US 20260000716A1

(19) **United States**

(12) **Patent Application Publication**

Sauer et al.

(10) **Pub. No.: US 2026/0000716 A1**

(43) **Pub. Date: Jan. 1, 2026**

(54) **METHODS AND COMPOSITIONS FOR THE TREATMENT OF CANCER**

(71) Applicant: **Wisconsin Alumni Research Foundation**, Madison, WI (US)

(72) Inventors: **John-Demian Sauer**, Madison, WI (US); **Paul Sondel**, Madison, WI (US); **Alexander Rakhmilevich**, Lake Mills, WI (US); **Kijeong Lee**, Madison, WI (US); **Zachary Morrow**, Madison, WI (US)

(21) Appl. No.: **19/215,947**

(22) Filed: **May 22, 2025**

Related U.S. Application Data

(60) Provisional application No. 63/651,513, filed on May 24, 2024.

Publication Classification

(51) **Int. Cl.**

A61K 35/741	(2015.01)
A61P 35/00	(2006.01)
C07K 14/54	(2006.01)
C07K 14/55	(2006.01)
C07K 14/705	(2006.01)
C12N 15/74	(2006.01)

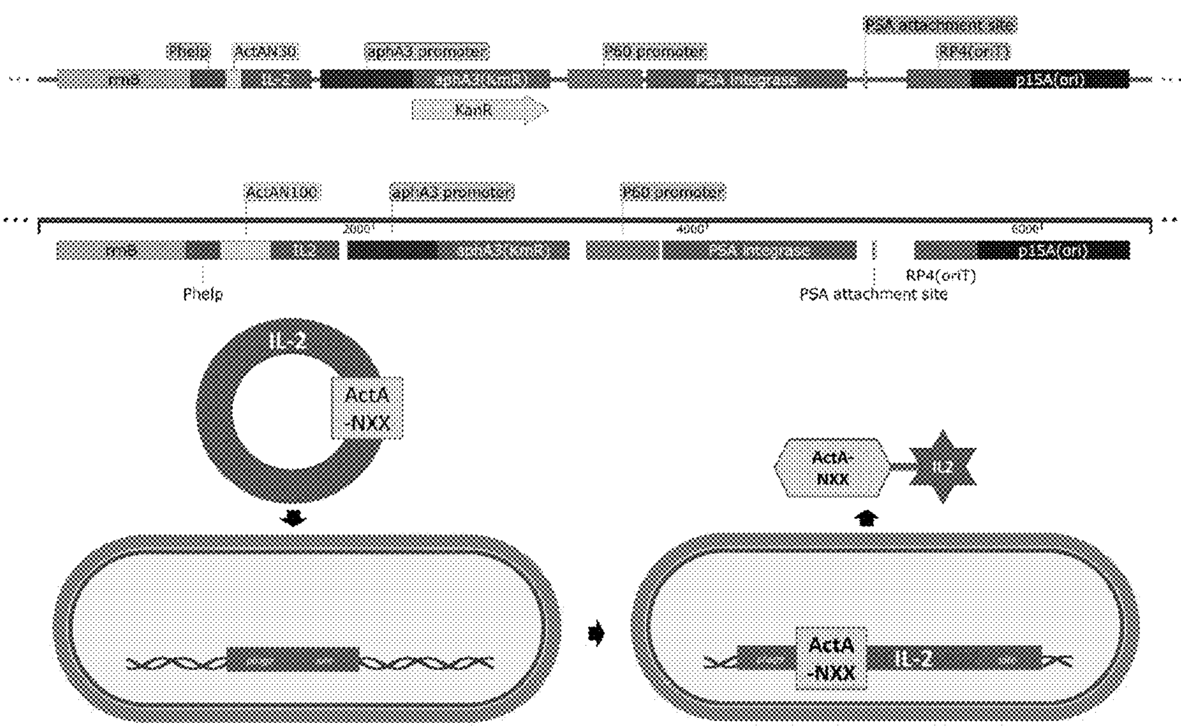
(52) **U.S. Cl.**

CPC ***A61K 35/741*** (2013.01); ***A61P 35/00*** (2018.01); ***C07K 14/5434*** (2013.01); ***C07K 14/55*** (2013.01); ***C07K 14/70575*** (2013.01); ***C12N 15/74*** (2013.01)

(57) **ABSTRACT**

Disclosed herein are genetically engineered *Listeria monocytogenes* strains and methods and compositions comprising the same for preventing, ameliorating, or treating cancer. In particular, the present technology relates to a genetically engineered *L. monocytogenes* bacterium, wherein the bacterium comprises a nucleic acid encoding IL-2, IL-12, or CD-40L, and methods of using said bacterium for preventing, ameliorating, or treating cancer.

Specification includes a Sequence Listing.



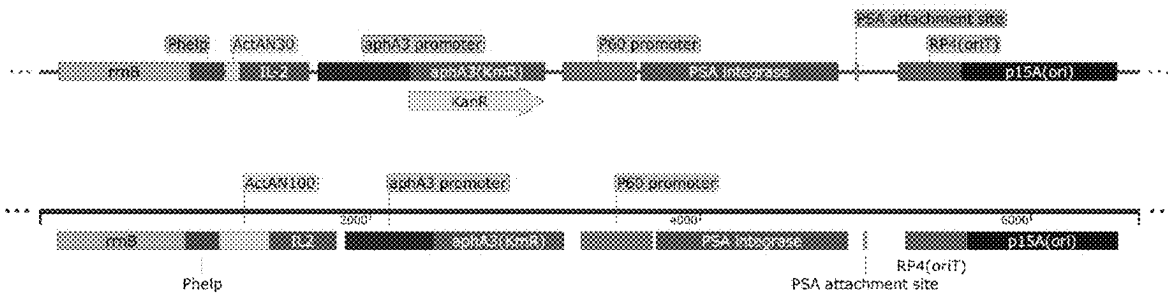


FIG. 1A

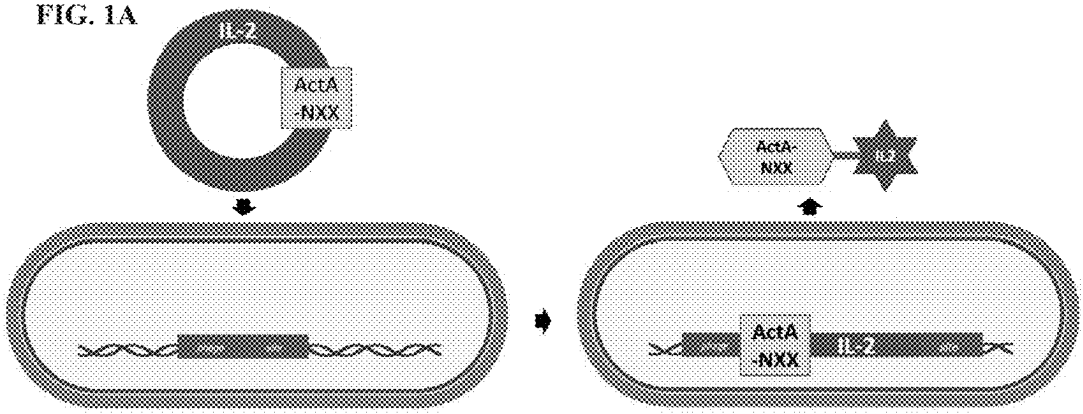


FIG. 1B

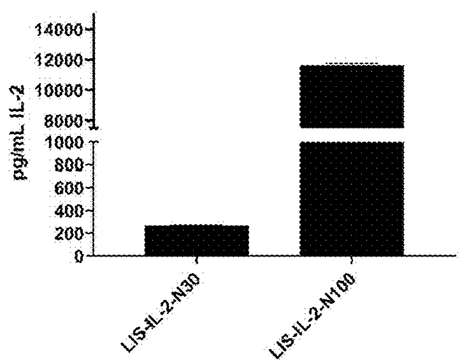


FIG. 1C

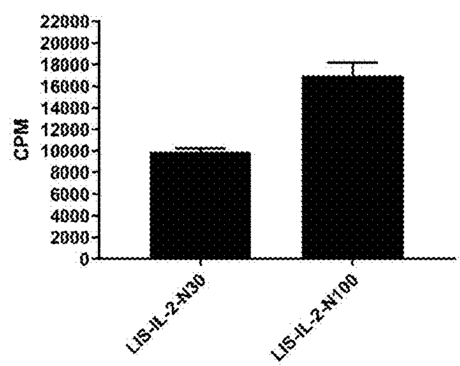


FIG. 1D

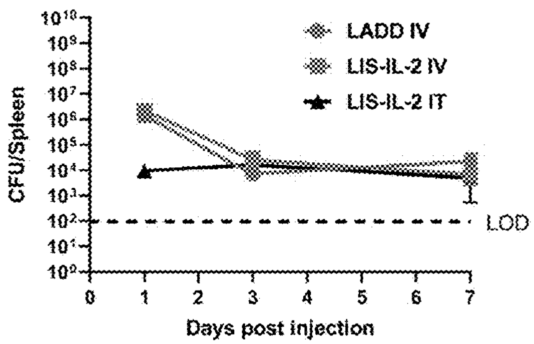


FIG. 2A

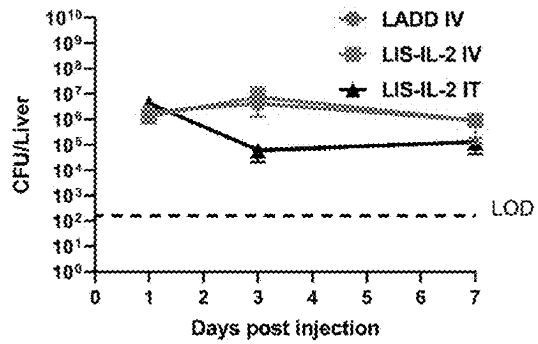


FIG. 2B

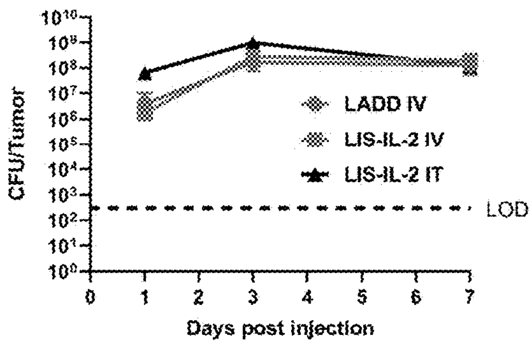


FIG. 2C

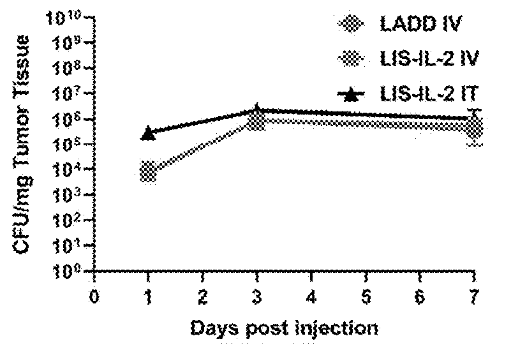


FIG. 2D

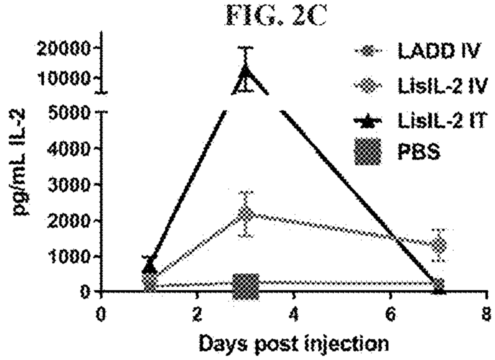


FIG. 2E

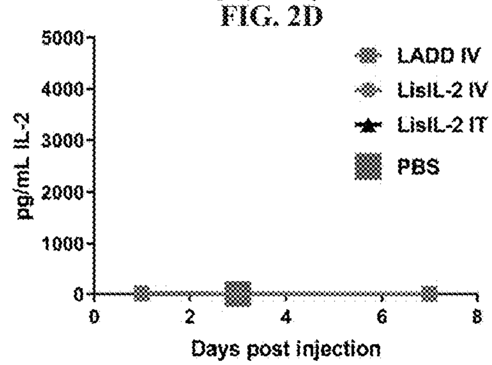


FIG. 2F

FIG. 3A

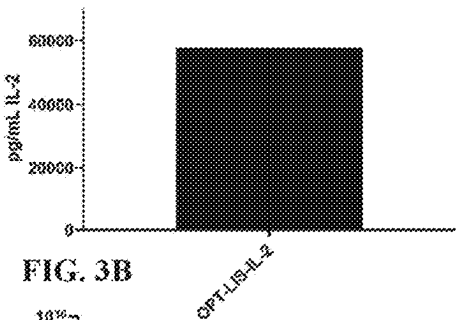


FIG. 3B

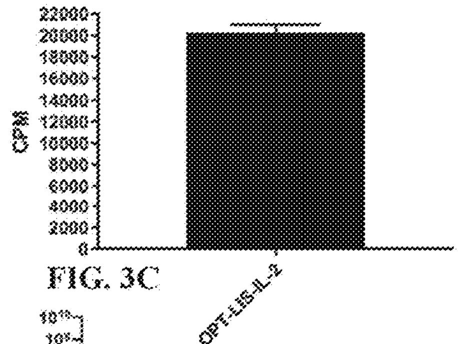


FIG. 3C

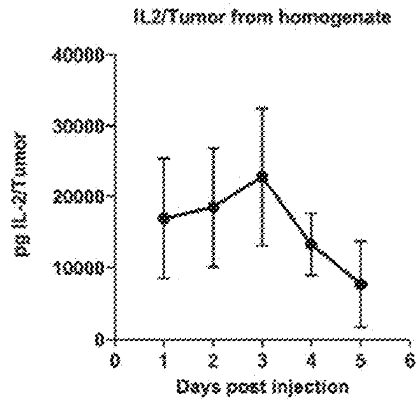


FIG. 3H

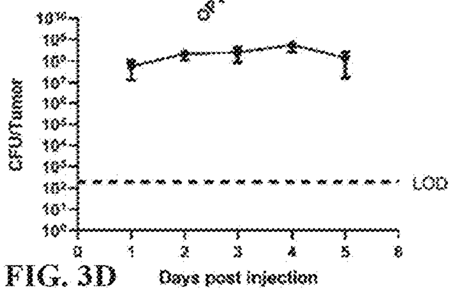


FIG. 3D

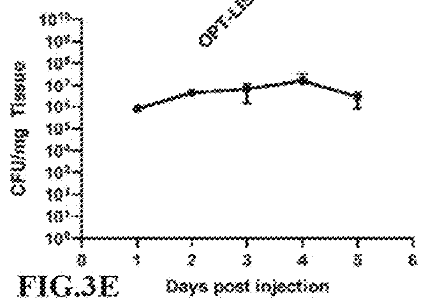


FIG. 3E

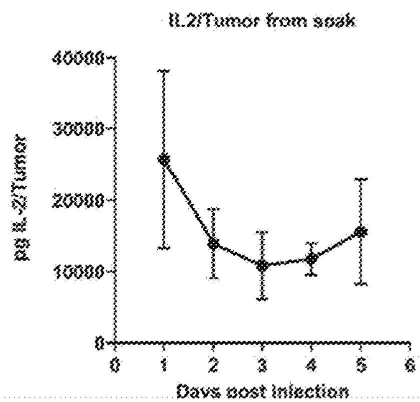


FIG. 3I

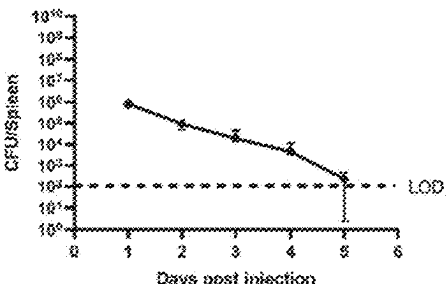


FIG. 3F

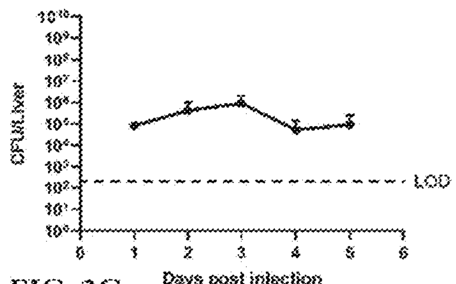


FIG. 3G

OPT-LIS-IL-2

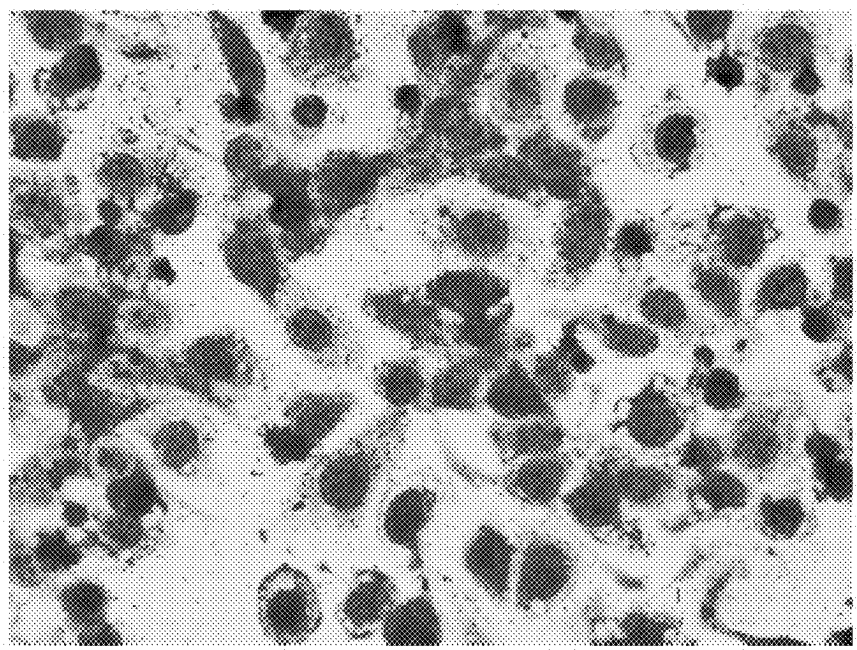


FIG. 3J

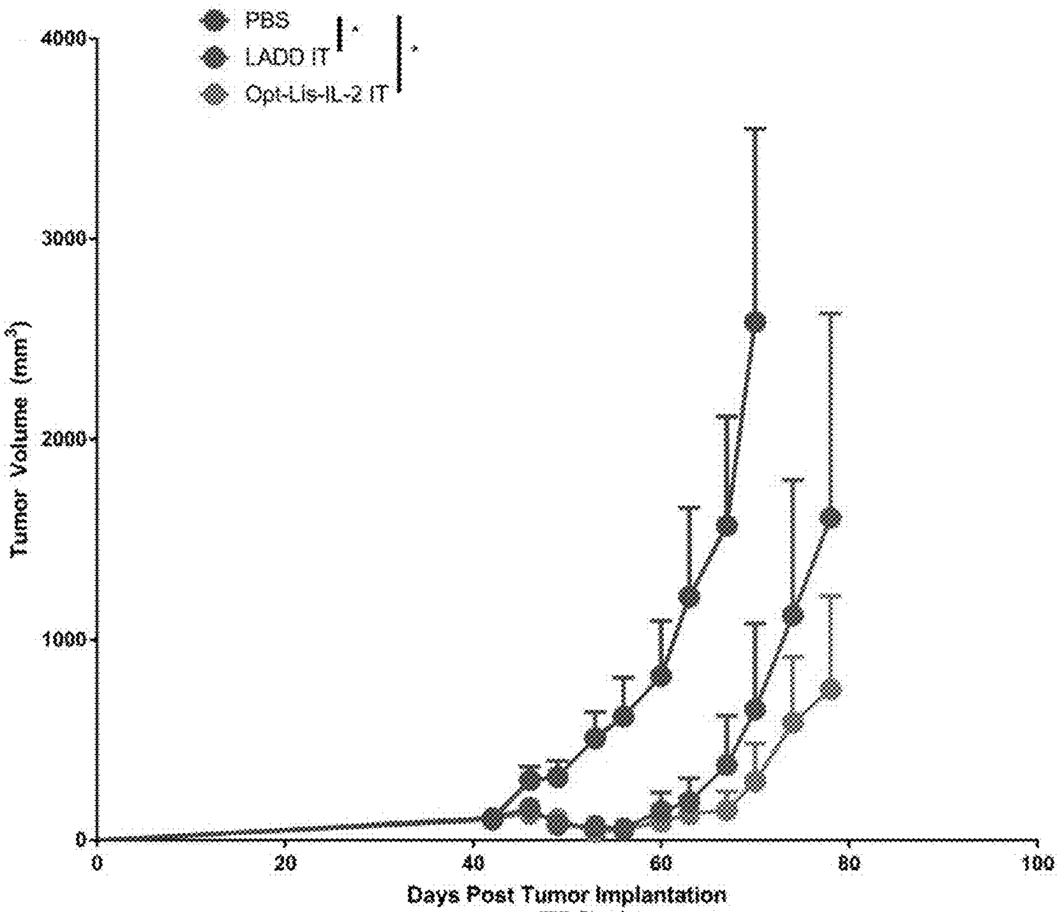

PBS
LADD IT
Opt-Lis-IL-2 IT
*
*
Tumor Volume (mm3)
4000
3000
2000
1000
0
0
20
40
60
80
100
Days Post Tumor Implantation

FIG. 4A

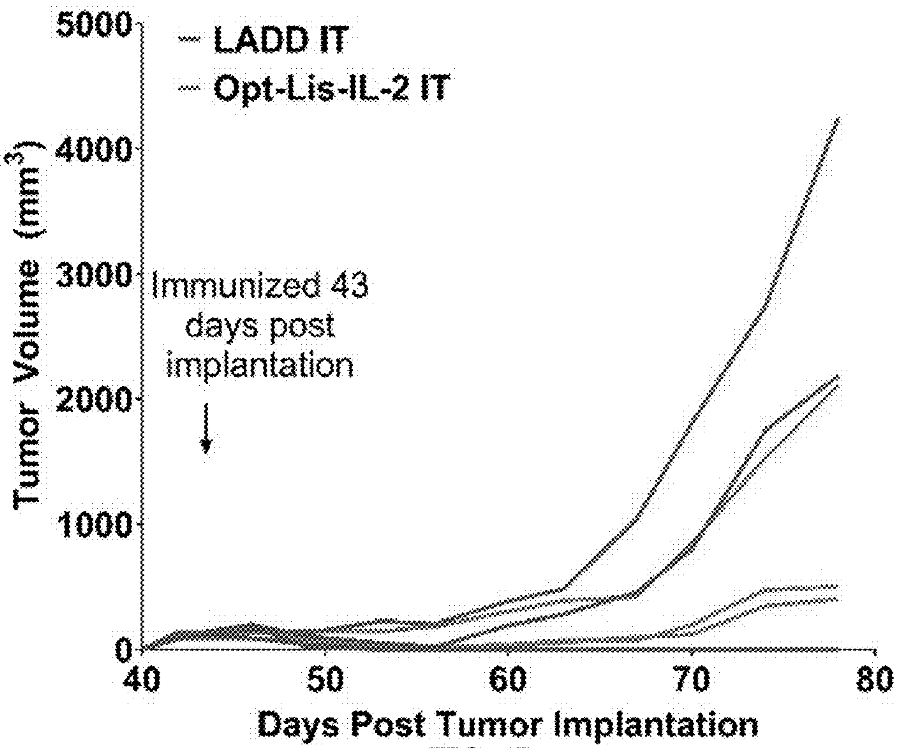

LADD IT
Opt-Lis-IL-2 IT
Tumor Volume (mm3)
5000
4000
3000
2000
1000
0
Immunized 43
days post
implantation
40
50
60
70
80
Days Post Tumor Implantation

FIG. 4B

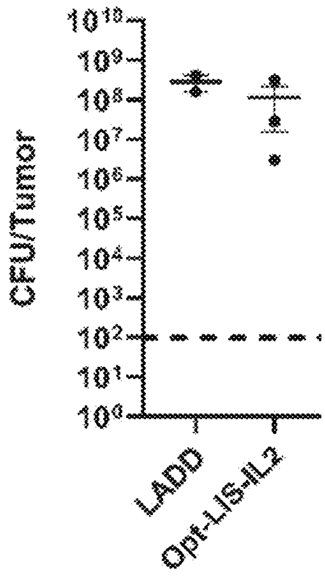


FIG. 4C

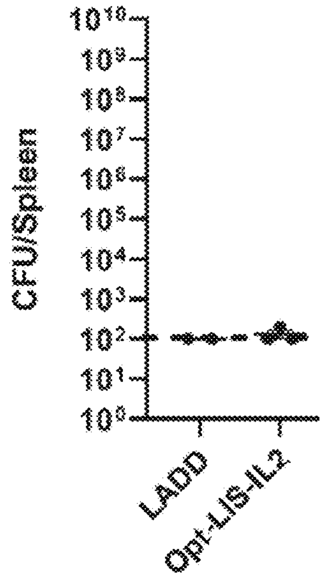


FIG. 4D

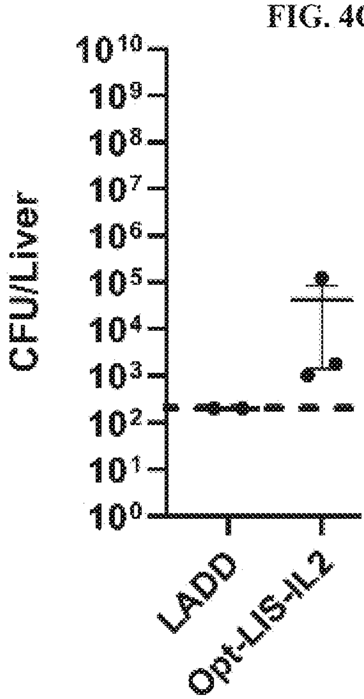


FIG. 4E

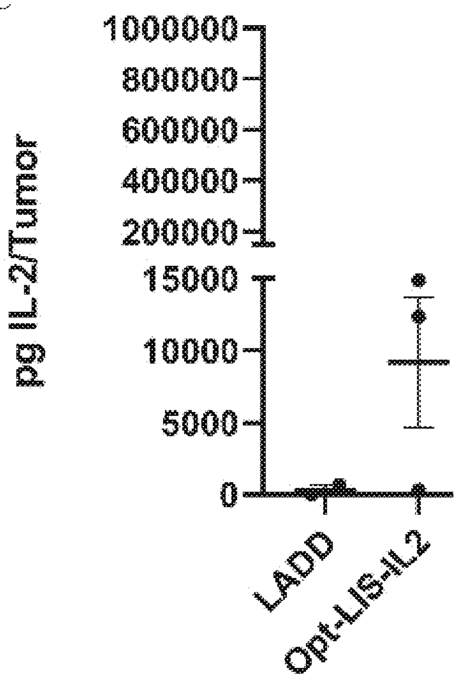


FIG. 4F

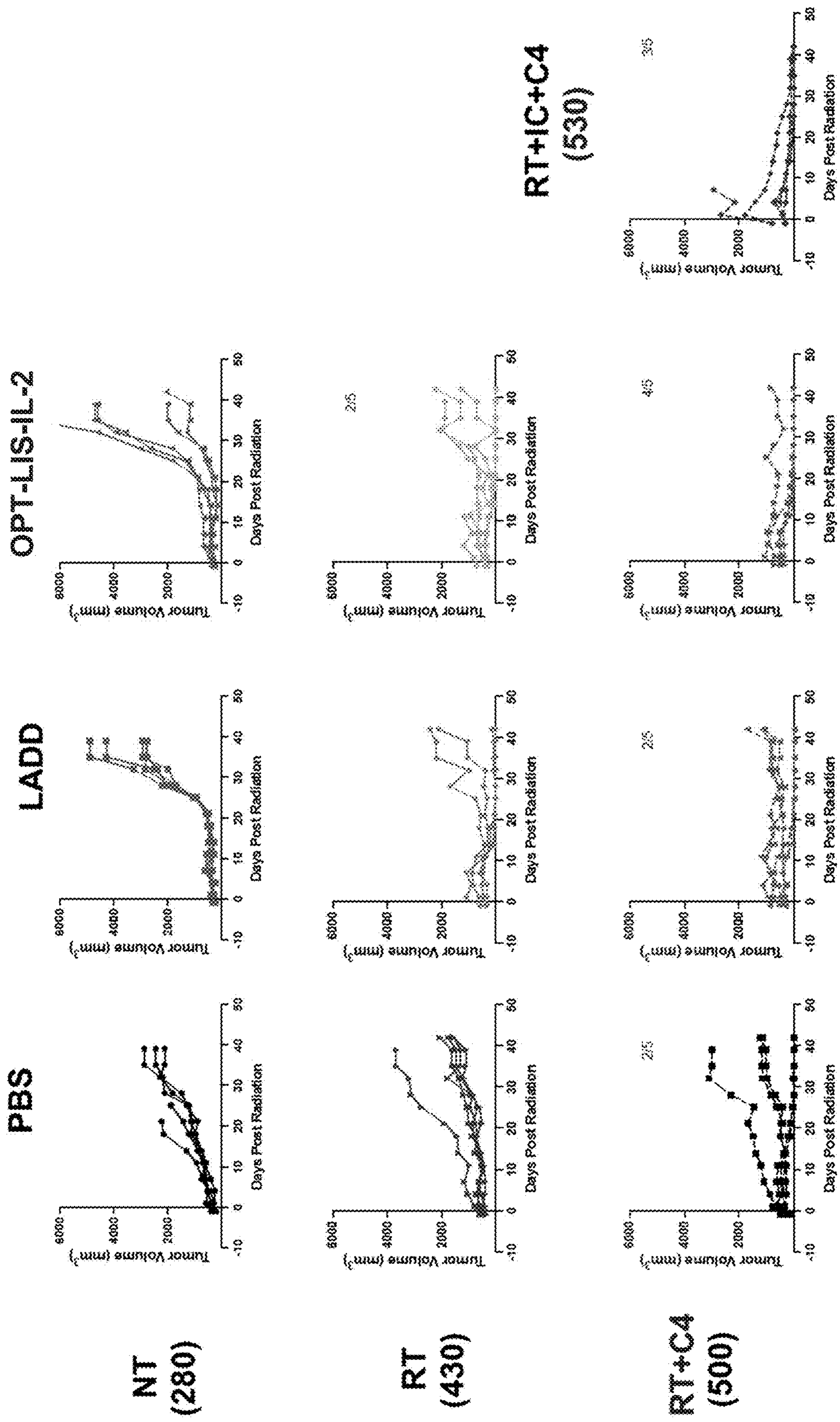

PBS
LADD
OPT-LIS-IL-2
NT
(280)
RT
(430)
RT+C4
(500)
RT+IC+C4
(530)
Tumor Volume (mm³)
Days Post Radiation
2/5
2/5
4/5
2/5
3/5

FIG. 5

METHODS AND COMPOSITIONS FOR THE TREATMENT OF CANCER

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This application claims the benefit of and priority to U.S. Provisional Patent Application No. 63/651,513, filed May 24, 2024, the entire contents of which are incorporated herein by reference.

SEQUENCE LISTING

[0002] The instant application contains a Sequence Listing which has been submitted electronically in XML format and is hereby incorporated by reference in its entirety. Said XML copy, created on Mar. 18, 2025, is named 032026-1554_SL.xml and is 37,528 bytes in size.

TECHNICAL FIELD

[0003] The present technology relates generally to genetically engineered *Listeria monocytogenes* and methods and compositions comprising the same for preventing, ameliorating, or treating cancer. In particular, the present technology relates to a genetically engineered *L. monocytogenes* bacterium, wherein the bacterium comprises a nucleic acid comprising a sequence encoding IL-2, IL-12, or CD-40L, and methods of using said bacterium for preventing, ameliorating, or treating cancer.

BACKGROUND

[0004] The following description is provided to assist the understanding of the reader. None of the information provided or references cited is admitted to be prior art to the compositions and methods disclosed herein.

[0005] The treatment of cancer is a pressing public health challenge. High dose cytokines have been deployed for cancer therapy since the late 1980's. However, challenges including systemic toxicity, frequency of treatment, and poor drug-like properties have limited their use despite some successes. Numerous strategies to overcome these challenges have been attempted, yet few have been successful. Accordingly, there is a need for new methods and compositions to deliver targeted cytokine therapies while minimizing undesirable aspects.

[0006] Attenuated *L. monocytogenes* has previously been employed in clinical trials as a vaccine due to its ability to stimulate a CD8+ T-cell response and engineering capacity to express tumor-specific antigens. In preclinical studies, it was observed that *L. monocytogenes* accumulates in the tumor microenvironment while being cleared from healthy tissues, indicating its potential as a vector for administering treatments to cancerous tissues. Methods and compositions described herein are directed to the use of *L. monocytogenes* to deliver anti-tumor agents to tumor microenvironments.

SUMMARY

[0007] In one aspect, the present disclosure provides a genetically engineered *Listeria monocytogenes* bacterium comprising a nucleic acid sequence encoding interleukin 2 (IL-2), interleukin 12 (IL-12), or Cluster of Differentiation 40 ligand (CD40L). In some embodiments, the nucleic acid sequence encodes IL-2. In some embodiments, the nucleic acid sequence encodes IL-12. In some embodiments, the nucleic acid sequence encodes CD40L. In some embodiments, the nucleic acid sequence further encodes a *Listeria monocytogenes* secretion signal. In some embodiments, the *Listeria monocytogenes* secretion signal comprises an ActA secretion signal. In some embodiments, the ActA secretion signal comprises amino acids 1 to 30 of the ActA protein or amino acids 1 to 100 of the ActA protein. In some embodiments, the ActA secretion signal comprises amino acids 1-100 of the ActA protein. In some embodiments, the *Listeria monocytogenes* secretion signal comprises the amino acid sequence set forth in SEQ ID NO: 9. In some embodiments, the nucleic acid sequence further comprises a 5' untranslated region. In some embodiments, the 5' untranslated region comprises the 5' untranslated region of an hly gene. In some embodiments, the 5' untranslated region comprises the nucleotide sequence set forth in SEQ ID NO: 11. In some embodiments, the nucleic acid sequence comprises SEQ ID NO: 10. In some embodiments, the nucleic acid sequence is integrated into the bacterial genome. In some embodiments, the nucleic acid sequence is integrated into the bacterial genome at the attBB' site in the $tRNA^{Arg}$ locus.

[0008] In one aspect, the present disclosure provides a composition for treating a cancer in a subject in need thereof comprising the genetically engineered *Listeria monocytogenes* bacterium of any one of the preceding embodiments.

[0009] In one aspect, the present disclosure provides a composition for expressing an immune potentiating agent in a tumor microenvironment of a tumor in a subject comprising the genetically engineered *Listeria monocytogenes* bacterium of any one of the preceding embodiments wherein the immune potentiating agent is selected from the group consisting of IL-2, IL-12, and CD40L.

[0010] In one aspect, the present disclosure provides a composition for expressing an immune potentiating agent in a tumor cell in a solid tumor in a subject comprising the genetically engineered *Listeria monocytogenes* bacterium of any one of the preceding embodiments, wherein the immune potentiating agent is selected from the group consisting of IL-2, IL-12, and CD40L.

[0011] In one aspect, the present disclosure provides a method of treating a cancer in a subject in need thereof comprising administering to the subject a composition comprising the genetically engineered *Listeria monocytogenes* bacterium of any one of the preceding embodiments. In some embodiments, the cancer comprises melanoma or neuroblastoma. In some embodiments, the cancer comprises one or more solid tumors. In some embodiments, the genetically engineered *Listeria monocytogenes* bacterium persists in the one or more solid tumors for about 1 day to about 3 weeks post administration. In some embodiments, the method is effective for reducing tumor volume or slowing tumor growth. In some embodiments, the composition is administered intravenously or intratumorally. In some embodiments, the genetically engineered *Listeria monocytogenes* bacterium comprises a nucleic acid sequence encoding IL-2. In some embodiments, the genetically engineered *Listeria monocytogenes* bacterium comprises a nucleic acid sequence encoding IL-12. In some embodiments, the genetically engineered *Listeria monocytogenes* bacterium comprises a nucleic acid sequence encoding CD40L. In some embodiments, the composition further comprises a pharmaceutically acceptable carrier. In some embodiments, the method further comprises administering to the subject an additional therapeutic agent. In some embodiments, the additional therapeutic agent is selected from the group consisting of radiation therapy and immune checkpoint blockade inhibitors. In some embodiments, the additional therapeutic agent is administered simultaneously, separately, or sequentially to the composition.

[0012] In one aspect, the present disclosure provides a method of expressing an immune potentiating agent in a tumor microenvironment of a solid tumor in a subject comprising administering to the subject a composition comprising the genetically engineered *Listeria monocytogenes* bacterium of any one of the preceding embodiments, wherein the immune potentiating agent is selected from the group consisting of IL-2, IL-12, and CD40L. In some embodiments, the cancer comprises melanoma or neuroblastoma. In some embodiments, the cancer comprises one or more solid tumors. In some embodiments, the genetically engineered *Listeria monocytogenes* bacterium persists in the one or more solid tumors for about 1 day to about 3 weeks post administration. In some embodiments, the method is effective for reducing tumor volume or slowing tumor growth. In some embodiments, the composition is administered intravenously or intratumorally. In some embodiments, the genetically engineered *Listeria monocytogenes* bacterium comprises a nucleic acid sequence encoding IL-2. In some embodiments, the genetically engineered *Listeria monocytogenes* bacterium comprises a nucleic acid sequence encoding IL-12. In some embodiments, the genetically engineered *Listeria monocytogenes* bacterium comprises a nucleic acid sequence encoding CD40L. In some embodiments, the composition further comprises a pharmaceutically acceptable carrier. In some embodiments, the method further comprises administering to the subject an additional therapeutic agent. In some embodiments, the additional therapeutic agent is selected from the group consisting of radiation therapy and immune checkpoint blockade inhibitors. In some embodiments, the additional therapeutic agent is administered simultaneously, separately, or sequentially to the composition.

[0013] In one aspect, the present disclosure provides a method of expressing an immune potentiating agent in a tumor cell in a solid tumor in a subject comprising administering to the subject a composition comprising the genetically engineered *Listeria monocytogenes* bacterium of any one of the preceding embodiments, wherein the immune potentiating agent is selected from the group consisting of IL-2, IL-12, and CD40L. In some embodiments, the cancer comprises melanoma or neuroblastoma. In some embodiments, the cancer comprises one or more solid tumors. In some embodiments, the genetically engineered *Listeria monocytogenes* bacterium persists in the one or more solid tumors for about 1 day to about 3 weeks post administration. In some embodiments, the method is effective for reducing tumor volume or slowing tumor growth. In some embodiments, the composition is administered intravenously or intratumorally. In some embodiments, the genetically engineered *Listeria monocytogenes* bacterium comprises a nucleic acid sequence encoding IL-2. In some embodiments, the genetically engineered *Listeria monocytogenes* bacterium comprises a nucleic acid sequence encoding IL-12. In some embodiments, the genetically engineered *Listeria monocytogenes* bacterium comprises a nucleic acid sequence encoding CD40L. In some embodiments, the composition further comprises a pharmaceutically acceptable carrier. In some embodiments, the method further comprises administering to the subject an additional therapeutic agent. In some embodiments, the additional therapeutic agent is selected from the group consisting of radiation therapy and immune checkpoint blockade inhibitors. In some embodiments, the additional therapeutic agent is administered simultaneously, separately, or sequentially to the composition.

BRIEF DESCRIPTION OF THE DRAWINGS

[0014] The patent or application file contains at least one drawing executed in color. Copies of this patent or patent application publication with color drawing(s) will be provided by the Office upon request and payment of necessary fee.

[0015] FIGS. **1**A-**1**D show *L. monocytogenes* engineered to express human IL-2. FIG. **1**A is a diagram showing design of ActA-IL-2 fusion constructs constructed for genomic integration into *L. monocytogenes*. FIG. **1**B is a schematic representation of *L. monocytogenes* expression construct, integration to the phage site of the genome, and expression of IL-2 fused to the n-terminus of ActA. FIG. **1**C shows the results of an IL-2 ELISA performed on RPMI media used to grow *L. monocytogenes* expressing IL-2 for 1 hour at 37° C. FIG. **1**D is a graph showing the CPM for CTLL-2 cells co-cultured in the presence of [^{3}H]thymidine with 50% RPMI used to grow *L. monocytogenes* expressing IL-2 for 1 hour at 37° C. and then subjected to liquid scintillation counting.

[0016] FIGS. **2**A-**2**F show that *L. monocytogenes* accumulates in the tumor microenvironment and can be engineered to express IL-2 in vivo. 2×10^6 B78 cells were implanted into the flank of syngeneic mice. When tumors reached ~300 mm^3, 1×10^7 LADD or LIS-IL-2 was injected IV or IT. Splenic (FIG. **2**A), liver (FIG. **2**B), and tumor (FIGS. **2**C-**2**D) burdens were assessed on the indicated days. IL-2 was quantified in the tumor homogenates (FIG. **2**E) and mouse serum (FIG. **2**F) at the indicated timepoints. N=3 mice/group at each timepoint.

[0017] FIGS. **3**A-**3**J show that OPT-LIS-IL-2 produces IL-2 and accumulates in tumors. FIG. **3**A shows the design of an ActAN100-IL-2 fusion construct with hly 5' UTR upstream of the fusion. OPT-LIS-IL-2 was grown for 1 hour in RPMI at 37° C. and clarified supernatants were tested for IL-2 by ELSIA (FIG. **3**B) and CTLL-2 proliferation induction capacity (FIG. **3**C). 2×10^6 B78 cells were implanted into syngeneic mice. When tumors reached ~300 mm^3, 1×10^7 OPT-LIS-IL-2 was injected IT and tissues were collected at the indicated timepoints. *L. monocytogenes* burdens were quantified in the tumor (FIGS. **3**D-**3**E), spleen (FIG. **3**F), and liver (FIG. **3**G). When tumors were collected, half was directly homogenized and homogenate was tested for IL-2 quantification (FIG. **3**H), and the other half was soaked in RPMI to allow IL-2 to passively diffuse from the tissue followed by IL-2 quantification from the tumor immersion (FIG. **3**I). B78 cells were co-cultured with OPT-LIS-IL-2 (FIG. **3**J) for 6 hours prior to standard DiffQuick staining for bacteria. N=3 mice/group.

[0018] FIGS. **4**A-**4**F show that OPT-LIS-IL-2 persist in tumors and offers tumor control. 2×10^6 B78 cells were implanted into the flank of syngeneic mice. When tumors reached ~100 mm^3, 1×10^7 of the indicated strain was injected IT and tumors (FIGS. **4**A-**4**B) were tracked for growth. At 35 days post treatment for OPT-LIS-IL-2 or

LADD, tumors (FIG. **4**C), spleens (FIG. **4**D) and livers (FIG. **4**E) were collected and quantified for *L. monocytogenes* burdens and tumor homogenates were quantified for IL-2 by ELISA (FIG. **4**F). N=4 mice/group except where individual data points are shown. Significance was determined by one-way ANOVA of area under the curve of tumor growth curves. * $p<0.05$.

[0019] FIG. **5** shows the antitumor effect of OPT-IL-2 in combination with radiotherapy (RT) and anti-CTLA-4 (C4), with PBS and the LADD strain acting as controls. The top row shows mouse tumor volumes after receiving PBS, LADD, or OPT-LIS-IL-2. The second row shows mouse tumor volumes after receiving RT plus PBS, LADD, or OPT-LIS-IL-2. The third row shows mouse tumor volumes after receiving C4, RT and PBS, LADD, or OPT-LIS-IL-2, with the graph at the far right showing mouse tumor volume after receiving RT, anti-CTLA-4 and 14.18-IL2 immunocytokine (IC).

DETAILED DESCRIPTION

[0020] It is to be appreciated that certain aspects, modes, embodiments, variations and features of the present technology are described below in various levels of detail in order to provide a substantial understanding of the present technology. The definitions of certain terms as used in this specification are provided below. Unless defined otherwise, all technical and scientific terms used herein generally have the same meaning as commonly understood by one of ordinary skill in the art to which this present technology belongs.

I. Definitions

[0021] The following terms are used herein, the definitions of which are provided for guidance.

[0022] As used herein, the singular forms "a," "an," and "the" designate both the singular and the plural, unless expressly stated to designate the singular only.

[0023] The term "about" and the use of ranges in general, whether or not qualified by the term about, means that the number comprehended is not limited to the exact number set forth herein, and is intended to refer to ranges substantially within the quoted range while not departing from the scope of the present technology. As used herein, "about" will be understood by persons of ordinary skill in the art and will vary to some extent on the context in which it is used. If there are uses of the term which are not clear to persons of ordinary skill in the art given the context in which it is used, "about" will mean up to plus or minus 10% of the particular term.

[0024] As used herein, "administration" of an agent, drug, bacterial strain(s), or composition of the present technology to a subject includes any route of introducing or delivering to a subject a compound to perform its intended function. Administration can be carried out by any suitable route, including intratumorally, orally, intranasally, parenterally (intravenously, intramuscularly, intraperitoneally, or subcutaneously), topically, or by inhalation. In some embodiments, the compositions are formulated for intravenous or intratumoral delivery. As used herein, administration includes self-administration and administration by another.

[0025] As used herein, "LADD" refers to a Live Attenuated Double Deleted *L. monocytogenes* strain known in the art. Attenuation of the strain is achieved by deletion of two virulence genes, actA and inlB, preventing cell-to-cell spread and hepatotoxicity, respectively.

[0026] As used herein, the terms "effective amount," or "therapeutically effective amount," and "pharmaceutically effective amount" refer to a quantity sufficient to achieve a desired therapeutic and/or prophylactic effect, e.g., an amount which results in the prevention of a disease, condition, and/or symptom(s) thereof. In the context of therapeutic or prophylactic applications, the amount of a composition administered to the subject will depend on the type and severity of the disease and on the characteristics of the subject, such as general health, age, sex, body weight, and tolerance to the composition drugs. It will also depend on the degree, severity, and type of disease or condition. The skilled artisan will be able to determine appropriate dosages depending on these and other factors. In some embodiments, multiple doses are administered. Additionally or alternatively, in some embodiments, multiple therapeutic compositions or compounds are used (e.g., pharmaceutical compositions comprising multiple bacterial strains alone or in combination with additional active agents, such as checkpoint blockade inhibitors or radiation therapies). In the methods described herein, compositions comprising the bacterial strains of the present technology may be administered to a subject having one or more signs, symptoms, or risk factors of cancer. For example, a "therapeutically effective amount" of the compositions of the present technology, includes levels at which the presence, frequency, or severity of one or more signs, symptoms, or risk factors of cancer are, at a minimum, ameliorated. In some embodiments, a therapeutically effective amount is achieved by multiple administrations. In some embodiments, a therapeutically effective amount is achieved with a single administration.

[0027] As used herein, "pharmaceutically acceptable carrier and/or diluent" or "pharmaceutically acceptable excipient" includes but is not limited to solvents, dispersion media, coatings, antibacterial and antifungal agents, isotonic and absorption delaying agents, and the like. In some embodiments, the pharmaceutically acceptable carrier comprises a polysaccharide, locust bean gum, an anionic polysaccharide, a starch, a protein, sodium ascorbate, glutathione, trehalose, sucrose, or pectin. In some embodiments, the polysaccharide comprises a plant, animal, algal, or microbial polysaccharide. In some embodiments, the polysaccharide comprises guar gum, inulin, amylose, chitosan, chondroitin sulphate, an alginate, or dextran. In some embodiments, the starch comprises rice starch. The use of such media and agents for biologically active substances is well known in the art. Further details of excipients are provided below. Supplementary active ingredients, such as antimicrobials, for example antifungal agents, can also be incorporated into the compositions.

[0028] As used herein, "pharmaceutically acceptable excipient" refers to substances and compositions that do not produce an adverse, allergic, or other untoward reaction when administered to an animal or a human. As used herein, the term includes all inert, non-toxic, liquid or solid fillers, or diluents that do not react with the therapeutic substance of the present technology in an inappropriate negative manner, solvents, dispersion media, coatings, antibacterial and antifungal agents, isotonic and absorption delaying agents, preservatives and the like, for example liquid pharmaceutical carriers e.g., sterile water, saline, sugar solutions, Tris buffer, ethanol and/or certain oils.

[0029] As used herein, "prevention," "prevent," or "preventing" of a disorder or condition refers to, in a statistical sample, reduction in the occurrence or recurrence of the disorder or condition in treated subjects/samples relative to an untreated controls, or refers delays the onset of one or more symptoms of the disorder or condition relative to the untreated controls.

[0030] As used herein "subject" and "patient" are used interchangeably. In some embodiments, the subject is an animal subject. In some embodiments, the animal subject is a mammal. In some embodiments, the mammalian subject is a human.

[0031] As used herein, the term "simultaneous" administration refers to the administration of at least two agents by the same route and at the same time or at substantially the same time.

[0032] As used herein, the term "separate" administration refers to an administration of at least two agents at the same time or at substantially the same time by different routes.

[0033] As used herein, the term "sequential" administration refers to administration of at least two agents at different times, the administration route being identical or different. More particularly, sequential use refers to the whole administration of one agent before administration of the other agent(s) commences. It is thus possible to administer one of the agents over several minutes, hours, or days before administering another.

[0034] A "synergistic therapeutic effect" refers to a greater-than-additive therapeutic effect which is produced by a combination of at least two therapeutic agents, and which exceeds that which would otherwise result from the individual administration of the agents. For example, use of bacterial strain(s) of the present technology in conjunction with other agents for the treatment of cancer may result in a greater than additive therapeutic effect. In some embodiments, the synergistic effect may permit the use of lower doses of bacterial strain(s) of the present technology and/or other agents than would be required if each were used alone.

[0035] "Treating," "treat," "treated," or "treatment" of a disease or disorder includes: (i) inhibiting the disease or disorder, i.e., arresting its development; (ii) relieving the disease or disorder, i.e., causing its regression; (iii) slowing progression of the disorder; and/or (iv) inhibiting, relieving, or slowing progression of one or more symptoms of the disease or disorder.

[0036] It is to be appreciated that the various modes of treatment or prevention of medical diseases and conditions as described are intended to mean "substantial," which includes total but also less than total treatment or prevention, and wherein some biologically or medically relevant result is achieved.

II. Cancer Treatment and IL-2, IL-12, and CD40L

[0037] Harnessing the immune system to treat cancer has become commonplace due to the revolutionizing impact of immunotherapy[1]. Despite these successes not all patients respond to immunotherapies and many eventually progress in their disease[2]. Thus, more work is needed to develop broadly efficacious treatments that lead to durable responses and help fill the gap between responders and non-responders. CD8+ T-cell frequency is typically associated with greater overall survival[3] and increased response to immunotherapy treatments such as checkpoint inhibitors (CPIs) 4. Yet for those with fewer CD8+ T-cells whose tumors are considered immunologically cold, the response to CPI treatments is less favorable[4]. Thus, treatments aimed at generating large numbers of highly functional, tumor-specific CD8+ T-cells and creating an immunologically hot tumor microenvironment to support those T-cells are needed to fill the gap.

[0038] IL-2 is a cytokine that stimulates the proliferation and survival of T-cells. High-dose recombinant IL-2 has been used as a treatment for renal cell carcinoma and metastatic melanoma with some success[5]. Despite some successes use of high-dose IL-2 has been limited due to severe treatment-related toxicities and a short in vivo half-life requiring many frequent administrations[6,7].

[0039] IL-12 is a cytokine that has a wide range of anti-tumor functions including activation of T-cells and NK cells to increase IFNγ production. Recombinant IL-12 has been demonstrated to potently induce tumor regression and has demonstrated synergy with IL-2 when delivered intratumorally[47-50]. Despite these promising results, the requirement for repeated intratumoral administration due to toxicity associated with systemic IL-12 delivery has limited further successful development of IL-12 in the clinic[51].

[0040] CD40L is an activating ligand for CD40 which has many functions including promoting macrophage activation and antigen presentation as well as promoting dendritic cell maturation and antigen presentation among other functions[52]. Activation of CD40 signaling by either antibody mediated or CD40L mediated activation has demonstrated a synergistic effect in combination with radiotherapy and chemotherapy[53-57]. Similar to IL-2 and IL-12 mediated therapies, full development of anti-CD40 or CD40L therapeutics have been limited by toxicity associated with systemic delivery and signaling.

III. *Listeria monocytogenes* Clinical Applications

[0041] *Listeria monocytogenes* is a gram-positive, facultative intracellular bacterium that has been employed in many clinical trials as an anti-cancer vaccine for numerous cancer types[9-11]. The bacterium has been used for decades to understand aspects of immunology and cell biology[12]. As a result, genetic tools have been developed that enable genetic engineering of the bacterium[13]. In clinical trials, *L. monocytogenes* has been engineered to express peptide fragment cancer antigens or larger proteins that are processed into antigenic fragments, and spurs a strong CD8+ T-cell response[14]. However, *L. monocytogenes* has not been shown to be an effective expression vector for full length, immune modulating proteins with secondary structures, and the cancer antigen expressing *L. monocytogenes* strains only produced cellular localized antigens.

[0042] Wild-type *L. monocytogenes* gains intracellular access through phagocytosis by professional phagocytes or by receptor mediated endocytosis mediated by two surface proteins, InlA and InlB into non-phagocytes[9,15,16]. Once internalized *L. monocytogenes* is initially encased in a vacuole where it uses a cholesterol-dependent pore forming protein called Listerialysin O (LLO) to break free of the vacuole and into the cytosol[17]. The expression and function of LLO is tightly regulated as strains that fail to compartmentalize LLO function lyse any cell they infect[18]. Once in the cytosol, *L. monocytogenes* spreads cell to cell by hijacking the host actin cytoskeleton using the secreted *L. monocytogenes* protein, ActA[19,20].

[0043] Strains of *L. monocytogenes* used in clinical settings have been attenuated to be safe for application in human patients. The vaccine strain of *L. monocytogenes* used in this study, LADD (Live Attenuated Double Deleted), is attenuated by deletion of two virulence genes, actA and inlB, preventing cell to cell spread and hepatotoxicity, respectively[10]. This strain is 1,000-fold less virulent than wild-type *L. monocytogenes* yet retains its immunogenicity[10]. Importantly, LADD has previously been engineered to express and secrete both, exogenous antigens to drive antigen specific CD8+ T-cell responses, as well as mammalian proteins to modulate host cell physiology[21-25]. During administration to a healthy mouse, LADD is taken into phagocytic cells of the liver and spleen where it establishes an intracellular niche and stimulates a robust CD8+ T-cell response[19,26]. When applied to a tumor-bearing mouse, it was observed that *L. monocytogenes* is cleared or persists at very low levels in the spleen and liver yet accumulates in the tumor microenvironment (TME)[27,28, and unpublished data].

[0044] The present technology takes advantage of *L. monocytogenes* accumulation in the tumor microenvironment (TME) to deliver tumor-targeted immunomodulatory cytokines. Specifically, to express human IL-2 from *L. monocytogenes* as an in-situ vaccine to drive proliferation of tumor-specific T-cells. This disclosure demonstrates the production of bioactive IL-2 and accumulation of IL-2 engineered *L. monocytogenes* in the TME. Accordingly, the technology is useful for therapeutic methods for the treatment of cancer.

IV. Therapeutic and Prophylactic Methods

[0045] The following discussion is presented by way of example only, and is not intended to be limiting.

[0046] One aspect of the present technology includes methods of treating or preventing cancer in a subject diagnosed as having, suspected as having, or at risk of having cancer. In therapeutic applications, compositions or medicaments comprising the *L. monocytogenes* bacterial strains of the present technology are administered to a subject suspected of, or already suffering from, cancer, in an amount sufficient to cure, or at least partially arrest, the signs or symptoms of cancer, including its complications and intermediate pathological phenotypes in development of the disease.

[0047] Subjects suffering from cancer can be identified by any or a combination of diagnostic or prognostic assays known in the art. For example, typical symptoms of cancer include, but are not limited to: tumor appearance, tumor growth, tumor metastasis, changes in weight, changes in skin coloration, sores that do not heal, changes in bowel or bladder habits, persistent cough, trouble breathing, difficult swallowing, hoarseness, indigestion or discomfort after eating, unexplained muscle pain, joint pain, fevers, night sweats, bleeding, or bruising, or any combination thereof. In some embodiments cancer is diagnosed using imaging technology, such as MRI, CT scans, ultrasounds, PET scans, and X-rays, for abnormalities. In some embodiments, cancer is diagnosed using genetic testing. In some embodiments, cancer is diagnosed by assaying tissue samples or biopsies for abnormalities.

[0048] In some embodiments, subjects with cancer treated with the bacterial strain(s) of the present technology, or spores thereof, will show amelioration or elimination of one or more of the following symptoms: tumor appearance, tumor growth, tumor metastasis, changes in weight, changes in skin coloration, sores that do not heal, changes in bowel or bladder habits, persistent cough, trouble breathing, difficult swallowing, hoarseness, indigestion or discomfort after eating, unexplained muscle pain, joint pain, fevers, night sweats, bleeding, or bruising, or any combination thereof.

V. Modes of Administration and Effective Dosages

[0049] Compositions of the present technology for use in preventing, ameliorating, or treating cancer and/or reducing the severity of one or more risk factors, signs, or symptoms associated with cancer include genetically engineered *L. monocytogenes* bacterium, wherein the bacterium comprises a nucleic acid comprising a sequence encoding IL-2, IL-12, or CD-40L. The compositions of the present technology are administered to the subject in effective amounts (i.e., amounts that have desired therapeutic effect). The dose and dosage regimen will depend upon the nature of the cancer, the degree of cancer progression and symptom severity in the subject, the characteristics of the strain used, e.g., its therapeutic index, the subject, and the subject's history. The effective amount may be determined during pre-clinical trials and clinical trials by methods familiar to physicians and clinicians.

[0050] Additional components of the compositions of the present technology may include a preservative selected from the group consisting of sucrose, sodium ascorbate, and glutathione. In some embodiments the preservative is a cryoprotectant selected from the group consisting of a nucleotide, a disaccharide, a polyol, and a polysaccharide. In some embodiments, the cryoprotectant is selected from the group consisting of inosine-5'-monophosphate (IMP), guanosine-5'-monophosphate (GMP), adenosine-5'-monophosphate (AMP), uranosine-5'-monophosphate (UMP), cytidine-5'-monophosphate (CMP), adenine, guanine, uracil, cytosine, guanosine, uridine, cytidine, hypoxanthine, xanthine, orotidine, thymidine, inosine, trehalose, maltose, lactose, sucrose, sorbitol, mannitol, dextrin, inulin, sodium ascorbate, glutathione, and skim milk.

[0051] The genetically engineered *L. monocytogenes* bacterium, wherein the bacterium comprises a nucleic acid comprising a sequence encoding IL-2, IL-12, or CD-40L, described herein can be incorporated into pharmaceutical compositions for administration, singly or in combination, and given to a subject for the treatment or prevention of a disorder described herein. Such compositions typically include the active agent and a pharmaceutically acceptable carrier. As used herein the term "pharmaceutically acceptable carrier" includes saline, solvents, dispersion media, coatings, antibacterial and antifungal agents, isotonic and absorption delaying agents, and the like, compatible with pharmaceutical administration. Supplementary active compounds can also be incorporated into the compositions. Carriers can be solid-based dry materials for formulations in powdered form, and can be liquid or gel-based materials for formulations in liquid or gel forms, which forms depend, in part, upon the routes or modes of administration. In some embodiments, the pharmaceutically acceptable carrier comprises a polysaccharide, locust bean gum, an anionic polysaccharide, a starch, a protein, sodium ascorbate, glutathione, trehalose, sucrose, or pectin. In some embodiments, the polysaccharide comprises a plant, animal, algal, or microbial polysaccharide. In some embodiments, the polysaccharide comprises guar gum, inulin, amylose, chitosan, chondroitin sulphate, an alginate, or dextran. In some embodiments, the starch comprises rice starch.

[0052] Pharmaceutical compositions are typically formulated to be compatible with the intended route of administration. Examples of routes of administration include intratumorally, orally, intranasally, parenterally (intravenously, intramuscularly, intraperitoneally, or subcutaneously), topically, or by inhalation. In some embodiments, the compositions of the present technology are formulated for intravenous administration. In some embodiments, the compositions of the present technology are formulated for intratumoral administration. Other formulations will be readily apparent to one skilled in the art.

[0053] Dosage, toxicity and therapeutic efficacy of any therapeutic agent can be determined by standard pharmaceutical procedures in cell cultures or experimental animals. The data obtained from the cell culture assays and animal studies can be used in formulating a range of dosage for use in humans. The dosage of such compounds may be within a range of circulating concentrations that include the ED50 with little or no toxicity. The dosage may vary within this range depending upon the dosage form employed and the route of administration utilized. For any compound used in the methods, the therapeutically effective dose can be estimated initially from cell culture assays. A dose can be formulated in animal models to achieve a circulating plasma concentration range that includes the IC50 (i.e., the concentration of the test compound which achieves a half-maximal inhibition of symptoms) as determined in cell culture. Such information can be used to determine useful doses in humans accurately.

[0054] In some embodiments, the compositions of the present technology comprise one or more genetically engineered *L. monocytogenes* strains comprising a nucleic acid comprising a sequence encoding IL-2, IL-12, or CD-40L, ranging from at least about 10^6 colony forming units (CFU)/mL to at least about 10^9 CFU/mL, or any value in between. In some embodiments, the compositions range from about 10^6 CFU/mL to about 10^9 CFU/mL. In some embodiments, the compositions range from about 10^7 CFU/mL to about 10^9 CFU/mL. In some embodiments, the compositions range from about 10^8 CFU/mL to about 10^9 CFU/mL.

[0055] An exemplary treatment regimen entails administration of at least a single dose of the one or more genetically engineered *L. monocytogenes* strains of the present technology to a subject. In some embodiments, the subject receives a single dose of the one or more genetically engineered *L. monocytogenes* strains. In some embodiments, the subject receives multiple doses of the one or more genetically engineered *L. monocytogenes* strains. In some embodiments, the multiple doses are administered about 1 day apart, about 2 days apart, about 3 days apart, about 4 days apart, about 5 days apart, about 6 days apart, about 1 week apart, about 2 weeks apart, about 3 weeks apart, about 4 weeks apart, about two months apart, about 3 months apart, about 4 months apart, about 5 months apart, about 6 months apart, about 7 months apart, about 8 months apart, about 9 months apart, about 10 months apart, about 1 year apart, or about 2 years apart. In some embodiments the multiple doses are administered at regular or irregular intervals. In some embodiments, the subject receives a single dose of the one or more genetically engineered *L. monocytogenes* strains of the present technology, and receives a second dose once the one or more genetically engineered *L. monocytogenes* strains are no longer detected in one or more tumors in the subject. In therapeutic applications, a relatively high dosage at relatively short intervals is sometimes required until progression of the disease is reduced or terminated, or until the subject shows partial or complete amelioration of symptoms of disease. Thereafter, the subject can be administered a prophylactic regime. In some embodiments, compositions of the present technology are administered multiple times per day. In some embodiments, compositions of the present technology are administered to a subject once, twice, or three times per day or more for a certain period of time or until the subject is deemed cured of primary disease, not to be at risk for recurrence of primary disease, or not to be at risk for the disease. In some embodiments, the compositions of the present technology may be administered to the subject for the remainder of the subject's life.

[0056] In some embodiments, administration is paired with an exposure to co-therapeutics (i.e., agents known in the art for the treatment of cancer, such as immune checkpoint blockade inhibitors or radiation therapies), either simultaneously, separately, or sequentially with dosing of the compositions of the present technology. In some embodiments, administration of radiation therapy is combined simultaneously, separately, or sequentially with dosing of the compositions of the present technology. In some embodiments, administration of immune checkpoint blockade inhibitor therapy is combined simultaneously, separately, or sequentially with dosing of the compositions of the present technology. In some embodiments, administration of radiation therapy and immune checkpoint blockade inhibitor therapy are combined simultaneously, separately, or sequentially with dosing of the compositions of the present technology. In some embodiments, compositions of the present technology, radiation therapy, and immune checkpoint blockade inhibitor therapy are administered simultaneously, separately, or sequentially to a subject. In any of the preceding embodiments, the one or more co-therapeutics and the compositions of the present technology can be administered by any appropriate route and can be formulated for any appropriate route of administration. For example, in some embodiments the one or more genetically engineered *L. monocytogenes* strains, the radiation therapy, and the immune checkpoint blockade therapy are formulated for intratumoral, intraperitoneally, or intravenous administration. In some embodiments, the one or more genetically engineered *L. monocytogenes* strains, the radiation therapy, and the immune checkpoint blockade therapy are administered intratumorally, intraperitoneally, or intravenously. In some embodiments, the radiation therapy is administered via an external beam. In some embodiments, any of the combination treatments described herein result in a synergistic therapeutic effect. For example, administration of compositions of the present technology with one or more additional therapeutic agents for the prevention or treatment of cancer will have greater than additive effects in the prevention or treatment of the disease and/or one or more of its signs or symptoms.

[0057] In any case, the multiple therapeutic agents may be administered in any order or even simultaneously. If simultaneously, the multiple therapeutic agents may be provided in a single, unified form, or in multiple forms (by way of example only, either as a single pill or as two separate pills). One of the therapeutic agents may be given in multiple doses, or both may be given as multiple doses. In addition, the combination methods, compositions and formulations are not to be limited to the use of only two agents.

[0058] The skilled artisan will appreciate that certain factors may influence the dosage and timing required to effectively treat a subject, including but not limited to, the severity of the disease or disorder, previous treatments, the general health and/or age of the subject, and other diseases present. Moreover, treatment of a subject with a therapeutically effective amount of the therapeutic compositions described herein can include a single treatment or a series of treatments.

EXAMPLES

Example 1: Materials and Methods

[0059] This example describes materials and methods used to generate and test *L. monocytogenes* strains described herein.

Bacterial Strains and Construction

[0060] *E. coli* was regularly grown at 37° C. in LB or on LB+1.5% agar plates and frozen in LB+40% glycerol. XL-1 Blue *E. coli* was used for subcloning and propagation of plasmids and S17 *E. coli* was used for conjugation into *L. monocytogenes. L. monocytogenes* were routinely grown at 37° C. in brain-heart infusion (BHI, [BD, 237500]) or on BHI+1.5% agar plates and frozen in BHI+40% glycerol. Antibiotics were used at the following concentrations: 200 μg/ml streptomycin or 30 μg/ml kanamycin.

[0061] Plasmids pIMK2-ActAN30-IL2 and pIMK2-ActAN100-IL2 were constructed by Gibson Assembly (New England Biolabs, E2621) using NcoI-HF (New England Biolabs, R3193) and SalI-HF (New England Biolabs, R3138) linearized pIMK2 and a gBlock (IDT) comprised of the ActA N30 or N100 amino acids fused to human IL2 (sequences in Table 1). Plasmid pIMK2-hly5'UTR-ActAN100-IL2 was constructed by Gibson Assembly using NcoI-HF linearized and Quick CIP (New England Biolabs, M0525) treated pIMK2-ActAN100-IL2, and a 46 base-pair fragment of the hly 5' UTR amplified from genomic DNA by primers phelp-hly5' utr_fwd and phelp-hly5' utr_rev (sequence in Table 1). Plasmids were cloned first into XL1-Blue *E. coli* and selected for on kanamycin plates and sequence confirmed by full-plasmid sequencing (plasmidsaurus) and then subcloned into S17 *E. coli* for subsequent conjugation. Conjugations were performed by spreading a single S17 colony onto a BHI-agar plate in a 1 cm square, followed by spreading of a corresponding *L. monocytogenes* colony in perpendicular fashion over the first colony. This was grown overnight at 37° C., and the patch was then scraped into 400 μL PBS, and diluted in 10-fold serial dilutions and plated onto antibiotic-containing plates to select against S17 and for plasmid integration into *L. monocytogenes*. Single colonies were screened by PCR for the hly gene (hly-F, and hly-R) and for the IL-2 construct. IL-2 production was then confirmed by ELISA.

[0062] All strains of *L. monocytogenes* used in this study were in the 10403S background. The attenuated strain used in this study, termed Live Attenuated Double Deleted (LADD) is genetically modified by in-frame deletion of the virulence factors, actA and inlB (ΔactA and ΔinlB) and is previously described[10]. Strain LIS-IL-2N100 (referred to as LIS-IL-2) was constructed by conjugating pIMK2-ActAN100-IL-2 into LADD. The strain LIS-IL-2N30 was constructed by conjugating pIMK2-ActAN30-IL-2 into LADD. The strain OPT-LIS-IL-2 was constructed by conjugating pIMK2-hly5'UTR-ActAN100-IL-2 into LADD.

Cell Lines and Culture

[0063] B78 cells were routinely cultured in RPMI (Fisher, #11875093), with 10% FBS (Fisher, SH30071), 1% L-Glutamine (Fisher, #25030081), and 1% Antibiotic/Antimycotic (Fisher, 15240062). For infection experiments, Antibiotic/Antimycotic was omitted. MC38 and CT26 cells were routinely cultured in DMEM (Invitrogen, 11965092) with 10% FBS, 1% L-Glutamine, and 1% Antibiotic/Antimycotic. For infection experiments, 5×10^5 B78 cells were plated in 24 well plates (Fisher, 12556006) onto flamed glass coverslips (VWR, 89015-725). Cells were then infected with indicated strains washed with PBS and grown overnight at 30° C. without agitation at an MOI=1. Infection proceeded until indicated timepoints, and media was filtered and stored at −80° C. until use, and coverslips were subjected to standard Diff-Quick staining protocols. Coverslips were then mounted and imaged under a brightfield microscope using a 50× objective.

[0064] CTLL-2 cells were maintained in RPMI+10% FBS, 1% L-Glutamine+200 U/mL IL-2. Cells were washed to remove residual IL-2 and 5×10^3 cells were added to 96 well plates and media was mixed ½ with 0.22 μM filtered RPMI media inoculated with 1×10^8 IL-2 secreting *L. monocytogenes* and grown for 1 hour at 37° C. with agitation. Cells were cultured for 24 hours in the presence of [^{3}H] thymidine and incorporation was measured by liquid scintillation counting.

Tumor Experiments and Treatments

[0065] 2×10^6 B78, or MC38 cells were injected intradermally into the right flank of syngeneic C57BL/6 mice (Taconic Farms) or 2×10^6 CT26 were injected intradermally into the right flank of syngeneic BALB/C mice (Taconic Farms). Tumors were measured at the indicated timepoints using electronic calipers and tumor volume was calculated according to tumor volume=0.5× ((small width) 2×(large width)) where the large width was the widest point in the tumor, and the small width was the widest corresponding perpendicular width. When tumors reached the indicated size or timepoint, treatment with *L. monocytogenes* was performed by injection of 1×10^7 bacteria resuspended in PBS in 50 μL directly into the tumor using a 27 g needle, or by injection of 1×10^7 bacteria resuspended in 200 μL PBS via the lateral tail vein using a 27 g needle. Tumor volumes were measured at the indicated timepoints until collection.

Enumeration of Bacterial Burdens in Tissues and Collection

[0066] At the indicated timepoints, mice were euthanized, and tumors were collected into cold, sterile PBS, while livers (including the gallbladder) and spleens were collected into cold, sterile PBS+0.1% IPEGAL (Sigma, I8896-50ML). Tissues were then homogenized using a tissue grinder (Polytron, Pt-3100). In some experiments, tumors were cut in half, weighed, and ½ was subjected to homogenization in PBS immediately. The other half was soaked in 500 μL RPMI at 4° C. for 18 hours to allow cytokines to passively diffuse from the tissue and avoid cellular lysis from homogenization. Homogenates were then plated on BHI+agar+

streptomycin plates and stored at 4° C. and CFU were enumerated the following day. CFU/tumor was determined by adjusting for percents of tumors homogenized versus used for soaking. Blood was collected by retroorbital bleed using heparinized capillary tubes (Fisher, 22260950) into serum separator tubes (Fisher, 365956). After a minimum of 10 minutes of coagulation at room temperature, separator tubes were spun at 3000×G for 10 minutes and cell free serum was collected into new tubes and plated for bacterial enumeration and frozen at −80° C. until use in ELISA.

ELISAs and LegendPlex Assays

[0067] Medias, tissue homogenates, or mouse serum were clarified by spinning at max speed and then subjected to filtration through Nalgene sterile 25 mm PES 0.2 um syringe filters (ThermoFisher, 725-2520). Human IL-2 ELISA was performed using ELISA MAX™ Standard Set Human IL-2 (Biolegend, 431801) according to manufactures instructions. LEGENDplex™ Mouse B Effector ½ (Be1/2) Panel (8-plex) w/VbP (Biolegend, 740821) was performed according to manufacturer's instructions.

ELISAs and LegendPlex Assays

[0068] Primers and other oligonucleotides used in these experiments are shown below in Table 1.

Statistics and Analysis

[0069] Statistical analysis was performed by GraphPad Prism Software (La Jolla, CA) and analyzed via Kruskal-Wallace, Mann Whitney or one-way ANOVA with Tukey's correction as indicated. For tumor experiments, Prism was used to calculate the area under the curve at the last timepoint with all mice surviving and one-way ANOVA with Tukey's correction was used to determine significance.

Example 2: Construction and Characterization of *L. monocytogenes* Expressing IL-2

[0070] This example demonstrates the engineering of *L. monocytogenes* strain for the production of biologically active IL-2.

[0071] To create *L. monocytogenes* capable of secreting human IL-2, a *Listeria*-codon optimized sequence of the human interleukin-2 (IL-2) gene was fused to the n-terminal 30 or 100 amino acids (termed N30 or N100) of the *L. monocytogenes* secreted protein ActA as previously described[21,36] Fusion to ActA N30 or N100 facilitates secretion from the bacterium; the N30 fusion results in an untagged IL-2 while N100 results in 70 amino acids of ActA remaining on the secreted IL-2. These constructs were cloned into an *L. monocytogenes* expression vector (pIMK2)

TABLE 1

gBlock and primer sequences used in this study

ActAN30-IL2	aggagagtgaaacccatggGtgggattaaatagatttatgcgtgcgatgatggtagttttcattactgc caactgcattacgattaaccccgacataatatttgcagcgCCAACATCTTCTTCCACTAAGAAGAC TCAGCTACAACTTGAACACCTTCTGTTGGACCTACAAATGATTCTA AATGGAATAAACAATTATAAAAATCCTAAATTAACTAGAATGCTCA CGTTCAAATTCTACATGCCGAAAAAAGCAACCGAGTTAAAACATTT ACAATGTCTTGAAGAAGAACTTAAACCATTGGAAGAAGTGCTTAA CTTGGCCCAAAGTAAAAATTTTCATTTACGTCCACGAGATTTAATT AGCAATATCAATGTCATTGTATTAGAATTAAAAGGTAGTGAAACCA CATTTATGTGCGAATATGCTGATGAGACAGCGACAATTGTTGAATT TTTAAACCGCTGGATTACATTTTGTCAATCAATTATCTCGACGTTAA CGTGAGTCGACCTCGAGGGG	SEQ ID NO: 1
ActAN100-IL2	gaaggagagtgaaacccatggGtgggattaaatagatttatgcgtgcgatgatggtagttttcattact gccaactgcattacgattaaccccgacataatatttgcagcgacagatagcgaagattccagtctaaa cacagatgaatgggaagaagaaaaaacagaagagcagccaagcgaggtaaatacgggaccaagatacga aactgcacgtgaagtaagttcacgtgatattgaggaactagaaaaatcgaataaagtgaaaaatacgaa caaagcagacctaatagcaatgttgaaagcaaaagcagagaaaggtggatccCCAACATCTTCTTCCAC TAAGAAGACTCAGCTACAACTTGAACACCTTCTGTTGGACCTACAAATGATTCTA AATGGAATAAACAATTATAAAAATCCTAAATTAACTAGAATGCTCA CGTTCAAATTCTACATGCCGAAAAAAGCAACCGAGTTAAAACATTT ACAATGTCTTGAAGAAGAACTTAAACCATTGGAAGAAGTGCTTAA CTTGGCCCAAAGTAAAAATTTTCATTTACGTCCACGAGATTTAATT AGCAATATCAATGTCATTGTATTAGAATTAAAAGGTAGTGAAACCA CATTTATGTGCGAATATGCTGATGAGACAGCGACAATTGTTGAATT TTTAAACCGCTGGATTACATTTTGTCAATCAATTATCTCGACGTTAA CGTGAGTCGACCTCGAGGGGGGG	SEQ ID NO: 2
phelp-hly5' utr fwd	agggaacaaaagctgggtacCCATTATGCTTTGGCAGTTTATTC	SEQ ID NO: 3
phelp-hly5' utr rev	agcctgacatGGGTTTCACTCTCCTTCTAC	SEQ ID NO: 4
hly-F	TCAACCAGATGTTCTCCCTGTA	SEQ ID NO: 5
hly-R	CACTGTAAGCCATTTCGTCATC	SEQ ID NO: 6

that integrates stably into the bacterium's genome at the attBB' site in the $tRNA^{Arg}$ locus[37, 46], generating pIMK2-ActAN100-IL-2 and pIMK2-ActAN30-IL-2 which were then conjugated into the attenuated *L. monocytogenes* background, LADD (FIGS. **1**A-**1**B).

[0072] To determine whether the engineered *L. monocytogenes* strains were capable of secreting IL-2, IL-2 production was assessed by ELISA from *L. monocytogenes* grown in RPMI media. *L. monocytogenes* carrying the ActA N30 and ActA N100 constructs secreted IL-2, however the N100 variant produced more IL-2 than the N30 variant (FIG. **1**C). To test the hypothesis that IL-2 secreted by *L. monocytogenes* retained biological activity, proliferation of IL-2 responsive CTLL-2 cells was assessed in response to supernatants from IL-2-secreting *L. monocytogenes* in the presence of [^{3}H]thymidine for 24 hours. Measuring [^{3}H]thymidine incorporation by liquid scintillation counting, it was found that both *L. monocytogenes* strains engineered to secrete IL-2 induced CTLL-2 proliferation and, consistent with the ELISA data, *L. monocytogenes* harboring the N100-IL-2 fusion induced greater [^{3}H]thymidine incorporation than the N30-IL-2 fusion (FIG. **1**D). Taken together, these data demonstrate that the *L. monocytogenes* constructs express and secrete functional IL-2 that retains functionality on mammalian cells expressing the cognate receptors. Based on the increased production of IL-2 by the N100 variant, combined with the retained biological activity of IL-2, the N100 variant, hereafter called LIS-IL-2, was used for subsequent experiments.

[0073] These results demonstrate that engineered *L. monocytogenes* of the present disclosure is effective for the production of biologically active IL-2. Accordingly, it is effective in therapeutic methods comprising the production of IL-2, such as in the treatment of cancer.

Example 3: Engineered *L. monocytogenes* Accumulates and Produces IL-2 in Tumor Microenvironments

[0074] This example demonstrates that engineered *L. monocytogenes* of the present disclosure accumulates and produces IL-2 in tumor microenvironments.

[0075] It was then determined whether LIS-IL-2 would accumulate in the tumors of B78-tumor bearing mice and secrete IL-2, while being cleared from normal *L. monocytogenes* target tissues. To test this hypothesis, B78 tumors were implanted into the flank of syngeneic mice, and when the tumors reached 300 mm^3 on average, mice were treated with 1×10^7LIS-IL-2 or LADD (which does not secrete IL-2) as a control by intratumoral (IT) or intravenous (IV) injection. At 1-, 3- and 7-days post treatment serum, livers, spleens, and tumor tissue were collected and assessed for *L. monocytogenes* burdens.

[0076] *L. monocytogenes* was detected in all tissues (FIGS. **2**A-C) of immunized mice, but not in the serum (data not shown), suggesting that the bacteria is rapidly cleared from circulation. Burdens decreased over time in the spleen following IV administration but remained constant following IT administration (FIG. **2**A). Splenic burdens were equivalent by day 7 post treatment for both treatments. Liver burdens were slightly higher than in the spleen. When treatment was applied IT, a decreased burden in the liver compared to IV was observed, however, as in the spleen, burdens eventually normalized (FIG. **2**B). There were no apparent differences between LADD and LIS-IL-2 *L. monocytogenes* tissue clearance. In contrast to the peripheral tissues, tumor burdens increased over time. When given IV, ~1×10^6 LIS-IL-2 or LADD were detected in tumors 24 hours post-treatment. IT administration yielded nearly 1×10^8 bacteria at this early timepoint. This number increased for both LADD and LIS-IL-2, reaching a maximum of nearly 1×10^9 bacteria per tumor (FIG. **2**C). It is possible that there is a maximum carrying capacity of bacteria that can be supported by a given mass of tumor, as burdens maxed out at 1×10^6 bacteria/mg of tumor tissue (FIG. **2**D). These data demonstrates that attenuated *L. monocytogenes* persists in the spleen and liver in tumor-bearing mice in contrast to administration of the LADD strain to naïve mice where the strains are fully cleared within 72 hours of immunization[10]. It is possible that this is due to the accumulation of bacteria in the immunosuppressed TME which results in recurrent seeding of peripheral organs. Nevertheless, LADD-based vaccines have been utilized in various clinical trials at doses of 1×10^9 bacteria/infusion in humans[14], demonstrating the safety of this approach.

[0077] IL-2 levels were assessed in the tumors of immunized mice using an ELISA for human IL-2. IL-2 levels were below detection limits in the tumor of all mice treated with LADD, or PBS as a control. In contrast, LIS-IL-2 treatment yielded peak IL-2 concentrations in the tumor at 3-days post treatment, which appeared to decline by day 7. IT administration yielded a greater peak of almost 10 ng IL-2/tumor compared to 3 ng IL-2/tumor for IV administration (FIG. **2**E). IL-2 was below the detection limit in all serum samples, suggesting that the LIS-IL-2 delivered IL-2 remains largely localized to sites of high bacterial burden (FIG. **2**F). No overt signs of distress or illnesses were observed in treated mice compared to PBS controls. Collectively, this data demonstrates that *L. monocytogenes* accumulates in B78 tumors while being controlled in healthy tissue and demonstrates that *L. monocytogenes* can deliver cytokines specifically to the TME.

[0078] These results demonstrate that engineered *L. monocytogenes* strains of the present disclosure accumulate and produce IL-2 in tumor microenvironments. Accordingly, the strains effective in therapeutic methods comprising the production of IL-2, such as in the treatment of cancer.

Example 4: Use of *L. monocytogenes* Expressing IL-2 for the Suppression of Tumor Growth

[0079] This example demonstrates the use of *L. monocytogenes* expressing IL-2 to slow tumor growth.

[0080] A second version of LIS-IL-2 with increased IL-2 expression was created named operational plasmid transformant LIS-IL-2 (OPT-LIS-IL-2). An expression construct was generated like that for LIS-IL-2 but with the 5'-UTR of the *L. monocytogenes* hly gene upstream of the ActAN100-IL-2 fusion construct (FIG. **3**A). This UTR addition enhances protein expression, increasing translation in the bacterium[38]. This construct was conjugated into LADD to generate OPT-LIS-IL-2. Increased IL-2 production was confirmed (~60,000 μg/mL for OPT-LIS-IL-2 compared to ~12,000 μg/mL for LIS-IL-2) and increased CTLL-2 proliferation (~20,000 μg/mL for OPT-LIS-IL-2 compared to ~18,000 μg/mL for LIS-IL-2) in supernatants from this strain compared to LIS-IL-2 (FIGS. **1**C, **1**D, **3**B, **3**C). OPT-LIS-IL-2 accumulates in tumor tissues reaching a max of ~1×10^9 bacteria/tumor (FIGS. **3**D-**3**E). The new strain is cleared from the liver and spleen with comparable kinetics to LIS-IL-2 (FIGS. **3**F-**3**G). OPT-LIS-IL-2 was cultured with B78 cells for 6 hours and cells had a normal appearance with what appear to be intracellular bacteria (FIG. **3**J).

[0081] To address whether IL-2 produced by OPT-LIS-IL-2 was retained intracellularly, tumors from one through five days post treatment were collected and half the tumor was soaked in media for 18 hours to allow passive diffusion of cytokines from the tumor, while the other half was directly homogenized. Relatively similar amounts of IL-2 were detected in samples of OPT-LIS-IL-2 treated tumors that were directly homogenized compared to soaked (FIGS. **3**H, **3**I). When tumor samples were soaked, an initial peak was observed at one day post treatment, which decreased until day four and began rising again at day five post treatment (FIG. **3**I). Overall, the levels of IL-2 detected in these samples was comparable to that of directly homogenized samples, indicating that IL-2 is predominantly extracellular.

[0082] It was hypothesized that enhanced IL-2 expression in OPT-LIS-IL-2 would result in better tumor control relative to LADD. To test this hypothesis, B78 tumors were implanted into the flank of syngeneic B6 mice, and 1×10^7 LADD, OPT-LIS-IL-2, or PBS was administered via IT injection when tumors reached ~100 mm^3. OPT-LIS-IL-2 treatment conferred enhanced tumor control compared to LADD, with diminished tumor volumes at multiple time points during the experiment (FIG. **4**A). Of four mice treated with LADD, two completely cleared the B78 tumor without recurrence, whereas only one of four OPT-LIS-IL-2 treated mice completely cleared the tumor (FIG. **4**B). Tumor shrinkage was observed for all mice treated with these two strains, and most tumors were nearly undetectable by day 10 post treatment. Of the mice that did not completely clear the tumors, those treated with LADD had more rapid tumor growth than the mice treated with OPT-LIS-IL-2 (FIG. **4**B), which may suggest that when tumors are not completely cleared, OPT-LIS-IL-2 may control regrowth better than LADD.

[0083] To assess bacterial burdens and IL-2 production, the tumors, livers, and spleens of LADD and OPT-LIS-IL-2 were collected at day thirty-five post treatment due to differences in tumor growth rates. All strains tested persisted in B78 tumors at high burdens for many weeks after initial treatment (FIG. **4**C). At these later timepoints, *L. monocytogenes* was nearly undetectable in the spleens (FIG. **4**D) and only OPT-LIS-IL-2 treated mice had low but detectible burdens in the liver (FIG. **4**E). At thirty-five days post treatment, there was an average of ~10 ng IL-2/tumor in the OPT-LIS-IL-2-treated tumor homogenates, whereas none was detected in LADD-treated mice (FIG. **4**F) suggesting that unlike the original LIS-IL-2 strains, IL-2 production in the TME persists with the OPT-LIS-IL2 strains. Taken together, these data demonstrate that the increased and persistent levels of IL-2 produced in the TME by OPT-LIS-IL-2 provide benefits to tumor control.

[0084] These results demonstrate that the delivery of IL-2 to tumor microenvironments by engineered *L. monocytogenes* of the present disclosure slows tumor growth. Accordingly, the engineered *L. monocytogenes* of the present disclosure are effective in therapeutic methods for the treatment of cancer.

Example 5: Synergistic Benefits from OPT-LIS-IL-2 Co-Therapies

[0085] This example demonstrates the use of engineered *L. monocytogenes* of the present disclosure in combination with standard cancer therapies. Briefly, 2×10^6 B78 tumor cells were injected on the right flank of mice. Mice were randomized 43 days later. The tumors were treated with either OPT-LIS-IL-2, OPT-LIS-IL-2 and radiation therapy (RT), or OPT-LIS-IL-2, RT, and anti-CTLA-4 (C4). The LADD strain and PBS were used as controls for the OPT-LIS-IL-2 combination therapies. The tumors were injected intratumorally with 0.1 ml of PBS, 1×10^7 OPT-LIS-IL-2, or 1×10^7 LADD. Tumors of RT treated mice were given 12 Gy of external beam radiation therapy (RT). C4 treated mice were injected intraperitoneally with 0.2 mg anti-CTLA-4 antibody (C4). Tumor growth was measured over time via calipers and tumor volume was estimated. FIG. **5** shows individual tumor volume over time, including noting how many of the tumors were rejected or cleared. Tumors receiving OPT-LIS-IL-2, RT, and anti-CTLA-4 had the greatest proportion of mice reject or clear their tumors, even in comparison with the standard treatment of RT, anti-CTLA-4 and 14.18-IL2 immunocytokine (IC) (RT+IC+C4). This experiment was repeated and showed similar results. In addition, B78 tumor-bearing mice treated with OPT-LIS-IL-2, RT, and anti-CTLA-4 had statistically significant longer survival ($p=0.038$) than mice treated with LADD, RT, and anti-CTLA-4 (data not shown).

Example 6: *L. monocytogenes* IL-12 Expression Strain

[0086] This example will demonstrate the engineering of *L. monocytogenes* strain for the production of biologically active IL-12.

[0087] An *L. monocytogenes* IL-12 expression strain (Lis-IL-12) will be generated and testing for therapeutic efficacy against cancer. IL-12 is a dimer of IL-12p35 and IL-12p40. Briefly, Lis-IL-12 will be generated by operably linking an IL-12p35 (NCBI Gene ID: 16159) encoding transcript and an IL-12p40 (NCBI Gene Id: 16160) encoding transcript to *L. monocyotgenes* secretion signals, such as the ActAN secretion signal. In some embodiments, the IL-12 encoding transcripts encodes full-length IL-12p35 and IL-12p40. In some embodiments, the IL-12p35 encoding transcript and the IL-12p40 transcript encode a portion of IL-12p35 and IL-12p40 respectively. The IL-12 encoding transcripts may further comprise one or more untranslated regions that promote IL-12 expression, such as the hly 5' UTR. The IL-12 encoding transcripts will then be incorporated into a vector for expression in *L. monocytogenes*, such as pIMK2, and a *L. monocytogenes* strain (e.g., LADD) will be transformed with said vector. The resulting Lis-IL-12 strain will then be assayed for release of biologically active IL-12 and therapeutic efficacy against one or more cancer models, optionally including the B78 tumor mouse models as described herein.

[0088] It is expected that these results will show that a Lis-IL-12 strain has been developed that releases biologically active IL-12 in vivo and that Lis-IL-12 is effective for treating or ameliorating one or more signs and symptoms of cancer. Accordingly, these results will show that Lis-IL-12 is effective in compositions and methods for the treatment of cancer.

Example 7: *L. monocytogenes* CD40L Expression Strain

[0089] This example will demonstrate the engineering of *L. monocytogenes* strain for the production of biologically active CD40L.

[0090] An *L. monocytogenes* CD40L expression strain (Lis-CD40L) will be generated and testing for therapeutic efficacy against cancer. Briefly, Lis-CD40L will be generated by operably linking a CD40L (NCBI Gene ID: 21947) encoding transcript to an *L. monocyotgenes* secretion signal, such as the ActAN secretion signal. In some embodiments, the CD40L encoding transcript encodes full-length CD40L. In some embodiments, the CD40L encoding transcript encodes a portion of IL-12. The CD40L encoding transcript may further comprise one or more untranslated regions that promote CD40L expression, such as the hly 5' UTR. The CD40L encoding transcript will then be incorporated into a vector for expression in *L. monocytogenes*, such as pIMK2, and a *L. monocytogenes* strain (e.g., LADD) will be transformed with said vector. The resulting Lis-CD40L strain will then be assayed for release of biologically active CD40L and therapeutic efficacy against one or more cancer models, optionally including the B78 tumor mouse models as described herein.

[0091] It is expected that these results will show that a Lis-CD40L strain has been developed that releases biologically active CD40L in vivo and that Lis-CD40L is effective for treating or ameliorating one or more signs and symptoms of cancer. Accordingly, these results will show that Lis-CD40L is effective in compositions and methods for the treatment of cancer.

Example 8: *L. monocytogenes* IL-2/IL-12/CD40L Expression Strains for Treating Cancer in Human Subjects

[0092] This example will demonstrate the efficacy of engineered *L. monocytogenes* strains of the present technology for treating cancer in human subjects.

[0093] The one or more engineered strains of the present technology will be assayed for their therapeutic potential in treating cancer in humans. Briefly, one or more subjects diagnosed with cancer will be administered one or more of the engineered *L. monocytogenes* strains of the present technology (e.g., OPT-Lis-IL-2, Lis-IL-12, Lis-CD40L). The one or more engineered *L. monocytogenes* strains will be administered by any suitable route, such as intratumorally or intravenously. The one or more engineered *L. monocytogenes* strains can be administered in combination with the standard of care treatment for the cancer the subjects are diagnosed with. Treatment efficacy will then be established by measuring production of IL-2, IL-12 and/or CD40L in tumors, tumor volume, tumor size, disease progression, tumor metastases, and survival.

[0094] It is anticipated that the results will demonstrate that *L. monocytogenes* strains of the present technology produce IL-2, IL-12, and/or CD40L in a human tumor and selectively persist in a human tumor. It is further expected that the results will show that the *L. monocytogenes* strains of the present technology ameliorate, mitigate, or improve one or more symptoms of cancer including reducing tumor size, slowing tumor growth, reducing or preventing metastases, and increasing survival. Accordingly, these results will show that *L. monocytogenes* strains of the present technology are useful in methods and compositions for treating cancer in humans.

REFERENCES

[0095] 1 Haslam, A. & Prasad, V. Estimation of the Percentage of US Patients With Cancer Who Are Eligible for and Respond to Checkpoint Inhibitor Immunotherapy Drugs. *JAMA Netw Open* 2, e192535-e192535 (2019).

[0096] 2. Carretero-González, A. et al. Analysis of response rate with anti-PD1/PDL1 antibodies in advanced solid tumors: A meta-analysis of randomized clinical trials (RCT). *Journal of Clinical Oncology* 35, 8706-8715 (2018).

[0097] 3. Barnes, T. A. & Amir, E. HYPE or HOPE: the prognostic value of infiltrating immune cells in cancer. *Br J Cancer* 117, 451 (2017).

[0098] 4. Li, F. et al. The association between CD8+ tumor-infiltrating lymphocytes and the clinical outcome of cancer immunotherapy: A systematic review and meta-analysis. *eClinicalMedicine* 41, 160 (2021).

[0099] 5. Jiang, T., Zhou, C. & Ren, S. Role of IL-2 in cancer immunotherapy. *Oncoimmunology* 5, (2016).

[0100] 6. Klapper, J. A. et al. High-dose Interleukin-2 for the Treatment of Metastatic Renal Cell Carcinoma: A Retrospective Analysis of Response and Survival in Patients Treated in the Surgery Branch at the National Cancer Institute Between 1986 and 2006. *Cancer* 113, 293 (2008).

[0101] 7. Atkins, M. B. et al. High-dose recombinant interleukin 2 therapy for patients with metastatic melanoma: analysis of 270 patients treated between 1985 and 1993. *J Clin Oncol* 17, 2105-2116 (1999).

[0102] 8. Pieper, A. A. et al. Combination of radiation therapy, bempegaldesleukin, and checkpoint blockade eradicates advanced solid tumors and metastases in mice. *J Immunother Cancer* 9, 2715 (2021).

[0103] 9. Morrow, Z. T., Powers, Z. M. & Sauer, J. D. *Listeria monocytogenes* Cancer Vaccines: Bridging Innate and Adaptive Immunity. *Current Clinical Microbiology Reports* vol. 6 213-224 Preprint at https://doi.org/10.1007/s40588-019-00133-4 (2019).

[0104] 10. Brocksted, D. G. et al. *Listeria*-based cancer vaccines that segregate immunogenicity from toxicity. *Proc Natl Acad Sci USA* 101, 13832-13837 (2004).

[0105] 11. Le, D. T., Dubenksy, T. W. & Brockstedt, D. G. Clinical development of *Listeria monocytogenes*-based immunotherapies. *Semin Oncol* 39, 311-322 (2012).

[0106] 12. Rolhion, N. & Cossart, P. How the study of *Listeria monocytogenes* has led to new concepts in biology. *Future Microbiol* 12, 621-638 (2017).

[0107] 13. Lauer, P., Chow, M. Y. N., Loessner, M. J., Portnoy, D. A. & Calendar, R. Construction, characterization, and use of two *Listeria monocytogenes* site-specific phage integration vectors. *J Bacteriol* 184, 4177-4186 (2002).

[0108] 14. Le, D. T. et al. A live-attenuated *listeria* vaccine (ANZ-100) and a live-attenuated *listeria* vaccine expressing mesothelin (CRS-207) for advanced cancers: Phase I studies of safety and immune induction. *Clinical Cancer Research* 18, 858-868 (2012).

[0109] 15. Mengaud, J., Ohayon, H., Gounon, P., Mege, R. M. & Cossart, P. E-cadherin is the receptor for internalin, a surface protein required for entry of *L. monocytogenes* into epithelial cells. *Cell* 84, 923-932 (1996).

[0110] 16. Shen, Y., Naujokas, M., Park, M. & Ireton, K. InIB-dependent internalization of *Listeria* is mediated by the Met receptor tyrosine kinase. Cell 103, 501-510 (2000).

[0111] 17. Hamon, M. A., Ribet, D., Stavru, F. & Cossart, P. Listeriolysin O: The Swiss army knife of *Listeria. Trends in Microbiology* vol. 20 360-368 Preprint at https://doi.org/10.1016/j.tim.2012.04.006 (2012).

[0112] 18. Glomski, I. J., Decatur, A. L. & Portnoy, D. A. *Listeria monocytogenes* Mutants That Fail to Compartmentalize Listerolysin O Activity Are Cytotoxic, Avirulent, and Unable to Evade Host Extracellular Defenses. *Infect Immun* 71, 6754-6765 (2003).

[0113] 19. Morrow, Z. T., Powers, Z. M. & Sauer, J. D. *Listeria monocytogenes* Cancer Vaccines: Bridging Innate and Adaptive Immunity. *Current Clinical Microbiology Reports* vol. 6 213-224 Preprint at https://doi.org/10.1007/s40588-019-00133-4 (2019).

[0114] 20. Kocks, C. et al. *L. monocytogenes*-induced actin assembly requires the actA gene product, a surface protein. *Cell* 68, 521-531 (1992).

[0115] 21. Sauer, J. D. et al. *Listeria monocytogenes* engineered to activate the Nlrc4 inflammasome are severely attenuated and are poor inducers of protective immunity. *Proc Natl Acad Sci USA* 108, 12419-24 (2011).

[0116] 22. Theisen, E. & Sauer, J. D. *Listeria monocytogenes*-induced cell death inhibits the generation of cell-mediated immunity. *Infect Immun* 85, e00733-16 (2017).

[0117] 23. Theisen, E. et al. Cyclooxygenase-1 and -2 Play Contrasting Roles in *Listeria*-Stimulated Immunity. *The Journal of Immunology* 200, 3729-3738 (2018).

[0118] 24. Deng, W. et al. Recombinant *Listeria* promotes tumor rejection by CD8+ T cell-dependent remodeling of the tumor microenvironment. *Proc Natl Acad Sci USA* 115, 8179-8184 (2018).

[0119] 25. Crittenden, M. et al. Phase I study of safety and immunogenicity of ADU-623, a live-attenuated *Listeria monocytogenes* vaccine (Δ actA/ΔinlB) expressing EGFRVIII and N Y-ESO-1, in patients with WHO grade III/IV astrocytomas. *Journal of Clinical Oncology* 33, TPS3106-TPS3106 (2015).

[0120] 26. Chávez-Arroyo, A. & Portnoy, D. A. Why is *Listeria monocytogenes* such a potent inducer of CD8+ T-cells? *Cell Microbiol* 22, e13175 (2020).

[0121] 27. Chandra, D., Jahangir, A., Quispe-Tintaya, W., Einstein, M. H. & Gravekamp, C. Myeloid-derived suppressor cells have a central role in attenuated *Listeria monocytogenes*-based immunotherapy against metastatic breast cancer in young and old mice. *Br J Cancer* 108, 2281-2290 (2013).

[0122] 28. Selvanesan, B. C. et al. *Listeria* delivers tetanus toxoid protein to pancreatic tumors and induces cancer cell death in mice. *Sci Transl Med* 14, 1600 (2022).

[0123] 29. Rosenberg, S. A. IL-2: the first effective immunotherapy for human cancer. *J Immunol* 192, 5451-5458 (2014).

[0124] 30. Canale, F. P. et al. Metabolic modulation of tumours with engineered bacteria for immunotherapy. 662 |*Nature*|598, (2021).

[0125] 31. Sun, H. K., Castro, F., Paterson, Y. & Gravekamp, C. High efficacy of a *Listeria*-based vaccine against metastatic breast cancer reveals a dual mode of action. *Cancer Res* 69, 5860-5866 (2009).

[0126] 32. Kim, S. H. et al. Mage-b vaccine delivered by recombinant *Listeria monocytogenes* is highly effective against breast cancer metastases. *Br J Cancer* 99, 741-749 (2008).

[0127] 33. Jahangir, A. et al. Immunotherapy with *Listeria* reduces metastatic breast cancer in young and old mice through different mechanisms. *Oncoimmunology* 6, (2017).

[0128] 34. Quispe-Tintaya, W. et al. Nontoxic radioactive Listeriaat is a highly effective therapy against metastatic pancreatic cancer. *Proc Natl Acad Sci USA* 110, 8668-8673 (2013).

[0129] 35. Chandra, D. et al. 32-Phosphorus selectively delivered by *listeria* to pancreatic cancer demonstrates a strong therapeutic effect. *Oncotarget* 8, 20729-20740 (2017).

[0130] 36. Theisen, E. & Sauer, J.-D. D. *Listeria monocytogenes*-Induced Cell Death Inhibits the Generation of Cell-Mediated Immunity. *Infect Immun* 85, (2017).

[0131] 37. Monk, I. R., Gahan, C. G. M. & Hill, C. Tools for functional postgenomic analysis of *Listeria monocytogenes. Appl Environ Microbiol* 74, (2008).

[0132] 38. Lebreton, A. & Cossart, P. RNA- and protein-mediated control of *Listeria monocytogenes* virulence gene expression. *RNA Biol* 14, 460 (2017).

[0133] 39. Boyman, O. & Sprent, J. The role of interleukin-2 during homeostasis and activation of the immune system. *Nature Reviews Immunology* 2012 12:3 12, 180-190 (2012).

[0134] 40. Morris, Z. S. et al. Tumor-specific inhibition of in situ vaccination by distant untreated tumor sites. *Cancer Immunol Res* 6, 825 (2018).

[0135] 41. Vazquez-Lombardi, R. et al. Potent antitumour activity of interleukin-2-Fc fusion proteins requires Fc-mediated depletion of regulatory T-cells. *Nat Commun* 8, (2017).

[0136] 42. Carmenate, T. et al. Human IL-2 mutein with higher antitumor efficacy than wild type IL-2. *J Immunol* 190, 6230-6238 (2013).

[0137] 43. Habiba, U. e. et al. The multifaceted role of IL-12 in cancer. *Advances in Cancer Biology—Metastasis* 5, 100053 (2022).

[0138] 44. Cirella, A. et al. Intratumoral Gene Transfer of mRNAs Encoding IL12 in Combination with Decoy-Resistant IL18 Improves Local and Systemic Antitumor Immunity. *Cancer Immunol Res* 11, 184-198 (2023).

[0139] 45. Kohli, K., Pillarisetty, V. G. & Kim, T. S. Key chemokines direct migration of immune cells in solid tumors. *Cancer Gene Therapy* 2021 29:1 29, 10-21 (2021).

[0140] 46. Lauer et al Construction, Characterization, and Use of Two *Listeria monocytogenes* Site-Specific Phage Integration Vectors. J. Bacteriology 2002.

[0141] 47. Rakhmilevich A L, et al. Gene gun-mediated skin transfection with interleukin 12 gene results in regression of established primary and metastatic murine tumors. Proc Natl Acad Sci USA 1996 93:6291-6296.

[0142] 48. Rakhmilevich A L, et al. Cytokine gene therapy of cancer using gene gun technology: superior antitumor activity of interleukin-12. Hum Gene Ther 1997 8:1303-1311.

[0143] 49. Cheng E M, et al. Interleukin-12 as an in situ cancer vaccine component: a review. Cancer Immunol Immunother. 2022 September; 71 (9): 2057-2065.

[0144] 50. Wigginton J M, et al. IL-12/IL-2 combination cytokine therapy for solid tumours: translation from bench to bedside. Expert Opin Biol Ther. 2002 June; 2 (5): 513-24.

[0145] 51. Cheng E M, et al. Interleukin-12 as an in situ cancer vaccine component: a review. Cancer Immunol Immunother. 2022 September; 71 (9): 2057-2065.

[0146] 52. Bullock, T. N. J., CD40 stimulation as a molecular adjuvant for cancer vaccines and other immunotherapies. Nature 2021.

[0147] 53. Voeller J. Erbe A K, Slowinski J, Rasmussen K, Carlson P M, Hoefges A, VandenHeuvel S Stuckwisch A, Wang X, Gillies S D, Patel R, Farrell A, Maris J M, Hank J A, Morris Z S, Rakhmilevich A L, Sondel P M. Combined Innate and Adaptive Immunotherapeutic Approach Overcomes Resistance of Immunologically Cold Syngeneic Murine Neuroblastoma to Checkpoint Inhibition. J Immunother Cancer. 2019 Dec. 6; 7 (1): 344.

[0148] 54. Aiken T J, et al. Mechanism of effective combination radio-immunotherapy against 9464D-GD2, an immunologically cold murine neuroblastoma. J Immunother Cancer. 2022 May; 10 (5): e004834.

[0149] 55. Richards D M, Sefrin J P, Gieffers C, Hill O, Merz C. Concepts for agonistic targeting of CD40 in immuno-oncology. Hum Vaccin Immunother. 2020; 16 (2): 377-387.

[0150] 56. Buhtoiarov I N, Sondel P M, Wigginton J M, Buhtoiarova T N, Yanke E M, Mahvi D A, Rakhmilevich A L. Antitumor Synergy of Cytotoxic Chemotherapy and Anti-CD40 Plus CpG-ODN Immunotherapy Through Repolarization of Tumor Associated Macrophages. Immunology. 132:226-239, 2011. PMCID: PMC3050446

[0151] 57. Qu X, Felder M A R, Perez Horta Z, Sondel P M, Rakhmilevich A L. Antitumor effects of anti-CD40/CpG immunotherapy combined with gemcitabine or 5-fluorouracil chemotherapy in the B16 melanoma model. International Immunopharmacology, 17:1141-1147, 2013. PMCID: PMC3873769

EQUIVALENTS

[0152] The present technology is not to be limited in terms of the particular embodiments described in this application, which are intended as single illustrations of individual aspects of the present technology. Many modifications and variations of this present technology can be made without departing from its spirit and scope, as will be apparent to those skilled in the art. Functionally equivalent methods and apparatuses within the scope of the present technology, in addition to those enumerated herein, will be apparent to those skilled in the art from the foregoing descriptions. Such modifications and variations are intended to fall within the scope of the appended claims. The present technology is to be limited only by the terms of the appended claims, along with the full scope of equivalents to which such claims are entitled. It is to be understood that this present technology is not limited to particular methods, reagents, compounds compositions or biological systems, which can, of course, vary. It is also to be understood that the terminology used herein is for the purpose of describing particular embodiments only, and is not intended to be limiting.

[0153] Each and every publication and patent mentioned in the above specification is herein incorporated by reference in its entirety for all purposes. Various modifications and variations of the described methods and system of the present technology will be apparent to those skilled in the art without departing from the scope and spirit of the present technology. Although the present technology has been described in connection with specific embodiments, the present technology as claimed should not be unduly limited to such specific embodiments. Indeed, various modifications of the described modes for carrying out the present technology which are obvious to those skilled in the art and in fields related thereto are intended to be within the scope of the following claims.

Sequence Listing

ActAN30-IL2 (SEQ ID NO: 1)
aggagagtgaaacccatggGtgggattaaatagatttatgcgtgcgatgatggtagtttt-
cattactgccaactgcattacgattaaccccgac
ataatatttgcagcgCCAACATCTTCTTCCACTAAGAAGACTCAGCTACAACTTGAACACCTT
CTGTTGGACCTACAAATGATTCTAAATGGAATAAACAATTATAAAAATCCTAAATTA
ACTAGAATGCTCACGTTCAAATTCTACATGCCGAAAAAAGCAACCGAGTTAAAACA
TTTACAATGTCTTGAAGAAGAACTTAAACCATTGGAAGAAGTGCTTAACTTGGCCCA
AAGTAAAAATTTTCATTTACGTCCACGAGATTTAATTAGCAATATCAATGTCATTGT
ATTAGAATTAAAAGGTAGTGAAACCACATTTATGTGCGAATATGCTGATGAGACAG
CGACAATTGTTGAATTTTTAAACCGCTGGATTACATTTTGTCAATCAATTATCTCGAC
GTTAACGTGAGTCGACCTCGAGGGG

ActAN100-IL2 (SEQ ID NO: 2)
gaaggagagtgaaacccatggGtgggattaaatagatttatgcgtgcgatgatggtagttttcattactgccaactgcattacgattaacccc
gacataatatttgcagcgacagatagcgaagattccagtctaaacacagatgaatgggaagaagaaaaaacagaagagcagccaagcga
ggtaaatacgggaccaagatacgaaactgcacgtgaagtaagttcacgtgatattgaggaactagaaaaatcgaataaagtgaaaaatacg
aacaaagcagacctaatagcaatgttgaaagcaaaagcagagaaaggtggatccCCAACATCTTCTTCCACTAAGA
AGACTCAGCTACAACTTGAACACCTTCTGTTGGACCTACAAATGATTCTAAATGGAA
TAAACAATTATAAAAATCCTAAATTAACTAGAATGCTCACGTTCAAATTCTACATGC
CGAAAAAAGCAACCGAGTTAAAACATTTACAATGTCTTGAAGAAGAACTTAAACCA
TTGGAAGAAGTGCTTAACTTGGCCCAAAGTAAAAATTTTCATTTACGTCCACGAGAT
TTAATTAGCAATATCAATGTCATTGTATTAGAATTAAAAGGTAGTGAAACCACATTT
ATGTGCGAATATGCTGATGAGACAGCGACAATTGTTGAATTTTTAAACCGCTGGATT
ACATTTTGTCAATCAATTATCTCGACGTTAACGTGAGTCGACCTCGAGGGGGGG

-continued

Sequence Listing

phelp-hly5' utr_fwd (SEQ ID NO: 3)
agggaacaaaagctgggtacCCATTATGCTTTGGCAGTTTATTC

phelp-hly5' utr_rev (SEQ ID NO: 4)
agcctgacatGGGTTTCACTCTCCTTCTAC

hly-F (SEQ ID NO: 5)
TCAACCAGATGTTCTCCCTGTA

hly-R (SEQ ID NO: 6)
CACTGTAAGCCATTTCGTCATC

pIMK2 (SEQ ID NO: 7)
TTGGCAGCATCACCCGACGCACTTTGCGCCGAATAAATACCTGTGACGGAAGATCA
CTTCGCAGAATAAATAAATCCTGGTGTCCCTGTTGATACCGGGAAGCCCTGGGACGT
CGGGCCCTTTCGTCTTCAAGAATTAATTCCCAATTCCAGGCATCAAATAAAACGAAA
GGCTCAGTCGAAAGACTGGGCCTTTCGTTTTATCTGTTGTTTGTCGGTGAACGCTCTC
CTGAGTAGGACAAATCCGCCGGGAGCGGATTTGAACGTTGCGAAGCAACGGCCCGG
AGGGTGGCGGGCAGGACGCCCGCCATAAACTGCCAGGAATTAATTCCAGGCATCAA
ATAAAACGAAAGGCTCAGTCGAAAGACTGGGCCTTTCGTTTTATCTGTTGTTTGTCG
GTGAACGCTCTCCTGAGTAGGACAAATCCGCCGGGAGCGGATTTGAACGTTGCGAA
GCAACGGCCCGGAGGGTGGCGGGCAGGACGCCCGCCATAAACTGCCAGGAATTAAT
TCCAGGCATCAAATAAAACGAAAGGCTCAGTCGAAAGACTGGGCCTTTCGTTTTATC
TGTTGTTTGTCGGTGAACGCTCTCCTGAGTAGGACAAATCCGCCGGGAGCGGATTTG
AACGTTGCGAAGCAACGGCCCGGAGGGTGGCGGGCAGGACGCCCGCCATAAACTGC
CAGGAATTAATTCCAGGCATCAAATAAAACGAAAGGCTCAGTCGAAAGACTGGGCC
TTTCGTTTTATCTGTTGTTTGTCGGTGAACGCTCTCCTGAGTAGGACAAATCCGCCGG
GAGCGGATTTGAACGTTGCGAAGCAACGGCCCGGAGGGTGGCGGGCAGGACGCCC
GCCATAAACTGCCAGGAATTGGGGATCGGAATTCGAGCTCcattatgctttggcagtttattcttgacat
gtagtgagggggctggtataatcacatacggccgataaagcaagcatataatattgcgtttcatctttagaagcgaatttcgccaatattat
aattatcaaaagagaggggtggcaaacggtatttggcattattaggttaaaaaatgtagaaggagagtgaaacccatggaaaaGGATCCC
CCGGGCTGCAGGAATTCGATATCAAGCTTATCGATACCGTCGACCTCGAGGGGGGG
CCCGGTACCCAGCTTTTGTTCCCTTTAGTGAGGGTTAATTCCCAGCGAACCATTTGAG
GTGATAGGTAAGATTATACCGAGGTATGAAAACGAGAATTGGACCTTTACAGAATT
ACTCTATGAAGCGCCATATTTAAAAAGCTACCAAGACGAAGAGGATGAAGAGGATG
AGGAGGCAGATTGCCTTGAATATATTGACAATACTGATAAGATAATATATCTTTTAT
ATAGAAGATATCGCCGTATGTAAGGATTTCAGGGGGCAAGGCATAGGCAGCGCGCT
TATCAATATATCTATAGAATGGGCAAAGCATAAAAACTTGCATGGACTAATGCTTGA
AACCCAGGACAATAACCTTATAGCTTGTAAATTCTATCATAATTGTGGTTTCAAAAT
CGGCTCCGTCGATACTATGTTATACGCCAACTTTGAAAACAACTTTGAAAAAGCTGT
TTTCTGGTATTTAAGGTTTTAGAATGCAAGGAACAGTGAATTGGAGTTCGTCTTGTT
ATAATTAGCTTCTTGGGGTATCTTTAAATACTGTAGAAAAGAGGAAGGAAATAATA
AATGGCTAAAATGAGAATATCACCGGAATTGAAAAAACTGATCGAAAAATACCGCT
GCGTAAAAGATACGGAAGGAATGTCTCCTGCTAAGGTATATAAGCTGGTGGGAGAA
AATGAAAACCTATATTTAAAAATGACGGACAGCCGGTATAAAGGGACCACCTATGA
TGTGGAACGGGAAAAGGACATGATGCTATGGCTGGAAGGAAAGCTGCCTGTTCCAA
AGGTCCTGCACTTTGAACGGCATGATGGCTGGAGCAATCTGCTCATGAGTGAGGCCG
ATGGCGTCCTTTGCTCGGAAGAGTATGAAGATGAACAAAGCCCTGAAAAGATTATC
GAGCTGTATGCGGAGTGCATCAGGCTCTTTCACTCCATCGACATATCGGATTGTCCC
TATACGAATAGCTTAGACAGCCGCTTAGCCGAATTGGATTACTTACTGAATAACGAT
CTGGCCGATGTGGATTGCGAAAACTGGGAAGAAGACACTCCATTTAAAGATCCGCG
CGAGCTGTATGATTTTTTAAAGACGGAAAAGCCCGAAGAGGAACTTGTCTTTTCCCA
CGGCGACCTGGGAGACAGCAACATCTTTGTGAAAGATGGCAAAGTAAGTGGCTTTA
TTGATCTTGGGAGAAGCGGCAGGGCGGACAAGTGGTATGACATTGCCTTCTGCGTCC
GGTCGATCAGGGAGGATATCGGGGAAGAACAGTATGTCGAGCTATTTTTTGACTTAC
TGGGGATCAAGCCTGATTGGGAGAAAATAAAATATTATATTTTACTGGATGAATTGT
TTTAGTACCTAGATTTAGATGTCTAAAAAGCTTTAACTACAAGCTTTTTAGACATCTA
ATCTTTTCTGAAGTACATCCGCAACTGTCCATACTCTGATGTTTCATATGATCATCAT
AATTCTGTCTCATTATATAACATCCTCCATACCTTCTATTATAGAATACCATAAACTC
ATCTGGCAATTCATTTCGAGTCACGAAGAACGGAAAAACTGCCGGTTTTTATATTAC
AAATGTATTAAGTTTTTCTATTAACAAAAAACAATAGGTTTCCCATAGCGAAAGTTG
TTGATTAACGTTCACATCCCACTTACACTATAAAGGTTTACCCAGCAATACATCTCA
AGCCCTAAGAATACACGTTCGCTTTTCAACTGTTACAGAATTATTACAAATAGTTGG
TATAGTCCTCTTTAGCCTTTGGAGCTATTATCTCATCATTTGTTTTTTAGGTGAAAAC
TGGGTAAACTTAGTATTAATCAATATAAAATTAATTCTCAAATACTTAATTACGTAC
TGGGATTTTCTGAAAAAAAGATCTCCAAAAATAAACAGGTGGTGGTATTAATGAAG
ATAAAAAAATTAGCAAACGGTAAATATTGTGTTCGCCTACGTATAAAAGTCGATGGT
GAATGGAAAGAAAAGCGTTTGACAGATACAAGTGAAACAAACTTAATGTATAAAGC
ATCTAAATTATTAAAACAAGTTCAGCATGATAGTAGTTCTCTGAAAGAATGGAACTT
CAAAGAATTTTATACGCTATTCATGAAAACATTTAAAGATGGGAAAAGTAGTCAATC
TACTATTAATTTATACGATCTTGCTTATAATCAATTCGTTGATTATTTCGATGAAAAA
ATTAAATTTAATTCGATTGATGCGGTTCAATATCAACAATTTATTAATCATTTATCTG
TAGACTATGCAATATCCACTGTAGACACCAGACACCGCAAAATTAGAGCGATTTTTA
ACAAGGCTGTTCATTTAGGTTACATGAAGAAAAACCCCACTATAGGGGCTCATATAA

-continued

Sequence Listing

GCGGACAGGACGTAGCGAAAAATAAAGCACAATTTATGGAAACAGACAAAGTTCAT
TTACTATTAGAAGAACTTGCAAAATTTCATTCTATATCACGAGCAGTTATCTTTCTAG
CTGTCCAGACAGGCATGAGGTTCGAAGAAATTATTGCACTAACAAAGAAGGATATT
AATTTCACTAAACGTTCAATAACTGTGAATAAAGCTTGGGATTACAAGTACACTAAT
ACATTCATTGATACCAAAACAAAAAAATCACGAGTGATCTATATTGATAACTCTACC
GCTCAATATTTACATTCGTATTTAAATTGGCATACTGAATATATGAAGGAACATGCT
ATTAAGAATCCATTGATGTTATTATTCATCACTTACCACAATAAGCCAGTAGACAAC
GCGTCTTGTAATAAAGCTTTGAAGAAGATATGTAGTACAATCAATTCTGAACCAGTG
ACATTACACAAGCTACGACATACGCATACAGGCTTATGTGTAGAAGCGGGTATGGA
TATTATTTATGTAGCTGATAGGCTTGGTCATGATGACATTAATACAACATTAAAATA
CTATAGTCATCTAAGCTCTAATTTAAGACAACATAATCAGTCCAAAGTAGATGCTTT
TTTCACACTAAAAACAGATGAAAATACCACAAATTTTACCACAAATGCCACAAAAA
CAACGGAATAAAACGGGTATTATACGATATAAAAAAAACTCCAAAACATTCATCCG
CCCTTTAATATCAAGGCTTTTCAACGTTTTAGAGATTTCTTTACATTACTATTTAACG
TCCTGAGAGGGATTAACACACACTGATATAAAGCCATTTAGGATATATATACCACAA
ATAATACCACAAACATTTTATGTAATAATAAATATTATTTATTATTACATTGAAATA
AATATTCGTTATAAATAGTTTTTATATCAAGATGTTTTTTCTCAAGGTTTTTATAAAA
TGACTTTAATTCTTTTGTTTCAAGTAGTCCAGAGAAGATTTTTTCAACAGCGTTCTTC
TTTCCCTCCACGCATGCCTCTCGCCTGTCCCCTCAGTTCAGTAATTTCCTGCATTTGC
CTGTTTCCAGTCGGTAGATATTCCACAAAACAGCAGGGAAGCAGCGCTTTTCCGCTG
CATAACCCTGCTTCGGGGTCATTATAGCGATTTTTTCGGTATATCCATCCTTTTTCGC
ACGATATACAGGATTTTGCCAAAGGGTTCGTGTAGACTTTCCTTGGTGTATCCAACG
GCGTCAGCCGGGCAGGATAGGTGAAGTAGGCCCACCCGCGAGCGGGTGTTCCTTCT
TCACTGTCCCTTATTCGCACCTGGCGGTGCTCAACGGGAATCCTGCTCTGCGAGGCT
GGCCGGCTACCGCCGGCGTAACAGATGAGGGCAAGCGGCGGAGAATTACAACTTAT
ATCGTATGGGGCTGACTTCAGGTGCTACATTTGAAGAGATAAATTGCACTGAAATCT
AGAAATATTTTATCTGATTAATAAGATGATCTTCTTGAGATCGTTTTGGTCTGCGCGT
AATCTCTTGCTCTGAAAACGAAAAAACCGCCTTGCAGGGCGGTTTTTCGAAGGTTCT
CTGAGCTACCAACTCTTTGAACCGAGGTAACTGGCTTGGAGGAGCGCAGTCACCAA
AACTTGTCCTTTCAGTTTAGCCTTAACCGGCGCATGACTTCAAGACTAACTCCTCTAA
ATCAATTACCAGTGGCTGCTGCCAGTGGTGCTTTTGCATGTCTTTCCGGGTTGGACTC
AAGACGATAGTTACCGGATAAGGCGCAGCGGTCGGACTGAACGGGGGGTTCGTGCA
TACAGTCCAGCTTGGAGCGAACTGCCTACCCGGAACTGAGTGTCAGGCGTGGAATG
AGACAAACGCGGCCATAACAGCGGAATGACACCGGTAAACCGAAAGGCAGGAACA
GGAGAGCGCACGAGGGAGCCGCCAGGGGGAAACGCCTGGTATCTTTATAGTCCTGT
CGGGTTTCGCCACCACTGATTTGAGCGTCAGATTTCGTGATGCTTGTCAGGGGGGCG
GAGCCTATGGAAAAACGGCTTTGCCGCGGCCCTCTCACTTCCCTGTTAAGTATCTTC
CTGGCATCTTCCAGGAAATCTCCGCCCCGTTCGTAAGCCATTTCCGCTCGCCGCAGT
CGAACGACCGAGCGTAGCGAGTCAGTGAGCGAGGAAGCGGAATATATCCTGTATCA
CATATTCTGCTGACGCACCGGTGCAGCCTTTTTTCTCCTGCCACATGAAGCACTTCAC
TGACACCCTCATCAGTGCCAACATAGTAAGCCAGTATACACTCCGCTAGCGCTGATG
TCCGGCGGTGCTTTTGCCGTTACGCACCACCCCGTCAGTAGCTGAACAGGAGGGACA
GCTGATAGAAACAGAAGCCACTGGAGCACCTCAAAAACACCATCATACACTAAATC
AGTAAG

pIMK2-ActAN30-IL-2 (SEQ ID NO: 8)
TTGGCAGCATCACCCGACGCACTTTGCGCCGAATAAATACCTGTGACGGAAGATCA
CTTCGCAGAATAAATAAATCCTGGTGTCCCTGTTGATACCGGGAAGCCCTGGGACGT
CGGGCCCTTTCGTCTTCAAGAATTAATTCCCAATTCCAGGCATCAAATAAAACGAAA
GGCTCAGTCGAAAGACTGGGCCTTTCGTTTTATCTGTTGTTTGTCGGTGAACGCTCTC
CTGAGTAGGACAAATCCGCCGGGAGCGGATTTGAACGTTGCGAAGCAACGGCCCGG
AGGGTGGCGGGCAGGACGCCCGCCATAAACTGCCAGGAATTAATTCCAGGCATCAA
ATAAAACGAAAGGCTCAGTCGAAAGACTGGGCCTTTCGTTTTATCTGTTGTTTGTCG
GTGAACGCTCTCCTGAGTAGGACAAATCCGCCGGGAGCGGATTTGAACGTTGCGAA
GCAACGGCCCGGAGGGTGGCGGGCAGGACGCCCGCCATAAACTGCCAGGAATTAAT
TCCAGGCATCAAATAAAACGAAAGGCTCAGTCGAAAGACTGGGCCTTTCGTTTTATC
TGTTGTTTGTCGGTGAACGCTCTCCTGAGTAGGACAAATCCGCCGGGAGCGGATTTG
AACGTTGCGAAGCAACGGCCCGGAGGGTGGCGGGCAGGACGCCCGCCATAAACTGC
CAGGAATTAATTCCAGGCATCAAATAAAACGAAAGGCTCAGTCGAAAGACTGGGCC
TTTCGTTTTATCTGTTGTTTGTCGGTGAACGCTCTCCTGAGTAGGACAAATCCGCCGG
GAGCGGATTTGAACGTTGCGAAGCAACGGCCCGGAGGGTGGCGGGCAGGACGCCC
GCCATAAACTGCCAGGAATTGGGGATCGGAATTCGAGCTCcattatgctttggcagtttattcttgacat
gtagtgagggggctggtataatcacatacggccgataaagcaagcatataatattgcgtttcatctttagaagcgaatttcgccaatattat
aattatcaaaagagaggggtggcaaacggtatttggcattattaggttaaaaaatgtagaaggagagtgaaacccatggGtgggattaaata
gatttatgcgtgcgatgatggtagttttcattactgccaactgcattacgattaaccccgacataatatttgcagcgCCAACATCTTCTTC
CACTAAGAAGACTCAGCTACAACTTGAACACCTTCTGTTGGACCTACAAATGATTCT
AAATGGAATAAACAATTATAAAAATCCTAAATTAACTAGAATGCTCACGTTCAAATT
CTACATGCCGAAAAAAGCAACCGAGTTAAAACATTTACAATGTCTTGAAGAAGAAC
TTAAACCATTGGAAGAAGTGCTTAACTTGGCCCAAAGTAAAAATTTTCATTTACGTC
CACGAGATTTAATTAGCAATATCAATGTCATTGTATTAGAATTAAAAGGTAGTGAAA
CCACATTTATGTGCGAATATGCTGATGAGACAGCGACAATTGTTGAATTTTTAAACC
GCTGGATTACATTTTGTCAATCAATTATCTCGACGTTAACGTGAGTCGACCTCGAGG
GGGGGCCCGGTACCCAGCTTTTGTTCCCTTTAGTGAGGGTTAATTCCCAGCGAACCA
TTTGAGGTGATAGGTAAGATTATACCGAGGTATGAAAACGAGAATTGGACCTTTAC

-continued

Sequence Listing
AGAATTACTCTATGAAGCGCCATATTTAAAAAGCTACCAAGACGAAGAGGATGAAG AGGATGAGGAGGCAGATTGCCTTGAATATATTGACAATACTGATAAGATAATATAT CTTTTATATAGAAGATATCGCCGTATGTAAGGATTTCAGGGGGCAAGGCATAGGCA GCGCGCTTATCAATATATCTATAGAATGGGCAAAGCATAAAAACTTGCATGGACTA ATGCTTGAAACCCAGGACAATAACCTTATAGCTTGTAAATTCTATCATAATTGTGGT TTCAAAATCGGCTCCGTCGATACTATGTTATACGCCAACTTTGAAAACAACTTTGAA AAAGCTGTTTTCTGGTATTTAAGGTTTTAGAATGCAAGGAACAGTGAATTGGAGTTC GTCTTGTTATAATTAGCTTCTTGGGGTATCTTTAAATACTGTAGAAAAGAGGAAGGA AATAATAAATGGCTAAAATGAGAATATCACCGGAATTGAAAAAACTGATCGAAAAA TACCGCTGCGTAAAAGATACGGAAGGAATGTCTCCTGCTAAGGTATATAAGCTGGT GGGAGAAAATGAAAACCTATATTTAAAAATGACGGACAGCCGGTATAAAGGGACCA CCTATGATGTGGAACGGGAAAAGGACATGATGCTATGGCTGGAAGGAAAGCTGCCT GTTCCAAAGGTCCTGCACTTTGAACGGCATGATGGCTGGAGCAATCTGCTCATGAGT GAGGCCGATGGCGTCCTTTGCTCGGAAGAGTATGAAGATGAACAAAGCCCTGAAAA GATTATCGAGCTGTATGCGGAGTGCATCAGGCTCTTTCACTCCATCGACATATCGGA TTGTCCCTATACGAATAGCTTAGACAGCCGCTTAGCCGAATTGGATTACTTACTGAA TAACGATCTGGCCGATGTGGATTGCGAAAACTGGGAAGAAGACACTCCATTTAAAG ATCCGCGCGAGCTGTATGATTTTTTAAAGACGGAAAAGCCCGAAGAGGAACTTGTCT TTTCCCACGGCGACCTGGGAGACAGCAACATCTTTGTGAAAGATGGCAAAGTAAGT GGCTTTATTGATCTTGGGAGAAGCGGCAGGGCGGACAAGTGGTATGACATTGCCTTC TGCGTCCGGTCGATCAGGGAGGATATCGGGGAAGAACAGTATGTCGAGCTATTTTTT GACTTACTGGGGATCAAGCCTGATTGGGAGAAAATAAAATATTATATTTTACTGGAT GAATTGTTTTAGTACCTAGATTTAGATGTCTAAAAAGCTTTAACTACAAGCTTTTTAG ACATCTAATCTTTTCTGAAGTACATCCGCAACTGTCCATACTCTGATGTTTCATATGA TCATCATAATTCTGTCTCATTATATAACATCCTCCATACCTTCTATTATAGAATACCA TAAACTCATCTGGCAATTCATTTCGAGTCACGAAGAACGGAAAAACTGCCGGTTTTT ATATTACAAATGTATTAAGTTTTTCTATTAACAAAAAACAATAGGTTTCCCATAGCG AAAGTTGTTGATTAACGTTCACATCCCACTTACACTATAAAGGTTTACCCAGCAATA CATCTCAAGCCCTAAGAATACACGTTCGCTTTTCAACTGTTACAGAATTATTACAAA TAGTTGGTATAGTCCTCTTTAGCCTTTGGAGCTATTATCTCATCATTTGTTTTTTAGGT GAAAACTGGGTAAACTTAGTATTAATCAATATAAAATTAATTCTCAAATACTTAATT ACGTACTGGGATTTTCTGAAAAAAAGATCTCCAAAAATAAACAGGTGGTGGTATTA ATGAAGATAAAAAAATTAGCAAACGGTAAATATTGTGTTCGCCTACGTATAAAAGT CGATGGTGAATGGAAAGAAAAGCGTTTGACAGATACAAGTGAAACAAACTTAATGT ATAAAGCATCTAAATTATTAAAACAAGTTCAGCATGATAGTAGTTCTCTGAAAGAAT GGAACTTCAAAGAATTTTATACGCTATTCATGAAAACATTTAAAGATGGGAAAAGT AGTCAATCTACTATTAATTTATACGATCTTGCTTATAATCAATTCGTTGATTATTTCG ATGAAAAAATTAAATTTAATTCGATTGATGCGGTTCAATATCAACAATTTATTAATC ATTTATCTGTAGACTATGCAATATCCACTGTAGACACCAGACACCGCAAAATTAGAG CGATTTTTAACAAGGCTGTTCATTTAGGTTACATGAAGAAAAACCCCACTATAGGGG CTCATATAAGCGGACAGGACGTAGCGAAAAATAAAGCACAATTTATGGAAACAGAC AAAGTTCATTTACTATTAGAAGAACTTGCAAAATTTCATTCTATATCACGAGCAGTT ATCTTTCTAGCTGTCCAGACAGGCATGAGGTTCGAAGAAATTATTGCACTAACAAAG AAGGATATTAATTTCACTAAACGTTCAATAACTGTGAATAAAGCTTGGGATTACAAG TACACTAATACATTCATTGATACCAAAACAAAAAAATCACGAGTGATCTATATTGAT AACTCTACCGCTCAATATTTACATTCGTATTTAAATTGGCATACTGAATATATGAAG GAACATGCTATTAAGAATCCATTGATGTTATTATTCATCACTTACCACAATAAGCCA GTAGACAACGCGTCTTGTAATAAAGCTTTGAAGAAGATATGTAGTACAATCAATTCT GAACCAGTGACATTACACAAGCTACGACATACGCATACAGGCTTATGTGTAGAAGC GGGTATGGATATTATTTATGTAGCTGATAGGCTTGGTCATGATGACATTAATACAAC ATTAAAATACTATAGTCATCTAAGCTCTAATTTAAGACAACATAATCAGTCCAAAGT AGATGCTTTTTTCACACTAAAAACAGATGAAAATACCACAAATTTTACCACAAATGC CACAAAAACAACGGAATAAAACGGGTATTATACGATATAAAAAAAACTCCAAAACA TTCATCCGCCCTTTAATATCAAGGCTTTTCAACGTTTTAGAGATTTCTTTACATTACT ATTTAACGTCCTGAGAGGGATTAACACACACTGATATAAAGCCATTTAGGATATATA TACCACAAATAATACCACAAACATTTTATGTAATAATAAATATTATTTATTATTACAT TGAAATAAATATTCGTTATAAATAGTTTTTATATCAAGATGTTTTTTCTCAAGGTTTT TATAAAATGACTTTAATTCTTTTGTTTCAAGTAGTCCAGAGAAGATTTTTTCAACAGC GTTCTTCTTTCCCTCCACGCATGCCTCTCGCCTGTCCCCTCAGTTCAGTAATTTCCTGC ATTTGCCTGTTTCCAGTCGGTAGATATTCCACAAAACAGCAGGGAAGCAGCGCTTTT CCGCTGCATAACCCTGCTTCGGGGTCATTATAGCGATTTTTTCGGTATATCCATCCTT TTTCGCACGATATACAGGATTTTGCCAAAGGGTTCGTGTAGACTTTCCTTGGTGTATC CAACGGCGTCAGCCGGGCAGGATAGGTGAAGTAGGCCCACCCGCGAGCGGGTGTTC CTTCTTCACTGTCCCTTATTCGCACCTGGCGGTGCTCAACGGGAATCCTGCTCTGCGA GGCTGGCCGGCTACCGCCGGCGTAACAGATGAGGGCAAGCGGCGGAGAATTACAAC TTATATCGTATGGGGCTGACTTCAGGTGCTACATTTGAAGAGATAAATTGCACTGAA ATCTAGAAATATTTTATCTGATTAATAAGATGATCTTCTTGAGATCGTTTTGGTCTGC GCGTAATCTCTTGCTCTGAAAACGAAAAAACCGCCTTGCAGGGCGGTTTTTCGAAGG TTCTCTGAGCTACCAACTCTTTGAACCGAGGTAACTGGCTTGGAGGAGCGCAGTCAC CAAAACTTGTCCTTTCAGTTTAGCCTTAACCGGCGCATGACTTCAAGACTAACTCCTC TAAATCAATTACCAGTGGCTGCTGCCAGTGGTGCTTTTGCATGTCTTTCCGGGTTGGA CTCAAGACGATAGTTACCGGATAAGGCGCAGCGGTCGGACTGAACGGGGGGTTCGT GCATACAGTCCAGCTTGGAGCGAACTGCCTACCCGGAACTGAGTGTCAGGCGTGGA ATGAGACAAACGCGGCCATAACAGCGGAATGACACCGGTAAACCGAAAGGCAGGA

-continued

Sequence Listing

ACAGGAGAGCGCACGAGGGAGCCGCCAGGGGGAAACGCCTGGTATCTTTATAGTCC
TGTCGGGTTTCGCCACCACTGATTTGAGCGTCAGATTTCGTGATGCTTGTCAGGGGG
GCGGAGCCTATGGAAAAACGGCTTTGCCGCGGCCCTCTCACTTCCCTGTTAAGTATC
TTCCTGGCATCTTCCAGGAAATCTCCGCCCCGTTCGTAAGCCATTTCCGCTCGCCGCA
GTCGAACGACCGAGCGTAGCGAGTCAGTGAGCGAGGAAGCGGAATATATCCTGTAT
CACATATTCTGCTGACGCACCGGTGCAGCCTTTTTTCTCCTGCCACATGAAGCACTTC
ACTGACACCCTCATCAGTGCCAACATAGTAAGCCAGTATACACTCCGCTAGCGCTGA
TGTCCGGCGGTGCTTTTGCCGTTACGCACCACCCCGTCAGTAGCTGAACAGGAGGGA
CAGCTGATAGAAACAGAAGCCACTGGAGCACCTCAAAAACACCATCATACACTAAA
TCAGTAAG

pIMK2-ActAN100-IL-2 (SEQ ID NO: 9)
TTGGCAGCATCACCCGACGCACTTTGCGCCGAATAAATACCTGTGACGGAAGATCA
CTTCGCAGAATAAATAAATCCTGGTGTCCCTGTTGATACCGGGAAGCCCTGGGACGT
CGGGCCCTTTCGTCTTCAAGAATTAATTCCCAATTCCAGGCATCAAATAAAACGAAA
GGCTCAGTCGAAAGACTGGGCCTTTCGTTTTATCTGTTGTTTGTCGGTGAACGCTCTC
CTGAGTAGGACAAATCCGCCGGGAGCGGATTTGAACGTTGCGAAGCAACGGCCCGG
AGGGTGGCGGGCAGGACGCCCGCCATAAACTGCCAGGAATTAATTCCAGGCATCAA
ATAAAACGAAAGGCTCAGTCGAAAGACTGGGCCTTTCGTTTTATCTGTTGTTTGTCG
GTGAACGCTCTCCTGAGTAGGACAAATCCGCCGGGAGCGGATTTGAACGTTGCGAA
GCAACGGCCCGGAGGGTGGCGGGCAGGACGCCCGCCATAAACTGCCAGGAATTAAT
TCCAGGCATCAAATAAAACGAAAGGCTCAGTCGAAAGACTGGGCCTTTCGTTTTATC
TGTTGTTTGTCGGTGAACGCTCTCCTGAGTAGGACAAATCCGCCGGGAGCGGATTTG
AACGTTGCGAAGCAACGGCCCGGAGGGTGGCGGGCAGGACGCCCGCCATAAACTGC
CAGGAATTAATTCCAGGCATCAAATAAAACGAAAGGCTCAGTCGAAAGACTGGGCC
TTTCGTTTTATCTGTTGTTTGTCGGTGAACGCTCTCCTGAGTAGGACAAATCCGCCGG
GAGCGGATTTGAACGTTGCGAAGCAACGGCCCGGAGGGTGGCGGGCAGGACGCCC
GCCATAAACTGCCAGGAATTGGGGATCGGAATTCGAGCTCcattatgctttggcagtttattcttgacat
gtagtgaggggggctggtataatcacatacggccgataaagcaagcatataatattgcgtttcatctttagaagcgaatttcgccaatattat
aattatcaaaagagaggggtggcaaacggtatttggcattattaggttaaaaaatgtagaaggagagtgaaacccatggGgggattaaatag
atttatgcgtgcgatgatggtagttttcattactgccaactgcattacgattaaccccgacataatatttgcagcgacagatagcgaagatt
ccagtctaaacacagatgaatgggaagaagaaaaaacagaagagcagccaagcgaggtaaatacgggaccaagatacgaaactgcacgtgaa
gtaagttcacgtgatattgaggaactagaaaaatcgaataaagtgaaaaatacgaacaaagcagacctaatagcaatgttgaaagcaaaagc
agagaaaggtggatccCCAACATCTTCTTCCACTAAGAAGACTCAGCTACAACTTGAACACCT
TCTGTTGGACCTACAAATGATTCTAAATGGAATAAACAATTATAAAAATCCTAAATT
AACTAGAATGCTCACGTTCAAATTCTACATGCCGAAAAAAGCAACCGAGTTAAAAC
ATTTACAATGTCTTGAAGAAGAACTTAAACCATTGGAAGAAGTGCTTAACTTGGCCC
AAAGTAAAAATTTTCATTTACGTCCACGAGATTTAATTAGCAATATCAATGTCATTG
TATTAGAATTAAAAGGTAGTGAAACCACATTTATGTGCGAATATGCTGATGAGACA
GCGACAATTGTTGAATTTTTAAACCGCTGGATTACATTTTGTCAATCAATTATCTCGA
CGTTAACGTGAGTCGACCTCGAGGGGGGGCCCGGTACCCAGCTTTTGTTCCCTTTAG
TGAGGGTTAATTCCCAGCGAACCATTTGAGGTGATAGGTAAGATTATACCGAGGTAT
GAAAACGAGAATTGGACCTTTACAGAATTACTCTATGAAGCGCCATATTTAAAAAG
CTACCAAGACGAAGAGGATGAAGAGGATGAGGAGGCAGATTGCCTTGAATATATTG
ACAATACTGATAAGATAATATATCTTTTATATAGAAGATATCGCCGTATGTAAGGAT
TTCAGGGGGCAAGGCATAGGCAGCGCGCTTATCAATATATCTATAGAATGGGCAAA
GCATAAAAACTTGCATGGACTAATGCTTGAAACCCAGGACAATAACCTTATAGCTTG
TAAATTCTATCATAATTGTGGTTTCAAAATCGGCTCCGTCGATACTATGTTATACGCC
AACTTTGAAAACAACTTTGAAAAAGCTGTTTTCTGGTATTTAAGGTTTTAGAATGCA
AGGAACAGTGAATTGGAGTTCGTCTTGTTATAATTAGCTTCTTGGGGTATCTTTAAAT
ACTGTAGAAAAGAGGAAGGAAATAATAAATGGCTAAAATGAGAATATCACCGGAA
TTGAAAAAACTGATCGAAAAATACCGCTGCGTAAAAGATACGGAAGGAATGTCTCC
TGCTAAGGTATATAAGCTGGTGGGAGAAAATGAAAACCTATATTTAAAAATGACGG
ACAGCCGGTATAAAGGGACCACCTATGATGTGGAACGGGAAAAGGACATGATGCTA
TGGCTGGAAGGAAAGCTGCCTGTTCCAAAGGTCCTGCACTTTGAACGGCATGATGGC
TGGAGCAATCTGCTCATGAGTGAGGCCGATGGCGTCCTTTGCTCGGAAGAGTATGAA
GATGAACAAAGCCCTGAAAAGATTATCGAGCTGTATGCGGAGTGCATCAGGCTCTTT
CACTCCATCGACATATCGGATTGTCCCTATACGAATAGCTTAGACAGCCGCTTAGCC
GAATTGGATTACTTACTGAATAACGATCTGGCCGATGTGGATTGCGAAAACTGGGA
AGAAGACACTCCATTTAAAGATCCGCGCGAGCTGTATGATTTTTTAAAGACGGAAA
AGCCCGAAGAGGAACTTGTCTTTTCCCACGGCGACCTGGGAGACAGCAACATCTTTG
TGAAAGATGGCAAAGTAAGTGGCTTTATTGATCTTGGGAGAAGCGGCAGGGCGGAC
AAGTGGTATGACATTGCCTTCTGCGTCCGGTCGATCAGGGAGGATATCGGGGAAGA
ACAGTATGTCGAGCTATTTTTTGACTTACTGGGGATCAAGCCTGATTGGGAGAAAAT
AAAATATTATATTTTACTGGATGAATTGTTTTAGTACCTAGATTTAGATGTCTAAAAA
GCTTTAACTACAAGCTTTTTAGACATCTAATCTTTTCTGAAGTACATCCGCAACTGTC
CATACTCTGATGTTTCATATGATCATCATAATTCTGTCTCATTATATAACATCCTCCA
TACCTTCTATTATAGAATACCATAAACTCATCTGGCAATTCATTTCGAGTCACGAAG
AACGGAAAAACTGCCGGTTTTTATATTACAAATGTATTAAGTTTTTCTATTAACAAA
AAACAATAGGTTTCCCATAGCGAAAGTTGTTGATTAACGTTCACATCCCACTTACAC
TATAAAGGTTTACCCAGCAATACATCTCAAGCCCTAAGAATACACGTTCGCTTTTCA
ACTGTTACAGAATTATTACAAATAGTTGGTATAGTCCTCTTTAGCCTTTGGAGCTATT
ATCTCATCATTTGTTTTTAGGTGAAAACTGGGTAAACTTAGTATTAATCAATATAAA
ATTAATTCTCAAATACTTAATTACGTACTGGATTTTCTGAAAAAAAGATCTCCAAA

-continued

Sequence Listing

AATAAACAGGTGGTGGTATTAATGAAGATAAAAAAATTAGCAAACGGTAAATATTG
TGTTCGCCTACGTATAAAAGTCGATGGTGAATGGAAAGAAAAGCGTTTGACAGATA
CAAGTGAAACAAACTTAATGTATAAAGCATCTAAATTATTAAAACAAGTTCAGCAT
GATAGTAGTTCTCTGAAAGAATGGAACTTCAAAGAATTTTATACGCTATTCATGAAA
ACATTTAAAGATGGGAAAAGTAGTCAATCTACTATTAATTTATACGATCTTGCTTAT
AATCAATTCGTTGATTATTTCGATGAAAAAATTAAATTTAATTCGATTGATGCGGTTC
AATATCAACAATTTATTAATCATTTATCTGTAGACTATGCAATATCCACTGTAGACA
CCAGACACCGCAAAATTAGAGCGATTTTTAACAAGGCTGTTCATTTAGGTTACATGA
AGAAAAACCCCACTATAGGGGCTCATATAAGCGGACAGGACGTAGCGAAAAATAA
AGCACAATTTATGGAAACAGACAAAGTTCATTTACTATTAGAAGAACTTGCAAAATT
TCATTCTATATCACGAGCAGTTATCTTTCTAGCTGTCCAGACAGGCATGAGGTTCGA
AGAAATTATTGCACTAACAAAGAAGGATATTAATTTCACTAAACGTTCAATAACTGT
GAATAAAGCTTGGGATTACAAGTACACTAATACATTCATTGATACCAAAACAAAAA
AATCACGAGTGATCTATATTGATAACTCTACCGCTCAATATTTACATTCGTATTTAAA
TTGGCATACTGAATATATGAAGGAACATGCTATTAAGAATCCATTGATGTTATTATT
CATCACTTACCACAATAAGCCAGTAGACAACGCGTCTTGTAATAAAGCTTTGAAGAA
GATATGTAGTACAATCAATTCTGAACCAGTGACATTACACAAGCTACGACATACGCA
TACAGGCTTATGTGTAGAAGCGGGTATGGATATTATTTATGTAGCTGATAGGCTTGG
TCATGATGACATTAATACAACATTAAAATACTATAGTCATCTAAGCTCTAATTTAAG
ACAACATAATCAGTCCAAAGTAGATGCTTTTTTCACACTAAAAACAGATGAAAATAC
CACAAATTTTACCACAAATGCCACAAAAACAACGGAATAAAACGGGTATTATACGA
TATAAAAAAAACTCCAAAACATTCATCCGCCCTTTAATATCAAGGCTTTTCAACGTT
TTAGAGATTTCTTTACATTACTATTTAACGTCCTGAGAGGGATTAACACACACTGAT
ATAAAGCCATTTAGGATATATATACCACAAATAATACCACAAACATTTTATGTAATA
ATAAATATTATTTATTATTACATTGAAATAAATATTCGTTATAAATAGTTTTTATATC
AAGATGTTTTTTCTCAAGGTTTTATAAAATGACTTTAATTCTTTTGTTTCAAGTAGT
CCAGAGAAGATTTTTTCAACAGCGTTCTTCTTTCCCTCCACGCATGCCTCTCGCCTGT
CCCCTCAGTTCAGTAATTTCCTGCATTTGCCTGTTTCCAGTCGGTAGATATTCCACAA
AACAGCAGGGAAGCAGCGCTTTTCCGCTGCATAACCCTGCTTCGGGGTCATTATAGC
GATTTTTTCGGTATATCCATCCTTTTTCGCACGATATACAGGATTTTGCCAAAGGGTT
CGTGTAGACTTTCCTTGGTGTATCCAACGGCGTCAGCCGGGCAGGATAGGTGAAGTA
GGCCCACCCGCGAGCGGGTGTTCCTTCTTCACTGTCCCTTATTCGCACCTGGCGGTG
CTCAACGGGAATCCTGCTCTGCGAGGCTGGCCGGCTACCGCCGGCGTAACAGATGA
GGGCAAGCGGCGGAGAATTACAACTTATATCGTATGGGGCTGACTTCAGGTGCTAC
ATTTGAAGAGATAAATTGCACTGAAATCTAGAAATATTTTATCTGATTAATAAGATG
ATCTTCTTGAGATCGTTTTGGTCTGCGCGTAATCTCTTGCTCTGAAAACGAAAAAAC
CGCCTTGCAGGGCGGTTTTTCGAAGGTTCTCTGAGCTACCAACTCTTTGAACCGAGG
TAACTGGCTTGGAGGAGCGCAGTCACCAAAACTTGTCCTTTCAGTTTAGCCTTAACC
GGCGCATGACTTCAAGACTAACTCCTCTAAATCAATTACCAGTGGCTGCTGCCAGTG
GTGCTTTTGCATGTCTTTCCGGGTTGGACTCAAGACGATAGTTACCGGATAAGGCGC
AGCGGTCGGACTGAACGGGGGGTTCGTGCATACAGTCCAGCTTGGAGCGAACTGCC
TACCCGGAACTGAGTGTCAGGCGTGGAATGAGACAAACGCGGCCATAACAGCGGAA
TGACACCGGTAAACCGAAAGGCAGGAACAGGAGAGCGCACGAGGGAGCCGCCAGG
GGGAAACGCCTGGTATCTTTATAGTCCTGTCGGGTTTCGCCACCACTGATTTGAGCG
TCAGATTTCGTGATGCTTGTCAGGGGGGCGGAGCCTATGGAAAAACGGCTTTGCCGC
GGCCCTCTCACTTCCCTGTTAAGTATCTTCCTGGCATCTTCCAGGAAATCTCCGCCCC
GTTCGTAAGCCATTTCCGCTCGCCGCAGTCGAACGACCGAGCGTAGCGAGTCAGTG
AGCGAGGAAGCGGAATATATCCTGTATCACATATTCTGCTGACGCACCGGTGCAGCC
TTTTTTCTCCTGCCACATGAAGCACTTCACTGACACCCTCATCAGTGCCAACATAGTA
AGCCAGTATACACTCCGCTAGCGCTGATGTCCGGCGGTGCTTTTGCCGTTACGCACC
ACCCCGTCAGTAGCTGAACAGGAGGGACAGCTGATAGAAACAGAAGCCACTGGAG
CACCTCAAAAACACCATCATACACTAAATCAGTAAG

pIMK2-hly5'UTR-ActAN100-IL2 (SEQ ID NO: 10)
TTGGCAGCATCACCCGACGCACTTTGCGCCGAATAAATACCTGTGACGGAAGATCA
CTTCGCAGAATAAATAAATCCTGGTGTCCCTGTTGATACCGGGAAGCCCTGGGACGT
CGGGCCCTTTCGTCTTCAAGAATTAATTCCCAATTCCAGGCATCAAATAAAACGAAA
GGCTCAGTCGAAAGACTGGGCCTTTCGTTTTATCTGTTGTTTGTCGGTGAACGCTCTC
CTGAGTAGGACAAATCCGCCGGGAGCGGATTTGAACGTTGCGAAGCAACGGCCCGG
AGGGTGGCGGGCAGGACGCCCGCCATAAACTGCCAGGAATTAATTCCAGGCATCAA
ATAAAACGAAAGGCTCAGTCGAAAGACTGGGCCTTTCGTTTTATCTGTTGTTTGTCG
GTGAACGCTCTCCTGAGTAGGACAAATCCGCCGGGAGCGGATTTGAACGTTGCGAA
GCAACGGCCCGGAGGGTGGCGGGCAGGACGCCCGCCATAAACTGCCAGGAATTAAT
TCCAGGCATCAAATAAAACGAAAGGCTCAGTCGAAAGACTGGGCCTTTCGTTTTATC
TGTTGTTTGTCGGTGAACGCTCTCCTGAGTAGGACAAATCCGCCGGGAGCGGATTTG
AACGTTGCGAAGCAACGGCCCGGAGGGTGGCGGGCAGGACGCCCGCCATAAACTGC
CAGGAATTAATTCCAGGCATCAAATAAAACGAAAGGCTCAGTCGAAAGACTGGGCC
TTTCGTTTTATCTGTTGTTTGTCGGTGAACGCTCTCCTGAGTAGGACAAATCCGCCGG
GAGCGGATTTGAACGTTGCGAAGCAACGGCCCGGAGGGTGGCGGGCAGGACGCCC
GCCATAAACTGCCAGGAATTGGGGATCGGAATTCGAGCTCcattatgctttggcagtttattcttgacat
gtagtgagggggctggtataatcacatacggccgataaagcaagcatataatattgcgtttcatctttagaagcgaatttcgccaatattat
aattatcaaaagagaggggtggcaaacggtatttggcattattaggttaaaaaatgtagaaggagagtgaaacccatgGATAAAGCAA
GCATATAATATTGCGTGTAGAAGGAGAGTGAAACCCGTGGGATTAAATAGATTTAT
GCGTGCGATGATGGTAGTTTTCATTACTGCCAACTGCATTACGATTAACCCCGACAT

-continued

Sequence Listing
AATATTTGCAGCGACAGATAGCGAAGATTCCAGTCTAAACACAGATGAATGGGAAG AAGAAAAAACAGAAGAGCAGCCAAGCGAGGTAAATACGGGACCAAGATACGAAAC TGCACGTGAAGTAAGTTCACGTGATATTGAGGAACTAGAAAAATCGAATAAAGTGA AAAATACGAACAAAGCAGACCTAATAGCAATGTTGAAAGCAAAAGCAGAGAAAGG TGGATCCCCAACATCTTCTTCCACTAAGAAGACTCAGCTACAACTTGAACACCTTCT GTTGGACCTACAAATGATTCTAAATGGAATAAACAATTATAAAAATCCTAAATTAAC TAGAATGCTCACGTTCAAATTCTACATGCCGAAAAAAGCAACCGAGTTAAAACATTT ACAATGTCTTGAAGAAGAACTTAAACCATTGGAAGAAGTGCTTAACTTGGCCCAAA GTAAAAATTTTCATTTACGTCCACGAGATTTAATTAGCAATATCAATGTCATTGTATT AGAATTAAAAGGTAGTGAAACCACATTTATGTGCGAATATGCTGATGAGACAGCGA CAATTGTTGAATTTTTAAACCGCTGGATTACATTTTGTCAATCAATTATCTCGACGTT AACGTGAGTCGACCTCGAGGGGGGGCCCGGTACCCAGCTTTTGTTCCCTTTAGTGAG GGTTAATTCCCAGCGAACCATTTGAGGTGATAGGTAAGATTATACCGAGGTATGAA AACGAGAATTGGACCTTTACAGAATTACTCTATGAAGCGCCATATTTAAAAAGCTAC CAAGACGAAGAGGATGAAGAGGATGAGGAGGCAGATTGCCTTGAATATATTGACAA TACTGATAAGATAATATATCTTTTATATAGAAGATATCGCCGTATGTAAGGATTTCA GGGGGCAAGGCATAGGCAGCGCGCTTATCAATATATCTATAGAATGGGCAAAGCAT AAAAACTTGCATGGACTAATGCTTGAAACCCAGGACAATAACCTTATAGCTTGTAAA TTCTATCATAATTGTGGTTTCAAAATCGGCTCCGTCGATACTATGTTATACGCCAACT TTGAAAACAACTTTGAAAAAGCTGTTTTCTGGTATTTAAGGTTTTAGAATGCAAGGA ACAGTGAATTGGAGTTCGTCTTGTTATAATTAGCTTCTTGGGGTATCTTTAAATACTG TAGAAAAGAGGAAGGAAATAATAAATGGCTAAAATGAGAATATCACCGGAATTGA AAAAACTGATCGAAAAATACCGCTGCGTAAAAGATACGGAAGGAATGTCTCCTGCT AAGGTATATAAGCTGGTGGGAGAAAATGAAAACCTATATTTAAAAATGACGGACAG CCGGTATAAAGGGACCACCTATGATGTGGAACGGGAAAAGGACATGATGCTATGGC TGGAAGGAAAGCTGCCTGTTCCAAAGGTCCTGCACTTTGAACGGCATGATGGCTGG AGCAATCTGCTCATGAGTGAGGCCGATGGCGTCCTTTGCTCGGAAGAGTATGAAGAT GAACAAAGCCCTGAAAAGATTATCGAGCTGTATGCGGAGTGCATCAGGCTCTTTCAC TCCATCGACATATCGGATTGTCCCTATACGAATAGCTTAGACAGCCGCTTAGCCGAA TTGGATTACTTACTGAATAACGATCTGGCCGATGTGGATTGCGAAAACTGGGAAGA AGACACTCCATTTAAAGATCCGCGCGAGCTGTATGATTTTTTAAAGACGGAAAAGCC CGAAGAGGAACTTGTCTTTTCCCACGGCGACCTGGGAGACAGCAACATCTTTGTGAA AGATGGCAAAGTAAGTGGCTTTATTGATCTTGGGAGAAGCGGCAGGGCGGACAAGT GGTATGACATTGCCTTCTGCGTCCGGTCGATCAGGGAGGATATCGGGGAAGAACAG TATGTCGAGCTATTTTTTGACTTACTGGGGATCAAGCCTGATTGGGAGAAAATAAAA TATTATATTTTACTGGATGAATTGTTTTAGTACCTAGATTTAGATGTCTAAAAAGCTT TAACTACAAGCTTTTTAGACATCTAATCTTTTCTGAAGTACATCCGCAACTGTCCATA CTCTGATGTTTCATATGATCATCATAATTCTGTCTCATTATATAACATCCTCCATACC TTCTATTATAGAATACCATAAACTCATCTGGCAATTCATTTCGAGTCACGAAGAACG GAAAAACTGCCGGTTTTTATATTACAAATGTATTAAGTTTTTCTATTAACAAAAAAC AATAGGTTTCCCATAGCGAAAGTTGTTGATTAACGTTCACATCCCACTTACACTATA AAGGTTTACCCAGCAATACATCTCAAGCCCTAAGAATACACGTTCGCTTTTCAACTG TTACAGAATTATTACAAATAGTTGGTATAGTCCTCTTTAGCCTTTGGAGCTATTATCT CATCATTTGTTTTTTAGGTGAAAACTGGGTAAACTTAGTATTAATCAATATAAAATT AATTCTCAAATACTTAATTACGTACTGGGATTTTCTGAAAAAAAGATCTCCAAAAAT AAACAGGTGGTGGTATTAATGAAGATAAAAAAATTAGCAAACGGTAAATATTGTGT TCGCCTACGTATAAAAGTCGATGGTGAATGGAAAGAAAAGCGTTTGACAGATACAA GTGAAACAAACTTAATGTATAAAGCATCTAAATTATTAAAACAAGTTCAGCATGATA GTAGTTCTCTGAAAGAATGGAACTTCAAAGAATTTTATACGCTATTCATGAAAACAT TTAAAGATGGGAAAAGTAGTCAATCTACTATTAATTTATACGATCTTGCTTATAATC AATTCGTTGATTATTTCGATGAAAAAATTAAATTTAATTCGATTGATGCGGTTCAAT ATCAACAATTTATTAATCATTTATCTGTAGACTATGCAATATCCACTGTAGACACCA GACACCGCAAAATTAGAGCGATTTTTAACAAGGCTGTTCATTTAGGTTACATGAAGA AAAACCCCACTATAGGGGCTCATATAAGCGGACAGGACGTAGCGAAAAATAAAGCA CAATTTATGGAAACAGACAAAGTTCATTTACTATTAGAAGAACTTGCAAAATTTCAT TCTATATCACGAGCAGTTATCTTTCTAGCTGTCCAGACAGGCATGAGGTTCGAAGAA ATTATTGCACTAACAAAGAAGGATATTAATTTCACTAAACGTTCAATAACTGTGAAT AAAGCTTGGGATTACAAGTACACTAATACATTCATTGATACCAAAACAAAAAAATC ACGAGTGATCTATATTGATAACTCTACCGCTCAATATTTACATTCGTATTTAAATTGG CATACTGAATATATGAAGGAACATGCTATTAAGAATCCATTGATGTTATTATTCATC ACTTACCACAATAAGCCAGTAGACAACGCGTCTTGTAATAAAGCTTTGAAGAAGAT ATGTAGTACAATCAATTCTGAACCAGTGACATTACACAAGCTACGACATACGCATAC AGGCTTATGTGTAGAAGCGGGTATGGATATTATTTATGTAGCTGATAGGCTTGGTCA TGATGACATTAATACAACATTAAAATACTATAGTCATCTAAGCTCTAATTTAAGACA ACATAATCAGTCCAAAGTAGATGCTTTTTTCACACTAAAAACAGATGAAAATACCAC AAATTTTACCACAAATGCCACAAAAACAACGGAATAAAACGGGTATTATACGATAT AAAAAAAACTCCAAAACATTCATCCGCCCTTTAATATCAAGGCTTTTCAACGTTTTA GAGATTTCTTTACATTACTATTTAACGTCCTGAGAGGGATTAACACACACTGATATA AAGCCATTTAGGATATATATACCACAAATAATACCACAAACATTTTATGTAATAATA AATATTATTTATTATTACATTGAAATAAATATTCGTTATAAATAGTTTTTATATCAAG ATGTTTTTTCTCAAGGTTTTTATAAAATGACTTTAATTCTTTTGTTTCAAGTAGTCCAG AGAAGATTTTTTCAACAGCGTTCTTCTTTCCCTCCACGCATGCCTCTCGCCTGTCCCC TCAGTTCAGTAATTTCCTGCATTTGCCTGTTTCCAGTCGGTAGATATTCCACAAAACA GCAGGGAAGCAGCGCTTTTCCGCTGCATAACCCTGCTTCGGGGTCATTATAGCGATT

-continued

Sequence Listing
TTTTCGGTATATCCATCCTTTTTCGCACGATATACAGGATTTTGCCAAAGGGTTCGTG TAGACTTTCCTTGGTGTATCCAACGGCGTCAGCCGGGCAGGATAGGTGAAGTAGGCC CACCCGCGAGCGGGTGTTCCTTCTTCACTGTCCCTTATTCGCACCTGGCGGTGCTCAA CGGGAATCCTGCTCTGCGAGGCTGGCCGGCTACCGCCGGCGTAACAGATGAGGGCA AGCGGCGGAGAATTACAACTTATATCGTATGGGGCTGACTTCAGGTGCTACATTTGA AGAGATAAATTGCACTGAAATCTAGAAATATTTTATCTGATTAATAAGATGATCTTC TTGAGATCGTTTTGGTCTGCGCGTAATCTCTTGCTCTGAAAACGAAAAAACCGCCTT GCAGGGCGGTTTTTCGAAGGTTCTCTGAGCTACCAACTCTTTGAACCGAGGTAACTG GCTTGGAGGAGCGCAGTCACCAAAACTTGTCCTTTCAGTTTAGCCTTAACCGGCGCA TGACTTCAAGACTAACTCCTCTAAATCAATTACCAGTGGCTGCTGCCAGTGGTGCTT TTGCATGTCTTTCCGGGTTGGACTCAAGACGATAGTTACCGGATAAGGCGCAGCGGT CGGACTGAACGGGGGGTTCGTGCATACAGTCCAGCTTGGAGCGAACTGCCTACCCG GAACTGAGTGTCAGGCGTGGAATGAGACAAACGCGGCCATAACAGCGGAATGACAC CGGTAAACCGAAAGGCAGGAACAGGAGAGCGCACGAGGGAGCCGCCAGGGGGAAA CGCCTGGTATCTTTATAGTCCTGTCGGGTTTCGCCACCACTGATTTGAGCGTCAGATT TCGTGATGCTTGTCAGGGGGGCGGAGCCTATGGAAAAACGGCTTTGCCGCGGCCCTC TCACTTCCCTGTTAAGTATCTTCCTGGCATCTTCCAGGAAATCTCCGCCCCGTTCGTA AGCCATTTCCGCTCGCCGCAGTCGAACGACCGAGCGTAGCGAGTCAGTGAGCGAGG AAGCGGAATATATCCTGTATCACATATTCTGCTGACGCACCGGTGCAGCCTTTTTTCT CCTGCCACATGAAGCACTTCACTGACACCCTCATCAGTGCCAACATAGTAAGCCAGT ATACACTCCGCTAGCGCTGATGTCCGGCGGTGCTTTTGCCGTTACGCACCACCCCGT CAGTAGCTGAACAGGAGGGACAGCTGATAGAAACAGAAGCCACTGGAGCACCTCA AAAACACCATCATACACTAAATCAGTAAG
hly 5' UTR (SEQ ID NO: 11) GATAAAGCAAGCATATAATATTGCGTGTAGAAGGAGAGTGAAACCC

SEQUENCE LISTING

Sequence total quantity: 11
SEQ ID NO: 1 moltype = DNA length = 523
FEATURE Location/Qualifiers
source 1..523
mol_type = other DNA
organism = synthetic construct
SEQUENCE: 1
aggagagtga aacccatggg tggattaaa tagatttatg cgtgcgatga tggtagtttt 60
cattactgcc aactgcatta cgattaaccc cgacataata tttgcagcgc caacatcttc 120
ttccactaag aagactcagc tacaacttga acaccttctg ttggacctac aaatgattct 180
aaatggaata aacaattata aaaatcctaa attaactaga atgctcacgt tcaaattcta 240
catgccgaaa aaagcaaccg agttaaaaca tttacaatgt cttgaagaag aacttaaacc 300
attggaagaa gtgcttaact tggcccaaag taaaaatttt catttacgtc cacgagattt 360
aattagcaat atcaatgtca ttgtattaga attaaaaggt agtgaaacca catttatgtg 420
cgaatatgct gatgagacag cgacaattgt tgaattttta aaccgctgga ttacattttg 480
tcaatcaatt atctcgacgt taacgtgagt cgacctcgag ggg 523

SEQ ID NO: 2 moltype = DNA length = 744
FEATURE Location/Qualifiers
source 1..744
mol_type = other DNA
organism = synthetic construct
SEQUENCE: 2
gaaggagagt gaaacccatg ggtgggatta aatagattta tgcgtgcgat gatggtagtt 60
ttcattactg ccaactgcat tacgattaac cccgacataa tatttgcagc gacagatagc 120
gaagattcca gtctaaacac agatgaatgg gaagaagaaa aaacagaaga gcagccaagc 180
gaggtaaata cgggaccaag atacgaaact gcacgtgaag taagttcacg tgatattgag 240
gaactagaaa aatcgaataa agtgaaaaat acgaacaaag cagacctaat agcaatgttg 300
aaagcaaaag cagagaaagg tggatcccca acatcttctt ccactaagaa gactcagcta 360
caacttgaac accttctgtt ggacctacaa atgattctaa atggaataaa caattataaa 420
aatcctaaat taactagaat gctcacgttc aaattctaca tgccgaaaaa agcaaccgag 480
ttaaaacatt tacaatgtct tgaagaagaa cttaaaccat tggaagaagt gcttaacttg 540
gcccaaagta aaaatttca tttacgtcca cgagatttaa ttagcaatat caatgtcatt 600
gtattagaat taaaaggtag tgaaaccaca tttatgtgcg aatatgctga tgagacagcg 660
acaattgttg aatttttaaa ccgctggatt acattttgtc aatcaattat ctcgacgtta 720
acgtgagtcg acctcgaggg gggg 744

SEQ ID NO: 3 moltype = DNA length = 44
FEATURE Location/Qualifiers
source 1..44
mol_type = other DNA

-continued

```
                         organism = synthetic construct
SEQUENCE: 3
agggaacaaa agctgggtac ccattatgct ttggcagttt attc                  44

SEQ ID NO: 4             moltype = DNA  length = 30
FEATURE                  Location/Qualifiers
source                   1..30
                         mol_type = other DNA
                         organism = synthetic construct
SEQUENCE: 4
agcctgacat gggtttcact ctccttctac                                  30

SEQ ID NO: 5             moltype = DNA  length = 22
FEATURE                  Location/Qualifiers
source                   1..22
                         mol_type = other DNA
                         organism = synthetic construct
SEQUENCE: 5
tcaaccagat gttctccctg ta                                          22

SEQ ID NO: 6             moltype = DNA  length = 22
FEATURE                  Location/Qualifiers
source                   1..22
                         mol_type = other DNA
                         organism = synthetic construct
SEQUENCE: 6
cactgtaagc catttcgtca tc                                          22

SEQ ID NO: 7             moltype = DNA  length = 5996
FEATURE                  Location/Qualifiers
source                   1..5996
                         mol_type = other DNA
                         organism = synthetic construct
SEQUENCE: 7
ttggcagcat cacccgacgc actttgcgcc gaataaatac ctgtgacgga agatcacttc  60
gcagaataaa taaatcctgg tgtccctgtt gataccggga agccctggga cgtcgggccc  120
tttcgtcttc aagaattaat tcccaattcc aggcatcaaa taaaacgaaa ggctcagtcg  180
aaagactggg cctttcgttt tatctgttgt ttgtcggtga acgctctcct gagtaggaca  240
aatccgccgg gagcggattt gaacgttgcg aagcaacggc ccggagggtg gcgggcagga  300
cgcccgccat aaactgccag gaattaattc caggcatcaa ataaaacgaa aggctcagtc  360
gaaagactgg gcctttcgtt ttatctgttg tttgtcggtg aacgctctcc tgagtaggac  420
aaatccgccg ggagcggatt tgaacgttgc gaagcaacgg cccggagggt ggcgggcagg  480
acgcccgcca taaactgcca ggaattaatt ccaggcatca aataaaacga aaggctcagt  540
cgaaagactg ggcctttcgt tttatctgtt gtttgtcggt gaacgctctc ctgagtagga  600
caaatccgcc gggagcggat ttgaacgttg cgaagcaacg gcccggaggg tggcgggcag  660
gacgcccgcc ataaactgcc aggaattaat tccaggcatc aaataaaacg aaaggctcag  720
tcgaaagact gggcctttcg ttttatctgt tgtttgtcgg tgaacgctct cctgagtagg  780
acaaatccgc cgggagcgga tttgaacgtt gcgaagcaac ggcccggagg gtggcgggca  840
ggacgcccgc cataaactgc caggaattgg ggatcggaat tcgagctcca ttatgctttg  900
gcagtttatt cttgacatgt agtgaggggg ctggtataat cacatacggc cgataaagca  960
agcatataat attgcgtttc atctttagaa gcgaatttcg ccaatattat aattatcaaa  1020
agagaggggt ggcaaacggt atttggcatt attaggttaa aaaatgtaga aggagagtga  1080
aacccatgga aaaggatccc ccgggctgca ggaattcgat atcaagctta tcgataccgt  1140
cgacctcgag ggggggcccg gtacccagct tttgttccct ttagtgaggg ttaattccca  1200
gcgaaccatt tgaggtgata ggtaagatta taccgaggta tgaaaacgag aattggacct  1260
ttacagaatt actctatgaa gcgccatatt taaaaagcta ccaagacgaa gaggatgaag  1320
aggatgagga ggcagattgc cttgaatata ttgacaatac tgataagata atatatcttt  1380
tatatagaag atatcgccgt atgtaaggat ttcagggggc aaggcatagg cagcgcgctt  1440
atcaatatat ctatagaatg ggcaaagcat aaaaacttgc atggactaat gcttgaaacc  1500
caggacaata accttatagc ttgtaaattc tatcataatt gtggtttcaa aatcggctcc  1560
gtcgatacta tgttatacgc caactttgaa aacaactttg aaaaagctgt tttctggtat  1620
ttaaggtttt agaatgcaag gaacagtgaa ttggagttcg tcttgttata attagcttct  1680
tggggtatct ttaaatactg tagaaaagag gaaggaaata ataaatggct aaaatgagaa  1740
tatcaccgga attgaaaaaa ctgatcgaaa aataccgctg cgtaaaagat acggaaggaa  1800
tgtctcctgc taaggtatat aagctggtgg gagaaaatga aaacctatat ttaaaaatga  1860
cggacagccg gtataaaggg accacctatg atgtggaacg ggaaaaggac atgatgctat  1920
ggctggaagg aaagctgcct gttccaaagg tcctgcactt tgaacggcat gatggctgga  1980
gcaatctgct catgagtgag gccgatggcg tcctttgctc ggaagagtat gaagatgaac  2040
aaagccctga aaagattatc gagctgtatg cggagtgcat caggctcttt cactccatcg  2100
acatatcgga ttgtccctat acgaatagct tagacagccg cttagccgaa ttggattact  2160
tactgaataa cgatctggcc gatgtggatt gcgaaaactg ggaagaagac actccattta  2220
aagatccgcg cgagctgtat gatttttttaa agacggaaaa gcccgaagag gaacttgtct  2280
tttcccacgg cgacctggga gacagcaaca tctttgtgaa agatggcaaa gtaagtggct  2340
ttattgatct tgggagaagc ggcagggcgg acaagtggta tgacattgcc ttctgcgtcc  2400
ggtcgatcag ggaggatatc ggggaagaac agtatgtcga gctatttttt gacttactgg  2460
ggatcaagcc tgattgggag aaaataaaat attatatttt actggatgaa ttgttttagt  2520
```

-continued

acctagattt agatgtctaa aaagctttaa ctacaagctt tttagacatc taatcttttc 2580
tgaagtacat ccgcaactgt ccatactctg atgtttcata tgatcatcat aattctgtct 2640
cattatataa catcctccat accttctatt atagaatacc ataaactcat ctggcaattc 2700
atttcgagtc acgaagaacg gaaaaactgc cggtttttat attacaaatg tattaagttt 2760
ttctattaac aaaaaacaat aggtttccca tagcgaaagt tgttgattaa cgttcacatc 2820
ccacttacac tataaaggtt tacccagcaa tacatctcaa gccctaagaa tacacgttcg 2880
cttttcaact gttacagaat tattacaaat agttggtata gtcctcttta gcctttggag 2940
ctattatctc atcatttgtt ttttaggtga aaactgggta aacttagtat taatcaatat 3000
aaaattaatt ctcaaatact taattacgta ctgggatttt ctgaaaaaaa gatctccaaa 3060
aataaacagg tggtggtatt aatgaagata aaaaaattag caaacggtaa atattgtgtt 3120
cgcctacgta taaaagtcga tggtgaatgg aaagaaaagc gtttgacaga tacaagtgaa 3180
acaaacttaa tgtataaagc atctaaatta ttaaaacaag ttcagcatga tagtagttct 3240
ctgaaagaat ggaacttcaa agaattttat acgctattca tgaaaacatt taaagatggg 3300
aaaagtagtc aatctactat taatttatac gatcttgctt ataatcaatt cgttgattat 3360
ttcgatgaaa aaattaaatt taattcgatt gatgcggttc aatatcaaca atttattaat 3420
catttatctg tagactatgc aatatccact gtagacacca gacaccgcaa aattagagcg 3480
atttttaaca aggctgttca tttaggttac atgaagaaaa accccactat aggggctcat 3540
ataagcggac aggacgtagc gaaaaataaa gcacaattta tggaaacaga caaagttcat 3600
ttactattag aagaacttgc aaaatttcat tctatatcac gagcagttat ctttctagct 3660
gtccagacag gcatgaggtt cgaagaaatt attgcactaa caaagaagga tattaatttc 3720
actaaacgtt caataactgt gaataaagct tgggattaca agtacactaa tacattcatt 3780
gataccaaaa caaaaaaatc acgagtgatc tatattgata actctaccgc tcaatattta 3840
cattcgtatt taaattggca tactgaatat atgaaggaac atgctattaa gaatccattg 3900
atgttattat tcatcactta ccacaataag ccagtagaca acgcgtcttg taataaagct 3960
ttgaagaaga tatgtagtac aatcaattct gaaccagtga cattacacaa gctacgacat 4020
acgcatacag gcttatgtgt agaagcgggt atggatatta tttatgtagc tgataggctt 4080
ggtcatgatg acattaatac aacattaaaa tactatagtc atctaagctc taatttaaga 4140
caacataatc agtccaaagt agatgctttt ttcacactaa aaacagatga aaataccaca 4200
aattttacca caaatgccac aaaaacaacg gaataaaacg ggtattatac gatataaaaa 4260
aaactccaaa acattcatcc gccctttaat atcaaggctt ttcaacgttt tagagatttc 4320
tttacattac tatttaacgt cctgagaggg attaacacac actgatataa agccatttag 4380
gatatatata ccacaaataa taccacaaac attttatgta ataataaata ttatttatta 4440
ttacattgaa ataaatattc gttataaata gtttttatat caagatgttt tttctcaagg 4500
tttttataaa atgactttaa ttcttttgtt tcaagtagtc cagagaagat tttttcaaca 4560
gcgttcttct ttccctccac gcatgcctct cgcctgtccc ctcagttcag taatttcctg 4620
catttgcctg tttccagtcg gtagatattc cacaaaacag cagggaagca gcgcttttcc 4680
gctgcataac cctgcttcgg ggtcattata gcgatttttt cggtatatcc atccttttc 4740
gcacgatata caggattttg ccaaagggtt cgtgtagact ttccttggtg tatccaacgg 4800
cgtcagccgg gcaggatagg tgaagtaggc ccacccgcga gcgggtgttc cttcttcact 4860
gtcccttatt cgcacctggc ggtgctcaac gggaatcctg ctctgcgagg ctggccggct 4920
accgccggcg taacagatga gggcaagcgg cggagaatta caacttatat cgtatggggc 4980
tgacttcagg tgctacattt gaagagataa attgcactga aatctagaaa tattttatct 5040
gattaataag atgatcttct tgagatcgtt ttggtctgcg cgtaatctct tgctctgaaa 5100
acgaaaaaac cgccttgcag ggcggttttt cgaaggttct ctgagctacc aactctttga 5160
accgaggtaa ctggcttgga ggagcgcagt caccaaaact tgtcctttca gtttagcctt 5220
aaccggcgca tgacttcaag actaactcct ctaaatcaat taccagtggc tgctgccagt 5280
ggtgcttttg catgtctttc cgggttggac tcaagacgat agttaccgga taaggcgcag 5340
cggtcggact gaacgggggg ttcgtgcata cagtccagct tggagcgaac tgcctacccg 5400
gaactgagtg tcaggcgtgg aatgagacaa acgcggccat aacagcggaa tgacaccggt 5460
aaaccgaaag gcaggaacag gagagcgcac gagggagccg ccagggggaa acgcctggta 5520
tctttatagt cctgtcgggt ttcgccacca ctgatttgag cgtcagattt cgtgatgctt 5580
gtcagggggg cggagcctat ggaaaaacgg ctttgccgcg gccctctcac ttccctgtta 5640
agtatcttcc tggcatcttc caggaaatct ccgccccgtt cgtaagccat ttccgctcgc 5700
cgcagtcgaa cgaccgagcg tagcgagtca gtgagcgagg aagcggaata tatcctgtat 5760
cacatattct gctgacgcac cggtgcagcc ttttttctcc tgccacatga agcacttcac 5820
tgacaccctc atcagtgcca acatagtaag ccagtataca ctccgctagc gctgatgtcc 5880
ggcggtgctt ttgccgttac gcaccacccc gtcagtagct gaacaggagg gacagctgat 5940
agaaacagaa gccactggag cacctcaaaa acaccatcat acactaaatc agtaag 5996

SEQ ID NO: 8 moltype = DNA length = 6436
FEATURE Location/Qualifiers
source 1..6436
mol_type = other DNA
organism = synthetic construct
SEQUENCE: 8
ttggcagcat cacccgacgc actttgcgcc gaataaatac ctgtgacgga agatcacttc 60
gcagaataaa taaatcctgg tgtccctgtt gataccggga agccctggga cgtcgggccc 120
tttcgtcttc aagaattaat tcccaattcc aggcatcaaa taaaacgaaa ggctcagtcg 180
aaagactggg cctttcgttt tatctgttgt ttgtcggtga acgctctcct gagtaggaca 240
aatccgccgg gagcggattt gaacgttgcg aagcaacggc ccggagggtg gcgggcagga 300
cgcccgccat aaactgccag gaattaattc caggcatcaa ataaaacgaa aggctcagtc 360
gaaagactgg gcctttcgtt ttatctgttg tttgtcggtg aacgctctcc tgagtaggac 420
aaatccgccg ggagcggatt tgaacgttgc gaagcaacgg cccggagggt ggcgggcagg 480
acgcccgcca taaactgcca ggaattaatt ccaggcatca aataaaacga aaggctcagt 540
cgaaagactg ggcctttcgt tttatctgtt gtttgtcggt gaacgctctc ctgagtagga 600
caaatccgcc gggagcggat ttgaacgttg cgaagcaacg gcccggaggg tggcgggcag 660

-continued

```
gacgcccgcc ataaactgcc aggaattaat tccaggcatc aaataaaacg aaaggctcag  720
tcgaaagact gggcctttcg ttttatctgt tgtttgtcgg tgaacgctct cctgagtagg  780
acaaatccgc cgggagcgga tttgaacgtt gcgaagcaac ggcccggagg gtggcgggca  840
ggacgcccgc cataaactgc caggaattgg ggatcggaat tcgagctcca ttatgctttg  900
gcagtttatt cttgacatgt agtgaggggg ctggtataat cacatacggc cgataaagca  960
agcatataat attgcgtttc atctttagaa gcgaatttcg ccaatattat aattatcaaa  1020
agagaggggt ggcaaacggt atttggcatt attaggttaa aaaatgtaga aggagagtga  1080
aacccatggg tgggattaaa tagatttatg cgtgcgatga tggtagtttt cattactgcc  1140
aactgcatta cgattaaccc cgacataata tttgcagcgc caacatcttc ttccactaag  1200
aagactcagc tacaacttga acaccttctg ttggacctac aaatgattct aaatggaata  1260
aacaattata aaaatcctaa attaactaga atgctcacgt tcaaattcta catgccgaaa  1320
aaagcaaccg agttaaaaca tttacaatgt cttgaagaag aacttaaacc attggaagaa  1380
gtgcttaact tggcccaaag taaaaatttt catttacgtc cacgagattt aattagcaat  1440
atcaatgtca ttgtattaga attaaaaggt agtgaaacca catttatgtg cgaatatgct  1500
gatgagacag cgacaattgt tgaattttta aaccgctgga ttacattttg tcaatcaatt  1560
atctcgacgt taacgtgagt cgacctcgag ggggggcccg gtacccagct tttgttccct  1620
ttagtgaggg ttaattccca gcgaaccatt tgaggtgata ggtaagatta taccgaggta  1680
tgaaaacgag aattggacct ttacagaatt actctatgaa gcgccatatt taaaaagcta  1740
ccaagacgaa gaggatgaag aggatgagga ggcagattgc cttgaatata ttgacaatac  1800
tgataagata atatatcttt tatatagaag atatcgccgt atgtaaggat ttcagggggc  1860
aaggcatagg cagcgcgctt atcaatatat ctatagaatg ggcaaagcat aaaaacttgc  1920
atggactaat gcttgaaacc caggacaata accttatagc ttgtaaattc tatcataatt  1980
gtggtttcaa aatcggctcc gtcgatacta tgttatacgc caactttgaa aacaactttg  2040
aaaaagctgt tttctggtat ttaaggtttt agaatgcaag gaacagtgaa ttggagttcg  2100
tcttgttata attagcttct tggggtatct ttaaatactg tagaaaagag gaaggaaata  2160
ataaatggct aaaatgagaa tatcaccgga attgaaaaaa ctgatcgaaa aataccgctg  2220
cgtaaaagat acggaaggaa tgtctcctgc taaggtatat aagctggtgg gagaaaatga  2280
aaacctatat ttaaaaatga cggacagccg gtataaaggg accacctatg atgtggaacg  2340
ggaaaaggac atgatgctat ggctggaagg aaagctgcct gttccaaagg tcctgcactt  2400
tgaacggcat gatggctgga gcaatctgct catgagtgag gccgatggcg tcctttgctc  2460
ggaagagtat gaagatgaac aaagccctga aaagattatc gagctgtatg cggagtgcat  2520
caggctcttt cactccatcg acatatcgga ttgtccctat acgaatagct tagacagccg  2580
cttagccgaa ttggattact tactgaataa cgatctggcc gatgtggatt gcgaaaactg  2640
ggaagaagac actccattta aagatccgcg cgagctgtat gattttttaa agacggaaaa  2700
gcccgaagag gaacttgtct tttcccacgg cgacctggga gacagcaaca tctttgtgaa  2760
agatggcaaa gtaagtggct ttattgatct tgggagaagc ggcagggcgg acaagtggta  2820
tgacattgcc ttctgcgtcc ggtcgatcag ggaggatatc ggggaagaac agtatgtcga  2880
gctatttttt gacttactgg ggatcaagcc tgattgggag aaaataaaat attatatttt  2940
actggatgaa ttgttttagt acctagattt agatgtctaa aaagctttaa ctacaagctt  3000
tttagacatc taatcttttc tgaagtacat ccgcaactgt ccatactctg atgtttcata  3060
tgatcatcat aattctgtct cattatataa catcctccat accttctatt atagaatacc  3120
ataaactcat ctggcaattc atttcgagtc acgaagaacg gaaaaactgc cggtttttat  3180
attacaaatg tattaagttt ttctattaac aaaaaacaat aggtttccca tagcgaaagt  3240
tgttgattaa cgttcacatc ccacttacac tataaaggtt tacccagcaa tacatctcaa  3300
gccctaagaa tacacgttcg cttttcaact gttacagaat tattacaaat agttggtata  3360
gtcctcttta gcctttggag ctattatctc atcatttgtt ttttaggtga aaactgggta  3420
aacttagtat taatcaatat aaaattaatt ctcaaatact taattacgta ctgggatttt  3480
ctgaaaaaaa gatctccaaa aataaacagg tggtggtatt aatgaagata aaaaaattag  3540
caaacggtaa atattgtgtt cgcctacgta taaaagtcga tggtgaatgg aaagaaaagc  3600
gtttgacaga tacaagtgaa acaaacttaa tgtataaagc atctaaatta ttaaaacaag  3660
ttcagcatga tagtagttct ctgaaagaat ggaacttcaa agaattttat acgctattca  3720
tgaaaacatt taaagatggg aaaagtagtc aatctactat taatttatac gatcttgctt  3780
ataatcaatt cgttgattat ttcgatgaaa aaattaaatt taattcgatt gatgcggttc  3840
aatatcaaca atttattaat catttatctg tagactatgc aatatccact gtagacacca  3900
gacaccgcaa aattagagcg atttttaaca aggctgttca tttaggttac atgaagaaaa  3960
accccactat aggggctcat ataagcggac aggacgtagc gaaaaataaa gcacaattta  4020
tggaaacaga caaagttcat ttactattag aagaacttgc aaaatttcat tctatatcac  4080
gagcagttat ctttctagct gtccagacag gcatgaggtt cgaagaaatt attgcactaa  4140
caaagaagga tattaatttc actaaacgtt caataactgt gaataaagct tgggattaca  4200
agtacactaa tacattcatt gataccaaaa caaaaaaatc acgagtgatc tatattgata  4260
actctaccgc tcaatattta cattcgtatt taaattggca tactgaatat atgaaggaac  4320
atgctattaa gaatccattg atgttattat tcatcactta ccacaataag ccagtagaca  4380
acgcgtcttg taataaagct ttgaagaaga tatgtagtac aatcaattct gaaccagtga  4440
cattacacaa gctacgacat acgcatacag gcttatgtgt agaagcgggt atggatatta  4500
tttatgtagc tgataggctt ggtcatgatg acattaatac aacattaaaa tactatagtc  4560
atctaagctc taatttaaga caacataatc agtccaaagt agatgctttt ttcacactaa  4620
aaacagatga aaataccaca aattttacca caaatgccac aaaaacaacg gaataaaacg  4680
ggtattatac gatataaaaa aaactccaaa acattcatcc gccctttaat atcaaggctt  4740
ttcaacgttt tagagatttc tttacattac tatttaacgt cctgagaggg attaacacac  4800
actgatataa agccatttag gatatatata ccacaaataa taccacaaac attttatgta  4860
ataataaata ttatttatta ttacattgaa ataaatattc gttataaata gtttttatat  4920
caagatgttt tttctcaagg tttttataaa atgactttaa ttcttttgtt tcaagtagtc  4980
cagagaagat tttttcaaca gcgttcttct ttccctccac gcatgcctct cgcctgtccc  5040
ctcagttcag taatttcctg catttgcctg tttccagtcg gtagatattc cacaaaacag  5100
cagggaagca gcgcttttcc gctgcataac cctgcttcgg ggtcattata gcgatttttt  5160
cggtatatcc atcctttttc gcacgatata caggattttg ccaaagggtt cgtgtagact  5220
```

-continued

```
ttccttggtg tatccaacgg cgtcagccgg gcaggatagg tgaagtaggc ccacccgcga  5280
gcgggtgttc cttcttcact gtcccttatt cgcacctggc ggtgctcaac gggaatcctg  5340
ctctgcgagg ctggccggct accgccggcg taacagatga gggcaagcgg cggagaatta  5400
caacttatat cgtatggggc tgacttcagg tgctacattt gaagagataa attgcactga  5460
aatctagaaa tattttatct gattaataag atgatcttct tgagatcgtt ttggtctgcg  5520
cgtaatctct tgctctgaaa acgaaaaaac cgccttgcag ggcggttttt cgaaggttct  5580
ctgagctacc aactctttga accgaggtaa ctggcttgga ggagcgcagt caccaaaact  5640
tgtcctttca gtttagcctt aaccggcgca tgacttcaag actaactcct ctaaatcaat  5700
taccagtggc tgctgccagt ggtgcttttg catgtctttc cgggttggac tcaagacgat  5760
agttaccgga taaggcgcag cggtcggact gaacgggggg ttcgtgcata cagtccagct  5820
tggagcgaac tgcctacccg gaactgagtg tcaggcgtgg aatgagacaa acgcggccat  5880
aacagcggaa tgacaccggt aaaccgaaag gcaggaacag gagagcgcac gagggagccg  5940
ccagggggaa acgcctggta tctttatagt cctgtcgggt ttcgccacca ctgatttgag  6000
cgtcagattt cgtgatgctt gtcagggggg cggagcctat ggaaaaacgg ctttgccgcg  6060
gccctctcac ttccctgtta agtatcttcc tggcatcttc caggaaatct ccgccccgtt  6120
cgtaagccat ttccgctcgc cgcagtcgaa cgaccgagcg tagcgagtca gtgagcgagg  6180
aagcggaata tatcctgtat cacatattct gctgacgcac cggtgcagcc tttttctcc  6240
tgccacatga agcacttcac tgacaccctc atcagtgcca acatagtaag ccagtataca  6300
ctccgctagc gctgatgtcc ggcggtgctt ttgccgttac gcaccacccc gtcagtagct  6360
gaacaggagg gacagctgat agaaacagaa gccactggag cacctcaaaa acaccatcat  6420
acactaaatc agtaag                                                 6436

SEQ ID NO: 9            moltype = DNA  length = 6652
FEATURE                 Location/Qualifiers
source                  1..6652
                        mol_type = other DNA
                        organism = synthetic construct
SEQUENCE: 9
ttggcagcat cacccgacgc actttgcgcc gaataaatac ctgtgacgga agatcacttc  60
gcagaataaa taaatcctgg tgtccctgtt gataccggga agccctggga cgtcgggccc  120
tttcgtcttc aagaattaat tcccaattcc aggcatcaaa taaaacgaaa ggctcagtcg  180
aaagactggg cctttcgttt tatctgttgt ttgtcggtga acgctctcct gagtaggaca  240
aatccgccgg gagcggattt gaacgttgcg aagcaacggc ccggagggtg gcgggcagga  300
cgcccgccat aaactgccag gaattaattc caggcatcaa ataaaacgaa aggctcagtc  360
gaaagactgg gcctttcgtt ttatctgttg tttgtcggtg aacgctctcc tgagtaggac  420
aaatccgccg ggagcggatt tgaacgttgc gaagcaacgg cccggagggt ggcgggcagg  480
acgcccgcca taaactgcca ggaattaatt ccaggcatca aataaaacga aaggctcagt  540
cgaaagactg ggcctttcgt tttatctgtt gtttgtcggt gaacgctctc ctgagtagga  600
caaatccgcc gggagcggat ttgaacgttg cgaagcaacg gcccggaggg tggcgggcag  660
gacgcccgcc ataaactgcc aggaattaat tccaggcatc aaataaaacg aaaggctcag  720
tcgaaagact gggcctttcg ttttatctgt tgtttgtcgg tgaacgctct cctgagtagg  780
acaaatccgc cgggagcgga tttgaacgtt gcgaagcaac ggcccggagg gtggcgggca  840
ggacgcccgc cataaactgc caggaattgg ggatcggaat tcgagctcca ttatgctttg  900
gcagtttatt cttgacatgt agtgaggggg ctggtataat cacatacggc cgataaagca  960
agcatataat attgcgtttc atctttagaa gcgaatttcg ccaatattat aattatcaaa  1020
agagaggggt ggcaaacggt atttggcatt attaggttaa aaaatgtaga aggagagtga  1080
aacccatggg tgggattaaa tagatttatg cgtgcgatga tggtagtttt cattactgcc  1140
aactgcatta cgattaaccc cgacataata tttgcagcga cagatagcga agattccagt  1200
ctaaacacag atgaatggga agaagaaaaa acagaagagc agccaagcga ggtaaatacg  1260
ggaccaagat acgaaactgc acgtgaagta agttcacgtg atattgagga actagaaaaa  1320
tcgaataaag tgaaaaatac gaacaaagca gacctaatag caatgttgaa agcaaaagca  1380
gagaaaggtg gatccccaac atcttcttcc actaagaaga ctcagctaca acttgaacac  1440
cttctgttgg acctacaaat gattctaaat ggaataaaca attataaaaa tcctaaatta  1500
actagaatgc tcacgttcaa attctacatg ccgaaaaaag caaccgagtt aaaacattta  1560
caatgtcttg aagaagaact taaaccattg gaagaagtgc ttaacttggc ccaaagtaaa  1620
aattttcatt tacgtccacg agatttaatt agcaatatca atgtcattgt attagaatta  1680
aaaggtagtg aaaccacatt tatgtgcgaa tatgctgatg agacagcgac aattgttgaa  1740
tttttaaacc gctggattac attttgtcaa tcaattatct cgacgttaac gtgagtcgac  1800
ctcgaggggg ggcccggtac ccagcttttg ttccctttag tgagggttaa ttcccagcga  1860
accatttgag gtgataggta agattatacc gaggtatgaa aacgagaatt ggacctttac  1920
agaattactc tatgaagcgc catatttaaa aagctaccaa gacgaagagg atgaagagga  1980
tgaggaggca gattgccttg aatatattga caatactgat aagataatat atcttttata  2040
tagaagatat cgccgtatgt aaggatttca gggggcaagg cataggcagc gcgcttatca  2100
atatatctat agaatgggca aagcataaaa acttgcatgg actaatgctt gaaacccagg  2160
acaataacct tatagcttgt aaattctatc ataattgtgg tttcaaaatc ggctccgtcg  2220
atactatgtt atacgccaac tttgaaaaca actttgaaaa agctgttttc tggtatttaa  2280
ggttttagaa tgcaaggaac agtgaattgg agttcgtctt gttataatta gcttcttggg  2340
gtatctttaa atactgtaga aaagaggaag gaaataataa atggctaaaa tgagaatatc  2400
accggaattg aaaaaactga tcgaaaaata ccgctgcgta aaagatacgg aaggaatgtc  2460
tcctgctaag gtatataagc tggtgggaga aaatgaaaac ctatatttaa aaatgacgga  2520
cagccggtat aaagggacca cctatgatgt ggaacgggaa aaggacatga tgctatggct  2580
ggaaggaaag ctgcctgttc caaaggtcct gcactttgaa cggcatgatg gctggagcaa  2640
tctgctcatg agtgaggccg atggcgtcct ttgctcggaa gagtatgaag atgaacaaag  2700
ccctgaaaag attatcgagc tgtatgcgga gtgcatcagg ctctttcact ccatcgacat  2760
atcggattgt ccctatacga atagcttaga cagccgctta gccgaattgg attacttact  2820
gaataacgat ctggccgatg tggattgcga aaactgggaa gaagacactc catttaaaga  2880
```

-continued

```
tccgcgcgag ctgtatgatt ttttaaagac ggaaaagccc gaagaggaac ttgtcttttc  2940
ccacggcgac ctgggagaca gcaacatctt tgtgaaagat ggcaaagtaa gtggctttat  3000
tgatcttggg agaagcggca gggcggacaa gtggtatgac attgccttct gcgtccggtc  3060
gatcagggag gatatcgggg aagaacagta tgtcgagcta ttttttgact tactggggat  3120
caagcctgat tgggagaaaa taaaatatta tattttactg gatgaattgt tttagtacct  3180
agatttagat gtctaaaaag ctttaactac aagcttttta gacatctaat cttttctgaa  3240
gtacatccgc aactgtccat actctgatgt ttcatatgat catcataatt ctgtctcatt  3300
atataacatc ctccatacct tctattatag aataccataa actcatctgg caattcattt  3360
cgagtcacga agaacggaaa aactgccggt ttttatatta caaatgtatt aagtttttct  3420
attaacaaaa aacaataggt ttcccatagc gaaagttgtt gattaacgtt cacatcccac  3480
ttacactata aaggtttacc cagcaataca tctcaagccc taagaataca cgttcgcttt  3540
tcaactgtta cagaattatt acaaatagtt ggtatagtcc tctttagcct ttggagctat  3600
tatctcatca tttgtttttt aggtgaaaac tgggtaaact tagtattaat caatataaaa  3660
ttaattctca aatacttaat tacgtactgg gatttctga aaaaaagatc tccaaaaata  3720
aacaggtggt ggtattaatg aagataaaaa aattagcaaa cggtaaatat tgtgttcgcc  3780
tacgtataaa agtcgatggt gaatggaaag aaaagcgttt gacagataca agtgaaacaa  3840
acttaatgta taaagcatct aaattattaa aacaagttca gcatgatagt agttctctga  3900
aagaatggaa cttcaaagaa ttttatacgc tattcatgaa aacatttaaa gatgggaaaa  3960
gtagtcaatc tactattaat ttatacgatc ttgcttataa tcaattcgtt gattatttcg  4020
atgaaaaaat taaatttaat tcgattgatg cggttcaata tcaacaattt attaatcatt  4080
tatctgtaga ctatgcaata tccactgtag acaccagaca ccgcaaaatt agagcgattt  4140
ttaacaaggc tgttcattta ggttacatga agaaaaaccc cactataggg gctcatataa  4200
gcggacagga cgtagcgaaa aataaagcac aatttatgga aacagacaaa gttcatttac  4260
tattagaaga acttgcaaaa tttcattcta tatcacgagc agttatcttt ctagctgtcc  4320
agacaggcat gaggttcgaa gaaattattg cactaacaaa gaaggatatt aatttcacta  4380
aacgttcaat aactgtgaat aaagcttggg attacaagta cactaataca ttcattgata  4440
ccaaaacaaa aaaatcacga gtgatctata ttgataactc taccgctcaa tatttacatt  4500
cgtatttaaa ttggcatact gaatatatga aggaacatgc tattaagaat ccattgatgt  4560
tattattcat cacttaccac aataagccag tagacaacgc gtcttgtaat aaagctttga  4620
agaagatatg tagtacaatc aattctgaac cagtgacatt acacaagcta cgacatacgc  4680
atacaggctt atgtgtagaa gcgggtatgg atattattta tgtagctgat aggcttggtc  4740
atgatgacat taatacaaca ttaaaatact atagtcatct aagctctaat ttaagacaac  4800
ataatcagtc caaagtagat gctttttca cactaaaaac agatgaaaat accacaaatt  4860
ttaccacaaa tgccacaaaa acaacggaat aaaacgggta ttatacgata taaaaaaaac  4920
tccaaaacat tcatccgccc tttaatatca aggcttttca acgttttaga gatttcttta  4980
cattactatt taacgtcctg agagggatta acacacactg atataaagcc atttaggata  5040
tatataccac aaataatacc acaaacattt tatgtaataa taaatattat ttattattac  5100
attgaaataa atattcgtta taaatagttt ttatatcaag atgtttttc tcaaggtttt  5160
tataaaatga ctttaattct tttgtttcaa gtagtccaga gaagattttt tcaacagcgt  5220
tcttctttcc ctccacgcat gcctctcgcc tgtcccctca gttcagtaat ttcctgcatt  5280
tgcctgtttc cagtcggtag atattccaca aaacagcagg gaagcagcgc ttttccgctg  5340
cataaccctg cttcggggtc attatagcga ttttttcggt atatccatcc tttttcgcac  5400
gatatacagg attttgccaa agggttcgtg tagactttcc ttggtgtatc caacggcgtc  5460
agccgggcag gataggtgaa gtaggcccac ccgcgagcgg gtgttccttc ttcactgtcc  5520
cttattcgca cctggcggtg ctcaacggga atcctgctct gcgaggctgg ccggctaccg  5580
ccggcgtaac agatgagggc aagcggcgga gaattacaac ttatatcgta tggggctgac  5640
ttcaggtgct acatttgaag agataaattg cactgaaatc tagaaatatt ttatctgatt  5700
aataagatga tcttcttgag atcgttttgg tctgcgcgta atctcttgct ctgaaaacga  5760
aaaaaccgcc ttgcagggcg gtttttcgaa ggttctctga gctaccaact ctttgaaccg  5820
aggtaactgg cttggaggag cgcagtcacc aaaacttgtc ctttcagttt agccttaacc  5880
ggcgcatgac ttcaagacta actcctctaa atcaattacc agtggctgct gccagtggtg  5940
cttttgcatg tctttccggg ttggactcaa gacgatagtt accggataag gcgcagcggt  6000
cggactgaac ggggggttcg tgcatacagt ccagcttgga gcgaactgcc tacccggaac  6060
tgagtgtcag gcgtggaatg agacaaacgc ggccataaca gcggaatgac accggtaaac  6120
cgaaaggcag gaacaggaga gcgcacgagg gagccgccag gggaaacgc ctggtatctt  6180
tatagtcctg tcgggtttcg ccaccactga tttgagcgtc agatttcgtg atgcttgtca  6240
ggggggcgga gcctatggaa aaacggcttt gccgcggccc tctcacttcc ctgttaagta  6300
tcttcctggc atcttccagg aaatctccgc cccgttcgta agccatttcc gctcgccgca  6360
gtcgaacgac cgagcgtagc gagtcagtga gcgaggaagc ggaatatatc ctgtatcaca  6420
tattctgctg acgcaccggt gcagcctttt ttctcctgcc acatgaagca cttcactgac  6480
accctcatca gtgccaacat agtaagccag tatacactcc gctagcgctg atgtccggcg  6540
gtgcttttgc cgttacgcac caccccgtca gtagctgaac aggagggaca gctgatagaa  6600
acagaagcca ctggagcacc tcaaaaacac catcatacac taaatcagta ag          6652
```

```
SEQ ID NO: 10           moltype = DNA  length = 6697
FEATURE                 Location/Qualifiers
source                  1..6697
                        mol_type = other DNA
                        organism = synthetic construct
SEQUENCE: 10
ttggcagcat cacccgacgc actttgcgcc gaataaatac ctgtgacgga agatcacttc  60
gcagaataaa taaatcctgg tgtccctgtt gataccggga agccctggga cgtcgggccc  120
tttcgtcttc aagaattaat tcccaattcc aggcatcaaa taaaacgaaa ggctcagtcg  180
aaagactggg cctttcgttt tatctgttgt ttgtcggtga acgctctcct gagtaggaca  240
aatccgccgg gagcggattt gaacgttgcg aagcaacggc ccggagggtg gcgggcagga  300
cgcccgccat aaactgccag gaattaattc caggcatcaa ataaaacgaa aggctcagtc  360
```

-continued

gaaagactgg gcctttcgtt ttatctgttg tttgtcggtg aacgctctcc tgagtaggac 420
aaatccgccg ggagcggatt tgaacgttgc gaagcaacgg cccggagggt ggcgggcagg 480
acgcccgcca taaactgcca ggaattaatt ccaggcatca aataaaacga aaggctcagt 540
cgaaagactg ggcctttcgt tttatctgtt gtttgtcggt gaacgctctc ctgagtagga 600
caaatccgcc gggagcggat ttgaacgttg cgaagcaacg gcccggaggg tggcgggcag 660
gacgcccgcc ataaactgcc aggaattaat tccaggcatc aaataaaacg aaaggctcag 720
tcgaaagact gggcctttcg ttttatctgt tgtttgtcgg tgaacgctct cctgagtagg 780
acaaatccgc cgggagcgga tttgaacgtt gcgaagcaac ggcccggagg gtggcgggca 840
ggacgcccgc cataaactgc caggaattgg ggatcggaat tcgagctcca ttatgctttg 900
gcagtttatt cttgacatgt agtgaggggg ctggtataat cacatacggc cgataaagca 960
agcatataat attgcgtttc atctttagaa gcgaatttcg ccaatattat aattatcaaa 1020
agagaggggt ggcaaacggt atttggcatt attaggttaa aaaatgtaga aggagagtga 1080
aacccatgga taaagcaagc atataatatt gcgtgtagaa ggagagtgaa acccgtggga 1140
ttaaatagat ttatgcgtgc gatgatggta gttttcatta ctgccaactg cattacgatt 1200
aaccccgaca taatatttgc agcgacagat agcgaagatt ccagtctaaa cacagatgaa 1260
tgggaagaag aaaaaacaga agagcagcca agcgaggtaa atacgggacc aagatacgaa 1320
actgcacgtg aagtaagttc acgtgatatt gaggaactag aaaaatcgaa taaagtgaaa 1380
aatacgaaca aagcagacct aatagcaatg ttgaaagcaa aagcagagaa aggtggatcc 1440
ccaacatctt cttccactaa gaagactcag ctacaacttg aacaccttct gttggaccta 1500
caaatgattc taaatggaat aaacaattat aaaaatccta aattaactag aatgctcacg 1560
ttcaaattct acatgccgaa aaaagcaacc gagttaaaac atttacaatg tcttgaagaa 1620
gaacttaaac cattggaaga agtgcttaac ttggcccaaa gtaaaaattt tcatttacgt 1680
ccacgagatt taattagcaa tatcaatgtc attgtattag aattaaaagg tagtgaaacc 1740
acatttatgt gcgaatatgc tgatgagaca gcgacaattg ttgaattttt aaaccgctgg 1800
attacatttt gtcaatcaat tatctcgacg ttaacgtgag tcgacctcga ggggggggcc 1860
ggtacccagc ttttgttccc tttagtgagg gttaattccc agcgaaccat ttgaggtgat 1920
aggtaagatt ataccgaggt atgaaaacga gaattggacc tttacagaat tactctatga 1980
agcgccatat ttaaaaagct accaagacga agaggatgaa gaggatgagg aggcagattg 2040
ccttgaatat attgacaata ctgataagat aatatatctt ttatatagaa gatatcgccg 2100
tatgtaagga tttcaggggg caaggcatag gcagcgcgct tatcaatata tctatagaat 2160
gggcaaagca taaaaacttg catggactaa tgcttgaaac ccaggacaat aaccttatag 2220
cttgtaaatt ctatcataat tgtggtttca aaatcggctc cgtcgatact atgttatacg 2280
ccaactttga aaacaacttt gaaaaagctg ttttctggta tttaaggttt tagaatgcaa 2340
ggaacagtga attggagttc gtcttgttat aattagcttc ttggggtatc tttaaatact 2400
gtagaaaaga ggaaggaaat aataaatggc taaaatgaga atatcaccgg aattgaaaaa 2460
actgatcgaa aaataccgct gcgtaaaaga tacggaagga atgtctcctg ctaaggtata 2520
taagctggtg ggagaaaatg aaaacctata tttaaaaatg acggacagcc ggtataaagg 2580
gaccacctat gatgtggaac gggaaaagga catgatgcta tggctggaag gaaagctgcc 2640
tgttccaaag gtcctgcact ttgaacggca tgatggctgg agcaatctgc tcatgagtga 2700
ggccgatggc gtcctttgct cggaagagta tgaagatgaa caaagccctg aaaagattat 2760
cgagctgtat gcggagtgca tcaggctctt tcactccatc gacatatcgg attgtcccta 2820
tacgaatagc ttagacagcc gcttagccga attggattac ttactgaata acgatctggc 2880
cgatgtggat tgcgaaaact gggaagaaga cactccattt aaagatccgc gcgagctgta 2940
tgattttta aagacggaaa agcccgaaga ggaacttgtc ttttcccacg gcgacctggg 3000
agacagcaac atctttgtga aagatggcaa agtaagtggc tttattgatc ttgggagaag 3060
cggcagggcg gacaagtggt atgacattgc cttctgcgtc cggtcgatca gggaggatat 3120
cggggaagaa cagtatgtcg agctattttt tgacttactg gggatcaagc ctgattggga 3180
gaaaataaaa tattatattt tactggatga attgttttag tacctagatt tagatgtcta 3240
aaaagcttta actacaagct ttttagacat ctaatctttt ctgaagtaca tccgcaactg 3300
tccatactct gatgtttcat atgatcatca taattctgtc tcattatata acatcctcca 3360
taccttctat tatagaatac cataaactca tctggcaatt catttcgagt cacgaagaac 3420
ggaaaaactg ccggttttta tattacaaat gtattaagtt tttctattaa caaaaaacaa 3480
taggtttccc atagcgaaag ttgttgatta acgttcacat cccacttaca ctataaaggt 3540
ttacccagca atacatctca agccctaaga atacacgttc gcttttcaac tgttacagaa 3600
ttattacaaa tagttggtat agtcctcttt agcctttgga gctattatct catcatttgt 3660
tttttaggtg aaaactgggt aaacttagta ttaatcaata taaaattaat tctcaaatac 3720
ttaattacgt actgggattt tctgaaaaaa agatctccaa aaataaacag gtggtggtat 3780
taatgaagat aaaaaaatta gcaaacggta aatattgtgt tcgcctacgt ataaaagtcg 3840
atggtgaatg gaaagaaaag cgtttgacag atacaagtga aacaaactta atgtataaag 3900
catctaaatt attaaaacaa gttcagcatg atagtagttc tctgaaagaa tggaacttca 3960
aagaatttta tacgctattc atgaaaacat ttaaagatgg gaaaagtagt caatctacta 4020
ttaatttata cgatcttgct tataatcaat tcgttgatta tttcgatgaa aaaattaaat 4080
ttaattcgat tgatgcggtt caatatcaac aatttattaa tcatttatct gtagactatg 4140
caatatccac tgtagacacc agacaccgca aaattagagc gatttttaac aaggctgttc 4200
atttaggtta catgaagaaa aaccccacta taggggctca tataagcgga caggacgtag 4260
cgaaaaataa agcacaattt atggaaacag acaaagttca tttactatta gaagaacttg 4320
caaaatttca ttctatatca cgagcagtta tctttctagc tgtccagaca ggcatgaggt 4380
tcgaagaaat tattgcacta acaaagaagg atattaattt cactaaacgt tcaataactg 4440
tgaataaagc ttgggattac aagtacacta atacattcat tgataccaaa acaaaaaaat 4500
cacgagtgat ctatattgat aactctaccg ctcaatattt acattcgtat ttaaattggc 4560
atactgaata tatgaaggaa catgctatta agaatccatt gatgttatta ttcatcactt 4620
accacaataa gccagtagac aacgcgtctt gtaataaagc tttgaagaag atatgtagta 4680
caatcaattc tgaaccagtg acattacaca agctacgaca tacgcataca ggcttatgtg 4740
tagaagcggg tatggatatt atttatgtag ctgataggct tggtcatgat gacattaata 4800
caacattaaa atactatagt catctaagct ctaatttaag acaacataat cagtccaaag 4860
tagatgcttt tttcacacta aaaacagatg aaaataccac aaattttacc acaaatgcca 4920

-continued

```
caaaaacaac ggaataaaac gggtattata cgatataaaa aaaactccaa aacattcatc  4980
cgccctttaa tatcaaggct tttcaacgtt ttagagattt ctttacatta ctatttaacg  5040
tcctgagagg gattaacaca cactgatata aagccattta ggatatatat accacaaata  5100
ataccacaaa cattttatgt aataataaat attatttatt attacattga aataaatatt  5160
cgttataaat agtttttata tcaagatgtt ttttctcaag gtttttataa aatgacttta  5220
attcttttgt ttcaagtagt ccagagaaga tttttcaac agcgttcttc tttccctcca  5280
cgcatgcctc tcgcctgtcc cctcagttca gtaatttcct gcatttgcct gtttccagtc  5340
ggtagatatt ccacaaaaca gcagggaagc agcgcttttc cgctgcataa ccctgcttcg  5400
gggtcattat agcgattttt tcggtatatc catccttttt cgcacgatat acaggatttt  5460
gccaaagggt tcgtgtagac tttccttggt gtatccaacg gcgtcagccg ggcaggatag  5520
gtgaagtagg cccacccgcg agcgggtgtt ccttcttcac tgtcccttat tcgcacctgg  5580
cggtgctcaa cgggaatcct gctctgcgag gctggccggc taccgccggc gtaacagatg  5640
agggcaagcg gcggagaatt acaacttata tcgtatgggg ctgacttcag gtgctacatt  5700
tgaagagata aattgcactg aaatctagaa atattttatc tgattaataa gatgatcttc  5760
ttgagatcgt tttggtctgc gcgtaatctc ttgctctgaa aacgaaaaaa ccgccttgca  5820
gggcggtttt tcgaaggttc tctgagctac caactctttg aaccgaggta actggcttgg  5880
aggagcgcag tcaccaaaac ttgtcctttc agtttagcct taaccggcgc atgacttcaa  5940
gactaactcc tctaaatcaa ttaccagtgg ctgctgccag tggtgctttt gcatgtcttt  6000
ccgggttgga ctcaagacga tagttaccgg ataaggcgca gcggtcggac tgaacggggg  6060
gttcgtgcat acagtccagc ttggagcgaa ctgcctaccc ggaactgagt gtcaggcgtg  6120
gaatgagaca aacgcggcca taacagcgga atgacaccgg taaaccgaaa ggcaggaaca  6180
ggagagcgca cgagggagcc gccaggggga aacgcctggt atctttatag tcctgtcggg  6240
tttcgccacc actgatttga gcgtcagatt tcgtgatgct tgtcaggggg gcggagccta  6300
tggaaaaacg gctttgccgc ggccctctca cttccctgtt aagtatcttc ctggcatctt  6360
ccaggaaatc tccgccccgt tcgtaagcca tttccgctcg ccgcagtcga acgaccgagc  6420
gtagcgagtc agtgagcgag gaagcggaat atatcctgta tcacatattc tgctgacgca  6480
ccggtgcagc ctttttctc ctgccacatg aagcacttca ctgacaccct catcagtgcc  6540
aacatagtaa gccagtatac actccgctag cgctgatgtc cggcggtgct tttgccgtta  6600
cgcaccaccc cgtcagtagc tgaacaggag ggacagctga tagaaacaga agccactgga  6660
gcacctcaaa aacaccatca tacactaaat cagtaag                           6697

SEQ ID NO: 11          moltype = DNA  length = 46
FEATURE                Location/Qualifiers
source                 1..46
                       mol_type = other DNA
                       organism = synthetic construct
SEQUENCE: 11
gataaagcaa gcatataata ttgcgtgtag aaggagagtg aaaccc                 46
```

1. A genetically engineered *Listeria monocytogenes* bacterium comprising a nucleic acid sequence encoding interleukin 2 (IL-2), interleukin 12 (IL-12), or Cluster of Differentiation 40 ligand (CD40L).

2. The bacterium of claim **1**, wherein the nucleic acid sequence encodes IL-2.

3. The bacterium of claim **1**, wherein the nucleic acid sequence encodes IL-12.

4. The bacterium of claim **1**, wherein the nucleic acid sequence encodes CD40L.

5. The bacterium of claim **1**, wherein the nucleic acid sequence further encodes a *Listeria monocytogenes* secretion signal.

6. The bacterium of claim **1**, wherein the *Listeria monocytogenes* secretion signal comprises an ActA secretion signal.

7. The bacterium of claim **6**, wherein the ActA secretion signal comprises amino acids 1 to 30 of the ActA protein or amino acids 1 to 100 of the ActA protein.

8. The bacterium of claim **7**, wherein the ActA secretion signal comprises amino acids 1-100 of the ActA protein.

9. The bacterium of claim **5**, wherein the *Listeria monocytogenes* secretion signal comprises the amino acid sequence set forth in SEQ ID NO: 9.

10. The bacterium of claim **1**, wherein the nucleic acid sequence further comprises a 5' untranslated region.

11. The bacterium of claim **10**, wherein the 5' untranslated region comprises the 5' untranslated region of an hly gene.

12. The bacterium of claim **10**, wherein the 5' untranslated region comprises the nucleotide sequence set forth in SEQ ID NO: 11.

13. The bacterium of claim **1**, wherein the nucleic acid sequence comprises SEQ ID NO: 10.

14. The bacterium of claim **1**, wherein the nucleic acid sequence is integrated into the bacterial genome.

15. The bacterium of claim **14**, wherein the nucleic acid sequence is integrated into the bacterial genome at the attBB' site in the $tRNA^{Arg}$ locus.

16.-18. (canceled)

19. A method of treating a cancer in a subject in need thereof comprising administering to the subject a composition comprising a genetically engineered *Listeria monocytogenes* bacterium comprising a nucleic acid sequence encoding interleukin 2 (IL-2), interleukin 12 (IL-12), or Cluster of Differentiation 40 ligand (CD40L).

20. The method of claim **19**, wherein the cancer comprises melanoma or neuroblastoma.

21. The method of claim **19**, wherein the cancer comprises one or more solid tumors.

22. The method of claim **20**, wherein the genetically engineered *Listeria monocytogenes* bacterium persists in the one or more solid tumors for about 1 day to about 3 weeks post administration.

23. The method of claim **19**, wherein the method is effective for reducing tumor volume or slowing tumor growth, and wherein the composition is administered intravenously or intratumorally.

24.-**57**. (canceled)

* * * * *