inferior al 95 % no se consideran parte de la misma OTU. Las OTU también se pueden caracterizar por cualquier combinación de marcadores de nucleótidos o genes, en particular genes altamente conservados (por ejemplo, genes de "mantenimiento"), o una combinación de los mismos. Las unidades taxonómicas operativas (OTU) con asignaciones taxonómicas realizadas a, por ejemplo, género, especie y clado filogenético se proporcionan en este documento.

Como se usa en el presente documento, "unión específica" se refiere a la capacidad de un anticuerpo para

unirse a un antígeno predeterminado o la capacidad de un polipéptido para unirse a su pareja de unión predeterminada. Típicamente, un anticuerpo o polipéptido se une específicamente a su antígeno predeterminado o pareja de unión con una afinidad correspondiente a una KD de aproximadamente 10-7 M o menos, y se une al antígeno/pareja de unión predeterminada con una afinidad (expresada por KD) que es al menos 10 veces menos, al menos 100 veces menos o al menos 1000 veces menor que su afinidad por unirse a un compañero antígeno/enlace no específico y no relacionado (por ejemplo, BSA, caseína). Alternativamente, la unión específica se aplica más ampliamente a un sistema de dos componentes donde un componente es una proteína, lípido o carbohidrato o una combinación de los mismos y se aplica al segundo componente que es una proteína, lípido, carbohidrato o combinación de los mismos de una manera específica.

Los términos "sujeto" o "paciente" se refieren a cualquier animal. Un sujeto o un paciente descrito como "en necesidad del mismo" se refiere a uno que necesita un tratamiento para una enfermedad. Los mamíferos (es decir, animales mamíferos) incluyen humanos, animales de laboratorio (por ejemplo, primates, ratas, ratones), ganado (por ejemplo, vacas, ovejas, cabras, cerdos) y mascotas domésticas (por ejemplo, perros, gatos, roedores). Por ejemplo, el sujeto puede ser un mamífero no humano que incluye, pero no está limitado a, un perro, un gato, una vaca, un caballo, un cerdo, un asno, una cabra, un camello, un ratón, una rata, una Guinea cerdo, oveja, llama, mono, gorila o chimpancé. El sujeto o paciente puede estar sano, o puede estar sufriendo de una neoplasia en cualquier etapa de desarrollo, donde cualquiera de las etapas sea causada

por o esté apoyado de manera oportunista por un patógeno causante o asociado con el cáncer, o puede estar en riesgo de desarrollar una neoplasia, o transmitir a otros un patógeno causante de cáncer o cáncer asociado. En algunas realizaciones, los pacientes tienen cáncer de pulmón, cáncer de vejiga, cáncer de próstata, cáncer de ovario y/o melanoma. Los pacientes pueden tener tumores que muestran una macropinocitosis mejorada con la genómica subyacente de este proceso, incluida la activación de Ras. En otras realizaciones, los pacientes sufren de otros cánceres. En algunas realizaciones, el sujeto ha sido sometido a una terapia contra el cáncer.

"Tensión" se refiere a un miembro de una especie bacteriana con una firma genética tal que se puede diferenciar de miembros estrechamente relacionados de la misma especie bacteriana. La firma genética puede ser la ausencia de todo o parte de al menos un gen, la ausencia de todo o parte de al menos en la región reguladora (por ejemplo, un promotor, un terminador, un riboswitch, un sitio de unión a ribosomas), la ausencia ("Curado") de al menos un plásmido nativo, la presencia de al menos un gen recombinante, la presencia de al menos un gen mutado, la presencia de al menos un gen extraño (un gen derivado de otra especie), la presencia al menos una región reguladora mutada (por ejemplo, un promotor, un terminador, un riboswitch, un sitio de unión a ribosoma), la presencia de al menos un plásmido no nativo, la presencia de al menos un casete de resistencia a antibióticos, o una combinación de los mismos. Las firmas genéticas entre diferentes cepas se pueden identificar mediante amplificación por PCR opcionalmente seguida por secuenciación de ADN de la (s) región (es) genómica (s) de interés o del genoma completo.

En el caso en que una cepa (en comparación con otra de la misma especie) ha ganado o perdido resistencia a los antibióticos o ganado o perdido una capacidad biosintética (como una cepa auxotrófica), las cepas pueden diferenciarse por selección o contraselección usando un antibiótico o nutriente/metabolito, respectivamente.

Como se usa en este documento, el término "tratar" una enfermedad en un sujeto o "tratar" a un sujeto que tiene o se sospecha que tiene una enfermedad se refiere a someter al sujeto a un tratamiento farmacéutico, por ejemplo, la administración de uno o más agentes, tales como al menos un síntoma de la enfermedad disminuye o se evita que empeore. Por lo tanto, en una realización, "tratar" se refiere, entre otros, a retrasar la progresión, acelerar la remisión, inducir la remisión, aumentar la remisión, acelerar la recuperación, aumentar la eficacia o disminuir la resistencia a terapias alternativas, o una combinación de los mismos. En ciertas realizaciones, un cáncer se trata si el sujeto experimenta una reducción en el tamaño tumoral, un número reducido de tumores, una reducción en el crecimiento tumoral, una reducción en la metástasis del cáncer y/o un número reducido de células cancerosas totales después del tratamiento de lo que sería esperado en ausencia de tratamiento.

Bacteria

En ciertos aspectos, se proporcionan en el presente documento composiciones bacterianas que comprenden *Bifidobacterium animalis ssp. lactis* (por ejemplo, una cantidad eficaz de *Bifidobacterium animalis ssp. lactis*) y métodos para usar una composición bacteriana que comprende *Bifidobacterium animalis ssp. lactis*. En algunas realizaciones, *Bifidobacterium animalis ssp. lactis* es la

cepa A de *Bifidobacterium animalis ssp. lactis* (número de depósito ATCC PTA-125097). En algunas realizaciones, la composición bacteriana comprende un PhAB hecho a partir de o que comprende un *Bifidobacterium animalis ssp. lactis* descrito en este documento. En algunas realizaciones, *Bifidobacterium animalis ssp. lactis* es una cepa que comprende al menos 99 % de identidad de secuencia, al menos 99,6 % de identidad de secuencia, al menos 99,6 % de identidad de secuencia, al menos 99,7 % de identidad de secuencia, al menos 99,8 % de identidad de secuencia, al menos 99,9 % de identidad de secuencia. la secuencia de nucleótidos de la cepa A de *Bifidobacterium animalis ssp. lactis*

Bajo los términos del Tratado de Budapest sobre el Reconocimiento Internacional del Depósito de Microorganismos para el Propósito del Procedimiento de Patente, el cepa A de *Bifidobacterium animalis ssp. lactis* se depositó el 27 de abril de 2018 en American Type Culture Collection (ATCC) de 10801 University Boulevard, Manassas, Virginia 20110-2209 USA y se le asignó el número de acceso ATCC PTA-125097.

El solicitante representa que la ATCC es un depósito que permite la permanencia del depósito y la accesibilidad inmediata del público si se concede una patente. Todas las restricciones sobre la disponibilidad para el público del material así depositado se eliminarán irrevocablemente al otorgar una patente. El material estará disponible durante la tramitación de la solicitud de patente a uno determinado por el Comisionado para tener derecho al mismo bajo 37 CFR 1,14 y 35 U.S.C. 122. El material depositado se mantendrá con todos los cuidados necesarios para mantenerlo viable y no contaminado durante un período de al menos cinco años

después de la última solicitud para el suministro de una muestra del plásmido depositado, y en cualquier caso, para un un período de al menos treinta (30) años después de la fecha de depósito o de la vigencia de la patente, el período que sea más largo. El solicitante reconoce su deber de reemplazar el depósito si el depositario no puede proporcionar una muestra cuando se solicita debido a la condición del depósito.

En algunas realizaciones, las bacterias descritas en el presente documentose modifican para mejorar la colonización y/o injerto en el tracto gastrointestinal de mamíferos (por ejemplo, metabolismo modificado, como degradación mejorada de mucina, perfil de competencia mejorado, motilidad incrementada, adhesión incrementada a células epiteliales intestinales, quimiotaxis modificada) En algunas realizaciones, las bacterias descritas en el presente documentose modifican para potenciar su efecto inmunomodulador y/o terapéutico (por ejemplo, solo o en combinación con otro agente terapéutico). En algunas realizaciones, las bacterias descritas en el presente documentose modifican para potenciar la activación inmune (por ejemplo, a través de la producción modificada de polisacáridos, pili, fimbrias, adhesinas, vesículas de membrana externa). En algunas realizaciones, las bacterias descritas en el presente documentose modifican para mejorar la fabricación bacteriana (por ejemplo, mayor tolerancia al oxígeno, tolerancia mejorada a la congelación-descongelación, tiempos de generación más cortos).

En ciertas realizaciones, *Bifidobacterium animalis ssp. lactis* se cultiva en medio de Clostridium reforzado (medio RCM) (ATCC 2107 o medio de otro fabricante), medio Lactobacilli MRS (medio LMRS) y/o agar Bifido.

Alternativamente, Bifidobactrium animalis ssp. Lactis se cultiva usando medio RCM ATCC 1053 y/o agar/caldo de soja Trypticase con sangre de oveja desfibrimada ATCC 260 según las instrucciones del fabricante.

La secuencia de nucleótidos de cepa A de *Bifidobacterium animalis ssp. lactis* se muestra en la Tabla 1.

Tabla 1: Secuencia de nucleótido de cepa A de *Bifidobacterium animalis ssp. lactis:*

Secuencia de nucleótido
>1 longitud 1944302 profundidad 1,00 xcircular= verdadero CAACGATGCCTCATCCACAGCAGCCAACTCAAAGACATCCTTACACACTAACCTGAGAA GAGCCAACAAA GGCATCCCATTGCAGCGCATCGCAGGCTTCCACGTCGACGGCGACGAGCAACGACTCGC CATGCTGCATC TGCGCGGCGAACTGTCGATCGACAGCAATATGAAACGCCTGCTGCTGATTCGTGCGAAC GAGCAGCTGGC AGGGGGCCGTATCACCCGACCGCGTAGTCTCAATGCGGTGCCGCTCATTCCCGTACGTA TACAGTCGGGG TCGGATTTTGCGGATTGGCCTGCCGGTCAGTGCGATTACTGGGCGGAGCGCATAGGCAA CTATGTGCTCA CCCAATCCACCCGCAAATCGATGGAGGGACTCGACGACTTCCAGACGCGACGAGCCCGC ATTCTGCGCAG CCCAAGCTCACGCCAGTTCCCGTTGACGCGCGTGATCGGCGAACTTGAGAAGCTGACGC CGCAGTTCCTG CAATTGCGGCAGCACCAGATCGTCGAGCTGATCGCACAGGCGTGGAACATCAATCCGGG GGAGCTGGGAT CCTTGGCAGAGCCGCAGGGGCAGTCCGATCGCACAGGCACATCCGCGCATCGCAGTCGA CGCACGTCGAA GCGTGTGACGGTGGGCGAAGTGGTGCGCGCCGGTCTGCTCACGGTGGGGGACCGATTCG TATGGAACCGC

CCACGCAAGCACGAGATCTGGCGTATCACCGTCACCGAGTCTGGGTTCCGGGGCGAGGA
CGGCACCGAAT
ATGCGACGCCGACGTCCGCCGCCCGCGCCATCGGAGGTTCCTCGGCGAGCCTGAACGTA
TGGAAGCGGGA
ATCCGACGGCCGTGCATTGAGCGATATCTGGAAGACCTATCGCACCTCGATGTGATGGT
CATGGATGTGA
CGGTCATGCGACGTCAGCCATTCGATGGCGAGCTTCGTGCGGTTCGAGATGTGCGTCTT
CGCCAGAATGC
CGCTGATGCGGTTGCGCACGGTGCCCTCGCTCAGGAACAGCTTCGCCGCAATCTCACGA
TTGTCGAGCTC
CTCGGCGACCAGCTCGACGATGTCATGCTCCCGGTCGGTGAGATGCGCGAATCTGGGAT
CATCCGGTAAT
GGGGACGCCTTTGGTTCCTCCACATTCCCGGACGCATCGTGCATCGGCAGGTTGCGCAG
CACGTCCGCGC
CCAACACCACCTGCCCGGCCATGACCGCCTGCACTGCTGGAATCACCGAAGCCACGTCC
TGTTTGATGAG
ATAGCCTTTCGTGCCCAATTCGATGGCACGGCGAATGTACTCCGGGTCGGCGAAAGTGG
TGAGCACGAGA
ATGCGCTCGCTGGCTTCAAGGCCGTCGGTGCCCGGCATCTGCACGTCGATGAGCAACAC
ATCCGGTTTCT
GACGTGCATAGGCCACCACGGCGGCGGCCCCGTCATGCGCAGTCCACACGACCTCGGCG
GTGCCGGTGGT
CGTGAGGATCGTGCCGATGGACGAGCAGACAATAGGGTCATCGTCAACAATCGCGACCT
TCATCGCATAT
TCCTTTCCGCTGTTTTCGGGACGGACATGAACACGCGCCAGCCGCCGTTATGGTAGCCG
CAGGTGGCTGT
GCCATCCAATGCGCGCGCCCGTTCCTCGATGTCGGCGATTCCAATGCCCATGCTCGCCA
CGCGGCCGCGT
TCCGGTACGCCGCCGTTGCCCTGCACGATCAGCTGCCACAACCCCGGCAAATCAATCAG
ATGCACGTGGG
CCTCAGAAGCTGCGCTATGCCGGATTGTGTTCGTCAACGCTCCGCGAATCACGGCCGTG
AATGCATGTGC

CACATGGGAAGGCACGGCATCGATGGCATTGGAGAGGCGAATCTCGACATCAGATTGCT
TGGCGGTGGCC
ATGGCATCCTCGACCATCGCCCGAAAATCAGTGCCTTCGTCTTTGAGACCATGCACGGA
TTTGCGAATCA
TCGTCATCGCCTCGTCGAGGGTATGACCAAGCTGCCCGAATTGATCGGCATGCAGCGTG
TCACCGGTGCT
GGTGGCAATCACTTGGTCCGCATTCGTCAGCATGATCGCACGAGTGAGCAGATGTCCCA
CATTGTCGTGG
ATCTCGCGTGCGATGCGTGTGCGTTCGGTGAGCCGCGCATGCTGCAGGTCCGCCGACCT
GGCGGATTCGA
CATCGGCGAGACGAGCGCGCGAACGGCGGATACGTTCCCGTTGGTCGTCTGCCAACGAT
TGGAAGCACCG
TTGTCTTCGTTGCGCGAGTGCGAATACCCAGCCGCCGAGAACTGCGAGGGAGGTGAGCG
CACATAGAATC
CAAAGGGCCATCACGAGCGGCGAGGTGGCTGCGGCTGACAGATCGTGCAATGCCGGACT
GTGCTGACCGG
CCACGATTCCGCAGACCAGTGGGGGGACGCAGACCAGCATTTGGAAAGACCTGCCCAAC
GACTGCACCCG
CAATCCCAGATCGAAGCCCACGATCGGCATCAGCGCGATCCATTGCGGAACAAACAGGC
AGACCATGCAG
AACATCATGGCGAGTGCGATCCAGCATCTGAGGGGAGCCAGACGGATCAACGAGCAGAG
GATCACGCAGA
ACAGCAGACCAATGACGAAGGTGGTATCGGTGTCGCCGCTCAGCATCACCGAGAACAGC
ACGCTGACGAC
GAACATCAGCGCATGAGCTCCCACGGAGATGATCATGCCGTTGTCTCTCCCCTCCTATT
CTGTTCCATTG
TTCATGACAACAGGGCGAATGTCATGCCGAATGGTGACAAATGTCACCATTCGTACAAA
AACGGTGATTC
CTGGCACCGTGTCGGCCGTTGCCTGTTCGGCAATATGGAGGCATGAACACAGCAGAGAA
CAACGTACAGC
GCGATGCCGCACCAGCGATCGAGGTGCGCGAACTGGTGAAACGATATGCCGACAAGCTC
GCGCTCGACTA

TTTGAACATGAACGTGCGCACTGGGGAGATTTACGGATTGCTGGGGCCGAACGGTTCCG
GCAAGACCACC
GCGATCAACTGCATTCTCGGGTTGCTCACCTATGATTCGGGCCAAATCCGTGCATTCGG
CGGCAAGATGG
GCCCCACCGCCTACGACCTGAAGCGCAGGATCGGCGTGGTGCCTCAGGACGTGGCTGTA
TTCAACGAGCT
CACCGTGCAGGAGAACATCGAGTACTTCTGCTCGCTGTACGTCGCTGACAAGATGAGAC
GTGCGCGGCTG
GTGGACGAAGCAATCGAATTCGTGGGACTCGAGGATTACCGCAGATTCCGCCCGCGCAA
GCTCTCAGGAG
GTCTGCCGCGCCGATTGAACATTGCATGCGGCATAGTGCACCAACCCGAACCCATCTTC
TTCGACGAGCC
GACGGTGGCGGTGGATCCGCAAAGCCGCAACTCGATTCTCGAAGGCATCAAACGGCTCA
ACGAGCGTGGC
GCGACCGTCGTGTACACGAGCCATTACATGGAGGAGGTCGAGCAGATCTGCGACCGCAT
CCAGATCATAG
ATCACGGCCGTCAGATCGCCATCGGCACGGCCGACGAGCTCAAGCGCATGATCGAGGCC
GGCGAGCGAAT
CACCGTCGAAGCGCCGGCGTTGAGCGCGAATGAGGCCCTGTGCCAACGGGCCGTCTCGA
TGTTGGGTGAC
TTGGAGGCTGTGCACGAGGTGACGTTCGAGAACGACGAGCTCGTGGTGGTCTGTTCGCA
GGGCGAGCACA
ATCTCATCGATGTGCTGCAGGTGCTGCGCTCGCTCGACATCCCCATGGGGCATGTGAGC
TCCCGTGCGCC
GACGTTGAACGACGTGTTCCTCGCCCTCACCGGGACCGACCTGCGCGATTGAGCGTGCG
GCACATACGAC
GAAGGAGGTGAATACATGTGGAGTACATTCCTCAACACCATGCGCGTGAATCTGCGTGA
GAAGAGTTCGG
TGTTCTGGCTCTTCTGCTTCCCGATCATTCTGTCGACGATGTTCATGGGCATGTTCGGC
AACATCGGCGA
GGTCTATGAAATACATACGATGAGGTTCGCAGTGGTCGCGGACGCGAACTTCTGCAGGG
CGACCAACGCG

CGTCAGATGCTGCATGCGATGCAGCGGAATCCACTGCGGAACGAGGGCTGCGGGAACGC
CATGGAAGGCG
CTGCGCCCGATACGGCGTCCGGGCAGGTGGGTTCCGAGCTCAGGAACCTGTTGGAATTG
CAGGCCGTGTC
AAACGTCAAGGAGGCCAAGGGCCTCATCAACGCCGACCACGGGGTGCGTGGCTACATCA
ACGCCACGGAA
GACGGGCTGCTGAACCTGACGATCAGTCGTGCCAGCGTCGCATCCGTCAACGATGACAC
CGGAAATTCCG
GCTTCAGTGCATCCCTCAACATTCTGCATGGTGCCGTAGGCATGTTCAATAGGCGAGCC
ATCACCGTGAC
CGAGCTGCTCAACACCGATTCGGGCGTGTTCCGTGATGCCACGTTCACCAAGGAGATGG
GCGACACCCCG
ACGTTCACGAAAGTTGTGAGCCCCACCCATTTCAAGCCGACCGAATCCGTCCGATTCTA
CTATGCGCTGC
TCGCCATGACCGCACTCATGTCGATGACCTTCGCCACCACGACCGTGTGCGCCACGCAG
GCGAATCTTTC
GGCGCTTGGTATGAGACGGTCGCTGGCCCCGCTCTCCAAGTTCAACCAGCTCATGGGAG
GTTTCCTCGCC
AGCTGGCTATGCGCATCGGTGTCGATGGTGGTTGCGACGCTCTACGTCCATTTCGTGTG
CAAGGTCGATT
TCGGCGGCCGTTGGCCCGTGCTCGTCCTGGCGCTGCTGCTCGCGTCCCTTACCGCCAAT
GCGCTCGGCAC
GCTCATAGGATCGTTGCCGAAGCTCACTTCCGGCGTGAAAATAGGCATCACCACCGCGC
TGTCGTGCGTG
CTCTCGATATTCAGTGGACTCTACGGCACCGGCGCAATGAACCTGAGCGACTGGACCCA
ACGCAACGCCC
CCGGATTCGCCATCGTCAATCCCACCCAGCAGATCACGAACCTGTTCTACGACTTGCTC
CACTACGATTC
CTACGCCCCGTCCCTACGCACGTCGGGCATTCTCCTCGCAATGACCGTGCTCGCTCTGA
CACTGTCCACG
ATCTTCCTCAGGAGGCAACGCTATGAGCACCTTTAGAACATGCTGGCGAGTCCTGTTCG
CCCAACGAACC

ATGATCCTCGTCTACATCGTCTACCTGAGCCTCATGATGTTCGGGCTGTCCTGGAGCAT
CATCCAGAACC
TTTCGAACACCGACAACTCCACCACCTATGAGACGCTGCGACCCGAAGTGGCGGTGGTC
GACCGGAATGG
GGGCGTCGGCTCAGGGTTCTCGGATTCGCTGAGGAAATTCCTGTCCAAGGACTGCGACC
TCGTCGATGTG
GGCACCACCTCCCAGGACCTGCAGACCGCTGCGGCCTCAAACTATGCCGACCTCATCGT
CATCATTCCCG
AACACTATGTCGATGACCTGGCTGCGGCATTCACCTCGGATTCCGCCGTCCCCGAGCTG
CAGATGGTCAC
CAGCTTCACCGGAGCCTACGGCTCCCTCGCCAAACTGCAGGTGGAGGATTTCCTGAACC
TCACCCGGATG
ACTGCGCTTGCAGATGCAGCGTCGTCGGGTTCCTCCGTTAGCGTCGCCTCATTGAATGC
GGCGGCCGGTT
CGGTGATCGCGGATCTTACCGGCAATGCCGACGCCTACCCGCAGATCGCCATCGCCGAG
GACCCGGCAGG
GACCGACGACCGGGAGGCGAACGGACGGTACGCGTTCCAGACGATGATGGACATGGGAG
CCTATCCGATG
GTCGCGGCAATGTTCACGATCACCGCAATGACGATGGGATCGTTCTCGGGGGCAAGGGT
ACGCAGACGCA
TGTATGCCTCACCGCAACGCACCGGTTCGATGCTGTTCCAGCAGTTCGCCTGTTGTGGC
TTGTTCGGTGT
GCTCGTGAGCCTGTTCTATCTGGCGCTCGCCCTGGCTCTTCCGGTGGCGGCCGGCTTGC
CGATCACCGGC
GTCGACCCGAGGGGATTCCTACTGTGCGCATTGACGATGGTCGCCTATAGTCTCACGGC
CGCGGCCAGTG
GCTTCATGGTGAGCATGATCGCCGTCAACAGTGCGGCAATCAACGCGATCGCCAATGTG
TATGGTCTGGT
CATCATGTTCACCTCGGGCATGGCGTTCCCGGTGGATCTGATGCCCAAGGTGATGATCG
TCATAGGCAAA
TGCACCCCGGGTTGGTGGTTCTGCCGGTCGATCAGCGCCGCGATGAATTCCGAAGGCAC
CGTGAGCGTAT

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Composiciones Bacterianas

En ciertos aspectos, se proporcionan en el presente documento composiciones bacterianas que comprenden *Bifidobacterium animalis ssp. lactis*. En algunas realizaciones, *Bifidobacterium animalis ssp. lactis* es la cepa A de *Bifidobacterium animalis ssp. lactis* (número de depósito ATCC PTA-125097). En algunas realizaciones, la composición bacteriana comprende un PhAB hecho a partir de o que comprende un *Bifidobacterium animalis ssp. lactis* descrito en este documento. En algunas realizaciones, *Bifidobacterium animalis ssp. lactis* es una cepa que comprende al menos 99 % de identidad de secuencia, al menos 99,6 % de identidad de secuencia, al menos 99,6 % de identidad de secuencia, al menos 99,7 % de identidad de secuencia, al menos 99,8 % de identidad de secuencia, al menos 99,9 % de identidad de secuencia. la secuencia de nucleótidos de la cepa A de *Bifidobacterium animalis ssp. lactis*. En algunas realizaciones, la formulación bacteriana comprende una bacteria y/o una combinación de bacterias descritas en el presente documento y un vehículo

farmacéuticamente aceptable.

En ciertas realizaciones, al menos 50 %, 55 %, 60 %, 65 %, 70 %, 75 %, 80 %, 85 %, 90 %, 91 %, 92 %, 93 %, 94 %, 95 %, 96 %, 97 %, 98 % o 99 % de las bacterias en la composición bacteriana son *Bifidobacterium animalis ssp. lactis*. En ciertas realizaciones, sustancialmente todas las bacterias en la composición bacteriana son *Bifidobacterium animalis ssp. lactis*. En ciertas realizaciones, la composición bacteriana comprende al menos 1x103 unidades formadoras de colonias (UFC), 1x104 unidades formadoras de colonias (UFC), $1x10^5$ unidades formadoras de colonias (UFC), $5x10^5$ unidades formadoras de colonias (UFC), 1 x 10 unidades formadoras de colonias (UFC), 2 x 10 unidades formadoras de colonias (UFC), 3 x 10 unidades formadoras de colonias (UFC), 4 x 10 unidades formadoras de colonias (UFC), 5 x 10 unidades formadoras de colonias (UFC) 6 x 10 unidades formadoras de colonias (UFC), $7x10^6$ unidades formadoras de colonias (UFC), $8x10^6$ unidades formadoras de colonias (UFC), $9x10^6$ unidades formadoras de colonias (UFC), $1x10^7$ unidades formadoras de colonias (UFC), 2 $x10^7$ unidades formadoras de colonias (UFC), 3 $x10^7$ unidades formadoras de colonias (UFC), 4 $x10^7$ unidades formadoras de colonias (UFC), 5 $x10^7$ unidades formadoras de colonias (UFC), 6 $x10^7$ unidades formadoras de colonias (UFC) 7 $x10^7$ unidades formadoras de colonias (UFC), 8 $x10^7$ unidades formadoras de colonias (UFC), 9 $x10^7$ unidades formadoras de colonias (UFC), 1 $x10^8$ unidades formadoras de colonias (UFC), 2 $x10^8$ unidades formadoras de colonias (UFC) 3 $x10^8$ unidades formadoras de colonias (UFC), 4 x 10 8 unidades formadoras de colonias (UFC), 5 $x10^8$ unidades formadoras de colonias (UFC), 6 $x10^8$ unidades formadoras de colonias (UFC), 7 $x10^8$ unidades formadoras de colonias (UFC), 8 $x10^8$

unidades formadoras de colonias (UFC), 9 10^8 unidades formadoras de colonias (UFC), 1 x10^9 unidades formadoras de colonias (UFC), 5 x10^9 unidades formadoras de colonias (UFC), 1 x 10^{10} unidades formadoras de colonias (UFC) 5 x 10^{10} unidades formadoras de colonias (UFC), 1 x 10^{11} unidades formadoras de colonias (UFC) 5 x 10^{11} unidades formadoras de colonias (UFC), 1 x 10^{12} unidades formadoras de colonias (UFC) 5 x 10^{12} unidades formadoras de colonias (UFC), 1 x 10^{13} unidades formadoras de colonias (UFC) de *Bifidobacterium animalis ssp. lactis.*

En algunas realizaciones, las formulaciones probióticas que contienen Bifidobacterium animalis ssp. las lactis se proporcionan como formas encapsuladas, con recubrimiento entérico, o en polvo, con dosis que varían hasta 10^{11} ufc (por ejemplo, hasta 10^{10} ufc). En algunas realizaciones, la composición comprende 5 x 10^{11} UFC de *Bifidobacterium animalis ssp. lactis* y almidón de maíz al 10 % (p/p) en una cápsula. La cápsula está recubierta entéricamente para la liberación duodenal a pH 5,5. En algunas realizaciones, la cápsula está recubierta entéricamente para liberación duodenal a pH 5,5. En algunas realizaciones, la composición comprende un polvo de *Bifidobacterium animalis ssp. lactis* liofilizado, que se considera estado de "Presunción de seguridad calificada" (QPS). En algunas realizaciones, la composición es estable a temperatura congelada o refrigerada.

Los métodos para producir composiciones microbianas pueden incluir tres etapas principales de procesamiento. Los pasos son: banca de organismos, producción de organismos y preservación. En ciertas realizaciones, una muestra que contiene una abundancia de *Bifidobacterium animalis ssp. lactis* se puede cultivar evitando un paso de

aislamiento.

Para la banca, las cepas incluidas en la composición microbiana pueden (1) aislarse directamente de una muestra o tomarse de una población almacenada, (2) cultivarse opcionalmente en un agar nutritivo o caldo que soporte el crecimiento para generar biomasa viable, y (3) la biomasa se conserva opcionalmente en alícuotas múltiples en almacenamiento a largo plazo.

En realizaciones que usan una etapa de cultivo, el agar o el caldo pueden contener nutrientes que proporcionan elementos esenciales y factores específicos que permiten el crecimiento. Un ejemplo sería un medio compuesto por 20 g/l de glucosa, 10 g/l de extracto de levadura, 10 g/l de peptona de soja, 2 g/l de ácido cítrico, 1,5 g/l de fosfato de sodio monobásico, 100 mg/l de citrato de amonio férrico, 80 mg/l de sulfato de magnesio, 10 mg/l de cloruro de hemina, 2 mg/l de cloruro de calcio, 1 mg/l de menadiona. Otro ejemplo sería un medio compuesto por 10 g/l de extracto de carne, 10 g/l de peptona, 5 g/l de cloruro de sodio, 5 g/l de dextrosa, 3 g/l de extracto de levadura, 3 g/l de acetato de sodio, 1 g/l de almidón soluble, y 0,5 g/l de L-cisteína HCl, a pH 6,8. Una variedad de medios microbiológicos y variaciones son bien conocidos en la técnica (por ejemplo, R.M. Atlas, Handbook of Microbiological Media (2010) CRC Press). Los medios de cultivo pueden agregarse al cultivo al comienzo, pueden agregarse durante el cultivo o pueden fluir de forma intermitente/continua a través del cultivo. Las cepas en la composición bacteriana se pueden cultivar solas, como un subconjunto de la composición microbiana, o como una colección completa que comprende la composición microbiana. Como ejemplo, se puede cultivar una primera cepa junto con

una segunda cepa en un cultivo continuo mixto, a una velocidad de dilución menor que la tasa de crecimiento máxima de cualquiera de las células para evitar que el cultivo se elimine del cultivo.

El cultivo inoculado se incuba en condiciones favorables durante un tiempo suficiente para construir biomasa. Para composiciones microbianas para uso humano, esto a menudo está a una temperatura de 37 °C, pH y otros parámetros con valores similares al nicho humano normal. El entorno puede controlarse activamente, controlarse pasivamente (por ejemplo, a través de memorias intermedias) o dejarse derivar. Por ejemplo, para composiciones bacterianas anaeróbicas, se puede emplear un entorno anóxico/reductor. Esto se puede lograr mediante la adición de agentes reductores tales como cisteína al caldo y/o quitándole oxígeno. Como ejemplo, puede cultivarse un cultivo de una composición bacteriana a 37 °C, pH 7, en el medio anterior, prerreducido con 1 g/l de cisteína-HCl.

Cuando el cultivo ha generado suficiente biomasa, puede conservarse para la banca. Los organismos pueden colocarse en un medio químico que proteja de la congelación (agregando "crioprotectores"), secado ("lioprotectores") y/o choque osmótico ("osmoprotectores"), distribuyéndolos en contenedores múltiples (opcionalmente idénticos) para crear un uniforme banco, y luego tratar la cultura para la preservación. Los contenedores generalmente son impermeables y tienen cierres que aseguran el aislamiento del medio ambiente. El tratamiento de crioconservación se logra congelando un líquido a temperaturas ultrabajas (por ejemplo, ao por debajo de -80 °C). La conservación seca elimina el agua del cultivo por evaporación (en el caso de secado por pulverización o "secado en frío") o por

sublimación (por ejemplo, para liofilización, secado por congelación por pulverización). La eliminación de agua mejora la estabilidad de almacenamiento de la composición microbiana a largo plazo a temperaturas elevadas por encima de las condiciones criogénicas. Si la composición microbiana comprende, por ejemplo, especies formadoras de esporas y da como resultado la producción de esporas, la composición final puede purificarse por medios adicionales tales como centrifugación en gradiente de densidad y conservarse usando las técnicas [\ alpha] descritas anteriormente [\ alpha]. La banca de composición microbiana se puede realizar cultivando y preservando las cepas individualmente, o mezclando las cepas para crear un banco combinado. Como un ejemplo de crioconservación, un cultivo de composición microbiana se puede recoger mediante centrifugación para sedimentar las células del medio de cultivo, el sobrenadante se decanta y se reemplaza con caldo de cultivo nuevo que contiene 15 % de glicerol. El cultivo se puede alicuotar en criotubos de 1 ml, se sella y se coloca a -80 °C para la retención de la viabilidad a largo plazo. Este procedimiento logra una viabilidad aceptable al recuperarse del almacenamiento congelado.

La producción microbiana puede realizarse usando etapas de cultivo similares a la banca, incluidas las condiciones de composición y cultivo del medio descritas anteriormente. Puede realizarse a mayores escalas de operación, especialmente para desarrollo clínico o producción comercial. En escalas mayores, puede haber varios subcultivos de la composición microbiana antes del cultivo final. Al final del cultivo, el cultivo se cosecha para permitir una formulación adicional en una forma de dosificación para administración. Esto puede implicar

concentración, eliminación de componentes del medio indeseables y/o introducción en un entorno químico que preserve la composición microbiana y la haga aceptable para la administración a través de la ruta elegida. Por ejemplo, una composición microbiana se puede cultivar a una concentración de 10^{10} UFC/ml, luego se concentra 20 veces mediante microfiltración de flujo tangencial; el medio gastado puede intercambiarse por diafiltración con un medio conservante que consiste en 2 % de gelatina, 100 mM de trehalosa y 10 mM de tampón de fosfato de sodio. La suspensión se puede liofilizar a un polvo y valorar.

Después de secar, el polvo se puede mezclar a una potencia apropiada, y mezclarlo con otros cultivos y/o una carga tal como celulosa microcristalina para lograr consistencia y facilidad de manipulación, y la composición bacteriana formulada como se proporciona en este documento.

En ciertos aspectos, se proporcionan composiciones bacterianas para sujetos de administración. En algunas realizaciones, las composiciones bacterianas se combinan con materiales activos y/o inactivos adicionales para producir un producto final, que puede estar en una sola unidad de dosificación o en un formato de múltiples dosis.

En algunas realizaciones, la composición comprende al menos un hidrato de carbono. Un "carbohidrato" se refiere a un azúcar o polímero de azúcares. Los términos "sacárido", "polisacárido", "carbohidrato" y "oligosacárido" se pueden usar indistintamente. La mayoría de los carbohidratos son aldehídos o cetonas con muchos grupos hidroxilo, generalmente uno en cada átomo de carbono de la molécula. Los carbohidratos generalmente tienen la fórmula molecular CnH2nOn. Un carbohidrato puede ser un monosacárido, un disacárido, trisacárido, oligosacárido o polisacárido. El

carbohidrato más básico es un monosacárido, como glucosa, sacarosa, galactosa, manosa, ribosa, arabinosa, xilosa y fructosa. Los disacáridos son dos monosacáridos unidos. Los disacáridos ejemplares incluyen sacarosa, maltosa, celobiosa y lactosa. Típicamente, un oligosacárido incluye entre tres y seis unidades de monosacáridos (por ejemplo, rafinosa, estaquiosa), y los polisacáridos incluyen seis o más unidades de monosacáridos. Polisacáridos ejemplares incluyen almidón, glucógeno y celulosa. Los carbohidratos pueden contener unidades sacáridas modificadas tales como 2'-desoxirribosa en donde se elimina un grupo hidroxilo, 2'-fluororibosa en donde un grupo hidroxilo se reemplaza por un flúor, o N-acetilglucosamina, una forma de glucosa que contiene nitrógeno (por ejemplo, 2 ' -fluororibosa, desoxirribosa y hexosa). Los carbohidratos pueden existir en muchas formas diferentes, por ejemplo, confórmeros, formas cíclicas, formas acíclicas, estereoisómeros, tautómeros, anómeros e isómeros.

En algunas realizaciones, la composición comprende al menos un lípido. Tal como se usa en el presente documento, un "lípido" incluye grasas, aceites, triglicéridos, colesterol, fosfolípidos, ácidos grasos en cualquier forma que incluye ácidos grasos libres. Las grasas, los aceites y los ácidos grasos pueden ser saturados, insaturados (cis o trans) o parcialmente insaturados (cis o trans). En algunas realizaciones, el lípido comprende al menos un ácido graso seleccionado 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 linolénico (18: 3), ácido octadecatetraenoico (18: 4), ácido aren el presente

documentodico (20: 0), ácido eicosenoico (20: 1), ácido eicosadienoico (20: 2), ácido eicosatetraenoico (20: 4), ácido eicosapentaenoico (20: 5) (EPA), ácido docosanoico (22: 0), ácido docosenoico (22: 1), ácido docosapentaenoico (22: 5), ácido docosahexaenoico (22: 6) (DHA) y ácido tetracosanoico (24: 0). En algunas realizaciones, la composición comprende al menos un lípido modificado, por ejemplo, un lípido que se ha modificado por cocción.

En algunas realizaciones, la composición comprende al menos una fuente mineral o mineral suplementaria. Los ejemplos de minerales incluyen, sin limitación: cloruro, sodio, calcio, hierro, cromo, cobre, yodo, zinc, magnesio, manganeso, molibdeno, fósforo, potasio y selenio. Las formas adecuadas de cualquiera de los minerales anteriores incluyen sales minerales solubles, sales minerales poco solubles, sales minerales insolubles, minerales quelados, complejos minerales, minerales no reactivos tales como minerales de carbonilo y minerales reducidos, y combinaciones de los mismos.

En algunas realizaciones, la composición comprende al menos una vitamina suplementaria. La al menos una vitamina puede ser soluble en grasas o vitaminas solubles en agua. Las vitaminas adecuadas incluyen, pero no se limitan a, vitamina C, vitamina A, vitamina E, vitamina B12, vitamina K, riboflavina, niacina, vitamina D, vitamina B6, ácido fólico, piridoxina, tiamina, ácido pantoténico y biotina. Las formas adecuadas de cualquiera de los anteriores son sales de la vitamina, derivados de la vitamina, compuestos que tienen la misma o similar actividad de la vitamina y metabolitos de la vitamina.

En algunas realizaciones, la composición comprende un excipiente. Los ejemplos no limitantes de excipientes

adecuados incluyen un agente tamponante, un conservante, un estabilizante, un aglutinante, un agente de compactación, un lubricante, un potenciador de la dispersión, un agente de desintegración, un agente aromatizante, un edulcorante y un agente colorante.

En algunas realizaciones, el excipiente es un agente tamponante. Los ejemplos no limitantes de agentes tamponantes adecuados incluyen citrato de sodio, carbonato de magnesio, bicarbonato de magnesio, carbonato de calcio y bicarbonato de calcio.

En algunas realizaciones, el excipiente comprende un conservante. Los ejemplos no limitantes de conservantes adecuados incluyen antioxidantes, tales como alfa-tocoferol y ascorbato, y antimicrobianos, tales como parabenos, clorobutanol y fenol.

En algunas realizaciones, la composición comprende un aglutinante como un excipiente. Ejemplos no limitantes de aglutinantes adecuados incluyen almidones, almidones pregelatinizados, gelatina, polivinilpirrolidona, celulosa, metilcelulosa, carboximetilcelulosa sódica, etilcelulosa, poliacrilamidas, poliviniloxazolidona, poli (alcoholes vinílicos), alcohol de ácidos grasos C12-C18, polietilenglicol, polioles, sacáridos, oligosacáridos y combinaciones en esto.

En algunas realizaciones, la composición comprende un lubricante como un excipiente. Los ejemplos no limitantes de lubricantes adecuados incluyen estearato de magnesio, estearato de calcio, estearato de zinc, aceites vegetales hidrogenados, sterotex, monoestearato de polioxietileno, talco, polietilenglicol, benzoato de sodio, lauril sulfato de sodio, lauril sulfato de magnesio y aceite mineral ligero.

En algunas realizaciones, la composición comprende un potenciador de la dispersión como un excipiente. Los ejemplos no limitantes de dispersantes adecuados incluyen almidón, ácido algínico, polivinilpirrolidonas, goma guar, caolín, bentonita, celulosa de madera purificada, glicolato de almidón sódico, silicato isoamorfo y celulosa microcristalina como tensioactivos emulsionantes de alto HLB.

En algunas realizaciones, la composición comprende un desintegrante como un excipiente. En algunas realizaciones, el desintegrante es un desintegrante no efervescente. Ejemplos no limitativos de desintegrantes no efervescentes adecuados incluyen almidones tales como almidón de maíz, almidón de patata, almidones pregelatinizados y modificados de los mismos, edulcorantes, arcillas, tales como bentonita, celulosa microcristalina, alginatos, glicolato de almidón sódico, gomas tales como agar, guar, algarrobo, karaya, pectina y tragacanto. En algunas realizaciones, el desintegrante es un desintegrante efervescente. Los ejemplos no limitantes de desintegrantes efervescentes adecuados incluyen bicarbonato sódico en combinación con ácido cítrico y bicarbonato sódico en combinación con ácido tartárico.

En algunas realizaciones, la formulación bacteriana comprende un recubrimiento entérico o microencapsulación. En ciertas realizaciones, el recubrimiento entérico o la microencapsulación mejora el direccionamiento a una región deseada del tracto gastrointestinal. Por ejemplo, en ciertas realizaciones, la composición bacteriana comprende un recubrimiento entérico y/o microcápsulas que se disuelve a un pH asociado con una región particular del tracto gastrointestinal. En algunas realizaciones, el

recubrimiento entérico y/o microcápsulas se disuelven a un pH de aproximadamente 5,5 - 6,2 para liberar en el duodeno, a un valor de pH de aproximadamente 7,2 - 7,5 para liberar en el íleon, y/o a un valor de pH de aproximadamente 5,6 - 6,2 para liberar en el colon. Los ejemplos de recubrimientos entéricos y microcápsulas se describen, por ejemplo, en la patente U.S. Pub. No. 2016/0022592, que se incorpora en el presente documentopor referencia en su totalidad.

En algunas realizaciones, la composición es un producto alimenticio (por ejemplo, un alimento o bebida) tal como un alimento o bebida saludable, un alimento o bebida para bebés, un alimento o bebida para mujeres embarazadas, atletas, personas mayores u otro grupo específico, un alimento funcional, una bebida, un alimento o bebida para un uso específico de la salud, un suplemento dietético, un alimento o bebida para pacientes o un alimento para animales. Los ejemplos específicos de alimentos y bebidas incluyen diversas bebidas como jugos, bebidas refrescantes, bebidas de té, preparaciones para bebidas, bebidas de gelatina y bebidas funcionales; bebidas alcohólicas tales como cervezas; alimentos que contienen carbohidratos tales como productos alimenticios de arroz, fideos, panes y pastas; pegar productos como jamones de pescado, salchichas, productos de pasta de mariscos; productos de bolsas de retorta como el curry, comida aderezada con gruesas salsas amiláceas y sopas chinas; sopas; productos lácteos tales como leche, bebidas lácteas, helados, quesos y yogures; productos fermentados tales como pastas fermentadas de soja, yogures, bebidas fermentadas y encurtidos; productos de frijol; varios productos de confitería, incluyendo galletas, galletas, y similares,

dulces, gomas de mascar, gomitas, postres fríos, incluyendo gelatinas, caramelos de crema y postres helados; alimentos instantáneos como sopas instantáneas y sopas instantáneas de soja; alimentos para microondas; y similares. Además, los ejemplos también incluyen alimentos y bebidas saludables preparados en forma de polvos, gránulos, tabletas, cápsulas, líquidos, pastas y gelatinas.

En ciertas realizaciones, las bacterias descritas en el presente documentose administran junto con un prebiótico para el sujeto. Los prebióticos son carbohidratos que generalmente no son digeribles por un animal huésped y son fermentados o metabolizados selectivamente por bacterias. Los prebióticos pueden ser carbohidratos de cadena corta (por ejemplo, oligosacáridos) y/o azúcares simples (por ejemplo, mono y disacáridos) y/o mucinas (proteínas fuertemente glucosiladas) que alteran la composición o el metabolismo de un microbioma en el huésped. Los carbohidratos de cadena corta también se denominan oligosacáridos, y normalmente contienen de 2 o 3 y hasta 8, 9, 10, 15 o más fracciones de azúcar. Cuando los prebióticos se introducen en un huésped, los prebióticos afectan a las bacterias dentro del huésped y no afectan directamente al huésped. En ciertos aspectos, una composición prebiótica puede estimular selectivamente el crecimiento y/o la actividad de uno de un número limitado de bacterias en un huésped. Los prebióticos incluyen oligosacáridos como fructooligosacáridos (FOS) (incluida la inulina), galactooligosacáridos (GOS), trans-galactooligosacáridos, xilooligosacáridos (XOS), quitooligosacáridos (COS), oligosacáridos de soja (por ejemplo, estaquiosa y rafinosa) gentiooligosacáridos, isomaltooligosacáridos, mannooligosacáridos,

maltooligosacáridos y mananoligosacáridos. Los oligosacáridos no son necesariamente componentes individuales, y pueden ser mezclas que contienen oligosacáridos con diferentes grados de oligomerización, que a veces incluyen el disacárido original y los azúcares monoméricos. Varios tipos de oligosacáridos se encuentran como componentes naturales en muchos alimentos comunes, como frutas, verduras, leche y miel. Ejemplos específicos de oligosacáridos son lactulosa, lactosacarosa, palatinosa, glicosil sacarosa, goma guar, goma arábiga, tagalosa, amilosa, amilopectina, pectina, xilano y ciclodextrinas. Los prebióticos también pueden ser purificados o sintetizados química o enzimáticamente.

Producción de PhABs

En ciertos aspectos, los PhAB descritos en el presente documentopueden prepararse usando cualquier método conocido en la técnica.

En algunas realizaciones, los PhAB descritos en el presente documentose preparan por fraccionamiento. Las células bacterianas y/o sobrenadantes de células de bacterias cultivadas se fraccionan en diversas biomasas farmacológicamente activas (PhAB) y/o productos derivados de las mismas. Las células bacterianas y/o sobrenadantes se fraccionan usando materiales y métodos conocidos en la técnica (véase, por ejemplo, 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: 3271-3279; Thein et al. Efficient subfractionation of gram-negative bacteria for proteomics studies. 2010. Am Chem Society. 9: 6135-6147; Hobb et al.

Evaluation of procedures for outer membrane isolation from Campylobacter jejuni. 2009. 155(Pt. 3): 979-988).

Adicionalmente, los PhAB obtenidos mediante los métodos proporcionados en la presente pueden purificarse adicionalmente mediante cromatografía en columna basada en el tamaño, mediante cromatografía de afinidad, y mediante ultracentrifugación en gradiente, usando métodos que pueden incluir, pero no se limitan a, uso de un gradiente de sacarosa o gradiente Optiprep. Brevemente, usando un método de gradiente de sacarosa, si se usó precipitación con sulfato de amonio o ultracentrifugación para concentrar los sobrenadantes filtrados, los sedimentos se resuspenden en 60 % de sacarosa, 30 mM de Tris, pH 8,0. Si se usó filtración para concentrar el sobrenadante filtrado, el concentrado se intercambió con tampón en sacarosa al 60 %, Tris 30 mM, pH 8,0, usando una columna Amicon Ultra. Las muestras se aplican a un gradiente de sacarosa discontinuo del 35-60 % y se centrifugan a 200,000 x g durante 3-24 horas a 4 °C. Brevemente, usando un método de gradiente Optiprep, si se usó precipitación con sulfato amónico o ultracentrifugación para concentrar los sobrenadantes filtrados, los sedimentos se resuspenden en Optiprep al 35 % en PBS. En algunas realizaciones, si se usó filtración para concentrar el sobrenadante filtrado, el concentrado se diluye usando 60 % de Optiprep hasta una concentración final de Optiprep de 35 %. Las muestras se aplican a un gradiente de sacarosa discontinuo del 35-60 % y se centrifugan a 200,000 x g durante 3-24 horas a 4 °C.

En algunas realizaciones, para confirmar la esterilidad y el aislamiento de las preparaciones de PhAB, los PhAB se diluyen en serie en un medio de agar usado para el cultivo de rutina de las bacterias que se están

ensayando, y se incuban usando condiciones de rutina. Las preparaciones no estériles se pasan a través de un filtro de 0,22 µm para excluir las células intactas. Para aumentar aún más la pureza, los PhAB aislados pueden tratarse con ADNasa o proteinasa K.

En algunas realizaciones, para la preparación de PhABs usados para inyecciones *in vivo*, los PhAB purificados se procesan como se describió previamente (G. Norheim, et al. *PLoS ONE*. 10(9): e0134353 (2015). Brevemente, después de la centrifugación en gradiente de sacarosa, las bandas que contienen PhAB se resuspenden hasta una concentración final de 50 µg/ml en una solución que contiene 3 % de sacarosa u otra solución adecuada para inyección *in vivo* conocida por los expertos en la técnica. Esta solución también puede contener adyuvante, por ejemplo hidróxido de aluminio a una concentración de 0-0,5 % (p/v).

En ciertas realizaciones, para hacer muestras compatibles con pruebas adicionales (por ejemplo, para eliminar sacarosa antes de la formación de imágenes TEM o ensayos *in vitro*), las muestras se intercambian en tampón en PBS o Tris 30 mM, pH 8,0 usando filtración (por ejemplo columnas Amicon Ultra), diálisis, o ultracentrifugación (200,000 xg, ≥ 3 horas, 4°C) y resuspensión.

En algunas realizaciones, la esterilidad de las preparaciones de PhAB puede confirmarse plaqueando una porción de los PhAB en un medio de agar usado para el cultivo estándar de las bacterias usadas en la generación de los PhAB e incubando usando condiciones estándar.

En algunas realizaciones, los PhAB seleccionados se aíslan y se enriquecen mediante cromatografía y restos de superficie de unión en PhAB. En otras realizaciones, los PhAB seleccionados se aíslan y/o se enriquecen mediante

clasificación de células fluorescentes mediante métodos que utilizan reactivos de afinidad, colorantes químicos, proteínas recombinantes u otros métodos conocidos por los expertos en la técnica.

Administración

En ciertos aspectos, se proporciona en la presente un método para suministrar una bacteria y/o una composición bacteriana descrita en el presente documentoa un sujeto. En algunas realizaciones de los métodos proporcionados en este documento, las bacterias se administran junto con la administración de un agente terapéutico contra el cáncer. En algunas realizaciones, la bacteria se coformula en una composición farmacéutica con el agente terapéutico contra el cáncer. En algunas realizaciones, la bacteria se coadministra con el agente terapéutico contra el cáncer. En algunas realizaciones, el agente terapéutico contra el cáncer se administra al sujeto antes de la administración de la bacteria (por ejemplo, aproximadamente 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25, 30, 35)., 40, 45, 50 o 55 minutos antes, aproximadamente 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22 o 23 horas antes, o aproximadamente 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13 o 14 días antes). En algunas realizaciones, el agente terapéutico contra el cáncer se administra al sujeto después de la administración de la bacteria (por ejemplo, aproximadamente 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25, 30, 35)., 40, 45, 50 o 55 minutos después, aproximadamente 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22 o 23 horas después, o aproximadamente 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13 o 14 días después). En algunas realizaciones, el mismo modo de administración se usa para administrar tanto la bacteria

como la terapéutica contra el cáncer. En algunas realizaciones, se usan diferentes modos de administración para administrar las bacterias y el agente terapéutico contra el cáncer. Por ejemplo, en algunas realizaciones, la bacteria se administra por vía oral mientras que la terapéutica contra el cáncer se administra mediante inyección (por ejemplo, una inyección intravenosa, intramuscular y/o intratumoral).

En ciertas realizaciones, las composiciones farmacéuticas, formas de dosificación y kits descritos en el presente documentopueden administrarse junto con cualquier otro tratamiento convencional contra el cáncer, tal como, por ejemplo, radioterapia y resección quirúrgica del tumor. Estos tratamientos se pueden aplicar según sea necesario y/o como se indique y pueden ocurrir antes, simultáneamente o después de la administración de las composiciones farmacéuticas, formas de dosificación y kits descritos en este documento.

El régimen de dosificación puede ser cualquiera de una variedad de métodos y cantidades, y puede determinarse por un experto en la técnica de acuerdo con factores clínicos conocidos. Como es sabido en las artes médicas, las dosis para un paciente pueden depender de muchos factores, que incluyen la especie, el tamaño, el área de superficie corporal, la edad, el sexo, la inmunocompetencia y la salud general del sujeto, el microorganismo particular a administrar, la duración y la ruta de la administración, el tipo y el estadio de la enfermedad, por ejemplo, el tamaño del tumor y otros compuestos, como las drogas que se administran al mismo tiempo. Además de los factores anteriores, tales niveles pueden verse afectados por la infectividad del microorganismo y la naturaleza del

microorganismo, como puede determinar un experto en la técnica. En los presentes métodos, niveles de dosificación mínima apropiados de microorganismos pueden ser niveles suficientes para que el microorganismo sobreviva, crezca y se replique en un tumor o metástasis. Los métodos de tratamiento descritos en el presente documentopueden ser adecuados para el tratamiento de un tumor primario, un tumor secundario o metástasis, así como para tumores recurrentes o cánceres. La dosis de las composiciones farmacéuticas descritas en la presente memoria puede ajustarse o ajustarse de manera apropiada de acuerdo con la forma de dosificación, la vía de administración, el grado o etapa de una enfermedad objetivo, y similares. Por ejemplo, la dosis eficaz general de los agentes puede oscilar entre 0,01 mg/kg de peso corporal/día y 1000 mg/kg de peso corporal/día, entre 0,1 mg/kg de peso corporal/día y 1000 mg/kg de peso corporal/día. 0,5 mg/kg de peso corporal/día y 500 mg/kg de peso corporal/día, 1 mg/kg de peso corporal/día y 100 mg/kg de peso corporal/día, o entre 5 mg/kg de peso corporal/día y 50 mg/kg de peso corporal/día. La dosis efectiva puede ser de 0,01, 0,05, 0,1, 0,5, 1, 2, 3, 5, 10, 20, 30, 40, 50, 60, 70, 80, 90, 100, 200, 500 o 1000 mg/kg peso corporal/día o más, pero la dosis no está limitada a eso.

En algunas realizaciones, la dosis administrada a un sujeto es suficiente para prevenir el cáncer, retrasar su aparición, o ralentizar o detener su progresión o prevenir una recaída de un cáncer. Un experto en la materia reconocerá que la dosificación dependerá de una variedad de factores que incluyen la resistencia del compuesto particular empleado, así como la edad, especie, estado y peso corporal del sujeto. El tamaño de la dosis también se

determinará por la ruta, el momento y la frecuencia de administración, así como la existencia, naturaleza y alcance de cualquier efecto secundario adverso que pueda acompañar la administración de un compuesto particular y el efecto fisiológico deseado.

Las dosis y los regímenes de dosificación adecuados se pueden determinar mediante técnicas convencionales de búsqueda de rango conocidas por los expertos en la técnica. En general, el tratamiento se inicia con dosis más pequeñas, que son menores que la dosis óptima del compuesto. A partir de entonces, la dosificación se incrementa en pequeños incrementos hasta que se alcanza el efecto óptimo bajo las circunstancias. Se puede determinar una dosis efectiva y un protocolo de tratamiento por medios rutinarios y convencionales, comenzando, por ejemplo, con una dosis baja en animales de laboratorio y luego aumentando la dosificación mientras se monitorean los efectos, y también variando sistemáticamente el régimen de dosificación. Los estudios en animales se usan comúnmente para determinar la dosis máxima tolerable ("MTD") de agente bioactivo por kilogramo de peso. Los expertos en la técnica extrapolan regularmente dosis para la eficacia, mientras evitan la toxicidad, en otras especies, incluidos los humanos.

De acuerdo con lo anterior, en aplicaciones terapéuticas, las dosificaciones de los agentes activos usados de acuerdo con la invención varían dependiendo del agente activo, la edad, el peso y el estado clínico del paciente receptor, y la experiencia y el juicio del médico. o el profesional que administra la terapia, entre otros factores que afectan la dosis seleccionada. En general, la dosis debe ser suficiente para dar como resultado la

desaceleración, y preferentemente la regresión, del crecimiento de los tumores y, lo más preferentemente, provocar la regresión completa del cáncer.

Las administraciones separadas pueden incluir cualquier cantidad de dos o más administraciones (por ejemplo, dosis), que incluyen dos, tres, cuatro, cinco o seis administraciones. Un experto en la técnica puede determinar fácilmente el número de administraciones que realizará, o la conveniencia de realizar una o más administraciones adicionales, de acuerdo con métodos conocidos en la técnica para monitorizar métodos terapéuticos y otros métodos de monitorización proporcionados en este documento. En algunas realizaciones, las dosis pueden separarse por al 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 o 30 días o 1, 2, 3 o 4 semanas. Por consiguiente, los métodos proporcionados en el presente documentoincluyen métodos para proporcionar al sujeto una o más administraciones de una bacteria, donde el número de administraciones puede determinarse controlando el sujeto y, sobre la base de los resultados de la monitorización, determinar si se proporciona o no una o más administraciones adicionales. La decisión de proporcionar una o más administraciones adicionales puede basarse en una variedad de resultados de monitoreo, que incluyen, entre otros, indicación de crecimiento tumoral o inhibición del crecimiento tumoral, aparición de nuevas metástasis o inhibición de la metástasis, el tema del sujeto título de anticuerpo anti-bacteria, el título de anticuerpo anti-tumoral del sujeto, la salud general del sujeto y/o el peso del sujeto.

El período de tiempo entre las administraciones puede

ser cualquiera de una variedad de períodos de tiempo. El período de tiempo entre las administraciones puede ser una función de cualquiera de una variedad de factores, incluyendo los pasos de monitoreo, como se describe en relación con el número de administraciones, el período de tiempo para que un sujeto monte una respuesta inmune y/o el período de tiempo para un sujeto a eliminar las bacterias del tejido normal. En un ejemplo, el período de tiempo puede ser una función del período de tiempo para que un sujeto monte una respuesta inmune; por ejemplo, el período de tiempo puede ser más que el período de tiempo para que un sujeto monte una respuesta inmune, tal como más de aproximadamente una semana, más de aproximadamente diez días, más de aproximadamente dos semanas, o más de aproximadamente un mes; en otro ejemplo, el período de tiempo puede ser menor que el período de tiempo para que un sujeto monte una respuesta inmune, tal como menos de aproximadamente una semana, menos de aproximadamente diez días, menos de aproximadamente dos semanas o menos de aproximadamente un mes. En otro ejemplo, el período de tiempo puede ser una función del período de tiempo para que un sujeto elimine las bacterias del tejido normal; por ejemplo, el período de tiempo puede ser más que el período de tiempo para que un sujeto elimine las bacterias del tejido normal, como más de aproximadamente un día, más de aproximadamente dos días, más de aproximadamente tres días, más de aproximadamente cinco días, o más de una semana

En algunas realizaciones, la administración de un agente terapéutico contra el cáncer en combinación con las bacterias descritas en el presente documentoreduce los efectos adversos y/o mejora la eficacia del agente terapéutico contra el cáncer.

La dosis efectiva de un agente terapéutico contra el cáncer descrito en el presente documentoes la cantidad de agente terapéutico que es efectiva para lograr la respuesta terapéutica deseada para un paciente particular, composición y modo de administración, con la menor toxicidad para el paciente. El nivel de dosificación efectivo puede identificarse usando los métodos descritos en el presente documentoy dependerá de una variedad de factores farmacocinéticos que incluyen la actividad de las composiciones particulares administradas, la ruta de administración, el tiempo de administración, la tasa de excreción del compuesto particular que se está empleando., la duración del tratamiento, otros fármacos, compuestos y/o materiales usados en combinación con las composiciones particulares empleadas, la edad, el sexo, el peso, el estado, la salud general y el historial médico previo del paciente que se está tratando, y factores similares bien conocidos en las artes médicas. En general, una dosis efectiva de una terapia contra el cáncer será la cantidad del agente terapéutico que sea la dosis más baja efectiva para producir un efecto terapéutico. Tal dosis efectiva generalmente dependerá de los factores descritos anteriormente.

La toxicidad de una terapia contra el cáncer es el nivel de efectos adversos experimentados por el sujeto durante y después del tratamiento. Los eventos adversos asociados con la toxicidad de la terapia contra el cáncer incluyen, entre otros, dolor abdominal, indigestión ácida, reflujo ácido, reacciones alérgicas, alopecia, anafilaxia, anemia, ansiedad, falta de apetito, artralgias, astenia, ataxia, azotemia, pérdida del equilibrio., dolor de huesos, sangrado, coágulos sanguíneos, presión arterial baja,

presión arterial elevada, dificultad para respirar, bronquitis, hematomas, recuento bajo de glóbulos blancos, recuento bajo de glóbulos rojos, recuento bajo de plaquetas, cardiotoxicidad, cistitis, cistitis hemorrágica, arritmias, corazón valvulopatía, cardiomiopatía, enfermedad de las arterias coronarias, cataratas, neurotoxicidad central, deterioro cognitivo, confusión, conjuntivitis, estreñimiento, tos, cólicos, cistitis, trombosis venosa profunda, deshidratación, depresión, diarrea, mareos, boca seca, piel seca, dispepsia, disnea, edema, desequilibrio electrolítico, esofagitis, fatiga, pérdida de fertilidad, fiebre, flatulencia, enrojecimiento, reflujo gástrico, enfermedad por reflujo gastroesofágico, dolor genital, granulocitos openia, ginecomastia, glaucoma, pérdida de cabello, síndrome mano-pie, dolor de cabeza, pérdida de audición, insuficiencia cardíaca, palpitaciones, ardor de estómago, hematoma, cistitis hemorrágica, hepatotoxicidad, hiperamilasemia, hipercalcemia, hipercloremia, hiperglucemia, hipercalemia, hiperlipemia, hipermagnesemia, hipernatremia, hiperfosfatemia, hiperpigmentación, hipertrigliceridemia, hiperuricemia, hipoalbuminemia, hipocalcemia, hipocloremia, hipoglucemia, hipocalemia, hipomagnesemia, hiponatremia, hipofosfatemia, impotencia, infección, reacciones en el lugar de la inyección, insomnio, deficiencia de hierro, picazón, dolor en las articulaciones, insuficiencia renal, leucopenia, disfunción hepática, pérdida de memoria, menopausia, llagas en la boca, mucositis, dolor muscular, mialgias, mielosupresión, miocarditis, fiebre neutropénica, náuseas, nefrotoxicidad, neutropenia, hemorragias nasales, entumecimiento, ototoxicidad, dolor, eritrodisestesia palmo-plantar, pancitopenia, pericarditis, neuropatía periférica,

faringitis fotofobia, fotosensibilidad, neumonía, neumonitis, pr oteinuria, émbolo pulmonar, fibrosis pulmonar, toxicidad pulmonar, erupción cutánea, latido rápido del corazón, sangrado rectal, inquietud, rinitis, convulsiones, dificultad para respirar, sinusitis, trombocitopenia, tinnitus, infección del tracto urinario, sangrado vaginal, sequedad vaginal, vértigo, retención de agua, debilidad, pérdida de peso, aumento de peso y xerostomía. En general, la toxicidad es aceptable si los beneficios para el sujeto logrados a través de la terapia superan los eventos adversos experimentados por el sujeto debido a la terapia.

En algunas realizaciones, la administración de la composición bacteriana trata el cáncer. En algunas realizaciones, la composición bacteriana induce una respuesta inmune antitumoral en el sujeto.

Agentes terapéuticos

En ciertos aspectos, los métodos proporcionados en el presente documentoincluyen la administración a un sujeto de una bacteria y/o una composición bacteriana descrita en el presente documento (por ejemplo, una composición bacteriana que contiene *Bifidobacterium animalis ssp. lactis*) sola o en combinación con otro agente terapéutico contra el cáncer. El otro agente terapéutico contra el cáncer puede incluir, por ejemplo, resección quirúrgica, radioterapia o un agente terapéutico contra el cáncer. En algunas realizaciones, la composición bacteriana y la otra terapia contra el cáncer se pueden administrar al sujeto en cualquier orden. En algunas realizaciones, la composición bacteriana y la otra terapia contra el cáncer se administran conjuntamente.

En algunas realizaciones, la bacteria se administra al

sujeto antes de que se administre la terapia contra el cáncer (por ejemplo, al menos 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23 o 24 horas antes o al 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 o 30 días antes). En algunas realizaciones, la bacteria se administra al sujeto después de administrarse el tratamiento terapéutico contra el cáncer (por ejemplo, al menos 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15)., 16, 17, 18, 19, 20, 21, 22, 23 o 24 horas después o al 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 o 30 días después). En algunas realizaciones, la bacteria y el agente terapéutico contra el cáncer se administran al sujeto simultáneamente o casi simultáneamente (por ejemplo, las administraciones se producen en una hora el uno del otro). En algunas realizaciones, al sujeto se le administra un antibiótico antes de que la bacteria se administre al sujeto (p. Ej., Al menos 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23 o 24 horas antes o al 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 o 30 días antes). En algunas realizaciones, al sujeto se le administra un antibiótico después de que la bacteria se administre al sujeto (p. Ej., Al menos 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23 o 24 horas antes o al 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 o 30 días después). En algunas realizaciones, la bacteria y el antibiótico se administran al sujeto simultáneamente o casi simultáneamente (por ejemplo, las administraciones se producen en una hora el

uno del otro).

En ciertas realizaciones, el sujeto puede someterse a cirugía. Los tipos de cirugía incluyen, pero no se limitan a, cirugía preventiva, diagnóstica o de estadificación, curativa y paliativa. La cirugía curativa es un tratamiento contra el cáncer que se puede usar junto con otras terapias, tales como el tratamiento de la presente invención, quimioterapia, radioterapia, terapia hormonal, terapia génica, inmunoterapia y/o terapias alternativas.

La cirugía curativa incluye la resección donde todo o parte del tejido canceroso se elimina, extirpa y/o destruye físicamente. La resección tumoral se refiere a la extirpación física de al menos parte de un tumor. Además de la resección tumoral, el tratamiento mediante cirugía incluye cirugía con láser, criocirugía, electrocirugía y cirugía controlada microscópicamente (cirugía de Mohs). Tras la extirpación de parte de todas las células cancerosas, tejido o tumor, se puede formar una cavidad en el cuerpo.

En ciertas realizaciones, el sujeto puede someterse a radioterapia. La radioterapia incluye la administración o aplicación de agentes y factores radioterapéuticos que incluyen, entre otros, además de bandejas, irradiación UV, microondas, emisiones electrónicas y radioisótopos. El sitio tumoral localizado puede ser irradiado, incluyendo por una o más formas de radiaciones descritas anteriormente. Todos estos factores pueden afectar un amplio rango de ADN dañado, en los precursores del ADN, la replicación y reparación del ADN y el ensamblaje y mantenimiento de los cromosomas.

Los rangos de dosificación para radiografías varían desde dosis diarias de 50 a 200 roentgens por períodos

prolongados (3 a 4 semanas), a dosis únicas de 2000 a 6000 roentgens. Los rangos de dosificación para los radioisótopos varían ampliamente, y dependen de la vida media del isótopo, la fuerza y el tipo de radiación emitida, y la absorción por las células neoplásicas.

En ciertos aspectos, los métodos proporcionados en la presente memoria comprenden además administrar otro agente terapéutico contra el cáncer al sujeto.

En algunas realizaciones, el agente terapéutico contra el cáncer es un agente quimioterapéutico. Los ejemplos de tales agentes quimioterapéuticos incluyen, pero no se limitan a, agentes alquilantes tales como tiotepa y ciclofosfamida; alquilsulfonatos tales como busulfan, improsulfan y piposulfan; aziridinas tales como benzodopa, carboquona, meturedopa y uredopa; etileniminas y metilamelaminas que incluyen altretamina, trietilenmelamina, trietilenfosforamida, trietilenotiofosforamida y trimetilolomelamina; acetogeninas (especialmente bullatacina y bullatacinona); una camptotecina (incluido el topotecan análogo sintético); bryostatin; callystatin; CC-1065 (incluidos sus análogos sintéticos de adozelesina, carzelesina y bizelesina); criptoficinas (particularmente criptoficina 1 y criptoficina 8); dolastatin; duocarmicina (incluidos los análogos sintéticos, KW-2189 y CB1-TM1); eleuterobin; pancratistatin; una sarcodictyin; espongistatina; mostazas nitrogenadas tales como clorambucilo, clonafazina, colofosfamida, estramustina, ifosfamida, mecloretamina, hidrocloruro de óxido de mecloretamina, melfalano, novembichina, fenestina, prednimustina, trofosfamida, mostaza de uracilo; nitrosureas tales como carmustina, clorozotocina, fotemustina, lomustina, nimustina y

ranimnustina; antibióticos como los antibióticos enediyne (p. ej., caliqueamicina, especialmente caliqueamicina gammalI y caliqueamicina omegal1; dinemicina, incluida la dianmicina A; bifosfonatos, como clodronato; una esperamicina; así como neocarzinostatin cromóforo y cromoproteína relacionada; enediyne, antibióticos, cromóforos, aclacinomisinas, actinomicina, authrarnycin, azaserina, bleomicinas, cactinomicina, carabicina, caminomicina, carzinofilina, cromomicina, dactinomicina, daunorrubicina, detorubicina, 6-diazo-5-oxo-L-norleucina, doxorrubicina (incluida la morfolino-doxorrubicina, cianomorfolino-doxorrubicina, 2-pirrolino-doxorrubicina y deoxydoxorubicin), epirrubicina, esorubicina, idarrubicina, marcellomicina, mitomicinas como mitomicina C, ácido micofenólico, nogalamicina, olivomicinas, peplomicina, potfiromicina, puromicina, quelamicina, rodorubicina, estreptonigrina, estreptozocina, tubercidina, ubenimex, zinostatin, zorubicin; antimetabolitos tales como metotrexato y 5-fluorouracilo (5-FU); análogos de ácido fólico tales como denopterina, metotrexato, pteropterina, trimerexato; análogos de purina tales como fludarabina, 6-mercaptopurina, tiamiprina, tioguanina; análogos de pirimidina tales como ancitabina, azacitidina, 6-azauridina, carmofur, citarabina, didesoxiuridina, doxifluridina, enocitabina, floxuridina; andrógenos tales como calusterona, propionato de dromostanolona, epitiostanol, mepitiostano, testolactona; anti-adrenales tales como aminoglutetimida, mitotano, trilostano; reabastecedor de ácido fólico tal como ácido frolínico; aceglatona; aldofosfamida glucósido; ácido aminolevulínico; eniluracilo; amsacrina; bestrabucil; bisantreno; edatraxate; defofamina; demecolcina; diaziquone;

elformitina; acetato de elliptinio; una epotilona; etoglucida; nitrato de galio; hidroxiurea; lentinan; lonidainina; maitansinoides tales como maitansina y ansamitocinas; mitoguazone; mitoxantrona; mopidanmol; nitraerina; pentostatina; phenamet; pirarrubicina; losoxantrona; ácido podofilínico; 2-etilhidrazida; procarbazina; Complejo de polisacárido PSK); razoxane; rizoxina; sizofuran; Spirogermanium; ácido tenuazónico; triaziquona; 2,2 ', 2' '- triclorotrietilamina; tricotecenos (especialmente toxina T-2, verracurina A, roridina A y anguidina); uretano; vindesina; dacarbazina; mannomustine; mitobronitol; mitolactol; pipobroman; gacytosine; arabinósido ("Ara-C"); ciclofosfamida; tiotepa; taxoides, por ejemplo, paclitaxel y doxetaxel; clorambucilo; gemcitabina; 6-tioguanina; mercaptopurina; metotrexato; complejos de coordinación de platino tales como cisplatino, oxaliplatino y carboplatino; vinblastina; platino; etopósido (VP-16); ifosfamida; mitoxantrona; vincristina; vinorelbina; novantrona; tenipósido; edatrexate; daunomicina; aminopterina; xeloda; ibandronato; irinotecán (por ejemplo, CPT-11); inhibidor de topoisomerasa RFS 2000; difluorometilomitina (DMFO); retinoides tales como ácido retinoico; capecitabina; y sales, ácidos o derivados farmacéuticamente aceptables de cualquiera de los anteriores.

En algunas realizaciones, el agente terapéutico contra el cáncer es un agente de inmunoterapia contra el cáncer. La inmunoterapia se refiere a un tratamiento que usa el sistema inmunológico de un sujeto para tratar el cáncer, por ejemplo, inhibidores del punto de control, vacunas contra el cáncer, citocinas, terapia celular, células CAR-T y terapia con células dendríticas. Ejemplos no limitantes

de inmunoterapias son los inhibidores de puntos de control incluyen Nivolumab (BMS, anti-PD-1), Pembrolizumab (Merck, anti-PD-1), Ipilimumab (BMS, anti-CTLA-4), MEDI4736 (AstraZeneca, anti-PD -L1) y MPDL3280A (Roche, anti-PD-L1). Otras inmunoterapias pueden ser vacunas tumorales, 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 péptido E75, IMA901, POL-103A, Belagenpumatucel-L, GSK1572932A, MDX-1279, GV1001 y Tecemotida. La inmunoterapia se puede administrar mediante inyección (por ejemplo, por vía intravenosa, intratumoral, subcutánea o en los ganglios linfáticos), pero también se puede administrar por vía oral, tópica o mediante aerosol. Las inmunoterapias pueden comprender adyuvantes tales como citocinas.

En algunas realizaciones, el agente de inmunoterapia es un inhibidor del punto de control inmune. La inhibición del punto de control inmunológico se refiere en general a la inhibición de los puntos de control que las células cancerígenas pueden producir para prevenir o regular negativamente una respuesta inmune. Los ejemplos de proteínas de punto de control inmune incluyen, pero sin limitación, CTLA4, PD-1, PD-L1, PD-L2, A2AR, B7-H3, B7-H4, BTLA, KIR, LAG3, TIM-3 o VISTA. Los inhibidores de punto de control inmunitario pueden ser anticuerpos o fragmentos de unión a antígeno de los mismos que se unen e inhiben una proteína de punto de control inmune. Los ejemplos de inhibidores del punto de control inmune incluyen, pero no se limitan a, nivolumab, pembrolizumab, pidilizumab, AMP-224, AMP-514, STI-A1110, TSR-042, RG-7446, BMS-936559, MEDI-4736, MSB-0020718C, AUR-012 y STI-A10^{10}. En algunas realizaciones, la inmunoterapia del cáncer incluye

administrar un inhibidor de punto de control inmune adicional (por ejemplo, administrando 2, 3, 4 o 5, inhibidores del punto de control inmune) al sujeto.

En ciertas realizaciones, los inhibidores del punto de control inmune pueden ser una molécula de ácido nucleico inhibidor (por ejemplo, una molécula de ARNip, una molécula de ARNhc o una molécula de ARN antisentido) que inhibe la expresión de una proteína de punto de control inmune que inhibe la expresión de una proteína de punto de control inmune.

En algunas realizaciones, el inhibidor del punto de control inmune es una molécula de ARNip. Dichas moléculas de ARNip deberían incluir una región de suficiente homología con la región diana, y tener una longitud suficiente en términos de nucleótidos, de modo que la molécula de ARNic regula por disminución el ARN diana (por ejemplo, ARN de una proteína de punto de control inmune). El término "ribonucleótido" o "nucleótido" puede, en el caso de un sustituto de ARN o nucleótido modificado, también referirse a un nucleótido modificado, o resto sustituto sustituto en una o más posiciones. No es necesario que exista una complementariedad perfecta entre la molécula de ARNip y el objetivo, pero la correspondencia debe ser suficiente para permitir que la molécula de ARNic dirija el silenciamiento específico de la secuencia, tal como mediante la escisión de ARNi del ARN diana. En algunas realizaciones, la cadena con sentido solo necesita ser suficientemente complementaria con la cadena antisentido para mantener el carácter general de doble cadena de la molécula.

Además, una molécula de ARNip puede modificarse o incluir sustitutos de nucleósidos. Las regiones

monocatenarias de una molécula de ARNip pueden modificarse o incluir sustitutos de nucleósidos, por ejemplo, la región o regiones no emparejadas de una estructura en horquilla, por ejemplo, una región que une dos regiones complementarias, pueden tener modificaciones o sustitutos de nucleósidos. La modificación para estabilizar uno o más extremos 3 'o 5' de una molécula de ARNip, por ejemplo, contra exonucleasas, o para favorecer que el agente de ARNic antisentido ingrese en RISC también es útil. Las modificaciones pueden incluir enlazadores de amino C3 (o C6, C7, C12), ligadores de tiol, ligadores de carboxilo, espaciadores no nucleotídicos (C3, C6, C9, C12, abasico, trietilenglicol, hexaetilenglicol), biotina especial o reactivos de fluoresceína que vienen como fosforamiditas y que tienen otro grupo hidroxilo protegido con DMT, permitiendo múltiples acoplamientos durante la síntesis de ARN.

Cada cadena de una molécula de ARNip puede ser igual o inferior a 35, 30, 25, 24, 23, 22, 21 o 20 nucleótidos de longitud. En algunas realizaciones, la cadena tiene al menos 19 nucleótidos de longitud. Por ejemplo, cada cadena puede tener entre 21 y 25 nucleótidos de longitud. En algunas realizaciones, los agentes de siRNA tienen una región dúplex de 17, 18, 19, 29, 21, 22, 23, 24 o 25 pares de nucleótidos, y uno o más salientes, tales como uno o dos 3 'voladizos, de 2- 3 nucleótidos.

En algunas realizaciones, el inhibidor del punto de control inmune es una molécula de ARNhc. Un "ARN de horquilla pequeño" o "ARN de horquilla corta" o "ARNhc" incluye una secuencia corta de ARN que hace un giro brusco que puede usarse para silenciar la expresión génica a través de la interferencia de ARN. Los shRNA proporcionados

en el presente documentopueden sintetizarse químicamente o transcribirse a partir de un casete de transcripción en un plásmido de DNA. La estructura de la horquilla de ARNhc es escindida por la maquinaria celular en ARNsi, que luego se une al complejo de silenciamiento inducido por ARN (RISC).

En algunas realizaciones, los shRNA son aproximadamente de 15-60, 15-50 o 15-40 (dúplex) nucleótidos de longitud, aproximadamente 15-30, 15-25 o 19-25 (dúplex) nucleótidos de longitud, o son aproximadamente 20 -24, 21-22, o 21-23 (duplex) nucleótidos de longitud (p. Ej., Cada secuencia complementaria del ARNhc bicatenario es 15-60, 15-50, 15-40, 15-30, 15-25, o 19-25 nucleótidos de longitud, o aproximadamente 20-24, 21-22, o 21-23 nucleótidos de longitud, y el ARNhc bicatenario es aproximadamente 15-60, 15-50, 15-40, 15-30, 15-25, o 19-25 pares de bases de longitud, o aproximadamente 18-22, 19-20 o 19-21 pares de bases de longitud). Los dúplex de shARN pueden comprender salientes en 3 'de aproximadamente 1 a aproximadamente 4 nucleótidos o aproximadamente 2 a aproximadamente 3 nucleótidos en la cadena antisentido y/o los extremos 5' - fosfato en la cadena con sentido. En algunas realizaciones, el shRNA comprende una cadena de sentido y/o una secuencia de cadena antisentido de aproximadamente 15 a aproximadamente 60 nucleótidos de longitud (por ejemplo, aproximadamente 15-60, 15-55, 15-50, 15-45, 15-40, 15-35, 15-30, o 15-25 nucleótidos de longitud), o de aproximadamente 19 a aproximadamente 40 nucleótidos de longitud (por ejemplo, aproximadamente 19-40, 19-35, 19-30 o 19-25 nucleótidos de longitud).), o de aproximadamente 19 a aproximadamente 23 nucleótidos de longitud (por ejemplo, 19, 20, 21, 22 o 23 nucleótidos de longitud).

Los ejemplos no limitantes de ARNhc incluyen una molécula de polinucleótido bicatenario ensamblada a partir de una molécula monocatenaria, donde las regiones sentido y antisentido están unidas por un enlazador basado en ácido nucleico o no ácido nucleico; y una molécula de polinucleótido bicatenario con una estructura secundaria en horquilla que tiene regiones sentido y antisentido autocomplementarias. En algunas realizaciones, las cadenas sentido y antisentido del shRNA están unidas por una estructura de bucle que comprende de aproximadamente 1 a aproximadamente 25 nucleótidos, de aproximadamente 2 a aproximadamente 20 nucleótidos, de aproximadamente 4 a aproximadamente 15 nucleótidos, de aproximadamente 5 a aproximadamente 12 nucleótidos., o 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, o más nucleótidos.

Se describen realizaciones adicionales relacionadas con los shRNA, así como con los métodos para diseñar y sintetizar dichos shRNA, en la publicación de la solicitud de patente de los EE.UU. número de publicación 2011/0071208, cuya descripción se incorpora en el presente documento como referencia en su totalidad para todos los fines.

En algunas realizaciones, el inhibidor del punto de control inmune es un compuesto oligonucleotídico antisentido que inhibe la expresión de una proteína de punto de control inmune. En ciertas realizaciones, el grado de complementariedad entre la secuencia diana y la secuencia diana antisentido es suficiente para formar un dúplex estable. La región de complementariedad de los oligonucleótidos antisentido con la secuencia de ARN diana puede ser tan corta como 8-11 bases, pero puede ser 12-15

bases o más, por ejemplo, 10-40 bases, 12-30 bases, 12-25 bases, 15-25 bases, 12-20 bases o 15-20 bases, incluidos todos los enteros entre estos rangos. Un oligonucleótido antisentido de aproximadamente 14-15 bases es generalmente suficientemente largo para tener una secuencia complementaria única.

En ciertas realizaciones, los oligonucleótidos antisentido pueden ser 100 % complementarios a la secuencia diana, o pueden incluir desapareamientos, por ejemplo, para mejorar el direccionamiento selectivo del alelo que contiene la mutación asociada a enfermedad, siempre que un heterodúplex formado entre el oligonucleótido y la secuencia diana sea suficientemente estable para resistir la acción de nucleasas celulares y otros modos de degradación que pueden producirse *in vivo*. Por lo tanto, ciertos oligonucleótidos pueden tener aproximadamente o al menos aproximadamente 70 % de complementariedad de secuencia, por ejemplo, 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 Complementariedad de secuencia %, 98 %, 99 % o 100 %, entre el oligonucleótido y la secuencia diana. Los esqueletos de oligonucleótidos que son menos susceptibles a la escisión por nucleasas se discuten en el presente documento. Los desajustes, si están presentes, son típicamente menos desestabilizadores hacia las regiones finales del dúplex híbrido que en el medio. El número de desapareamientos permitidos dependerá de la longitud del oligonucleótido, el porcentaje de pares de bases G: C en el dúplex y la posición del (los) emparejamiento (s) incorrecto (s) en el dúplex, de acuerdo con principios bien entendidos de estabilidad dúplex.

La molécula de ácido nucleico inhibidor puede prepararse, por ejemplo, mediante síntesis química, transcripción *in vitro* o digestión de dsRNA largo por Rnase III o Dicer. Estos pueden introducirse en las células mediante transfección, electroporación u otros métodos conocidos en la técnica. Véase Hannon, GJ, 2002, RNA Interference, Nature 418: 244-251; Bernstein E et al., 2002, The rest is silence. RNA 7: 1509-1521; Hutvagner G et al., RNAi: Nature abhors a double-strand. Curr. Opin. Genetics & Development 12: 225-232; Brummelkamp, 2002, A system for stable expression of short interfering RNAs in mammalian cells. Science 296: 550-553; Lee NS, Dohjima T, Bauer G, Li H, Li M-J, Ehsani A, Salvaterra P, and Rossi J. (2002). Expression of small interfering RNAs targeted against HIV-1 rev transcripts in human cells. Nature Biotechnol. 20:500-505; Miyagishi M, and Taira K. (2002). U6-promoter-driven siRNAs with four uridine 3' overhangs efficiently suppress targeted gene expression in mammalian cells. Nature Biotechnol. 20:497-500; Paddison PJ, Caudy AA, Bernstein E, Hannon GJ, and Conklin DS. (2002). Short hairpin RNAs (shRNAs) induce sequence-specific silencing in mammalian cells. Genes & Dev. 16:948-958; Paul CP, Good PD, Winer I, and Engelke DR. (2002). Effective expression of small interfering RNA in human cells. Nature Biotechnol. 20:505-508; Sui G, Soohoo C, Affar E-B, Gay F, Shi Y, Forrester WC, and Shi Y. (2002). A DNA vector-based RNAi technology to suppress gene expression in mammalian cells. Proc. Natl. Acad. Sci. USA 99(6):5515-5520; Yu J-Y, DeRuiter SL, and Turner DL. (2002). RNA interference by expression of short-interfering RNAs and hairpin RNAs in mammalian cells. Proc. Natl. Acad. Sci. USA 99(9):6047-6052.

En los presentes métodos, la molécula de ácido nucleico inhibidor puede administrarse al sujeto, por ejemplo, como ácido nucleico desnudo, en combinación con un reactivo de administración, y/o como un ácido nucleico que comprende secuencias que expresan una molécula de ácido nucleico interferente. En algunas realizaciones, el ácido nucleico que comprende secuencias que expresan las moléculas de ácido nucleico interferentes se administran dentro de vectores, p. plásmido, vectores virales y bacterianos. Cualquier método de administración de ácido nucleico conocido en la técnica se puede usar en los métodos descritos en este documento. Los reactivos de administración adecuados incluyen, pero no se limitan a, por ejemplo, el reactivo lipófilo Mirus Transit TKO; lipofectina; lipofectamina; Cellfectin; policationes (por ejemplo, polilisina), atelocolágeno, nanoplastos y liposomas. El uso de atelocolágeno como vehículo de administración para moléculas de ácido nucleico se describe en Minakuchi *et al.* Nucleic Acids Res., 32(13):e109 (2004); Hanai *et al.* Ann NY Acad Sci., 1082:9-17 (2006); and Kawata *et al.* Mol Cancer Ther., 7(9):2904-12 (2008); cada uno de los cuales se incorpora en el presente documento en su totalidad. Los ejemplos de sistemas de administración de ácido nucleico que interfieren se proporcionan en las patentes de los Estados Unidos números 8,283,461, 8,313,772, 8,501,930. 8,426,554, 8,268,798 y 8,324,366, cada una de las cuales se incorpora en el presente documentopor referencia en su totalidad.

En algunas realizaciones, el agente de inmunoterapia es un anticuerpo o fragmento de unión a antígeno del mismo que, por ejemplo, se une a un antígeno asociado al cáncer. Los ejemplos de antígenos asociados al cáncer incluyen,

pero no se limitan a, adipophilin, AIM-2, ALDH1A1, alfa-actinin-4, alfa-fetoprotein ("AFP"), ARTC1, B-RAF, BAGE-1, BCLX (L), Proteína de fusión BCR-ABL b3a2, beta-catenina, BING-4, CA-125, CALCA, antígeno carcinoembrionario ("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 fusión dek-can, DKK1, EFTUD2, factor de elongación 2, ENAH (hMena), Ep-CAM, EpCAM, EphA3, antígeno tumoral epitelial ("ETA"), proteína de fusión ETV6-AML1, EZH2, FGF5, FLT3-ITD, FN1, G250/MN/CAIX, GAGE-1,2,8, GAGE-3,4,5,6, 7, GAS7, glypican-3, GnTV, gp100/Pmel17, GPNMB, HAUS3, Hepsin, HER-2/neu, HERV-K-MEL, HLA-A11, HLA-A2, HLA-DOB, hsp70-2, IDO1, IGF2B3, IL13Ralpha2, carboxiloesterasa intestinal, K-ras, Kallikrein 4, KIF20A, KK-LC-1, KKLC1, KM-HN-1, KMHN1 también conocida como CCDC110, LAGE-1, proteína de fusión LDLR-fucosiltransferasaAS, 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, Melan-A/MART -1, Meloe, Midkine, MMP-2, MMP-7, MUC1, MUC5AC, mucina, MUM-1, MUM-2, MUM-3, miosina, miosina clase I, N-raw, NA88-A, neo-PAP, NFYC, NY-BR-1, NY-ESO-1/lAGE-2, OA1, OGT, OS-9, polipéptido P, p53, PAP, PAX5, PBF, proteína de fusión pml-RARalpha, 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, Proteínas de fusión SPA17, SSX-2, SSX-4, STEAP1, survivina, SYT-SSX1 o -SSX2, TAG-1, TAG-2, telomerasa, TGF-betaRII, TPBG, TRAG-3, isofosfato de triosafosfato, TRP-1/gp75, TRP-2, TRP2-INT2, tirosinasa, tirosinasa ("TYR"), VEGF, WT1, XAGE-1b/GAGED2a. En algunas

realizaciones, el antígeno es un neoantígeno.

En algunas realizaciones, el agente de inmunoterapia es una vacuna contra el cáncer y/o un componente de una vacuna contra el cáncer (por ejemplo, un péptido y/o proteína antigénica). La vacuna contra el cáncer puede ser una vacuna proteica, una vacuna de ácido nucleico o una combinación de las mismas. Por ejemplo, en algunas realizaciones, la vacuna contra el cáncer comprende un polipéptido que comprende un epítopo de un antígeno asociado al cáncer. En algunas realizaciones, la vacuna contra el cáncer comprende un ácido nucleico (por ejemplo, ADN o ARN, tal como ARNm) que codifica un epítopo de un antígeno asociado al cáncer. En algunas realizaciones, el ácido nucleico es un vector (por ejemplo, un vector bacteriano, vector viral). Los ejemplos de vectores bacterianos incluyen, pero sin limitación, Mycobacterium bovis (BCG), Salmonella Typhimurium ssp., Salmonella Typhi ssp., Clostridium sp. esporas, Escherichia coli Nissle 1917, Escherichia coli K-12/IL, Listeria monocytogenes y Shigella flexneri. Los ejemplos de vectores víricos incluyen, pero no se limitan a virus vaccinia, adenovirus, ARN y avipox defectuoso en replicación, viruela aviar defectuosa en la replicación, viruela de canario defectuosa en la replicación, MVA defectivo en la replicación y adenovirus defectuoso en la replicación.

En algunas realizaciones, la inmunoterapia del cáncer comprende la administración de una célula presentadora de antígeno (APC) cebada con un antígeno específico de cáncer. En algunas realizaciones, la APC es una célula dendrítica, un macrófago o una célula B.

Los ejemplos de antígenos asociados al cáncer incluyen, pero no se limitan a, adipophilin, AIM-2,

ALDH1A1, alfa-actinin-4, alfa-fetoprotein ("AFP"), ARTC1, B-RAF, BAGE-1, BCLX (L), Proteína de fusión BCR-ABL b3a2, beta-catenina, BING-4, CA-125, CALCA, antígeno carcinoembrionario ("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 fusión dek-can, DKK1, EFTUD2, factor de elongación 2, ENAH (hMena), Ep-CAM, EpCAM, EphA3, antígeno tumoral epitelial ("ETA"), proteína de fusión ETV6-AML1, EZH2, FGF5, FLT3-ITD, FN1, G250/MN/CAIX, GAGE-1,2,8, GAGE-3,4,5,6, 7, GAS7, glypican-3, GnTV, gp100/Pmel17, GPNMB, HAUS3, Hepsin, HER-2/neu, HERV-K-MEL, HLA-A11, HLA-A2, HLA-DOB, hsp70-2, IDO1, IGF2B3, IL13Ralpha2, carboxiloesterasa intestinal, K-ras, Kallikrein 4, KIF20A, KK-LC-1, KKLC1, KM-HN-1, KMHN1 también conocida como CCDC110, LAGE-1, proteína de fusión LDLR-fucosiltransferasaAS, 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, Melan-A/MART -1, Meloe, Midkine, MMP-2, MMP-7, MUC1, MUC5AC, mucina, MUM-1, MUM-2, MUM-3, miosina, miosina clase I, N-raw, NA88-A, neo-PAP, NFYC, NY-BR-1, NY-ESO-1/lAGE-2, OA1, OGT, OS-9, polipéptido P, p53, PAP, PAX5, PBF, proteína de fusión pml-RARalpha, 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, Proteínas de fusión SPA17, SSX-2, SSX-4, STEAP1, survivina, SYT-SSX1 o -SSX2, TAG-1, TAG-2, telomerasa, TGF-betaRII, TPBG, TRAG-3, isofosfato de triosafosfato, TRP-1/gp75, TRP-2, TRP2-INT2, tirosinasa, tirosinasa ("TYR"), VEGF, WT1, XAGE-1b/GAGED2a. En algunas realizaciones, el antígeno es un neoantígeno.

En algunas realizaciones, la inmunoterapia contra el cáncer comprende la administración de un receptor de antígeno quimérico específico para cáncer (CAR). En algunas realizaciones, el CAR se administra en la superficie de una célula T. En algunas realizaciones, el CAR se une específicamente a un antígeno asociado al cáncer.

En algunas realizaciones, la inmunoterapia del cáncer comprende la administración de un linfocito T específico para el cáncer al sujeto. En algunas realizaciones, la célula T es una célula T CD4 +. En algunas realizaciones, la célula T CD4 + es una célula T TH1, una célula T TH2 o una célula T TH17. En algunas realizaciones, la célula T expresa un receptor de células T específico para un antígeno asociado al cáncer.

En algunas realizaciones, la vacuna contra el cáncer se administra con un adyuvante. Los ejemplos de adyuvantes incluyen, pero no se limitan a, una proteína inmunomoduladora, Adyuvante 65, α-GalCer, fosfato de aluminio, hidróxido de aluminio, fosfato de calcio, péptido β-glucano, ADN CpG, GPI-0100, lípido A, lipopolisacárido, Lipovant, Montanide, N-acetil-muramil-L-alanil-D-isoglutamina, Pam3CSK4, quil A y dimicolato de trehalosa.

En algunas realizaciones, el agente de inmunoterapia es una proteína inmunomoduladora para el sujeto. En algunas realizaciones, la proteína inmunomoduladora es una citocina. Los ejemplos de proteínas inmunomoduladoras incluyen, pero no se limitan a, quimioatrayentes de linfocitos B ("BLC"), quimiocina CC mot motif 11 ("Eotaxin-1"), proteína quimiotáctica de eosinófilos 2 ("Eotaxin-2"), estimulante de colonias de granulocitos factor ("G-CSF"), factor estimulante de colonias de granulocitos macrófagos ("GM-CSF"), 1-309, molécula de adhesión intercelular 1

("ICAM-1"), interferón gamma ("IFN-gamma"), Interleucina -1 alfa ("IL-1 alfa"), Interleucina-1 beta ("IL-1 beta"), antagonista del receptor de interleucina 1 ("IL-1 ra"), Interleucina-2 ("IL-2"), Interleucina-4 ("IL-4"), Interleucina-5 ("IL-5"), Interleucina-6 ("IL-6"), receptor soluble de Interleucina-6 ("IL-6 sR"), interleucina-7 ("IL-7"), interleucina-8 ("IL-8"), interleucina-10 ("IL-10"), interleucina-11 ("IL-11"), Subunidad beta de Interleucina-12 ("IL-12 p40" o "IL-12 p70"), Interleucina-13 ("IL-13"), Interleucina-15 ("IL-15"), Interleucina-16 ("IL-16"), interleuquina-17 ("IL-17"), quimiocina (motivo CC), ligando 2 ("MCP-1"), factor estimulante de colonias de macrófagos ("M-CSF"), monocina inducida por gamma interferón ("MIG"), quimioquina (motivo CC) ligando 2 ("MIP-1 alfa"), quimiocina (motivo CC) ligando 4 ("MIP-1 beta"), proteína 1-delta inflamatoria de macrófago ("MIP- 1 delta "), subunidad B del factor de crecimiento derivado de plaquetas (" PDGF-BB "), ligando quimioquina (motivo CC) 5, regulado en la activación, células T normales expresadas y secretadas (" RANTES "), inhibidor 1 de la metalopeptidasa TIMP (" TIMP-1 "), inhibidor de metalopeptidasa TIMP 2 (" TIMP-2 "), factor de necrosis tumoral, linfotoxina-alfa (" TNF alfa "), Factor de necrosis tumoral, linfotoxina beta ("TNF beta"), receptor soluble de TNF tipo 1 ("sTNFRI"), sTNFRIIAR, factor neurotrófico derivado del cerebro ("BDNF"), factor de crecimiento de fibroblastos básico ("bFGF"), hueso proteína morfogenética 4 ("BMP-4"), proteína morfogenética ósea 5 ("BMP-5"), proteína morfogenética ósea 7 ("BMP-7"), factor de crecimiento nervioso ("b-NGF"), factor de crecimiento epidérmico ("EGF"), receptor del factor de crecimiento epidérmico ("EGFR"), factor de crecimiento endotelial vascular

derivado de la glándula endocrina ("EG-VEGF"), factor de crecimiento fibroblástico 4 ("FGF-4"), factor de crecimiento de queratinocitos ("FGF" -7 "), Factor de diferenciación de crecimiento 15 (" GDF-15 "), Factor neurotrófico derivado de células gliales (" GDNF "), Hormona de crecimiento, Factor de crecimiento similar a EGF de unión a heparina (" HB-EGF "), Crecimiento de hepatocitos factor ("HGF"), proteína de unión al factor de crecimiento similar a insulina 1 ("IGFBP-1"), proteína 2 de unión al factor de crecimiento similar a insulina ("IGFBP-2"), proteína 3 de unión al factor de crecimiento similar a insulina ("IGFBP" -3 "), proteína 4 de unión al factor de crecimiento similar a la insulina (" IGFBP-4 "), insulina proteína de unión al factor de crecimiento in-like 6 ("IGFBP-6"), factor de crecimiento insulínico 1 ("IGF-1"), insulina, factor estimulante de colonias de macrófagos ("M-CSF R"), receptor del factor de crecimiento nervioso ("NGF R"), Neurotrofina-3 ("NT-3"), Neurotrofina-4 ("NT-4"), factor inhibidor de la osteoclastogénesis ("Osteoprotegerin"), receptores del factor de crecimiento derivado de plaquetas ("PDGF-AA"), Biosíntesis de fosfatidilinositol-glicano ("PIGF"), Skp, Cullin, F-box que contiene comples ("SCF"), receptor del factor de células madre ("SCF R"), factor de crecimiento alfa transformante ("TGFalpha"), crecimiento transformante factor beta-1 ("TGF beta 1"), factor de crecimiento beta-3 transformante ("TGF beta 3"), factor de crecimiento endotelial vascular ("VEGF"), receptor 2 del factor de crecimiento endotelial vascular ("VEGFR2"), factor de creecimiento endotelial vascular receptor 3 ("VEGFR3"), VEGF-D 6Ckine, receptor de tirosina-proteína quinasa UFO ("Axl"), Betacellulin ("BTC"), quimioquina epitelial asociada a mucosa ("CCL28"),

quimioquina (motivo CC) ligando 27 ("CTACK"), quimiocina (motivo CXC) li gand 16 ("CXCL16"), motivo CXC quimioquina 5 ("ENA-78"), quimioquina (motivo CC) ligando 26 ("Eotaxin-3"), proteína quimiotáctica granulocito 2 ("GCP-2"), GRO, quimioquina (Motivo CC) ligando 14 ("HCC-1"), ligando quimiocina (motivo CC) 16 ("HCC-4"), interleucina-9 ("IL-9"), interleucina-17 F ("IL-17F")), Proteína de unión a interleucina-18 ("IL-18 BPa"), interleucina-28 A ("IL-28A"), interleucina 29 ("IL-29"), interleucina 31 ("IL-31"), CXC motivo quimioquina 10 ("IP-10"), receptor de quimioquina CXCR3 ("I-TAC"), factor inhibidor de la leucemia ("LIF"), ligando de luz, quimiocina (motivo C) ("linfotactina"), proteína 2 quimiotáctica de monocitos ("MCP-2"), proteína chemoattractant de monocitos 3 ("MCP-3"), proteína chemoattractant de monocitos 4 ("MCP-4"), quimioquina derivada de macrófagos ("MDC"), factor inhibidor de migración de macrófagos ("MIF"), Ligando 20 de quimiocina (motivo CC) ("MIP-3 alfa"), quimioquina CC mot motif 19 ("MIP-3 beta"), ligando 23 de quimioquina (motivo CC) ("MPIF-1"), cadena alfa de proteína estimulante de macrófagos ("MSPalpha"), Nucleoso me ensamble proteína 1-like 4 ("NAP-2"), fosfoproteína secretada 1 ("Osteopontin"), citocina pulmonar y regulada por activación ("PARC"), factor plaquetario 4 ("PF4"), factor derivado de células del Stroma -1 alfa ("SDF-1 alpha"), quimioquina (motivo CC) ligando 17 ("TARC"), quimioquina expresada en el timo ("TECK"), linfopoyetina del estroma tímico ("TSLP 4-IBB"), antígeno CD 166 ("ALCAM"), grupo de diferenciación 80 ("B7-1"), miembro de la superfamilia 17 del receptor del factor de necrosis tumoral ("BCMA"), grupo de diferenciación 14 ("CD14 "), Grupo de diferenciación 30 (" CD30 "), grupo de diferenciación 40 (" ligando CD40 "),

molécula 1 de adhesión celular relacionada con antígeno carcinoembrionario (glucoproteína biliar) (" CEACAM-1 "), receptor de muerte 6 (" DR6 " "), Desoxitimidina quinasa (" Dtk "), glicoproteína de membrana tipo 1 (" Endoglina "), receptor tirosina-proteína quinasa erbB-3 (" ErbB3 "), molécula de adhesión leucocitaria endotelial 1 (" E-Selectina "), apoptosis antígeno 1 ("Fas"), tirosina quinasa 3 similar a Fms ("Flt-3L"), miembro 1 de la superfamilia del receptor del factor de necrosis tumoral ("GITR"), miembro 14 de la superfamilia del receptor del factor de necrosis tumoral ("HVEM"), adhesión intercelular molécula 3 ("ICAM-3"), IL-1 R4, IL-1 RI, IL-10 Rbeta, IL-17R, IL-2Rgamma, IL-21R, proteína de membrana Lysosome 2 ("LIMPII"), gelatinasa de neutrófilos- lipocalina asociada ("Lipocalin-2"), CD62L ("L-Selectin"), Lymp endotelio hepático ("LYVE-1"), secuencia A relacionada con el polipéptido MHC de clase I ("MICA"), secuencia B relacionada con polipéptido MHC de clase I ("MICB"), NRG1-betal, factor de crecimiento derivado de plaquetas de tipo beta receptor ("PDGF Rbeta"), molécula de adhesión de células endoteliales plaquetarias ("PECAM-1"), RAGE, receptor 1 del virus de la hepatitis A ("TIM-1"), miembro de la superfamilia del receptor del factor de necrosis tumoral ("TRAIL R3"), Dominio de unión a transglutaminasa de proteína Trappin ("Trappin-2"), receptor Urokinase ("uPAR"), proteína 1 de adhesión celular vascular ("VCAM-1"), XedARActivin A, proteína relacionada con Agouti ("AgRP"), ribonucleasa 5 ("Angiogenina"), angiopoyetina 1, angiostatina, cateprina S, CD40, proteína Cryptic de la familia IB ("Cripto-1"), DAN, proteína relacionada con Dickkopf 1 ("DKK-1"), E-Cadherina, adhesión de células epiteliales molécula ("EpCAM"), Fas Ligando (FasL o CD95L),

Fcg RIIB/C, FoUistatin, Galectin-7, molécula de adhesión intercelular 2 ("ICAM-2"), IL-13 R1, IL-13R2, IL-17B, IL-2 Ra, IL-2 Rb, IL-23, LAP, molécula de adhesión de células neuronales (" NrCAM "), inhibidor del activador del plasminógeno-1 (" PAI-1 "), receptores del factor de crecimiento derivado de plaquetas (" PDGF-AB "), Resistina, factor 1 derivado de las células estromales (" SDF-1 beta "), sgpl30, Secreted proteína 2 relacionada con frizzled ("ShhN"), lectinas de tipo inmunoglobulina de unión a ácido siálico ("Siglec-5"), ST2, factor de crecimiento transformante-beta 2 ("TGF beta 2"), Tie-2, trombopoyetina (" TPO "), miembro 10D de la superfamilia del receptor del factor de necrosis tumoral (" TRAIL R4 "), receptor desencadenante expresado en células mieloides 1 (" TREM-1 "), factor C vascular del crecimiento endotelial (" VEGF-C "), VEGFRlAdiponectina, Adipsina ("Y"), alfa-fetoproteína ("AFP"), angiopoyetina-4 ("ANGPTL4"), beta-2-microglobulina ("B2M"), molécula de adhesión de células basales ("BCAM"), antígeno 125 de carbohidratos (" CA125 "), antígeno del cáncer 15-3 (" CA15-3 "), antígeno carcinoembrionario (" CEA "), proteína del receptor cAMP (" CRP "), receptor del factor de crecimiento epidérmico humano 2 ("ErbB2"), Follistatin, hormona foliculoestimulante ("FSH"), quimioquina (motivo CXC) ligando 1 ("GRO alfa"), gonadotropina coriónica humana ("beta HCG"), receptor del factor 1 de crecimiento similar a la insulina ("IGF-1 sR"), IL-1 sRII, IL-3, IL-18 Rb, IL-21, Leptina, Metaloproteinasa-1 de la matriz ("MMP-1"), Metaloproteinasa-2 de la matriz ("MMP-2"), Metaloproteinasa-3 de la matriz ("MMP-3"), Metaloproteinasa-8 de la matriz ("MMP-8"), Metaloproteinasa-9 de la matriz ("MMP-9"),

Metaloproteinasa-10 de la matriz ("MMP-10"), Metaloproteinasa-13 de la matriz (" MMP-13 "), Molécula de Adhesión de Células Neurales (" NCAM-1 "), Entactina (" Nidogen-1 "), enolasa específica de neuronas (" NSE "), Oncostatina M (" OSM "), Procalcitonina, Prolactina, Específico de próstata antígeno ("PSA"), lectina 9 similar a Ig de unión a ácido siálico ("Siglec-9"), ADAM 17 endopeptidasa ("TACE"), tiroglobulina, inhibidor de metaloproteinasa 4 ("TIMP-4"), TSH2B4, desintegrina y proteína que contiene el dominio metaloproteinasa 9 ("ADAM-9"), angiopoyetina 2, miembro de la superfamilia del ligando del factor de necrosis tumoral 13/grupo ácido rico en leucina fosfoproteína clara 32 miembro de la familia B ("APRIL"), proteína morfogenética ósea 2 ("BMP-2"), proteína morfogenética ósea 9 ("BMP-9"), componente del complemento 5a ("C5a"), catepsina l,CD200, CD97, Chemerin, miembro de la superfamilia del receptor del factor de necrosis tumoral 6B ("DcR3"), proteína 2 de unión al ácido graso ("FABP2"), proteína de activación del fibroblasto, alfa ("FAP"), factor de crecimiento fibroblástico 19 ("FGF-19"), Galectin-3, receptor del factor de crecimiento hepatocítico ("HGF R"), IFN-gammalpha/beta R2, factor de crecimiento similar a la insulina 2 ("IGF-2"), receptor del factor de crecimiento 2 similar a la insulina ("IGF-2 R "), receptor 6 de interleucina-1 (" IL-1R6 "), interleucina 24 (" IL-24 "), interleucina 33 (" IL-33 ", calicreína 14, asparaginil endopeptidasa (" Legumain "), oxidado receptor de lipoproteína de densidad 1 ("LOX-1"), lectina de unión a manosa ("MBL"), neprilisina ("NEP"), homólogo de Notch 1, asociado a translocación (Drosophila) ("Notch-1"), sobreexpresión de nefroblastoma ("NOV"), Osteoactivina, proteína de muerte celular programada 1 ("PD-1"), N-

acetilmuramoil-L-alanina a midasa ("PGRP-5"), Serpin A4, proteína relacionada con Frizzled secretada 3 ("sFRP-3"), trombomodulina, receptor Tolllike 2 ("TLR2"), miembro de la superfamilia 10A del receptor del factor de necrosis tumoral ("TRAIL R1"), Transferrina ("TRF"), WIF-lACE-2, albúmina, AMICA, angiopoyetina 4, factor activador de células B ("BAFF"), antígeno carbohidratado 19-9 ("CA19-9"), CD 163, clusterina, CRT AM, quimiocina (motivo CXC) ligando 14 ("CXCL14"), cistatina C, Decorin ("DCN"), proteína relacionada con Dickkopf 3 ("Dkk-3"), proteína tipo Delta ("DLL1"), Fetuin A, factor de crecimiento de unión a heparina 1 ("aFGF"), receptor de folato alfa ("FOLR1"), Furin, proteína de clasificación 1 asociada a GPCR ("GASP-1"), proteína de clasificación 2 asociada a GPCR ("GASP-2" "), Receptor de factor estimulante de colonias de granulocitos (" GCSF R "), serina proteasa hepsina (" HAI-2 "), receptor de interleucina-17B (" IL-17B R "), interleucina 27 (" IL-27 "), El gen 3 de activación de linfocitos ("LAG-3"), la apolipoproteína AV ("LDL R"), el pepsinógeno I, la proteína 4 de unión a retinol ("RBP4"), el SOST, el proteoglicano sulfato de heparán (" Syndecan-1 "), miembro de la superfamilia del receptor del factor de necrosis tumoral 13B (" TACI "), inhibidor de la vía del factor tisular (" TFPI "), TSP-1, superfamilia del receptor del factor de necrosis tumoral, miembro 10b (" TRAIL R2 "), TRANCE, Troponina I, activador del plasminógeno Urokinase ("uPA"), Cadherin 5, tipo 2 o VE-cadherina (endotelio vascular) también conocida como CD144 ("VE-Cadherin"), proteína 1 de la ruta de señalización inducible por WNTl ("WISP-1 ") y el activador del receptor del factor nuclear κ B (" RANK ").

En algunas realizaciones, el agente terapéutico contra

el cáncer es un resto radiactivo que comprende un radionúclido. Ejemplos de radionucleidos incluyen, pero no están limitados a 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, and Y-90.

En algunas realizaciones, el agente terapéutico contra el cáncer es un inhibidor de la angiogénesis para el sujeto. Los ejemplos de tales inhibidores de angiogénesis incluyen, pero no se limitan a, Bevacizumab (Avastin®), Ziv-aflibercept (Zaltrap®), Sorafenib (Nexavar®), Sunitinib (Sutent®), Pazopanib (Votrient®), Regorafenib (Stivarga®), y Cabozantinib (Cometriq ™).

En algunas realizaciones, el agente terapéutico contra el cáncer es un antibiótico. Por ejemplo, si se detecta la presencia de una bacteria asociada al cáncer y/o un perfil de microbioma asociado al cáncer de acuerdo con los métodos proporcionados en este documento, se pueden administrar antibióticos para eliminar las bacterias asociadas al cáncer del sujeto. "Antibióticos" se refiere ampliamente a compuestos capaces de inhibir o prevenir una infección bacteriana. Los antibióticos se pueden clasificar de varias maneras, incluido su uso para infecciones específicas, su mecanismo de acción, su biodisponibilidad o su espectro de microbios objetivo (p. Ej., Bacterias Gram negativas frente a Gram positivas, bacterias aerobias frente a anaerobias,

etc.) y pueden usarse para matar bacterias específicas en áreas específicas del huésped ("nichos") (Leekha, et al 2011. General Principles of Antimicrobial Therapy. Mayo Clin Proc. 86(2): 156-167). En ciertas realizaciones, los antibióticos pueden usarse para dirigirse selectivamente a las bacterias de un nicho específico. En algunas realizaciones, los antibióticos conocidos para tratar una infección particular que incluye un nicho de cáncer pueden usarse para dirigirse a microbios asociados al cáncer, incluyendo bacterias asociadas al cáncer en ese nicho. En otras realizaciones, los antibióticos se administran después del tratamiento bacteriano. En algunas realizaciones, se administran antibióticos después del tratamiento bacteriano para eliminar el injerto.

En algunos aspectos, los antibióticos se pueden seleccionar en función de sus propiedades bactericidas o bacteriostáticas. Los antibióticos bactericidas incluyen mecanismos de acción que interrumpen la pared celular (por ejemplo, \ beta - lactamas), la membrana celular (por ejemplo, daptomicina) o el ADN bacteriano (por ejemplo, fluoroquinolonas). Los agentes bacteriostáticos inhiben la replicación bacteriana e incluyen sulfonamidas, tetraciclinas y macrólidos, y actúan inhibiendo la síntesis de proteínas. Además, aunque algunos fármacos pueden ser bactericidas en ciertos organismos y bacteriostáticos en otros, conocer al organismo diana permite a un experto en la técnica seleccionar un antibiótico con las propiedades apropiadas. En ciertas condiciones de tratamiento, los antibióticos bacteriostáticos inhiben la actividad de los antibióticos bactericidas. Por lo tanto, en ciertas realizaciones, los antibióticos bactericidas y bacteriostáticos no se combinan.

Los antibióticos incluyen, a modo no taxativo, aminoglucósidos, ansamicinas, carbacefemas, carbapenémicos, cefalosporinas, glucopéptidos, lincosamidas, lipopéptidos, macrólidos, monobactámicos, nitrofuranos, oxazolidononas, penicilinas, antibióticos polipeptídicos, quinolonas, fluoroquinolonas, sulfonamidas, tetraciclinas y compuestos antimicobacterianos. y combinaciones de los mismos

Los aminoglucósidos incluyen, pero no se limitan a, amikacina, gentamicina, kanamicina, neomicina, netilmicina, tobramicina, paromomicina y espectinomicina. Los aminoglucósidos son efectivos, por ejemplo, contra bacterias Gram-negativas, tales como Escherichia coli, Klebsiella, Pseudomonas aeruginosa y Francisella tularensis, y contra ciertas bacterias aeróbicas, pero menos eficaces contra anaerobios obligados/facultativos. Se cree que los aminoglucósidos se unen a la subunidad ribosómica bacteriana 30S o 50S, inhibiendo así la síntesis de proteínas bacterianas.

Las ansamicinas incluyen, pero no se limitan a, Geldanamicina, Herbimicina, Rifamicina y Estreptovaricina. Se cree que Geldanamycin and Herbimycin inhiben o alteran la función de Heat Shock Protein 90.

Los carbaceofes incluyen, pero no se limitan a, Loracarbef. Se cree que los carbaceofens inhiben la síntesis bacteriana de la pared celular.

Los carbapenémicos incluyen, pero no se limitan a, Ertapenem, Doripenem, Imipenem/Cilastatin y Meropenem. Los carbapenémicos son bactericidas tanto para bacterias Gram-positivas como Gram-negativas como antibióticos de amplio espectro. Se cree que los carbapenémicos inhiben la síntesis bacteriana de la pared celular.

Las cefalosporinas incluyen, entre otras, cefadroxilo,

cefazolina, cefalotina, cefalotina, cefalexina, cefaclor, cefamandol, cefoxitina, cefprozil, cefuroxima, cefixima, cefdinir, cefditoren, cefoperazona, cefotaxima, cefpodoxima, ceftazidima, ceftibuten, ceftizoxima, ceftriaxona, cefepima., Ceftaroline fosamil y Ceftobiprole. Las Cefalosporinas seleccionadas son efectivas, por ejemplo, contra bacterias Gram-negativas y contra bacterias Gram-positivas, incluyendo Pseudomonas, ciertas Cefalosporinas son eficaces contra Staphylococcus aureus resistente a la meticilina (SARM). Se cree que las cefalosporinas inhiben la síntesis bacteriana de la pared celular al alterar la síntesis de la capa de peptidoglicanos de las paredes celulares bacterianas.

Los glucopéptidos incluyen, pero no se limitan a, Teicoplanina, Vancomicina y Telavancina. Los glucopéptidos son eficaces, por ejemplo, contra bacterias Gram-positivas aerobias y anaeróbicas, incluyendo MRSA y Clostridium difficile. Se cree que los glucopéptidos inhiben la síntesis bacteriana de la pared celular al alterar la síntesis de la capa de peptidoglicano de las paredes celulares bacterianas.

Las lincosamidas incluyen, entre otras, clindamicina y lincomicina. Las lincosamidas son efectivas, por ejemplo, contra bacterias anaeróbicas, así como contra Staphylococcus y Streptococcus. Se cree que las lincosamidas se unen a la subunidad ribosómica 50S bacteriana, inhibiendo así la síntesis de proteínas bacterianas.

Los lipopéptidos incluyen, pero no están limitados a, Daptomicina. Los lipopéptidos son efectivos, por ejemplo, contra bacterias Gram-positivas. Se cree que los lipopéptidos se unen a la membrana bacteriana y causan una

despolarización rápida.

Los macrólidos incluyen, pero no se limitan a, azitromicina, claritromicina, diritromicina, eritromicina, roxitromicina, troleandomicina, telitromicina y espiramicina. Los macrólidos son efectivos, por ejemplo, contra Streptococcus y Mycoplasma. Se cree que los macrólidos se unen a la subunidad bacteriana o ribosómica 50S, lo que inhibe la síntesis de proteínas bacterianas.

Los monobactámicos incluyen, pero no se limitan a, Aztreonam. Los monobactámicos son efectivos, por ejemplo, contra bacterias Gram-negativas. Se cree que los monobactámicos inhiben la síntesis bacteriana de la pared celular al alterar la síntesis de la capa de peptidoglicano de las paredes celulares bacterianas..

Los nitrofuranos incluyen, entre otros, Furazolidona y Nitrofurantoína.

Las oxazolidononas incluyen, pero sin limitación, Linezolid, Posizolid, Radezolid y Torezolid. Se cree que las oxazolidononas son inhibidores de la síntesis de proteínas.

Las penicilinas incluyen, pero no están limitadas a, Amoxicilina, Ampicilina, Azlocilina, Carbenicilina, Cloxacilina, Dicloxacilina, Flucloxacilina, Mezlocilina, Meticilina, Nafcilina, Oxacilina, Penicilina G, Penicilina V, Piperacilina, Temocilina y Ticarcilina. Las penicilinas son efectivas, por ejemplo, contra bacterias Gram-positivas, anaerobios facultativos, por ejemplo, Streptococcus, Borrelia y Treponema. Se cree que las penicilinas inhiben la síntesis bacteriana de la pared celular al alterar la síntesis de la capa de peptidoglucano de las paredes celulares bacterianas.

Las combinaciones de penicilina incluyen, pero no se

limitan a, amoxicilina/clavulánico, ampicilina/sulbactam, piperacilina/tazobactam y ticarcilina/clavulanato.

Los antibióticos polipeptídicos incluyen, pero no se limitan a, Bacitracin, Colistin, y Polymyxin B y E. Polipéptido Los antibióticos son efectivos, por ejemplo, contra bacterias Gram-negativas. Se cree que ciertos antibióticos polipeptídicos inhiben el isoprenil pirofosfato implicado en la síntesis de la capa de peptidoglucano de las paredes celulares bacterianas, mientras que otros desestabilizan la membrana bacteriana externa al desplazar contraiones bacterianos.

Las quinolonas y la fluoroquinolona incluyen, entre otras, ciprofloxacina, enoxacina, gatifloxacina, gemifloxacina, levofloxacina, lomefloxacina, moxifloxacina, ácido nalidíxico, norfloxacina, ofloxacina, trovafloxacina, grepafloxacina, esparfloxacina y temafloxacina. Las quinolonas/fluoroquinolona son efectivas, por ejemplo, contra Streptococcus y Neisseria. Se cree que las quinolonas/fluoroquinolonas inhiben la ADN girasa bacteriana o la topoisomerasa IV, lo que inhibe la replicación y la transcripción del ADN.

Las sulfonamidas incluyen, pero no se limitan a, mafenida, sulfacetamida, sulfadiazina, sulfadiazina de plata, sulfadimetoxina, sulfamethizole, sulfametoxazol, sulfanilimida, sulfasalazina, sulfisoxazol, trimetoprim-sulfametoxazol (cotrimoxazol) y sulfonamidocrysoidina. Se cree que las sulfonamidas inhiben la síntesis de folato mediante la inhibición competitiva de la dihidropteroato sintetasa, inhibiendo así la síntesis de ácidos nucleicos.

Las tetraciclinas incluyen, pero no se limitan a Demeclocycline, Doxycycline, Minocycline, Oxytetracycline y Tetracycline. Las tetraciclinas son efectivas, por ejemplo,

contra bacterias Gram-negativas. Se cree que las tetraciclinas se unen a la subunidad ribosómica 30S bacteriana, inhibiendo así la síntesis de proteínas bacterianas.

Los compuestos anti-micobacterianos incluyen, pero sin limitación, Clofazimina, Dapsona, Capreomicina, Cicloserina, Etambutol, Etionamida, Isoniacida, Pirazinamida, Rifampicina, Rifabutina, Rifapentina y Estreptomicina.

Los antibióticos adecuados también incluyen arsfenamina, cloranfenicol, fosfomicina, ácido fusídico, metronidazol, mupirocina, platensimicina, quinupristina/dalfopristina, tigeciclina, tinidazol, amoxicilina trimetoprim/clavulánico, ampicilina/sulbactam, anftoximis ristocetina, azitromicina, bacitracina, buforina II, carbomicina, cecropina Pl, claritromicina, eritromicinas, furazolidona, ácido fusídico, Na fusidato, gramicidina, imipenem, indolicidina, josamicina, magainan II, metronidazol, nitroimidazoles, mikamicina, mutacina B-Ny266, mutacina B-JH1 140, mutacina J-T8, nisina, nisina A, novobiocina, oleandomicina, ostreogrycin, piperacilina/tazobactam, pristinamicina, ramoplanina, ranalexina, reuterina, rifaximina, rosamicina, rosaramicina, espectinomicina, espiramicina, estafylomicina, estreptogramina, estreptogramina A, sinergistina, taurolidina, teicoplanina, telitromicina, ticarcilina/ácido clavulánico, triacetiloleandomicina, tilosina, tirocidina, tirotricina, vancomicina, vemamicina y virginiamicina.

En algunas realizaciones, la terapia contra el cáncer comprende administrar una bacteria terapéutica y/o una combinación terapéutica de bacterias al sujeto de modo que

se puede reconstituir un microbioma saludable en el sujeto. En algunas realizaciones, la bacteria terapéutica es una bacteria no asociada al cáncer. En algunas realizaciones, la bacteria terapéutica es una bacteria probiótica.

Cáncer

En algunas realizaciones, los métodos y composiciones descritos en la presente memoria se refieren al tratamiento del cáncer. Los ejemplos de cánceres que pueden tratarse mediante los métodos descritos en el presente documentoincluyen, pero no se limitan a, neoplasia maligna hematológica, leucemia no linfocítica aguda, leucemia linfocítica crónica, leucemia granulocítica aguda, leucemia granulocítica crónica, leucemia promielocítica aguda, leucemia de células T en adultos, leucemia aleucémica, leucemia leucocítica, leucemia basófila, leucemia de células blásticas, leucemia bovina, leucemia mielocítica crónica, leucemia cutánea, leucemia embrionaria, leucemia eosinofílica, leucemia gruesa, leucemia de células Rieder, leucemia de Schilling, leucemia de células madre, leucemia subleucémica, leucemia celular indiferenciada, peluda - leucemia celular, leucemia hemoblástica, leucemia hemocitoblástica, leucemia histiocítica, leucemia de células madre, leucemia monocítica aguda, leucemia leucopénica, leucemia linfática, leucemia linfoblástica, leucemia linfocítica, leucemia linfoide, leucemia linfoide, leucemia de células de linfosarcoma, leucemia de mastocitos, leucemia megacariocítica leucemia micromieloblástica, monocito tic leucemia, leucemia mieloblástica, leucemia mielocítica, leucemia mieloide granulocítica, leucemia mielomonocítica, leucemia de Naegeli, leucemia de células plasmáticas, leucemia plasmocítica, leucemia promielocítica, carcinoma acinar,

carcinoma acelular, carcinoma adenocístico, carcinoma quístico adenoide, carcinoma adenomatoso, carcinoma de la corteza suprarrenal, carcinoma alveolar, carcinoma de células alveolares, carcinoma basocelular, carcinoma basocelular, carcinoma basaloide, carcinoma basoescamoso, carcinoma bronquioalveolar, carcinoma bronquiolar, carcinoma broncogénico, carcinoma cerebriforme, carcinoma colangiocelular, carcinoma coriónico, carcinoma coloide, carcinoma comedo, carcinoma corpuscreático, carcinoma cribiforme, carcinoma en cuirasse, carcinoma cutáneo, carcinoma cilíndrico, carcinoma de células cilíndricas, carcinoma ductal, carcinoma durum, carcinoma embrionario, carcinoma encefaloide, carcinoma epienoide, adenoides epiteliales del carcinoma, carcinoma exofítico, carcinoma ex ulcerado, carcinoma fibroso, gelatinos formar carcinoma, carcinoma gelatinoso, carcinoma de células gigantes, carcinoma de células en anillo de sello, carcinoma simple, carcinoma de células pequeñas, carcinoma solanoide, carcinoma de células esferoidales, carcinoma de células fusiformes, carcinoma esponjoso, carcinoma escamoso, carcinoma de células escamosas, carcinoma de cadena, carcinoma telangiectásico, carcinoma telangiectásico, carcinoma de células transicionales, carcinoma tuberoso, carcinoma tuberoso, carcinoma verrucoso, carcinoma villoso, carcinoma gigantocelular, carcinoma glandular, carcinoma de células de la granulosa, carcinoma de la matriz del cabello, carcinoma hematoide, carcinoma hepatocelular, carcinoma de células de Hurthle, carcinoma hialino, carcinoma hipernefroide, carcinoma embrionario infantil, carcinoma in situ, carcinoma intraepidérmico, carcinoma intraepitelial, carcinoma de Krompecher, carcinoma de células Kulchitzky, carcinoma de células grandes, carcinoma

lenticular, carcinoma lenticular, carcinoma lipomatoso, carcinoma linfoepitelial, carcinoma medular, carcinoma medular, carcinoma melanótico, carcino inoma molle, carcinoma mucinoso, carcinoma muciparum, carcinoma mucocelular, carcinoma mucoepidermoide, carcinoma mucoso, carcinoma mucoso, mixomatodes de carcinoma, carcinoma nasofaríngeo, carcinoma de células de avena, carcinoma osificante, carcinoma osteoide, carcinoma papilar, carcinoma periportal, carcinoma preinvasivo, carcinoma de células de prickle, carcinoma pituitario, carcinoma de riñón de células renales, carcinoma de células de reserva, sarcomatoides de carcinoma, carcinoma de schneider, carcinoma de esclerótica, carcinoma de escroto, condrosarcoma, fibrosarcoma, linfosarcoma, melanosarcoma, miosarcoma, osteosarcoma, sarcoma de endometrio, sarcoma de estroma, sarcoma de Ewing, sarcoma de fascia, sarcoma fibroblástico, sarcoma de células gigantes, sarcoma de Abemethy, sarcoma adiposo, liposarcoma, sarcoma de parte blanda alveolar, sarcoma ameloblástico, sarcoma de botryoid, sarcoma de cloroma, carcinoma de corio, sarcoma embrionario, sarcoma de tumor de Wilms, sarcoma granulocítico, sarcoma de Hodgkin, pigmentación múltiple idiopática sarcoma hemorrágico, inmun sarcoma obtástico de células B, linfoma, sarcoma inmunoblástico de células T, sarcoma de Jensen, sarcoma de Kaposi, sarcoma de células de Kupffer, angiosarcoma, leucosarcoma, sarcoma mesenquimoma maligno, sarcoma parosteal, sarcoma reticulocítico, rabdosarcoma, sarcoma serosocístico, sarcoma sinovial, sarcoma telangiectásico, enfermedad de Hodgkin, linfoma no Hodgkin, mieloma múltiple, neuroblastoma, cáncer de vejiga, cáncer de mama, cáncer de ovario, cáncer de pulmón, cáncer colorrectal, rabdomiosarcoma, trombocitosis primaria,

macroglobulinemia primaria, los tumores de pulmón de células pequeñas, tumores cerebrales primarios, cáncer de estómago, cáncer de colon, insulanoma maligno pancreático, carcinoide maligno, lesiones de la piel premalignas, cáncer testicular, linfomas, cáncer de tiroides, neuroblastoma, cáncer de esófago, cáncer del tracto genitourinario, hipercalcemia maligna, cáncer cervical, cáncer endometrial, cáncer de la corteza suprarrenal, Harding-Passey melanoma, melanoma juvenil, melanoma lentigo maligno, melanoma maligno, melanoma acral-lentiginoso, melanoma amelanótico, melanoma juvenil benigno, melanoma de Cloudman, S91 melanoma, melanoma nodular subungueal melanoma y melanoma de extensión superficial.

En algunas realizaciones, los métodos y composiciones proporcionados en el presente documentose refieren al tratamiento de una leucemia. El término "leucemia" se entiende enfermedades ampliamente progresivos, malignas de los órganos/sistemas hematopoyéticos y se caracteriza generalmente por una proliferación y desarrollo distorsionados de los leucocitos y sus precursores en la sangre y la médula ósea. ejemplos de enfermedades leucemia incluyen, leucemia aguda no linfocítica, leucemia linfocítica crónica, leucemia granulocítica aguda, leucemia granulocítica crónica, leucemia promielocítica aguda, leucemia de células T adultas, leucemia aleucémica, una leucemia leukocythemic, leucemia basófila, la leucemia de células blásticas, bovino no limitantes leucemia, leucemia mielocítica crónica, leucemia cutánea, leucemia embolígena, leucemia eosinofílica, leucemia gruesa, leucemia de células Rieder, leucemia de Schilling, leucemia de células madre, leucemia subleucémica, leucemia celular indiferenciada, leucemia de células pilosas, leucemia hemoblástica,

leucemia hemocitoblástica, leucemia histiocítica, leucemia de células madre, leucemia monocítica aguda, leucemia leucopénica, leucemia linfoblástica, leucemia linfoblástica, leucemia linfocítica, leucemia linfoide, leucemia linfoide, leucemia de células de linfosarcoma, leucemia de mastocitos, leucemia megacariocítica, leucemia micromieloblástica, leucemia monocítica, leucemia mieloblástica, leucemia mielocítica, granul mieloide leucemia ocítica, leucemia mielomonocítica, leucemia de Naegeli, leucemia de células plasmáticas, leucemia plasmocítica y leucemia promielocítica.

En algunas realizaciones, los métodos y composiciones proporcionados en el presente documentose refieren al tratamiento de un carcinoma. El término "carcinoma" se refiere a un crecimiento maligno formado por células epiteliales que tienden a infiltrarse en los tejidos circundantes y/o a resistir señales de muerte celular fisiológicas y no fisiológicas y da lugar a metástasis. Los tipos ejemplares no limitativos de carcinomas incluyen, carcinoma acinar, carcinoma acelular, carcinoma adenocístico, carcinoma quístico adenoide, carcinoma adenomatoso, carcinoma de corteza suprarrenal, carcinoma alveolar, carcinoma de células alveolares, carcinoma de células basales, carcinoma basocelular, carcinoma basaloide, carcinoma de células basoescamosas, carcinoma bronquioalveolar, carcinoma bronquiolar, carcinoma broncogénico, carcinoma cerebriforme, carcinoma colangiocelular, carcinoma coriónico, carcinoma coloidal, carcinoma comedo, carcinoma corpus, carcinoma cribiforme, carcinoma en cuirasse, carcinoma cutáneo, carcinoma cilíndrico, carcinoma cilíndrico, carcinoma ductal, carcinoma durum, carcinoma embrionario, carcinoma

encefaloide, carcinoma epienoide, adenoides epiteliales del carcinoma, carcinoma exofítico, carcinoma ex ulcerado, carcinoma fibroso, carcinoma gelatiniforme, carcinoma gelatinoso, carcinoma de células gigantes, carcinoma de células en anillo de sello, carcinoma simple, carcinoma de células pequeñas, carcinoma solanoide, carcinoma de células fusiformes, carcinoma de células fusiformes, carcinoma esponjoso, carcinoma escamoso, carcinoma de células escamosas, carcinoma de cadena, carcinoma telangiectásico, carcinoma telangiectásico, carcinoma de células transicionales, carcinoma tuberoso, carcinoma tuberoso, carcinoma verrucoso, carcinoma villoso, carcinoma gigantocelular, carcinoma glandular, granulosa carcinoma de células, carcinoma de la matriz del cabello, carcinoma hematoide, carcinoma hepatocelular, carcinoma de células de Hurthle, carcinoma hialino, carcinoma hipernefroideo, carcinoma embrionario infantil, carcinoma in situ, carcinoma intraepidérmico, carcinoma intraepitelial, carcinoma de Krompecher, carcinoma de células Kulchitzky, carcinoma de células grandes, carcinoma lenticular, carcinoma lenticular, carcinoma lipomatoso, carcinoma linfoepitelial, carcinoma medular, carcinoma medular, carcinoma melanótico, carcinoma molle, carcinoma mucinoso, carcinoma muciparum, carcinoma mucocelular, carcinoma mucoepidermoide, carcinoma mucoso, carcinoma mucoso, carcinoma oma mixomas, carcinoma nasofaríngeo, carcinoma de avena, carcinoma osificante, carcinoma osteoide, carcinoma papilar, carcinoma periportal, carcinoma preinvasivo, carcinoma espinocelular, carcinoma pultaceo, carcinoma de riñón de células renales, carcinoma de células de reserva, sarcomatoides de carcinoma, carcinoma schneideriano, carcinoma escirótico y carcinoma scroti.

En algunas realizaciones, los métodos y composiciones proporcionados en el presente documentose refieren al tratamiento de un sarcoma. El término "sarcoma" generalmente se refiere a un tumor que se compone de una sustancia como el tejido conectivo embrionario y generalmente está compuesto por células estrechamente empaquetadas incrustadas en una sustancia fibrilar, heterogénea u homogénea. Los sarcomas incluyen, pero no se limitan a, condrosarcoma, fibrosarcoma, linfosarcoma, melanosarcoma, myxosarcoma, osteosarcoma, sarcoma endometrial, sarcoma estromal, sarcoma de Ewing, sarcoma fascial, sarcoma fibroblástico, sarcoma de células gigantes, sarcoma de Abemethy, sarcoma adiposo, liposarcoma, sarcoma de parte blanda alveolar, sarcoma ameloblástico, sarcoma de botryoid, sarcoma de cloroma, carcinoma de corio, sarcoma embrionario, sarcoma de tumor de Wilms, sarcoma granulocítico, sarcoma de Hodgkin, sarcoma hemorrágico pigmentado múltiple idiopático, sarcoma inmunoblástico de células B, linfoma, sarcoma de células T inmunoblástico, sarcoma de Jensen, sarcoma de Kaposi, sarcoma de células de Kupffer, angiosarcoma, leucosarcoma, maligno mesenquimoma sarcoma, sarcoma de parosteal, sarcoma reticulocítico, sarcoma de Rous, sarcoma seroquístico, sarcoma sinovial y sarcoma telangiectaltic.

Neoplasias ejemplares adicionales que pueden tratarse usando los métodos y composiciones descritos en el presente documentoincluyen enfermedad de Hodgkin, linfoma no Hodgkin, mieloma múltiple, neuroblastoma, cáncer de mama, cáncer de ovario, cáncer de pulmón, rabdomiosarcoma, trombocitosis primaria, macroglobulinemia primaria, tumores de pulmón de células pequeñas, tumores cerebrales primarios, cáncer de estómago, cáncer de colon, insulanoma

pancreático maligno, carcinoide maligno, lesiones premalignas de la piel, cáncer testicular, linfomas, cáncer de tiroides, neuroblastoma, cáncer de esófago, cáncer del tracto genitourinario, hipercalcemia maligna, cáncer de cuello uterino, cáncer de endometrio y suprarrenal cáncer cortical

En algunas realizaciones, el cáncer tratado es un melanoma. El término "melanoma" se refiere a un tumor que surge del sistema melanocítico de la piel y otros órganos. Los ejemplos no limitantes de melanomas son melanoma Harding-Passey, melanoma juvenil, melanoma lentigo maligno, melanoma maligno, melanoma acral-lentiginoso, melanoma amelanótico, melanoma juvenil benigno, melanoma Cloudman, melanoma S91, melanoma nodular, melanoma subungular y melanoma de extensión superficial.

Las categorías particulares de tumores que pueden tratarse usando métodos y composiciones descritos en el presente documentoincluyen trastornos linfoproliferativos, cáncer de mama, cáncer de ovario, cáncer de próstata, cáncer de cuello uterino, cáncer de endometrio, cáncer de hueso, cáncer de hígado, cáncer de estómago, cáncer de colon, cáncer colorrectal, cáncer de páncreas, cáncer de tiroides, cáncer de cabeza y cuello, cáncer del sistema nervioso central, cáncer del sistema nervioso periférico, cáncer de piel, cáncer de riñón, así como metástasis de todo lo anterior. Tipos particulares de tumores incluyen carcinoma hepatocelular, hepatoma, hepatoblastoma, rabdomiosarcoma, carcinoma esofágico, carcinoma tiroideo, ganglioblastoma, fibrosarcoma, mixosarcoma, liposarcoma, condrosarcoma, sarcoma osteogénico, cordoma, angiosarcoma, endoteliosarcoma, tumor de Ewing, leimiosarcoma, rhabdotheliosarcoma, carcinoma ductal invasivo,

adenocarcinoma papilar, melanoma, carcinoma epidermoide pulmonar, carcinoma de células basales, adenocarcinoma (bien diferenciado, moderadamente diferenciado, poco diferenciado o indiferenciado), carcinoma bronquioloalveolar, carcinoma de células renales, hipernefroma, adenocarcinoma hipernefroid, carcinoma del conducto biliar, coriocarcinoma, seminoma, carcinoma embrionario, Tumor de Wilms, tumor testicular, carcinoma de pulmón, incluyendo carcinoma de células pequeñas, carcinoma de pulmón no microcítico y de células grandes, carcinoma de vejiga, glioma, astrocitoma, meduloblastoma, craneofaringioma, ependimoma, pinealoma, retinoblastoma, neuroblastoma, carcinoma de colon, carcinoma rectal neoplasias hematopoyéticas incluyendo todos los tipos de leucemia y linfoma que incluyen: leucemia mielógena aguda, leucemia mielocítica aguda, leucemia linfocítica aguda, leucemia mielógena crónica, leucemia linfocítica crónica, leucemia de mastocitos, mieloma múltiple, linfoma mieloide, linfoma de Hodgkin, no Hodgkin ' s linfoma.

Los cánceres tratados en ciertas realizaciones también incluyen lesiones precancerosas, por ejemplo, queratosis actínica (queratosis solar), lunares (nevos displásicos), quelitis acitínica (labio del granjero), cuernos cutáneos, esófago de Barrett, gastritis atrófica, disqueratosis congénita, disfagia sideropenica, liquen plano, fibrosis submucosa oral, elastosis actínica (solar) y displasia cervical.

Los cánceres tratados en algunas realizaciones incluyen tumores benignos o no cancerosos, por ejemplo, de origen endodérmico, ectodérmico o mesenquimal, que incluyen, pero no se limitan a, colangioma, pólipo de colon, adenoma, papiloma, cistadenoma, adenoma de células

hepáticas, mola hidatidiforme, adenoma tubular renal, papiloma de células escamosas, pólipo gástrico, hemangioma, osteoma, condroma, lipoma, fibroma, linfangioma, leiomioma, rabdomioma, astrocitoma, nevus, meningioma y ganglioneuroma.

EJEMPLOS

Ejemplo 1: Producción de *Bifidobacterium animalis ssp. lactis*

Los medios enriquecidos se utilizan para cultivar y preparar *Bifidobacterium animalis ssp. lactis* para uso *in vitro* e *in vivo*. Por ejemplo, los medios pueden contener azúcar, extractos de levadura, peptonas, oligoelementos y vitaminas. Alternativamente, se pueden preparar medios y *Bifidobacterium animalis ssp. lactis* crecida como muestran Saarela *et al., J. Applied Microbiology*. 2005. 99: 1330-1339, que se incorpora en el presente documento como referencia. Influencia del tiempo de fermentación, crioprotector y neutralización del concentrado de células en la supervivencia de liofilización, estabilidad de almacenamiento y exposición ácida y biliar de células de *Bifidobacterium animalis ssp. lactis* producidas sin ingredientes a base de leche,

A gran escala, los medios están esterilizados. La esterilización puede realizarse mediante un proceso de temperatura ultra alta (UHT). El procesamiento UHT se realiza a temperatura muy alta durante cortos períodos de tiempo. El rango UHT puede ser de 135-180 °C. Por ejemplo, el medio puede esterilizarse entre 10 y 30 segundos a 135 °C.

El inóculo se puede preparar en matraces o en biorreactores más pequeños y se controla el crecimiento. Por ejemplo, el tamaño del inóculo puede estar entre

aproximadamente 0,5 y 3 % del volumen total del biorreactor. Según la aplicación y la necesidad de material, el volumen del biorreactor puede ser de al menos 2 l,10 l,80 l,100 l,250 l,1000 l,2500 l,5000 l,10 000 l.

Antes de la inoculación, el biorreactor está preparado y listo con medio a pH, temperatura y concentración de oxígeno deseados. Por ejemplo, el pH puede establecerse entre 4,5 y 8,0. Durante la fermentación, el pH puede controlarse mediante el uso de hidróxido de sodio, hidróxido de potasio o hidróxido de amonio. La temperatura puede controlarse de 25 °C a 45 °C, por ejemplo a 37 °C. Las condiciones anaeróbicas se crean al reducir el nivel de oxígeno en el caldo de cultivo de alrededor de 8 mg/l a 0 mg/l. Por ejemplo, se pueden usar mezclas de nitrógeno o gas (N_2, CO_2 y H_2) para establecer condiciones anaeróbicas. Alternativamente, no se utilizan gases y las condiciones anaeróbicas se establecen por las células que consumen el oxígeno restante del medio. Dependiendo de la tensión y del tamaño del inóculo, el tiempo de fermentación del biorreactor puede variar. Por ejemplo, el tiempo de fermentación puede variar de aproximadamente 5 horas a 48 horas.

La cosecha puede realizarse por centrifugación continua. El producto se resuspenderá con diversos excipientes hasta la concentración final deseada. Se pueden agregar excipientes para protección criogénica o para protección durante la liofilización. Los excipientes pueden incluir, pero sin limitación, sacarosa, trehalosa o lactosa, y estos pueden mezclarse alternativamente con tampón y antioxidantes. Antes de la liofilización, las gotas de pellets celulares mezcladas con excipientes se sumergen en nitrógeno líquido.

La liofilización del material, incluidas las bacterias vivas, comienza con el secado primario. Durante la fase de secado primario, se retira el hielo. En el presente documento, se genera un vacío y se suministra una cantidad apropiada de calor al material para que el hielo sublime. Durante la fase de secado secundaria, se eliminan las moléculas de agua unidas al producto. En el presente documento, la temperatura se eleva más que en la fase de secado primario para romper cualquier interacción físico-química que se haya formado entre las moléculas de agua y el material del producto. La presión también puede reducirse aún más para mejorar la desorción durante esta etapa. Después de que se completa el proceso de liofilización, la cámara puede llenarse con un gas inerte, tal como nitrógeno. El producto puede sellarse dentro del liofilizador en condiciones secas, evitando la exposición al agua atmosférica y a los contaminantes.

Ejemplo 2: Bifidobacterium animalis ssp. lactis administrado por vía oral inhibe el crecimiento tumoral del carcinoma colorrectal

Se obtuvieron ratones Balb/c hembra 6-8 semanas de edad de Taconic (Germantown, NY). Se resuspendieron 100.000 células tumorales colorrectales CT-26 (ATCC CRL-2638) en PBS estéril y se inocularon en presencia de Matrigel al 50 %. Las células tumorales CT-26 se inyectaron por vía subcutánea en un flanco posterior de cada ratón. Cuando los volúmenes tumorales alcanzaron un promedio de 100 mm^3 (aproximadamente 10-12 días después de la inoculación de las células tumorales), los animales se distribuyeron en los siguientes grupos: 1) anticuerpo IgG2a control del isotipo vehículo +; y 3) la cepa A de *Bifidobacterium animalis ssp. lactis* (número de acceso ATCC PTA 125097) +

IgG2a. Las bacterias de *Bifidobacterium animalis ssp. lactis* ($1x10^9$) se administraron por sonda oral (p.o.) diariamente, comenzando el día 1 hasta la conclusión del estudio. El grupo de *Bifidobacterium animalis ssp. lactis* + IgG2a mostró una inhibición significativa del crecimiento tumoral en comparación con el grupo vehículo + IgG2a (ver figura 1).

Ejemplo 3: Bifidobacterium animalis ssp. lactis administrado por vía oral inhibe el crecimiento tumoral del carcinoma colorrectal comparable a la inhibición observada con el tratamiento con anti-PD-1

Las células tumorales CT-26 se inyectaron por vía subcutánea en un flanco posterior de cada ratón Balb/c como anteriormente. Cuando los volúmenes tumorales alcanzaron un promedio de 100 mm^3 (aproximadamente 10-12 días después de la inoculación de células tumorales), los animales se distribuyeron en los siguientes grupos: 1) Vehículo + PBS; 2) Vehículo + isotipo de control de anticuerpos IgG2a; y 3) Vehículo + anti-PD-1; 4) la cepa A de *Bifidobacterium animalis ssp. lactis* (número de acceso ATCC PTA-125097) + IgG2a; y 5) cepa A de *Bifidobacterium animalis ssp. lactis*+ anti-PD-1. Los anticuerpos se administraron por vía intraperitoneal (i.p.) a 100 µg/ratón (100 µl de volumen final) cada cuatro días, comenzando el día 1, y Las bacterias de Bifidobacterium animalis ssp. lactis ($1x10^9$) se administraron por sonda oral (p.o.) diariamente, comenzando el día 1 hasta la conclusión del estudio. El gripo de *Bifidobacterium animalis ssp. lactis* + IgG2a mostró una inhibición del crecimiento tumoral comparable a la observada en el grupo vehículo + anti-PD-1 (véase la Figura 2).

Ejemplo 4: Tratamiento del carcinoma colorrectal usando

diferentes cepas de Bifidobacterium animalis ssp. lactis

Como se describe en los Ejemplos 2 y 3, las células tumorales CT-26 se inyectaron por vía subcutánea en un flanco posterior de ratones y los animales se asignaron en grupos que recibieron los siguientes tratamientos: 1) Vehículo; 2) la cepa A de *Bifidobacterium animalis ssp. lactis* (número de acceso ATCC PTA-125097); 3) *B. animalis lactis*, cepa B; 4) *B. animalis ssp. lactis*, cepa C; y 5) anti-PD-1. Se administraron $1x10^9$ células bacterianas diariamente (p.o.), comenzando el día 1 de la asignación del grupo animal, y los tumores se midieron como se describió anteriormente. Se administraron anticuerpos anti-PD-1 por vía intraperitoneal (ip) a 100 µg/ratón (100 µl de volumen final) cada cuatro días, comenzando el día 1. Como se ve en la Tabla 2, la cepa A mostró una inhibición significativa del crecimiento tumoral en comparación con los otros grupos de tratamiento con *B. animalis lactis* (Tabla 2 y Figura 3).

Tabla 2. Volumen tumoral de carcinoma colorrectal (mm^3) el día 22 (12° día de administración))

	Anti PD-1	**Vehículo**	**Cepa C**	**Cepa B**	**Cepa A**
Mediana del vol. tumoral. (mm^3)	1096,97	1775,89	1294,94	1819,68	1788,83
Vol. tumoral medio (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

Ejemplo 5: *Bifidobacterium animalis ssp. lactis* administrada intratumoralmente inhibe el crecimiento tumoral del carcinoma colorrectal

Como se describe en los Ejemplos 2 y 3, las células

tumorales CT-26 se inyectaron por vía subcutánea en un flanco posterior de ratones y los animales se asignaron en grupos que recibieron los siguientes tratamientos: 1) Vehículo; 2) la cepa A de *Bifidobacterium animalis ssp. lactis* (número de acceso ATCC PTA-125097); y 3) anti-PD-1. Se administraron $2x10^9$ células bacterianas intratumoralmente (IT) en el día 1 de la asignación del grupo animal, y los tumores se midieron como se describió anteriormente. Los ratones recibieron una segunda dosis el día 4. Los anticuerpos anti-PD-1 se administraron por vía intraperitoneal (i.p.) a 100 µg/ratón (100 µl de volumen final) cada cuatro días, comenzando el día 1. El grupo de la cepa A de *Bifidobacterium animalis ssp. lactis* mostró una inhibición significativa del crecimiento tumoral en comparación con el grupo de control (ver Figura 4).

Ejemplo 6: *Bifidobacterium animalis ssp. lactis* en un modelo de melanoma de ratón

B. animalis ssp. Lactis se prueba para determinar su eficacia en un modelo de melanoma de ratón. Por ejemplo, ratones C57Bl/6 hembras de 6-8 semanas de edad se obtienen de Taconic (Germantown, NY). Entre 1x10 ^ 4 y 1x10 ^ 6, se pueden usar células tumorales B16-F10 (ATCC CRL-6475) para inocular varios grupos de ratones. Las células tumorales B16-F10 pueden resuspenderse en PBS estéril que contiene Matrigel al 50 % e inocularse en un volumen final de 100 µl en un flanco posterior (el primer flanco) de cada ratón. El tratamiento con *Bifidobacterium animalis ssp. lactis* se inicia en algún momento después de la inoculación de células tumorales en dosis variadas y a intervalos definidos. Por ejemplo, algunos ratones reciben entre 1-$5x10^9$ UFC (100µl de volumen final) por dosis. Las posibles vías de administración incluyen sonda oral (p.o.),

inyección intravenosa, inyección intratumoral (IT) o inyección peritumoral o subtumoral o subcutánea. Con el fin de evaluar los efectos sistémicos antitumorales del tratamiento con B. animalis lactis, se pueden inocular ratones adicionales con células tumorales en el flanco contralateral (no tratado, segundo) antes del tratamiento IT, peritumoral o subtumoral con B. animalis lactis en el primer flanco.

Algunos ratones pueden recibir *Bifidobacterium animalis ssp. lactis* (p.o.) el día 1 (el día después de la inyección de la célula tumoral). Otros ratones pueden recibir siete (7) dosis consecutivas de *Bifidobacterium animalis ssp. lactis* (una dosis por día los días 14-21). Otros ratones reciben una dosis diaria o, alternativamente, algunos ratones reciben la dosificación cada dos días. Alternativamente, los ratones se aleatorizan en diversos grupos de tratamiento en un punto de tiempo definido (por ejemplo, en el día 13) o cuando los tumores alcanzan un cierto tamaño (por ejemplo, 100 mm ^ {3}) y luego se inicia el tratamiento en consecuencia. Por ejemplo, cuando los volúmenes tumorales alcanzan un promedio de 100 mm^3 (aproximadamente 10-12 días después de la inoculación de células tumorales), los animales se distribuyen en grupos y se tratan con vehículo o *Bifidobacterium animalis ssp. lactis* (p.o. o IT). Algunos grupos adicionales de ratones se pueden tratar con un anticuerpo adicional terapéutico o un anticuerpo de control apropiado. Un ejemplo de un agente terapéutico contra el cáncer que se puede administrar es un inhibidor de un punto de control inmune, por ejemplo anti-PD-1, anti-PD-L1 u otro tratamiento que bloquea la unión de un punto de control inmune a su (s) ligando (s). Los inhibidores del punto de control anti-PD-1 y anti-PD-L1

pueden formularse en PBS y administrarse por vía intraperitoneal (i.p.) en dosis efectivas. Por ejemplo, los ratones reciben 100 µg de anti-PD-1 (i.p.) cada cuatro días a partir del día 1, y continúan durante la duración del estudio.

Además, algunos ratones son tratados con antibióticos antes del tratamiento. Por ejemplo, vancomicina (0,5 g/l), ampicilina (1,0 g/l), gentamicina (1,0 g/l) y anfotericina B (0,2 g/l) se agregan al agua potable, y el tratamiento con antibióticos se detiene en el momento de tratamiento o unos días antes del tratamiento. Algunos ratones se inoculan con células tumorales sin recibir tratamiento previo con antibióticos. Además, en algunos grupos, las células B16-f10 pueden cultivarse con 100 U/ml de penicilina y 10 mg/ml de estreptomicina antes de la inoculación.

Por ejemplo, las células B16-F10 se expandieron usando la técnica estándar de cultivo de tejidos, mantenidas en medio DMEM suplementado con 10 % de suero fetal bovino (FBS), 100 U/ml de penicilina y 10 mg/ml de estreptomicina en una incubadora a 37°C con 5 % CO2. El día de la implantación de células tumorales, las células B16-F10 se resuspendieron en HBSS frío a 1x10 ^ 6 células tumorales/ml y a cada ratón se le inyectaron 100 ul de células tumorales resuspendidas. El crecimiento tumoral se midió tres veces por semana usando calibres digitales de acuerdo con métodos conocidos en la técnica. Cuando el tamaño promedio del tumor alcanzó aproximadamente 100 mm^3, los ratones se separaron aleatoriamente en grupos de tratamiento y se inició el tratamiento de acuerdo con la Tabla 3 a continuación.

Tabla 3. Tratamientos

Grupo, N=20	Treatamiento n.° 1	Tratamiento n.° 2
1	Vehículo (PO, 100 µl vol. CD)	PBS (IP, 100 µl, C4D)
2	Vehículo (PO, 100 µl vol. CD)	anti-PD-L1 (IP, 200 µg/100 µl vol, C4D)
3	B. animalis ssp. Lactis, Cepa A (PO, $6,5x10^9$ UFC in 100 µl vol. CD)	PBS (IP, 100 µl, C4D)
4	B. animalis ssp. Lactis, Cepa A (PO, $6,5x10^9$ UFC in 100 µl vol. CD)	anti-PD-L1 (IP, 200 µg/100 µl vol, C4D)

Los animales recibieron el Tratamiento n.° 1 (Vehículo o B. animalis ssp. Lactis, cepa A) mediante sonda oral todos los días. Los ratones recibieron el tratamiento n.° 2 (PBS o anti-PD-L1 por vía intraperitoneal cada 4 días hasta la finalización del estudio.) El tratamiento de ratones inoculados con tumor con una combinación de cepa A de *Bifidobacterium animalis ssp. lactis* y anti-PD-L1 inhibe el crecimiento tumoral en una manera superior a La cepa A de Bifidobacterium animalis ssp. lactis o anti-PD-L1 solo (figura 5).

Ejemplo 7: la infiltración de células CD3+ aumenta significativamente en tumores CT26 en ratones alimentados oralmente con la cepa A de B. animalis lactis.

En algunos estudios, en diversos momentos, los ratones se sacrifican y los tumores, ganglios linfáticos u otros tejidos se pueden eliminar para el análisis de citometría de flujo ex vivo usando métodos conocidos en la técnica.

Por ejemplo, los tumores se disocian usando un cóctel de enzima de disociación del tumor Miltenyi de acuerdo con las instrucciones del fabricante. Los pesos tumorales se registran y los tumores se cortan y luego se colocan en tubos de 15 ml que contienen el cóctel de enzimas y se colocan en hielo. Luego, las muestras se colocan en un agitador suave a 37 °C durante 45 minutos y se apagan con hasta 15 ml de RPMI completo. Cada suspensión celular se filtra a través de un filtro de 70 µm en un tubo halcón de 50 ml y se centrifuga a 1000 rpm durante 10 minutos. Las células se resuspenden en tampón FACS y se lavan para eliminar los restos restantes. Si es necesario, las muestras vuelven a filtrarse a través de un segundo filtro de 70 µm en un nuevo tubo. Las células se tiñen para el análisis mediante citometría de flujo usando técnicas conocidas en la técnica. Los anticuerpos de tinción pueden incluir anti-CD11c (células dendríticas), anti-CD80, anti-CD86, anti-CD40, anti-MHCII, anti-CD8a, anti-CD4 y anti-CD103. Otros marcadores que pueden analizarse incluyen el marcador de células paninmunitarias CD45, marcadores de células T (CD3, CD4, CD8, CD25, Foxp3, T-bet, Gata3, Rorγt, Granzyme B, CD69, PD-1, CTLA-4), y marcadores de macrófagos/mieloides (CD11b, MHCII, CD206, CD40, CSF1R, PD-L1, Gr-1). Además del inmunofenotipo, se analizan las citocinas, que incluyen, entre otras, 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 y MCP-1, usando técnicas conocidas en la técnica. El análisis de citocinas puede analizarse en células inmunitarias obtenidas de ganglios linfáticos u otros tejidos, secciones tumorales, células tumorales disociadas, y/o en células inmunes infiltradas tumorales CD45 +

purificadas obtenidas ex vivo. Los niveles de citocina pueden determinarse usando diversas técnicas de tinción, qPCR u otras técnicas conocidas en las artes. La inmunohistoquímica también puede realizarse en secciones tumorales para medir células T, macrófagos, células dendríticas y moléculas de puntos de control u otras expresiones de proteínas usando técnicas conocidas en la técnica.

Los ratones se inocularon con células tumorales CT26, se dividieron en grupos como se describió anteriormente, y se sometieron a sonda nasogástrica con la cepa A de *Bifidobacterium animalis ssp. lactis* $8{,}3x10^{9}$ UFC/ratón por día. Otros ratones recibieron vehículo como control negativo. Los ratones de este estudio se sacrificaron el día 10 y los tumores se cosecharon para el análisis. Usando técnicas conocidas descritas anteriormente, las secciones tumorales se tiñeron con anticuerpos anti-CD3 (marcador de células T) para evaluar las células CD3+ promedio por sección tumoral y la expresión del gen tumoral se analizó mediante qPCR usando un ensayo TaqMan de la siguiente manera: Se aisló el ARN tumoral El kit RNeasy Mini de Qiagen (n.° de catálogo 74104), utilizando el protocolo del fabricante. El ADNc se aisló usando el kit de síntesis de ADNc iScript ™ de BioRad de acuerdo con el protocolo del fabricante (n° de catálogo 1708891). La reacción de qPCR se realizó usando SsoAdvanced ™ Universal Probes Supermix 1725281 usando el protocolo del fabricante en el instrumento BioRad CFX384. Las sondas específicas de ratón TaqMan eran de Invitrogen, MHC I (b2m) Mm00437762_m1 y β-actina Mm01205647_g1 para normalizar la expresión génica. El valor delta Ct para cada tumor se calculó para MHC I usando β-actina como el gen de referencia. La expresión de

MHC I se calculó usando la fórmula 2 ^ - delta Ct para cada tumor de ratón y este valor se dividió por su volumen final de tumor respectivo al final del estudio.

Los volúmenes tumorales se tomaron antes del infiltrado de células inmunitarias CD3+ y del análisis de expresión de MHC de clase I. Como se describe en la Figura 1, el volumen tumoral disminuyó notablemente el día 9 en B. animalis ssp. grupo lactis (Figura 6A). La infiltración de células inmunitarias CD3+ aumentó significativamente en *El grupo de la cepa A de Bifidobacterium animalis ssp. lactis* relativo al vehículo (Figura 6B), y B. animalis ssp. La cepa A de lactis indujo una sorprendente regulación de la expresión de MHC de Clase I (Figura 6C).

En lugar de sacrificarse, algunos ratones pueden volver a someterse a la inyección de células tumorales en el flanco contralateral (u otra área) para determinar el impacto de la respuesta de la memoria del sistema inmunológico sobre el crecimiento tumoral.

Ejemplo 8: *Bifidobacterium animalis ssp. lactis* en un modelo de cáncer de pulmón de ratón

Bifidobacterium animalis ssp. lactis se prueba para determinar su eficacia en el modelo de cáncer de pulmón de ratón, ya sea solo o en combinación con otras terapias contra el cáncer, incluido el/los inhibidor (es) del punto de control. Los ratones se dividen en grupos que reciben *Bifidobacterium animalis ssp. lactis*, con o sin tratamiento de inhibidor de punto de control. Como se describe en el Ejemplo 6, *Bifidobacterium animalis ssp. lactis* se administra en dosis variadas a intervalos definidos. Por ejemplo, algunos ratones reciben *Bifidobacterium animalis ssp. lactis* (p.o.) el día después de la inyección de la célula tumoral (día 1). Algunos ratones reciben siete (7)

dosis consecutivas de *Bifidobacterium animalis ssp. lactis* (una dosis por día los días 14-21). Otros ratones reciben una dosis diaria o, alternativamente, algunos ratones reciben la dosificación cada dos días. Alternativamente, los ratones se aleatorizan en diversos grupos de tratamiento en un punto de tiempo definido (por ejemplo, en el día 13) o cuando los tumores alcanzan un cierto tamaño (por ejemplo, 100 mm ^ {3}) y luego se inicia el tratamiento en consecuencia.

Se inyectan $1x10^6$ células LLC1, o un número apropiado de células de cáncer de pulmón de otra línea celular de cáncer de pulmón, en el flanco posterior de ratones singénicos. Los tumores de los diversos grupos de tratamiento se miden con pinzas a intervalos regulares. Como se describe en el Ejemplo 6, algunos ratones se sacrifican para el análisis tumoral ex vivo utilizando citometría de flujo. Otros ratones se pueden volver a estimular con la inyección de células tumorales en el flanco contralateral para determinar el impacto de la respuesta de la memoria del sistema inmunológico sobre el crecimiento tumoral.

Ejemplo 9: *Bifidobacterium animalis ssp. lactis* en un modelo de cáncer de mama de ratón

Bifidobacterium animalis ssp. lactis se prueba para determinar su eficacia en el modelo de cáncer de mama de ratón, ya sea solo o en combinación con otras terapias contra el cáncer, incluido el/los inhibidor (es) del punto de control. Los ratones se dividen en grupos que reciben *Bifidobacterium animalis ssp. lactis*, con o sin tratamiento de inhibidor de punto de control. Como se describe en el Ejemplo 6, *Bifidobacterium animalis ssp. lactis* se administra en dosis variadas a intervalos definidos. Por

ejemplo, algunos ratones reciben *Bifidobacterium animalis ssp. lactis* (p.o.) el día después de la inyección de la célula tumoral (día 1). Algunos ratones reciben siete (7) dosis consecutivas de *Bifidobacterium animalis ssp. lactis* (una dosis por día los días 14-21). Otros ratones reciben una dosis diaria o, alternativamente, algunos ratones reciben la dosificación cada dos días. Alternativamente, los ratones se distribuyen aleatoriamente en varios grupos de tratamiento en un punto de tiempo definido (por ejemplo, en el día 13) o cuando los tumores alcanzan un cierto tamaño (por ejemplo, 100 mm^3) y luego se inicia el tratamiento en consecuencia.

Las células de carcinoma de mama 4T1 se obtienen de ATCC y $1x10^6$ células en 50 ul de PBS se inyectan por vía subcutánea en una o ambas extremidades traseras de ratones hembra Balb/c (como describe Wang *et al.* 2003, Systemic dissemination of viral vectors during intratumoral injection. Molecular Cancer Therapeutics; 2(11). Alternativamente, las células de carcinoma mamario de ratón EMT6 se obtienen de ATCC y se inyectan $1x10^6$ células en 50 µl de PBS por vía subcutánea en una o ambas extremidades posteriores de ratones hembra Balb/c de 6-8 semanas de edad (como describen Guo *et al.* 2014, Combinatorial Photothermal and Immuno Cancer Therapy Using Chitosan-Coated Hollow Copper Sulfide Nanoparticles. ASC Nano.; 8(6): 5670-5681). Además, se pueden usar otras líneas de células mamarias de ratón disponibles.

Los tumores de los diversos grupos de tratamiento se miden con pinzas a intervalos regulares. Como se describe en el Ejemplo 6, *Bifidobacterium animalis ssp. lactis* se administra en dosis variadas a intervalos definidos. Por ejemplo, algunos ratones se sacrifican para el análisis

tumoral ex vivo utilizando citometría de flujo. Otros ratones se pueden volver a estimular con la inyección de células tumorales en el flanco contralateral para determinar el impacto de la respuesta de la memoria del sistema inmunológico sobre el crecimiento tumoral.

Alternativamente, las células 4T1 se pueden usar en un modelo murino ortotópico de cáncer de mama como se describe por Tao *et al.* (Tao *et al.* 2008. Imagable 4T1 model for the study of late stage breast cancer. 8: 288). Los ratones se sacrifican para el análisis tumoral ex vivo. Los tumores se analizan mediante citometría de flujo e inmunohistoquímica.

Ejemplo 10: *Bifidobacterium animalis ssp. lactis* en un modelo de cáncer de páncreas de ratón

Bifidobacterium animalis ssp. lactis se prueba para determinar su eficacia en el modelo de ratón de cáncer de páncreas, solo o en combinación con otras terapias contra el cáncer, incluido el/los inhibidor (es) del punto de control. Los ratones se dividen en grupos que reciben *Bifidobacterium animalis ssp. lactis*, con o sin tratamiento de inhibidor de punto de control. Como se describe en el Ejemplo 6, algunos ratones reciben *Bifidobacterium animalis ssp. lactis* (p.o.) el día después de la inyección de la célula tumoral (día 1). Algunos ratones reciben siete (7) dosis consecutivas de *Bifidobacterium animalis ssp. lactis* (una dosis por día los días 14-21). Otros ratones reciben una dosis diaria o, alternativamente, algunos ratones reciben la dosificación cada dos días. Alternativamente, los ratones se aleatorizan en diversos grupos de tratamiento en un punto de tiempo definido (por ejemplo, en el día 13) o cuando los tumores alcanzan un cierto tamaño (por ejemplo, 100 mm ^ {3}) y luego se inicia el tratamiento en consecuencia.

Las células Panc02 se mantienen en DMEM, se suplementan con 10 % de suero bovino fetal y 1 % de penicilina/estreptomicina, y se incuban a 37 °C con 5 % de CO2. Ratones hembra C57Bl/6 de 8-10 semanas de edad se obtienen de Charles River, Inc. u otro proveedor certificado. Ratones hembra C57Bl/6 se inyectan por vía subcutánea en el flanco trasero derecho con $1x10^6$ células Panc02. Este protocolo se basa en modelos de tumores Panc02 estándar (Maletzki *et al*. 2008. Pancreatic cancer regression by intratumoral injection of live streptococcus pyogenes in a syngeneic mouse model. Gut. 57:483-491). Los tumores de los diversos grupos de tratamiento se miden con pinzas a intervalos regulares. Como se describe en el Ejemplo 6, algunos ratones se sacrifican para el análisis de tumores ex vivo utilizando citometría de flujo, mientras que otros ratones se vuelven a poner a prueba para determinar el impacto de la respuesta de la memoria sobre el crecimiento tumoral.

Alternativamente, se pueden usar líneas de células Panc02, 6606PDA o Capan-1 en un modelo murino ortotópico de cáncer de páncreas como se describe por Partecke et al. (Partecke *et al.* (Partecke *et al.* 2011. A syngeneic orthotopic murine model of pancreatic adenocarcinoma in the C57/Bl6 mouse using the Panc02 and 6606PDA cell lines. Eur. Surg. Res. 47(2):98-107) o Chai *et al.* (Chai *et al.* 2013. Bioluminescent orthotopic model of pancreatic cancer progression. J. Vis. Exp. 76: 50395). Los ratones se sacrifican para el análisis tumoral ex vivo. Los tumores se analizan mediante citometría de flujo e inmunohistoquímica.

Ejemplo 11: *Bifidobacterium animalis ssp. lactis* en un modelo de ratón de carcinoma hepatocelular

Bifidobacterium animalis ssp. lactis se prueba para

determinar su eficacia en el modelo de carcinoma hepatocelular en ratones, ya sea solo o en combinación con otras terapias contra el cáncer, incluido el/los inhibidor (es) del punto de control. Los ratones se dividen en grupos que reciben *Bifidobacterium animalis ssp. lactis*, con o sin tratamiento de inhibidor de punto de control. Como se describe en el Ejemplo 6, *Bifidobacterium animalis ssp. lactis* se administra en dosis variadas a intervalos definidos. Por ejemplo, algunos ratones reciben *Bifidobacterium animalis ssp. lactis* (p.o.) el día después de la inyección de la célula tumoral (día 1). Algunos ratones reciben siete (7) dosis consecutivas de *Bifidobacterium animalis ssp. lactis* (una dosis por día los días 14-21). Otros ratones reciben una dosis diaria o, alternativamente, algunos ratones reciben la dosificación cada dos días. Alternativamente, los ratones se aleatorizan en diversos grupos de tratamiento en un punto de tiempo definido (por ejemplo, en el día 13) o cuando los tumores alcanzan un cierto tamaño (por ejemplo, 100 mm ^ {3}) y luego se inicia el tratamiento en consecuencia.

El carcinoma hepatocelular se induce en ratones mediante inoculación subcutánea de $1x10^6$ células Hepa129 (obtenidas de NCI u otra fuente), o un número apropiado de células de otra línea celular de carcinoma hepatocelular (como se describe en Gonzalez-Carmona *et al*. 2008. CD40 ligand-expressing dendritic cells induce regression of hepatocellular carcinoma by activating innate and acquired immunity *in vivo*. Hepatology. 48(1):157-168). Las células tumorales se inoculan en uno o ambos flancos. Los tumores de los diversos grupos de tratamiento se miden con pinzas a intervalos regulares. Como se describe en el Ejemplo 6, algunos ratones se sacrifican para el análisis de tumores

ex vivo utilizando citometría de flujo, mientras que otros ratones se vuelven a poner a prueba para determinar el impacto de la respuesta de la memoria sobre el crecimiento tumoral.

Ejemplo 12: *Bifidobacterium animalis ssp. lactis* en un modelo de linfoma de ratón

Bifidobacterium animalis ssp. lactis se prueba para determinar su eficacia en el modelo de linfoma de ratón, ya sea solo o en combinación con otras terapias contra el cáncer, incluido el/los inhibidor (es) del punto de control. Los ratones se dividen en grupos que reciben *Bifidobacterium animalis ssp. lactis*, con o sin tratamiento de inhibidor de punto de control. Como se describe en el Ejemplo 6, *Bifidobacterium animalis ssp. lactis* se administra en dosis variadas a intervalos definidos. Por ejemplo, algunos ratones reciben *Bifidobacterium animalis ssp. lactis* (p.o.) el día después de la inyección de la célula tumoral (día 1). Algunos ratones reciben siete (7) dosis consecutivas de *Bifidobacterium animalis ssp. lactis* (una dosis por día los días 14-21). Otros ratones reciben una dosis diaria o, alternativamente, algunos ratones reciben la dosificación cada dos días. Alternativamente, los ratones se aleatorizan en diversos grupos de tratamiento en un punto de tiempo definido (por ejemplo, en el día 13) o cuando los tumores alcanzan un cierto tamaño (por ejemplo, 100 mm ^ {3}) y luego se inicia el tratamiento en consecuencia.

Una línea celular de linfoma es el linfoma A20, aunque pueden usarse otras líneas celulares de linfoma con ratones singénicos. Las células de linfoma A20 se obtienen de ATCC y $5x10^6$ células en 50 ul de PBS se inyectan por vía subcutánea en una o ambas extremidades posteriores de

ratones hembra Balb/c (como se describe en Houot *et al.* 2009. T-cell modulation combined with intratumoral CpG cures lymphoma in a mouse model without the need for chemotherapy. Blood. 113(15): 3546-3552). Los tumores de los diversos grupos de tratamiento se miden con pinzas a intervalos regulares. Como se describe en el Ejemplo 6, algunos ratones se sacrifican para el análisis de tumores ex vivo utilizando citometría de flujo, mientras que otros ratones se vuelven a poner a prueba para determinar el impacto de la respuesta de la memoria sobre el crecimiento tumoral.

Ejemplo 13: *Bifidobacterium animalis ssp. lactis* en un modelo de cáncer de próstata de ratón

Bifidobacterium animalis ssp. lactis se prueba para determinar su eficacia en el modelo de cáncer de próstata en ratones, ya sea solo o en combinación con otras terapias contra el cáncer, incluido el inhibidor (es) de punto de control. Los ratones se dividen en grupos que reciben *Bifidobacterium animalis ssp. lactis*, con o sin tratamiento de inhibidor de punto de control. Como se describe en el Ejemplo 6, *Bifidobacterium animalis ssp. lactis* se administra en dosis variadas a intervalos definidos. Por ejemplo, algunos ratones reciben *Bifidobacterium animalis ssp. lactis* (p.o.) el día después de la inyección de la célula tumoral (día 1). Algunos ratones reciben siete (7) dosis consecutivas de *Bifidobacterium animalis ssp. lactis* (una dosis por día los días 14-21). Otros ratones reciben una dosis diaria o, alternativamente, algunos ratones reciben la dosificación cada dos días. Alternativamente, los ratones se aleatorizan en diversos grupos de tratamiento en un punto de tiempo definido (por ejemplo, en el día 13) o cuando los tumores alcanzan un cierto tamaño

(por ejemplo, 100 mm ^ {3}) y luego se inicia el tratamiento en consecuencia.

Las células de cáncer de próstata de ratón ($1x10^5$ células RM-1 o un número apropiado de células de otra línea celular de cáncer de próstata) se inyectan en ratones singénicos. Los tumores de los diversos grupos de tratamiento se miden con pinzas a intervalos regulares. Como se describe en el Ejemplo 6, algunos ratones se sacrifican para el análisis de tumores ex vivo utilizando citometría de flujo, mientras que otros ratones se vuelven a poner a prueba para determinar el impacto de la respuesta de la memoria sobre el crecimiento tumoral.

Ejemplo 14: *Bifidobacterium animalis ssp. lactis* en un modelo de plasmacitoma de ratón

Bifidobacterium animalis ssp. lactis se prueba para determinar su eficacia en el modelo de plasmacitoma en ratones, ya sea solo o en combinación con otras terapias contra el cáncer, incluido el/los inhibidor (es) del punto de control. Los ratones se dividen en grupos que reciben *Bifidobacterium animalis ssp. lactis*, con o sin tratamiento de inhibidor de punto de control. Como se describe en el Ejemplo 6, *Bifidobacterium animalis ssp. lactis* se administra en dosis variadas a intervalos definidos. Por ejemplo, algunos ratones reciben *Bifidobacterium animalis ssp. lactis* (p.o.) el día después de la inyección de la célula tumoral (día 1). Algunos ratones reciben siete (7) dosis consecutivas de *Bifidobacterium animalis ssp. lactis* (una dosis por día los días 14-21). Otros ratones reciben una dosis diaria o, alternativamente, algunos ratones reciben la dosificación cada dos días. Alternativamente, los ratones se aleatorizan en diversos grupos de tratamiento en un punto de tiempo definido (por ejemplo, en

el día 13) o cuando los tumores alcanzan un cierto tamaño (por ejemplo, 100 mm ^ {3}) y luego se inicia el tratamiento en consecuencia.

Modelo de plasmacitoma inducido por aceite mineral

Para examinar la eficacia de *Bifidobacterium animalis ssp. lactis* en un modelo de plasmacitoma o mieloma múltiple, los ratones se inyectan por vía intraperitoneal tres veces con 500 ul de 2,6,10,12-tetrametilpentadecano ("aceite de pristano") en diversos puntos de tiempo entre 0 y 60 días, según lo descrito por Potter *et al.* 1983. Peritoneal plasmacytomagenesis in mice: comparison of different pristane dose regimens. J. Natl. Cancer Inst. 71(2):391-5 (véase también Lattanzio *et al*. 1997. Defective Development of Pristane-Oil Induced Plasmacytomas in Interleukin-6-Deficient BALB/C Mice. Am. J. Pathology: 151(3):689696). La progresión de la enfermedad se mide por el grado de hinchazón abdominal y las células y partículas inmunes en la ascitis. El fluido de ascitis se analiza para determinar el fenotipo de células inmunes mediante citometría de flujo como se describe en el Ejemplo 2.

Modelo de plasmacitoma inducido por línea celular

Para examinar la eficacia de *Bifidobacterium animalis ssp. lactis* en un modelo de plasmacitoma o mieloma múltiple, se inyectan células MOPC-104E o células de plasmacitoma J558 (TIB-6 ATCC) por vía subcutánea en uno o más flancos posteriores de ratones Balb/c ($5x10^6$ células), según el modelo descrito por Bhoopalam *et al.* 1980. Effect of dextran-S (alpha, 1-3 dextran) on the growth of plasmacytomas MOPC-104E and J558. J. Immunol. 125(4):1454-8 (see also Wang *et al.* 2015. IL-10 enhances CTL-mediated tumor rejection by inhibiting highly suppressive CD4+ T cells and promoting CTL persistence in a murine model of

plasmacytoma. OncoImmunology. 4(7): e1014232-1-9). Los ratones se dividen en grupos que reciben *Bifidobacterium animalis ssp. lactis* por sonda oral, y con o sin tratamiento de inhibidor de punto de control. Los tumores de los diversos grupos de tratamiento se miden con pinzas a intervalos regulares. Como se describe en el Ejemplo 6, algunos ratones se sacrifican para el análisis de tumores ex vivo usando citometría de flujo, mientras que otros ratones se vuelven a poner a prueba para determinar el impacto de la respuesta de la memoria sobre el crecimiento tumoral.

Ejemplo 15: *Bifidobacterium animalis ssp. lactis* en un modelo de ratón SCID de mieloma de ratón

Bifidobacterium animalis ssp. lactis se prueba para determinar su eficacia en el modelo SCID de mieloma en ratones, ya sea solo o en combinación con otras terapias contra el cáncer, incluido el/los inhibidor (es) del punto de control. Los ratones se dividen en grupos que reciben *Bifidobacterium animalis ssp. lactis*, con o sin tratamiento de inhibidor de punto de control. Como se describe en el Ejemplo 6, *Bifidobacterium animalis ssp. lactis* se administra en dosis variadas a intervalos definidos. Por ejemplo, algunos ratones reciben *Bifidobacterium animalis ssp. lactis* (p.o.) el día después de la inyección de la célula tumoral (día 1). Algunos ratones reciben siete (7) dosis consecutivas de *Bifidobacterium animalis ssp. lactis* (una dosis por día los días 14-21). Otros ratones reciben una dosis diaria o, alternativamente, algunos ratones reciben la dosificación cada dos días. Alternativamente, los ratones se aleatorizan en diversos grupos de tratamiento en un punto de tiempo definido (por ejemplo, en el día 13) o cuando los tumores alcanzan un cierto tamaño

(por ejemplo, 100 mm ^ {3}) y luego se inicia el tratamiento en consecuencia.

Para examinar la eficacia de *Bifidobacterium animalis ssp. lactis* usando una leucemia de células plasmáticas humanas, se usan $1x10^7$ líneas celulares de mieloma humano, células ARH77 (ARH77-ATCC CRL-1621, o un número apropiado de células de otra línea celular de mieloma tal como KPMM2). Las células de mieloma se inyectan por vía subcutánea en uno o ambos flancos traseros de ratones SCID (ver Caers *et al*. 2004. Of mice and men: disease models of multiple myeloma. Drug Discovery Today: Disease Models. 1(4):373-380. Tumores de los diversos grupos de tratamiento se miden con calibradores a intervalos regulares. Como se describe en el Ejemplo 6, algunos ratones se sacrifican para análisis tumoral ex vivo utilizando citometría de flujo, mientras que otros ratones se vuelven a provocar para determinar el impacto de la respuesta de la memoria sobre el crecimiento tumoral.

Ejemplo 16: *Bifidobacterium animalis ssp. lactis* en un modelo de carcinoma de células renales de ratón

Bifidobacterium animalis ssp. lactis se prueba para determinar su eficacia en el modelo murino de carcinoma de células renales, ya sea solo o en combinación con otras terapias contra el cáncer, incluido el/los inhibidor (es) del punto de control. Los ratones se dividen en grupos que reciben *Bifidobacterium animalis ssp. lactis*, con o sin tratamiento de inhibidor de punto de control. Como se describe en el Ejemplo 6, *Bifidobacterium animalis ssp. lactis* se administra en dosis variadas a intervalos definidos. Por ejemplo, algunos ratones reciben *Bifidobacterium animalis ssp. lactis* (p.o.) el día después de la inyección de la célula tumoral (día 1). Algunos

ratones reciben siete (7) dosis consecutivas de *Bifidobacterium animalis ssp. lactis* (una dosis por día los días 14-21). Otros ratones reciben una dosis diaria o, alternativamente, algunos ratones reciben la dosificación cada dos días. Alternativamente, los ratones se aleatorizan en diversos grupos de tratamiento en un punto de tiempo definido (por ejemplo, en el día 13) o cuando los tumores alcanzan un cierto tamaño (por ejemplo, 100 mm ^ {3}) y luego se inicia el tratamiento en consecuencia.

Para examinar la eficacia de *Bifidobacterium animalis ssp. lactis* en un modelo de ratón de carcinoma de células renales, células de Renca (ATCC CRL-2947) u otras células de carcinoma de células renales inyectadas subcutáneamente en uno o ambos flancos de ratones Balb/c singénicos de 7-8 semanas de edad ($5x10^6$ en 0,1 ml de PBS). Los tumores de los diversos grupos de tratamiento se miden con pinzas a intervalos regulares. Como se describe en el Ejemplo 6, algunos ratones se sacrifican para el análisis de tumores ex vivo utilizando citometría de flujo, mientras que otros ratones se vuelven a poner a prueba para determinar el impacto de la respuesta de la memoria sobre el crecimiento tumoral.

Ejemplo 17: *Bifidobacterium animalis ssp. lactis* en un modelo de cáncer de vejiga del ratón

Bifidobacterium animalis ssp. lactis se prueba para determinar su eficacia en el modelo de cáncer de vejiga de ratón, ya sea solo o en combinación con otras terapias contra el cáncer, incluido el/los inhibidor (es) del punto de control. Los ratones se dividen en grupos que reciben *Bifidobacterium animalis ssp. lactis*, con o sin tratamiento de inhibidor de punto de control. Como se describe en el Ejemplo 6, *Bifidobacterium animalis ssp. lactis* se

administra en dosis variadas a intervalos definidos. Por ejemplo, algunos ratones reciben *Bifidobacterium animalis ssp. lactis* (p.o.) el día después de la inyección de la célula tumoral (día 1). Algunos ratones reciben siete (7) dosis consecutivas de *Bifidobacterium animalis ssp. lactis* (una dosis por día los días 14-21). Otros ratones reciben una dosis diaria o, alternativamente, algunos ratones reciben la dosificación cada dos días. Alternativamente, los ratones se aleatorizan en diversos grupos de tratamiento en un punto de tiempo definido (por ejemplo, en el día 13) o cuando los tumores alcanzan un cierto tamaño (por ejemplo, 100 mm ^ {3}) y luego se inicia el tratamiento en consecuencia.

El día de la inoculación, las células MBT-2 (u otra línea celular de cáncer de vejiga) se recogen y resuspenden en una mezcla 1: 1 de PBS/Matrigel. Se suspenden $2x10^5$ células MBT-2 en 100 µl de mezcla y se inyectan por vía subcutánea en uno o ambos flancos posteriores de ratones singénicos. Los tumores se miden con pinzas a intervalos regulares.

Como se describe en el Ejemplo 6, algunos ratones se sacrifican para el análisis de tumores ex vivo utilizando citometría de flujo, mientras que otros ratones se vuelven a poner a prueba para determinar el impacto de la respuesta de la memoria sobre el crecimiento tumoral.

Ejemplo 18: La eficacia de la cepa de *Bifidobacterium animalis ssp. lactis* se compara con otras cepas de Bifidobacterium para la eficacia en modelos de cáncer

Usando los métodos descritos anteriormente, varios *Bifidobacterium animalis ssp. lactis* y otras cepas de Bifidobacterium se prueban y se comparan para determinar su eficacia en varios modelos de cáncer. Tales modelos pueden

incluir, entre otros, melanoma, cáncer de pulmón, cáncer de mama, cáncer de colon, cáncer colorrectal, cáncer de páncreas, cáncer hepatocelular, linfoma, cáncer de próstata, plasmacitoma, un modelo SCI de mieloma, carcinoma de células renales y/o cáncer de vejiga.

Ejemplo 19: un estudio abierto de la seguridad, tolerabilidad y eficacia de un cepa A de *Bifidobacterium animalis ssp. lactis* terapéutico oral en pacientes con carcinoma colorrectal metastásico

Estudio clínico multicéntrico y abierto con aumentos de dosis y expansiones de dosis para evaluar la seguridad, tolerabilidad y eficacia preliminares de cepa A de *Bifidobacterium animalis ssp. lactis* se realiza. El estudio se desarrolla en tres partes: Parte A: aumento de la dosis en el carcinoma colorrectal (CRC) metastásico estable de microsatélites (MSS) y las indicaciones alternativas, Parte B: CRC de MSS e indicaciones alternativas, y Parte C: CRC de microsatélites inestable (MSI). La escalada de dosis ocurre en un diseño estándar 3 + 3. En la parte A, de 9 a 18 pacientes están inscritos. Las partes B y C están inscritas concurrentemente. En la Parte B, se inscriben hasta 15 y no menos de 10 patentes. En la Parte C, se inscribieron no menos de 5 y hasta 10 pacientes. En base a los estudios *in vivo* de ratones portadores de aloinjertos, una dosis eficaz humana estimada ejemplar es de $2x10^{11}$ organismos por día, administrados por cápsula entérica. Una descripción esquemática del estudio se representa en la Figura 7.

Los objetivos principales del estudio incluyen la determinación de la dosis máxima tolerada dentro del rango de dosis probado para cepa A de *Bifidobacterium animalis ssp. lactis* en pacientes con neoplasias malignas avanzadas;

la determinación de la dosis recomendada de fase 2 para la cepa A de *Bifidobacterium animalis ssp. lactis* en CRC; la evaluación de la seguridad y la tolerabilidad, incluidas las toxicidades limitantes de la dosis de Bifidobacterium animalis ssp. cepa de lactis; y la demostración de la actividad antitumoral de cepa A de *Bifidobacterium animalis ssp. lactis* en pacientes con CRC (MSI y MSS). La actividad antitumoral se evaluará monitorizando los cambios en las células tumorales circulantes, los subconjuntos de células inmunes en sangre y tumor y la viabilidad del tumor; tasa de respuesta objetiva y duración de la respuesta; supervivencia libre de progresión; sobrevivencia promedio; tasa de beneficio clínico y duración de la tasa de beneficio clínico; y tasa de control de la enfermedad y duración de la tasa de control de la enfermedad. El objetivo secundario es determinar la distribución y eliminación humana de la cepa A de *Bifidobacterium animalis ssp. lactis*. Los objetivos exploratorios es evaluar las correlaciones de Bifidobacterium animalis ssp. Respuesta de la cepa A de lactis con marcadores moleculares relevantes para cada tipo de tumor e identificar posibles mecanismos de resistencia a la cepa A de *Bifidobacterium animalis ssp. lactis*

Los criterios de inclusión y exclusión:

Los criterios de inclusión para todas las partes del estudio incluyen lo siguiente:

1. Consentimiento informado por escrito obtenido antes de cualquier procedimiento de evaluación y de acuerdo con las pautas federales, locales e institucionales.
2. Edad ≥ 18 años.
3. Función hepática adecuada:
   a. bilirrubina total ≤ 2 veces el límite superior de

la normalidad (LSN) (excepto los pacientes con síndrome de Gilbert [hiperbilirrubinemia indirecta hereditaria] que deben tener una bilirrubina total ≤ 3 veces el LSN),

b. aspartato aminotransferasa (AST) y alanina aminotransferasa (ALT) ≤ 2,5 veces el LSN (excepto los pacientes con afectación hepática conocida de su tumor que deben tener su AST y ALT ≤ 5,0 veces el LSN).

4. Función renal adecuada: aclaramiento de creatinina estimada de ≥ 30 ml/min, calculada con la fórmula de Cockcroft y Gault (140-Edad) • Masa (kg)/(72 • creatinina mg/dl); multiplicar por 0,85 si es mujer.

5. Todos los pacientes en la parte B + C deben estar dispuestos a recibir nuevas biopsias al inicio de la terapia y después de 4 semanas de terapia.

6. Anticoncepción: Las mujeres en edad fértil deben aceptar el uso de métodos anticonceptivos duales (incluido un método anticonceptivo altamente eficaz y efectivo) y tener una prueba negativa de embarazo en el suero durante el cribado, y los pacientes varones deben usar un método de barrera efectivo de la anticoncepción si es sexualmente activo con una mujer en edad fértil. Tanto para pacientes masculinos como femeninos, se deben usar métodos anticonceptivos efectivos durante todo el estudio y durante los 3 meses posteriores a la última dosis.

Para las partes del estudio que evalúan el cáncer colorrectal recidivante/refactorio, los criterios de inclusión también incluyen lo siguiente:

7. Documentación histológica o citológica de adenocarcinoma de colon o recto.

8. Estado conocido de MSI/MSS.

9. Enfermedad medible por RECIST v1,1.

10. La enfermedad metastásica no es adecuada para la cirugía de intención curativa inicial.

11. Evidencia documentada de enfermedad progresiva de acuerdo con RECIST v1,1.

12. Tratamiento previo (con la finalización de un curso de terapia o progresión de la enfermedad o intolerancia) con cada uno de los siguientes:

a. Quimioterapias basadas en fluoropirimidina, oxaliplatino, irinotecán (por ejemplo, FOLFOX y FOLFIRI)

b. si KRAS wild-type, una terapia anti-EGFR,

c. Regorafenib o TAS 102 (se requiere el Pasado A, partes B + C opcional)

d. La radiación y la cirugía no se consideran regímenes anticáncer previos

13. Los pacientes no deben ser dependientes de la transfusión.

14. Función hematopoyética adecuada: ANC ≥ 1000/mm^3, hemoglobina (Hb) ≥ 9,0 g/dl y recuento de plaquetas ≥ 100.000/mm^3.

15. Estado funcional del Eastern Cooperative Oncology Group (ECOG) de ≤ 1.

16. Esperanza de vida de ≥ 3 meses.

Las siguientes categorías de pacientes están excluidas del estudio:

1. Pacientes mujeres que están embarazadas o lactando.

2. Cirugía mayor dentro de 4 semanas antes de C1D1.

3. Deterioro de la función cardíaca o enfermedades cardíacas clínicamente significativas, incluidas cualquiera de las siguientes:

a. Angina inestable o infarto agudo de miocardio ≤ 3

meses antes de C1D1;

b. Cardiopatía clínicamente significativa (p. Ej., Insuficiencia cardíaca congestiva sintomática [p. Ej., Clase NYHA 2], arritmia incontrolada o hipertensión, antecedentes de hipertensión lábil o cumplimiento deficiente con un régimen antihipertensivo).

4. Infección sistémica grave activa no controlada que requiere antibióticos parenterales, antivirales o antifúngicos dentro de una semana antes de C1D1.

5. Cualquier tratamiento antibiótico en curso que no haya sido descontinuado al menos 3 días antes del inicio de la terapia

6. Los pacientes con metástasis cerebral sintomática conocida no son adecuados para la inscripción. Los pacientes con metástasis cerebrales estables, tratadas y asintomáticas son elegibles para el ingreso al estudio.

7. Pacientes con antecedentes conocidos de virus de la inmunodeficiencia humana (VIH); No se requieren pruebas de VIH como parte de este estudio.

8. infección conocida, activa de hepatitis A, B o C; o se sabe que es positivo para el ARN del VHC o HBsAg (antígeno de superficie del VHB).

9. Neoplasias malignas previas:

a. Los pacientes con carcinoma de células escamosas o de células escamosas adecuadamente resecado in situ (es decir, cuello uterino) pueden matricularse independientemente del momento del diagnóstico.

b. Neoplasias previas que pueden interferir con la interpretación del estudio. No se permitirá el cáncer tratado con intención curativa <5 años antes a menos que lo apruebe el Patrocinador. Se permitirá el cáncer tratado con intención curativa> 5 años antes y sin

evidencia de recurrencia.

10. Pacientes con neoplasia maligna activa del sistema nervioso central (SNC). Los pacientes que solo han recibido quimioterapia intratecal o intravenosa profiláctica contra la enfermedad del SNC son elegibles.

11. Pacientes con enfermedad del tracto gastrointestinal (o vómitos o diarrea incontrolados) que podrían interferir con la absorción de EVP001.

12. Enfermedades psiquiátricas o médicas graves que, en opinión del investigador, podrían interferir con el tratamiento, el cumplimiento o la capacidad para dar su consentimiento.

13. Pacientes que no están dispuestos a cumplir con el protocolo, incluidas las biopsias necesarias y las colecciones de muestras necesarias para medir la enfermedad.

14.Radioterapia dentro de las dos semanas previas a la detección. Los pacientes deben haberse recuperado de toxicidades clínicamente significativas.

Se permite el uso de cualquier medicación/terapia concomitante, incluidos los medicamentos de venta libre (OTC) que se consideran necesarios para el cuidado del paciente durante el estudio. Se permiten los medicamentos necesarios para tratar EA, controlar los síntomas del cáncer, enfermedades estables concurrentes y agentes de atención de apoyo (por ejemplo, transfusiones de productos sanguíneos), analgésicos, antieméticos y antidiarreicos. Se permite la terapia concurrente con factores de crecimiento. El uso de cualquier agente inmunosupresor debe discutirse entre el Investigador y el Monitor médico caso por caso. Debe registrarse cualquier procedimiento de diagnóstico, terapéutico o quirúrgico realizado durante el período de

estudio, incluidas las fechas, la descripción del procedimiento (s) y los hallazgos clínicos, si corresponde. Todos los antibióticos están contraindicados.

Los anticonceptivos hormonales están permitidos en mujeres en edad fértil. Los anticonceptivos hormonales incluyen cualquier agente anticonceptivo comercializado que incluya un estrógeno y/o un agente progestacional.

No está permitido el uso de agentes anticancerosos en investigación o comerciales distintos de la cepa A de *Bifidobacterium animalis ssp. lactis* durante el estudio. El inicio de cualquier tratamiento antitumoral específico no relacionado con el protocolo se considera una indicación de recidiva/progresión de la enfermedad y se debe registrar de forma adecuada en los formularios electrónicos de informe de casos.

Se permite la radioterapia paliativa a las lesiones no objetivo, pero el tratamiento del estudio se mantiene durante ≥1 día antes del inicio de la radioterapia paliativa y ≥ 1 día después de cada dosis de radioterapia paliativa. El tratamiento con cepa A de *Bifidobacterium animalis ssp. lactis* no se suspende únicamente debido a la radiación paliativa.

Las medidas de apoyo para la atención médica óptima se deben proporcionar a los pacientes durante la participación en este estudio. Estos deben basarse en pautas institucionales y/o nacionales de la Red Integral del Cáncer (NCCN).

Estudio de escalada de dosis

Los pacientes reciben todas las cepa A de *Bifidobacterium animalis ssp. lactis* dosis durante el período de tratamiento de 7 días, o ha tenido una toxicidad limitante de la dosis (DLT) dentro del período de

tratamiento para ser considerada evaluable para las decisiones de escalada de dosis. La cepa A de *Bifidobacterium animalis ssp. lactis* se administra por vía oral en forma de comprimidos o cápsulas con recubrimiento entérico. Las decisiones de escalamiento de dosis ocurren cuando la cohorte de pacientes cumple con estos criterios.

Una TLD se define como un acontecimiento adverso (AA) o un valor de laboratorio anormal que ocurre dentro de los primeros 7 días de tratamiento con la cepa A de *Bifidobacterium animalis ssp. lactis*, excepto aquellas que son claras e incontrovertibles debido a enfermedad subyacente, progresión de la enfermedad o causas extrañas, y cumple con cualquiera de los criterios incluidos en la Tabla 4. Criterios comunes de terminología para eventos adversos del National Cancer Institute (NCI CTCAE) (Versión 4,03) se usa para todas las calificaciones. Además,> 4 dosis olvidadas de cepa A de *Bifidobacterium animalis ssp. lactis* se considerará TLD. Las decisiones de escalamiento de dosis ocurren cuando la cohorte de pacientes cumple con estos criterios.

Tabla 4: Criterios para definir la TLD

Toxicidad	**Cualquiera de los criterios siguientes (basados en los CTCAE [Versión 4.03]):**
No hematológica	Grado ≥ 3 náuseas/vómitos, mientras toma medicamentos de apoyo óptimos.
	Cualquier otro grado ≥ 3 toxicidad no hematológica excepto alopecia o anormalías electrolíticas corregibles con terapia de apoyo.
Hematológica	Neutropenia de grado 4 que dura más de 5 días.

	Neutropenia febril (recuento absoluto de neutrófilos [ANC] < 1 x10^9/l, fiebre > 38,5 °C.).
	Trombocitopenia de grado 4 o trombocitopenia de grado 3 con sangrado o cualquier requerimiento de transfusión de plaquetas.
Se usará la version 4.30 de los CTCAE para clasificar todas las anomalías de laboratorio. Los pacientes pueden recibir atención de apoyo según las pautas institucionales locales.	

Para implementar decisiones de escalamiento de dosis, la información de toxicidad disponible (es decir, todos los EA y todas las anormalidades de laboratorio independientemente de la evaluación de DLT) es evaluada por el monitor médico Investigators and Sponsor en una reunión de decisión de dosis o teleconferencia. Las decisiones se basan en una evaluación de todos los datos relevantes disponibles de todas las cohortes de dosis evaluadas en el estudio en curso, incluida información de seguridad, TLD, todos los datos de toxicidad de NCI CTCAE, versión 4,03 durante el ciclo 1 de pacientes evaluables. La administración de medicamentos en la siguiente cohorte de dosis más alta no puede continuar hasta que el investigador reciba una confirmación por escrito del patrocinador que indique que se evaluaron los resultados de la cohorte de dosis previa y que es permisible pasar a la siguiente cohorte de dosis más alta.

La Tabla 5 a continuación describe la dosis inicial y los niveles de dosis que pueden evaluarse durante el estudio para todas las partes del estudio.

Tabla 5: Niveles de aumento de la dosis de la cepa A de

Bifidobacterium animalis ssp. lactis

Cohorte	**Nivel de dosis** *Dosificación CD; 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

Una escalada de dosis estándar 3 + 3 se lleva a cabo de la siguiente manera:

- Si 0 de 3 pacientes experimentan una TLD, escale a la próxima cohorte de dosis más alta.
- Si 1 de 3 pacientes experimenta una TLD, esa cohorte se expandirá a 6 pacientes. Si 1 de 6 pacientes experimenta una TLD, escale a la siguiente cohorte de dosis más alta;
- Si ≥ 2 de 3 o ≥ 2 de 6 pacientes experimentan una TLD, se excede la dosis máxima tolerada (MTD).
- Si se excede la DMT, la inscripción de pacientes adicionales tendrá un nivel de dosis menor. Si una dosis inicial no elimina la evaluación TLD, la disminución de la dosis se realizará utilizando las reglas estándar 3 + 3.

Se permiten escaladas de dosis intrapacientes para todas las cohortes después de que se haya demostrado que el nivel de dosis deseado es seguro (es decir, todos los pacientes tratados con el nivel de dosis deseado completaron las evaluaciones TLD y ≤ 1 paciente experimentó una DLT).

Para pacientes con escalada de dosis después de 7 días y pacientes con expansión de dosis, si se observa un evento

que cumple la definición de TLD, pero no necesariamente dentro de los primeros 21 días, en> 33 % de los pacientes en cualquier momento, o si> 33 % de Los pacientes tratados han retirado el consentimiento debido a la toxicidad, se realizará la inscripción y se llevará a cabo una reunión con todos los investigadores y el patrocinador para revisar los eventos y analizar su importancia clínica. Con base en esta revisión, el patrocinador puede optar por reducir la dosis para los pacientes inscritos y reanudar la inscripción de la cohorte de expansión a esta dosis más baja, o puede suspenderse la inscripción en la cohorte de expansión.

La seguridad y la tolerabilidad se evalúan mediante TLD (cohortes de escalada de dosis solamente), informes de AE, exámenes físicos, electrocardiogramas y evaluaciones de seguridad de laboratorio.

El investigador evalúa la actividad antitumoral de acuerdo con los criterios de respuesta específicos de la enfermedad y se describe en términos de tasa de respuesta objetiva, duración de la respuesta, supervivencia libre de progresión, tasa de beneficio clínico, supervivencia global y tasa de control de la enfermedad.

En la Parte A: los subconjuntos y las citocinas de WBC de sangre periférica se analizan al inicio y durante 7 días. Las citocinas se analizan adicionalmente a las 24 y 48 horas.

En las partes B y C: subconjuntos de glóbulos blancos y citocinas de sangre periférica se analizan al inicio del estudio y 7 y 21 días. Las biopsias tumorales basal y del día 28 se analizan para la infiltración de subconjuntos inmunes, así como para los fabricantes de angiogénesis, proliferación y muerte.

Los pacientes continúan recibiendo cepa A de *Bifidobacterium animalis ssp. lactis* hasta que el paciente haya confirmado la farmacodinamia, retire el consentimiento, se pierda durante el seguimiento, experimenta toxicidad intolerable que impida un tratamiento posterior con la cepa A de *Bifidobacterium animalis ssp. lactis*, o el tratamiento se suspende a discreción del paciente, investigador o patrocinador. Los pacientes con progresión objetiva de la enfermedad pero con evidencia de beneficio clínico general pueden, a solicitud del médico tratante, continuar el tratamiento con cepa A de *Bifidobacterium animalis ssp. lactis* después de la discusión con el monitor médico.

Ejemplo 20: Bifidobacterium animalis ssp. Administrado intratumoralmente. Lactis inhibe el crecimiento tumoral del carcinoma colorrectal

Como se describe en los Ejemplos 2 y 3, las células tumorales CT-26 se inyectaron por vía subcutánea en un flanco posterior de ratones y los animales se asignaron en grupos que recibieron los siguientes tratamientos: 1) vehículo de sacarosa; 2) anti-PD-1 y anti-CTLA4; y 3) la cepa A de *Bifidobacterium animalis ssp. lactis*, anti-PD-1 y anti-CTLA4. Se administraron $2x10^9$ células bacterianas por sonda oral (p.o.) todos los días durante 21 días. Los anticuerpos anti-PD-1 se administraron por vía intraperitoneal (i.p.) a 200 µg/ratón (100 µl de volumen final) cada cuatro días, comenzando el día 1, seis veces en total. Los anticuerpos anti-CTLA4 se administraron por vía intraperitoneal (i.p.) a 100 µg/ratón (100 µl de volumen final) cada cuatro días, comenzando el día 1, seis veces en total. Los volúmenes del tumor se midieron como se describió anteriormente. El grupo de triple combinación de

la cepa A de *Bifidobacterium animalis ssp. lactis*, anti-PD-1 y anti-CTLA4 mostraron una inhibición significativa del crecimiento tumoral en comparación con el grupo control y el grupo anti-PD-1 y anti-CTLA4 (véase la Figura 8).

Ejemplo 21: Análisis genómico de cepa A de Bifidobacterium animalis ssp. lactis

Durante la duración de los experimentos del modelo CT26 como se describió anteriormente, el volumen tumoral varió cuando algunas manchas de *Bifidobacterium animalis ssp. lactis* se administraron. Los microbios con resultados negativos se determinaron a través de una comparación tridimensional de los volúmenes tumorales en la terminación de los grupos tratados con microbios, anti-PD1 y vehículo. Las distribuciones de volúmenes tumorales en la terminación del grupo anti-PD1 frente al grupo de vehículo se utilizaron como punto de referencia para clasificar el rendimiento negativo de los microbios. Si las distribuciones se superpusieron más estrechamente con el vehículo que el grupo tratado con anti-PD1, la cepa se consideró un microbio de rendimiento negativo.

Por ejemplo, se encontró que el volumen tumoral era mayor para los grupos tratados con dos especies de Bifidobacterium animalis lactis, cepa B y cepa C, en comparación con el grupo tratado con cepa A.

De acuerdo con estos hallazgos, una comparación de firmas *in vitro* de las tres cepas de un ensayo de células dendríticas de ratón muestra que la cepa A muestra un perfil inmune con una disminución de la inducción de citocinas pro-tumorigénicas en comparación con la cepa B y la cepa C (véanse las figuras 9A y 9B). Se ha encontrado que varias de estas citocinas están asociadas con un pronóstico más precario en el suero de pacientes con CCR

(Mager et al., (2016) Cytokine-Induced Modulation of Colorectal Cancer. Frontiers in Oncology. 6. 96. 10.3389).

Para dilucidar las diferencias entre las cepas, las tinciones A, B y C se secuenciaron a ~ 500x usando Illumina MiSeq. El genoma completo y circular de la cepa A (tabla 1) se usó como referencia para denominar polimorfismos de un solo nucleótido (SNP) entre las cepas. Se predijeron las regiones de codificación y se registraron los SNP que ocurrieron en las regiones de codificación, junto con la naturaleza del cambio de codificación (sinónimo, missense, frameshift, etc.) usando agrupaciones establecidas a través de los resultados en el modelo tumoral CT26, SNP que estaban presentes en negativo cepas (cepas B y C) cuando se compararon con la cepa A de referencia, y se identificaron estructuras únicas para cepas positivas (p. ej., cepa A).

Cuando se compara con cepas no homólogas altamente homólogas, la cepa A contiene polimorfismos de un solo nucleótido (SNP) y/o eventos de inserción/deleción en los siguientes genes enumerados en las tablas 6 y 7.

Tabla 6: SNP y/o inserciones/deleciones en la cepa A en comparación con las cepas no eficaces

gen	**producto**	**Tipo de variante**	**Posición en el gen**	**Cambio de base en la cepa A**	**Cambio de aminoácido en la cepa A**
glcU	Supuesta proteína de captación de glucose GlcU	Sentido erróneo	301	C	Arg

gen	producto	Tipo de variante	Posición en el gen	Cambio de base en la cepa A	Cambio de aminoácido en la cepa A
glcU	Supuesta proteína de captación de glucose GlcU	Sentido erróneo	814	C	Pro
relA	(p)ppGpp sintasa/hidrolasa RelA bifuncional	Sentido erróneo	251	G	Gly
relA	p)ppGpp sintasa/hidrolasa RelA bifuncional	Sentido erróneo	2053	G	Val
metF	5,10-metilenétertetraahidrofolato reductasa	Sentido erróneo	527	T	Phe

gen	producto	Tipo de variante	Posición en el gen	Cambio de base en la cepa A	Cambio de aminoácido en la cepa A
nnr	Enzima Nnr de reparación de NAD(P)H-hidrato	Sentido erróneo	574	C	Pro
ykoE_1	Supuesta HMP/tiamina permeasa proteína YkoE	Sentido erróneo	533	C	Ala
tcrY	Supuesta histidina quinasa sensor TcrY	Sentido erróneo	300	G	Gln
tcrY	Supuesta histidina quinasa sensor TcrY	Sentido erróneo	49	C	Pro
dac	D-alanyl-D-alanina carboxipeptidasa	Cambio de marco	199	DELECIÓN	His

gen	producto	Tipo de variante	Posición en el gen	Cambio de base en la cepa A	Cambio de aminoácido en la cepa A
proteína hipotética	proteína hipotética	Sentido erróneo	739	A	Ile
proteína hipotética	proteína hipotética	Cambio de marco	990	DELECIÓN	His
rdgB	dITP/XTP pirofosfatasa	Sentido erróneo	547	A	Ser
proteína hipotética	proteína hipotética	Sentido erróneo	290	C	Ala
glnB	Proteína reguladora de nitrógeno P-II	Sentido erróneo	161	A	Asp
accC	Biotina carboxilasa	Cambio de marco	826	DELECIÓN	-
trxB_2	Tioredoxina reductasa	Sentido erróneo	926	T	Val

gen	producto	Tipo de variante	Posición en el gen	Cambio de base en la cepa A	Cambio de aminoácido en la cepa A
afsK	Serina/treonina-proteína quinasa AfsK	Sentido erróneo	686	G	Gly
nhaK	Antiporte de sodio, potasio, litio y rubidio /H(+)	Sentido erróneo	989	T	Val
proteína hipotética	proteína hipotética	Inserción dentro del marco	45-77	INSERCIÓN (GGCGCTCGGCACCCTGGCGATTGGCGCGGCAAC)	-
oxc_1	Oxalil-CoA descarboxilasa	Cambio de marco	183-184	DELECIÓN	-
wbgU	UDP-N-acetilglucosamina 4-epimerasa	Sentido erróneo	7	G	Val

gen	**producto**	**Tipo de variante**	**Posición en el gen**	**Cambio de base en la cepa A**	**Cambio de aminoácido en la cepa A**
wcaJ	UDP-glucosa:undecaprenil-fosfato glucosa-1-fosfato transferasa	Sentido erróneo	292	G	Gly
proteína hipotética	proteína hipotética	Deleción dentro del marco	126-128	DELECIÓN	-
yciV	5'-3' exorribonucleasa	Sentido erróneo	100	T	Ser
aspA	Aspartato amoníaco liasa	Sentido erróneo	1355	C	Ala
tcrY	Supuesta histidina quinasa sensor TcrY	Sentido erróneo	368	T	Phe
tcrY	Supuesta histidina quinasa sensor TcrY	Sentido erróneo	278	C	Pro

gen	producto	Tipo de variante	Posición en el gen	Cambio de base en la cepa A	Cambio de aminoácido en la cepa A
nagC_2	Represor de N-acetilglucosamina	Sentido erróneo	457	G	Ala

Tabla 7: Genes con variantes sinónimas

gen	producto	Posición en el gen	Cambio de base en la cepa A	Cambio de aminoácido en la cepa A
Proteína hipotéticaO	proteína hipotética	753	T	Leu
hemN	Proteína similar a la coproporphirinogen-III oxidase independiente de oxígeno	912	A	Gly
proteína hipotética	proteína hipotética	951	G	Pro
proteína hipotética	proteína hipotética	1722	T	Pro
cytR_2	HTH-type transcriptional repressor CytR	372	C	Ile
egtC	Gamma-glutamyl-	135	C	Ile

gen	producto	Posición en el gen	Cambio de base en la cepa A	Cambio de aminoácido en la cepa A
	hercynylcysteine sulfoxide hydrolase			
proteína hipotética	proteína hipotética	30	G	Arg

Incorporación por referencia

Todas las solicitudes de patentes de publicaciones mencionadas en el presente documentose incorporan por referencia en su totalidad como si cada publicación individual o solicitud de patente se indicara específica e individualmente para ser incorporada por referencia. En caso de conflicto, prevalecerá la presente solicitud, incluidas las definiciones en el presente documento.

Equivalentes

Los expertos en la técnica reconocerán, o serán capaces de determinar usando solo la experimentación de rutina, muchos equivalentes a las realizaciones específicas de la invención descrita en el presente documento. Tales equivalentes están destinados a estar abarcados por las siguientes reivindicaciones.

Se hace constar que con relación a esta fecha, el mejor método conocido por la solicitante para llevar a la práctica la citada invención, es el que resulta claro de la presente descripción de la invención.