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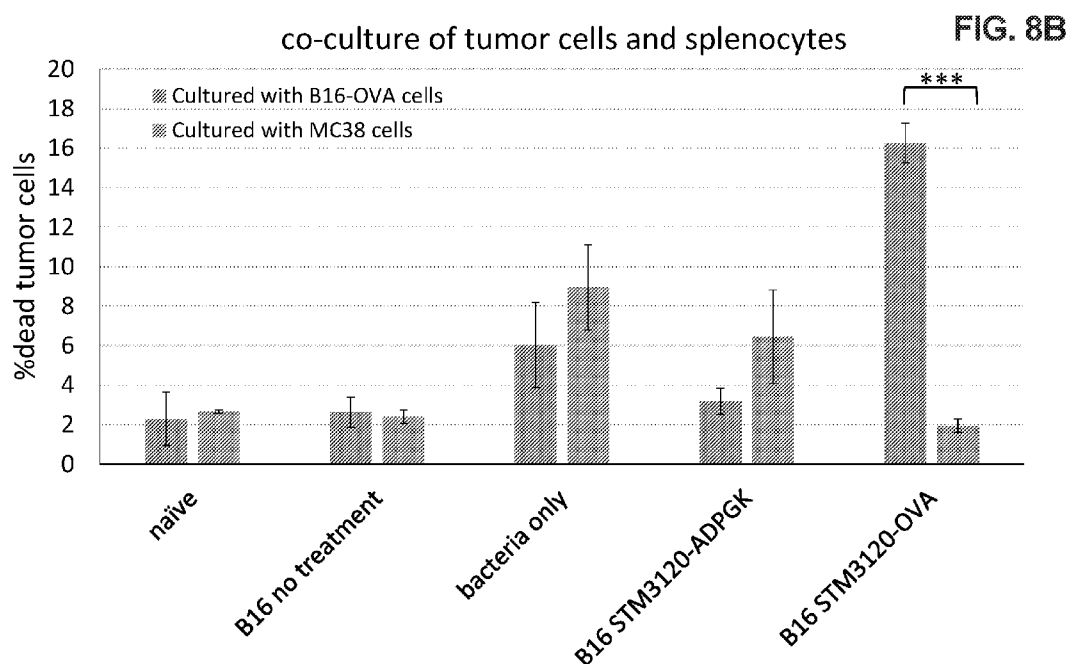
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(54) Title: GENETICALLY MODIFIED BACTERIA FOR GENERATING VACCINES



(57) Abstract: A vaccine comprising tumor-homing bacteria which are genetically modified to express at least one cancer-associated antigen and a pharmaceutically acceptable carrier is disclosed. Uses thereof are also disclosed.



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GENETICALLY MODIFIED BACTERIA FOR GENERATING VACCINES

RELATED APPLICATION/S

This application claims the benefit of priority of U.S. Provisional Patent Application
5 No. 63/150,681 filed on 18 February 2021, the contents of which are incorporated herein by
reference in their entirety.

SEQUENCE LISTING STATEMENT

The ASCII file, entitled 91248 SequenceListing.txt, created on
10 February 15, 2022, comprising 567,458 bytes, submitted concurrently with the filing of this
application is incorporated herein by reference.

FIELD AND BACKGROUND OF THE INVENTION

The present invention, in some embodiments thereof, relates to bacterial vaccines which
15 may be manipulated to comprise disease-associated antigens.

Advances in the understanding of molecular biology, the ability to predict immunogenic
neoantigens by next generation sequencing and prediction algorithms, the lifestyles of pathogenic
bacteria, bacterial engineering and synthetic biology tools have significantly accelerated the
rational design of bacteria as antigen delivery vectors. Being a strong immunogen, bacteria may
20 trigger a vast immune response against itself and consequently against the delivered neoantigen.
Indeed, bacterial vectors that deliver antigenic messages are also able to deliver a strong danger
signal mediated by their pathogen-associated molecular patterns (PAMPs), such as
lipopolysaccharides, lipoproteins, flagellin and CpG. PAMPs derived from different classes of
pathogens bind to diverse families of pathogen recognition receptors (PRRs) that include Toll-like
25 receptors (TLRs), C-type lectin-like receptors (CLRs), retinoic acid- inducible gene (RIG)-like
receptors (RLRs) and nucleotide-binding oligomerization domain (NOD)-like receptors (NLRs).
These interactions according to each pathogen trigger distinct signaling pathways to differentially
activate antigen presenting cells (APCs), thereby directing the adaptive effector response in a
manner that is specifically adapted to the microbe and hence to the antigen delivered by the
30 bacteria. Moreover, specialized toxins that bacteria use for their own virulence can reinforce
effector or memory responses.

Background art includes US Patent Application Nos. 20200087703, 20200054739 and
20190365830, Gopalakrishnan V et al, Science. 2018 Jan 5; 359(6371): 97–103; Geller et al.,

Science, Vol 357, Issue 6356
 15 September 2017; Riquelme E et al Cell. 2019 Aug 8;178(4):795-806.e12. doi:
 10.1016/j.cell.2019.07.008; Straussman R et al., Nature. 2012 Jul 26;487(7408):500-4. doi:
 10.1038/nature11183.

5 SUMMARY OF THE INVENTION

According to an aspect of the present invention there is provided a vaccine comprising tumor-homing bacteria which are genetically modified to express at least one cancer-associated antigen and a pharmaceutically acceptable carrier.

10 According to an aspect of the present invention there is provided a method of treating cancer of a subject in need thereof comprising administering to the subject a therapeutically effective amount of the vaccine of any one of claims 1-14, thereby treating the cancer.

According to an aspect of the present invention there is provided a method of treating cancer of a subject in need thereof comprising administering to the subject a therapeutically effective amount of:

- 15 (i) a first vaccine comprising a first bacteria which is genetically modified to express at least one cancer-associated antigen; and subsequently;
- (ii) a second vaccine comprising a second bacteria which is genetically modified to express at least one cancer-associated antigen, thereby treating the cancer.

20 According to an aspect of the present invention there is provided a method of preventing cancer of a subject in need thereof the method comprising administering to the subject a prophylatically effective amount of the vaccine described herein, thereby preventing the cancer.

Accordance to embodiments of the present invention, the bacteria is a gram positive bacteria.

25 Accordance to embodiments of the present invention, the bacteria is a gram negative bacteria.

Accordance to embodiments of the present invention, the bacteria is aerobic.

Accordance to embodiments of the present invention, the bacteria is anaerobic.

Accordance to embodiments of the present invention, the bacteria are live bacteria.

Accordance to embodiments of the present invention, the bacteria are attenuated bacteria.

30 Accordance to embodiments of the present invention, the bacteria is of a species or genus set forth in any of Tables 1-3.

Accordance to embodiments of the present invention, the genome of the bacteria comprises a 16S rRNA sequence as set forth in any one of SEQ ID NOs: 24-310.

Accordance to embodiments of the present invention, the cancer-associated antigen is a neoantigen.

5 Accordance to embodiments of the present invention, the bacteria are genetically modified to express a therapeutic protein.

Accordance to embodiments of the present invention, the therapeutic protein is a cytokine.

Accordance to embodiments of the present invention, the vaccine is devoid of an aluminium salt.

10 Accordance to embodiments of the present invention, the carrier is devoid of adjuvant.

Accordance to embodiments of the present invention, the first bacteria are viable bacteria and the second bacteria are non-viable bacteria.

Accordance to embodiments of the present invention, the first bacteria comprises a first strain of bacteria that is genetically modified to express a cancer-associated antigen and the second
15 bacteria comprises a second strain of bacteria that is non-identical to the first strain of bacteria, the second bacteria being genetically modified to express the cancer-associated antigen.

Accordance to embodiments of the present invention, the cancer is selected from the group consisting of breast, melanoma, colorectal cancer, lung cancer, gastric cancer, pancreatic cancer, ovarian cancer, bone cancer and brain cancer.

20 Accordance to embodiments of the present invention, the brain cancer comprises glioblastoma.

Accordance to embodiments of the present invention, the at least one cancer-associated antigen of the first vaccine is identical to the at least one cancer-associated antigen of the second vaccine.

25 Accordance to embodiments of the present invention, the at least one cancer-associated antigen of the first vaccine is non-identical to the at least one cancer-associated antigen of the second vaccine.

Accordance to embodiments of the present invention, the cancer is selected from the group consisting of breast, melanoma, colorectal cancer, gastric cancer, lung cancer, pancreatic cancer,
30 ovarian cancer, bone cancer and brain cancer.

Accordance to embodiments of the present invention, the brain cancer comprises glioblastoma.

Unless otherwise defined, all technical and/or scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which the invention pertains. Although methods and materials similar or equivalent to those described herein can be used in the practice or testing of embodiments of the invention, exemplary methods and/or materials are described below. In case of conflict, the patent specification, including definitions, will control. In addition, the materials, methods, and examples are illustrative only and are not intended to be necessarily limiting.

BRIEF DESCRIPTION OF THE SEVERAL VIEWS OF THE DRAWING(S)

Some embodiments of the invention are herein described, by way of example only, with reference to the accompanying drawings. With specific reference now to the drawings in detail, it is stressed that the particulars shown are by way of example and for purposes of illustrative discussion of embodiments of the invention. In this regard, the description taken with the drawings makes apparent to those skilled in the art how embodiments of the invention may be practiced.

In the drawings:

FIGs. 1A-B. Long-term efficacy of vaccination by OVA expressing bacteria in B16-OVA tumor model. (A) Experiment time line. (B) Tumor growth curves from start of treatment.

FIGs. 2A-G: Short-term efficacy and immunogenicity of vaccination by OVA expressing bacteria in B16-OVA tumor model. (A) Experiment time line. (B) Tumor growth curves from start of treatment. N=5 for all mice cohorts. (C) Tumor volume percentage at day 16 relative to day 0. P-values were obtained by two sided Mann-Whitney test. Below are the percentage values of fully cured mice per cohort. (D) Mice from the cohort treated with Anti-PD1 or Anti-PD1 together with PACMAN-OVA. While the tumor of the mouse treated with PACMAN-OVA gradually disappears, the tumor of the mouse treated with anti-PD1 only continued to grow exponentially. (E) CFU count of tumor and liver extracts. Following 16 days from vaccination, bacteria from tumors and livers were seeded on LB plates with resistance to AMP. Per mouse, CFU count and tumor volumes are given. Of note, 4 out of 5 mice of the PACMAN-OVA cohort exhibited complete clearance of bacteria. Bacteria were present in the mouse with the biggest tumor, suggesting that the tumor tissue enables bacteria proliferation. (F) Sera of mice cohorts were subjected to IFN γ ELISA. The PACMAN-OVA cohort exhibited the highest IFN γ serum level indicating high systemic immune activation. Green dot refers to mouse 836 in F. Mouse 836 was the only case where bacteria were present in the liver, probably resulting in the highest serum level of IFN γ . (G) Quantification of SIINFEKL (SEQ ID NO: 11) specific TCR by Flow Cytometry.

To quantify neoantigen specific T cell clones, splenocytes were co-incubated with Tetramer presenting the OVA neoantigen (SIINFEKL - SEQ ID NO: 11)) and conjugated to a fluorescent dye. Thus, splenocytes which are positive to the Tetramer dye possess a TCR that can bind the OVA neoantigen. Following FACS, percentage of SIINFEKL (SEQ ID NO: 11) positive T cells out of CD3+CD8+ population was the highest among mice vaccinated with the PACMAN-OVA. P-values were obtained by Two sided Mann-Whitney test.

FIGs. 3A-D. Alternate administration of different bacterial vaccines may overcome acquired immunity. (A) Experiment time table. (B) Tumor growth curves. (C) Weight change (percentage of initial weight) during the first 24 days of treatment. Mice cohorts are: STM3210 +aPD1(N=3), STM-pagC-SspH1-OVA+aPD1 (N=3), STM-SspH2-OVA+aPD1 (N=5). Dashed lines demarcate day of bacteria administration. (D) Weight change (percentage of initial weight) during the first 40 days of treatment. Mice cohorts are: STM3210 +aPD1(N=3), STM-pagC-SspH1-OVA+aPD1 (N=3), STM-SspH2-OVA+aPD1 (N=5), CHA-OST. Dashed lines demarcate day of bacteria administration.

FIGs. 4A-B. Immune memory of vaccination by OVA expressing bacteria in B16-OVA tumor model. (A) Experiment time table. (B) Tumor growth curves.

FIGs. 5A-C. Long-term efficacy of vaccination by Adpgk expressing bacteria in MC38 CRC tumor model. (A) Experiment time line. (B) Tumor growth curves from treatment start. As shown, tumor growth was significantly inhibited in the PACMAN-Adpgk cohort relative to the other mice cohorts. Following two cycles of immunization, one mouse in the PACMAN-Adpgk cohort was fully cured. (C) To test the immune memory of mice vaccinated with PACMAN-Adpgk, the mice exhibiting full cure following vaccination with PACMAN-Adpgk or VNP20009 (w/o adpgk) were re-challenged with 10^5 MC38 cells and tumor growth was compared to naïve mice injected with the same amount of cells. While naïve mice exhibited exponentially growing tumors shortly after injection, re-challenged mouse which was vaccinated with PACMAN-Adpgk remained tumor free, indicating the establishment of long term immune memory against MC38 cells. Of note, the fully cured mouse following vaccination only with the VNP20009, exhibited tumor growth following re-challenge indicating that the immune memory was a consequence of Adpgk presentation by the bacteria.

FIG. 6 is a graph illustrating tumor homing of attenuated (STM3120) Salmonella bacteria.

FIG. 7 is a graph illustrating toxicity of i.v. administration of attenuated (STM3120) vs parental (14028) Salmonella.

FIGs. 8A-B are graphs illustrating splenocytes immune profiling following vaccination by OVA expressing bacteria in B16-OVA tumor model. Figure 8A - Quantification of IFN γ positive CD8 T-cells by FACS. Figure 8B - Quantification of T cells killing capacity.

FIGs. 9A-C illustrate long-term efficacy of vaccination by Adpgk expressing *P. Aeruginosa* in MC38 tumor model. Figure 9A - Experiment timetable. Figure 9B - Tumor growth curves. Mice treated with PACMAN-ADPGK i.v, exhibited a considerable delayed tumor growth. Per treatment, indicated number of fully cured mice. Figure 9C - Average tumor growth curves of the mice cohorts in Fig 9B. Whiskers indicate standard error.

FIGs. 10A-B illustrates long-term efficacy of vaccination by Adpgk expressing *Bacillus Subtilis* spores in MC38 tumor model. Figure 10A - Experimental timetable. Figure 10B. Tumor growth curves. Mice treated with PACMAN-ADPGK spores p.o or i.v, exhibited a considerable delayed tumor growth. Per treatment, indicated number of fully cured mice.

FIGs. 11A-B illustrates long-term efficacy of vaccination by Adpgk expressing attenuated Salmonella (STM3120) in MC38 tumor model. Figure 11A. Experiment timetable. Figure 11B - Tumor growth curves. Mice treated with PACMAN-ADPGK exhibited a considerable delayed tumor growth. Per treatment, indicated number of fully cured mice.

DESCRIPTION OF SPECIFIC EMBODIMENTS OF THE INVENTION

The present invention, in some embodiments thereof, relates to bacterial vaccines which may be manipulated to contain disease-associated antigens on their outer surface.

Before explaining at least one embodiment of the invention in detail, it is to be understood that the invention is not necessarily limited in its application to the details set forth in the following description or exemplified by the Examples. The invention is capable of other embodiments or of being practiced or carried out in various ways.

In vivo therapeutic cancer vaccine strategies based on bacterial vectors that directly deliver antigens or nucleic acids encoding antigens to the cytosol of APCs, have been developed in academic laboratories and pharmaceutical industry due to their ease of use. Typically, the bacteria is genetically modified to express (and even secrete) the disease antigen. Alternatively, the bacteria may be used to deliver plasmid cDNA which encode the disease antigen to the immune system.

The present inventors have now conceived of a novel vaccine which includes tumor-homing bacteria. The bacteria are genetically modified to express disease associated antigens. These vaccines are referred to herein as **Personalized Anti-Cancer Microbiome-Assisted Vaccination (PACMAN)**.

As is illustrated herein under and in the examples section which follows, the present inventors show that it is possible to genetically modify bacteria to express tumor antigens. The genetically modified bacteria serve two purposes 1) as a targeting vehicle – homing to the tumor site and 2) as an adjuvant, stimulating the immune system.

The Inventors demonstrated the ability to produce effective vaccines using a number of different bacteria including *Salmonella Typhimurium* (Figures 1A-B, 2A-G, 3A-D, 4A-B, 5A-C), *P. aeruginosa* (Figures 9A-B) and *B. Subtilis* (Figures 10A-C). The bacteria were genetically modified to express various tumor specific antigens including OVA (Figures 1A-B, 2A-G, 3A-D, 4A-B) and ADPGK (Figures 5A-C, 9A-B, 10A-C and 11A-B).

Whilst further reducing the present invention to practice, the present inventors showed that alternate administration of different bacterial vaccines can overcome acquired immunity (see Figures 3A-C).

Thus, according to an aspect of the present invention there is provided a vaccine comprising tumor-homing bacteria which are genetically modified to express at least one cancer-associated antigen and a pharmaceutically acceptable carrier.

As used herein, the term “vaccine” refers to a pharmaceutical preparation (pharmaceutical composition) that upon administration induces an immune response, in particular a cellular immune response, which recognizes and attacks a cancer cell. Preferably, the vaccine results in the formation of long-term immune memory towards the targeted antigen. The vaccine of the present invention preferably also includes a pharmaceutically acceptable carrier (e.g. a liquid composition which carries the bacteria). In one embodiment, the carrier is one which retains the viability of the bacteria.

The isolated bacteria of this aspect of the present invention may be gram positive or gram negative bacteria or may be a combination of both.

The bacteria may be aerobic or anaerobic bacteria.

As mentioned, the bacteria are capable of homing to a tumor site.

In another embodiment, the bacteria which are capable of homing to a tumor are present in a tumor microbiome of the subject.

The term “tumor microbiome” refers to the totality of microbes (bacteria, fungi, protists), their genetic elements (genomes) in a defined environment, e.g. within the tumor of a host. In a particular embodiment, the microbiome refers only to the totality of bacteria in a defined environment, e.g. within the tumor of a host. The tumor may be a primary tumor or a secondary tumor (i.e. metastasized tumor).

Examples of bacteria known to be present in a breast tumor microbiome are set forth in Table 1, herein below. Such bacteria may be particular relevant for use in vaccines for treating breast cancer. The sequence provided refers to the 16S rRNA sequence for each bacteria.

Table 1

<u>king dom</u>	<u>phylum</u>	<u>class</u>	<u>order</u>	<u>family</u>	<u>genus</u>	<u>species</u>	<u>SE Q ID</u>
Bact eria	Actinob acteria	Actinobacter ia	Actinomyc etales	Actinomyce taceae	Trueperella		24
Bact eria	Actinob acteria	Actinobacter ia	Actinomyc etales	Bogoriellace ae	Georgenia		25
Bact eria	Actinob acteria	Actinobacter ia	Actinomyc etales	Cellulomon adaceae	Cellulomon as		26
Bact eria	Actinob acteria	Actinobacter ia	Actinomyc etales	Cellulomon adaceae	Oerskovia		27
Bact eria	Actinob acteria	Actinobacter ia	Actinomyc etales	Corynebacte riaceae	Corynebact erium	Corynebact erium tuberculos tearicum	29
Bact eria	Actinob acteria	Actinobacter ia	Actinomyc etales	Corynebacte riaceae	Corynebact erium	Corynebact erium tuberculos tearicum	31
Bact eria	Actinob acteria	Actinobacter ia	Actinomyc etales	Corynebacte riaceae	Corynebact erium	Corynebact erium variabile	32
Bact eria	Actinob acteria	Actinobacter ia	Actinomyc etales	Corynebacte riaceae	Corynebact erium		33
Bact eria	Actinob acteria	Actinobacter ia	Actinomyc etales	Corynebacte riaceae	Corynebact erium		35
Bact eria	Actinob acteria	Actinobacter ia	Actinomyc etales	Corynebacte riaceae	Corynebact erium		36
Bact eria	Actinob acteria	Actinobacter ia	Actinomyc etales	Corynebacte riaceae	Corynebact erium		37
Bact eria	Actinob acteria	Actinobacter ia	Actinomyc etales	Dermabacte raceae	Dermabact er		38

Bact eria	Actinob acteria	Actinobacter ia	Actinomyc etales	Dermacocca ceae	Dermacocc us		39
Bact eria	Actinob acteria	Actinobacter ia	Actinomyc etales	Dermacocca ceae	Dermacocc us		40
Bact eria	Actinob acteria	Actinobacter ia	Actinomyc etales	Dietziaceae	Dietzia		41
Bact eria	Actinob acteria	Actinobacter ia	Actinomyc etales	Geodermato philaceae	Blastococc us		42
Bact eria	Actinob acteria	Actinobacter ia	Actinomyc etales	Intrasporang iaceae	Janibacter		43
Bact eria	Actinob acteria	Actinobacter ia	Actinomyc etales	Intrasporang iaceae	Ornithinimi crobium		44
Bact eria	Actinob acteria	Actinobacter ia	Actinomyc etales	Microbacter iaceae	Agrococcu s		45
Bact eria	Actinob acteria	Actinobacter ia	Actinomyc etales	Microbacter iaceae	Agrococcu s		46
Bact eria	Actinob acteria	Actinobacter ia	Actinomyc etales	Microbacter iaceae	Microbacte rium		47
Bact eria	Actinob acteria	Actinobacter ia	Actinomyc etales	Microbacter iaceae	Microbacte rium		48
Bact eria	Actinob acteria	Actinobacter ia	Actinomyc etales	Microbacter iaceae	Microbacte rium		49
Bact eria	Actinob acteria	Actinobacter ia	Actinomyc etales	Microbacter iaceae	Microbacte rium		50
Bact eria	Actinob acteria	Actinobacter ia	Actinomyc etales	Microbacter iaceae	Microbacte rium		51
Bact eria	Actinob acteria	Actinobacter ia	Actinomyc etales	Micrococca ceae	Arthrobact er	Arthrobact er aureus	52
Bact eria	Actinob acteria	Actinobacter ia	Actinomyc etales	Micrococca ceae	Micrococc us	Micrococc us luteus	53
Bact eria	Actinob acteria	Actinobacter ia	Actinomyc etales	Micrococca ceae	Micrococc us	Micrococc us luteus	54

Bact eria	Actinob acteria	Actinobacter ia	Actinomyc etales	Micrococca ceae	Micrococc us	Micrococc us luteus	55
Bact eria	Actinob acteria	Actinobacter ia	Actinomyc etales	Micrococca ceae	Micrococc us	Micrococc us luteus	56
Bact eria	Actinob acteria	Actinobacter ia	Actinomyc etales	Micrococca ceae	Micrococc us	Micrococc us luteus	57
Bact eria	Actinob acteria	Actinobacter ia	Actinomyc etales	Micrococca ceae	Micrococc us	Micrococc us luteus	58
Bact eria	Actinob acteria	Actinobacter ia	Actinomyc etales	Micrococca ceae	Micrococc us	Micrococc us luteus	59
Bact eria	Actinob acteria	Actinobacter ia	Actinomyc etales	Micrococca ceae	Micrococc us	Micrococc us luteus	60
Bact eria	Actinob acteria	Actinobacter ia	Actinomyc etales	Micrococca ceae	Micrococc us	Micrococc us luteus	61
Bact eria	Actinob acteria	Actinobacter ia	Actinomyc etales	Micrococca ceae	Micrococc us	Micrococc us luteus	62
Bact eria	Actinob acteria	Actinobacter ia	Actinomyc etales	Micrococca ceae	Micrococc us	Micrococc us luteus	63
Bact eria	Actinob acteria	Actinobacter ia	Actinomyc etales	Micrococca ceae	Micrococc us	Micrococc us luteus	64
Bact eria	Actinob acteria	Actinobacter ia	Actinomyc etales	Micrococca ceae	Micrococc us	Micrococc us luteus	65
Bact eria	Actinob acteria	Actinobacter ia	Actinomyc etales	Micrococca ceae	Micrococc us	Micrococc us luteus	66
Bact eria	Actinob acteria	Actinobacter ia	Actinomyc etales	Micrococca ceae	Micrococc us	Micrococc us luteus	67
Bact eria	Actinob acteria	Actinobacter ia	Actinomyc etales	Micrococca ceae	Micrococc us	Micrococc us luteus	68
Bact eria	Actinob acteria	Actinobacter ia	Actinomyc etales	Micrococca ceae	Micrococc us	Micrococc us luteus	69
Bact eria	Actinob acteria	Actinobacter ia	Actinomyc etales	Micrococca ceae	Micrococc us	Micrococc us luteus	70

Bact eria	Actinob acteria	Actinobacter ia	Actinomyc etales	Micrococca ceae	Micrococc us	Micrococc us luteus	71
Bact eria	Actinob acteria	Actinobacter ia	Actinomyc etales	Micrococca ceae	Arthrobact er		72
Bact eria	Actinob acteria	Actinobacter ia	Actinomyc etales	Micrococca ceae	Kocuria		73
Bact eria	Actinob acteria	Actinobacter ia	Actinomyc etales	Micrococca ceae	Microbispo ra		74
Bact eria	Actinob acteria	Actinobacter ia	Actinomyc etales	Micrococca ceae	Microbispo ra		75
Bact eria	Actinob acteria	Actinobacter ia	Actinomyc etales	Micrococca ceae	Micrococc us		76
Bact eria	Actinob acteria	Actinobacter ia	Actinomyc etales	Micrococca ceae	Micrococc us		77
Bact eria	Actinob acteria	Actinobacter ia	Actinomyc etales	Micrococca ceae	Micrococc us		78
Bact eria	Actinob acteria	Actinobacter ia	Actinomyc etales	Micrococca ceae	Micrococc us		79
Bact eria	Actinob acteria	Actinobacter ia	Actinomyc etales	Mycobacteri aceae	Mycobacte rium		80
Bact eria	Actinob acteria	Actinobacter ia	Actinomyc etales	Nocardiacea e	Rhodococc us	Rhodococ cus erythropol is	81
Bact eria	Actinob acteria	Actinobacter ia	Actinomyc etales	Propionibact eriaceae	Propioniba cterium	Propioniba cterium acnes	82
Bact eria	Actinob acteria	Actinobacter ia	Actinomyc etales	Propionibact eriaceae	Propioniba cterium	Propioniba cterium acnes	83
Bact eria	Actinob acteria	Actinobacter ia	Actinomyc etales	Propionibact eriaceae	Propioniba cterium	Propioniba cterium acnes	84

Bact eria	Actinob acteria	Actinobacter ia	Actinomyc etales	Propionibact eriaceae	Propioniba cterium	Propioniba cterium avidum	85
Bact eria	Actinob acteria	Actinobacter ia	Actinomyc etales	Propionibact eriaceae	Propioniba cterium	Propioniba cterium avidum	86
Bact eria	Firmicut es	Bacilli	Bacillales	Bacillaceae	Bacillus	Bacillus flexus	87
Bact eria	Firmicut es	Bacilli	Bacillales	Bacillaceae	Bacillus	Bacillus flexus	88
Bact eria	Firmicut es	Bacilli	Bacillales	Bacillaceae	Bacillus	Bacillus muralis	89
Bact eria	Firmicut es	Bacilli	Bacillales	Bacillaceae	Bacillus		90
Bact eria	Firmicut es	Bacilli	Bacillales	Bacillaceae 1	Bacillus	Bacillus subtilis	91
Bact eria	Firmicut es	Bacilli	Bacillales	Bacillaceae 1	Bacillus	Bacillus subtilis	92
Bact eria	Firmicut es	Bacilli	Bacillales	Bacillaceae 1	Bacillus	Bacillus subtilis	93
Bact eria	Firmicut es	Bacilli	Bacillales	Bacillaceae 1	Bacillus	Bacillus foraminis	94
Bact eria	Firmicut es	Bacilli	Bacillales	Bacillaceae 1	Bacillus	Bacillus nealsonii	95
Bact eria	Firmicut es	Bacilli	Bacillales	Bacillaceae 2	Terribacillu s		96
Bact eria	Firmicut es	Bacilli	Bacillales	Planococcac eae	Chryseomi crobium	Chryseomi crobium imtechens e	97
Bact eria	Firmicut es	Bacilli	Bacillales	Planococcac eae	Chryseomi crobium		98
Bact eria	Firmicut es	Bacilli	Bacillales	Planococcac eae	Sporosarci na		99

Bact eria	Firmicut es	Bacilli	Bacillales	Planococcac eae	Sporosarci na		100
Bact eria	Firmicut es	Bacilli	Bacillales	Staphylococ caceae	Staphyloco ccus	Staphyloc occus epidermidi s	101
Bact eria	Firmicut es	Bacilli	Bacillales	Staphylococ caceae	Staphyloco ccus	Staphyloc occus epidermidi s	102
Bact eria	Firmicut es	Bacilli	Bacillales	Staphylococ caceae	Staphyloco ccus	Staphyloc occus epidermidi s	103
Bact eria	Firmicut es	Bacilli	Bacillales	Staphylococ caceae	Staphyloco ccus	Staphyloc occus epidermidi s	104
Bact eria	Firmicut es	Bacilli	Bacillales	Staphylococ caceae	Staphyloco ccus	Staphyloc occus epidermidi s	105
Bact eria	Firmicut es	Bacilli	Bacillales	Staphylococ caceae	Staphyloco ccus	Staphyloc occus epidermidi s	106
Bact eria	Firmicut es	Bacilli	Bacillales	Staphylococ caceae	Staphyloco ccus	Staphyloc occus epidermidi s	107
Bact eria	Firmicut es	Bacilli	Bacillales	Staphylococ caceae	Staphyloco ccus	Staphyloc occus epidermidi s	108
Bact eria	Firmicut es	Bacilli	Bacillales	Staphylococ caceae	Staphyloco ccus	Staphyloc occus epidermidi s	109

Bact eria	Firmicut es	Bacilli	Bacillales	Staphylococ caceae	Staphyloco ccus	Staphyloc occus epidermidi s	110
Bact eria	Firmicut es	Bacilli	Bacillales	Staphylococ caceae	Staphyloco ccus	Staphyloc occus epidermidi s	111
Bact eria	Firmicut es	Bacilli	Bacillales	Staphylococ caceae	Staphyloco ccus	Staphyloc occus haemolyti cus	112
Bact eria	Firmicut es	Bacilli	Bacillales	Staphylococ caceae	Staphyloco ccus	Staphyloc occus hominis	113
Bact eria	Firmicut es	Bacilli	Bacillales	Staphylococ caceae	Staphyloco ccus	Staphyloc occus hominis	114
Bact eria	Firmicut es	Bacilli	Bacillales	Staphylococ caceae	Staphyloco ccus	Staphyloc occus hominis	115
Bact eria	Firmicut es	Bacilli	Bacillales	Staphylococ caceae	Staphyloco ccus	Staphyloc occus lugdunensi s	116
Bact eria	Firmicut es	Bacilli	Bacillales	Staphylococ caceae	Staphyloco ccus	Staphyloc occus lugdunensi s	117
Bact eria	Firmicut es	Bacilli	Bacillales	Staphylococ caceae	Staphyloco ccus	Staphyloc occus lugdunensi s	118
Bact eria	Firmicut es	Bacilli	Bacillales	Staphylococ caceae	Staphyloco ccus	Staphyloc occus lugdunensi s	119

Bact eria	Firmicut es	Bacilli	Bacillales	Staphylococ caceae	Staphyloco ccus	Staphyloc occus lugdunensi s	120
Bact eria	Firmicut es	Bacilli	Bacillales	Staphylococ caceae	Staphyloco ccus	Staphyloc occus lugdunensi s	121
Bact eria	Firmicut es	Bacilli	Bacillales	Staphylococ caceae	Staphyloco ccus	Staphyloc occus lugdunensi s	122
Bact eria	Firmicut es	Bacilli	Bacillales	Staphylococ caceae	Staphyloco ccus	Staphyloc occus succinus	123
Bact eria	Firmicut es	Bacilli	Bacillales	Staphylococ caceae	Staphyloco ccus	Staphyloc occus succinus	124
Bact eria	Firmicut es	Bacilli	Bacillales	Staphylococ caceae	Staphyloco ccus	Staphyloc occus succinus	125
Bact eria	Firmicut es	Bacilli	Bacillales	Staphylococ caceae	Staphyloco ccus		126
Bact eria	Firmicut es	Bacilli	Bacillales	Unknown species	Exiguobact erium	Exiguobac terium mexicanu m	127
Bact eria	Firmicut es	Bacilli	Bacillales	Unknown species	Exiguobact erium	Exiguobac terium profundu m	128
Bact eria	Firmicut es	Bacilli	Bacillales	Unknown species	Exiguobact erium		129
Bact eria	Firmicut es	Bacilli	Lactobacill ales	Aerococcac eae	Aerococcus	Aerococcu s viridans	130
Bact eria	Firmicut es	Bacilli	Lactobacill ales	Enterococca ceae	Enterococc us	Enterococ	131

						cus faecalis	
Bact eria	Firmicut es	Bacilli	Lactobacill ales	Streptococc aceae	Streptococc us	Streptococ cus infantis	132
Bact eria	Firmicut es	Bacilli	Lactobacill ales	Streptococc aceae	Streptococc us	Streptococ cus infantis	133
Bact eria	Firmicut es	Bacilli	Lactobacill ales	Streptococc aceae	Streptococc us	Streptococ cus infantis	134
Bact eria	Firmicut es	Bacilli	Lactobacill ales	Streptococc aceae	Streptococc us	Streptococ cus infantis	135
Bact eria	Firmicut es	Bacilli	Lactobacill ales	Streptococc aceae	Streptococc us	Streptococ cus oralis	136
Bact eria	Firmicut es	Bacilli	Lactobacill ales	Streptococc aceae	Streptococc us	Streptococ cus pneumoni ae	137
Bact eria	Firmicut es	Bacilli	Lactobacill ales	Streptococc aceae	Streptococc us	Streptococ cus pneumoni ae	138
Bact eria	Firmicut es	Bacilli	Lactobacill ales	Streptococc aceae	Streptococc us	Streptococ cus sanguinis	139
Bact eria	Firmicut es	Bacilli	Lactobacill ales	Streptococc aceae	Streptococc us	Streptococ cus vestibulari s	140
Bact eria	Firmicut es	Bacilli	Lactobacill ales	Streptococc aceae	Streptococc us	Streptococ cus vestibulari s	141
Bact eria	Firmicut es	Bacilli	Lactobacill ales	Streptococc aceae	Streptococc us	Assigned species179 1	142

Bacteria	Proteobacteria	Alphaproteobacteria	Rhodobacterales	Rhodobacteraceae	Paracoccus	Paracoccus aminovorans	143
Bacteria	Proteobacteria	Alphaproteobacteria	Rhodobacterales	Rhodobacteraceae	Paracoccus		144
Bacteria	Proteobacteria	Alphaproteobacteria	Rhodospirillales	Acetobacteraceae	Roseomonas	Roseomonas mucosa	145
Bacteria	Proteobacteria	Alphaproteobacteria	Rhodospirillales	Acetobacteraceae	Roseomonas		146
Bacteria	Proteobacteria	Alphaproteobacteria	Sphingomonadales	Sphingomonadaceae	Sphingomonas	Sphingomonas desiccabilis	147
Bacteria	Proteobacteria	Betaproteobacteria	Burkholderiales	Oxalobacteraceae	Massilia		148
Bacteria	Proteobacteria	Betaproteobacteria	Neisseriales	Neisseriaceae	Neisseria	Neisseria macacae	149
Bacteria	Proteobacteria	Betaproteobacteria	Neisseriales	Neisseriaceae	Neisseria	Neisseria subflava	150
Bacteria	Proteobacteria	Betaproteobacteria	Neisseriales	Neisseriaceae	Neisseria	Neisseria subflava	151
Bacteria	Proteobacteria	Gammaproteobacteria	Enterobacterales	Enterobacteriaceae	Enterobacter	Enterobacter cloacae	152
Bacteria	Proteobacteria	Gammaproteobacteria	Enterobacterales	Enterobacteriaceae	Proteus	Proteus mirabilis	153
Bacteria	Proteobacteria	Gammaproteobacteria	Enterobacterales	Enterobacteriaceae	Proteus	Proteus mirabilis	154
Bacteria	Proteobacteria	Gammaproteobacteria	Enterobacterales	Enterobacteriaceae	Proteus	Proteus mirabilis	155
Bacteria	Proteobacteria	Gammaproteobacteria	Enterobacterales	Enterobacteriaceae	Erwinia		156
Bacteria	Proteobacteria	Gammaproteobacteria	Enterobacterales	Enterobacteriaceae	Erwinia		157

Bact eria	Proteob acteria	Gammaprote obacteria	Enterobact eriales	Enterobacter iaceae	Erwinia		158
Bact eria	Proteob acteria	Gammaprote obacteria	Enterobact eriales	Enterobacter iaceae	Erwinia		159
Bact eria	Proteob acteria	Gammaprote obacteria	Pseudomo nadales	Moraxellace ae	Acinetobac ter	Acinetoba cter radioresist ens	160
Bact eria	Proteob acteria	Gammaprote obacteria	Pseudomo nadales	Moraxellace ae	Enhydroba cter	Enhydroba cter aerosaccus	161
Bact eria	Proteob acteria	Gammaprote obacteria	Pseudomo nadales	Moraxellace ae	Enhydroba cter	Enhydroba cter aerosaccus	162
Bact eria	Proteob acteria	Gammaprote obacteria	Pseudomo nadales	Moraxellace ae	Enhydroba cter	Enhydroba cter aerosaccus	163
Bact eria	Proteob acteria	Gammaprote obacteria	Pseudomo nadales	Moraxellace ae	Enhydroba cter		164
Bact eria	Proteob acteria	Gammaprote obacteria	Pseudomo nadales	Pseudomona daceae	Pseudomon as		165
Bact eria	Proteob acteria	Gammaprote obacteria	Pseudomo nadales	Pseudomona daceae	Pseudomon as		166
Fung i	Ascomy cota	Eurotiomyce tes	Eurotiales	Trichocoma ceae	Aspergillus	Aspergillu s kawachii	167
Fung i	Ascomy cota	Eurotiomyce tes	Eurotiales	Trichocoma ceae	Aspergillus	Aspergillu s niger	168
Fung i	Ascomy cota	Eurotiomyce tes	Eurotiales	Trichocoma ceae	Aspergillus	Aspergillu s pseudogla ucus	169
Fung i	Ascomy cota	Saccharomy cetes	Saccharom ycetales	Saccharomy cetaceae	Saccharom yces	Saccharo myces cerevisiae	170

Table 2 includes bacterial taxa that may be particularly relevant for use in a vaccine for treating breast, lung or ovarian cancers. Bacteria are sorted according to their p-values (lowest to highest) for enrichment per tumor type.

5

Table 2

bac t_I D	phylu m	class	order	family	genus	species	Tumo r type	SEQ ID NO:
12873	Proteo bacteri a	Alphaprot eobacteria	Sphingo monadal es	Sphingom onadaceae	Sphingo monas	Unknown species602	Breast	171
13620	Proteo bacteri a	Betaproteo bacteria	Burkhold eriales	Comamon adaceae	Tepidimo nas	Unknown species11	Breast	172
32080	Proteo bacteri a	Betaproteo bacteria	Burkhold eriales	Comamon adaceae	Tepidimo nas		Breast	
11657	Proteo bacteri a	Alphaprot eobacteria	Rhizobia les	Methyloba acteriaceae	Methylob acterium	Methylobacteriu m organophilum	Breast	173
11656	Proteo bacteri a	Alphaprot eobacteria	Rhizobia les	Methyloba acteriaceae	Methylob acterium	Methylobacteriu m mesophilicum	Breast	174
30362	Bacter oidetes	Bacteroidi a	Bacteroi dales	Prevotella ceae	Prevotella		Breast	
50030	Bacter oidetes	Bacteroidi a	Bacteroi dales				Breast	
30867	Firmic utes	Bacilli	Lactobac illales	Streptococ aceae	Streptoco ccus		Breast	
50075	Firmic utes	Bacilli	Bacillale s				Breast	
50148	Proteo bacteri a	Gammapr oteobacteri a	Pseudom onadales				Breast	
31477	Firmic utes	Clostridia	Clostridi ales	Tissierella ceae	Finegoldi a		Breast	
40195	Firmic utes	Clostridia	Clostridi ales	Tissierella ceae			Breast	
70016	Firmic utes						Breast	
30663	Cyano bacteri a	Chloroplas t	Streptop hyta	Unknown family	Unknown genus116		Breast	
4523	Cyano bacteri a	Chloroplas t	Streptop hyta	Unknown family	Unknown genus116	Unknown species19	Breast	175
40168	Firmic utes	Bacilli	Bacillale s	Staphyloc occaceae			Breast	
9900	Firmic utes	Clostridia	Clostridi ales	Tissierella ceae	Finegoldi a	Unknown species11	Breast	176

5302	Firmicutes	Bacilli	Bacillales	Staphylococcaceae	Staphylococcus	Unknown species8	Breast	178
5324	Firmicutes	Bacilli	Bacillales	Staphylococcaceae	Staphylococcus	Staphylococcus haemolyticus	Breast	179
15324	Proteobacteria	Gammaproteobacteria	Pseudomonadales	Moraxellaceae	Acinetobacter	Acinetobacter ursingii	Breast	180
30817	Firmicutes	Bacilli	Bacillales	Staphylococcaceae	Staphylococcus		Breast	
30858	Firmicutes	Bacilli	Lactobacillales	Lactobacillaceae	Lactobacillus		Breast	
31799	Proteobacteria	Alphaproteobacteria	Rhizobiales	Methylobacteriaceae	Methylobacterium		Breast	
40245	Proteobacteria	Alphaproteobacteria	Rhizobiales	Methylobacteriaceae			Breast	
60052	Firmicutes	Bacilli					Breast	
60078	Proteobacteria	Betaproteobacteria					Breast	
13182	Proteobacteria	Betaproteobacteria	Burkholderiales	Burkholderiaceae	Ralstonia	Ralstonia mannitolilytica	Breast	181
31786	Proteobacteria	Alphaproteobacteria	Rhizobiales	Hyphomicrobiaceae	Devosia		Breast	
40181	Firmicutes	Bacilli	Lactobacillales	Streptococcaceae			Breast	
40330	Proteobacteria	Gammaproteobacteria	Pseudomonadales	Pseudomonadaceae			Breast	
969	Actinobacteria	Actinobacteria	Actinomycetales	Corynebacteriaceae	Corynebacterium	Corynebacterium stationis	Breast	182
32410	Proteobacteria	Gammaproteobacteria	Pseudomonadales	Pseudomonadaceae	Pseudomonas		Breast	
230	Actinobacteria	Actinobacteria	Actinomycetales	Actinomycetaceae	Actinomyces	Actinomyces oris	Breast	183
5687	Firmicutes	Bacilli	Lactobacillales	Lactobacillaceae	Lactobacillus	Lactobacillus iners	Breast	184
40176	Firmicutes	Bacilli	Lactobacillales	Aerococcaceae			Breast	
50079	Firmicutes	Clostridia	Clostridiales				Breast	
40179	Firmicutes	Bacilli	Lactobacillales	Lactobacillaceae			Breast	
3941	Bacteroidetes	Flavobacteriia	Flavobacteriales	Weeksellaceae	Wautersiella	Unknown species18	Breast	187

30113	Actinobacteria	Actinobacteria	Actinomycetales	Cellulomonadaceae	Cellulomonas		Breast	
6166	Firmicutes	Bacilli	Lactobacillales	Streptococcaceae	Streptococcus	Streptococcus cristatus	Breast	188
15055	Proteobacteria	Gammaproteobacteria	Enterobacteriales	Enterobacteriaceae	Klebsiella	Klebsiella pneumoniae	Breast	189
30866	Firmicutes	Bacilli	Lactobacillales	Streptococcaceae	Lactococcus		Breast	
40144	Cyanobacteria	Chloroplast	Streptophyta	Unknown family			Breast	
40276	Proteobacteria	Betaproteobacteria	Neisseriales	Neisseriaceae			Breast	
50085	Fusobacteria	Fusobacteriia	Fusobacteriales				Breast	
40046	Actinobacteria	Actinobacteria	Actinomycetales	Propionibacteriaceae			Breast	
50077	Firmicutes	Bacilli	Lactobacillales				Breast	
60081	Proteobacteria	Gammaproteobacteria					Breast	
1021	Actinobacteria	Actinobacteria	Actinomycetales	Geodermatophilaceae	Blastococcus	Unknown species13	Breast	190
4719	Firmicutes	Bacilli	Bacillales	Bacillaceae	Anoxybacillus	Anoxybacillus kestanbolensis	Breast	191
40042	Actinobacteria	Actinobacteria	Actinomycetales	Nocardiaceae			Breast	
2556	Bacteroidetes	Bacteroidia	Bacteroidales	Paraprevotellaceae	Prevotella	Prevotella tanneriae	Breast	192
30099	Actinobacteria	Actinobacteria	Actinomycetales	Actinomycetaceae	Actinomyces		Breast	
9261	Firmicutes	Clostridia	Clostridiales	Ruminococcaceae	Faecalibacterium	Faecalibacterium prausnitzii	Breast	195
4521	Cyanobacteria	Chloroplast	Streptophyta	Unknown family	Unknown genus116	Unknown species17	Breast	197
30190	Actinobacteria	Actinobacteria	Actinomycetales	Mycobacteriaceae	Mycobacterium		Breast	
30225	Actinobacteria	Actinobacteria	Actinomycetales	Propionibacteriaceae	Propionibacterium		Breast	
32336	Proteobacteria	Gammaproteobacteria	Enterobacteriales	Enterobacteriaceae	Enterobacter		Breast	

40021	Actinobacteria	Actinobacteria	Actinomycetales	Actinomycetaceae			Breast	
40038	Actinobacteria	Actinobacteria	Actinomycetales	Micrococaceae			Breast	
40193	Firmicutes	Clostridia	Clostridiales	Ruminococcaceae			Breast	
40198	Firmicutes	Clostridia	Clostridiales	Veillonellaceae			Breast	
40278	Proteobacteria	Betaproteobacteria	Rhodocyclales	Rhodocyclaceae			Breast	
30845	Firmicutes	Bacilli	Lactobacillales	Aerococcaceae	Alloiococcus		Breast	
9771	Firmicutes	Clostridia	Clostridiales	Tissierellaceae	1-68	1-68 Unknown	Breast	198
5687	Firmicutes	Bacilli	Lactobacillales	Lactobacillaceae	Lactobacillus	Lactobacillus	Lung	199
40260	Proteobacteria	Alphaproteobacteria	Sphingomonadales	Erythrobacteraceae			Lung	

Table 3 summarizes the different bacterial species that are prevalent in specific tumor types.

Table 3

<u>Tumor type</u>	<u>Accession ID</u>	<u>phylum</u>	<u>class</u>	<u>order</u>	<u>family</u>	<u>genus</u>	<u>species</u>	<u>Prevalence in specific tumor type</u>	<u>SEQ ID NO:</u>
Breast	1824	Actinobacteria	Actinobacteria	Actinomycetales	Propionibacteriaceae	Propionibacterium	Propionibacterium granulosum	38%	201
Breast	1346	Actinobacteria	Actinobacteria	Actinomycetales	Micrococaceae	Rothia	Rothia mucilaginosa	37%	202
Breast	5687	Firmicutes	Bacilli	Lactobacillales	Lactobacillaceae	Lactobacillus	Lactobacillus iners	37%	203
Breast	6175	Firmicutes	Bacilli	Lactobacillales	Streptococcaceae	Streptococcus	Streptococcus infantis	36%	204
Breast	10545	Firmicutes	Clostridia	Clostridiales	Veillonellaceae	Veillonella	Veillonella dispar	36%	205
Breast	1344	Actinobacteria	Actinobacteria	Actinomycetales	Micrococaceae	Rothia	Rothia dentocariosa	32%	206
Breast	587	Actinobacteria	Actinobacteria	Actinomycetales	Corynebacteriaceae	Corynebacterium	Unknown species1715	28%	207

Breast	5330	Firmicutes	Bacilli	Bacillales	Staphylococcaceae	Staphylococcus	Staphylococcus pasteurii	28%	208
Breast	3046	Bacteroidetes	Bacteroidia	Bacteroidales	Prevotellaceae	Prevotella	Prevotella melaninogenica	27%	209
Breast	10726	Fusobacteriia	Fusobacteriia	Fusobacteriales	Fusobacteriaceae	Fusobacterium	Fusobacterium nucleatum	24%	210
Breast	4523	Cyanobacteria	Chloroplast	Streptophyta	Unknown family	Unknown genus 116	Unknown species 19	23%	211
Breast	9900	Firmicutes	Clostridia	Clostridiales	Tissierellaceae	Finegoldia	Unknown species 11	23%	212
Breast	15324	Proteobacteria	Gamma proteobacteria	Pseudomonadales	Moraxellaceae	Acinetobacter	Acinetobacter ursingii	23%	213
Breast	6184	Firmicutes	Bacilli	Lactobacillales	Streptococcaceae	Streptococcus	Streptococcus pneumoniae	22%	214
Breast	2555	Bacteroidetes	Bacteroidia	Bacteroidales	Paraprevotellaceae	Prevotella	Prevotella Unknown	22%	215
Breast	5286	Firmicutes	Bacilli	Bacillales	Staphylococcaceae	Staphylococcus	Unknown species 691	22%	216
Breast	10546	Firmicutes	Clostridia	Clostridiales	Veillonellaceae	Veillonella	Veillonella parvula	22%	217
Breast	12106	Proteobacteria	Alphaproteobacteria	Rhodobacterales	Rhodobacteraceae	Paracoccus	Paracoccus chinensis	21%	218
Breast	13904	Proteobacteria	Betaproteobacteria	Burkholderiales	Oxalobacteraceae	Massilia	Massilia timonae	21%	219
Breast	12109	Proteobacteria	Alphaproteobacteria	Rhodobacterales	Rhodobacteraceae	Paracoccus	Paracoccus marcusii	20%	220
Lung	1824	Actinobacteria	Actinobacteria	Actinomycetales	Propionibacteriaceae	Propionibacterium	Propionibacterium granulosum	19%	221
Lung	12551	Proteobacteria	Alphaproteobacteria	Sphingomonadales	Sphingomonadaceae	Kaistobacter	Kaistobacter Unknown	16%	222
Lung	10545	Firmicutes	Clostridia	Clostridiales	Veillonellaceae	Veillonella	Veillonella dispar	16%	223
Lung	587	Actinobacteria	Actinobacteria	Actinomycetales	Corynebacteriaceae	Corynebacterium	Unknown species 1715	16%	224

Lun g	1346	Actinobacteria	Actinobacteria	Actinomyces	Micrococcales	Rothia	Rothia mucilaginosa	16%	225
Lun g	2555	Bacteroidetes	Bacteroidia	Bacteroidales	Paraprevotellaceae	Prevotella	Prevotella Unknown	14%	226
Lun g	5687	Firmicutes	Bacilli	Lactobacillales	Lactobacillaceae	Lactobacillus	Lactobacillus iners	14%	227
Lun g	12937	Proteobacteria	Alphaproteobacteria	Sphingomonadales	Sphingomonadaceae	Sphingomonas	Sphingomonas yunnanensis	13%	228
Lun g	1766	Actinobacteria	Actinobacteria	Actinomyces	Propionibacteriaceae	Unknown genus24	Unknown species 1	12%	229
Lun g	12109	Proteobacteria	Alphaproteobacteria	Rhodobacterales	Rhodobacteraceae	Paracoccus	Paracoccus marcusii	11%	230
Lun g	12289	Proteobacteria	Alphaproteobacteria	Rhodospirillales	Acetobacteraceae	Roseomonas	Roseomonas mucosa	10%	231
Lun g	15666	Proteobacteria	Gamma proteobacteria	Pseudomonadales	Pseudomonadaceae	Pseudomonas	Pseudomonas baetica	9%	232
Lun g	1815	Actinobacteria	Actinobacteria	Actinomyces	Propionibacteriaceae	Propionibacterium	Unknown species 18	8%	233
Lun g	12808	Proteobacteria	Alphaproteobacteria	Sphingomonadales	Sphingomonadaceae	Sphingomonas	Unknown species 45	8%	234
Lun g	5338	Firmicutes	Bacilli	Bacillales	Staphylococcaceae	Staphylococcus	Staphylococcus warneri	7%	235
Lun g	13018	Proteobacteria	Betaproteobacteria	Burkholderiales	Alcaligenaceae	Alcaligenes	Alcaligenes faecalis	7%	236
Lun g	13194	Proteobacteria	Betaproteobacteria	Burkholderiales	Comamonadaceae	Acidovorax	Acidovorax temperans	7%	237
Lun g	545	Actinobacteria	Actinobacteria	Actinomyces	Corynebacteriaceae	Corynebacterium	Unknown species 1626	7%	238
Lun g	6066	Firmicutes	Bacilli	Lactobacillales	Streptococcaceae	Streptococcus	Unknown species 346	7%	239
Lun g	9900	Firmicutes	Clostridia	Clostridiales	Tissierellaceae	Finegoldia	Unknown species 11	7%	240

Melano ma	12 10 9	Proteobacteria	Alphaproteobacteria	Rhodobacterales	Rhodobacteraceae	Paracoccus	Paracoccus marcusii		20%	241
Melano ma	53 15	Firmicutes	Bacilli	Bacillales	Staphylococcaceae	Staphylococcus	Staphylococcus aureus		14%	242
Melano ma	24 95	Bacteroidetes	Bacteroidia	Bacteroidales	Bacteroidaceae	Bacteroides	Bacteroides dorei		10%	243
Melano ma	15 60 7	Proteobacteria	Gamma proteobacteria	Pseudomonadales	Pseudomonadaceae	Pseudomonas	Unknown species632		5%	244
Melano ma	10 44 4	Firmicutes	Clostridia	Clostridiales	Veillonellaceae	Selenomonas	Unknown species 18		5%	245
Melano ma	15 73 3	Proteobacteria	Gamma proteobacteria	Pseudomonadales	Pseudomonadaceae	Pseudomonas	Pseudomonas viridiflava		4%	246
Melano ma	48 86	Firmicutes	Bacilli	Bacillales	Bacillaceae	Geobacillus	Unknown species208		4%	247
Melano ma	15 04 3	Proteobacteria	Gamma proteobacteria	Enterobacteriales	Enterobacteriaceae	Klebsiella	Unknown species 25		4%	248
Melano ma	15 60 8	Proteobacteria	Gamma proteobacteria	Pseudomonadales	Pseudomonadaceae	Pseudomonas	Unknown species643		4%	249
Melano ma	46 19	Cyanobacteria	Chloroplast	Streptophyta	Unknown family	Unknown genus39	Unknown species 8		3%	250
Melano ma	15 95 6	Proteobacteria	Gamma proteobacteria	Xanthomonadales	Xanthomonadaceae	Xanthomonas	Xanthomonas arboricola		3%	251
Melano ma	43 6	Actinobacteria	Actinobacteria	Actinomycetales	Corynebacteriaceae	Corynebacterium	Unknown species1061		2%	252
Melano ma	62 91	Firmicutes	Clostridia	Clostridiales	Clostridiaceae	Clostridium	Unknown species 19		2%	253
Melano ma	15 36 8	Proteobacteria	Gamma proteobacteria	Pseudomonadales	Moraxellaceae	Acinetobacter	Unknown species 31		2%	254
Melano ma	13 81 0	Proteobacteria	Betaproteobacteria	Burkholderiales	Oxalobacteraceae	Massilia	Unknown species177		2%	255
Melano ma	67 03	Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	Unknown	Unknown		2%	256

						genus3008	species1			
Melano ma	14087	Proteobacteria	Betaproteobacteria	Neisseriales	Neisseriaceae	Eikenella	Eikenella corrodens		2%	257
Melano ma	2437	Bacteroidetes	Bacteroidia	Bacteroidales	Bacteroidaceae	Bacteroides	Unknown species388		2%	258
Melano ma	10458	Firmicutes	Clostridia	Clostridiales	Veillonellaceae	Selenomonas	Unknown species208		2%	259
Melano ma	8343	Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	Lachnospirillum	Eubacterium saburreum		1%	260
Melano ma	10504	Firmicutes	Clostridia	Clostridiales	Veillonellaceae	Selenomonas	Unknown species59		1%	261
Pancreas	14775	Proteobacteria	Gamma proteobacteria	Enterobacteriales	Enterobacteriaceae	Citrobacter	Citrobacter freundii		45%	262
Pancreas	15055	Proteobacteria	Gamma proteobacteria	Enterobacteriales	Enterobacteriaceae	Klebsiella	Klebsiella pneumoniae		42%	263
Pancreas	14847	Proteobacteria	Gamma proteobacteria	Enterobacteriales	Enterobacteriaceae	Enterobacter	Enterobacter asburiae		33%	264
Pancreas	10545	Firmicutes	Clostridia	Clostridiales	Veillonellaceae	Veillonella	Veillonella dispar		19%	265
Pancreas	10726	Fusobacteria	Fusobacteriia	Fusobacteriales	Fusobacteriaceae	Fusobacterium	Fusobacterium nucleatum		18%	266
Pancreas	14849	Proteobacteria	Gamma proteobacteria	Enterobacteriales	Enterobacteriaceae	Enterobacter	Enterobacter cloacae		18%	267
Pancreas	15054	Proteobacteria	Gamma proteobacteria	Enterobacteriales	Enterobacteriaceae	Klebsiella	Klebsiella oxytoca		15%	268
Pancreas	14846	Proteobacteria	Gamma proteobacteria	Enterobacteriales	Enterobacteriaceae	Enterobacter	Enterobacter aerogenes		13%	269
Pancreas	6161	Firmicutes	Bacilli	Lactobacillales	Streptococcaceae	Streptococcus	Streptococcus anginosus		13%	270
Pancreas	15695	Proteobacteria	Gamma proteobacteria	Pseudomonadales	Pseudomonadaceae	Pseudomonas	Pseudomonas mendocina		12%	271
Pancreas	1346	Actinobacteria	Actinobacteria	Actinomycetales	Micrococaceae	Rothia	Rothia mucilaginosa		10%	272

Panc reas	14680	Proteobacteria	Gamma proteobacteria	Alteromonadales	Shewanellaceae	Shewanella	Shewanella decolorationis	10%	273
Panc reas	5583	Firmicutes	Bacilli	Lactobacillales	Enterococcaceae	Enterococcus	Enterococcus gallinarum	10%	274
Panc reas	5552	Firmicutes	Bacilli	Lactobacillales	Carnobacteriaceae	Granulicatella	Granulicatella adiacens	9%	275
Panc reas	986	Actinobacteria	Actinobacteria	Actinomycetales	Dermabacteraceae	Brachybacterium	Brachybacterium conglomeratum	7%	276
Panc reas	14174	Proteobacteria	Betaproteobacteria	Neisseriales	Neisseriaceae	Neisseria	Neisseria subflava	7%	277
Panc reas	2555	Bacteroidetes	Bacteroidia	Bacteroidales	Paraprevotellaceae	Prevotella	Prevotella Unknown	6%	278
Panc reas	5582	Firmicutes	Bacilli	Lactobacillales	Enterococcaceae	Enterococcus	Enterococcus faecium	6%	279
Panc reas	1344	Actinobacteria	Actinobacteria	Actinomycetales	Micrococcaceae	Rothia	Rothia dentocariosa	4%	280
Panc reas	1075	Fusobacteria	Fusobacteriia	Fusobacteriales	Leptotrichiaceae	Leptotrichia	Unknown species235	4%	281
Ovary	12289	Proteobacteria	Alphaproteobacteria	Rhodospirillales	Acetobacteraceae	Roseomonas	Roseomonas mucosa	20%	282
Ovary	12873	Proteobacteria	Alphaproteobacteria	Sphingomonadales	Sphingomonadaceae	Sphingomonas	Unknown species602	20%	283
Ovary	5319	Firmicutes	Bacilli	Bacillales	Staphylococcaceae	Staphylococcus	Staphylococcus cohnii	9%	284
Ovary	16227	Thermi	Deinococci	Deinococcales	Deinococcaceae	Deinococcus	Unknown species124	7%	285
Ovary	5627	Firmicutes	Bacilli	Lactobacillales	Lactobacillaceae	Lactobacillus	Unknown species479	5%	286
Bone	12674	Proteobacteria	Alphaproteobacteria	Sphingomonadales	Sphingomonadaceae	Sphingobium	Sphingomonas yanoikuyae	36%	287
Bone	1824	Actinobacteria	Actinobacteria	Actinomycetales	Propionibacteriaceae	Propionibacterium	Propionibacterium granulosum	28%	288
Bone	225	Actinobacteria	Actinobacteria	Actinomycetales	Actinomycetaceae	Actinomyces	Actinomyces massiliensis	18%	289

Bone	15662	Proteobacteria	Gamma proteobacteria	Pseudomonadales	Pseudomonadales	Pseudomonas	Pseudomonas argentinensis	13%	290
Bone	14847	Proteobacteria	Gamma proteobacteria	Enterobacteriales	Enterobacteriaceae	Enterobacter	Enterobacter asburiae	10%	291
Bone	15568	Proteobacteria	Gamma proteobacteria	Pseudomonadales	Pseudomonadales	Pseudomonas	Unknown species 39	10%	292
Bone	5968	Firmicutes	Bacilli	Lactobacillales	Streptococcaceae	Streptococcus	Unknown species 2029	8%	293
Bone	16119	TM7	TM7-3	CW040	Unknown family	Unknown genus 3	Unknown species 7	5%	294
Bone	16025	Spirochaetes	Spirochaetes	Spirochaetales	Spirochaetales	Treponema	Treponema socranskii	5%	295
Bone	4868	Firmicutes	Bacilli	Bacillales	Bacillaceae	Bacillus	Bacillus clausii	5%	296
Bone	527	Actinobacteria	Actinobacteria	Actinomycetales	Corynebacteriaceae	Corynebacterium	Unknown species 1534	5%	297
GBM	14849	Proteobacteria	Gamma proteobacteria	Enterobacteriales	Enterobacteriaceae	Enterobacter	Enterobacter cloacae	10%	298
GBM	14169	Proteobacteria	Betaproteobacteria	Neisseriales	Neisseriaceae	Neisseria	Neisseria macacae	8%	299
GBM	1311	Actinobacteria	Actinobacteria	Actinomycetales	Micrococaceae	Kocuria	Kocuria atrinae	8%	300
GBM	15409	Proteobacteria	Gamma proteobacteria	Pseudomonadales	Moraxellaceae	Acinetobacter	Unknown species 424	8%	301
GBM	14934	Proteobacteria	Gamma proteobacteria	Enterobacteriales	Enterobacteriaceae	Escherichia/Shigella	Unknown species 231	8%	302
GBM	14795	Proteobacteria	Gamma proteobacteria	Enterobacteriales	Enterobacteriaceae	Enterobacter	Unknown species 196	8%	303
GBM	1129	Actinobacteria	Actinobacteria	Actinomycetales	Microbacteriaceae	Agromyces	Agromyces mediolanus	5%	304
GBM	11842	Proteobacteria	Alphaproteobacteria	Rhizobiales	Rhizobiaceae	Agrobacterium	Unknown species 298	5%	305
GBM	15853	Proteobacteria	Gamma proteobacteria	Xanthomonadales	Xanthomonadales	Luteimonas	Unknown species 76	5%	306

GB M	51 06	Firmi cutes	Bacilli	Bacilla les	Planoco ccaceae	Lysiniba cillus	Lysinibacillus boronitolerans	5%	307
GB M	50 09	Firmi cutes	Bacilli	Bacilla les	Exiguo bactera ceae	Exiguob acterium	Unkno wn species 29	5%	308
GB M	15 33 3	Prote obact eria	Gamma proteob acteria	Pseudo monad ales	Moraxe llaceae	Acineto bacter	Unknown species127	5%	309
GB M	15 49 4	Prote obact eria	Gamma proteob acteria	Pseudo monad ales	Moraxe llaceae	Psychro bacter	Unkno wn species 28	5%	310

According to a particular embodiment, the bacteria is Salmonella Typhimurium - e.g. the Salmonella Typhimurium attenuated strain VNP20009, Salmonella Typhimurium 14028 strain STM3120, Salmonella Typhimurium 14028 strain STM1414, Pseudomonas aeruginosa (strain 5 CHA-OST) and/or Bacillus Subtillis (strain PY79).

The term "isolated" or "enriched" encompasses bacteria that has been (1) separated from at least some of the components with which it was associated when initially produced (whether in nature or in an experimental setting), and/or (2) produced, prepared, purified, and/or manufactured by the hand of man. Isolated microbes may be separated from at least about 10%, about 20%, about 10 30%, about 40%, about 50%, about 60%, about 70%, about 80%, about 90%, or more of the other components with which they were initially associated. In some embodiments, isolated microbes are more than about 80%, about 85%, about 90%, about 91%, about 92%, about 93%, about 94%, about 95%, about 96%, about 97%, about 98%, about 99%, or more than about 99% pure. As used herein, a substance is "pure" if it is substantially free of other components. The terms "purify," 15 "purifying" and "purified" refer to a microbe or other material that has been separated from at least some of the components with which it was associated either when initially produced or generated (e.g., whether in nature or in an experimental setting), or during any time after its initial production. A microbe or a microbial population may be considered purified if it is isolated at, or after production, such as from a material or environment containing the microbe or microbial population, 20 and a purified microbe or microbial population may contain other materials up to about 10%, about 20%, about 30%, about 40%, about 50%, about 60%, about 70%, about 80%, about 90%, or above about 90% and still be considered "isolated." In some embodiments, purified microbes or microbial population are more than about 80%, about 85%, about 90%, about 91%, about 92%, about 93%, about 94%, about 95%, about 96%, about 97%, about 98%, about 99%, or more than about 99% 25 pure. In the instance of microbial compositions provided herein, the one or more microbial types

present in the composition can be independently purified from one or more other microbes produced and/or present in the material or environment containing the microbial type. Microbial compositions and the microbial components thereof are generally purified from residual habitat products.

5 In certain embodiments, at least 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% of the bacteria in the vaccine are of a genus, species or strain listed in Tables 1-3.

According to a specific embodiment, the genome of the bacteria comprises a 16S rRNA sequence at least 90 %, 91 %, 92 %, 93 %, 94 %, 95 %, 96 %, 97 %, 98 %, 99 %, 99.1 %, 99.2 %, 10 99.3 %, 99.4 %, 99.5 %, 99.6 %, 99.7 %, 99.8 %, 99.9 %, 99.95 % identical to any one of the sequences as set forth in SEQ ID NO: 24-310.

As used herein, "percent homology", "percent identity", "sequence identity" or "identity" or grammatical equivalents as used herein in the context of two nucleic acid or polypeptide sequences includes reference to the residues in the two sequences which are the same when 15 aligned. When percentage of sequence identity is used in reference to proteins it is recognized that residue positions which are not identical often differ by conservative amino acid substitutions, where amino acid residues are substituted for other amino acid residues with similar chemical properties (*e.g.* charge or hydrophobicity) and therefore do not change the functional properties of the molecule. Where sequences differ in conservative substitutions, the percent sequence identity 20 may be adjusted upwards to correct for the conservative nature of the substitution. Sequences which differ by such conservative substitutions are considered to have "sequence similarity" or "similarity". Means for making this adjustment are well-known to those of skill in the art. Typically this involves scoring a conservative substitution as a partial rather than a full mismatch, thereby increasing the percentage sequence identity. Thus, for example, where an identical amino 25 acid is given a score of 1 and a non-conservative substitution is given a score of zero, a conservative substitution is given a score between zero and 1. The scoring of conservative substitutions is calculated, *e.g.*, according to the algorithm of Henikoff S and Henikoff JG. [Amino acid substitution matrices from protein blocks. Proc. Natl. Acad. Sci. U.S.A. 1992, 89(22): 10915-9].

30 Percent identity can be determined using any homology comparison software, including for example, the BlastN software of the National Center of Biotechnology Information (NCBI) such as by using default parameters.

Other exemplary sequence alignment programs that may be used to determine % homology or identity between two sequences include, but are not limited to, the FASTA package (including rigorous (SSEARCH, LALIGN, GGSEARCH and GLSEARCH) and heuristic (FASTA, FASTX/Y, TFASTX/Y and FASTS/M/F) algorithms, the EMBOSS package (Needle, stretcher, water and matcher), the BLAST programs (including, but not limited to BLASTN, BLASTX, TBLASTX, BLASTP, TBLASTN), megablast and BLAT. In some embodiments, the sequence alignment program is BLASTN. For example, 95% homology refers to 95% sequence identity determined by BLASTN, by combining all non-overlapping alignment segments (BLAST HSPs), summing their numbers of identical matches and dividing this sum with the length of the shorter sequence.

In some embodiments, the sequence alignment program is a basic local alignment program, e.g., BLAST. In some embodiments, the sequence alignment program is a pairwise global alignment program. In some embodiments, the pairwise global alignment program is used for protein-protein alignments. In some embodiments, the pairwise global alignment program is Needle. In some embodiments, the sequence alignment program is a multiple alignment program. In some embodiments, the multiple alignment program is MAFFT. In some embodiments, the sequence alignment program is a whole genome alignment program. In some embodiments, the whole genome alignment is performed using BLASTN. In some embodiments, BLASTN is utilized without any changes to the default parameters.

According to some embodiments of the invention, the identity is a global identity, *i.e.*, an identity over the entire nucleic acid sequences of the invention and not over portions thereof.

Methods of qualifying which bacteria are present in a tumor microbiome are described herein below. Care should be taken to take a sufficient number of measurements when analyzing which microbes are present in the microbiome to minimize and control for contaminations.

In some embodiments, determining a presence of one or more bacteria or components or products thereof comprises determining a level or set of levels of one or more DNA sequences. In some embodiments, one or more DNA sequences comprises any DNA sequence that can be used to differentiate between different bacterial types. In certain embodiments, one or more DNA sequences comprises 16S rRNA gene sequences. In certain embodiments, one or more DNA sequences comprises 18S rRNA gene sequences. In some embodiments, 1, 2, 3, 4, 5, 10, 15, 20, 25, 50, 100, 1,000, 5,000 or more sequences are amplified.

In some embodiments, a microbiota sample (e.g. tumor sample) is directly assayed for a presence, a level or set of levels of one or more DNA sequences. In some embodiments, DNA is

isolated from a tumor microbiota sample and isolated DNA is assayed for a level or set of levels of one or more DNA sequences. Methods of isolating microbial DNA are well known in the art. Examples include but are not limited to phenol-chloroform extraction and a wide variety of commercially available kits, including QIAamp DNA Stool Mini Kit (Qiagen, Valencia, Calif.).

5 In some embodiments, a presence, a level or set of levels of one or more DNA sequences is determined by amplifying DNA sequences using PCR (e.g., standard PCR, semi-quantitative, or quantitative PCR). In some embodiments, a level or set of levels of one or more DNA sequences is determined by amplifying DNA sequences using quantitative PCR. These and other basic DNA amplification procedures are well known to practitioners in the art and are described in Ausubel et
10 al. (Ausubel F M, Brent R, Kingston R E, Moore D, Seidman J G, Smith J A, Struhl K (eds). 1998. Current Protocols in Molecular Biology. Wiley: New York).

In some embodiments, DNA sequences are amplified using primers specific for one or more sequence that differentiate(s) individual microbial types from other, different microbial types. In some embodiments, 16S rRNA gene sequences or fragments thereof are amplified using primers
15 specific for 16S rRNA gene sequences. In some embodiments, 18S DNA sequences are amplified using primers specific for 18S DNA sequences.

In some embodiments, a presence, a level or set of levels of one or more 16S rRNA gene sequences is determined using phylochip technology. Use of phylochips is well known in the art and is described in Hazen et al. ("Deep-sea oil plume enriches indigenous oil-degrading bacteria."
20 Science, 330, 204-208, 2010), the entirety of which is incorporated by reference. Briefly, 16S rRNA genes sequences are amplified and labeled from DNA extracted from a microbiota sample. Amplified DNA is then hybridized to an array containing probes for microbial 16S rRNA genes. Level of binding to each probe is then quantified providing a sample level of microbial type corresponding to 16S rRNA gene sequence probed. In some embodiments, phylochip analysis is
25 performed by a commercial vendor. Examples include but are not limited to Second Genome Inc. (San Francisco, Calif.).

In some embodiments, determining a presence, a level or set of levels of one or more types of microbes or components or products thereof comprises determining a presence, a level or set of levels of one or more microbial RNA molecules (e.g., transcripts). Methods of quantifying levels
30 of RNA transcripts are well known in the art and include but are not limited to northern analysis, semi-quantitative reverse transcriptase PCR, quantitative reverse transcriptase PCR, and microarray analysis.

In some embodiments, determining a presence, a level or set of levels of one or more types of microbes or components or products thereof comprises determining a presence, a level or set of levels of one or more microbial polypeptides. Methods of quantifying polypeptide levels are well known in the art and include but are not limited to Western analysis and mass spectrometry. These and all other basic polypeptide detection procedures are described in Ausubel et al.

In some embodiments, determining a presence, a level or set of levels of one or more types of microbes or components or products thereof comprises determining a presence, a level or set of levels of one or more microbial metabolites. In some embodiments, levels of metabolites are determined by mass spectrometry. In some embodiments, levels of metabolites are determined by nuclear magnetic resonance spectroscopy. In some embodiments, levels of metabolites are determined by enzyme-linked immunosorbent assay (ELISA). In some embodiments, levels of metabolites are determined by colorimetry. In some embodiments, levels of metabolites are determined by spectrophotometry.

In certain embodiments, the vaccine comprises at least 1×10^3 colony forming units (CFUs), 1×10^4 colony forming units (CFUs), 1×10^5 colony forming units (CFUs), 1×10^6 colony forming units (CFUs), 1×10^7 colony forming units (CFUs), 1×10^8 colony forming units (CFUs), 1×10^9 colony forming units (CFUs), 1×10^{10} colony forming units (CFUs) of bacteria of a family/genus/species/strain listed in Tables 1-3.

Methods for producing bacteria may include three main processing steps. The steps are: organism banking, organism production, and preservation.

For banking, the strains included in the bacteria may be (1) isolated directly from a specimen or taken from a banked stock, (2) optionally cultured on a nutrient agar or broth that supports growth to generate viable biomass, and (3) the biomass optionally preserved in multiple aliquots in long-term storage.

In embodiments using a culturing step, the agar or broth may contain nutrients that provide essential elements and specific factors that enable growth. An example would be a medium composed of 20 g/L glucose, 10 g/L yeast extract, 10 g/L soy peptone, 2 g/L citric acid, 1.5 g/L sodium phosphate monobasic, 100 mg/L ferric ammonium citrate, 80 mg/L magnesium sulfate, 10 mg/L hemin chloride, 2 mg/L calcium chloride, 1 mg/L menadione. Another examples would be a medium composed of 10 g/L beef extract, 10 g/L peptone, 5 g/L sodium chloride, 5 g/L dextrose, 3 g/L yeast extract, 3 g/L sodium acetate, 1 g/L soluble starch, and 0.5 g/L L-cysteine HCl, at pH 6.8. A variety of microbiological media and variations are well known in the art (e.g., R. M. Atlas, Handbook of Microbiological Media (2010) CRC Press). Culture media can be added to the culture

at the start, may be added during the culture, or may be intermittently/continuously flowed through the culture. The strains in the vaccine may be cultivated alone, as a subset of the microbial composition, or as an entire collection comprising the microbial composition. As an example, a first strain may be cultivated together with a second strain in a mixed continuous culture, at a dilution rate lower than the maximum growth rate of either cell to prevent the culture from washing out of the cultivation.

The inoculated culture is incubated under favorable conditions for a time sufficient to build biomass. For microbial compositions for human use this is often at 37 °C temperature, pH, and other parameter with values similar to the normal human niche. The environment may be actively controlled, passively controlled (e.g., via buffers), or allowed to drift. For example, for anaerobic bacterial compositions, an anoxic/reducing environment may be employed. This can be accomplished by addition of reducing agents such as cysteine to the broth, and/or stripping it of oxygen. As an example, a culture of a bacterial composition may be grown at 37 °C, pH 7, in the medium above, pre-reduced with 1 g/L cysteine-HCl.

When the culture has generated sufficient biomass, it may be preserved for banking. The organisms may be placed into a chemical milieu that protects from freezing (adding 'cryoprotectants'), drying ('lyoprotectants'), and/or osmotic shock ('osmoprotectants'), dispensing into multiple (optionally identical) containers to create a uniform bank, and then treating the culture for preservation. Containers are generally impermeable and have closures that assure isolation from the environment. Cryopreservation treatment is accomplished by freezing a liquid at ultra-low temperatures (e.g., at or below -80 °C). Dried preservation removes water from the culture by evaporation (in the case of spray drying or 'cool drying') or by sublimation (e.g., for freeze drying, spray freeze drying). Removal of water improves long-term microbial composition storage stability at temperatures elevated above cryogenic. If the microbial composition comprises, for example, spore forming species and results in the production of spores, the final composition may be purified by additional means such as density gradient centrifugation preserved using the techniques described above. Microbial composition banking may be done by culturing and preserving the strains individually, or by mixing the strains together to create a combined bank. As an example of cryopreservation, a microbial composition culture may be harvested by centrifugation to pellet the cells from the culture medium, the supernatant decanted and replaced with fresh culture broth containing 15 % glycerol. The culture can then be aliquoted into 1 mL cryotubes, sealed, and placed at -80 °C for long-term viability retention. This procedure achieves acceptable viability upon recovery from frozen storage.

Microbial production may be conducted using similar culture steps to banking, including medium composition and culture conditions. It may be conducted at larger scales of operation, especially for clinical development or commercial production. At larger scales, there may be several subcultivations of the microbial composition prior to the final cultivation. At the end of cultivation, the culture is harvested to enable further formulation into a dosage form for administration. This can involve concentration, removal of undesirable medium components, and/or introduction into a chemical milieu that preserves the microbial composition and renders it acceptable for administration via the chosen route. After drying, the powder may be blended to an appropriate potency, and mixed with other cultures and/or a filler such as microcrystalline cellulose for consistency and ease of handling, and the bacterial composition formulated as provided herein.

In certain aspects, provided are vaccines (i.e. bacterial compositions) for administration to subjects. In some embodiments, the bacteria are combined with additional active and/or inactive materials in order to produce a final product, which may be in single dosage unit or in a multi-dose format.

The bacteria present in the vaccine may be viable (e.g. capable of propagating when cultured in the appropriate medium, or inside the body, following administration).

In another embodiment, the bacteria present in the vaccine are non-viable.

In still another embodiment, the bacteria are attenuated such that they are not capable of causing disease.

As mentioned, the bacteria of the vaccine disclosed herein express at least one cancer associated antigen.

Cancer-associated antigens are typically short peptides corresponding to one or more antigenic determinants of a protein. The cancer-associated antigen typically binds to a class I or II MHC receptor thus forming a ternary complex that can be recognized by a T-cell bearing a matching T-cell receptor binding to the MHC/peptide complex with appropriate affinity. Peptides binding to MHC class I molecules are typically about 8-14 amino acids in length. T-cell epitopes that bind to MHC class II molecules are typically about 12-30 amino acids in length. In the case of peptides that bind to MHC class II molecules, the same peptide and corresponding T cell epitope may share a common core segment, but differ in the overall length due to flanking sequences of differing lengths upstream of the amino-terminus of the core sequence and downstream of its carboxy terminus, respectively. A T-cell epitope may be classified as an antigen if it elicits an immune response.

The antigens for cancers can be antigens from testicular cancer, ovarian cancer, brain cancer such as glioblastoma, pancreatic cancer, melanoma, lung cancer, prostate cancer, hepatic cancer, breast cancer, rectal cancer, colon cancer, esophageal cancer, gastric cancer, renal cancer, sarcoma, neuroblastoma, Hodgkins and non-Hodgkins lymphoma and leukemia.

In one embodiment, the cancer-associated antigen is a cancer testis antigen (e.g. a member of the melanoma antigen protein (MAGE) family, Squamous Cell Carcinoma-1 (NY-ESO-1), BAGE (B melanoma antigen), LAGE-1 antigen, Brother of the Regulator of Imprinted Sites (BORIS) and members of the GAGE family).

In another embodiment, the cancer-associated antigen is derived from MART-1/Melan-A protein e.g. (MART1 MHC class I peptides (Melan-A:26-35(L27), ELAGIGILTV; SEQ ID NO: 1) and MHC class II peptides (Melan-A:51-73(RR-23) RNGYRALMDKSLHVGTTQCALTRR; SEQ ID NO: 2).

In another embodiment, the cancer-associated antigen is derived from glycoprotein 70, glycoprotein 100 (gp100:25-33 (MHC class I (EGSRNQDWL - SEQ ID NO: 7)) or gp100:44-59 MHC class II (WNRQLYPEWTEAQRLLD - SEQ ID NO: 8) peptides).

In still another embodiment, the cancer-associated antigen is derived from tyrosinase, tyrosinase-related protein 1 (TRP1), tyrosinase-related protein 2 (TRP-2) or TRP-2/INT2 (TRP-2/intron2).

In still another embodiment, the cancer-associated antigen comprises MUT30 (mutation in Kinesin family member 18B, Kif18b – PSKPSFQEFVDWENVSPELNSTDQPFL - SEQ ID NO: 9) or MUT44 (cleavage and polyadenylation specific factor 3-like, Cpsf3l – EFKHIKAFDRTFANNPGPMVVFATPGM - SEQ ID NO: 10).

In still another embodiment, the cancer-associated antigen is derived from stimulator of prostatic adenocarcinoma-specific T cells- SPAS-1.

In still another embodiment, the cancer-associated antigen is derived from human telomerase reverse transcriptase (hTERT) or hTRT (human telomerase reverse transcriptase).

In still another embodiment, the cancer-associated antigen is derived from ovalbumin (OVA) for example OVA₂₅₇₋₂₆₄ MHCI H-2Kb (SIINFEKL – SEQ ID NO: 11) and OVA₃₂₃₋₃₃₉ MHCII I-A(d) (ISQAVHAAHAEINEAGR SEQ ID NO: 12), a RAS mutation, mutant oncogenic forms of p53 (TP53) (p53mut (peptide antigen of mouse mutated p53_{R172H} sequence VVRHCPHHER - SEQ ID NO: 4 (human mutated p53_{R175H} sequence EVVRHCPHHE – SEQ ID NO: 5)), or from BRAF-V600E peptide (GDFGLATEKSRWSGS – SEQ ID NO: 13).

According to a particular embodiment, the cancer associated antigen is set forth in SEQ ID NO: 11.

In still another embodiment, the cancer-associated antigen is a breast cancer associated disease antigen including but not limited to α -Lactalbumin (α -Lac), Her2/neu, BRCA-2 or BRCA-1 (RNF53), KNG1K438-R457 (kininogen-1 peptide) and C3fS1304-R1320 (peptides that distinguish BRCA1 mutated from other BC and non-cancer mutated BRCA1).

In still another embodiment, the cancer-associated antigen is a colorectal cancer associated disease antigen including but not limited to MUC1, KRAS, CEA (CAP-1-6-D [Asp6]; YLSGADLNL - SEQ ID NO: 14) and Adpgk_{R304M} MC38 (MHCII-Adpgk: ASMTNMELM SEQ ID NO: 15; MHCII-Adpgk: GIPVHLELASMTNMELMSSIVHQQVFPT SEQ ID NO: 16).

In still another embodiment, the cancer-associated antigen is a pancreatic cancer associated disease antigen including but not limited to CEA, CA 19-9, MUC1, KRAS, p53mut (peptide antigen of mouse mutated p53_{R172H} sequence VVRHCPHHER - SEQ ID NO: 4 (human mutated p53_{R175H} sequence EVVRHCPHHE - SEQ ID NO: 5)) and MUC4 or MUC13, MUC3A or CEACAM5, KRAS peptides (e.g. KRAS-G12R, KRAS-G13D, p5-21 sequence KLVVVGAGGVGKSALTI (SEQ ID NO: 17), p5-21 G12D sequence KLVVVGADGVGKSALTI (SEQ ID NO: 18), p17-31 sequence SALTILIQNHVFDE (SEQ ID NO: 19), p78-92 sequence FLCVFAINNTKSFED (SEQ ID NO: 20), p156-170 sequence FYTLVREIRKHKEKM (SEQ ID NO: 21), NRAS (e.g. NRAS-Q61R), PI3K (e.g. PIK3CA-H1047R), C-Kit-D816V, and BRCA mutated epitopes YIHTHTFYV (SEQ ID NO: 22) and SQIWNLNPV (SEQ ID NO: 23) HLA-A*02:01 restricted neoepitopes.

In still another embodiment, the cancer-associated antigen is a lung cancer associated disease antigen including but not limited to Sperm Protein 17 (SP17), A-kinase anchor protein 4 (AKAP4) and Pituitary Tumor Transforming Gene 1 (PTTG1), Aurora kinase A, HER2/neu, and p53mut.

In still another embodiment, the cancer-associated antigen is a prostate cancer associated disease antigen such as prostate cancer antigen (PCA), prostate-specific antigen (PSA) or prostate-specific membrane antigen (PSMA).

In still another embodiment, the cancer-associated antigen is a brain cancer, specifically glioblastoma cancer associated disease antigen such as GL261 neoantigen (mImp3 D81N AALLNKLYA - SEQ ID NO: 6).

In another embodiment, the cancer-associated antigen is a neoantigen.

As used herein the term "neoantigen" is an epitope that has at least one alteration that makes it distinct from the corresponding wild-type, parental antigen, e.g., via mutation in a tumor cell or post-translational modification specific to a tumor cell. A neoantigen can include a polypeptide sequence or a nucleotide sequence. A mutation can include a frameshift or nonframeshift indel, missense or nonsense substitution, splice site alteration, genomic rearrangement or gene fusion, or any genomic or expression alteration giving rise to a neoORF. A mutation can also include a splice variant. Post-translational modifications specific to a tumor cell can include aberrant phosphorylation. Post-translational modifications specific to a tumor cell can also include a proteasome-generated spliced antigen.

An example of a mutant APC antigen is QATEAERSF (SEQ ID NO: 3).

Examples of BRCA mutated epitopes are YIHTHTFYV (SEQ ID NO: 22) and SQIWNLNPV (SEQ ID NO: 23) HLA-A*02:01 restricted neoepitopes.

An examples of a universal HLA-DR-binding T helper synthetic epitope (AKFVAAWTLKAAA, SEQ ID NO: 311) is the pan DR-biding epitope (PADRE), which is a 13 amino acid peptide that activates CD4⁺ T cells.

Another contemplated cancer-associated neoantigen is the GL261 neoantigen (mImp3 D81N, sequence AALLNKLYA – SEQ ID NO: 6).

The bacteria described herein are genetically modified to express the cancer associated antigen, intracellularly and/or on the bacterial surface (i.e., genetic surface display). In another embodiment, the bacteria are genetically modified to secrete the cancer associated antigen.

For example, in some embodiments, the bacteria comprises a nucleic acid encoding the cancer-associated antigen operably linked to transcriptional regulatory elements, such as a bacterial promotor. The transcriptional regulatory element can further comprise a secretion signal. In some embodiments, the cancer-associated antigen is constitutively expressed by the bacteria. In some embodiments, the cancer-associated antigen is inducibly expressed by the bacteria (e.g., it is expressed upon exposure to a sugar or an environmental stimulus like low pH or an anaerobic environment). In some embodiments, the bacteria comprises a plurality of nucleic acid sequences that encode for multiple different cancer-associated antigens that can be expressed by the same bacterial cell.

In some embodiments, the bacteria displays a recombinantly produced cancer-associated antigen on its surface using a bacterial surface display system. Examples of bacterial surface display systems include outer membrane protein systems (e.g., LamB, FhuA, OmpL, OmpA, OmpC, OmpT, eCPX derived from OmpX, OprF, and PgsA), surface appendage systems (e.g., F

pillin, FimH, FimA, FliC, and FliD), lipoprotein systems (e.g., INP, Lpp-OmpA, PAL, Tat-dependent, and TraT), and virulence factor-based systems (e.g., AIDA-1, EaeA, EstA, EspP, MSP1a, and invasins). Exemplary surface display systems are described, for example, in van Bloois, E., et al., Trends in Biotechnology, 2011, 29:79-86, which is hereby incorporated by reference.

5 Examples of bacterial promoters include but are not limited to STM1787 promoter, pepT promoter, pflE promoter, ansB promoter, vhb promoter, FF+20* promoter or p(luxI) promoter.

 In some embodiments, the genetically modified bacteria described herein comprise a cancer therapeutic (e.g., the cancer therapeutic is loaded into the bacteria prior to administration to a subject, or is genetically modified to express the cancer therapeutic).

10 In some embodiments, the cancer therapeutic is loaded into the bacteria by growing the bacteria in a medium that contains a high concentration (e.g., at least 1 mM) of the cancer therapeutic, which leads to either uptake of the cancer therapeutic during cell growth or binding of the cancer therapeutic to the outside of the bacteria. The cancer therapeutic can be taken up passively (e.g. by diffusion and/or partitioning into the lipophilic cell membrane) or actively
15 through membrane channels or transporters. In some embodiments, drug loading is improved by adding additional substances to the growth medium that either increase uptake of the molecule of interest (e.g., Pluronic F-127) or prevent extrusion of the molecules after uptake by the bacterium (e.g., efflux pump inhibitors like Verapamil, Reserpine, Carsonic acid, or Piperine). In some
20 embodiments, the bacteria is loaded with the cancer therapeutic by mixing the bacteria with the cancer therapeutic and then subjecting the mixture to electroporation, for example, as described in Sustarsic M., et al., Cell Biol., 2014, 142(1):113-24, which is hereby incorporated by reference. In some embodiments, the cells can also be treated with an efflux pump inhibitor (see above) after the electroporation to prevent extrusion of the loaded molecules.

25 In still further embodiments, the bacteria is genetically modified to express the cancer therapeutic.

 In some embodiments the bacteria of the vaccine comprise an inhibitory antibody or small molecule directed against the immune checkpoint protein - e.g. anti-CTLA4, anti-CD40, anti-41BB, anti-OX40, anti-PD1 and anti-PDL1.

30 The present inventors further contemplate that the bacteria of the vaccine may comprise therapeutic agents attached to the outside of the bacteria using an attachment method such as CLICK chemistry. Such methods are further described in US Patent Application No. 20200087703 and US Patent Application No.20200054739, the contents of which are incorporated herein by reference.

Examples of therapeutic agents include immune modulatory proteins, such as a cytokine. Examples of immune modulating proteins include, but are not limited to, B lymphocyte chemoattractant ("BLC"), C--C motif chemokine 11 ("Eotaxin-1"), Eosinophil chemotactic protein 2 ("Eotaxin-2"), Granulocyte colony-stimulating factor ("G-CSF"), Granulocyte macrophage colony-stimulating factor ("GM-CSF"), 1-309, Intercellular Adhesion Molecule 1 ("ICAM-1"), Interferon gamma ("IFN-gamma"), Interlukin-1 alpha ("IL-1 alpha"), Interlukin-1 beta ("IL-1 beta"), Interleukin 1 receptor antagonist ("IL-1ra"), Interleukin-2 ("IL-2"), Interleukin-4 ("IL-4"), Interleukin-5 ("IL-5"), Interleukin-6 ("IL-6"), Interleukin-6 soluble receptor ("IL-6 sR"), Interleukin-7 ("IL-7"), Interleukin-8 ("IL-8"), Interleukin-10 ("IL-10"), Interleukin-11 ("IL-11"), Subunit beta of Interleukin- 12 ("IL-12 p40" or "IL-12 p70"), Interleukin-13 ("IL-13"), Interleukin-15 ("IL-15"), Interleukin-16 ("IL-16"), Interleukin-17 ("IL-17"), Chemokine (C--C motif) Ligand 2 ("MCP-1"), Macrophage colony-stimulating factor ("M-CSF"), Monokine induced by gamma interferon ("MIG"), Chemokine (C--C motif) ligand 2 ("MIP-1 alpha"), Chemokine (C--C motif) ligand 4 ("MIP-1 beta"), Macrophage inflammatory protein- 1 -delta ("MIP-1 delta"), Platelet-derived growth factor subunit B ("PDGF-BB"), Chemokine (C--C motif) ligand 5, Regulated on Activation, Normal T cell Expressed and Secreted ("RANTES"), TIMP metalloproteinase inhibitor 1 ("TIMP-1"), TIMP metalloproteinase inhibitor 2 ("TIMP-2"), Tumor necrosis factor, lymphotoxin-alpha ("TNF alpha"), Tumor necrosis factor, lymphotoxin-beta ("TNF beta"), Soluble TNF receptor type 1 ("sTNFRI"), sTNFRIIAR, Brain-derived neurotrophic factor ("BDNF"), Basic fibroblast growth factor ("bFGF"), Bone morphogenetic protein 4 ("BMP-4"), Bone morphogenetic protein 5 ("BMP-5"), Bone morphogenetic protein 7 ("BMP-7"), Nerve growth factor ("b-NGF"), Epidermal growth factor ("EGF"), Epidermal growth factor receptor ("EGFR"), Endocrine-gland-derived vascular endothelial growth factor ("EG-VEGF"), Fibroblast growth factor 4 ("FGF-4"), Keratinocyte growth factor ("FGF-7"), Growth differentiation factor 15 ("GDF-15"), Glial cell-derived neurotrophic factor ("GDNF"), Growth Hormone, Heparin-binding EGF-like growth factor ("HB-EGF"), Hepatocyte growth factor ("HGF"), Insulin-like growth factor binding protein 1 ("IGFBP-1"), Insulin-like growth factor binding protein 2 ("IGFBP-2"), Insulin-like growth factor binding protein 3 ("IGFBP-3"), Insulin-like growth factor binding protein 4 ("IGFBP-4"), Insulin-like growth factor binding protein 6 ("IGFBP-6"), Insulin-like growth factor 1 ("IGF-1"), Insulin, Macrophage colony-stimulating factor ("M-CSF R"), Nerve growth factor receptor ("NGF R"), Neurotrophin-3 ("NT-3"), Neurotrophin-4 ("NT-4"), Osteoclastogenesis inhibitory factor ("Osteoprotegerin"), Platelet-derived growth factor receptors ("PDGF-AA"), Phosphatidylinositol-glycan biosynthesis ("PIGF"), Skp, Cullin, F-box containing complex ("SCF"), Stem cell factor

receptor ("SCF R"), Transforming growth factor alpha ("TGFalpha"), Transforming growth factor beta-1 ("TGF beta 1"), Transforming growth factor beta-3 ("TGF beta 3"), Vascular endothelial growth factor ("VEGF"), Vascular endothelial growth factor receptor 2 ("VEGFR2"), Vascular endothelial growth factor receptor 3 ("VEGFR3"), VEGF-D 6Ckine, Tyrosine-protein kinase receptor UFO ("Axl"), Betacellulin ("BTC"), Mucosae-associated epithelial chemokine ("CCL28"), Chemokine (C--C motif) ligand 27 ("CTACK"), Chemokine (C--X--C motif) ligand 16 ("CXCL16"), C--X--C motif chemokine 5 ("ENA-78"), Chemokine (C--C motif) ligand 26 ("Eotaxin-3"), Granulocyte chemotactic protein 2 ("GCP-2"), GRO, Chemokine (C--C motif) ligand 14 ("HCC-1"), Chemokine (C--C motif) ligand 16 ("HCC-4"), Interleukin-9 ("IL-9"), Interleukin-17 F ("IL-17F"), Interleukin-18-binding protein ("IL-18 BPa"), Interleukin-28 A ("IL-28A"), Interleukin 29 ("IL-29"), Interleukin 31 ("IL-31"), C--X--C motif chemokine 10 ("IP-10"), Chemokine receptor CXCR3 ("I-TAC"), Leukemia inhibitory factor ("LIF"), Light, Chemokine (C motif) ligand ("Lymphotactin"), Monocyte chemoattractant protein 2 ("MCP-2"), Monocyte chemoattractant protein 3 ("MCP-3"), Monocyte chemoattractant protein 4 ("MCP-4"), Macrophage-derived chemokine ("MDC"), Macrophage migration inhibitory factor ("MIF"), Chemokine (C--C motif) ligand 20 ("MIP-3 alpha"), C--C motif chemokine 19 ("MIP-3 beta"), Chemokine (C--C motif) ligand 23 ("MPIF-1"), Macrophage stimulating protein alpha chain ("MSPalpha"), Nucleosome assembly protein 1-like 4 ("NAP-2"), Secreted phosphoprotein 1 ("Osteopontin"), Pulmonary and activation-regulated cytokine ("PARC"), Platelet factor 4 ("PF4"), Stroma cell-derived factor-1 alpha ("SDF-1 alpha"), Chemokine (C--C motif) ligand 17 ("TARC"), Thymus-expressed chemokine ("TECK"), Thymic stromal lymphopoietin ("TSLP 4-IBB"), CD 166 antigen ("ALCAM"), Cluster of Differentiation 80 ("B7-1"), Tumor necrosis factor receptor superfamily member 17 ("BCMA"), Cluster of Differentiation 14 ("CD14"), Cluster of Differentiation 30 ("CD30"), Cluster of Differentiation 40 ("CD40 Ligand"), Carcinoembryonic antigen-related cell adhesion molecule 1 (biliary glycoprotein) ("CEACAM-1"), Death Receptor 6 ("DR6"), Deoxythymidine kinase ("Dtk"), Type 1 membrane glycoprotein ("Endoglin"), Receptor tyrosine-protein kinase erbB-3 ("ErbB3"), Endothelial-leukocyte adhesion molecule 1 ("E-Selectin"), Apoptosis antigen 1 ("Fas"), Fms-like tyrosine kinase 3 ("Flt-3L"), Tumor necrosis factor receptor superfamily member 1 ("GITR"), Tumor necrosis factor receptor superfamily member 14 ("HVEM"), Intercellular adhesion molecule 3 ("ICAM-3"), IL-1 R4, IL-1 RI, IL-10 Rbeta, IL-17R, IL-2Rgamma, IL-21R, Lysosome membrane protein 2 ("LIMP2"), Neutrophil gelatinase-associated lipocalin ("Lipocalin-2"), CD62L ("L-Selectin"), Lymphatic endothelium ("LYVE-1"), MEC class I polypeptide-related sequence A ("MICA"), MEC class I polypeptide-

related sequence B ("MICB"), NRG1-beta1, Beta-type platelet-derived growth factor receptor ("PDGF Rbeta"), Platelet endothelial cell adhesion molecule ("PECAM-1"), RAGE, Hepatitis A virus cellular receptor 1 ("TIM-1"), Tumor necrosis factor receptor superfamily member IOC ("TRAIL R3"), Trappin protein transglutaminase binding domain ("Trappin-2"), Urokinase receptor ("uPAR"), Vascular cell adhesion protein 1 ("VCAM-1"), XEDARActivin A, Agouti-related protein ("AgRP"), Ribonuclease 5 ("Angiogenin"), Angiopoietin 1, Angiostatin, Cathepsin S, CD40, Cryptic family protein IB ("Cripto-1"), DAN, Dickkopf-related protein 1 ("DKK-1"), E-Cadherin, Epithelial cell adhesion molecule ("EpCAM"), Fas Ligand (FasL or CD95L), Fcg RIIB/C, FoUistatin, Galectin-7, Intercellular adhesion molecule 2 ("ICAM-2"), IL-13 R1, IL-13R2, IL-17B, IL-2 Ra, IL-2 Rb, IL-23, LAP, Neuronal cell adhesion molecule ("NrCAM"), Plasminogen activator inhibitor-1 ("PAI-1"), Platelet derived growth factor receptors ("PDGF-AB"), Resistin, stromal cell-derived factor 1 ("SDF-1 beta"), sgp130, Secreted frizzled-related protein 2 ("ShhN"), Sialic acid-binding immunoglobulin-type lectins ("Siglec-5"), ST2, Transforming growth factor-beta 2 ("TGF beta 2"), Tie-2, Thrombopoietin ("TPO"), Tumor necrosis factor receptor superfamily member 10D ("TRAIL R4"), Triggering receptor expressed on myeloid cells 1 ("TREM-1"), Vascular endothelial growth factor C ("VEGF-C"), VEGFR1Adiponectin, Adipsin ("AND"), Alpha-fetoprotein ("AFP"), Angiopoietin-like 4 ("ANGPTL4"), Beta-2-microglobulin ("B2M"), Basal cell adhesion molecule ("BCAM"), Carbohydrate antigen 125 ("CA125"), Cancer Antigen 15-3 ("CA15-3"), Carcinoembryonic antigen ("CEA"), cAMP receptor protein ("CRP"), Human Epidermal Growth Factor Receptor 2 ("ErbB2"), Follistatin, Follicle-stimulating hormone ("FSH"), Chemokine (C-X-C motif) ligand 1 ("GRO alpha"), human chorionic gonadotropin ("beta HCG"), Insulin-like growth factor 1 receptor ("IGF-1 sR"), IL-1 sRII, IL-3, IL-18 Rb, IL-21, Leptin, Matrix metalloproteinase-1 ("MMP-1"), Matrix metalloproteinase-2 ("MMP-2"), Matrix metalloproteinase-3 ("MMP-3"), Matrix metalloproteinase-8 ("MMP-8"), Matrix metalloproteinase-9 ("MMP-9"), Matrix metalloproteinase-10 ("MMP-10"), Matrix metalloproteinase-13 ("MMP-13"), Neural Cell Adhesion Molecule ("NCAM-1"), Entactin ("Nidogen-1"), Neuron specific enolase ("NSE"), Oncostatin M ("OSM"), Procalcitonin, Prolactin, Prostate specific antigen ("PSA"), Sialic acid-binding Ig-like lectin 9 ("Siglec-9"), ADAM 17 endopeptidase ("TACE"), Thyroglobulin, Metalloproteinase inhibitor 4 ("TIMP-4"), TSH2B4, Disintegrin and metalloproteinase domain-containing protein 9 ("ADAM-9"), Angiopoietin 2, Tumor necrosis factor ligand superfamily member 13/ Acidic leucine-rich nuclear phosphoprotein 32 family member B ("APRIL"), Bone morphogenetic protein 2 ("BMP-2"), Bone morphogenetic protein 9 ("BMP-9"), Complement component 5a ("C5a"), Cathepsin L, CD200, CD97, Chemerin,

Tumor necrosis factor receptor superfamily member 6B ("DcR3"), Fatty acid-binding protein 2 ("FABP2"), Fibroblast activation protein, alpha ("FAP"), Fibroblast growth factor 19 ("FGF-19"), Galectin-3, Hepatocyte growth factor receptor ("HGF R"), IFN-gamma/beta R2, Insulin-like growth factor 2 ("IGF-2"), Insulin-like growth factor 2 receptor ("IGF-2 R"), Interleukin-1 receptor 6 ("IL-1R6"), Interleukin 24 ("IL-24"), Interleukin 33 ("IL-33"), Kallikrein 14, Asparaginyl endopeptidase ("Legumain"), Oxidized low-density lipoprotein receptor 1 ("LOX-1"), Mannose-binding lectin ("MBL"), Neprilysin ("NEP"), Notch homolog 1, translocation-associated (Drosophila) ("Notch-1"), Nephroblastoma overexpressed ("NOV"), Osteoactivin, Programmed cell death protein 1 ("PD-1"), N-acetylmuramoyl-L-alanine amidase ("PGRP-5"), Serpin A4, Secreted frizzled related protein 3 ("sFRP-3"), Thrombomodulin, Toll-like receptor 2 ("TLR2"), Tumor necrosis factor receptor superfamily member 10A ("TRAIL R1"), Transferrin ("TRF"), WIF-1/ACE-2, Albumin, AMICA, Angiopoietin 4, B-cell activating factor ("BAFF"), Carbohydrate antigen 19-9 ("CA19-9"), CD 163, Clusterin, CRT AM, Chemokine (C-X-C motif) ligand 14 ("CXCL14"), Cystatin C, Decorin ("DCN"), Dickkopf-related protein 3 ("Dkk-3"), Delta-like protein 1 ("DLL1"), Fetuin A, Heparin-binding growth factor 1 ("aFGF"), Folate receptor alpha ("FOLR1"), Furin, GPCR-associated sorting protein 1 ("GASP-1"), GPCR-associated sorting protein 2 ("GASP-2"), Granulocyte colony-stimulating factor receptor ("G-CSF R"), Serine protease hepsin ("HAI-2"), Interleukin-17B Receptor ("IL-17B R"), Interleukin 27 ("IL-27"), Lymphocyte-activation gene 3 ("LAG-3"), Apolipoprotein A-V ("LDL R"), Pepsinogen I, Retinol binding protein 4 ("RBP4"), SOST, Heparan sulfate proteoglycan ("Syndecan-1"), Tumor necrosis factor receptor superfamily member 13B ("TACI"), Tissue factor pathway inhibitor ("TFPI"), TSP-1, Tumor necrosis factor receptor superfamily, member 10b ("TRAIL R2"), TRANCE, Troponin I, Urokinase Plasminogen Activator ("uPA"), Cadherin 5, type 2 or VE-cadherin (vascular endothelial) also known as CD144 ("VE-Cadherin"), WNT1-inducible-signaling pathway protein 1 ("WISP-1"), and Receptor Activator of Nuclear Factor κ . B ("RANK"). The immune modulatory protein can be made recombinantly using methods known to one skilled in the art. The immune modulatory protein can be presented on the surface of a bacterium using bacterial surface display, where the bacterium expresses a genetically engineered protein-protein fusion of e.g., a membrane protein and the immune modulatory protein.

The bacteria of the vaccine of the present invention may serve as an adjuvant, thereby rendering the use of additional adjuvant not relevant.

In one embodiment, the vaccine is devoid of adjuvant (other than the bacteria itself).

In another embodiment, the vaccine comprises an adjuvant additional to the bacteria.

Adjuvants are substance that can be added to an immunogen or to a vaccine formulation to enhance the immune-stimulating properties of the immunogenic moiety. Examples of adjuvants or agents that may add to the effectiveness of proteinaceous immunogens include aluminum hydroxide, aluminum phosphate, aluminum potassium sulfate (alum), beryllium sulfate, silica, kaolin, carbon, water-in-oil emulsions, and oil-in-water emulsions. A particular type of adjuvant is muramyl dipeptide (MDP) and various MDP derivatives and formulations, e.g., N-acetyl-D-glucosaminyl-(.beta.1-4)-N-acetylmuramyl-L-alanyl-D-isoglutamine (GMDP) (Hornung, R L et al. Ther Immunol 1995 2:7-14) or ISAF-1 (5% squalene, 2.5% pluronic L121, 0.2% Tween 80 in phosphate-buffered solution with 0.4 mg of threonyl-muramyl dipeptide; see Kwak, L W et al. (1992) N. Engl. J. Med., 327:1209-1238). Other useful adjuvants are, or are based on, cholera toxin, bacterial endotoxin, lipid X, whole organisms or subcellular fractions of the bacteria *Propionobacterium acnes* or *Bordetella pertussis*, polyribonucleotides, sodium alginate, lanolin, lysolecithin, vitamin A, saponin and saponin derivatives such as QS21 (White, A. C. et al. (1991) Adv. Exp. Med. Biol., 303:207-210) which is now in use in the clinic (Helling, F et al. (1995) Cancer Res., 55:2783-2788; Davis, T A et al. (1997) Blood, 90: 509), levamisole, DEAE-dextran, blocked copolymers or other synthetic adjuvants. A number of adjuvants are available commercially from various sources, for example, Merck Adjuvant 65 (Merck and Company, Inc., Rahway, N.J.) or Freund's Incomplete Adjuvant and Complete Adjuvant (Difco Laboratories, Detroit, Mich.), Amphigen (oil-in-water), Alhydrogel (aluminum hydroxide), or a mixture of Amphigen and Alhydrogel. Aluminum is approved for human use.

As mentioned, the vaccines described herein may be used to treat and/or prevent cancer.

As used herein, the term "treating" includes abrogating, substantially inhibiting, slowing or reversing the progression of a condition, substantially ameliorating clinical or aesthetical symptoms of a condition.

According to a particular embodiment, the term preventing refers to substantially preventing the appearance of clinical or aesthetical symptoms of a condition.

Particular subjects which are treated are mammalian subjects - e.g. humans.

According to a particular embodiment, the subject has been diagnosed as having cancer.

Cancer

The term "cancer" as used herein refers to an uncontrolled, abnormal growth of a host's own cells which may lead to invasion of surrounding tissue and potentially tissue distal to the initial site of abnormal cell growth in the host. Major classes include carcinomas which are cancers of the epithelial tissue (e.g., skin, squamous cells); sarcomas which are cancers of the connective tissue

(e.g., bone, cartilage, fat, muscle, blood vessels, etc.); leukemias which are cancers of blood forming tissue (e.g., bone marrow tissue); lymphomas and myelomas which are cancers of immune cells; and central nervous system cancers which include cancers from brain and spinal tissue. "Cancer(s)," "neoplasm(s)," and "tumor(s)" are used herein interchangeably. As used herein, "cancer" refers to all types of cancer or neoplasm or malignant tumors including leukemias, carcinomas and sarcomas, whether new or recurring.

Specific examples of cancers that may be treated using the bacteria described herein include, but are not limited to adrenocortical carcinoma, hereditary; bladder cancer; breast cancer; breast cancer, ductal; breast cancer, invasive intraductal; breast cancer, sporadic; breast cancer, susceptibility to; breast cancer, type 4; breast cancer, type 4; breast cancer-1; breast cancer-3; breast-ovarian cancer; triple negative breast cancer, Burkitt's lymphoma; cervical carcinoma; colorectal adenoma; colorectal cancer; colorectal cancer, hereditary nonpolyposis, type 1; colorectal cancer, hereditary nonpolyposis, type 2; colorectal cancer, hereditary nonpolyposis, type 3; colorectal cancer, hereditary nonpolyposis, type 6; colorectal cancer, hereditary nonpolyposis, type 7; dermatofibrosarcoma protuberans; endometrial carcinoma; esophageal cancer; gastric cancer; fibrosarcoma; glioblastoma multiforme; glomus tumors, multiple; hepatoblastoma; hepatocellular cancer; hepatocellular carcinoma; leukemia, acute lymphoblastic; leukemia, acute myeloid; leukemia, acute myeloid, with eosinophilia; leukemia, acute nonlymphocytic; leukemia, chronic myeloid; Li-Fraumeni syndrome; liposarcoma; lung cancer; lung cancer, small cell; lymphoma, non-Hodgkin's; lynch cancer family syndrome II; male germ cell tumor; mast cell leukemia; medullary thyroid; medulloblastoma; melanoma, malignant melanoma, meningioma; multiple endocrine neoplasia; multiple myeloma, myeloid malignancy, predisposition to; myxosarcoma, neuroblastoma; osteosarcoma; osteocarcinoma, ovarian cancer; ovarian cancer, serous; ovarian carcinoma; ovarian sex cord tumors; pancreatic cancer; pancreatic endocrine tumors; paraganglioma, familial nonchromaffin; pilomatricoma; pituitary tumor, invasive; prostate adenocarcinoma; prostate cancer; renal cell carcinoma, papillary, familial and sporadic; retinoblastoma; rhabdoid predisposition syndrome, familial; rhabdoid tumors; rhabdomyosarcoma; small-cell cancer of lung; soft tissue sarcoma, squamous cell carcinoma, basal cell carcinoma, head and neck; T-cell acute lymphoblastic leukemia; Turcot syndrome with glioblastoma; tylosis with esophageal cancer; uterine cervix carcinoma, Wilms' tumor, type 2; and Wilms' tumor, type 1, and the like.

According to a particular embodiment, the cancer is selected from the group consisting of breast, melanoma, pancreatic cancer, ovarian cancer, bone cancer and brain cancer (e.g. glioblastoma).

According to another embodiment, the cancer is melanoma.

5 Malignant melanomas are clinically recognized based on the ABCD(E) system, where A stands for asymmetry, B for border irregularity, C for color variation, D for diameter >5 mm, and E for evolving. Further, an excision biopsy can be performed in order to corroborate a diagnosis using microscopic evaluation. Infiltrative malignant melanoma is traditionally divided into four principal histopathological subgroups: superficial spreading melanoma (SSM), nodular malignant
10 melanoma (NMM), lentigo maligna melanoma (LMM), and acral lentiginous melanoma (ALM). Other rare types also exist, such as desmoplastic malignant melanoma. A substantial subset of malignant melanomas appear to arise from melanocytic nevi and features of dysplastic nevi are often found in the vicinity of infiltrative melanomas. Melanoma is thought to arise through stages of progression from normal melanocytes or nevus cells through a dysplastic nevus stage and further
15 to an in situ stage before becoming invasive. Some of the subtypes evolve through different phases of tumor progression, which are called radial growth phase (RGP) and vertical growth phase (VGP).

In a particular embodiment, the melanoma is resistant to treatment with inhibitors of BRAF and/or MEK.

20 The tumor may be a primary tumor or a secondary tumor (i.e. metastasized tumor).

The compositions may be administered using any route such as for example oral administration, rectal administration, topical administration, inhalation (nasal) or injection. Administration by injection includes intravenous (IV), intramuscular (IM), intratumoral (IT), subcutaneous (ST), peritumoral (PT), and subcutaneous (SC) administration. The pharmaceutical
25 compositions described herein can be administered in any form by any effective route, including but not limited to intratumoral, oral, parenteral, enteral, intravenous, intraperitoneal, topical, transdermal (e.g., using any standard patch), intradermal, ophthalmic, (intra)nasally, local, non-oral, such as aerosol, inhalation, subcutaneous, intramuscular, buccal, sublingual, (trans)rectal, vaginal, intra-arterial, and intrathecal, transmucosal (e.g., sublingual, lingual, (trans)buccal,
30 (trans)urethral, vaginal (e.g., trans- and perivaginally), intravesical, intrapulmonary, intraduodenal, intragastric, and intrabronchial. In preferred embodiments, the pharmaceutical compositions described herein are administered orally, rectally, intratumorally, topically, intravesically, by

injection into or adjacent to a draining lymph node, intravenously, by inhalation or aerosol, or subcutaneously.

According to another aspect of the present invention there is provided a method of treating cancer of a subject in need thereof comprising administering to the subject a therapeutically effective amount of:

(i) a first vaccine comprising a first bacteria which is genetically modified to express at least one cancer-associated antigen; and subsequently;

(ii) a second vaccine comprising a second bacteria which is genetically modified to express at least one cancer-associated antigen, thereby treating the cancer.

The present invention contemplates at least 2 different vaccination cycles for the treatment of cancer, wherein at least one of the vaccination cycles includes one strain of genetically modified bacteria and at least another of the vaccination cycles includes a second (non-identical) genetically modified strain of bacteria. The two strains of bacteria may be genetically modified to express the same cancer associated antigens or different cancer associated antigens. Additionally, or alternatively, the present inventors contemplate at least one of the vaccination cycles includes viable bacteria (e.g, the first vaccination) and at least another of the vaccination cycles (e.g. a subsequent vaccination) includes attenuated (or dead) bacteria.

The vaccine of the present invention may be administered with additional anti-cancer agents.

In some embodiments the additional anti-cancer agent is an inhibitory antibody or small molecule directed against the immune checkpoint protein - e.g. anti-CTLA4, anti-CD40, anti-41BB, anti-OX40, anti-PD1 and anti-PDL1.

Other contemplated anti-cancer agents which may be administered to the subject in combination with the bacteria described herein include, but are not limited to Acivicin; Aclarubicin; Acodazole Hydrochloride; Acronine; Adriamycin; Adozelesin; Aldesleukin; Altretamine; Ambomycin; Ametantrone Acetate; Aminoglutethimide; Amsacrine; Anastrozole; Anthramycin; Asparaginase; Asperlin; Azacitidine; Azetepa; Azotomycin; Batimastat; Benzodepa; Bicalutamide; Bisantrene Hydrochloride; Bisnafide Dimesylate; Bizelesin; Bleomycin Sulfate; Brequinar Sodium; Bropirimine; Busulfan; Cactinomycin; Calusterone; Caracemide; Carbetimer; Carboplatin; Carmustine; Carubicin Hydrochloride; Carzelesin; Cedefingol; Chlorambucil; Cirolemycin; Cisplatin; Cladribine; Crisnatol Mesylate; Cyclophosphamide; Cytarabine; Dacarbazine; Dactinomycin; Daunorubicin Hydrochloride; Decitabine; Dexormaplatin; Dezaguanine; Dezaguanine Mesylate; Diaziquone; Docetaxel;

Doxorubicin; Doxorubicin Hydrochloride; Droloxifene; Droloxifene Citrate; Dromostanolone Propionate; Duazomycin; Edatrexate; Eflornithine Hydrochloride; Elsamitrucin; Enloplatin; Enpromate; Epipropidine; Epirubicin Hydrochloride; Erbulozole; Esorubicin Hydrochloride; Estramustine; Estramustine Phosphate Sodium; Etanidazole; Etoposide; Etoposide Phosphate; 5 Etoprine; Fadrozole Hydrochloride; Fazarabine; Fenretinide; Floxuridine; Fludarabine Phosphate; Fluorouracil; Flurocitabine; Fosquidone; Fostriecin Sodium; Gemcitabine; Gemcitabine Hydrochloride; Hydroxyurea; Idarubicin Hydrochloride; Ifosfamide; Ilmofofosine; Interferon Alfa-2a; Interferon Alfa-2b; Interferon Alfa-n1; Interferon Alfa-n3; Interferon Beta- I a; Interferon Gamma- I b; Iproplatin; Irinotecan Hydrochloride; Lanreotide Acetate; Letrozole; Leuprolide 10 Acetate; Liarozole Hydrochloride; Lometrexol Sodium; Lomustine; Losoxantrone Hydrochloride; Masoprocol; Maytansine; Mechlorethamine Hydrochloride; Megestrol Acetate; Melengestrol Acetate; Melphalan; Menogaril; Mercaptopurine; Methotrexate; Methotrexate Sodium; Metoprine; Meturedepa; Mitindomide; Mitocarcin; Mitocromin; Mitogillin; Mitomalcin; Mitomycin; Mitosper; Mitotane; Mitoxantrone Hydrochloride; Mycophenolic Acid; Nocodazole; 15 Nogalamycin; Ormaplatin; Oxisuran; Paclitaxel; Pegaspargase; Peliomycin; Pentamustine; Peplomycin Sulfate; Perfosfamide; Pipobroman; Pipo sulfan; Piroxantrone Hydrochloride; Plicamycin; Plomestane; Porfimer Sodium; Porfiromycin; Prednimustine; Procarbazine Hydrochloride; Puromycin; Puromycin Hydrochloride; Pyrazofurin; Riboprine; Rogletimide; Safingol; Safingol Hydrochloride; Semustine; Simtrazene; Sparfosate Sodium; Sparsomycin; 20 Spirogermanium Hydrochloride; Spiromustine; Spiroplatin; Streptonigrin; Streptozocin; Sulofenur; Talisomycin; Taxol; Tecogalan Sodium; Tegafur; Teloxantrone Hydrochloride; Temoporfin; Teniposide; Teroxirone; Testolactone; Thiamiprine; Thioguanine; Thiotepa; Tiazofuirin; Tirapazamine; Topotecan Hydrochloride; Toremifene Citrate; Trestolone Acetate; Triciribine Phosphate; Trimetrexate; Trimetrexate Glucuronate; Triptorelin; Tubulozole 25 Hydrochloride; Uracil Mustard; Uredepa; Vapreotide; Verteporfin; Vinblastine Sulfate; Vincristine Sulfate; Vindesine; Vindesine Sulfate; Vinepidine Sulfate; Vinglycinate Sulfate; Vinleurosine Sulfate; Vinorelbine Tartrate; Vinrosidine Sulfate; Vinzolidine Sulfate; Vorozole; Zeniplatin; Zinostatin; Zorubicin Hydrochloride. Additional antineoplastic agents include those disclosed in Chapter 52, Antineoplastic Agents (Paul Calabresi and Bruce A. Chabner), and the 30 introduction thereto, 1202-1263, of Goodman and Gilman's "The Pharmacological Basis of Therapeutics", Eighth Edition, 1990, McGraw-Hill, Inc. (Health Professions Division).

As used herein the term "about" refers to $\pm 10\%$

The terms "comprises", "comprising", "includes", "including", "having" and their conjugates mean "including but not limited to".

The term "consisting of" means "including and limited to".

5 The term "consisting essentially of" means that the composition, method or structure may include additional ingredients, steps and/or parts, but only if the additional ingredients, steps and/or parts do not materially alter the basic and novel characteristics of the claimed composition, method or structure.

10 As used herein, the singular form "a", "an" and "the" include plural references unless the context clearly dictates otherwise. For example, the term "a compound" or "at least one compound" may include a plurality of compounds, including mixtures thereof.

15 Throughout this application, various embodiments of this invention may be presented in a range format. It should be understood that the description in range format is merely for convenience and brevity and should not be construed as an inflexible limitation on the scope of the invention. Accordingly, the description of a range should be considered to have specifically disclosed all the possible subranges as well as individual numerical values within that range. For example, description of a range such as from 1 to 6 should be considered to have specifically disclosed subranges such as from 1 to 3, from 1 to 4, from 1 to 5, from 2 to 4, from 2 to 6, from 3 to 6 etc., as well as individual numbers within that range, for example, 1, 2, 3, 4, 5, and 6. This applies regardless of the breadth of the range.

20 Whenever a numerical range is indicated herein, it is meant to include any cited numeral (fractional or integral) within the indicated range. The phrases "ranging/ranges between" a first indicate number and a second indicate number and "ranging/ranges from" a first indicate number "to" a second indicate number are used herein interchangeably and are meant to include the first and second indicated numbers and all the fractional and integral numerals therebetween.

25 As used herein the term "method" refers to manners, means, techniques and procedures for accomplishing a given task including, but not limited to, those manners, means, techniques and procedures either known to, or readily developed from known manners, means, techniques and procedures by practitioners of the chemical, pharmacological, biological, biochemical and medical arts.

30 It is appreciated that certain features of the invention, which are, for clarity, described in the context of separate embodiments, may also be provided in combination in a single embodiment. Conversely, various features of the invention, which are, for brevity, described in the context of a single embodiment, may also be provided separately or in any suitable subcombination or as

suitable in any other described embodiment of the invention. Certain features described in the context of various embodiments are not to be considered essential features of those embodiments, unless the embodiment is inoperative without those elements.

Various embodiments and aspects of the present invention as delineated hereinabove and as claimed in the claims section below find experimental support in the following examples.

EXAMPLES

Reference is now made to the following examples, which together with the above descriptions illustrate some embodiments of the invention in a non limiting fashion.

Generally, the nomenclature used herein and the laboratory procedures utilized in the present invention include molecular, biochemical, microbiological and recombinant DNA techniques. Such techniques are thoroughly explained in the literature. See, for example, "Molecular Cloning: A laboratory Manual" Sambrook et al., (1989); "Current Protocols in Molecular Biology" Volumes I-III Ausubel, R. M., ed. (1994); Ausubel et al., "Current Protocols in Molecular Biology", John Wiley and Sons, Baltimore, Maryland (1989); Perbal, "A Practical Guide to Molecular Cloning", John Wiley & Sons, New York (1988); Watson et al., "Recombinant DNA", Scientific American Books, New York; Birren et al. (eds) "Genome Analysis: A Laboratory Manual Series", Vols. 1-4, Cold Spring Harbor Laboratory Press, New York (1998); methodologies as set forth in U.S. Pat. Nos. 4,666,828; 4,683,202; 4,801,531; 5,192,659 and 5,272,057; "Cell Biology: A Laboratory Handbook", Volumes I-III Cellis, J. E., ed. (1994); "Culture of Animal Cells - A Manual of Basic Technique" by Freshney, Wiley-Liss, N. Y. (1994), Third Edition; "Current Protocols in Immunology" Volumes I-III Coligan J. E., ed. (1994); Stites et al. (eds), "Basic and Clinical Immunology" (8th Edition), Appleton & Lange, Norwalk, CT (1994); Mishell and Shiigi (eds), "Selected Methods in Cellular Immunology", W. H. Freeman and Co., New York (1980); available immunoassays are extensively described in the patent and scientific literature, see, for example, U.S. Pat. Nos. 3,791,932; 3,839,153; 3,850,752; 3,850,578; 3,853,987; 3,867,517; 3,879,262; 3,901,654; 3,935,074; 3,984,533; 3,996,345; 4,034,074; 4,098,876; 4,879,219; 5,011,771 and 5,281,521; "Oligonucleotide Synthesis" Gait, M. J., ed. (1984); "Nucleic Acid Hybridization" Hames, B. D., and Higgins S. J., eds. (1985); "Transcription and Translation" Hames, B. D., and Higgins S. J., eds. (1984); "Animal Cell Culture" Freshney, R. I., ed. (1986); "Immobilized Cells and Enzymes" IRL Press, (1986); "A Practical Guide to Molecular Cloning" Perbal, B., (1984) and "Methods in Enzymology" Vol. 1-317, Academic Press; "PCR Protocols: A Guide To Methods And Applications", Academic Press, San Diego, CA

(1990); Marshak et al., "Strategies for Protein Purification and Characterization - A Laboratory Course Manual" CSHL Press (1996); all of which are incorporated by reference as if fully set forth herein. Other general references are provided throughout this document. The procedures therein are believed to be well known in the art and are provided for the convenience of the reader. All the information contained therein is incorporated herein by reference.

MATERIALS AND METHODS

Plasmids:

To generate backbone plasmids for *Salmonella Typhimurium* strains, Ssph2 promoter and secretion signal (aa:1-200), or the pagC promoter and Ssph1 secretion signal (aa:1-208) were amplified from the *Salmonella Typhimurium* attenuated strain VNP20009. Ssph2 and pagC-Ssph1 were inserted into pQE60 by NEBuilder cloning kit (cat. E5520S).

Proteins of interest were fused with either Ssph1 or Ssph2. To generate a backbone plasmid for *Pseudomonas aeruginosa*, proteins of interest were fused with the N-terminal 54 amino acids of ExoS in plasmid pEAI3-S54 (a courtesy of Bertrand Toussaint, PMID: 17010670). To generate a backbone plasmid for *Bacillus Subtilis* spores, proteins of interest were fused with CotC (amplified from *Bacillus Subtilis* 168) and cloned into pDG364 plasmid. In addition, 6His tag element was inserted to allow detection of the protein product.

Neoantigens:

To obtain a neoantigen of B16-OVA tumors, the C-terminal of Ovalbumin (aa 252-386) was amplified from pcDNA-OVA (Addgene 64599). The amplified oligo contains the sequence which corresponds to SIINFEKL (SEQ ID NO: 11), the epitope of Ovalbumin.

To obtain a neoantigen of MC38 tumors, a section of Adpgk (aa 289-421) was amplified from cDNA of MC38 cells. The amplified oligo contains a sequence which corresponds to a validated neoantigen of MC38, based on Yadav et al. (PMID: 25428506).

Both neoantigens were inserted to the backbone plasmids by NEBuilder cloning kit.

Bacteria:

The attenuated *Salmonella Typhimurium* strains VNP20009 (also named YS1646, ATCC, cat. 202165) and STM3210 were transformed with the relevant plasmids by electroporation. Briefly, bacteria were cultured to OD of 0.6-0.8, washed 3 times with Hepes 1mM and suspended in 10% glycerol in DDW. Suspension was electroporated with 0.2 cm, cuvette (BioRad, EC2) and moved to 1 ml cold SOC. Following 1 hour incubation in 37 °C, bacteria were seeded on LB

agar plate containing ampicilin. Selected colonies were verified by Sanger sequencing and Western blot using anti-6His tag (Cell Signalling, 2365S).

The attenuated *pseudomonas aeruginosa* (CHA-OST) was transformed as described by Diver et al. PMID: 2126169. The *Bacillus Subtilis* strain PY79 was transformed following incubation in minimal medium and 0.01M MgSO_4 in DDW (MC: 80 mM K_2HPO_4 , 30 mM KH_2PO_4 , 2% Glucose, 30 mM Trisodium citrate, 22 $\mu\text{g/ml}$ Ferric ammonium citrate, 0.1% Casein Hydrolysate (CAA), 0.2 % potassium glutamate) for 3 hours to induce competent bacteria. Next, plasmid pDG364 which contains an antigen fused to CotC protein was cut with Xba and incubated with competent bacteria for 3 hours. Upon integration into the amylase gene, colonies were selected by resistance to chloramphenicol 5 $\mu\text{g/ml}$.

In all transformations, selected colonies were verified by Sanger sequencing and Western blot using anti-6His tag (Cell Signalling, 2365S).

Freezing working stocks of Salmonella typhimurium:

Exponentially growing culture (OD 0.6-0.8), was washed twice in cold PBS. Bacteria pellet was suspended in 25 % glycerol in PBS. A sample from the bacterial stock was serially diluted and seeded on LB agar plate, while the rest of the pool was aliquoted and stored in – 80 °C. To verify viability of bacteria, a frozen aliquot was defrosted and seeded on LB agar plate. Recovery rate following freezing was quantified by calculating the ratio of frozen/fresh CFU count. Calculation of bacteria dosage in mice experiment was based on the CFU count of the frozen culture.

Sporulation of Bacillus Subtilis PY79:

PY79 were grown in LB, at 37 °C to OD 0.8. LB medium was removed and replaced by half volume of DSM exhaustion medium. Culture was incubated at 37 °C, whilst shaking for 60 hrs. Finally, bacteria were washed twice in cold water. To quantify spores, and sporulation rate, a sample from the washed sample was seeded on LB agar plate pre- and post 1 hour heating at 65 °C. The ration of heated/non heated CFU count is indicative of sporulation rate. Exhaustion medium preparaion (per 1 liter): dissolve 8g Difco nutrient broth (BD, cat. 234000), 1g KCl and 1mM MgSO_4 in DDW. Titrate with NaOH to PH7.6 and autoclave. Before usage, add 10 μM MnCl_2 , 1mM $\text{Ca}(\text{NO}_3)_2$ and 1mM FeSO_4 .

Mice models:

B16-OVA mouse melanoma cell line (10^6) or MC38 mouse CRC cell line (10^5) were injected s.c. to the right flank of 7 weeks C57BL/6 females. Tumor volume was calculated as $\text{width}^2 \times \text{length} / 2$.

Immune profiling of splenocytes by FACS:

Freshly resected spleens were mashed on a 70 micron strainer into cold PBS. To lyse red blood cells, the splenocytes were incubated with ACK lysis buffer (Quality Biological, cat. 118-156-101), then washed thoroughly in PBS and suspended in FACS labeling buffer. 100 µl of splenocytes were incubated for 1 hour at 4 °C with a mixture containing Fc blocker (BD, cat. 553142, 1:100), SIINFEKL (SEQ ID NO: 11) Tetramer (NIH Tetramer Core Facility, 1:500), anti-CD4 (BioLegend, cat. 100438, 1:800), anti-CD8 (Invitrogen, cat. 2021-05-05, 1:400), anti-CD3 (Invitrogen, cat. 2023-07-31, 1:1000) and Brilliant Buffer (BD, cat. 566349, 1:5). Next, cells were washed twice in labeling buffer and fixed with CytoFix/CytoPerm solution (BD, cat. 51-2090KZ) for 20 mins at 4 °C. Finally, cells were washed twice in Perm/Wash buffer (BD, cat. 51-2091KZ, diluted in DDW 1:10) suspended in labeling buffer and subjected to FACS.

Quantification of activated CD8 T cells by Peptide stimulation

Splenocytes were produced as described above. Next, splenocytes were incubated with OVA peptide (final conc. 2.5 µg/ml) for 2 hours at 37 °C. Next, Brafeldin A (BD, 51-2301kz) was added to the cells and incubated for additional 4 hours at 4 °C. FACS staining for CD3, CD8 and INFg were performed the next day as described above.

Ex vivo killing assay

MC38 or B16-OVA cells were seeded on 48 well plate. Cells were stained with CFSE (5µM) for 20 min at 37 °C, then quenched with culture medium (RPMI with 10% FCS) for 10 min at 37 °C and washed twice with culture medium.

The next day, spleens were resected as described above and cells were counted. Next, 10⁵ splenocytes were co-cultured with the tumor cells and incubated for 72 hours at 37 °C.

Following incubation, FACS staining for DEAD/LIVE (Invitrogen, L34962) and CFSE positive (tumor cells) were performed the next day as described.

IFNg quantification by ELISA:

To quantify serum level of IFNg, mice were bled into Eppendorf tube containing 20 µl Heparin (10mg/ml). Following centrifugation for 10 mins, 10,000g, sera were transferred to new tubes for long term storage at -20 °C. ELISA was performed according to manufacturer instructions (R&D, cat. DY485) using sera diluted 1:4.

Bacteria quantification in liver and tumor:

Slices of tumors and livers were suspended in sterile tubes containing LB and metal beads. Following vortex for 10 minutes at max speed, 200 µl of sup, were seeded on LB plates with the relevant antibiotics and incubated over night at 37 °C.

RESULTS

To demonstrate the efficacy of the **Personalized Anti-Cancer Microbiome-Assisted Vaccination** (PACMAN) vaccine, bacteria expressing the Ovalbumin known neoantigen SIINFEKL (SEQ ID NO: 11) were administered to mice bearing the B16 melanoma tumors which express the Ovalbumin protein (B16-OVA). To generate the OVA expressing bacteria vaccine, the OVA neoantigen SIINFEKL (SEQ ID NO: 11) was fused to SspH2 secretion signal of *Salmonella Typhimurium*. The resulted oligomer was transformed into the attenuated *Salmonella Typhimurium* strain VNP20009 (VNP-OVA). C57BL/6 mice were injected with 10^6 B16 OVA expressing cells in the right flank. When tumors reached a volume of $\sim 100\text{mm}^3$, mice were shuffled into the following treatment cohorts: No treatment control, mice receiving the checkpoint inhibitor, anti-PD1 (75 μg per mouse, i.p, once a week), and mice receiving anti-PD1 together with PACMAN-OVA (10^6 CFU, tail vein). The experiment time line is shown in Figure 1A. Tumor growth curves from treatment start are shown in Figure 1B. Tumor growth was completely stopped for 20 days in the PACMAN-OVA cohort versus the exponential growth observed in the other mice cohorts. Following two cycles of immunization, all mice in the VNP-OVA cohort survived significantly longer than the mice in the other cohorts.

To demonstrate the immunogenicity of the vaccine, splenocytes were profiled from mice bearing the B16-OVA tumor following the administration of the PACMAN vaccine. The PACMAN-OVA contained the OVA neoantigen SIINFEKL (SEQ ID NO: 11) fused to SspH2 secretion signal of *Salmonella Typhimurium* in the attenuated strain STM3210. For a negative control the OVA neoantigen was replaced by the MC38 neoantigen, Adpgk (PACMAN-Adpgk), which is not present in B16-OVA cells.

C57BL/6 mice were injected with 10^6 B16 OVA expressing cells in the right flank. When tumors reached a volume of $\sim 100\text{mm}^3$, mice were shuffled into the following treatment cohorts: No treatment control, mice receiving the checkpoint inhibitor, anti-PD1 (75 μg per mouse, i.p, once a week), mice receiving anti-PD1 together with PACMAN-OVA (10^6 CFU, tail vein) and mice receiving anti-PD1 with PACMAN-Adpgk (10^6 CFU, tail vein). Sixteen days post immunization spleens and liver were harvested for further analysis. The results are illustrated in Figures 2A-D.

To test the effect of alternate administration of PACMAN-OVA which is based on different attenuated bacteria, mice bearing B16-Ova tumor were vaccinated consecutively with two attenuated bacteria expressing the OVA neoantigen. The first bacteria is the *Salmonella* attenuated strain STM3210 expressing Ova neoantigen fused to either SspH2 secretion signal

under its endogenous promoter or to SspH1 secretion signal under pagC promoter which is induced upon phagocytosis by macrophages (STM-OVA). The second bacteria is the *Pseudomonas aeruginosa* attenuated strain, CHA-OST, expressing Ova neoantigen fused to the secretion signal of ExoS, a toxin of the type-three secretion system (TTSS). ExoS promoter is activated by the TTSS regulator ExsA, following induction by IPTG (CHA-OST-OVA).

C57BL/6 mice were injected with 10^6 B16 OVA expressing cells in the right flank. When tumors reached a volume of $\sim 100\text{mm}^3$, mice were shuffled into the following treatment cohorts: No treatment control, mice receiving the checkpoint inhibitor, anti-PD1 (75 μg per mouse, i.p, once a week), mice receiving anti-PD1 together with STM-SspH2-OVA and mice receiving anti-PD1 together with STM-pagC-SspH1-OVA. The vaccinated mice were treated with 3 doses of STM-OVA (10^6 CFU, tail vein), followed by anti-PD1 (75 μg per mouse, i.p, once a week). Two weeks since the last STM-OVA vaccine, the mice were treated with 2 doses of CHA-OST-OVA (10^7 CFU, tail vein, following 3 hours incubation with IPTG 0.5 mM). As illustrated in Figure 3B, tumor growth was significantly delayed in the mice which were vaccinated with STM-OVA compared to non-vaccinated mice. The majority of tumors in the vaccinated mice regained growth 20-30 days post vaccination, suggesting that the additional injections of the same bacteria did not contribute enough to anti-tumor immunity. Strikingly, vaccinating the mice with CHA-OST-OVA slowed down tumor growth and in some cases even caused exponential decay. As illustrated in Figure 3C and Figure 3D, weight decrease is observed following each bacteria administration, however, weight loss is less pronounced after additional vaccination with the same bacteria, further supporting the hypothesis that the mice develop immunity towards the bacteria resulting in fast clearance and thus less effect on body weight.

To test the immune memory of mice vaccinated with PACMAN-OVA, fully cured mice from the experiment described in Figure 3A were re-challenged with 10^6 B16-Ova cells and tumor growth was compared to naïve mice injected with the same amount of cells. As illustrated in Figure 4B, while naïve mice exhibited exponentially growing tumors shortly after injection, re-challenged mice remained tumor free, indicating the establishment of long term immune memory against B16-Ova cells.

To demonstrate the efficacy of the PACMAN vaccine with naturally occurring neoantigen in another mouse model, the effect of bacteria expressing the Adpgk neoantigen of MC38 model was tested on mice bearing the MC38 CRC tumors. To generate the Adpgk expressing bacteria vaccine, the Adpgk neoantigen was fused to SspH1 secretion signal of *Salmonella Typhimurium* under pagC promoter which is induced upon phagocytosis by macrophages. Next, the oligomer

was transformed into the attenuated *Salmonella* Typhimurium strain VNP20009 (PACMAN-Adpgk). C57BL/6 mice were injected with 10^5 MC38 cells in the right flank. When tumors reached a volume of $\sim 100 \text{ mm}^3$, mice were shuffled into the following treatment cohorts: mice receiving the checkpoint inhibitor, anti-PD1 (75 μg per mouse, i.p, once a week), mice receiving anti-PD1 together with VNP20009 and mice receiving anti-PD1 together with PACMAN-Adpgk (10^6 CFU, tail vein).

To test the immune memory of mice vaccinated with PACMAN-Adpgk, the mice exhibiting full cure following vaccination with PACMAN-Adpgk or VNP20009 (w/o adpgk) were re-challenged with 10^5 MC38 cells and tumor growth was compared to naïve mice injected with the same amount of cells. While naïve mice exhibited exponentially growing tumors shortly after injection, re-challenged mouse which was vaccinated with PACMAN-Adpgk remained tumor free, indicating the establishment of long term immune memory against MC38 cells. Of note, the fully cured mouse following vaccination only with the VNP20009, exhibited tumor growth following re-challenge indicating that the immune memory was a consequence of Adpgk presentation by the bacteria (Figure 5C).

To demonstrate selective homing of *Salmonella* to MC38 tumors, attenuated *Salmonella* (STM3120) was injected to the tail vein of mice bearing the MC38 CRC tumors at the indicated numbers. After 9 days, tumors, livers and spleens were resected and vigorously shaken in 1 ml LB and a metal ball. Supernatant was seeded on LB plates and colonies were counted following 24 hrs incubation at 37 °C. CFU was normalized to the dilution factor and tissue mass. For 1×10^6 , 1×10^5 N=4, for 1×10^4 N=3. As illustrated in Figure 6, the bacteria selectively homed to the tumors as compared to livers and spleens.

To compare the maximal tolerable dose of attenuated *Salmonella* (STM3120) vs parental *Salmonella* (14028), *Salmonella* were injected to the tail vein at various concentrations and body weight was monitored. As illustrated in Figure 7, in all doses but STM3120 1×10^6 , all mice in the cohort (N=4-5) died (indicated by X).

In order to quantify the amount of active T cells following PACMAN vaccination, splenocytes were harvested from the following cohorts: naïve mice (N=3), B16-OVA tumor bearing mice (N=5), mice injected with attenuated *Salmonella* STM3120 (N=4), B16 OVA tumor bearing mice injected with STM3120 expressing the unrelated neoantigen ADPGK (N=3), B16-OVA tumor bearing mice injected with STM3120 expressing the OVA neoantigen (N=5). In all cases, 1×10^6 bacteria were injected to the tail vein. Sixteen days post injection, splenocytes were

harvested. Figure 8A illustrates the increase in IFN γ positive CD8 T-cells following vaccination with the appropriate neoantigen.

In a further experiment to quantify T cell killing capacity, MC38 or B16-OVA tumor cells were pre-incubated with CFSE (green) to distinguish them from immune cells. Harvested splenocytes were co-cultured with tumor cells. Following 72 hours, dead tumor cells (CFSE positive) were quantified by FACS using Live/dead staining. Significant B16-OVA specific killing was observed in splenocytes originating from mice vaccinated with STM3120 expressing the OVA neoantigen (Two-tail t-test, Pval <0.001).

To demonstrate the immune mediated efficacy of *P. aeruginosa* based PACMAN vaccine in MC38 colorectal cancer model, the attenuated *P. aeruginosa*, CHA-OST either naïve or expressing Adpgk neoantigen was injected to the tail vein, followed by anti PD1 treatment. C57BL/6 mice were injected with 1×10^5 MC38 colorectal cancer cells in the right flank. When tumors reached a volume of $\sim 100 \text{ mm}^3$, mice were injected with CHA-OST naïve or PACMAN-ADPGK (1×10^6 CFU, i.v) followed by weekly administration of 150 μg anti-PD1, i.p. Figure 9A is a graphic representation of the treatment protocol. As illustrated in Figure 9B, only mice which where injected with the PACMAN-ADPGK vaccine showed a full cure. Of note, the cured mouse was re-challenged with MC38 cells, however no tumor growth was observed.

To demonstrate the immune mediated efficacy of *Bacillus Subtilis* based PACMAN vaccine in MC38 colorectal cancer model, the spores of the lab strain PY79 expressing Adpgk neoantigen were injected to the tail vein or administered orally (os), followed by aPD1 treatment. C57BL/6 mice were injected with 5×10^5 MC38 colorectal cancer cells in the right flank. When tumors reached a volume of $\sim 100 \text{ mm}^3$, mice were injected with bacillus spores of PACMAN-ADPGK (5×10^8 - 1×10^9 CFU, i.v) or given per os (5×10^9 CFU, p.o) followed by weekly administration of 150 μg anti-PD1, i.p. Figure 10A is a graphic representation of the treatment protocol. As illustrated in Figure 10B, mice which where injected with the PACMAN-ADPGK vaccine showed a full cure.

To demonstrate the immune mediated efficacy of Salmonella based PACMAN vaccine (which has previously been frozen) in MC38 colorectal cancer model, the attenuated Salmonella Typhimurium STM3210 expressing related (Adpgk) and unrelated (OVA) neoantigen was injected to the tail vein followed by aPD1 treatment. C57BL/6 mice were injected with 10^6 B16 OVA expressing cells in the right flank. When tumors reached a volume of $\sim 100 \text{ mm}^3$, mice were injected with PACMAN-ADPGK or PACMAN-OVA (3×10^6 CFU, i.v) followed by weekly administration of 75/150 μg anti-PD1, i.p. Figure 11A is a graphic representation of the treatment protocol. As

illustrated in Figure 11B, mice treated with PACMAN-ADPGK exhibited a considerable delayed tumor growth.

Although the invention has been described in conjunction with specific embodiments thereof, it is evident that many alternatives, modifications and variations will be apparent to those skilled in the art. Accordingly, it is intended to embrace all such alternatives, modifications and variations that fall within the spirit and broad scope of the appended claims.

It is the intent of the applicant(s) that all publications, patents and patent applications referred to in this specification are to be incorporated in their entirety by reference into the specification, as if each individual publication, patent or patent application was specifically and individually noted when referenced that it is to be incorporated herein by reference. In addition, citation or identification of any reference in this application shall not be construed as an admission that such reference is available as prior art to the present invention. To the extent that section headings are used, they should not be construed as necessarily limiting. In addition, any priority document(s) of this application is/are hereby incorporated herein by reference in its/their entirety.

WHAT IS CLAIMED IS:

1. A vaccine comprising tumor-homing bacteria which are genetically modified to express at least one cancer-associated antigen and a pharmaceutically acceptable carrier.
2. The vaccine of claim 1, wherein said bacteria is a gram positive bacteria.
3. The vaccine of claim 1, wherein said bacteria is a gram negative bacteria.
4. The vaccine of any one of claims 1-3, wherein said bacteria is aerobic.
5. The vaccine of any one of claims 1-3, wherein said bacteria is anaerobic.
6. The vaccine of any one of claims 1-3, wherein said bacteria are live bacteria.
7. The vaccine of any one of claims 1-3, wherein said bacteria are attenuated bacteria.
8. The vaccine of any one of claims 1-7, wherein the bacteria is of a species or genus set forth in any of Tables 1-3.
9. The vaccine of any one of claims 1-8, wherein a genome of the bacteria comprises a 16S rRNA sequence as set forth in any one of SEQ ID NOs: 24-310.
10. The vaccine of any one of claims 1-8, wherein said cancer-associated antigen is a neoantigen.
11. The vaccine of any one of claims 1-10, wherein said bacteria are genetically modified to express a therapeutic protein.
12. The vaccine of claim 11, wherein said therapeutic protein is a cytokine.
13. The vaccine of any one of claims 1-12, being devoid of an aluminium salt.

14. The vaccine of any one of claims 1-12, wherein said carrier is devoid of adjuvant.
15. A method of treating cancer of a subject in need thereof comprising administering to the subject a therapeutically effective amount of the vaccine of any one of claims 1-14, thereby treating the cancer.
16. A method of treating cancer of a subject in need thereof comprising administering to the subject a therapeutically effective amount of:
 - (i) a first vaccine comprising a first bacteria which is genetically modified to express at least one cancer-associated antigen; and subsequently;
 - (ii) a second vaccine comprising a second bacteria which is genetically modified to express at least one cancer-associated antigen, thereby treating the cancer.
17. The method of claim 16, wherein said first bacteria are viable bacteria and said second bacteria are non-viable bacteria.
18. The method of claim 16, wherein said first bacteria comprises a first strain of bacteria that is genetically modified to express a cancer-associated antigen and said second bacteria comprises a second strain of bacteria that is non-identical to said first strain of bacteria, said second bacteria being genetically modified to express said cancer-associated antigen.
19. The method of claims 15 or 16, wherein the cancer is selected from the group consisting of breast, melanoma, colorectal cancer, lung cancer, gastric cancer, pancreatic cancer, ovarian cancer, bone cancer and brain cancer.
20. The method of claim 19, wherein said brain cancer comprises glioblastoma.
21. The method of claim 16, wherein said at least one cancer-associated antigen of said first vaccine is identical to said at least one cancer-associated antigen of said second vaccine.
22. The method of claim 16, wherein said at least one cancer-associated antigen of said first vaccine is non-identical to said at least one cancer-associated antigen of said second vaccine.

23. A method of preventing cancer of a subject in need thereof the method comprising administering to the subject a prophylactically effective amount of the vaccine of any one of claims 1-14, thereby preventing the cancer.

24. The method of claim 23, wherein the cancer is selected from the group consisting of breast, melanoma, colorectal cancer, gastric cancer, lung cancer, pancreatic cancer, ovarian cancer, bone cancer and brain cancer.

25. The method of claim 24, wherein said brain cancer comprises glioblastoma.

FIG. 1A

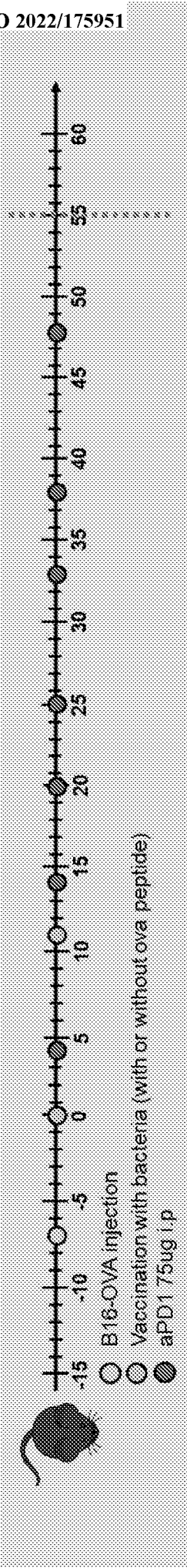


FIG. 1B

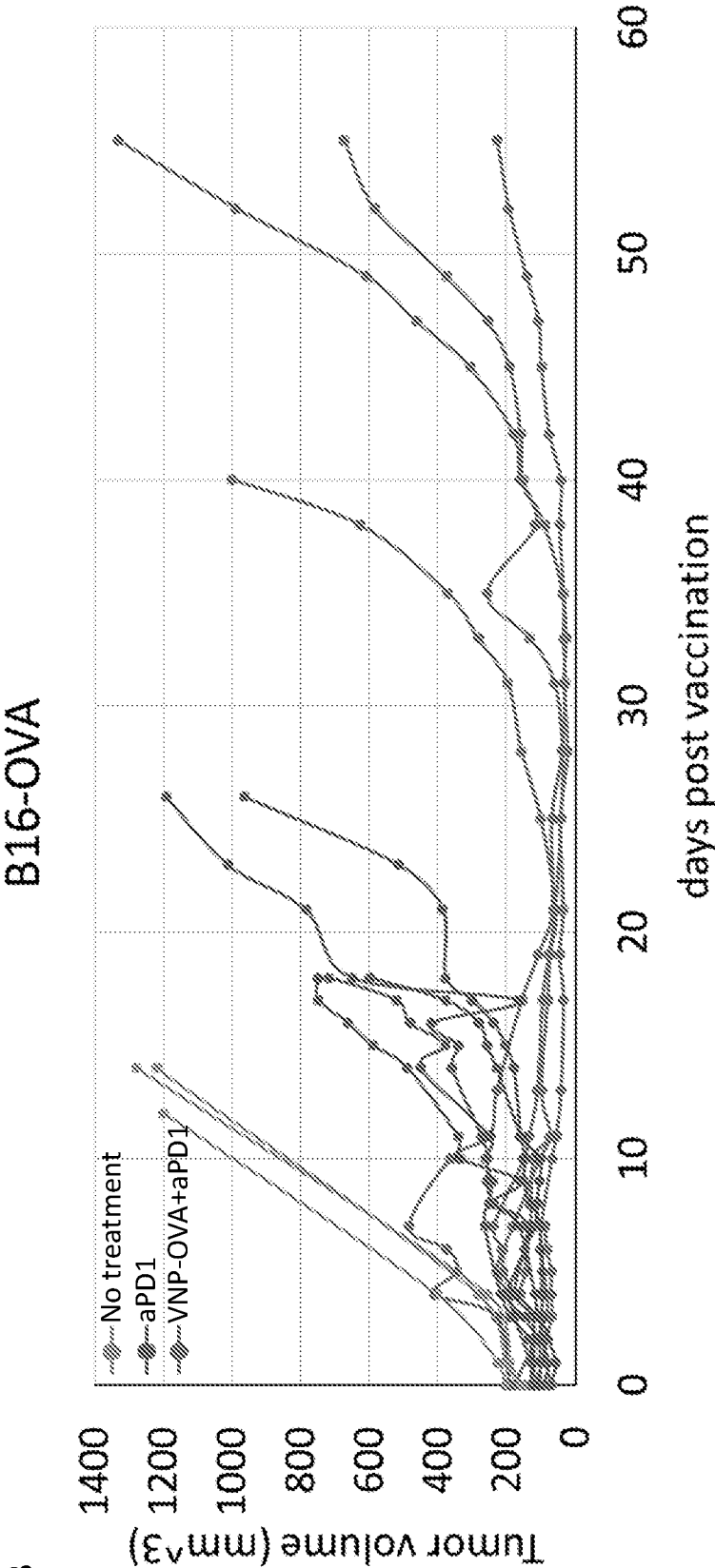


FIG. 2A

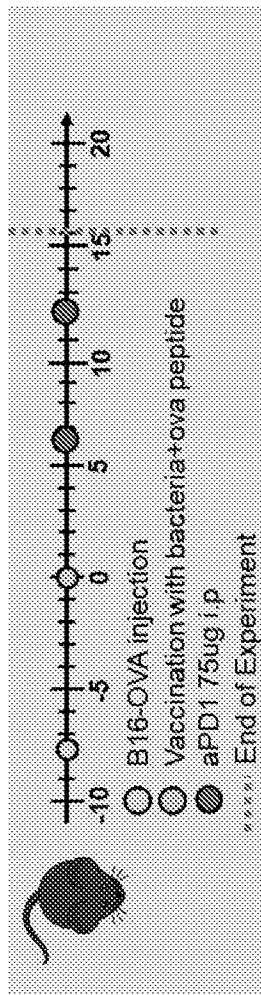


FIG. 2B

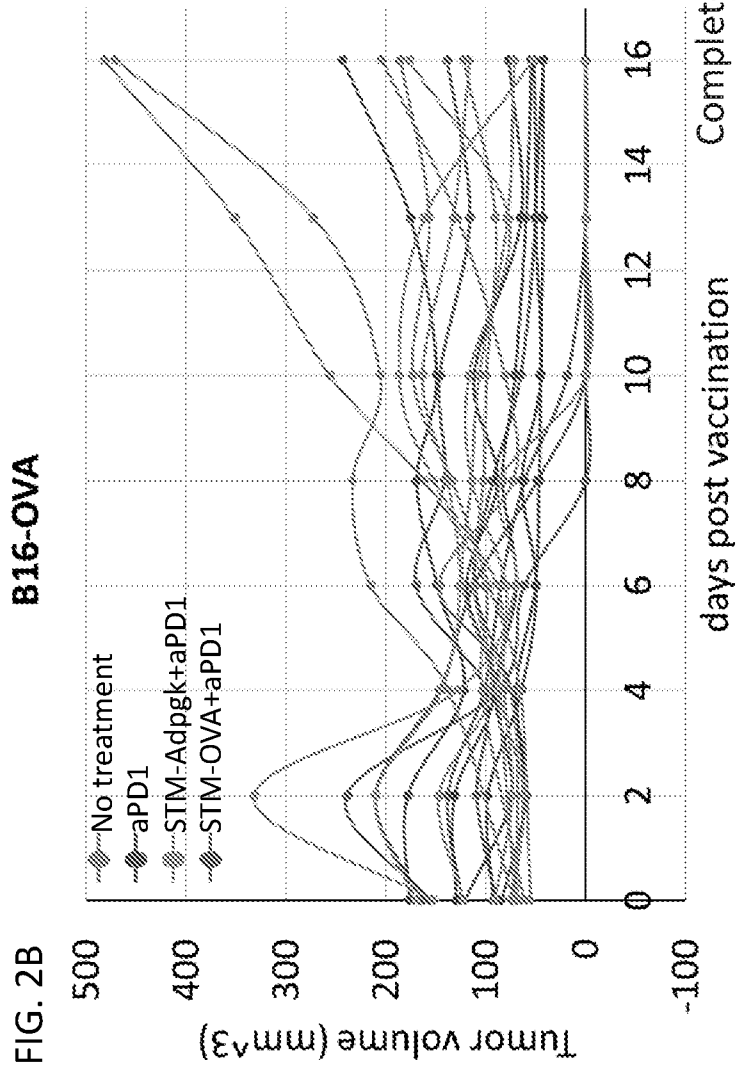


FIG. 2C

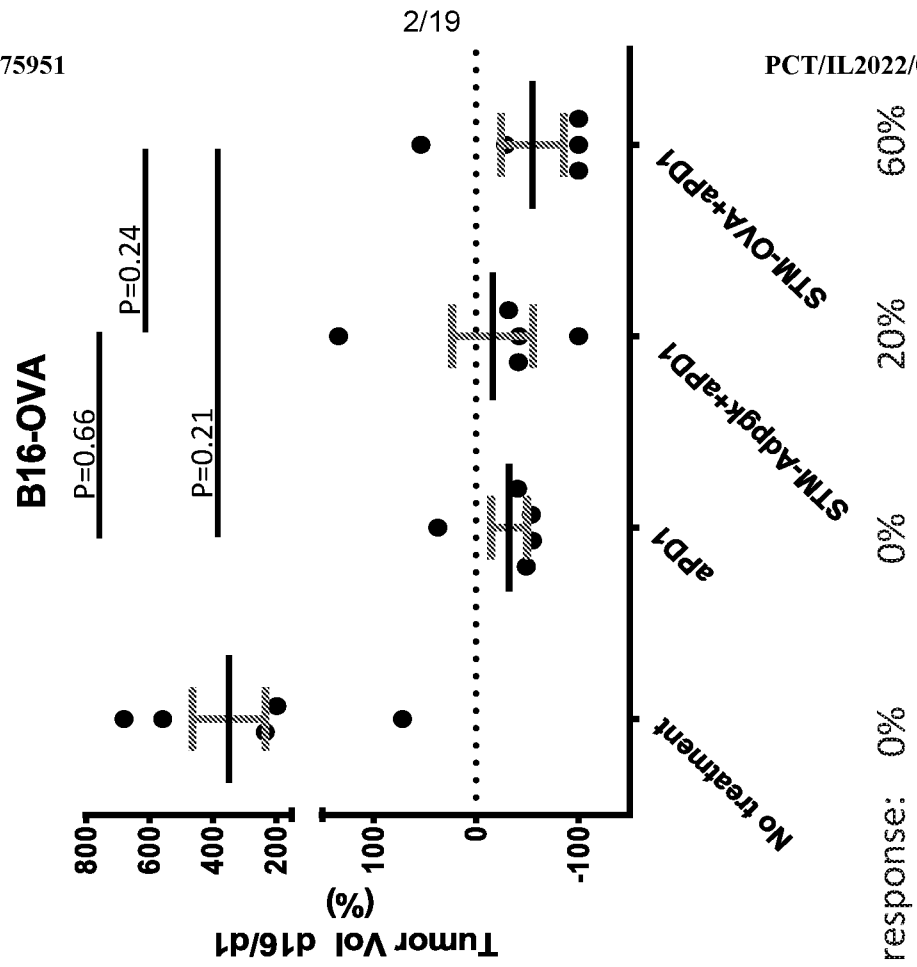


FIG. 2D

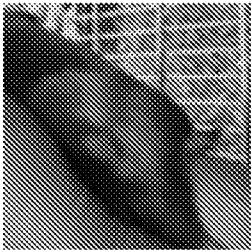
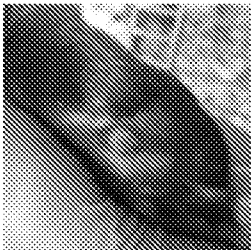
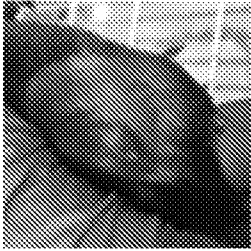
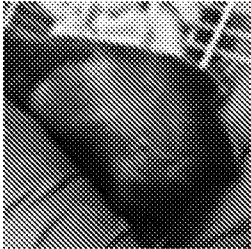
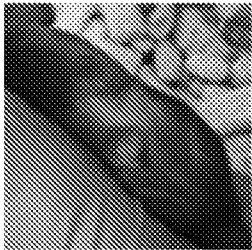
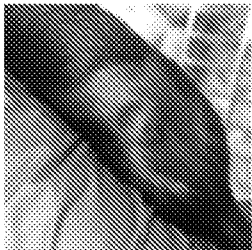
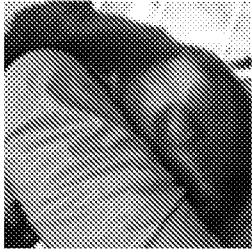
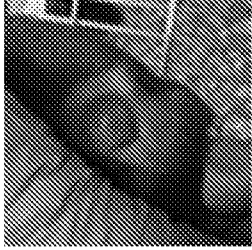
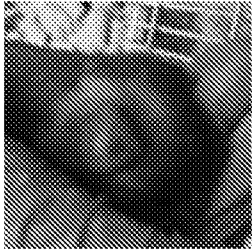
	Day-1 post Vac	Day-3 post Vac	Day-5 post Vac	Day-12 post Vac	Day-15 post Vac
STM-OVA + Anti-PD1					
	138	102	85	19	0
Tumor Vol mm ³	178	123	148	175	243
					
Anti-PD1					

FIG. 2F

IFN γ in serum

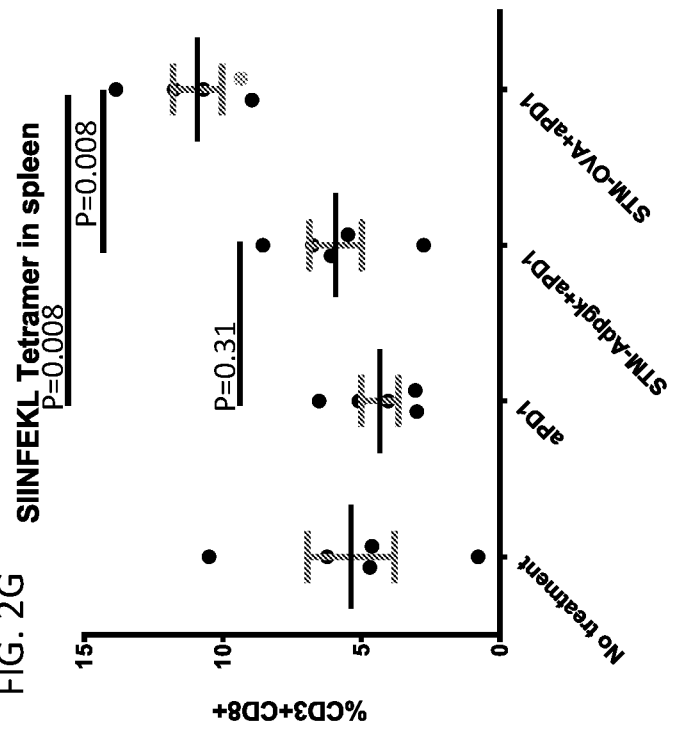
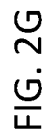


FIG. 3A

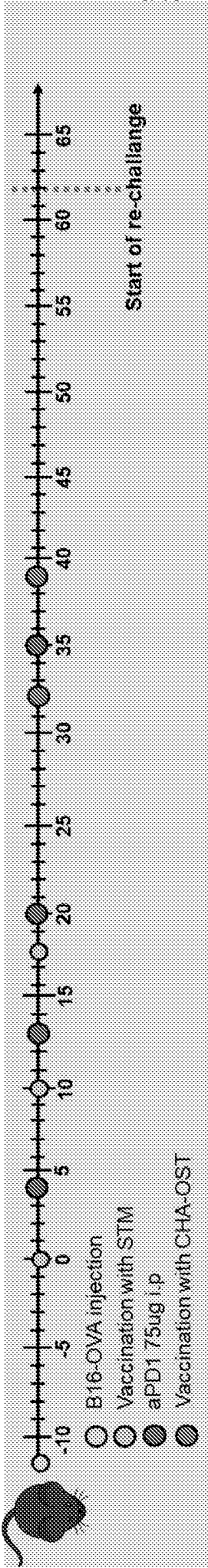


FIG. 3B

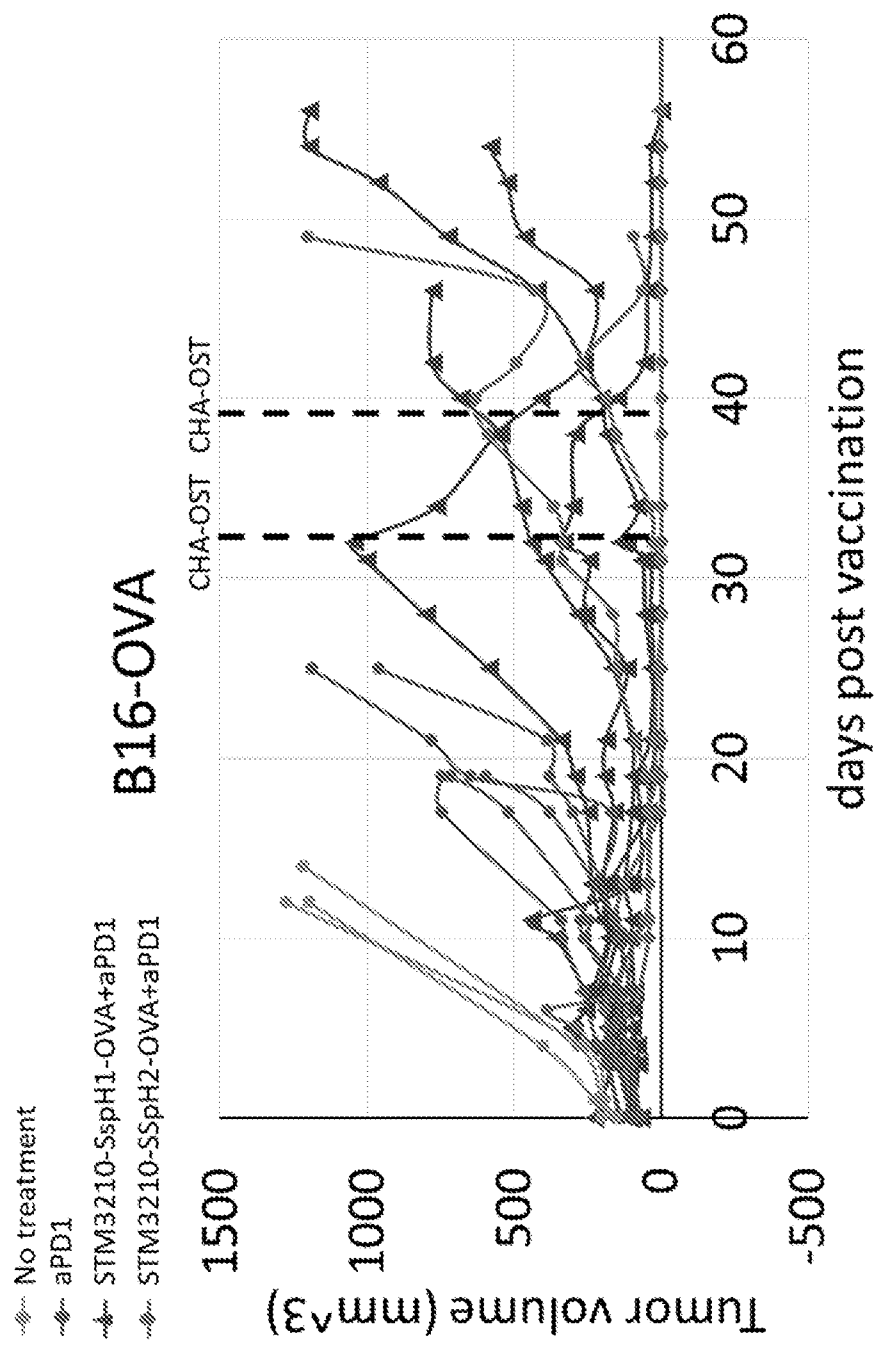


FIG. 3C

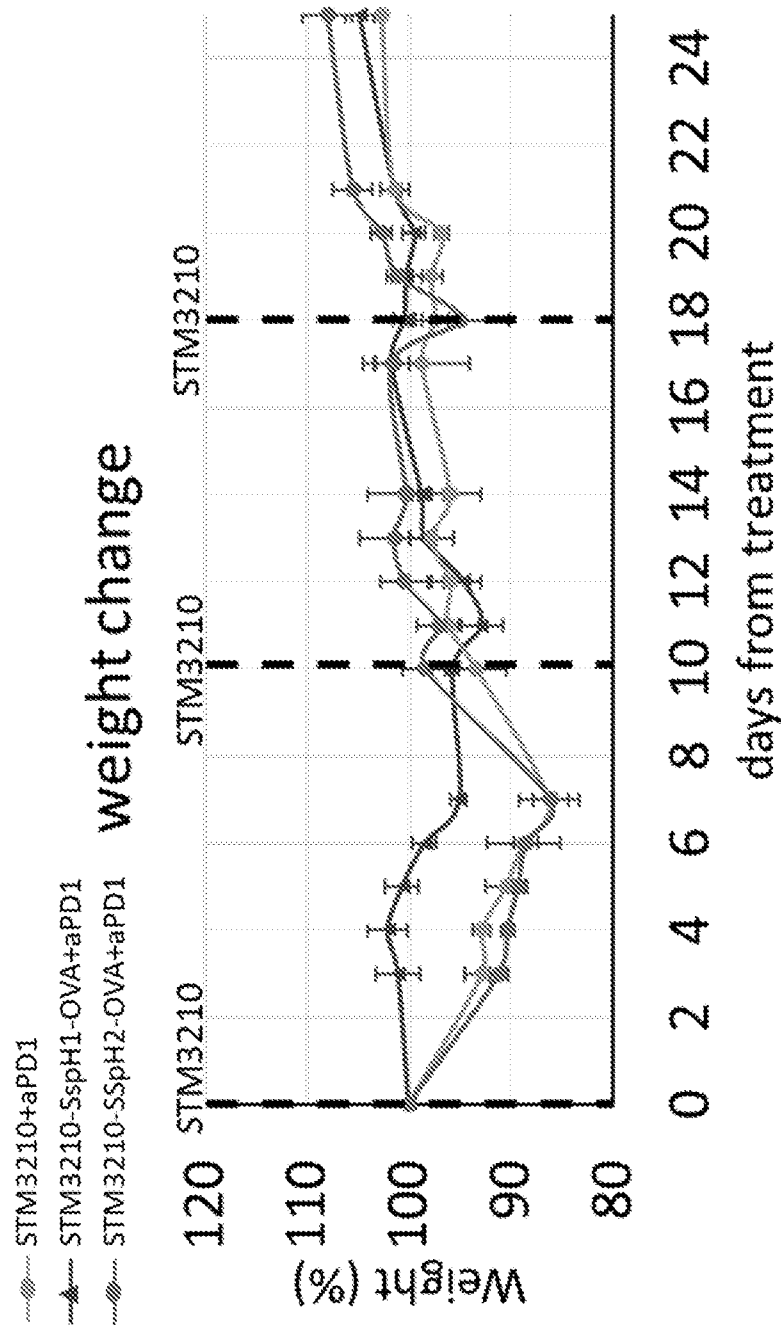


FIG. 3D

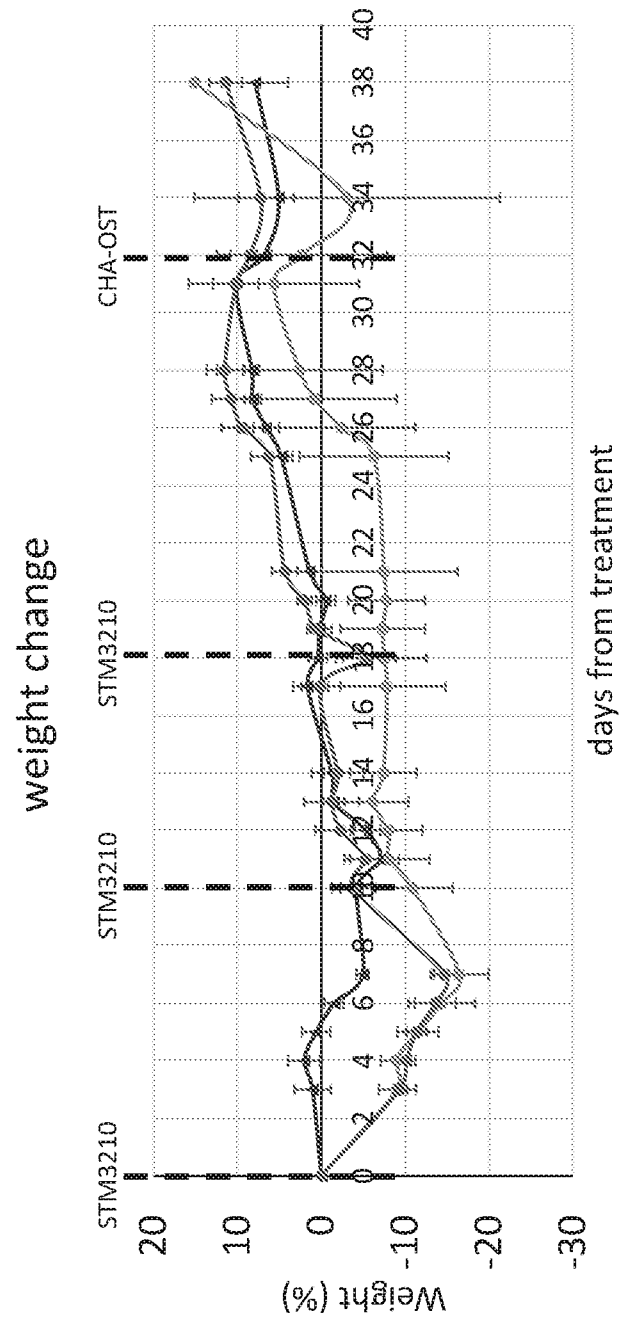


FIG. 4A

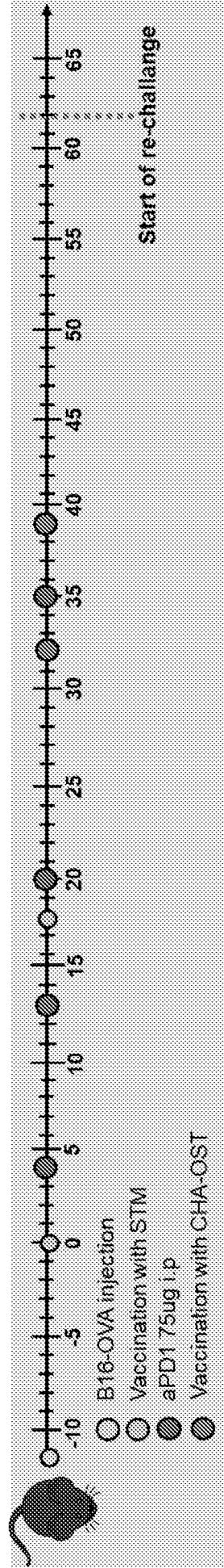


FIG. 4B

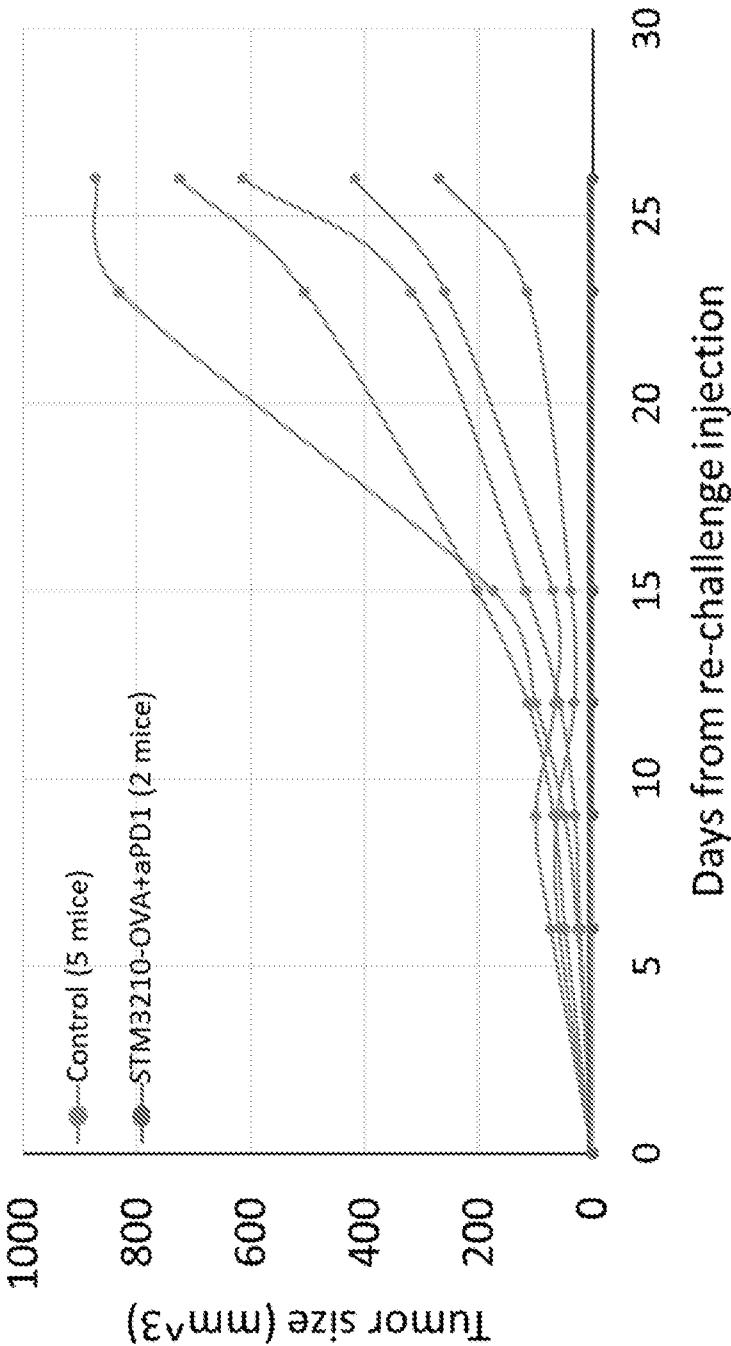


FIG. 5A

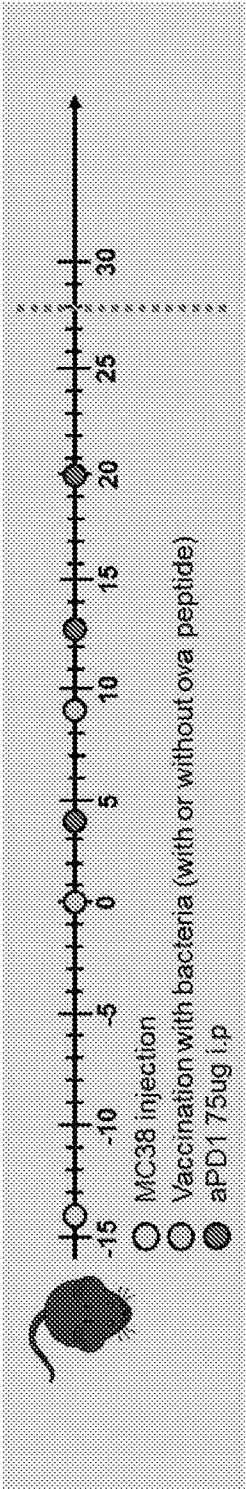
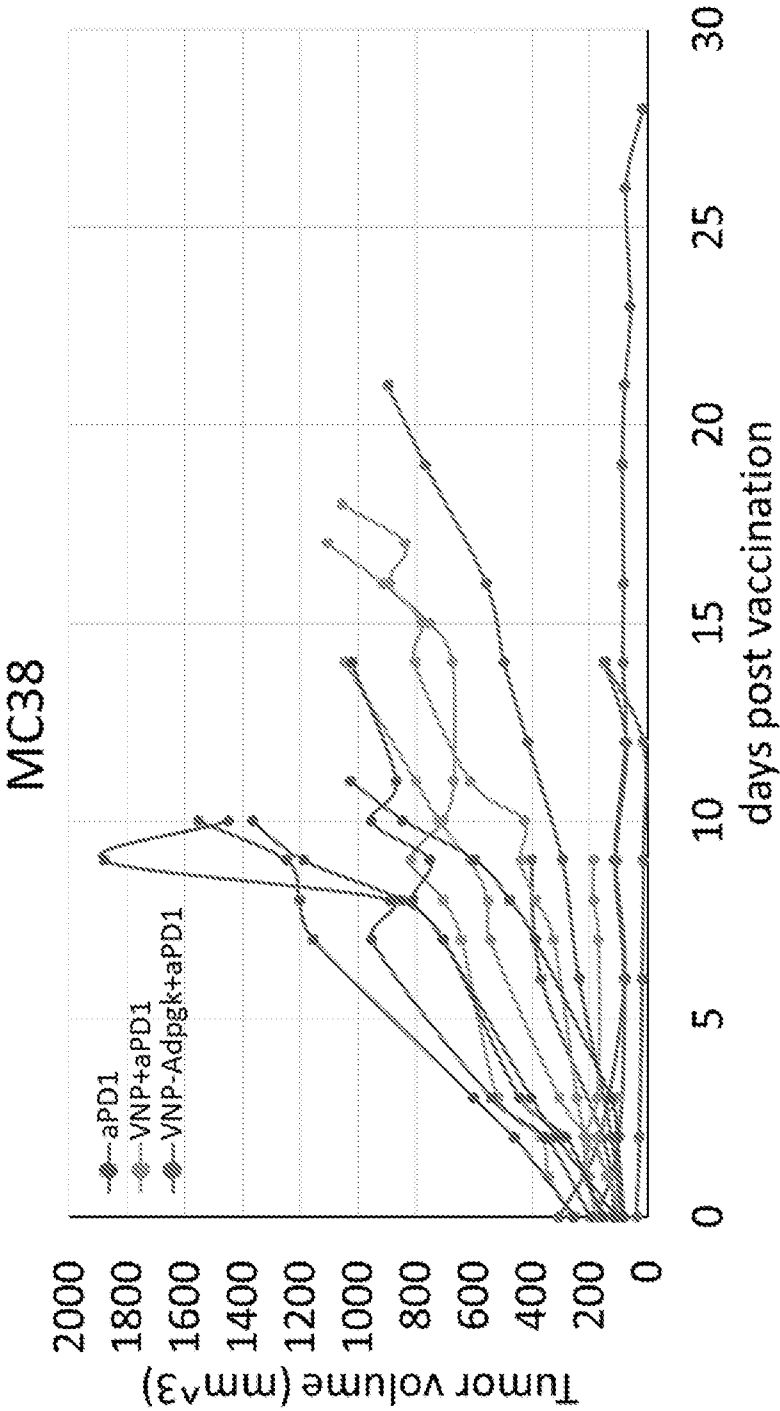
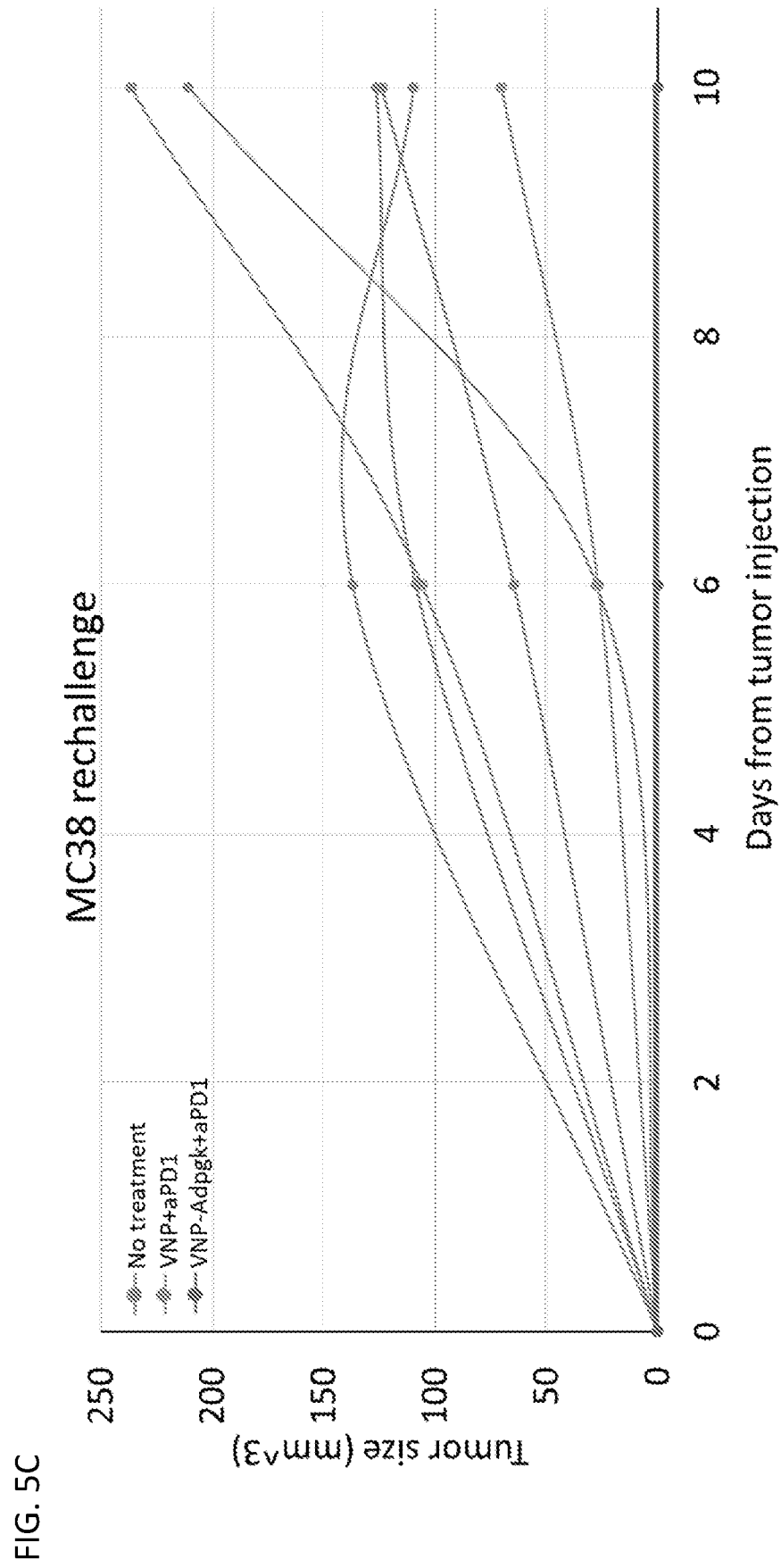


FIG. 5B





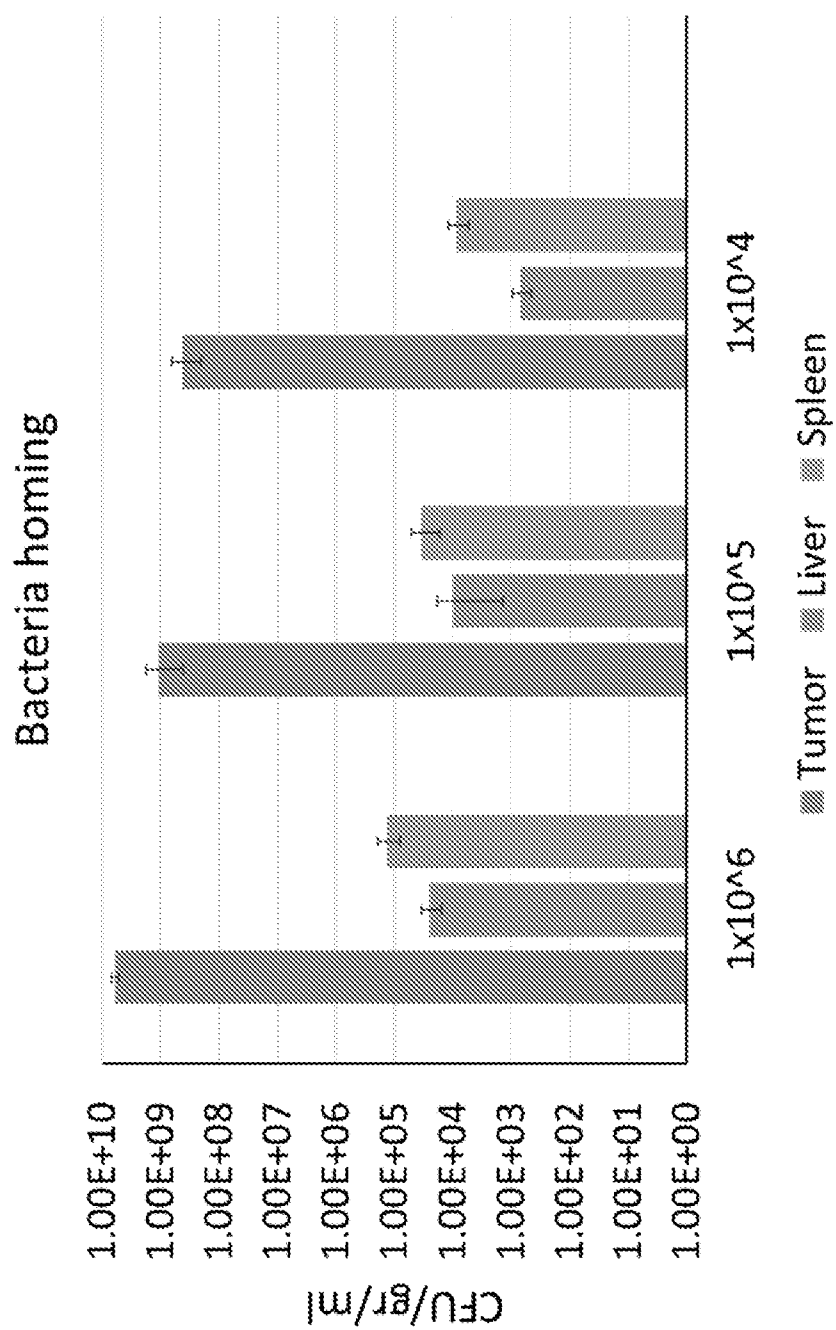


FIG. 6

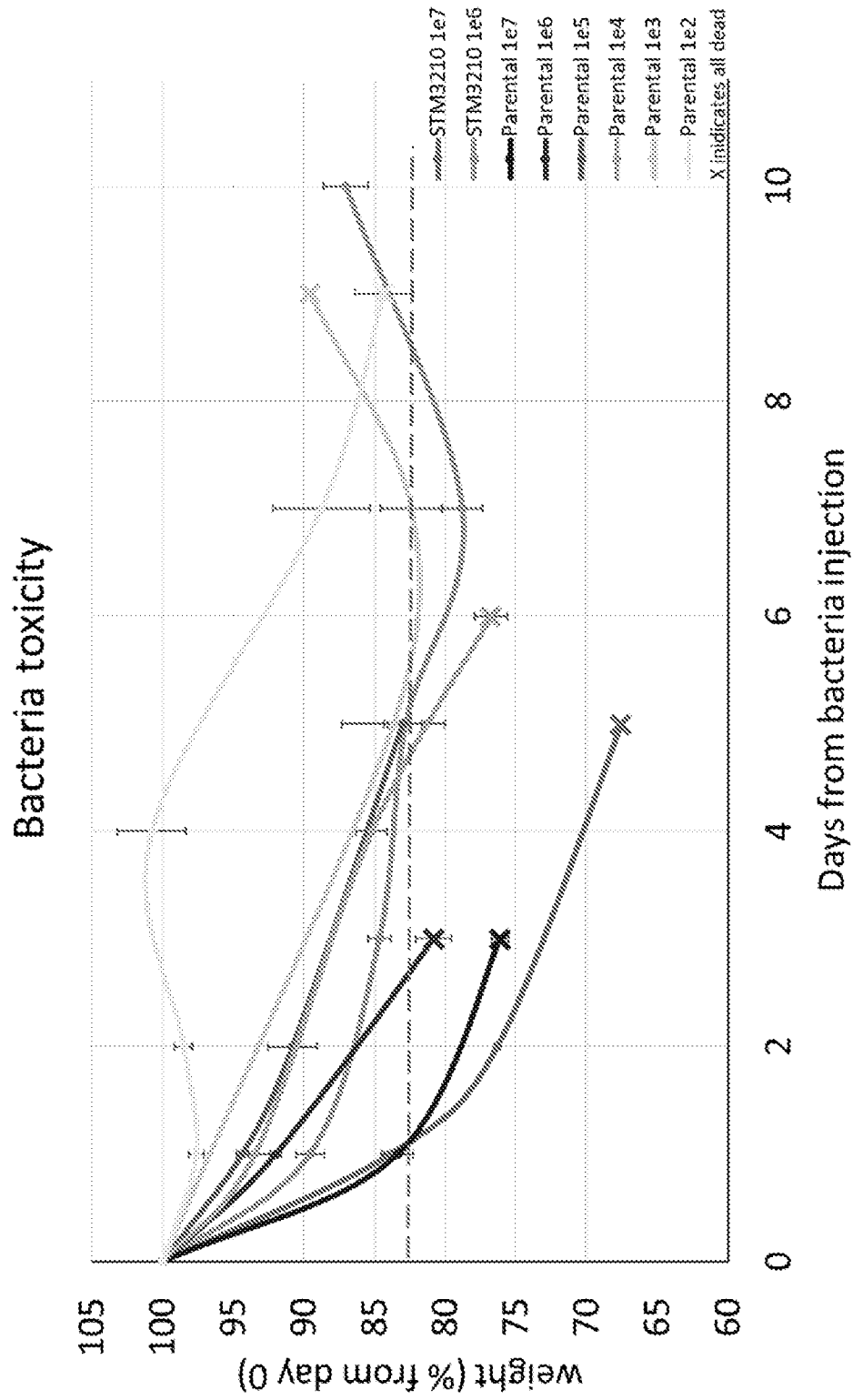


FIG. 7

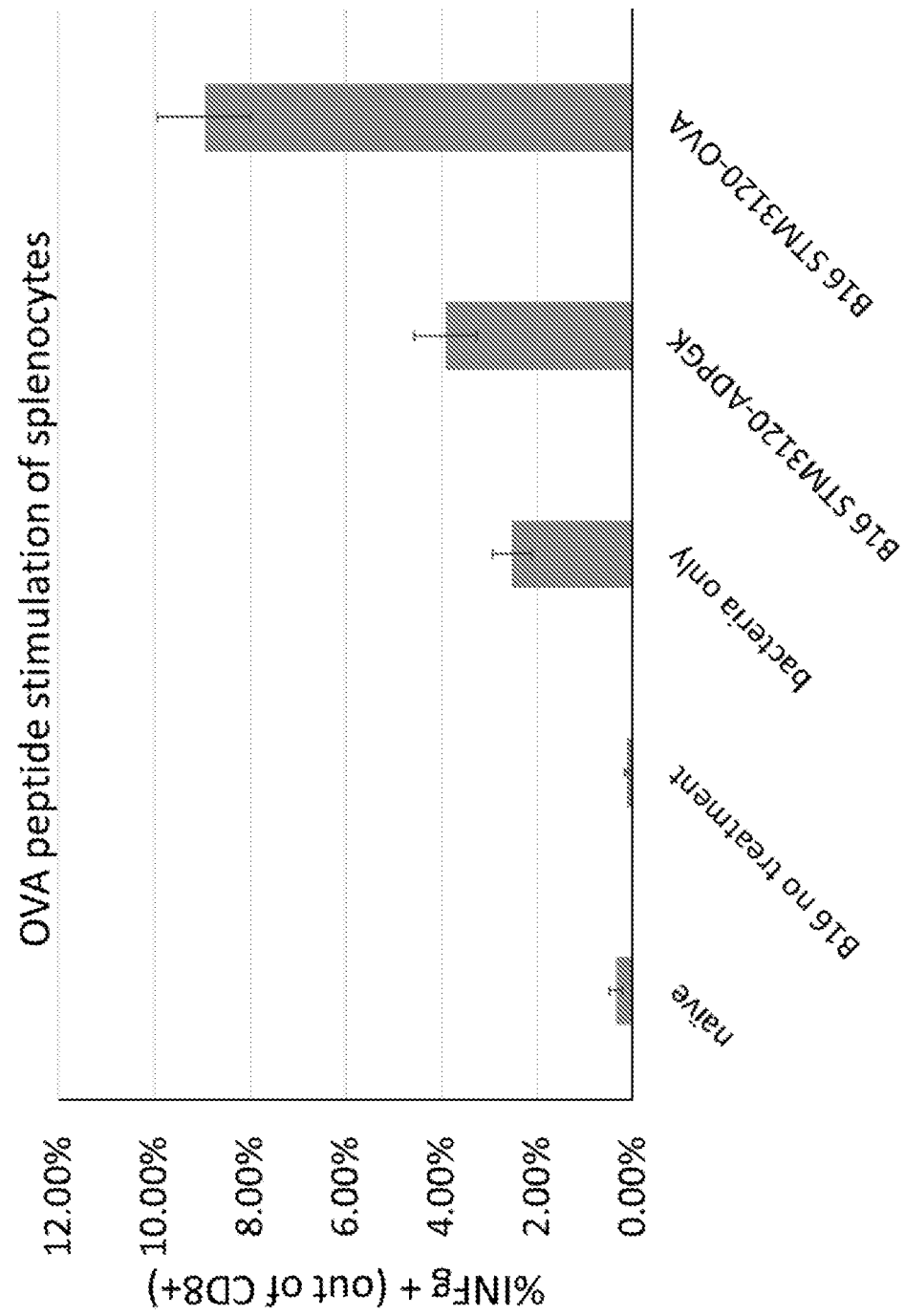


FIG. 8A

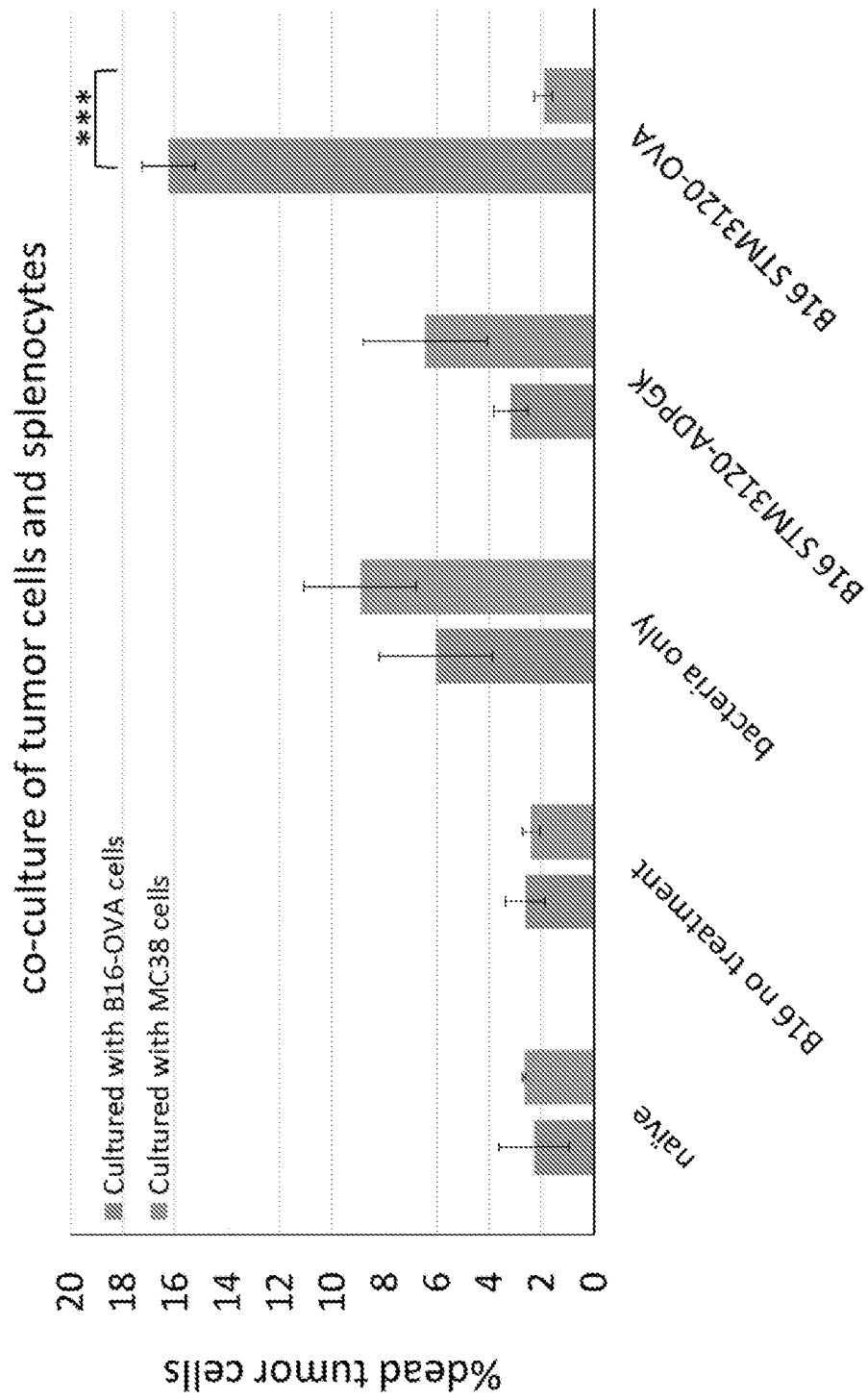


FIG. 8B

FIG. 9A

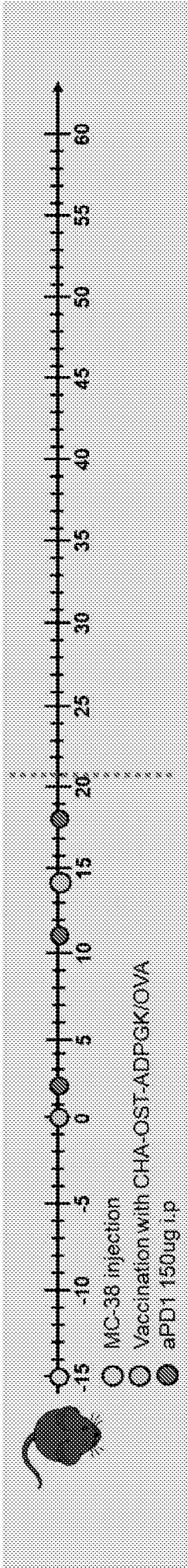


FIG. 9B

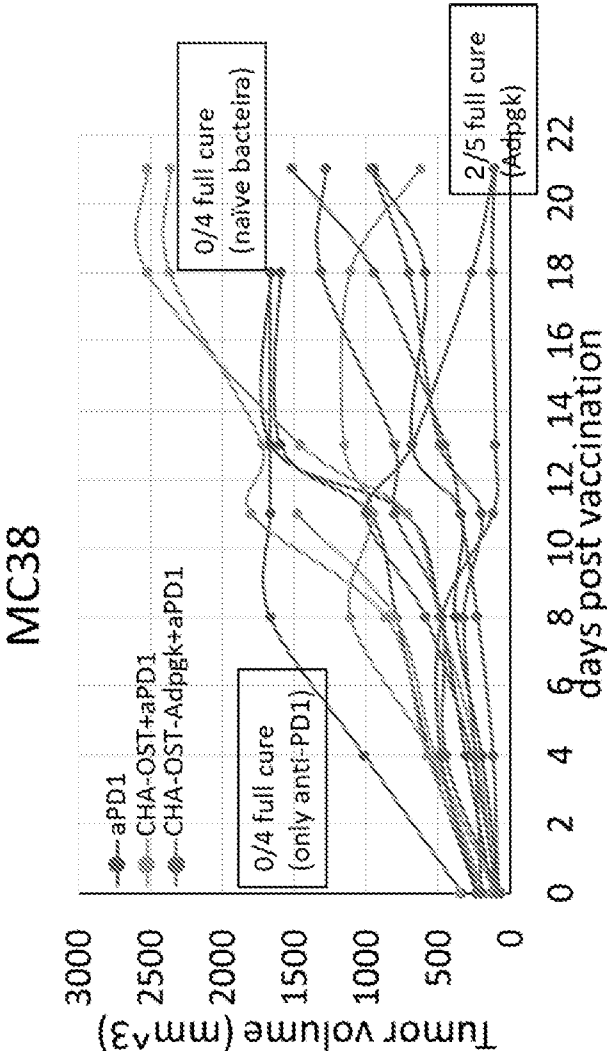


FIG. 9C

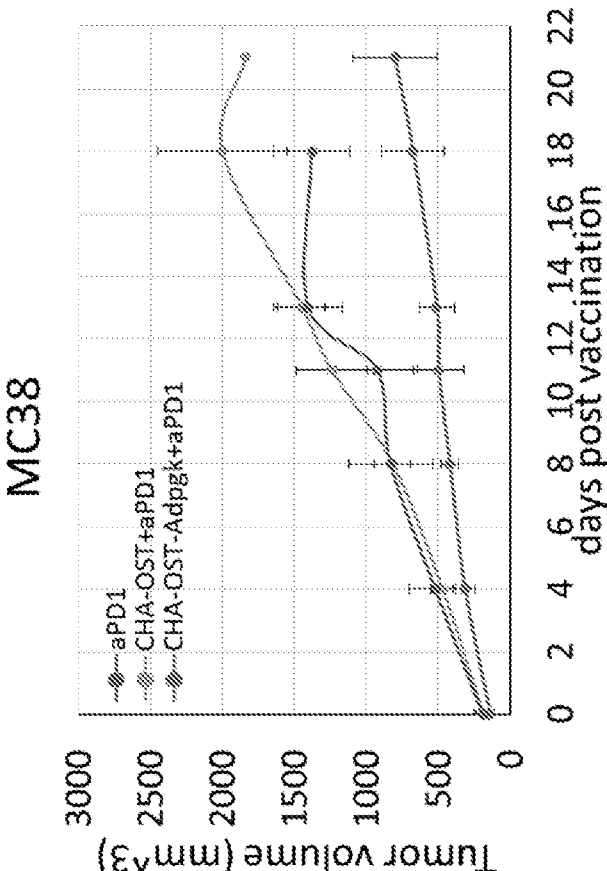


FIG. 10A

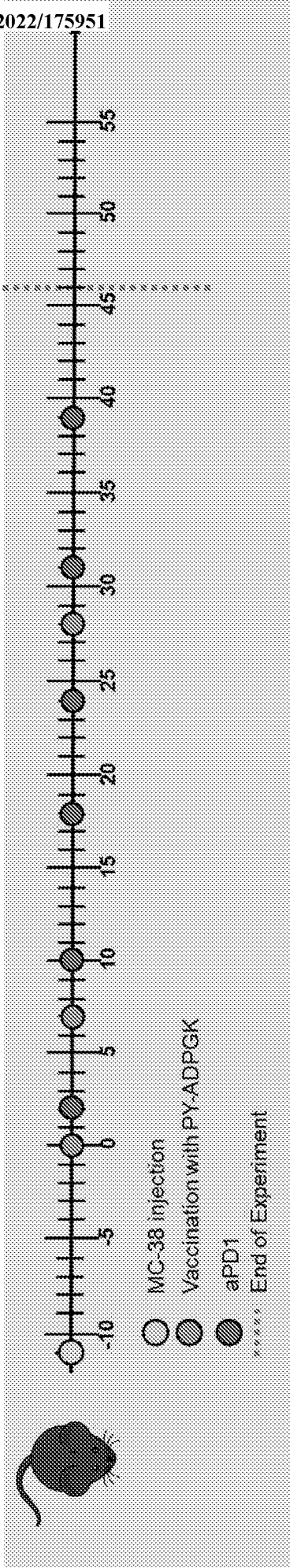


FIG. 10B

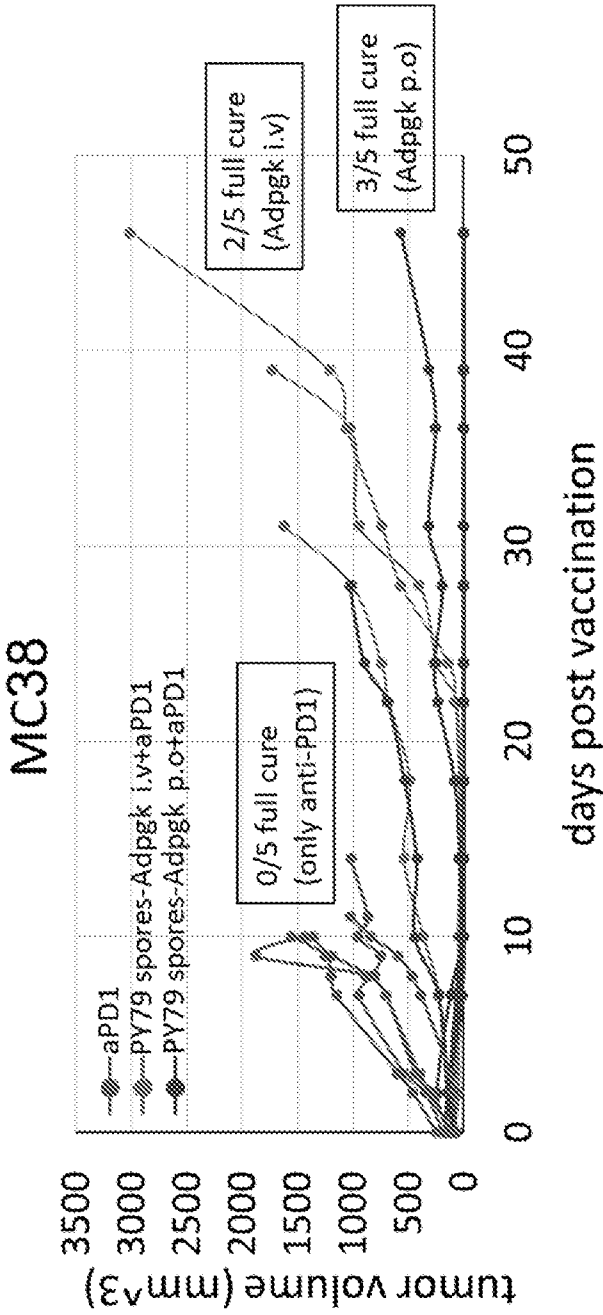


FIG. 11A

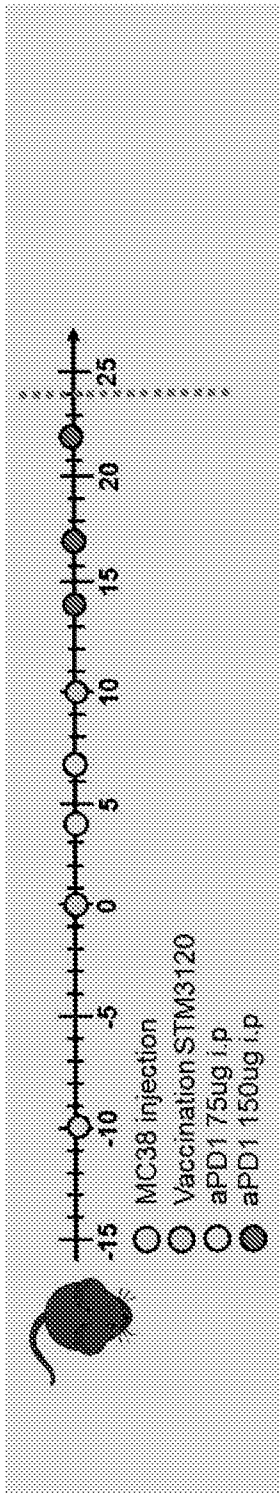
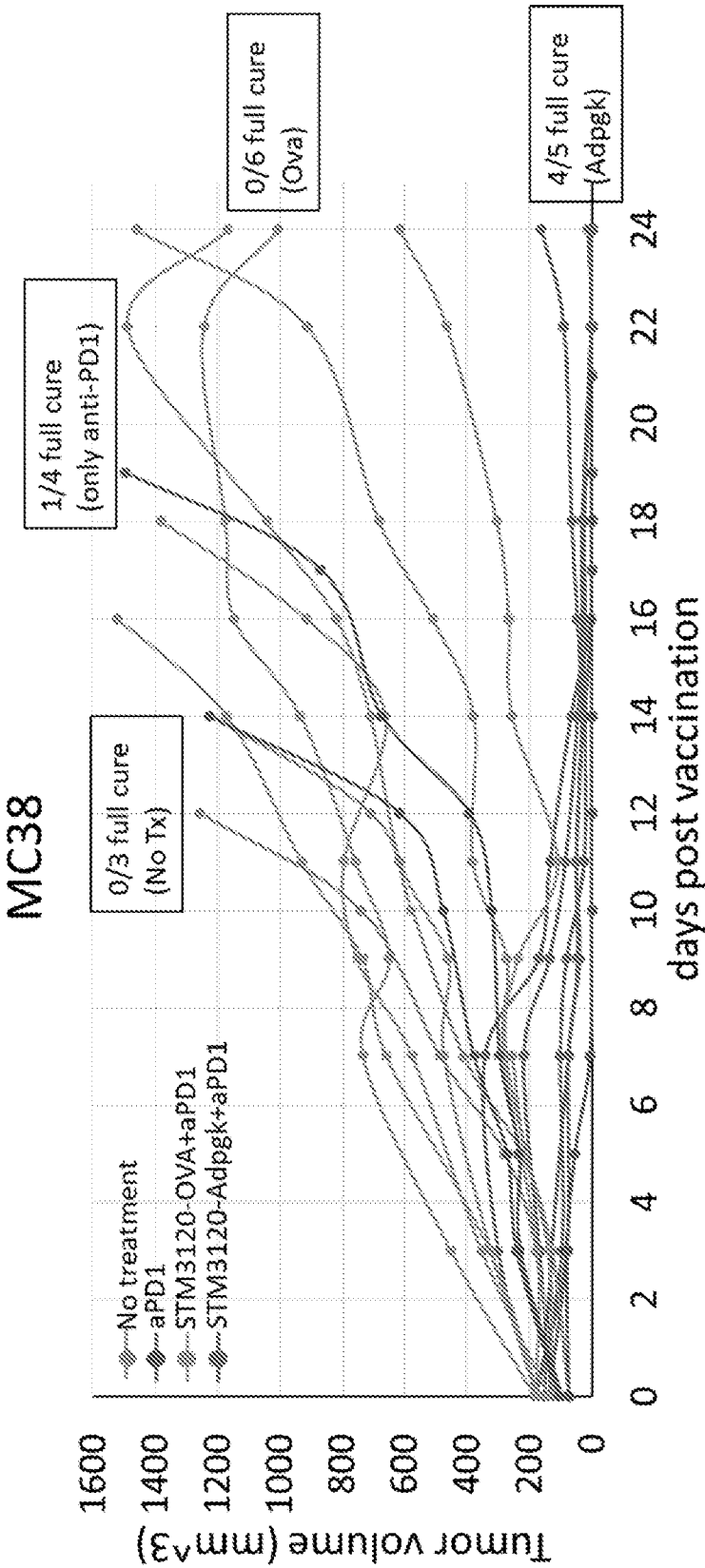


FIG. 11B



INTERNATIONAL SEARCH REPORT

International application No

PCT/IL2022/050191

A. CLASSIFICATION OF SUBJECT MATTER

INV. A61K39/00 A61P35/00 C07K16/00
ADD.

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

A61K C07K A61P

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

EPO-Internal

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 2020/049036 A1 (VAXIMM AG [CH]) 12 March 2020 (2020-03-12) abstract paragraphs [0010], [0012] - [0014], [0017] - [0020], [0028] - [0053], [0086], [0090] ----- -/--	1, 3-7, 10, 13-16, 18-20, 22-25

☒ Further documents are listed in the continuation of Box C.

☒ See patent family annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

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Date of the actual completion of the international search

20 June 2022

Date of mailing of the international search report

28/06/2022

Name and mailing address of the ISA/
European Patent Office, P.B. 5818 Patentlaan 2
NL - 2280 HV Rijswijk
Tel. (+31-70) 340-2040,
Fax: (+31-70) 340-3016

Authorized officer

Noë, Veerle

INTERNATIONAL SEARCH REPORT

International application No

PCT/IL2022/050191

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	ZHOU SHIBIN ET AL: "Tumour-targeting bacteria engineered to fight cancer", NATURE REVIEWS CANCER, NATURE PUB. GROUP, LONDON, vol. 18, no. 12, 7 November 2018 (2018-11-07), pages 727-743, XP036642899, ISSN: 1474-175X, DOI: 10.1038/S41568-018-0070-Z [retrieved on 2018-11-07] the whole document -----	1, 2, 4-7, 11-16, 19, 21, 23, 24
X	WO 2013/087667 A1 (UNIV GRENOBLE 1 [FR]; CT HOSPITALIER UNIVERSITAIRE GRENOBLE [FR]) 20 June 2013 (2013-06-20) examples 1, 2, 6 -----	1, 3, 4, 6-8, 13-16, 19, 21, 23, 24
X	WO 2007/044406 A2 (LUDWIG INST CANCER RES [US]; OLD LLOYD J [US] ET AL.) 19 April 2007 (2007-04-19) the whole document -----	1-8, 13-15, 19, 20
X	WO 2008/072895 A1 (BIOLEADERS CORP [KR]; SUNG MOON-HEE [KR] ET AL.) 19 June 2008 (2008-06-19) abstract example 3 page 3, paragraph 2 - page 4, paragraph 2 page 8, paragraph 3 -----	1-8, 13-16, 21, 23
X	M. LOEFFLER ET AL: "Attenuated Salmonella engineered to produce human cytokine LIGHT inhibit tumor growth", PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES, vol. 104, no. 31, 31 July 2007 (2007-07-31), pages 12879-12883, XP055504234, ISSN: 0027-8424, DOI: 10.1073/pnas.0701959104 abstract -----	11, 12

INTERNATIONAL SEARCH REPORT

International application No.

PCT/IL2022/050191

Box No. I Nucleotide and/or amino acid sequence(s) (Continuation of item 1.c of the first sheet)

1. With regard to any nucleotide and/or amino acid sequence disclosed in the international application, the international search was carried out on the basis of a sequence listing:
 - a. ☒ forming part of the international application as filed.
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 - ☐ on paper or in the form of an image file.
 - b. ☐ furnished together with the international application under PCT Rule 13ter.1(a) for the purposes of international search only in the form of an Annex C/ST.25 text file.
 - c. ☐ furnished subsequent to the international filing date for the purposes of international search only:
 - ☐ in the form of an Annex C/ST.25 text file (Rule 13ter.1(a)).
 - ☐ on paper or in the form of an image file (Rule 13ter.1(b) and Administrative Instructions, Section 713).
2. ☐ In addition, in the case that more than one version or copy of a sequence listing has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that forming part of the application as filed or does not go beyond the application as filed, as appropriate, were furnished.
3. Additional comments:

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No

PCT/IL2022/050191

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 2020049036 A1	12-03-2020	AU 2019334131 A1	04-03-2021
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