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APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
15/718,735	09/28/2017	Thomas F. Gajewski	UCHI-34458/US-4/CON	5538

72960 7590 08/24/2018

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EXAMINER	
HINES, JANA A	

ART UNIT	PAPER NUMBER
1645	

NOTIFICATION DATE	DELIVERY MODE
08/24/2018	ELECTRONIC

Please find below and/or attached an Office communication concerning this application or proceeding.

The time period for reply, if any, is set in the attached communication.

Notice of the Office communication was sent electronically on above-indicated "Notification Date" to the following e-mail address(es):

docketing@casimirjones.com
pto.correspondence@casimirjones.com

Office Action Summary	Application No. 15/718,735	Applicant(s) Gajewski et al.	
	Examiner JA-NA A HINES	Art Unit 1645	AIA Status Yes

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE 3 MONTHS FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133). Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

1)☑ Responsive to communication(s) filed on 7/24/2018.

☐ A declaration(s)/affidavit(s) under **37 CFR 1.130(b)** was/were filed on _____.

2a)☐ This action is **FINAL.** 2b) ☑ This action is non-final.

3)☐ An election was made by the applicant in response to a restriction requirement set forth during the interview on _____; the restriction requirement and election have been incorporated into this action.

4)☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims*

5) ☑ Claim(s) 20-49 is/are pending in the application.

5a) Of the above claim(s) _____ is/are withdrawn from consideration.

6) ☐ Claim(s) _____ is/are allowed.

7) ☑ Claim(s) 20-49 is/are rejected.

8) ☐ Claim(s) _____ is/are objected to.

9) ☐ Claim(s) _____ are subject to restriction and/or election requirement

* If any claims have been determined allowable, you may be eligible to benefit from the **Patent Prosecution Highway** program at a participating intellectual property office for the corresponding application. For more information, please see http://www.uspto.gov/patents/init_events/pph/index.jsp or send an inquiry to **PPHfeedback@uspto.gov.**

Application Papers

10)☐ The specification is objected to by the Examiner.

11)☐ The drawing(s) filed on _____ is/are: a)☐ accepted or b)☐ objected to by the Examiner.

Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).

Replacement drawing sheet(s) including the correction is required if the drawing(s) is objected to. See 37 CFR 1.121(d).

Priority under 35 U.S.C. § 119

12)☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

Certified copies:

a)☐ All b)☐ Some** c)☐ None of the:

1.☐ Certified copies of the priority documents have been received.

2.☐ Certified copies of the priority documents have been received in Application No. _____.

3.☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

** See the attached detailed Office action for a list of the certified copies not received.

Attachment(s)

1) ☑ Notice of References Cited (PTO-892)

2) ☐ Information Disclosure Statement(s) (PTO/SB/08a and/or PTO/SB/08b) Paper No(s)/Mail Date _____.

3) ☐ Interview Summary (PTO-413) Paper No(s)/Mail Date _____.

4) ☐ Other: _____.

DETAILED CORRESPONDENCE

Notice of Pre-AIA or AIA Status

1. The present application is being examined under the pre-AIA first to invent provisions.

Continued Examination Under 37 CFR 1.114

2. A request for continued examination under 37 CFR 1.114, including the fee set forth in 37 CFR 1.17(e), was filed in this application after final rejection. Since this application is eligible for continued examination under 37 CFR 1.114, and the fee set forth in 37 CFR 1.17(e) has been timely paid, the finality of the previous Office action has been withdrawn pursuant to 37 CFR 1.114. Applicant's submission filed on July 24, 2018 has been entered.

Claim Amendments

3. The amendment filed July 24, 2018 has been entered. Claims 1-19 were canceled. Claims 20-24, 27, and 40-42 have been amended. Claims 20-49 are under consideration in this Office Action.

Withdrawal of Rejections

4. The following rejections have been withdrawn in view of applicants' amendments:

a) The rejection of claims 20-39 under pre-AIA 35 U.S.C. 103(a) as being unpatentable over Korman et al., in view of Mohania et al., and Prakash et al; and

b) The rejection of claims 40-49 under 35 U.S.C. 103 as being unpatentable over Korman et al., Mohania et al., and Prakash et al., as applied to claims 20-39 above, and further in view of Duncan et al.

Response to Arguments

5. Applicant's arguments, filed July 24, 2018, with respect to the rejection(s) of claims 20-49 under Korman et al., in view of Mohania et al., and Prakash et al., have been fully considered and are persuasive. Therefore, the rejection has been withdrawn. However, upon further consideration, a new ground of rejection is made in view of Stritzker et al., (US Patent Pub. 2008/0193373 published August 2008).

New Grounds of Rejection Necessitated By Applicants Amendment

Claim Rejections - 35 USC § 103

In the event the determination of the status of the application as subject to AIA 35 U.S.C. 102 and 103 (or as subject to pre-AIA 35 U.S.C. 102 and 103) is incorrect, any correction of the statutory basis for the rejection will not be considered a new ground of rejection if the prior art relied upon, and the rationale supporting the rejection, would be the same under either status.

The following is a quotation of pre-AIA 35 U.S.C. 103(a) which forms the basis for all obviousness rejections set forth in this Office action:

> (a) A patent may not be obtained though the invention is not identically disclosed or described as set forth in section 102, if the differences between the subject matter sought to be patented and the prior art are such that the subject matter as a whole would have been obvious at the

> time the invention was made to a person having ordinary skill in the art to which said subject matter pertains. Patentability shall not be negatived by the manner in which the invention was made.

The factual inquiries set forth in *Graham v. John Deere Co.*, 383 U.S. 1, 148 USPQ 459 (1966), that are applied for establishing a background for determining obviousness under pre-AIA 35 U.S.C. 103(a) are summarized as follows:

1. Determining the scope and contents of the prior art.

2. Ascertaining the differences between the prior art and the claims at issue.

3. Resolving the level of ordinary skill in the pertinent art.

4. Considering objective evidence present in the application indicating obviousness or nonobviousness.

6. Claims 20-49 are rejected under pre-AIA 35 U.S.C. 103(a) as being unpatentable over Stritzker et al., (US Patent Pub. 2008/0193373 published August 2008) in view of Korman et al., (US Patent Publication 2009/0217401 published Aug. 2009).

The claims are drawn to a method of treating cancer in a human subject comprising co-administering to the subject an immune checkpoint inhibitor and a bacterial formulation comprising bacteria of the genus *Adlercreutzia, Oscillopira, Mollicutes, Butyrivibrio, Bacteroides, Clostridium, Fusobacterium, Eubacterium, Ruminococcus, Peptococcus, Peptostreptococcus, Rikenella, Alistipes, Marinilabilia, or Escherichia*.

Stritzker et al., teach methods which use microorganisms or cells for treating a disease, disorder or condition. Such sites, diseases and disorders include sites of cell proliferation, proliferative conditions, neoplasms, tumors and neoplastic diseases [abstract]. Further described are microorganisms and cells for use in the methods and compositions, combinations and kits, including diagnostic and pharmaceutical

compositions, containing a microorganism or cell [abstract]. Stritzker et al., uses of microorganisms or cells (e.g. Nissle) in the methods provided herein for detecting and/or treating a site of proliferation or a proliferative condition, such as a tumor, tumor tissue, cancer or metastasis [para. 0016]. Bacteria employed in the methods provided herein include, but are not limited to, mutual or commensal strains of *Escherichia coli, Bacteroides, Eubacterium, and Fusobacterium* [para. 0020]. And/or one that is a probiotic strain of *Escherichia coli, Lactococcus, Lactobacillus reuteri, Lactobacillus amylovorus, Lactobacillus crispatus, Lactobacillus gallinarum, Lactobacillus gasseri, Lactobacillus johnsonii, Lactobacillus bifidum, Lactobacillus helveticus,* [para. 0021]. Bacteria can also be used in the methods provided herein. Any of a variety of bacteria possessing the desired characteristics can be used. Exemplary bacteria provided herein include *Oscillopira, Butyrivibrio, Bacteroides, Clostridium, Fusobacterium, Eubacterium, Ruminococcus, Peptococcus, Peptostreptococcus, Rikenella, Alistipes, Marinilabilia, or Escherichia* [para 489]. The microbes used in the methods provided herein are typically attenuated [para. 478].

The dosage regimen can be any of a variety of methods and amounts, and can be determined by one skilled in the art according to known clinical factors [para 616]. Exemplary routes of administration, such as topical, local, or systemic administration can differ in the dosage given. For example, dosages for injections intravenously, intraperitoneally, or intratumorally can differ. Thus, dosages delivered directly into a tumor (i.e. intratumoral injection) can be administered at lower effective dosages [para. 616]. Exemplary levels for administering a bacterium to a 65 kg human can include 1×10^6 or about 1×10^6 cfu, 1×10^7 or about 1×10^7 cfu, 5×10^7 or about 5×10^7 cfu [para.

616]. The methods provided herein can include multiple administrations of a microorganism or cell to a subject [para. 618]. Separate administrations can include any number of two or more administrations, including two, three, four, five or six administrations [para. 620]. The time period can be a function of the time period for a subject to mount an immune response; for example, the time period can be more than the time period for a subject to mount an immune response, such as more than about one week [para 621]. Modes of administration for a co-administered substance can be the same mode of administration as the microorganism or cell or can be via a different mode of administration. Modes of administration can include, but are not limited to, intravenous, intraperitoneal, subcutaneous, intramuscular, topical, intratumor, multipuncture, inhalation, intranasal, oral, intracavity (e.g., administering to the bladder via a catheter, administering to the gut by suppository or enema), aural, ocular, transdermal, subcutaneous, intra-arterial (e.g. hepatic artery infusion), intravesicular perfusion, or intrapleural administration [para 624].

Therapeutic agents for the compositions, methods and uses provided herein can be, for example, an anti-cancer agent. Anti-cancer agents provided herein include, but are not limited to, anti-cancer antibiotics, anti-cancer antibodies [para. 0040]. Stritzker et al., teach a combination, comprising a Nissle bacterium; and an anti-tumor or anti-cancer agent [claim 131]. An anti-cancer agent or compound (used interchangeably with "anti-tumor or anti-neoplastic agent") refers to any agents, or compounds, used in anti-cancer treatment. These include any agents, when used alone or in combination with other compounds, that can alleviate, reduce, ameliorate, prevent, or place or maintain in a state of remission of clinical symptoms or diagnostic markers associated with

neoplastic disease, tumors and cancer, and can be used in methods, combinations [para. 0072].

Therefore Stritzker et al., teach a method of treating cancer in a human subject comprising co-administering to the subject an immune checkpoint inhibitor and a bacterial formulation comprising bacteria of the genus *Oscillopira, Butyrivibrio, Bacteroides, Clostridium, Fusobacterium, Eubacterium, Ruminococcus, Peptococcus, Peptostreptococcus, Rikenella, Alistipes, Marinilabilia, Anacrostipcs, or Escherichia*, wherein the immune checkpoint inhibitor is an anti-cancer antibody.

Korman et al., teach methods for treating cancer, using anti-PD-1 antibodies. The methods provide for using a combination immunotherapy, such as the combination of anti-CTLA-4 and anti-PD-1 antibodies, to treat hyperproliferative disease, such as cancer [abstract]. Korman et al., teach the use of anti-PD-1 antibodies and the use of combination immunotherapy, including the combination of anti-CTLA-4 and anti-PD-1 antibodies, to treat cancer [para. 0001]. The method of inhibiting growth of tumor cells in a subject, comprising administering to a subject a therapeutically effective amount of an anti-PD-1 antibody, or antigen-binding portion thereof [para. 0133]. The "subject" includes any human or nonhuman animal [page 232]. Korman et al., commercially available anti-PD-1 antibodies. Compositions comprising an antibody, or antigen-binding portion thereof, or immunoconjugate or bispecific molecule of the invention, and a pharmaceutically acceptable carrier, are also provided [para. 0130]. The antibodies of the invention exhibit one or more desirable functional properties, such as high affinity binding to PD-1, lack of cross-reactivity to other CD28 family members, the ability to stimulate T cell proliferation, IFN-γ and/or IL-2 secretion in mixed lymphocyte reactions,

the ability to inhibit binding of one or more PD-1 ligands (e.g., PD-L1 and/or PD-L2), the ability to cross-react with monkey PD-1, the ability to stimulate antigen-specific memory responses, the ability to stimulate antibody responses and/or the ability to inhibit growth of tumor cells *in vivo* [para. 202]. Dosage regimens are adjusted to provide the optimum desired response (e.g., a therapeutic response). For example, a single bolus may be administered, several divided doses may be administered over time or the dose may be proportionally reduced or increased as indicated by the exigencies of the therapeutic situation [para. 450]. An exemplary treatment regime entails administration once per week, once every two weeks, once every three weeks, once every four weeks, once a month, once every 3 months or once every three to 6 months [para 451]. Preferred routes of administration for antibodies of the invention include intravenous, intramuscular, intradermal, intraperitoneal, subcutaneous, spinal or other parenteral routes of administration, for example by injection [para. 458].

Therefore, it would have been *prima facie* obvious at the time of applicants' invention to incorporate Korman et al., anti-cancer (anti-PD-1 antibodies) to treat cancer within Stritzker et al., method of treating cancer in a human subject comprising co-administering to the subject an immune checkpoint inhibitor and a bacterial formulation when Stritzker et al., already it was known to treat cancer in a human subject comprising administering to the subject bacterial formulations in combination with other anti-cancer antibody therapies to treat cancer. One of ordinary skill in the art would have a reasonable expectation of success by combining both components because the prior art teach combination therapy was well known to produce beneficial cancer treating

results by interfering with PD-1 and reducing PD-1 expression and using bacteria to treat the site of cancer proliferation or a proliferative cancer condition.

Additionally, *KSR International Co. v. Teleflex Inc.,* 127 S. Ct. 1727, 1741 (2007), discloses combining prior art elements according to known methods to yield predictable results, thus the combination is obvious unless its application is beyond that person's skill. *KSR International Co. v. Teleflex Inc.,* 127 S. Ct. 1727, 1741 (2007) also discloses that "The combination of familiar element according to known methods is likely to be obvious when it does no more than yield predictable results". It is well known to take a method of treating cancer when each ingredient is well-known to treat cancer, and where there is no change in the respective function of immune check point inhibitor or the bacteria thus the combination would have yielded a reasonable expectation or success along with predictable results to one of ordinary skill in the art at the time of the invention. Therefore, it would have been obvious to a person of ordinary skill in the art to combine prior art elements according to known methods that is ready for improvement to yield predictable results. The claimed invention is *prima facie* obvious in view of the teachings of the prior art, absent any convincing evidence to the contrary.

Claim Rejections - 35 USC § 103

7. Claims 20-22, 25-29 and 32-39 are rejected under 35 U.S.C. 103 as being unpatentable over Langermann (US Patent Pub. 2013/0017199 published Jan. 2013) in view of Stritzker et al., (US Patent Pub 2008/0193373 published August 2008).

The claims are drawn to a method of treating cancer in a human subject comprising co-administering to the subject an immune checkpoint inhibitor and a bacterial formulation comprising bacteria of the genus *Adlercreutzia, Oscillopira, Mollicutes, Butyrivibrio, Bacteroides, Clostridium, Fusobacterium, Eubacterium, Ruminococcus, Peptococcus, Peptostreptococcus, Rikenella, Alistipes, Marinilabilia, or Escherichia*.

Langermann teach methods and compositions for treating cancer that results from (1) failure to elicit rapid T cell mediated responses, (2) induction of T cell exhaustion, T cell anergy or both, or (3) failure to activate monocytes, macrophages, dendritic cells and/or other APCs, for example, as required to kill intracellular pathogens. The method and compositions solve the problem of undesired T cell inhibition by simultaneously inhibiting the PD-1 ligands, PD-L1 and PD-L2. The immune response can be modulated by providing antagonists which bind with different affinity, by varying the dosage of agent which is administered, by intermittent dosing over a regime, and combinations thereof, that provides for dissociation of agent from the molecule to which it is bound prior to being administered again [abstract]. The immunomodulatory compositions and methods for treating diseases such as cancer or infections, in particular to diseases inducing T cell exhaustion, T cell anergy, or both, or diseases [para. 0001]. A preferred embodiment, the compositions simultaneously block both PD-L1 and PD-L2 mediated signal transduction in T cells, which have differential effects on T cell activity. Blocking PD-L1 mediated signal transduction induces robust effector cell responses, such as increasing the number of infiltrating IFNγ producing T cells and M1 macrophages. Blocking PD-L2 mediated signal transduction decreases the

number of infiltrating Tregs. This decrease in Tregs can increase the number of Th17 cells and the level of IL-17 production, and also reduce the number of PD-1 positive cells. Therefore, simultaneous blocking of two independent PD-1 ligands can enhance two different beneficial T cell activities. Preferred compositions include immunomodulatory agents that bind directly to PD-1, PD-L1, PD-L2, or a combination thereof and increase or activate T cell responses, such as T cell proliferation or activation [para. 0016]. Preferred immunomodulatory agents interfere with or inhibit the interaction between the endogenous ligands of PD-1 and PD-1. For example, the immunomodulatory agent interferes with, inhibits, or blocks PD-L1 (also known as B7-H1), PD-L2 (also known as B7-DC), or both ligands from interacting with PD-1 [para 0061]. Additional embodiments include antibodies that bind to PD-L2, PD-L1, PD-1 or B7-1 polypeptides, and variants and/or fragments thereof [para 0065]. The immunomodulatory agents are administered intermittently over a period of days, weeks or months to elicit periodic enhanced immune response which are allowed to diminish prior to the next administration, which may serve to initiate an immune response, stimulate an immune response, or enhance an immune response. In some aspects, the immunomodulating agent is AMP-224. AMP-224 can be administered as a bolus dose at a specific dosage. In another aspect, AMP-224 is administered over the period of about a week [para. 0482].

The compositions can be administered in combination or alternation with a vaccine containing one or more antigens such as bacterial antigens [para 0024]. Vaccines require strong T cell response to eliminate infected cells. Immunomodulatory agents described herein can be administered as a component of a vaccine to promote,

augment, or enhance the primary immune response and effector cell activity and numbers. Vaccines include antigens, the immunomodulatory agent (or a source thereof) and optionally other adjuvants and targeting molecules. Sources of immunomodulatory agent include any of the disclosed PD-L1, PD-L2 or PD-1 polypeptides, fusion proteins, or variants thereof, nucleic acids encoding any of these polypeptides, or host cells containing vectors that express any of these polypeptides [para. 418]. The antigen can be derived from a bacterium, and can be a whole cell [para. 420]. The antigens are whole inactivated or attenuated organisms. These organisms may be infectious organisms such as bacteria [para. 421]. Bacterial antigens can originate from any bacteria including, but not limited to, *Bacteroides, Clostridium, Escherichia,* and *Oscillatoria* [page 427].

Pharmaceutical compositions containing peptides or polypeptides may be for administration by parenteral (intramuscular, intraperitoneal, intravenous (IV) or subcutaneous injection), transdermal (either passively or using iontophoresis or electroporation), or transmucosal (nasal, vaginal, rectal, or sublingual) routes of administration [para 403]. Therapeutic uses for the disclosed compositions include the treatment of one or more symptoms of cancer and/or induction of tumor immunity. Exemplary tumor cells that can be treated, include but not limited to, sarcoma, melanoma, lymphoma, leukemia, neuroblastoma, or carcinoma cells [para. 0022]. The dose of immunomodulatory agent enhances an immune response to an antigen in a human [para 468]. Therefore, Langermann teaches a method of treating cancer in a human subject comprising co-administering to the subject an immune checkpoint inhibitor wherein the immune checkpoint protein is PD-1 or PD-L1 and the inhibitor is

AMP-224 and a whole cell bacterial formulation comprising *Bacteroides, Clostridium, Escherichia* or *Oscillatoria*

Stritzker et al., has been discussed above as teaching a method of treating cancer in a human subject comprising co-administering to the subject an immune checkpoint inhibitor which is an anti-cancer antibody and a bacterial formulation comprising bacteria of the genus *Oscillopira, Butyrivibrio, Bacteroides, Clostridium, Fusobacterium, Eubacterium, Ruminococcus, Peptococcus, Peptostreptococcus, Rikenella, Alistipes, Marinilabilia, Anacrostipcs, or Escherichia*.

Therefore, it would have been *prima facie* obvious at the time of applicants' invention to incorporate Stritzker et al's whole cell bacterial formulation to treat cancer within the method of treating cancer as taught by Langermann in order to treat cancer using combination therapy. One of ordinary skill in the art would have a reasonable expectation of success by combining the components because the prior art teach combination therapy was well known to produce beneficial by promoting, augmenting, or enhancing the primary immune response and effector cell activity and numbers while also decreasing PD-1 expression and blocking PD-1.

Additionally, *KSR International Co. v. Teleflex Inc.,* 127 S. Ct. 1727, 1741 (2007), discloses combining prior art elements according to known methods to yield predictable results, thus the combination is obvious unless its application is beyond that person's skill. *KSR International Co. v. Teleflex Inc.,* 127 S. Ct. 1727, 1741 (2007) also discloses that "The combination of familiar element according to known methods is likely to be obvious when it does no more than yield predictable results". It is well known to take a

method of treating cancer when each ingredient is well-known to treat cancer, and where there is no change in the respective function of immune check point inhibitor, or the bacterial formulation; thus the combination would have yielded a reasonable expectation or success along with predictable results to one of ordinary skill in the art at the time of the invention. Therefore, it would have been obvious to a person of ordinary skill in the art to combine prior art elements according to known methods that is ready for improvement to yield predictable results. The claimed invention is *prima facie* obvious in view of the teachings of the prior art, absent any convincing evidence to the contrary.

Pertinent Art

8. The prior art made of record and not relied upon is considered pertinent to applicant's disclosure. Cojocaru et al., (US Pat. Pub 2014/0294765) teach antibodies and antigen binding fragments and conjugates containing same, and/or alternative scaffolds, specific for LSR molecules, which are suitable drugs for immunotherapy and treatment of specific cancer. Lee et al., (US Patent Pub. 2014/0271557) teach methods of treating cancer comprising administering a therapeutically effective dose of a probiotic organism in combination with anti PD-1 antibodies. The bacterium *Bacteroidetes fragilis* is a probiotic organism that exerts a protective effect by modulating inflammatory immune responses and *E. coli* is a well-known probiotic.

Conclusion

9. No claims allowed.

10. Any inquiry concerning this communication or earlier communications from the examiner should be directed to JA-NA A HINES whose telephone number is (571)272-0859. The examiner can normally be reached Monday thru Thursday.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor Gary Nickol, can be reached on 571-272-0835. The fax phone number for the organization where this application or proceeding is assigned is 571-273-8300.

Information regarding the status of an application may be obtained from the Patent Application Information Retrieval (PAIR) system. Status information for published applications may be obtained from either Private PAIR or Public PAIR. Status information for unpublished applications is available through Private PAIR only. For more information about the PAIR system, see http://pair-direct.uspto.gov. Should you have questions on access to the Private PAIR system, contact the Electronic Business Center (EBC) at 866-217-9197 (toll-free).

/JANA A HINES/
Primary Examiner, Art Unit 1645

Notice of References Cited	Application/Control No. 15/718,735	Applicant(s)/Patent Under Reexamination Gajewski et al.	
	Examiner JA-NA A HINES	Art Unit 1645	Page 1 of 1

U.S. PATENT DOCUMENTS

*		Document Number Country Code-Number-Kind Code	Date MM-YYYY	Name	CPC Classification	US Classification
*	A	US-20130017199-A1	01-2013	Langermann; Solomon	A61K38/17	424/134.1
*	B	US-20080193373-A1	08-2008	Stritzker; Jochen Harald	A61K33/24	424/1.17
*	C	US-20090217401-A1	08-2009	Korman; Alan J.	A61K47/6849	800/18
	D					
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FOREIGN PATENT DOCUMENTS

*		Document Number Country Code-Number-Kind Code	Date MM-YYYY	Country	Name	CPC Classification
	N					
	O					
	P					
	Q					
	R					
	S					
	T					

NON-PATENT DOCUMENTS

*		Include as applicable: Author, Title Date, Publisher, Edition or Volume, Pertinent Pages)
	U	
	V	
	W	
	X	

*A copy of this reference is not being furnished with this Office action. (See MPEP § 707.05(a).)
Dates in MM-YYYY format are publication dates. Classifications may be US or foreign.

Search Notes	Application/Control No.	Applicant(s)/Patent Under Reexamination
	15/718,735	Gajewski et al.
	Examiner	Art Unit
	JA-NA A HINES	1645

CPC - Searched*		
Symbol	**Date**	**Examiner**
A61K39/02; A61P1/00; A61P1/02; A61P1/04; A61P1/12; A61P1/16; A61P3/04 ; A61P3/10; A61P5/00; A61P7/02; A61P7/06; A61P9/00; A61P9/10; A61P11/ 00; A61P11/06; A61P13/12; A61P15/00; A61P17/00 ; A61P17/02;	11/09/2017	jah
A61K35/74; C12N1/20; C12P7/52	01/03/2018	jah
search updated	04/25/2018	jah
updated searches based on claim amendments	08/21/2018	jah

CPC Combination Sets - Searched*		
Symbol	**Date**	**Examiner**

US Classification - Searched*			
Class	**Subclass**	**Date**	**Examiner**
424	234.1	11/09/2017	jah
435	252.1		
424	93.4	01/03/2018	jah
435	252.1		

* See search history printout included with this form or the SEARCH NOTES box below to determine the scope of the search.

Search Notes		
Search Notes	**Date**	**Examiner**
searched inventors, applications, patents. Commercial database search of claim text	11/09/2017	jah
Search based upon claim amendments	01/03/2018	jah
updated searches	04/25/2018	jah
search updated based on claim amdts	08/21/2018	jah

/JANA A HINES/ Primary Examiner, Art Unit 1645	

Search Notes	Application/Control No.	Applicant(s)/Patent Under Reexamination
	15/718,735	Gajewski et al.
	Examiner	Art Unit
	JA-NA A HINES	1645

Interference Search			
US Class/CPC Symbol	US Subclass/CPC Group	Date	Examiner

/JANA A HINES/ Primary Examiner, Art Unit 1645	

Doc code: RCEX
Doc description: Request for Continued Examination (RCE)

PTO/SB/30EFS (02-18)
Approved for use through 11/30/2020. OMB 0651-0031
U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE
Under the Paperwork Reduction Act of 1995, no persons are required to respond to a collection of information unless it contains a valid OMB control number.

REQUEST FOR CONTINUED EXAMINATION(RCE)TRANSMITTAL
(Submitted Only via EFS-Web)

Application Number	15718735	Filing Date	2017-09-28	Docket Number (if applicable)	UCHI-34458/US-4/CON	Art Unit	1645
First Named Inventor	Gajewski			Examiner Name	Hines		

This is a Request for Continued Examination (RCE) under 37 CFR 1.114 of the above-identified application.
Request for Continued Examination (RCE) practice under 37 CFR 1.114 does not apply to any utility or plant application filed prior to June 8, 1995, or to any design application. The Instruction Sheet for this form is located at WWW.USPTO.GOV

SUBMISSION REQUIRED UNDER 37 CFR 1.114

Note: If the RCE is proper, any previously filed unentered amendments and amendments enclosed with the RCE will be entered in the order in which they were filed unless applicant instructs otherwise. If applicant does not wish to have any previously filed unentered amendment(s) entered, applicant must request non-entry of such amendment(s).

☐ Previously submitted. If a final Office action is outstanding, any amendments filed after the final Office action may be considered as a submission even if this box is not checked.

- ☐ Consider the arguments in the Appeal Brief or Reply Brief previously filed on _____
- ☐ Other _____

☒ Enclosed

- ☒ Amendment/Reply
- ☐ Information Disclosure Statement (IDS)
- ☐ Affidavit(s)/ Declaration(s)
- ☐ Other _____

MISCELLANEOUS

☐ Suspension of action on the above-identified application is requested under 37 CFR 1.103(c) for a period of months _____ (Period of suspension shall not exceed 3 months; Fee under 37 CFR 1.17(i) required)

☐ Other _____

FEES

☒ **The RCE fee under 37 CFR 1.17(e) is required by 37 CFR 1.114 when the RCE is filed.**
The Director is hereby authorized to charge any underpayment of fees, or credit any overpayments, to Deposit Account No 54302

SIGNATURE OF APPLICANT, ATTORNEY, OR AGENT REQUIRED

☒ Patent Practitioner Signature

Applicant Signature

PTO/SB/30EFS (02-18)
Approved for use through 11/30/2020. OMB 0651-0031
U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE

Signature of Registered U.S. Patent Practitioner			
Signature	/David W. Staple/	Date (YYYY-MM-DD)	2018-07-24
Name	David W. Staple	Registration Number	65903

This collection of information is required by 37 CFR 1.114. The information is required to obtain or retain a benefit by the public which is to file (and by the USPTO to process) an application. Confidentiality is governed by 35 U.S.C. 122 and 37 CFR 1.11 and 1.14. This collection is estimated to take 12 minutes to complete, including gathering, preparing, and submitting the completed application form to the USPTO. Time will vary depending upon the individual case. Any comments on the amount of time you require to complete this form and/or suggestions for reducing this burden, should be sent to the Chief Information Officer, U.S. Patent and Trademark Office, U.S. Department of Commerce, P.O. Box 1450, Alexandria, VA 22313-1450.

If you need assistance in completing the form, call 1-800-PTO-9199 and select option 2.

Privacy Act Statement

The Privacy Act of 1974 (P.L. 93-579) requires that you be given certain information in connection with your submission of the attached form related to a patent application or patent. Accordingly, pursuant to the requirements of the Act, please be advised that: (1) the general authority for the collection of this information is 35 U.S.C. 2(b)(2); (2) furnishing of the information solicited is voluntary; and (3) the principal purpose for which the information is used by the U.S. Patent and Trademark Office is to process and/or examine your submission related to a patent application or patent. If you do not furnish the requested information, the U.S. Patent and Trademark Office may not be able to process and/or examine your submission, which may result in termination of proceedings or abandonment of the application or expiration of the patent.

The information provided by you in this form will be subject to the following routine uses:

1. The information on this form will be treated confidentially to the extent allowed under the Freedom of Information Act (5 U.S.C. 552) and the Privacy Act (5 U.S.C. 552a). Records from this system of records may be disclosed to the Department of Justice to determine whether the Freedom of Information Act requires disclosure of these records.

2. A record from this system of records may be disclosed, as a routine use, in the course of presenting evidence to a court, magistrate, or administrative tribunal, including disclosures to opposing counsel in the course of settlement negotiations.

3. A record in this system of records may be disclosed, as a routine use, to a Member of Congress submitting a request involving an individual, to whom the record pertains, when the individual has requested assistance from the Member with respect to the subject matter of the record.

4. A record in this system of records may be disclosed, as a routine use, to a contractor of the Agency having need for the information in order to perform a contract. Recipients of information shall be required to comply with the requirements of the Privacy Act of 1974, as amended, pursuant to 5 U.S.C. 552a(m).

5. A record related to an International Application filed under the Patent Cooperation Treaty in this system of records may be disclosed, as a routine use, to the International Bureau of the World Intellectual Property Organization, pursuant to the Patent Cooperation Treaty.

6. A record in this system of records may be disclosed, as a routine use, to another federal agency for purposes of National Security review (35 U.S.C. 181) and for review pursuant to the Atomic Energy Act (42 U.S.C. 218(c)).

7. A record from this system of records may be disclosed, as a routine use, to the Administrator, General Services, or his/her designee, during an inspection of records conducted by GSA as part of that agency's responsibility to recommend improvements in records management practices and programs, under authority of 44 U.S.C. 2904 and 2906. Such disclosure shall be made in accordance with the GSA regulations governing inspection of records for this purpose, and any other relevant (i.e., GSA or Commerce) directive. Such disclosure shall not be used to make determinations about individuals.

8. A record from this system of records may be disclosed, as a routine use, to the public after either publication of the application pursuant to 35 U.S.C. 122(b) or issuance of a patent pursuant to 35 U.S.C. 151. Further, a record may be disclosed, subject to the limitations of 37 CFR 1.14, as a routine use, to the public if the record was filed in an application which became abandoned or in which the proceedings were terminated and which application is referenced by either a published application, an application open to public inspections or an issued patent.

9. A record from this system of records may be disclosed, as a routine use, to a Federal, State, or local law enforcement agency, if the USPTO becomes aware of a violation or potential violation of law or regulation.

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant:	University of Chicago	Confirmation No.:	5538
Serial No.:	15/718,735	Art Unit:	1645
Filed:	28-Sep-2017	Examiner:	Hines, Jana A.
Title:	**TREATMENT OF CANCER BY MANIPULATION OF COMMENSAL MICROFLORA**		

RESPONSE TO ADVISORY ACTION MAILED JULY 20, 2018

VIA EFS-WEB
COMMISSIONER FOR PATENTS
P.O. BOX 1450
ALEXANDRIA, VA 22313-1450

Examiner Hines:

This communication is in response to the Final Office Action mailed April 30, 2018, and to the Advisory Action mailed July 20, 2018, and is filed with a Request for Continued Examination (RCE).

The Commissioner is authorized by this paper to charge any fees during the entire pendency of this application, including fees due under 37 C.F.R. §§ 1.16 and 1.17 that may be required, including any required extension of time fees, or credit any overpayment to Deposit Account 50-4302, referencing Attorney Docket No.: UCHI-34458/US-4/CON. This paragraph is intended to be a CONSTRUCTIVE PETITION FOR EXTENSION OF TIME in accordance with 37 C.F.R. § 1.136(a)(3).

CLAIMS

1-19. (cancelled)

20. (currently amended) A method of treating cancer in a human subject comprising co-administering to the subject an immune checkpoint inhibitor and a bacterial formulation comprising bacteria of the genus *Adlercreutzia, Oscillopira, Mollicutes, Butyrivibrio, Bacteroides, Clostridium, Fusobacterium, Eubacterium, Ruminococcus, Peptococcus, Peptostreptococcus, Rikenella, Alistipes, Marinilabilia, ~~Anaerostipes,~~ or Escherichia~~, or Lactobacillus~~*.

21. (currently amended) The method of claim 20, wherein at least 50% of the bacteria in the bacterial formulation are of the genus *Adlercreutzia, Oscillopira, Mollicutes, Butyrivibrio, Bacteroides, Clostridium, Fusobacterium, Eubacterium, Ruminococcus, Peptococcus, Peptostreptococcus, Rikenella, Alistipes, Marinilabilia, ~~Anaerostipes,~~ or Escherichia~~, or Lactobacillus~~*.

22. (currently amended) The method of claim 20, wherein at least 90% of the bacteria in the bacterial formulation are of the genus *Adlercreutzia, Oscillopira, Mollicutes, Butyrivibrio, Bacteroides, Clostridium, Fusobacterium, Eubacterium, Ruminococcus, Peptococcus, Peptostreptococcus, Rikenella, Alistipes, Marinilabilia, ~~Anaerostipes,~~ or Escherichia~~, or Lactobacillus~~*.

23. (currently amended) The method of claim 20, ~~wherein the~~ further comprising bacteria [[are]] of the genus *Lactobacillus.*

24. (currently amended) The method of claim 23, wherein the bacteria of the genus *Lactobacillus* are of the species *Lactobacillus acidophilus, Lactobacillus casei, Lactobacillus kefiri, Lactobacillus bifidus, Lactobacillus brevis, Lactobacillus helveticus, Lactobacillus*

paracasei , Lactobacillus rhamnosus, Lactobacillus salivarius, Lactobacillus curvatus, Lactobacillus bulgaricus, Lactobacillus sakei, Lactobacillus reuteri, Lactobacillus fermentum, Lactobacillus farciminis, Lactobacillus lac tis, Lactobacillus delbrueckii, Lactobacillus plantarum, Lactobacillus paraplantarum, Lactobacillus crispatus, Lactobacillus gasseri, Lactobacillus johnsonii or *Lactobacillus jensenii.*

25. (previously presented) The method of claim 20, wherein the bacterial formulation is administered by oral administration or rectal administration.

26. (previously presented) The method of claim 25, wherein the bacterial formulation is administered by oral administration.

27. (currently amended) The method of claim 20, wherein the bacterial formulation comprises at least $5x10^6$ CFU of bacteria of the genus *Adlercreutzia, Oscillopira, Mollicutes, Butyrivibrio, Bacteroides, Clostridium, Fusobacterium, Eubacterium, Ruminococcus, Peptococcus, Peptostreptococcus, Rikenella, Alistipes, Marinilabilia, ~~Anaerostipes,~~ or Escherichia~~, or Lactobacillus~~*.

28. (previously presented) The method of claim 20, wherein the bacterial formulation is administered to the subject in two or more doses.

29. (previously presented) The method of claim 28, wherein the administration of the two or more doses are separated by at least 1 week.

30. (previously presented) The method of claim 20, further comprising administering to the subject an antibiotic prior to the administration of the bacterial formulation.

31. (previously presented) The method of claim 29, wherein the antibiotic is administered to the subject at least 1 day before the bacterial formulation is administered to the subject.

32. (previously presented) The method of claim 20, wherein the immune checkpoint inhibitor is a protein or polypeptide that binds to an immune checkpoint protein.

33. (previously presented) The method of claim 32, wherein the immune checkpoint protein is CTLA4, PD-1, PD-L1, PD-L2, A2AR, B7-H3, B7-H4, BTLA, KIR, LAG3, TIM-3 or VISTA.

34. (previously presented) The method of claim 33, wherein the immune checkpoint protein is PD-1 or PD-L1.

35. (previously presented) The method of claim 20, wherein the immune checkpoint inhibitor is an antibody or antigen binding fragment thereof that binds to an immune checkpoint protein.

36. (previously presented) The method of claim 35, wherein the immune checkpoint protein is CTLA4, PD-1, PD-L1, PD-L2, A2AR, B7-H3, B7-H4, BTLA, KIR, LAG3, TIM-3 or VISTA.

37. (previously presented) The method of claim 36, wherein the immune checkpoint protein is PD-1 or PD-L1.

38. (previously presented) The method of claim 20, wherein the immune checkpoint inhibitor is nivolumab, pembrolizumab, pidilizumab, AMP-224, AMP-514, STI-A1110, TSR-

042, RG-7446, BMS-936559, BMS-936558, MK-3475, CT Ol l, MPDL3280A, MEDI-4736, MSB-0020718C, AUR-012 and STI-A1010.

39. (previously presented) The method of claim 20, wherein the immune checkpoint inhibitor is administered by intravenous injection, intramuscular injection, intratumoral injection or subcutaneous injection.

40. (currently amended) The method of claim 20, wherein the bacteria are of the genus *Rikenella, Alistipes, or Marinilabilia ~~or Anaerostipes~~.*

41. (currently amended) The method of claim 40, wherein the bacterial formulation comprises at least $5x10^6$ CFU of bacteria of the genus *Rikenella, Alistipes, or Marinilabilia ~~or Anaerostipes,~~* and wherein at least 50% of the bacteria in the bacterial formulation are of the genus *Rikenella, Alistipes, or Marinilabilia ~~or Anaerostipes~~*.

42. (currently amended) The method of claim 41, wherein at least 90% of the bacteria in the bacterial formulation are of the genus *Rikenella, Alistipes, or Marinilabilia ~~or Anaerostipes~~*.

43. (previously presented) The method of claim 40, wherein the bacterial formulation is administered by oral administration or rectal administration.

44. (previously presented) The method of claim 43, wherein the bacterial formulation is administered by oral administration.

45. (previously presented) The method of claim 40, wherein the bacterial formulation is administered to the subject in two or more doses.

46. (previously presented) The method of claim 40, further comprising administering to the subject an antibiotic before the bacterial formulation is administered to the subject.

47. (previously presented) The method of claim 40, wherein the immune checkpoint inhibitor is an antibody or antigen binding fragment thereof that binds to CTLA4, PD-1, PD-L1, PD-L2, A2AR, B7-H3, B7-H4, BTLA, KIR, LAG3, TIM-3 or VISTA.

48. (previously presented) The method of claim 40, wherein the immune checkpoint inhibitor is an antibody or antigen binding fragment thereof that binds to PD-1 or PD-L1.

49. (previously presented) The method of claim 40, wherein the immune checkpoint inhibitor is nivolumab, pembrolizumab, pidilizumab, AMP-224, AMP-514, STI-A1110, TSR-042, RG-7446, BMS-936559, BMS-936558, MK-3475, CT O11, MPDL3280A, MEDI-4736, MSB-0020718C, AUR-012 and STI-A1010.

REMARKS

Claims 20-24, 27, and 40-42 are amended in the present communication. Amendments are made without acquiescing to the Examiner's arguments, in order to further prosecution, and while retaining the right to pursue unamended or similar claims in the future. Support for the amendments to the claims can be found throughout the specification; no new matter is added.

Claims 20-39 are rejected under 35 U.S.C. 103(a) as allegedly being unpatentable over U.S. Pat. Pub. No. 2009/0217401 ("Korman") in view of Mohania *et al., Acta. BioMed.* 2013 84:102-109 ("Mohania") and U.S. Pat. Pub. No. 2010/0028449 ("Prakish"). Specifically, the office action states that "Korman et al., teach methods for treating cancer, using anti-PD-1 antibodies"[1] while "Mohania et al., teach modulation of expression of Programmed Death-1 by administration of probiotic Dahi in DMH-induced colorectal carcinogenesis in rats"[2] and "Prakash et al., teach [a] method of treating a patient [who] is suffering from a cancer disease or disorder including colorectal cancer, cancer breast cancer, prostate cancer, [or] lung cancer."[3] According to the Office Action, "it would have been *prima facie* obvious at the time of applicants' invention to incorporate Mohania and Prakash et al.'s *Lactobacillus* to treat cancer to Korman's method for treating cancer in a human subject comprising administering to the subject an immune checkpoint inhibitor."[4] Applicant respectfully disagrees. However, without acquiescing to the Examiner's arguments, Applicant has amended claim 20 to recite "A method of treating cancer in a human subject comprising co-administering to the subject an immune checkpoint inhibitor and a bacterial formulation comprising bacteria of the genus *Adlercreutzia, Oscillopira, Mollicutes, Butyrivibrio, Bacteroides, Clostridium, Fusobacterium, Eubacterium, Ruminococcus, Peptococcus, Peptostreptococcus, Rikenella, Alistipes, Marinilabilia, ~~Anaerostipes,~~ <u>or</u> Escherichia~~, or Lactobacillus~~*," thereby removing *Lactobacillus* from the claim. In light of the amendment, the cited references do not, individually or in combination, teach or suggest the subject matter of claim 20, or any claims dependent thereupon. As such, Applicant submits that the claims are not obvious over the alleged combination of references, and respectfully requests withdrawal of the rejection.

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[1] Pending Office Action, at p. 3.
[2] *Id.,* at p. 5.
[3] *Id.,* at p. 6.
[4] *Id.,* at p. 7.

Claims 40-49 are rejected under 35 U.S.C. 103(a) as allegedly being unpatentable over Korman, Mohania and Prakish, in further view of U.S. Pat. Pub. No. 2007/0258953 ("Duncan"). Specifically, the Office Action states that "Korman et al., Mohania et al., and Prakash et al., have been discussed above as teaching a method of treating cancer in a human subject comprising co-administering to the subject an immune checkpoint inhibitor and a bacterial formulation comprising a bacterium, but the bacteria is not of the genus *Rikenella, Alistipes, Marinilabilia,* or *Anaerostipes,*" but that this deficiency is cured by the disclosure of Duncan.[5] Applicant respectfully disagrees. As noted above, claim 20, from which claims 40-49 depend, has been amended to remove *Anaerostipes* from the claims; claims 40-49 have been amended similarly. In light of the amendments, the cited references do not, individually or in combination, teach or suggest the subject matter of claims 40-49. As such, Applicant submits that the claims are not obvious over the alleged combination of references, and respectfully requests withdrawal of the rejection.

CONCLUSION

Applicant respectfully submits that the remarks herein overcome the Office's rejections and place the claims in condition for allowance. If the Examiner wishes to discuss this case, Applicants encourage the Examiner to call the undersigned at 608-662-1277 at the Examiner's convenience.

Respectfully submitted,

Date: July 24, 2018

/David W. Staple/

David W. Staple
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[5] *Id.,* at p. 9.

Electronic Patent Application Fee Transmittal

Application Number:	15718735
Filing Date:	28-Sep-2017
Title of Invention:	TREATMENT OF CANCER BY MANIPULATION OF COMMENSAL MICROFLORA
First Named Inventor/Applicant Name:	Thomas F. Gajewski
Filer:	David William Staple
Attorney Docket Number:	UCHI-34458/US-4/CON

Filed as Small Entity

Filing Fees for Utility under 35 USC 111(a)

Description	Fee Code	Quantity	Amount	Sub-Total in USD($)
Basic Filing:				
Pages:				
Claims:				
Miscellaneous-Filing:				
Petition:				
Patent-Appeals-and-Interference:				
Post-Allowance-and-Post-Issuance:				
Extension-of-Time:				

Description	Fee Code	Quantity	Amount	Sub-Total in USD($)
Miscellaneous:				
RCE- 1ST REQUEST	2801	1	650	650
		Total in USD ($)		**650**

Electronic Acknowledgement Receipt

EFS ID:	33263139
Application Number:	15718735
International Application Number:	
Confirmation Number:	5538
Title of Invention:	TREATMENT OF CANCER BY MANIPULATION OF COMMENSAL MICROFLORA
First Named Inventor/Applicant Name:	Thomas F. Gajewski
Customer Number:	72960
Filer:	David William Staple/Jesse Owens
Filer Authorized By:	David William Staple
Attorney Docket Number:	UCHI-34458/US-4/CON
Receipt Date:	24-JUL-2018
Filing Date:	28-SEP-2017
Time Stamp:	15:49:41
Application Type:	Utility under 35 USC 111(a)

Payment information:

Submitted with Payment	yes
Payment Type	CARD
Payment was successfully received in RAM	$650
RAM confirmation Number	072518INTEFSW15500200
Deposit Account	
Authorized User	

The Director of the USPTO is hereby authorized to charge indicated fees and credit any overpayment as follows:

File Listing:

Document Number	Document Description	File Name	File Size(Bytes)/ Message Digest	Multi Part /.zip	Pages (if appl.)
1	Request for Continued Examination (RCE)	2018-07-24_34458US4CON_RCE.pdf	1350070 15186a9f7eefff410bc8ff0ee3e2778a9043355f	no	3
Warnings:					
Information:					
2		2018-07-24_34458US4CON_RFOA.pdf	46780 a35b977e85a5190193803a63cd41857cd67e82ff	yes	8

Multipart Description/PDF files in .zip description

Document Description	Start	End
Amendment Submitted/Entered with Filing of CPA/RCE	1	1
Claims	2	6
Applicant Arguments/Remarks Made in an Amendment	7	8

Warnings:					
Information:					
3	Fee Worksheet (SB06)	fee-info.pdf	30622 cf94b9dc2084b3d5fdb64328e37b021b8317bf0f	no	2
Warnings:					
Information:					
		Total Files Size (in bytes):	1427472		

This Acknowledgement Receipt evidences receipt on the noted date by the USPTO of the indicated documents, characterized by the applicant, and including page counts, where applicable. It serves as evidence of receipt similar to a Post Card, as described in MPEP 503.

New Applications Under 35 U.S.C. 111

If a new application is being filed and the application includes the necessary components for a filing date (see 37 CFR 1.53(b)-(d) and MPEP 506), a Filing Receipt (37 CFR 1.54) will be issued in due course and the date shown on this Acknowledgement Receipt will establish the filing date of the application.

National Stage of an International Application under 35 U.S.C. 371

If a timely submission to enter the national stage of an international application is compliant with the conditions of 35 U.S.C. 371 and other applicable requirements a Form PCT/DO/EO/903 indicating acceptance of the application as a national stage submission under 35 U.S.C. 371 will be issued in addition to the Filing Receipt, in due course.

New International Application Filed with the USPTO as a Receiving Office

If a new international application is being filed and the international application includes the necessary components for an international filing date (see PCT Article 11 and MPEP 1810), a Notification of the International Application Number and of the International Filing Date (Form PCT/RO/105) will be issued in due course, subject to prescriptions concerning national security, and the date shown on this Acknowledgement Receipt will establish the international filing date of the application.

PTO/SB/06 (09-11)
Approved for use through 1/31/2014. OMB 0651-0032
U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE

PATENT APPLICATION FEE DETERMINATION RECORD Substitute for Form PTO-875	Application or Docket Number 15/718,735	Filing Date 09/28/2017	☐ To be Mailed

ENTITY: ☐ LARGE ☑ SMALL ☐ MICRO

APPLICATION AS FILED - PART I

FOR	(Column 1) NUMBER FILED	(Column 2) NUMBER EXTRA		RATE ($)	FEE ($)
☐ BASIC FEE (37 CFR 1.16(a), (b), or (c))	N/A	N/A		N/A	
☐ SEARCH FEE (37 CFR 1.16(k), (i), or (m))	N/A	N/A		N/A	
☐ EXAMINATION FEE (37 CFR 1.16(o), (p), or (q))	N/A	N/A		N/A	
TOTAL CLAIMS (37 CFR 1.16(i))	minus 20 =	*		x $40 =	
INDEPENDENT CLAIMS (37 CFR 1.16(h))	minus 3 =	*		x $210 =	
☐ APPLICATION SIZE FEE (37 CFR 1.16(s))	If the specification and drawings exceed 100 sheets of paper, the application size fee due is $310 ($155 for small entity) for each additional 50 sheets or fraction thereof. See 35 U.S.C. 41(a)(1)(G) and 37 CFR 1.16(s).				
☐ MULTIPLE DEPENDENT CLAIM PRESENT (37 CFR 1.16(j))					
* If the difference in column 1 is less than zero, enter "0" in column 2.				TOTAL	

APPLICATION AS AMENDED - PART II

		(Column 1)		(Column 2)	(Column 3)			
AMENDMENT	07/24/2018	CLAIMS REMAINING AFTER AMENDMENT		HIGHEST NUMBER PREVIOUSLY PAID FOR	PRESENT EXTRA		RATE ($)	ADDITIONAL FEE ($)
	Total (37 CFR 1.16(i))	* 30	Minus	** 30	= 0		x $50 =	0
	Independent (37 CFR 1.16(h))	* 1	Minus	*** 3	= 0		x $230 =	0
	☐ Application Size Fee (37 CFR 1.16(s))							
	☐ FIRST PRESENTATION OF MULTIPLE DEPENDENT CLAIM (37 CFR 1.16(j))							
							TOTAL ADD'L FEE	0

		(Column 1)		(Column 2)	(Column 3)			
AMENDMENT		CLAIMS REMAINING AFTER AMENDMENT		HIGHEST NUMBER PREVIOUSLY PAID FOR	PRESENT EXTRA		RATE ($)	ADDITIONAL FEE ($)
	Total (37 CFR 1.16(i))	*	Minus	**	=		x $ 0 =	
	Independent (37 CFR 1.16(h))	*	Minus	***	=		x $ 0 =	
	☐ Application Size Fee (37 CFR 1.16(s))							
	☐ FIRST PRESENTATION OF MULTIPLE DEPENDENT CLAIM (37 CFR 1.16(j))							
							TOTAL ADD'L FEE	

* If the entry in column 1 is less than the entry in column 2, write "0" in column 3.	LIE
** If the "Highest Number Previously Paid For" IN THIS SPACE is less than 20, enter "20".	marsha R richards
*** If the "Highest Number Previously Paid For" IN THIS SPACE is less than 3, enter "3".	
The "Highest Number Previously Paid For" (Total or Independent) is the highest number found in the appropriate box in column 1.	

UNITED STATES PATENT AND TRADEMARK OFFICE

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www.uspto.gov

APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
15/718,735	09/28/2017	Thomas F. Gajewski	UCHI-34458/US-4/CON	5538

72960 7590 07/20/2018

Casimir Jones, S.C.
2275 Deming Way Ste 310
Middleton, WISCONSIN 53562
UNITED STATES OF AMERICA

EXAMINER	
HINES, JANA A	

ART UNIT	PAPER NUMBER
1645	

NOTIFICATION DATE	DELIVERY MODE
07/20/2018	ELECTRONIC

Please find below and/or attached an Office communication concerning this application or proceeding.

The time period for reply, if any, is set in the attached communication.

Notice of the Office communication was sent electronically on above-indicated "Notification Date" to the following e-mail address(es):

docketing@casimirjones.com
pto.correspondence@casimirjones.com

Advisory Action Before the Filing of an Appeal Brief	Application No. 15/718,735	Applicant(s) Gajewski et al.	
	Examiner JA-NA A HINES	Art Unit 1645	AIA Status Yes

--The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

THE REPLY FILED 29 June 2018 FAILS TO PLACE THIS APPLICATION IN CONDITION FOR ALLOWANCE.

NO NOTICE OF APPEAL FILED

1. ☑ The reply was filed after a final rejection. No Notice of Appeal has been filed. To avoid abandonment of this application, applicant must timely file one of the following replies: (1) an amendment, affidavit, or other evidence, which places the application in condition for allowance; (2) a Notice of Appeal (with appeal fee) in compliance with 37 CFR 41.31; or (3) a Request for Continued Examination (RCE) in compliance with 37 CFR 1.114 if this is a utility or plant application. Note that RCEs are not permitted in design applications. The reply must be filed within one of the following time periods:
   a) ☐ The period for reply expires ____ months from the mailing date of the final rejection.
   b) ☑ The period for reply expires on: (1) the mailing date of this Advisory Action; or (2) the date set forth in the final rejection, whichever is later. In no event, however, will the statutory period for reply expire later than SIX MONTHS from the mailing date of the final rejection.
   c) ☐ A prior Advisory Action was mailed more than 3 months after the mailing date of the final rejection in response to a first after-final reply filed within 2 months of the mailing date of the final rejection.The current period for reply expires ____ months from the mailing date of *the prior Advisory Action* or SIX MONTHS from the mailing date of the final rejection, whichever is earlier.
   Examiner Note: If box 1 is checked, check either box (a), (b) or (c). ONLY CHECK BOX (b) WHEN THIS ADVISORY ACTION IS THE FIRST RESPONSE TO APPLICANTS FIRST AFTER-FINAL REPLY WHICH WAS FILED WITHIN TWO MONTHS OF THE FINAL REJECTION. ONLY CHECK BOX (c) IN THE LIMITED SITUATION SET FORTH UNDER BOX (c). See MPEP 706.07(f).

Extensions of time may be obtained under 37 CFR 1.136(a). The date on which the petition under 37 CFR 1.136(a) and the appropriate extension fee have been filed is the date for purposes of determining the period of extension and the corresponding amount of the fee. The appropriate extension fee under 37 CFR 1.17(a) is calculated from: (1) the expiration date of the shortened statutory period for reply originally set in the final Office action; or (2) as set forth in (b) or (c) above, if checked. Any reply received by the Office later than three months after the mailing date of the final rejection, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

NOTICE OF APPEAL

2. ☐ The Notice of Appeal was filed on _____. A brief in compliance with 37 CFR 41.37 must be filed within two months of the date of filing the Notice of Appeal (37 CFR 41.37(a)), or any extension thereof (37 CFR 41.37(e)), to avoid dismissal of the appeal. Since a Notice of Appeal has been filed, any reply must be filed within the time period set forth in 37CFR 41.37(a).

AMENDMENTS

3. ☑ The proposed amendments filed after a final rejection, but prior to the date of filing a brief, will not be entered because
   a) ☑ They raise new issues that would require further consideration and/or search (see NOTE below);
   b) ☐ They raise the issue of new matter (see NOTE below);
   c) ☑ They are not deemed to place the application in better form for appeal by materially reducing or simplifying the issues for appeal; and/or
   d) ☐ They present additional claims without canceling a corresponding number of finally rejected claims.
   NOTE: See Continuation Sheet (See 37CFR 1.116 and 41.33(a)).
4. ☐ The amendments are not in compliance with 37CFR 1.121. See attached Notice of Non-Compliant Amendment (PTOL-324).
5. ☐ Applicants reply has overcome the following rejection(s): _____
6. ☐ Newly proposed or amended claim(s) _____ would be allowable if submitted in a separate, timely filed amendment canceling the non-allowable claim(s).
7. ☑ For purposes of appeal, the proposed amendment(s):(a) ☑ will not be entered, or (b) ☐ will be entered, and an explanation of how the new or amended claims would be rejected is provided below or appended.

AFFIDAVIT OR OTHER EVIDENCE

8. ☐ A declaration(s)/affidavit(s) under **37 CFR 1.130(b)** was/were filed on _____
9. ☐ The affidavit or other evidence filed after final action, but before or on the date of filing a Notice of Appeal will not be entered because applicant failed to provide a showing of good and sufficient reasons why the affidavit or other evidence is necessary and was not earlier presented. See 37 CFR 1.116(e).
10. ☐ The affidavit or other evidence filed after the date of filing the Notice of Appeal, but prior to the date of filing a brief, will not be entered because the affidavit or other evidence failed to overcome all rejections under appeal and/or appellant fails to provide a showing of good and sufficient reasons why it is necessary and was not earlier presented. See 37 CFR 41.33(d)(1).
11. ☐ The affidavit or other evidence is entered. An explanation of the status of the claims after entry is below or attached.

REQUEST FOR RECONSIDERATION/OTHER

12. ☐ The request for reconsideration has been considered but does NOT place the application in condition for allowance because: _____.
13. ☐ Note the attached Information *Disclosure Statement*(s). (PTO/SB/08) Paper No(s). _____
14. ☐ Other: _____.

STATUS OF CLAIMS

15. The status of the claim(s) is (or will be) as follows:
    Claim(s) allowed:_____.
    Claim(s) objected to:_____.
    Claim(s) rejected:20-49.
    Claim(s) withdrawn from consideration:_____.

/JANA A HINES/ Primary Examiner, Art Unit 1645	

The proposed after final amendment now excludes two previously claimed bacterial genera. The after final proposed amendments requires further search and consideration. Moreover, the proposed after final amendment does not place the application in better form for appeal and it increases the issues for appeal. Therefore, the proposed after final amendments with not be entered.

In view of the proposed after final amendments not being entered, applicants arguments will not be addressed. Therefore the previous rejections of record are maintained.

CLAIMS

1-19. (cancelled)

20. (currently amended) A method of treating cancer in a human subject comprising co-administering to the subject an immune checkpoint inhibitor and a bacterial formulation comprising bacteria of the genus *Adlercreutzia, Oscillopira, Mollicutes, Butyrivibrio, Bacteroides, Clostridium, Fusobacterium, Eubacterium, Ruminococcus, Peptococcus, Peptostreptococcus, Rikenella, Alistipes, Marinilabilia, ~~Anaerostipes,~~ or Escherichia~~, or Lactobacillus~~*.

21. (currently amended) The method of claim 20, wherein at least 50% of the bacteria in the bacterial formulation are of the genus *Adlercreutzia, Oscillopira, Mollicutes, Butyrivibrio, Bacteroides, Clostridium, Fusobacterium, Eubacterium, Ruminococcus, Peptococcus, Peptostreptococcus, Rikenella, Alistipes, Marinilabilia, ~~Anaerostipes,~~ or Escherichia~~, or Lactobacillus~~*.

22. (currently amended) The method of claim 20, wherein at least 90% of the bacteria in the bacterial formulation are of the genus *Adlercreutzia, Oscillopira, Mollicutes, Butyrivibrio, Bacteroides, Clostridium, Fusobacterium, Eubacterium, Ruminococcus, Peptococcus, Peptostreptococcus, Rikenella, Alistipes, Marinilabilia, ~~Anaerostipes,~~ or Escherichia~~, or Lactobacillus~~*.

23. (currently amended) The method of claim 20, ~~wherein the~~ further comprising bacteria [[are]] of the genus *Lactobacillus.*

24. (currently amended) The method of claim 23, wherein the bacteria of the genus *Lactobacillus* are of the species *Lactobacillus acidophilus, Lactobacillus casei, Lactobacillus kefiri, Lactobacillus bifidus, Lactobacillus brevis, Lactobacillus helveticus, Lactobacillus*

paracasei , Lactobacillus rhamnosus, Lactobacillus salivarius, Lactobacillus curvatus, Lactobacillus bulgaricus, Lactobacillus sakei, Lactobacillus reuteri, Lactobacillus fermentum, Lactobacillus farciminis, Lactobacillus lac tis, Lactobacillus delbrueckii, Lactobacillus plantarum, Lactobacillus paraplantarum, Lactobacillus crispatus, Lactobacillus gasseri, Lactobacillus johnsonii or *Lactobacillus jensenii.*

25. (previously presented) The method of claim 20, wherein the bacterial formulation is administered by oral administration or rectal administration.

26. (previously presented) The method of claim 25, wherein the bacterial formulation is administered by oral administration.

27. (currently amended) The method of claim 20, wherein the bacterial formulation comprises at least $5x10^6$ CFU of bacteria of the genus *Adlercreutzia, Oscillopira, Mollicutes, Butyrivibrio, Bacteroides, Clostridium, Fusobacterium, Eubacterium, Ruminococcus, Peptococcus, Peptostreptococcus, Rikenella, Alistipes, Marinilabilia, ~~Anaerostipes,~~ or Escherichia~~, or Lactobacillus~~*.

28. (previously presented) The method of claim 20, wherein the bacterial formulation is administered to the subject in two or more doses.

29. (previously presented) The method of claim 28, wherein the administration of the two or more doses are separated by at least 1 week.

30. (previously presented) The method of claim 20, further comprising administering to the subject an antibiotic prior to the administration of the bacterial formulation.

31. (previously presented) The method of claim 29, wherein the antibiotic is administered to the subject at least 1 day before the bacterial formulation is administered to the subject.

32. (previously presented) The method of claim 20, wherein the immune checkpoint inhibitor is a protein or polypeptide that binds to an immune checkpoint protein.

33. (previously presented) The method of claim 32, wherein the immune checkpoint protein is CTLA4, PD-1, PD-L1, PD-L2, A2AR, B7-H3, B7-H4, BTLA, KIR, LAG3, TIM-3 or VISTA.

34. (previously presented) The method of claim 33, wherein the immune checkpoint protein is PD-1 or PD-L1.

35. (previously presented) The method of claim 20, wherein the immune checkpoint inhibitor is an antibody or antigen binding fragment thereof that binds to an immune checkpoint protein.

36. (previously presented) The method of claim 35, wherein the immune checkpoint protein is CTLA4, PD-1, PD-L1, PD-L2, A2AR, B7-H3, B7-H4, BTLA, KIR, LAG3, TIM-3 or VISTA.

37. (previously presented) The method of claim 36, wherein the immune checkpoint protein is PD-1 or PD-L1.

38. (previously presented) The method of claim 20, wherein the immune checkpoint inhibitor is nivolumab, pembrolizumab, pidilizumab, AMP-224, AMP-514, STI-A1110, TSR-

042, RG-7446, BMS-936559, BMS-936558, MK-3475, CT Ol l, MPDL3280A, MEDI-4736, MSB-0020718C, AUR-012 and STI-A1010.

39. (previously presented) The method of claim 20, wherein the immune checkpoint inhibitor is administered by intravenous injection, intramuscular injection, intratumoral injection or subcutaneous injection.

40. (currently amended) The method of claim 20, wherein the bacteria are of the genus *Rikenella, Alistipes, or Marinilabilia ~~or Anaerostipes~~*.

41. (currently amended) The method of claim 40, wherein the bacterial formulation comprises at least $5x10^6$ CFU of bacteria of the genus *Rikenella, Alistipes, or Marinilabilia ~~or Anaerostipes,~~* and wherein at least 50% of the bacteria in the bacterial formulation are of the genus *Rikenella, Alistipes, or Marinilabilia ~~or Anaerostipes~~*.

42. (currently amended) The method of claim 41, wherein at least 90% of the bacteria in the bacterial formulation are of the genus *Rikenella, Alistipes, or Marinilabilia ~~or Anaerostipes~~*.

43. (previously presented) The method of claim 40, wherein the bacterial formulation is administered by oral administration or rectal administration.

44. (previously presented) The method of claim 43, wherein the bacterial formulation is administered by oral administration.

45. (previously presented) The method of claim 40, wherein the bacterial formulation is administered to the subject in two or more doses.

46. (previously presented) The method of claim 40, further comprising administering to the subject an antibiotic before the bacterial formulation is administered to the subject.

47. (previously presented) The method of claim 40, wherein the immune checkpoint inhibitor is an antibody or antigen binding fragment thereof that binds to CTLA4, PD-1, PD-L1, PD-L2, A2AR, B7-H3, B7-H4, BTLA, KIR, LAG3, TIM-3 or VISTA.

48. (previously presented) The method of claim 40, wherein the immune checkpoint inhibitor is an antibody or antigen binding fragment thereof that binds to PD-1 or PD-L1.

49. (previously presented) The method of claim 40, wherein the immune checkpoint inhibitor is nivolumab, pembrolizumab, pidilizumab, AMP-224, AMP-514, STI-A1110, TSR-042, RG-7446, BMS-936559, BMS-936558, MK-3475, CT Ol l, MPDL3280A, MEDI-4736, MSB-0020718C, AUR-012 and STI-A1010.

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant:	University of Chicago	Confirmation No.:	5538
Serial No.:	15/718,735	Art Unit:	1645
Filed:	28-Sep-2017	Examiner:	Hines, Jana A.
Title:	**TREATMENT OF CANCER BY MANIPULATION OF COMMENSAL MICROFLORA**		

RESPONSE TO FINAL OFFICE ACTION MAILED APRIL 30, 2018

VIA EFS-WEB
COMMISSIONER FOR PATENTS
P.O. BOX 1450
ALEXANDRIA, VA 22313-1450

Examiner Hines:

This communication is in response to the Final Office Action mailed April 30, 2018, and is filed within the two-month time period to provoke an Advisory Action.

The Commissioner is authorized by this paper to charge any fees during the entire pendency of this application, including fees due under 37 C.F.R. §§ 1.16 and 1.17 that may be required, including any required extension of time fees, or credit any overpayment to Deposit Account 50-4302, referencing Attorney Docket No.: UCHI-34458/US-4/CON. This paragraph is intended to be a CONSTRUCTIVE PETITION FOR EXTENSION OF TIME in accordance with 37 C.F.R. § 1.136(a)(3).

CLAIMS

1-19. (cancelled)

20. (currently amended) A method of treating cancer in a human subject comprising co-administering to the subject an immune checkpoint inhibitor and a bacterial formulation comprising bacteria of the genus *Adlercreutzia, Oscillopira, Mollicutes, Butyrivibrio, Bacteroides, Clostridium, Fusobacterium, Eubacterium, Ruminococcus, Peptococcus, Peptostreptococcus, Rikenella, Alistipes, Marinilabilia, ~~Anaerostipes,~~ or Escherichia~~, or Lactobacillus~~*.

21. (currently amended) The method of claim 20, wherein at least 50% of the bacteria in the bacterial formulation are of the genus *Adlercreutzia, Oscillopira, Mollicutes, Butyrivibrio, Bacteroides, Clostridium, Fusobacterium, Eubacterium, Ruminococcus, Peptococcus, Peptostreptococcus, Rikenella, Alistipes, Marinilabilia, ~~Anaerostipes,~~ or Escherichia~~, or Lactobacillus~~*.

22. (currently amended) The method of claim 20, wherein at least 90% of the bacteria in the bacterial formulation are of the genus *Adlercreutzia, Oscillopira, Mollicutes, Butyrivibrio, Bacteroides, Clostridium, Fusobacterium, Eubacterium, Ruminococcus, Peptococcus, Peptostreptococcus, Rikenella, Alistipes, Marinilabilia, ~~Anaerostipes,~~ or Escherichia~~, or Lactobacillus~~*.

23. (currently amended) The method of claim 20, ~~wherein the~~ further comprising bacteria [[are]] of the genus *Lactobacillus.*

24. (currently amended) The method of claim 23, wherein the bacteria of the genus *Lactobacillus* are of the species *Lactobacillus acidophilus, Lactobacillus casei, Lactobacillus kefiri, Lactobacillus bifidus, Lactobacillus brevis, Lactobacillus helveticus, Lactobacillus*

paracasei , Lactobacillus rhamnosus, Lactobacillus salivarius, Lactobacillus curvatus, Lactobacillus bulgaricus, Lactobacillus sakei, Lactobacillus reuteri, Lactobacillus fermentum, Lactobacillus farciminis, Lactobacillus lac tis, Lactobacillus delbrueckii, Lactobacillus plantarum, Lactobacillus paraplantarum, Lactobacillus crispatus, Lactobacillus gasseri, Lactobacillus johnsonii or *Lactobacillus jensenii.*

25. (previously presented) The method of claim 20, wherein the bacterial formulation is administered by oral administration or rectal administration.

26. (previously presented) The method of claim 25, wherein the bacterial formulation is administered by oral administration.

27. (currently amended) The method of claim 20, wherein the bacterial formulation comprises at least $5x10^6$ CFU of bacteria of the genus *Adlercreutzia, Oscillopira, Mollicutes, Butyrivibrio, Bacteroides, Clostridium, Fusobacterium, Eubacterium, Ruminococcus, Peptococcus, Peptostreptococcus, Rikenella, Alistipes, Marinilabilia, ~~Anaerostipes,~~ or Escherichia~~, or Lactobacillus~~*.

28. (previously presented) The method of claim 20, wherein the bacterial formulation is administered to the subject in two or more doses.

29. (previously presented) The method of claim 28, wherein the administration of the two or more doses are separated by at least 1 week.

30. (previously presented) The method of claim 20, further comprising administering to the subject an antibiotic prior to the administration of the bacterial formulation.

31. (previously presented) The method of claim 29, wherein the antibiotic is administered to the subject at least 1 day before the bacterial formulation is administered to the subject.

32. (previously presented) The method of claim 20, wherein the immune checkpoint inhibitor is a protein or polypeptide that binds to an immune checkpoint protein.

33. (previously presented) The method of claim 32, wherein the immune checkpoint protein is CTLA4, PD-1, PD-L1, PD-L2, A2AR, B7-H3, B7-H4, BTLA, KIR, LAG3, TIM-3 or VISTA.

34. (previously presented) The method of claim 33, wherein the immune checkpoint protein is PD-1 or PD-L1.

35. (previously presented) The method of claim 20, wherein the immune checkpoint inhibitor is an antibody or antigen binding fragment thereof that binds to an immune checkpoint protein.

36. (previously presented) The method of claim 35, wherein the immune checkpoint protein is CTLA4, PD-1, PD-L1, PD-L2, A2AR, B7-H3, B7-H4, BTLA, KIR, LAG3, TIM-3 or VISTA.

37. (previously presented) The method of claim 36, wherein the immune checkpoint protein is PD-1 or PD-L1.

38. (previously presented) The method of claim 20, wherein the immune checkpoint inhibitor is nivolumab, pembrolizumab, pidilizumab, AMP-224, AMP-514, STI-A1110, TSR-

042, RG-7446, BMS-936559, BMS-936558, MK-3475, CT Ol l, MPDL3280A, MEDI-4736, MSB-0020718C, AUR-012 and STI-A1010.

39. (previously presented) The method of claim 20, wherein the immune checkpoint inhibitor is administered by intravenous injection, intramuscular injection, intratumoral injection or subcutaneous injection.

40. (currently amended) The method of claim 20, wherein the bacteria are of the genus *Rikenella, Alistipes, or Marinilabilia ~~or Anaerostipes~~*.

41. (currently amended) The method of claim 40, wherein the bacterial formulation comprises at least $5x10^6$ CFU of bacteria of the genus *Rikenella, Alistipes, or Marinilabilia ~~or Anaerostipes,~~* and wherein at least 50% of the bacteria in the bacterial formulation are of the genus *Rikenella, Alistipes, or Marinilabilia ~~or Anaerostipes~~*.

42. (currently amended) The method of claim 41, wherein at least 90% of the bacteria in the bacterial formulation are of the genus *Rikenella, Alistipes, or Marinilabilia ~~or Anaerostipes~~*.

43. (previously presented) The method of claim 40, wherein the bacterial formulation is administered by oral administration or rectal administration.

44. (previously presented) The method of claim 43, wherein the bacterial formulation is administered by oral administration.

45. (previously presented) The method of claim 40, wherein the bacterial formulation is administered to the subject in two or more doses.

46. (previously presented) The method of claim 40, further comprising administering to the subject an antibiotic before the bacterial formulation is administered to the subject.

47. (previously presented) The method of claim 40, wherein the immune checkpoint inhibitor is an antibody or antigen binding fragment thereof that binds to CTLA4, PD-1, PD-L1, PD-L2, A2AR, B7-H3, B7-H4, BTLA, KIR, LAG3, TIM-3 or VISTA.

48. (previously presented) The method of claim 40, wherein the immune checkpoint inhibitor is an antibody or antigen binding fragment thereof that binds to PD-1 or PD-L1.

49. (previously presented) The method of claim 40, wherein the immune checkpoint inhibitor is nivolumab, pembrolizumab, pidilizumab, AMP-224, AMP-514, STI-A1110, TSR-042, RG-7446, BMS-936559, BMS-936558, MK-3475, CT Ol l, MPDL3280A, MEDI-4736, MSB-0020718C, AUR-012 and STI-A1010.

REMARKS

Claims 20-24, 27, and 40-42 are amended in the present communication. Amendments are made without acquiescing to the Examiner's arguments, in order to further prosecution, and while retaining the right to pursue unamended or similar claims in the future. Support for the amendments to the claims can be found throughout the specification; no new matter is added.

Claims 20-39 are rejected under 35 U.S.C. 103(a) as allegedly being unpatentable over U.S. Pat. Pub. No. 2009/0217401 ("Korman") in view of Mohania *et al., Acta. BioMed.* 2013 84:102-109 ("Mohania") and U.S. Pat. Pub. No. 2010/0028449 ("Prakish"). Specifically, the office action states that "Korman et al., teach methods for treating cancer, using anti-PD-1 antibodies"[1] while "Mohania et al., teach modulation of expression of Programmed Death-1 by administration of probiotic Dahi in DMH-induced colorectal carcinogenesis in rats"[2] and "Prakash et al., teach [a] method of treating a patient [who] is suffering from a cancer disease or disorder including colorectal cancer, cancer breast cancer, prostate cancer, [or] lung cancer."[3] According to the Office Action, "it would have been *prima facie* obvious at the time of applicants' invention to incorporate Mohania and Prakash et al.'s *Lactobacillus* to treat cancer to Korman's method for treating cancer in a human subject comprising administering to the subject an immune checkpoint inhibitor."[4] Applicant respectfully disagrees. However, without acquiescing to the Examiner's arguments, Applicant has amended claim 20 to recite "A method of treating cancer in a human subject comprising co-administering to the subject an immune checkpoint inhibitor and a bacterial formulation comprising bacteria of the genus *Adlercreutzia, Oscillopira, Mollicutes, Butyrivibrio, Bacteroides, Clostridium, Fusobacterium, Eubacterium, Ruminococcus, Peptococcus, Peptostreptococcus, Rikenella, Alistipes, Marinilabilia, ~~Anaerostipes,~~ or Escherichia, ~~or Lactobacillus~~*," thereby removing *Lactobacillus* from the claim. In light of the amendment, the cited references do not, individually or in combination, teach or suggest the subject matter of claim 20, or any claims dependent thereupon. As such, Applicant submits that the claims are not obvious over the alleged combination of references, and respectfully requests withdrawal of the rejection.

[1] Pending Office Action, at p. 3.
[2] *Id.*, at p. 5.
[3] *Id.*, at p. 6.
[4] *Id.*, at p. 7.

Claims 40-49 are rejected under 35 U.S.C. 103(a) as allegedly being unpatentable over Korman, Mohania and Prakish, in further view of U.S. Pat. Pub. No. 2007/0258953 ("Duncan"). Specifically, the Office Action states that "Korman et al., Mohania et al., and Prakash et al., have been discussed above as teaching a method of treating cancer in a human subject comprising co-administering to the subject an immune checkpoint inhibitor and a bacterial formulation comprising a bacterium, but the bacteria is not of the genus *Rikenella, Alistipes, Marinilabilia,* or *Anaerostipes,*" but that this deficiency is cured by the disclosure of Duncan.[5] Applicant respectfully disagrees. As noted above, claim 20, from which claims 40-49 depend, has been amended to remove *Anaerostipes* from the claims; claims 40-49 have been amended similarly. In light of the amendments, the cited references do not, individually or in combination, teach or suggest the subject matter of claims 40-49. As such, Applicant submits that the claims are not obvious over the alleged combination of references, and respectfully requests withdrawal of the rejection.

CONCLUSION

Applicant respectfully submits that the remarks herein overcome the Office's rejections and place the claims in condition for allowance. If the Examiner wishes to discuss this case, Applicants encourage the Examiner to call the undersigned at 608-662-1277 at the Examiner's convenience.

Respectfully submitted,

Date: June 29, 2018

/David W. Staple/

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[5] *Id.,* at p. 9.

Electronic Acknowledgement Receipt

EFS ID:	33051568
Application Number:	15718735
International Application Number:	
Confirmation Number:	5538
Title of Invention:	TREATMENT OF CANCER BY MANIPULATION OF COMMENSAL MICROFLORA
First Named Inventor/Applicant Name:	Thomas F. Gajewski
Customer Number:	72960
Filer:	David William Staple/Stephanie Filandrinos
Filer Authorized By:	David William Staple
Attorney Docket Number:	UCHI-34458/US-4/CON
Receipt Date:	29-JUN-2018
Filing Date:	28-SEP-2017
Time Stamp:	16:44:18
Application Type:	Utility under 35 USC 111(a)

Payment information:

Submitted with Payment	no

File Listing:

Document Number	Document Description	File Name	File Size(Bytes)/ Message Digest	Multi Part /.zip	Pages (if appl.)
1		2018-06-29_34458US4CON_RFOA.pdf	120879 64dc59cf6c9f69d5be745385b4611df1cabcd872	yes	8

	Multipart Description/PDF files in .zip description		
	Document Description	**Start**	**End**
	Response After Final Action	1	1
	Claims	2	6
	Applicant Arguments/Remarks Made in an Amendment	7	8
Warnings:			
Information:			
	Total Files Size (in bytes):	120879	

This Acknowledgement Receipt evidences receipt on the noted date by the USPTO of the indicated documents, characterized by the applicant, and including page counts, where applicable. It serves as evidence of receipt similar to a Post Card, as described in MPEP 503.

New Applications Under 35 U.S.C. 111

If a new application is being filed and the application includes the necessary components for a filing date (see 37 CFR 1.53(b)-(d) and MPEP 506), a Filing Receipt (37 CFR 1.54) will be issued in due course and the date shown on this Acknowledgement Receipt will establish the filing date of the application.

National Stage of an International Application under 35 U.S.C. 371

If a timely submission to enter the national stage of an international application is compliant with the conditions of 35 U.S.C. 371 and other applicable requirements a Form PCT/DO/EO/903 indicating acceptance of the application as a national stage submission under 35 U.S.C. 371 will be issued in addition to the Filing Receipt, in due course.

New International Application Filed with the USPTO as a Receiving Office

If a new international application is being filed and the international application includes the necessary components for an international filing date (see PCT Article 11 and MPEP 1810), a Notification of the International Application Number and of the International Filing Date (Form PCT/RO/105) will be issued in due course, subject to prescriptions concerning national security, and the date shown on this Acknowledgement Receipt will establish the international filing date of the application.

PTO/SB/06 (09-11)
Approved for use through 1/31/2014. OMB 0651-0032
U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE
Under the Paperwork Reduction Act of 1995, no persons are required to respond to a collection of information unless it displays a valid OMB control number.

PATENT APPLICATION FEE DETERMINATION RECORD Substitute for Form PTO-875	Application or Docket Number 15/718,735	Filing Date 09/28/2017	☐ To be Mailed

ENTITY: ☐ LARGE ☒ SMALL ☐ MICRO

APPLICATION AS FILED – PART I

FOR	(Column 1) NUMBER FILED	(Column 2) NUMBER EXTRA	RATE ($)	FEE ($)
☐ BASIC FEE (37 CFR 1.16(a), (b), or (c))	N/A	N/A	N/A	
☐ SEARCH FEE (37 CFR 1.16(k), (i), or (m))	N/A	N/A	N/A	
☐ EXAMINATION FEE (37 CFR 1.16(o), (p), or (q))	N/A	N/A	N/A	
TOTAL CLAIMS (37 CFR 1.16(i))	minus 20 =	*	X $ =	
INDEPENDENT CLAIMS (37 CFR 1.16(h))	minus 3 =	*	X $ =	
☐ APPLICATION SIZE FEE (37 CFR 1.16(s))	If the specification and drawings exceed 100 sheets of paper, the application size fee due is $310 ($155 for small entity) for each additional 50 sheets or fraction thereof. See 35 U.S.C. 41(a)(1)(G) and 37 CFR 1.16(s).			
☐ MULTIPLE DEPENDENT CLAIM PRESENT (37 CFR 1.16(j))				
* If the difference in column 1 is less than zero, enter "0" in column 2.			TOTAL	

APPLICATION AS AMENDED – PART II

AMENDMENT **06/29/2018**

	(Column 1) CLAIMS REMAINING AFTER AMENDMENT		(Column 2) HIGHEST NUMBER PREVIOUSLY PAID FOR	(Column 3) PRESENT EXTRA	RATE ($)	ADDITIONAL FEE ($)
Total (37 CFR 1.16(i))	* 30	Minus	** 30	= 0	X $50 =	0
Independent (37 CFR 1.16(h))	* 1	Minus	***3	= 0	X $230 =	0
☐ Application Size Fee (37 CFR 1.16(s))						
☐ FIRST PRESENTATION OF MULTIPLE DEPENDENT CLAIM (37 CFR 1.16(j))						
					TOTAL ADD'L FEE	0

AMENDMENT

	(Column 1) CLAIMS REMAINING AFTER AMENDMENT		(Column 2) HIGHEST NUMBER PREVIOUSLY PAID FOR	(Column 3) PRESENT EXTRA	RATE ($)	ADDITIONAL FEE ($)
Total (37 CFR 1.16(i))	*	Minus	**	=	X $ =	
Independent (37 CFR 1.16(h))	*	Minus	***	=	X $ =	
☐ Application Size Fee (37 CFR 1.16(s))						
☐ FIRST PRESENTATION OF MULTIPLE DEPENDENT CLAIM (37 CFR 1.16(j))						
					TOTAL ADD'L FEE	

* If the entry in column 1 is less than the entry in column 2, write "0" in column 3.
** If the "Highest Number Previously Paid For" IN THIS SPACE is less than 20, enter "20".
*** If the "Highest Number Previously Paid For" IN THIS SPACE is less than 3, enter "3".
The "Highest Number Previously Paid For" (Total or Independent) is the highest number found in the appropriate box in column 1.

LIE
YOLANDA CHADWICK

UNITED STATES PATENT AND TRADEMARK OFFICE

UNITED STATES DEPARTMENT OF COMMERCE
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Alexandria, Virginia 22313-1450
www.uspto.gov

APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
15/718,735	09/28/2017	Thomas F. Gajewski	UCHI-34458/US-4/CON	5538

72960 7590 04/30/2018

Casimir Jones, S.C.
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UNITED STATES OF AMERICA

EXAMINER	
HINES, JANA A	

ART UNIT	PAPER NUMBER
1645	

NOTIFICATION DATE	DELIVERY MODE
04/30/2018	ELECTRONIC

Please find below and/or attached an Office communication concerning this application or proceeding.

The time period for reply, if any, is set in the attached communication.

Notice of the Office communication was sent electronically on above-indicated "Notification Date" to the following e-mail address(es):

docketing@casimirjones.com
pto.correspondence@casimirjones.com

Office Action Summary	Application No. 15/718,735	Applicant(s) Gajewski et al.	
	Examiner JA-NA A HINES	Art Unit 1645	AIA Status Yes

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE 3 MONTHS FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133). Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

1)☑ Responsive to communication(s) filed on 3/8/18.

☐ A declaration(s)/affidavit(s) under **37 CFR 1.130(b)** was/were filed on _____.

2a)☑ This action is **FINAL.** 2b) ☐ This action is non-final.

3)☐ An election was made by the applicant in response to a restriction requirement set forth during the interview on _____; the restriction requirement and election have been incorporated into this action.

4)☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims*

5) ☑ Claim(s) 20-49 is/are pending in the application.

5a) Of the above claim(s) _____ is/are withdrawn from consideration.

6) ☐ Claim(s) _____ is/are allowed.

7) ☑ Claim(s) 20-49 is/are rejected.

8) ☐ Claim(s) _____ is/are objected to.

9) ☐ Claim(s) _____ are subject to restriction and/or election requirement

* If any claims have been determined allowable, you may be eligible to benefit from the **Patent Prosecution Highway** program at a participating intellectual property office for the corresponding application. For more information, please see http://www.uspto.gov/patents/init_events/pph/index.jsp or send an inquiry to **PPHfeedback@uspto.gov.**

Application Papers

10)☐ The specification is objected to by the Examiner.

11)☐ The drawing(s) filed on _____ is/are: a)☐ accepted or b)☐ objected to by the Examiner.

Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).

Replacement drawing sheet(s) including the correction is required if the drawing(s) is objected to. See 37 CFR 1.121(d).

Priority under 35 U.S.C. § 119

12)☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

Certified copies:

a)☐ All b)☐ Some** c)☐ None of the:

1.☐ Certified copies of the priority documents have been received.

2.☐ Certified copies of the priority documents have been received in Application No. _____.

3.☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

** See the attached detailed Office action for a list of the certified copies not received.

Attachment(s)

1) ☐ Notice of References Cited (PTO-892)

2) ☐ Information Disclosure Statement(s) (PTO/SB/08a and/or PTO/SB/08b) Paper No(s)/Mail Date _____.

3) ☐ Interview Summary (PTO-413) Paper No(s)/Mail Date _____.

4) ☐ Other: _____.

DETAILED CORRESPONDENCE

Notice of Pre-AIA or AIA Status

The present application is being examined under the pre-AIA first to invent provisions.

Claim Status

1. Claims 1-19 are canceled. Claims 20-49 are under consideration in this Office Action.

Maintained Grounds of Rejection

Claim Rejections - 35 USC § 103

In the event the determination of the status of the application as subject to AIA 35 U.S.C. 102 and 103 (or as subject to pre-AIA 35 U.S.C. 102 and 103) is incorrect, any correction of the statutory basis for the rejection will not be considered a new ground of rejection if the prior art relied upon, and the rationale supporting the rejection, would be the same under either status.

The following is a quotation of pre-AIA 35 U.S.C. 103(a) which forms the basis for all obviousness rejections set forth in this Office action:

> (a) A patent may not be obtained though the invention is not identically disclosed or described as set forth in section 102, if the differences between the subject matter sought to be patented and the prior art are such that the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art to which said subject matter pertains. Patentability shall not be negatived by the manner in which the invention was made.

The factual inquiries set forth in *Graham v. John Deere Co.*, 383 U.S. 1, 148 USPQ 459 (1966), that are applied for establishing a background for determining obviousness under pre-AIA 35 U.S.C. 103(a) are summarized as follows:

1. Determining the scope and contents of the prior art.

2. Ascertaining the differences between the prior art and the claims at issue.

3. Resolving the level of ordinary skill in the pertinent art.

4. Considering objective evidence present in the application indicating obviousness or nonobviousness.

2. Claims 20-39 are rejected under pre-AIA 35 U.S.C. 103(a) as being unpatentable over Korman et al., (US Patent Publication 2009/0217401 published Aug. 2009) in view of Mohania et al., (Acta. BioMed. 2013. 84:102-109) and Prakash et al., (US Patent Publication 2010/0028449 published Feb, 2010).

The claims are drawn to a method of treating cancer in a human subject comprising co-administering to the subject an immune checkpoint inhibitor and a bacterial formulation comprising bacteria of the genus *Adlercreutzia, Oscillopira, Mollicutes, Butyrivibrio, Bacteroides, Clostridium, Fusobacterium, Eubacterium, Ruminococcus, Peptococcus, Peptostreptococcus, Rikenella, Alistipes, Marinilabilia, Anaerostipes, Escherichia,* or *Lactobacillus.*

Korman et al., teach methods for treating cancer, using anti-PD-1 antibodies. The methods provide for using a combination immunotherapy, such as the combination of anti-CTLA-4 and anti-PD-1 antibodies, to treat hyperproliferative disease, such as cancer [abstract]. Korman et al., teach the use of anti-PD-1 antibodies and the use of combination immunotherapy, including the combination of anti-CTLA-4

and anti-PD-1 antibodies, to treat cancer [para. 0001]. The method of inhibiting growth of tumor cells in a subject, comprising administering to a subject a therapeutically effective amount of an anti-PD-1 antibody, or antigen-binding portion thereof [para. 0133]. The "subject" includes any human or nonhuman animal [page 232]. Korman et al., commercially available anti-PD-1 antibodies. Compositions comprising an antibody, or antigen-binding portion thereof, or immunoconjugate or bispecific molecule of the invention, and a pharmaceutically acceptable carrier, are also provided [para. 0130]. The antibodies of the invention exhibit one or more desirable functional properties, such as high affinity binding to PD-1, lack of cross-reactivity to other CD28 family members, the ability to stimulate T cell proliferation, IFN-γ and/or IL-2 secretion in mixed lymphocyte reactions, the ability to inhibit binding of one or more PD-1 ligands (e.g., PD-L1 and/or PD-L2), the ability to cross-react with monkey PD-1, the ability to stimulate antigen-specific memory responses, the ability to stimulate antibody responses and/or the ability to inhibit growth of tumor cells *in vivo* [para. 202]. Dosage regimens are adjusted to provide the optimum desired response (e.g., a therapeutic response). For example, a single bolus may be administered, several divided doses may be administered over time or the dose may be proportionally reduced or increased as indicated by the exigencies of the therapeutic situation [para. 450]. An exemplary treatment regime entails administration once per week, once every two weeks, once every three weeks, once every four weeks, once a month, once every 3 months or once every three to 6 months [para 451]. Preferred routes of administration for antibodies of the invention include intravenous, intramuscular, intradermal, intraperitoneal, subcutaneous, spinal or other parenteral routes of administration, for example by

injection [para. 458]. However Korman et al., does not teach the bacterial formulation comprising bacteria of the genus *Adlercreutzia, Oscillopira, Mollicutes, Butyrivibrio, Bacteroides, Clostridium, Fusobacterium, Eubacterium, Ruminococcus, Peptococcus, Peptostreptococcus, Rikenella, Alistipes, Marinilabilia, Anaerostipes, Escherichia,* or *Lactobacillus*.

Mohania et al., teach modulation of expression of Programmed Death-1 by administration of probiotic Dahi in DMH-induced colorectal carcinogenesis in rats. Interaction of probiotic bacteria with the host immune system elicits beneficial immune modulating effects. Although, there are many published studies on interaction of probiotics with immune system focusing on activation of immune system by bacterial cell wall through the engagement of Toll-like receptor family, very few studies have focused on molecules involved in the T-cell activation, and not much work has been executed to study the correlation of probiotics and programmed death-1 in colorectal carcinogenesis in animal models. Hence, the present study was carried out to assess the effect of probiotic Dahi on expression of programmed death (PD-1) in colorectum of 1, 2-dimethylhydrazine treated rats [abstract]. Mohania et al., prepared probiotic fermented milk namely probiotic Dahi (LaLp Dahi or LaBb Dahi) along with mixed Dahi cultures of lactococci for the delivery of probiotic strains that can provide protection against colorectal cancer in animal models [page 106, col. 1]. The Dahi preparation LaBb contained *Lactobacillus acidophilus* LaVK2 and *Bifidobacterium bifidum* BbVK3 and the isolates had probiotic attributes tested through *in vitro* tests as per FAO/WHO guidelines [page 103, col. 2]. The final product contained, *L. acidophilus,*

2-20x10^8 cfu/g and *B. bifidus*, 2- 20x10^8cfu/g [page 103, col. 2]. PD-1 expression was observed in colorectal tissues of normal and DMH-treated rats. An increase in PD-1 expression upon DMH treatment was observed and its reversion by two different preparations of the probiotic Dahi [abstract]. Mohania et al., feed rats with probiotic Dahi treatment decreased the expression of PD-1 in DMH-induced colorectal mucosa. Combined treatment with probiotic Dahi was significantly more effective in reducing the expression of PD-1 [abstract]. PD-1 is expressed independently of carcinogen administration in normal colonic mucosa and may play a role in immune response modulation in DMH-induced colorectal carcinogenesis. It was concluded that PD-1 is expressed independent of carcinogen administration and is upregulated by DMH administration in colorectal carcinogenesis. The findings of this study indicate that consumption of probiotic Dahi (LaBb Dahi) decreased expression of PD-1antigen [page 107, col. 2]. Mohania et al., teach that probiotic Dahi can be used as an effective chemopreventive agent in the management of colorectal cancer [abstract].

Prakash et al., teach method of treating a patient is suffering from a cancer disease or disorder including colorectal cancer, breast cancer, prostate cancer, lung cancer, [para. 0018]. The bacteria may be chosen from Lactobacilli cells, *Lactobacillus plantarum 80, Lactobacillus delbrueckii subsp. Lactis, Lactobacillus Rhamnosus,* Lactobacillus, more particularly from *Lactobacillus acidophilus, Lactobacillus casei, Lactobacillus plantarum 80, Lactobacillus delbrueckii subsp. Lactis, Lactobacillus Rhamnosus, Lactobacillus GG* [para. 0011]. Prakash et al., teach an oral formulation [para. 0012]. The oral formulation comprises a microcapsule containing bacteria; and a fermented milk carrier [para. 0019-21].

The bacteria may be live and the bacteria may be present in a range from 10^9 to 10^{12} colony forming units (CFU) [para. 0031-0032]. The subject or patient may be a mammal, optionally a human [para.0027]. It is estimated that a decrease of at least 60-70 percent in breast, colorectal, and prostate cancers and 40-50 percent in lung cancer would occur when a diet is complied with (according to the anti-cancer diet guidelines) which includes probiotic yogurt products. In order to be labeled probiotic, yogurt must contain a cell load of at least 10^7 cfu/g at the time of manufacture [para. 005]. Therefore Prakash et al., teach a method of treating cancer, such as breast cancer, colorectal cancer, prostate cancer, lung cancer, colon cancer and inflammation-related colon cancer, including adenoma, carcinoma, leiomyosarcoma, carcinoid tumor, or squamous cell carcinoma in a human comprising administering a bacterial formulation comprising *Lactobacillus* just as instantly claimed.

Therefore, it would have been *prima facie* obvious at the time of applicants' invention to incorporate Mohania and Prakash et al's *Lactobacillus* to treat cancer to Korman's method for treating cancer in a human subject comprising administering to the subject an immune checkpoint inhibitor when Mohania and Prakash et al., already it was known to treat cancer in a human subject comprising administering to the subject *Lactobacillus* in combination with other therapies that also treat cancer. One of ordinary skill in the art would have a reasonable expectation of success by combining both components because the prior art teach combination therapy was well known to produce beneficial cancer treating results by interfering with PD-1 and reducing PD-1 expression.

Additionally, *KSR International Co. v. Teleflex Inc.,* 127 S. Ct. 1727, 1741 (2007), discloses combining prior art elements according to known methods to yield predictable results, thus the combination is obvious unless its application is beyond that person's skill. *KSR International Co. v. Teleflex Inc.,* 127 S. Ct. 1727, 1741 (2007) also discloses that "The combination of familiar element according to known methods is likely to be obvious when it does no more than yield predictable results". It is well known to take a method of treating cancer when each ingredient is well-known to treat cancer, and where there is no change in the respective function of immune check point inhibitor or the *Lactobacillus*; thus the combination would have yielded a reasonable expectation or success along with predictable results to one of ordinary skill in the art at the time of the invention. Therefore, it would have been obvious to a person of ordinary skill in the art to combine prior art elements according to known methods that is ready for improvement to yield predictable results. The claimed invention is *prima facie* obvious in view of the teachings of the prior art, absent any convincing evidence to the contrary.

Claim Rejections - 35 USC § 103

3. Claims 40-49 are rejected under 35 U.S.C. 103 as being unpatentable over Korman et al., Mohania et al., and Prakash et al., as applied to claims 20-39 above, and further in view of Duncan et al., (US Patent publication 2007/0258953 published Nov. 2007).

The claims are drawn to a method of treating cancer in a human subject comprising co-administering to the subject an immune checkpoint inhibitor and a

bacterial formulation comprising bacteria of the genus *Rikenella, Alistipes, Marinilabilia,* or *Anaerostipes*.

Korman et al., Mohania et al., and Prakash et al., have been discussed above as teaching a method of treating cancer in a human subject comprising co-administering to the subject an immune checkpoint inhibitor and a bacterial formulation comprising a bacterium; but the bacteria is not of the genus *Rikenella, Alistipes, Marinilabilia,* or *Anaerostipes*.

Duncan et al., teach a prophylactic method to reduce the incidence or severity of colorectal cancer in mammals caused in part by high lactic acid and low butyric acid concentrations, which method comprises the administration of a therapeutically effective dose of at least one strain of live lactic acid utilising bacteria selected from the group consisting of *Anaerostipes caccae* strain [claim 17]. Duncan et al., teach a prophylactic method to reduce the incidence or severity of colorectal cancer or colitis in mammals caused in part by high lactic acid and low butyric acid concentrations, which method comprises the administration of a therapeutically effective dose of at least one above identified strains of live lactic acid utilising bacteria and/or butyric acid producing bacteria mentioned above or of *Anaerostipes caccae* [para. 0031]. The use of live *Anaerostipes caccae* or at least one of the above mentioned lactic acid utilising bacteria as a medicament. Advantageously the strain chosen may produce butyric acid as its sole or predominant fermentation product from lactate [para. 0032]. Another aspect taught by Duncan et al., is where at least one lactate-utilising strain of bacteria, *Anaerostipes caccae* are used in combination with lactic acid producing bacteria including those such as *Lactobacillus* spp. or other additives or growth enhancing

supplement currently used as probiotics [para.0033]. The combination of strains would potentially enhance the health-promoting benefits of the lactic acid bacterium by converting its fermentation products (lactic acid alone or lactic acid plus acetic acid) into butyrate. Indeed, it is possible that certain health-promoting properties currently ascribed to lactic acid bacteria might actually be due to stimulation of other species such as lactate-consumers in vivo, particularly where probiotic approaches (see below) are used to boost native populations in the gut. Furthermore, the presence of the lactic acid producing bacteria in a combined inoculum could help to protect the lactate consumer against oxygen prior to ingestion [para. 0034]. For the use, prevention or treatment of conditions described herein, the bacteria or prebiotic(s) or symbiotic(s) are preferentially delivered to the site of action in the gastro-intestinal tract by oral or rectal administration in any appropriate formulae or carrier or excipient or diluent or stabiliser. Such modes of delivery may be of any formulation included but not limited to solid formulations such as tablets or capsules; liquid solutions such as yoghurts or drinks or suspensions. Ideally, the delivery mechanism delivers the bacteria or prebiotic or symbiotic without harm through the acid environment of the stomach and through the rumen to the site of action within the gastro-intestinal tract [para. 0038]. Example 5 teach the total viable count for *Anaerostipes caccae* was at 2.4 x 10^8 CFU/ml.

Therefore, it would have been *prima facie* obvious at the time of applicants' invention to incorporate Duncan's *Anaerostipes caccae* bacteria to treat cancer to the method of Korman, Mohania and Prakash et al's using lactic-acid bacteria to treat cancer. Korman, and Mohania already teach the modulation of PD-1 to treat colorectal cancer; while Mohania, Prakash and Duncan all teach using lactic acid bacteria such as

Lactobacillus and/or *Anaerostipes* to treat colorectal cancer. One of ordinary skill in the art would have a reasonable expectation of success by combining the components because the prior art teach combination therapy was well known to produce beneficial by decreasing PD-1 expression and blocking PD-1.

Additionally, *KSR International Co. v. Teleflex Inc.,* 127 S. Ct. 1727, 1741 (2007), discloses combining prior art elements according to known methods to yield predictable results, thus the combination is obvious unless its application is beyond that person's skill. *KSR International Co. v. Teleflex Inc.,* 127 S. Ct. 1727, 1741 (2007) also discloses that "The combination of familiar element according to known methods is likely to be obvious when it does no more than yield predictable results". It is well known to take a method of treating cancer when each ingredient is well-known to treat cancer, and where there is no change in the respective function of immune check point inhibitor, or the *Anaerostipes*; thus the combination would have yielded a reasonable expectation or success along with predictable results to one of ordinary skill in the art at the time of the invention. Therefore, it would have been obvious to a person of ordinary skill in the art to combine prior art elements according to known methods that is ready for improvement to yield predictable results. The claimed invention is *prima facie* obvious in view of the teachings of the prior art, absent any convincing evidence to the contrary.

Response to Arguments

4. Applicant's arguments filed March 8, 2018 have been fully considered but they are not persuasive. The rejection of claims 20-39 under pre-AIA 35 U.S.C. 103(a) as

being unpatentable over Korman et al., in view of Mohania et al., and Prakash et al., is maintained for reasons of record. The rejection of claims 40-49 under 35 U.S.C. 103 as being unpatentable over Korman et al., Mohania et al., and Prakash et al., as applied to claims 20-39 above, and further in view of Duncan et al., is maintained for reasons of record.

Applicants argue that Korman teach the administration of anti-PD-1 inhibit growth of tumor cells in vivo and Mohania teaches that administration of the Lactobacillus comprising formulation results in reduced expression of PD-1 while Korman teach the PD-1. Therefore, while both the Mohania probiotic and the Korman antibodies act on PD-1, they do so through different, mutually counter-productive mechanisms. However, it is the position of the Office, that the action of PD-1 is not counterproductive. Nothing in Korman et al., Mohania et al., and/or Prakash et al., have the opposite of the desired effect wherein the desired effect is to treat cancer. Furthermore, the administered composition of Korman et al., Mohania et al., and/or Prakash et al., all work to decrease PD-1 or inhibit PD-1 function. Those purposes are not counter-productive. Rather as the rejection explained the action on PD-1 is a two-prong measure wherein Korman administers anti-PD-1 antibodies to bind PD-1 present on tumor cells while Mohania and Prakash administer Lactobacillus to decrease PD-1 expression.

Applicants argue that Prakash discloses nothing regarding the effect of Lactobacillus on gastrointestinal PD-1 expression and therefore not change the expectation established in Mohania and Korman that delivery of an anti-PD-1 antibody in the presence of Lactobacillus would be counter-productive. It is well-known that PD-1 is found on cancer cells. Therefore, it would have been *prima facie* obvious at the time

of applicants' invention to incorporate Mohania and Prakash et al's *Lactobacillus* known to decrease PD-1 expression while Korman's method administers anti-PD-1 to inhibit/interfere with the remaining or present PD-1. One of ordinary skill in the art would have a reasonable expectation of success by combining the components because the prior art teach combination therapy was well known to produce beneficial cancer treating methods by inhibiting PD-1 already present on tumor cells and simultaneously reducing further PD-1 expression.

Applicants argue Prakash discloses nothing regarding the effect of Lactobacillus on gastrointestinal PD-1 expression. In response to applicant's arguments against the Prakash et al., reference individually, one cannot show nonobviousness by attacking references individually where the rejections are based on combinations of references. See *In re Keller*, 642 F.2d 413, 208 USPQ 871 (CCPA 1981); *In re Merck & Co.,* 800 F.2d 1091, 231 USPQ 375 (Fed. Cir. 1986). In this case, none of the claims are drawn to gastrointestinal PD-1 expression. Furthermore, Mohania et al., already teach effect of *Lactobacillus* on PD-1 expression. Therefore, this argument is not persuasive.

Applicants combat arguments about *In re Kerkhoven* using *Ex Parte Bokisa*, 1997 WL 1897871, *3 (Bd. Pat.App. & Interf, 1997) (combination of known compounds not obvious when prior art indicates that *Ex Parte Bokisa*, 1997 WL 1897871, *3 (Bd. Pat.App. & Interf, 1997) is drawn to the combination of known compounds not obvious when the prior art indicates that one compound exhibits undesirable effects while the other compound minimizes these effects. The instant facts are unlike *Ex Parte Bokisa*. First, neither compound exhibits any under undesirable effect and Applicants have not

asserted such. Second, neither compound minimizes these effects of the other. Unlike Applicants arguments, the agents do not have opposite effects. In this case, combination therapy was well known to produce beneficial cancer treating methods. Furthermore, anti-PD-1 inhibits PD-1 present on tumor cells. Anti-PD-1 is not known to increase the expression of PD-1. Thus, there are no opposite or undesirable effects. Lactobacillus is known to reduce PD-1 expression. *Lactobacillus* is not known to interfere with anti-PD-1 binding of PD-1. Therefore, the combination does not increase and at the same time decrease the expression of PD-1. There is no teaching of one compound exhibiting undesirable effects while the other compound minimizes these effects. Accordingly, the reasoning of *Bokisa* is not applicable. Applicants' assertion is not persuasive and the rejections are maintained.

In response to applicant's argument that there is no teaching, suggestion, or motivation to combine the Duncan references, the examiner recognizes that obviousness may be established by combining or modifying the teachings of the prior art to produce the claimed invention where there is some teaching, suggestion, or motivation to do so found either in the references themselves or in the knowledge generally available to one of ordinary skill in the art. See *In re Fine*, 837 F.2d 1071, 5 USPQ2d 1596 (Fed. Cir. 1988), *In re Jones*, 958 F.2d 347, 21 USPQ2d 1941 (Fed. Cir. 1992), and *KSR International Co. v. Teleflex, Inc.*, 550 U.S. 398, 82 USPQ2d 1385 (2007). In this case, Applicants have not provided any evidence that administering anti-PD-1 antibodies and administering *Lactobacillus* to decrease PD-1 expression is counterproductive. Applicants have not pointed to an undesirable effect. Applicants has not shown that administering anti-PD-1 antibodies and *Lactobacillus* function with

opposing mechanisms. At best, Applicants have proven why the combination would have been obvious, by pointing out that *Lactobacillus* only decreases PD-1 expression, it does not eliminate PD-1. Furthermore, Applicant concedes that administered anti-PD-1 would bind and interfere with the PD-1 present in the host system. In addition, obviously, less PD-1 within the host system obviously treats cancer.

Lactobacillus decreases PD-1 expression is does not affect the PD-1 already present. Therefore, contrary to applicants' assertions, one of ordinary skill in the art would be motivated to combine compositions known to treat cancer by negatively affecting PD-1. Therefore, the rejections are maintained.

Conclusion

5. No claims allowed.

6. **THIS ACTION IS MADE FINAL.** Applicant is reminded of the extension of time policy as set forth in 37 CFR 1.136(a).

A shortened statutory period for reply to this final action is set to expire THREE MONTHS from the mailing date of this action. In the event a first reply is filed within TWO MONTHS of the mailing date of this final action and the advisory action is not mailed until after the end of the THREE-MONTH shortened statutory period, then the shortened statutory period will expire on the date the advisory action is mailed, and any extension fee pursuant to 37 CFR 1.136(a) will be calculated from the mailing date of

the advisory action. In no event, however, will the statutory period for reply expire later than SIX MONTHS from the mailing date of this final action.

7. Any inquiry concerning this communication or earlier communications from the examiner should be directed to JA-NA A HINES whose telephone number is (571)272-0859. The examiner can normally be reached Monday thru Thursday.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor Gary Nickol, can be reached on 571-272-0835. The fax phone number for the organization where this application or proceeding is assigned is 571-273-8300.

Information regarding the status of an application may be obtained from the Patent Application Information Retrieval (PAIR) system. Status information for published applications may be obtained from either Private PAIR or Public PAIR. Status information for unpublished applications is available through Private PAIR only. For more information about the PAIR system, see http://pair-direct.uspto.gov. Should you have questions on access to the Private PAIR system, contact the Electronic Business Center (EBC) at 866-217-9197 (toll-free).

/JANA A HINES/
Primary Examiner, Art Unit 1645

Search Notes	Application/Control No.	Applicant(s)/Patent Under Reexamination
	15/718,735	Gajewski et al.
	Examiner	Art Unit
	JA-NA A HINES	1645

CPC - Searched*		
Symbol	**Date**	**Examiner**
A61K39/02; A61P1/00; A61P1/02; A61P1/04; A61P1/12; A61P1/16; A61P3/04 ; A61P3/10; A61P5/00; A61P7/02; A61P7/06; A61P9/00; A61P9/10; A61P11/ 00; A61P11/06; A61P13/12; A61P15/00; A61P17/00 ; A61P17/02;	11/09/2017	jah
A61K35/74; C12N1/20; C12P7/52	01/03/2018	jah
search updated	04/25/2018	jah

CPC Combination Sets - Searched*		
Symbol	**Date**	**Examiner**

US Classification - Searched*			
Class	**Subclass**	**Date**	**Examiner**
424	234.1	11/09/2017	jah
435	252.1		
424	93.4	01/03/2018	jah
435	252.1		

* See search history printout included with this form or the SEARCH NOTES box below to determine the scope of the search.

Search Notes		
Search Notes	**Date**	**Examiner**
searched inventors, applications, patents. Commercial database search of claim text	11/09/2017	jah
Search based upon claim amendments	01/03/2018	jah
updated searches	04/25/2018	jah

/JANA A HINES/ Primary Examiner, Art Unit 1645	

Search Notes	Application/Control No.	Applicant(s)/Patent Under Reexamination
	15/718,735	Gajewski et al.
	Examiner	Art Unit
	JA-NA A HINES	1645

Interference Search			
US Class/CPC Symbol	US Subclass/CPC Group	Date	Examiner

/JANA A HINES/ Primary Examiner, Art Unit 1645	

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant:	University of Chicago	Confirmation No.:	5538
Serial No.:	15/718,735	Art Unit:	1645
Filed:	28-Sep-2017	Examiner:	Hines, Jana A.
Title:	**TREATMENT OF CANCER BY MANIPULATION OF COMMENSAL MICROFLORA**		

RESPONSE TO NOTICE TO OFFICE ACTION MAILED JANUARY 8, 2018

VIA EFS-WEB
COMMISSIONER FOR PATENTS
P.O. BOX 1450
ALEXANDRIA, VA 22313-1450

Examiner Hines:

This communication is responsive the Office Action mailed January 8, 2018.

The Commissioner is authorized by this paper to charge any fees during the entire pendency of this application, including fees due under 37 C.F.R. §§ 1.16 and 1.17 that may be required, including any required extension of time fees, or credit any overpayment to Deposit Account 50-4302, referencing Attorney Docket No.: UCHI-34458/US-4/CON. This paragraph is intended to be a CONSTRUCTIVE PETITION FOR EXTENSION OF TIME in accordance with 37 C.F.R. § 1.136(a)(3).

CLAIMS

1-19. (cancelled)

20. (previously presented) A method of treating cancer in a human subject comprising co-administering to the subject an immune checkpoint inhibitor and a bacterial formulation comprising bacteria of the genus *Adlercreutzia, Oscillopira, Mollicutes, Butyrivibrio, Bacteroides, Clostridium, Fusobacterium, Eubacterium, Ruminococcus, Peptococcus, Peptostreptococcus, Rikenella, Alistipes, Marinilabilia, Anaerostipes, Escherichia,* or *Lactobacillus*.

21. (previously presented) The method of claim 20, wherein at least 50% of the bacteria in the bacterial formulation are of the genus *Adlercreutzia, Oscillopira, Mollicutes, Butyrivibrio, Bacteroides, Clostridium, Fusobacterium, Eubacterium, Ruminococcus, Peptococcus, Peptostreptococcus, Rikenella, Alistipes, Marinilabilia, Anaerostipes, Escherichia,* or *Lactobacillus*.

22. (previously presented) The method of claim 20, wherein at least 90% of the bacteria in the bacterial formulation are of the genus *Adlercreutzia, Oscillopira, Mollicutes, Butyrivibrio, Bacteroides, Clostridium, Fusobacterium, Eubacterium, Ruminococcus, Peptococcus, Peptostreptococcus, Rikenella, Alistipes, Marinilabilia, Anaerostipes, Escherichia,* or *Lactobacillus*.

23. (previously presented) The method of claim 20, wherein the bacteria are of the genus *Lactobacillus.*

24. (previously presented) The method of claim 23, wherein the bacteria are of the species *Lactobacillus acidophilus, Lactobacillus casei, Lactobacillus kefiri, Lactobacillus bifidus, Lactobacillus brevis, Lactobacillus helveticus, Lactobacillus paracasei , Lactobacillus*

rhamnosus, Lactobacillus salivarius, Lactobacillus curvatus, Lactobacillus bulgaricus, Lactobacillus sakei, Lactobacillus reuteri, Lactobacillus fermentum, Lactobacillus farciminis, Lactobacillus lac tis, Lactobacillus delbrueckii, Lactobacillus plantarum, Lactobacillus paraplantarum, Lactobacillus crispatus, Lactobacillus gasseri, Lactobacillus johnsonii or *Lactobacillus jensenii.*

25. (previously presented) The method of claim 20, wherein the bacterial formulation is administered by oral administration or rectal administration.

26. (previously presented) The method of claim 25, wherein the bacterial formulation is administered by oral administration.

27. (previously presented) The method of claim 20, wherein the bacterial formulation comprises at least $5x10^6$ CFU of bacteria of the genus *Adlercreutzia, Oscillopira, Mollicutes, Butyrivibrio, Bacteroides, Clostridium, Fusobacterium, Eubacterium, Ruminococcus, Peptococcus, Peptostreptococcus, Rikenella, Alistipes, Marinilabilia, Anaerostipes, Escherichia,* or *Lactobacillus*.

28. (previously presented) The method of claim 20, wherein the bacterial formulation is administered to the subject in two or more doses.

29. (previously presented) The method of claim 28, wherein the administration of the two or more doses are separated by at least 1 week.

30. (previously presented) The method of claim 20, further comprising administering to the subject an antibiotic prior to the administration of the bacterial formulation.

31. (previously presented) The method of claim 29, wherein the antibiotic is administered to the subject at least 1 day before the bacterial formulation is administered to the subject.

32. (previously presented) The method of claim 20, wherein the immune checkpoint inhibitor is a protein or polypeptide that binds to an immune checkpoint protein.

33. (previously presented) The method of claim 32, wherein the immune checkpoint protein is CTLA4, PD-1, PD-L1, PD-L2, A2AR, B7-H3, B7-H4, BTLA, KIR, LAG3, TIM-3 or VISTA.

34. (previously presented) The method of claim 33, wherein the immune checkpoint protein is PD-1 or PD-L1.

35. (previously presented) The method of claim 20, wherein the immune checkpoint inhibitor is an antibody or antigen binding fragment thereof that binds to an immune checkpoint protein.

36. (previously presented) The method of claim 35, wherein the immune checkpoint protein is CTLA4, PD-1, PD-L1, PD-L2, A2AR, B7-H3, B7-H4, BTLA, KIR, LAG3, TIM-3 or VISTA.

37. (previously presented) The method of claim 36, wherein the immune checkpoint protein is PD-1 or PD-L1.

38. (previously presented) The method of claim 20, wherein the immune checkpoint inhibitor is nivolumab, pembrolizumab, pidilizumab, AMP-224, AMP-514, STI-A1110, TSR-

042, RG-7446, BMS-936559, BMS-936558, MK-3475, CT Ol l, MPDL3280A, MEDI-4736, MSB-0020718C, AUR-012 and STI-A1010.

39. (previously presented) The method of claim 20, wherein the immune checkpoint inhibitor is administered by intravenous injection, intramuscular injection, intratumoral injection or subcutaneous injection.

40. (previously presented) The method of claim 20, wherein the bacteria are of the genus *Rikenella, Alistipes, Marinilabilia* or *Anaerostipes.*

41. (previously presented) The method of claim 40, wherein the bacterial formulation comprises at least $5x10^6$ CFU of bacteria of the genus *Rikenella, Alistipes, Marinilabilia* or *Anaerostipes,* and wherein at least 50% of the bacteria in the bacterial formulation are of the genus *Rikenella, Alistipes, Marinilabilia or Anaerostipes*.

42. (previously presented) The method of claim 41, wherein at least 90% of the bacteria in the bacterial formulation are of the genus *Rikenella, Alistipes, Marinilabilia or Anaerostipes*.

43. (previously presented) The method of claim 40, wherein the bacterial formulation is administered by oral administration or rectal administration.

44. (previously presented) The method of claim 43, wherein the bacterial formulation is administered by oral administration.

45. (previously presented) The method of claim 40, wherein the bacterial formulation is administered to the subject in two or more doses.

46. (previously presented) The method of claim 40, further comprising administering to the subject an antibiotic before the bacterial formulation is administered to the subject.

47. (previously presented) The method of claim 40, wherein the immune checkpoint inhibitor is an antibody or antigen binding fragment thereof that binds to CTLA4, PD-1, PD-L1, PD-L2, A2AR, B7-H3, B7-H4, BTLA, KIR, LAG3, TIM-3 or VISTA.

48. (previously presented) The method of claim 40, wherein the immune checkpoint inhibitor is an antibody or antigen binding fragment thereof that binds to PD-1 or PD-L1.

49. (previously presented) The method of claim 40, wherein the immune checkpoint inhibitor is nivolumab, pembrolizumab, pidilizumab, AMP-224, AMP-514, STI-A1110, TSR-042, RG-7446, BMS-936559, BMS-936558, MK-3475, CT Ol l, MPDL3280A, MEDI-4736, MSB-0020718C, AUR-012 and STI-A1010.

REMARKS

No claims are amended, cancelled or added in the present communication.

Claims 20-39 are rejected under 35 U.S.C. 103(a) as allegedly being unpatentable over U.S. Pat. Pub. No. 2009/0217401 ("Korman") in view of Mohania *et al., Acta. BioMed.* 2013 84:102-109 ("Mohania") and U.S. Pat. Pub. No. 2010/0028449 ("Prakish"). Specifically, the office action states that "Korman et al., teach methods for treating cancer, using anti-PD-1 antibodies"[1] while "Mohania et al., teach modulation of expression of Programmed Death-1 by administration of probiotic Dahi in DMH-induced colorectal carcinogenesis in rats"[2] and "Prakash et al., teach [a] method of treating a patient [who] is suffering from a cancer disease or disorder including colorectal cancer, cancer breast cancer, prostate cancer, [or] lung cancer."[3] According to the Office Action, "it would have been *prima facie* obvious at the time of applicants' invention to incorporate Mohania and Prakash et al.'s *Lactobacillus* to treat cancer to Korman's method for treating cancer in a human subject comprising administering to the subject an immune checkpoint inhibitor."[4] Applicant respectfully disagrees.

One of skill in the art would not have been motivated to combine the administration of the anti-PD-1 antibodies disclosed in Korman with the bacterial formulations disclosed in Mohania and Prakish for the treatment of cancer. As noted in the Office Action, the therapeutic methods disclosed in Korman are premised on the idea that the binding of certain anti-PD-1 antibodies to PD-1 results in them exhibiting certain "desirable functional properties," including, "the ability to stimulate T cell proliferation, IFN-y and/or IL-2 secretion in mixed lymphocyte reactions…, the ability to stimulate antigen-specific memory responses, the ability to stimulate antibody responses and/or the ability to inhibit growth of tumor cells *in vivo*."[5] However, Mohania discloses that administration of a probiotic formulation comprising *Lactobacillus* results in a reduction in the expression of PD-1in colorectal tissues of DMH-treated rats.[6] Thus, Mohania teaches that administration of the disclosed *Lactobacillus*-comprising formulation results in reduced expression of the very target of the

[1] Pending Office Action, at p. 3.
[2] *Id.,* at p. 5.
[3] *Id.,* at p. 6.
[4] *Id.,* at p. 7.
[5] *Id.,* at p. 4.
[6] Mohania, at p. 105.

antibodies disclosed in Korman. In light of this, one of skill in the art would expect administration of such a *Lactobacillus* formulation would eliminate or, at the very least, reduce the "desirable functional properties" (e.g., stimulation of T cells, stimulation of antibody response, stimulation of antigen-specific memory responses, etc.) attributed to the anti-PD-1 antibodies of Korman.. In other words, while both the Mohania probiotic and the Korman antibodies act on PD-1, they do so through different, mutually counter-productive mechanisms.

The deficiencies in the disclosures of Korman and Mohania are not cured by Prakash, which is directed to the microencapsulation of *Lactobacillus acidophilus* in order to improve their mechanical stability and survival.[7] Prakash discloses nothing regarding the effect of *Lactobacillus* on gastrointestinal PD-1 expression and therefore not change the expectation established in Mohania and Korman that delivery of an anti-PD-1 antibody in the presence of *Lactobacillus* would be counter-productive.

The Office Action argues that that the administration of a combination of anti-PD-1 antibodies and a *Lactobacillus* formulation because it "would have been obvious to a person of ordinary skill in the art to combine prior art elements according to known methods that is ready for improvement to yield predictable results."[8] This reasoning flows from *In re Kerkhoven*, which held that "[i]t is *prima facie* obvious to combine two compositions each of which is taught by the prior art to be useful for the same purpose, in order to form a third composition to be used for the very same purpose." (MPEP §2144.06). However, Applicant submits that the reasoning of *Kerkhoven* is not applicable to the current facts in which the methods taught in the references being combined are disclosed to produce counter-productive effects. In this way the current facts are analogous to those of *Ex Parte Bokisa*. In *Bokisa,* the Examiner had argued that:

> [I]t would be [sic: have been] obvious to one of ordinary skill in the art at the time the invention was made to use both alkane or alkanol sulfonic acid and fluoboric acid in tin plating baths of Nobel because it has been held to be obvious to use a mixture of two materials each of which has been used separately for the same purpose, *In re Kerkhoven*, [626 F.2d 846, 850,] 205USPQ 1269, [sic: 1069,] 1072 (CCPA 1980).

However, the BPAI majority in *Bokisa* held that:

> The combination of the plating baths suggested by the dissent does not flow from the teachings in Nobel showing a difference in the precipitation for fluoboric acid and alkyl

[7] Prakash, at ¶[0009].
[8] Pending Office Action, at p. 8.

> or alkylol sulfonic acid even at low temperatures in example 1. Clearly, the purpose of Nobel's disclosure is to contrast the effect of the named antioxidants in a comparison of the plating baths. The effect of the comparison is to view each plating bath as having different characteristics. As pointed out by the dissent, even at low temperatures one causes more precipitation than the other. **One of ordinary skill in the art would not have combined a plating bath causing more precipitation with one causing less. *Kerkhoven* is not applicable on these facts.**

Thus, as was the case in *Bokisa*, though both of the agents being applied to the claimed methods are potentially applicable to the same general purpose, the references disclose that the agents have different and opposing underlying effects. Thus, applying the BPAI's reasoning in *Bokisa* to the instant case, one of ordinary skill in the art would not have combined that administration of the anti-PD-1 antibody of Korman, which requires the presence of PD-1 to mediate its effect, with the administration of the *Lactobacillus*-containing formulations of Mohania and Prakash, which Mohania teaches reduce PD-1 expression.

In light of the opposing mechanisms taught in Korman and Mohania (e.g., binding to PD-1 vs. reducing the amount of PD-1), and consistent with the applicable case law, Applicant submits that the claims are not obvious over the alleged combination of references, and respectfully requests withdrawal of the rejection.

Claims 40-49 are rejected under 35 U.S.C. 103(a) as allegedly being unpatentable over Korman, Mohania and Prakish, in further view of U.S. Pat. Pub. No. 2007/0258953 ("Duncan"). Specifically, the Office Action states that "Korman et al., Mohania et al., and Prakash et al., have been discussed above as teaching a method of treating cancer in a human subject comprising co-administering to the subject an immune checkpoint inhibitor and a bacterial formulation comprising a bacterium, but the bacteria is not of the genus *Rikenella, Alistipes, Marinilabilia,* or *Anaerostipes.*," but that this deficiency is cured by the disclosure of Duncan.[9] Applicant respectfully traverses the rejection.

As discussed above, in light of the counter-productive therapeutic mechanisms disclosed by Korman and Mohania, one of skill in the art would not have been motivated to combine the administration of the anti-PD-1 antibody of Korman with the bacterial formulations of Mohania and Prakash. This deficiency in the prior art is not cured by Duncan. Specifically, Duncan discloses

[9] *Id.,* at p. 9.

nothing regarding the effect of bacterial formulations on gastrointestinal PD-1 expression and therefore not change the expectation established in Mohania and Korman that delivery of an anti-PD-1 antibody in the presence of the disclosed bacterial formulations would be counter-productive. Accordingly, the disclosures of Mohania, Korman, Prakash and Duncan, considered individually or in combination, do not render the present claims obvious and Applicant respectfully requests withdrawal of the rejection.

CONCLUSION

Applicant respectfully submits that the remarks herein overcome the Office's rejections and place the claims in condition for allowance. If the Examiner wishes to discuss this case, Applicants encourage the Examiner to call the undersigned at 608-662-1277 at the Examiner's convenience.

Respectfully submitted,

Date: March 8, 2018

/David W. Staple/

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Electronic Acknowledgement Receipt

EFS ID:	31997691
Application Number:	15718735
International Application Number:	
Confirmation Number:	5538
Title of Invention:	TREATMENT OF CANCER BY MANIPULATION OF COMMENSAL MICROFLORA
First Named Inventor/Applicant Name:	Thomas F. Gajewski
Customer Number:	72960
Filer:	David William Staple/Stephanie Filandrinos
Filer Authorized By:	David William Staple
Attorney Docket Number:	UCHI-34458/US-4/CON
Receipt Date:	08-MAR-2018
Filing Date:	28-SEP-2017
Time Stamp:	16:53:19
Application Type:	Utility under 35 USC 111(a)

Payment information:

Submitted with Payment	no

File Listing:

Document Number	Document Description	File Name	File Size(Bytes)/ Message Digest	Multi Part /.zip	Pages (if appl.)
1		2018-03-08_34458US4CON_ROA.pdf	196252 0c440c35fe8185dc5eb149a67e37b272b953e743	yes	10

	Multipart Description/PDF files in .zip description		
	Document Description	**Start**	**End**
	Amendment/Req. Reconsideration-After Non-Final Reject	1	1
	Claims	2	6
	Applicant Arguments/Remarks Made in an Amendment	7	10
Warnings:			
Information:			
	Total Files Size (in bytes):	196252	

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New Applications Under 35 U.S.C. 111

If a new application is being filed and the application includes the necessary components for a filing date (see 37 CFR 1.53(b)-(d) and MPEP 506), a Filing Receipt (37 CFR 1.54) will be issued in due course and the date shown on this Acknowledgement Receipt will establish the filing date of the application.

National Stage of an International Application under 35 U.S.C. 371

If a timely submission to enter the national stage of an international application is compliant with the conditions of 35 U.S.C. 371 and other applicable requirements a Form PCT/DO/EO/903 indicating acceptance of the application as a national stage submission under 35 U.S.C. 371 will be issued in addition to the Filing Receipt, in due course.

New International Application Filed with the USPTO as a Receiving Office

If a new international application is being filed and the international application includes the necessary components for an international filing date (see PCT Article 11 and MPEP 1810), a Notification of the International Application Number and of the International Filing Date (Form PCT/RO/105) will be issued in due course, subject to prescriptions concerning national security, and the date shown on this Acknowledgement Receipt will establish the international filing date of the application.

PTO/SB/06 (09-11)
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PATENT APPLICATION FEE DETERMINATION RECORD Substitute for Form PTO-875	Application or Docket Number 15/718,735	Filing Date 09/28/2017	☐ To be Mailed

ENTITY: ☐ LARGE ☒ SMALL ☐ MICRO

APPLICATION AS FILED – PART I

FOR	(Column 1) NUMBER FILED	(Column 2) NUMBER EXTRA	RATE ($)	FEE ($)
☐ BASIC FEE (37 CFR 1.16(a), (b), or (c))	N/A	N/A	N/A	
☐ SEARCH FEE (37 CFR 1.16(k), (i), or (m))	N/A	N/A	N/A	
☐ EXAMINATION FEE (37 CFR 1.16(o), (p), or (q))	N/A	N/A	N/A	
TOTAL CLAIMS (37 CFR 1.16(i))	minus 20 =	*	X $ =	
INDEPENDENT CLAIMS (37 CFR 1.16(h))	minus 3 =	*	X $ =	
☐ APPLICATION SIZE FEE (37 CFR 1.16(s))	If the specification and drawings exceed 100 sheets of paper, the application size fee due is $310 ($155 for small entity) for each additional 50 sheets or fraction thereof. See 35 U.S.C. 41(a)(1)(G) and 37 CFR 1.16(s).			
☐ MULTIPLE DEPENDENT CLAIM PRESENT (37 CFR 1.16(j))				
* If the difference in column 1 is less than zero, enter "0" in column 2.			TOTAL	

APPLICATION AS AMENDED – PART II

AMENDMENT **03/08/2018**

	(Column 1) CLAIMS REMAINING AFTER AMENDMENT		(Column 2) HIGHEST NUMBER PREVIOUSLY PAID FOR	(Column 3) PRESENT EXTRA	RATE ($)	ADDITIONAL FEE ($)
Total (37 CFR 1.16(i))	* 30	Minus	** 30	= 0	X $50 =	0
Independent (37 CFR 1.16(h))	* 1	Minus	***3	= 0	X $230 =	0
☐ Application Size Fee (37 CFR 1.16(s))						
☐ FIRST PRESENTATION OF MULTIPLE DEPENDENT CLAIM (37 CFR 1.16(j))						
					TOTAL ADD'L FEE	0

AMENDMENT

	(Column 1) CLAIMS REMAINING AFTER AMENDMENT		(Column 2) HIGHEST NUMBER PREVIOUSLY PAID FOR	(Column 3) PRESENT EXTRA	RATE ($)	ADDITIONAL FEE ($)
Total (37 CFR 1.16(i))	*	Minus	**	=	X $ =	
Independent (37 CFR 1.16(h))	*	Minus	***	=	X $ =	
☐ Application Size Fee (37 CFR 1.16(s))						
☐ FIRST PRESENTATION OF MULTIPLE DEPENDENT CLAIM (37 CFR 1.16(j))						
					TOTAL ADD'L FEE	

* If the entry in column 1 is less than the entry in column 2, write "0" in column 3.
** If the "Highest Number Previously Paid For" IN THIS SPACE is less than 20, enter "20".
*** If the "Highest Number Previously Paid For" IN THIS SPACE is less than 3, enter "3".
The "Highest Number Previously Paid For" (Total or Independent) is the highest number found in the appropriate box in column 1.

LIE
CORALIA BETANCOURT

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APPLICATION NUMBER	FILING OR 371(C) DATE	FIRST NAMED APPLICANT	ATTY. DOCKET NO./TITLE
15/718,735	09/28/2017	Thomas F. Gajewski	UCHI-34458/US-4/CON

CONFIRMATION NO. 5538

72960
Casimir Jones, S.C.
2275 DEMING WAY, SUITE 310
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PUBLICATION NOTICE

OC000000096755482

Title:TREATMENT OF CANCER BY MANIPULATION OF COMMENSAL MICROFLORA

Publication No.US-2018-0015131-A1
Publication Date:01/18/2018

NOTICE OF PUBLICATION OF APPLICATION

The above-identified application will be electronically published as a patent application publication pursuant to 37 CFR 1.211, et seq. The patent application publication number and publication date are set forth above.

The publication may be accessed through the USPTO's publically available Searchable Databases via the Internet at www.uspto.gov. The direct link to access the publication is currently http://www.uspto.gov/patft/.

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In addition, information on the status of the application, including the mailing date of Office actions and the dates of receipt of correspondence filed in the Office, may also be accessed via the Internet through the Patent Electronic Business Center at www.uspto.gov using the public side of the Patent Application Information and Retrieval (PAIR) system. The direct link to access this status information is currently https://portal.uspto.gov/pair/PublicPair. Prior to publication, such status information is confidential and may only be obtained by applicant using the private side of PAIR.

Further assistance in electronically accessing the publication, or about PAIR, is available by calling the Patent Electronic Business Center at 1-866-217-9197.

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Alexandria, Virginia 22313-1450
www.uspto.gov

APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
15/718,735	09/28/2017	Thomas F. Gajewski	UCHI-34458/US-4/CON	5538

72960 7590 01/08/2018

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EXAMINER	
HINES, JANA A	

ART UNIT	PAPER NUMBER
1645	

NOTIFICATION DATE	DELIVERY MODE
01/08/2018	ELECTRONIC

Please find below and/or attached an Office communication concerning this application or proceeding.

The time period for reply, if any, is set in the attached communication.

Notice of the Office communication was sent electronically on above-indicated "Notification Date" to the following e-mail address(es):

docketing@casimirjones.com
pto.correspondence@casimirjones.com

Office Action Summary	Application No. 15/718,735	Applicant(s) Gajewski et al.	
	Examiner JA-NA A HINES	Art Unit 1645	AIA Status Yes

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE 3 MONTHS FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133). Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

1)☑ Responsive to communication(s) filed on 12/13/17 .

☐ A declaration(s)/affidavit(s) under **37 CFR 1.130(b)** was/were filed on _____ .

2a)☐ This action is **FINAL.** 2b) ☑ This action is non-final.

3)☐ An election was made by the applicant in response to a restriction requirement set forth during the interview on _____; the restriction requirement and election have been incorporated into this action.

4)☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims*

5) ☑ Claim(s) 20-49 is/are pending in the application.

5a) Of the above claim(s) _____ is/are withdrawn from consideration.

6) ☐ Claim(s) _____ is/are allowed.

7) ☑ Claim(s) 20-49 is/are rejected.

8) ☐ Claim(s) _____ is/are objected to.

9) ☐ Claim(s) _____ are subject to restriction and/or election requirement

* If any claims have been determined allowable, you may be eligible to benefit from the **Patent Prosecution Highway** program at a participating intellectual property office for the corresponding application. For more information, please see http://www.uspto.gov/patents/init_events/pph/index.jsp or send an inquiry to **PPHfeedback@uspto.gov.**

Application Papers

10)☐ The specification is objected to by the Examiner.

11)☐ The drawing(s) filed on _____ is/are: a)☐ accepted or b)☐ objected to by the Examiner.

Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).

Replacement drawing sheet(s) including the correction is required if the drawing(s) is objected to. See 37 CFR 1.121(d).

Priority under 35 U.S.C. § 119

12)☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

Certified copies:

a)☐ All b)☐ Some** c)☐ None of the:

1.☐ Certified copies of the priority documents have been received.

2.☐ Certified copies of the priority documents have been received in Application No. _____.

3.☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

** See the attached detailed Office action for a list of the certified copies not received.

Attachment(s)

1) ☑ Notice of References Cited (PTO-892)

2) ☐ Information Disclosure Statement(s) (PTO/SB/08a and/or PTO/SB/08b) Paper No(s)/Mail Date _____.

3) ☐ Interview Summary (PTO-413) Paper No(s)/Mail Date _____.

4) ☐ Other: _____.

DETAILED CORRESPONDENCE

Notice of Pre-AIA or AIA Status

The present application is being examined under the pre-AIA first to invent provisions.

Claim Status

1. Claims filed December 13, 2017 have been entered. Claims 1-19 have been canceled. Claims 20-49 have been newly added. Claims 20-49 are under consideration in this Office Action.

Claim Rejections - 35 USC § 103

In the event the determination of the status of the application as subject to AIA 35 U.S.C. 102 and 103 (or as subject to pre-AIA 35 U.S.C. 102 and 103) is incorrect, any correction of the statutory basis for the rejection will not be considered a new ground of rejection if the prior art relied upon, and the rationale supporting the rejection, would be the same under either status.

The following is a quotation of pre-AIA 35 U.S.C. 103(a) which forms the basis for all obviousness rejections set forth in this Office action:

> (a) A patent may not be obtained though the invention is not identically disclosed or described as set forth in section 102, if the differences between the subject matter sought to be patented and the prior art are such that the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art to which said subject matter pertains. Patentability shall not be negatived by the manner in which the invention was made.

The factual inquiries set forth in *Graham v. John Deere Co.*, 383 U.S. 1, 148 USPQ 459 (1966), that are applied for establishing a background for determining obviousness under pre-AIA 35 U.S.C. 103(a) are summarized as follows:

1. Determining the scope and contents of the prior art.

2. Ascertaining the differences between the prior art and the claims at issue.

3. Resolving the level of ordinary skill in the pertinent art.

4. Considering objective evidence present in the application indicating obviousness or nonobviousness.

2. Claims 20-39 are rejected under pre-AIA 35 U.S.C. 103(a) as being unpatentable over Korman et al., (US Patent Publication 2009/0217401 published Aug. 2009) in view of Mohania et al., (Acta. BioMed. 2013. 84:102-109) and Prakash et al., (US Patent Publication 2010/0028449 published Feb, 2010).

The claims are drawn to a method of treating cancer in a human subject comprising co-administering to the subject an immune checkpoint inhibitor and a bacterial formulation comprising bacteria of the genus *Adlercreutzia, Oscillopira, Mollicutes, Butyrivibrio, Bacteroides, Clostridium, Fusobacterium, Eubacterium, Ruminococcus, Peptococcus, Peptostreptococcus, Rikenella, Alistipes, Marinilabilia, Anaerostipes, Escherichia,* or *Lactobacillus.*

Korman et al., teach methods for treating cancer, using anti-PD-1 antibodies. The methods provide for using a combination immunotherapy, such as the combination of anti-CTLA-4 and anti-PD-1 antibodies, to treat hyperproliferative disease, such as cancer [abstract]. Korman et al., teach the use of anti-PD-1 antibodies and the use of combination immunotherapy, including the combination of anti-CTLA-4

and anti-PD-1 antibodies, to treat cancer [para. 0001]. The method of inhibiting growth of tumor cells in a subject, comprising administering to a subject a therapeutically effective amount of an anti-PD-1 antibody, or antigen-binding portion thereof [para. 0133]. The "subject" includes any human or nonhuman animal [page 232]. Korman et al., commercially available anti-PD-1 antibodies. Compositions comprising an antibody, or antigen-binding portion thereof, or immunoconjugate or bispecific molecule of the invention, and a pharmaceutically acceptable carrier, are also provided [para. 0130]. The antibodies of the invention exhibit one or more desirable functional properties, such as high affinity binding to PD-1, lack of cross-reactivity to other CD28 family members, the ability to stimulate T cell proliferation, IFN-γ and/or IL-2 secretion in mixed lymphocyte reactions, the ability to inhibit binding of one or more PD-1 ligands (e.g., PD-L1 and/or PD-L2), the ability to cross-react with monkey PD-1, the ability to stimulate antigen-specific memory responses, the ability to stimulate antibody responses and/or the ability to inhibit growth of tumor cells *in vivo* [para. 202]. Dosage regimens are adjusted to provide the optimum desired response (e.g., a therapeutic response). For example, a single bolus may be administered, several divided doses may be administered over time or the dose may be proportionally reduced or increased as indicated by the exigencies of the therapeutic situation [para. 450]. An exemplary treatment regime entails administration once per week, once every two weeks, once every three weeks, once every four weeks, once a month, once every 3 months or once every three to 6 months [para 451]. Preferred routes of administration for antibodies of the invention include intravenous, intramuscular, intradermal, intraperitoneal, subcutaneous, spinal or other parenteral routes of administration, for example by

injection [para. 458]. However Korman et al., does not teach the bacterial formulation comprising bacteria of the genus *Adlercreutzia, Oscillopira, Mollicutes, Butyrivibrio, Bacteroides, Clostridium, Fusobacterium, Eubacterium, Ruminococcus, Peptococcus, Peptostreptococcus, Rikenella, Alistipes, Marinilabilia, Anaerostipes, Escherichia,* or *Lactobacillus.*

Mohania et al., teach modulation of expression of Programmed Death-1 by administration of probiotic Dahi in DMH-induced colorectal carcinogenesis in rats. Interaction of probiotic bacteria with the host immune system elicits beneficial immune modulating effects. Although, there are many published studies on interaction of probiotics with immune system focusing on activation of immune system by bacterial cell wall through the engagement of Toll-like receptor family, very few studies have focused on molecules involved in the T-cell activation, and not much work has been executed to study the correlation of probiotics and programmed death-1 in colorectal carcinogenesis in animal models. Hence, the present study was carried out to assess the effect of probiotic Dahi on expression of programmed death (PD-1) in colorectum of 1, 2-dimethylhydrazine treated rats [abstract]. Mohania et al., prepared probiotic fermented milk namely probiotic Dahi (LaLp Dahi or LaBb Dahi) along with mixed Dahi cultures of lactococci for the delivery of probiotic strains that can provide protection against colorectal cancer in animal models [page 106, col. 1]. The Dahi preparation LaBb contained *Lactobacillus acidophilus* LaVK2 and *Bifidobacterium bifidum* BbVK3 and the isolates had probiotic attributes tested through *in vitro* tests as per FAO/WHO guidelines [page 103, col. 2]. The final product contained, *L. acidophilus,*

2-20x10^8 cfu/g and *B. bifidus*, 2- 20x10^8cfu/g [page 103, col. 2]. PD-1 expression was observed in colorectal tissues of normal and DMH-treated rats. An increase in PD-1 expression upon DMH treatment was observed and its reversion by two different preparations of the probiotic Dahi [abstract]. Mohania et al., feed rats with probiotic Dahi treatment decreased the expression of PD-1 in DMH-induced colorectal mucosa. Combined treatment with probiotic Dahi was significantly more effective in reducing the expression of PD-1 [abstract]. PD-1 is expressed independently of carcinogen administration in normal colonic mucosa and may play a role in immune response modulation in DMH-induced colorectal carcinogenesis. It was concluded that PD-1 is expressed independent of carcinogen administration and is upregulated by DMH administration in colorectal carcinogenesis. The findings of this study indicate that consumption of probiotic Dahi (LaBb Dahi) decreased expression of PD-1antigen [page 107, col. 2]. Mohania et al., teach that probiotic Dahi can be used as an effective chemopreventive agent in the management of colorectal cancer [abstract].

Prakash et al., teach method of treating a patient is suffering from a cancer disease or disorder including colorectal cancer, breast cancer, prostate cancer, lung cancer, [para. 0018]. The bacteria may be chosen from Lactobacilli cells, *Lactobacillus plantarum 80, Lactobacllus delbrueckii subsp. Lactis, Lactobacillus Rhamnosus,* Lactobacillus, more particularly from *Lactobacillus acidophilus, Lactobacillus casei, Lactobacillus plantarum 80, Lactobacillus delbrueckii subsp. Lactis, Lactobacillus Rhamnosus, Lactobacillus GG* [para. 0011]. Prakash et al., teach an oral formulation [para. 0012]. The oral formulation comprises a microcapsule containing bacteria; and a fermented milk carrier [para. 0019-21].

The bacteria may be live and the bacteria may be present in a range from 10^9 to 10^{12} colony forming units (CFU) [para. 0031-0032]. The subject or patient may be a mammal, optionally a human [para.0027]. It is estimated that a decrease of at least 60-70 percent in breast, colorectal, and prostate cancers and 40-50 percent in lung cancer would occur when a diet is complied with (according to the anti-cancer diet guidelines) which includes probiotic yogurt products. In order to be labeled probiotic, yogurt must contain a cell load of at least 10^7 cfu/g at the time of manufacture [para. 005]. Therefore Prakash et al., teach a method of treating cancer, such as breast cancer, colorectal cancer, prostate cancer, lung cancer, colon cancer and inflammation-related colon cancer, including adenoma, carcinoma, leiomyosarcoma, carcinoid tumor, or squamous cell carcinoma in a human comprising administering a bacterial formulation comprising *Lactobacillus* just as instantly claimed.

Therefore, it would have been *prima facie* obvious at the time of applicants' invention to incorporate Mohania and Prakash et al's *Lactobacillus* to treat cancer to Korman's method for treating cancer in a human subject comprising administering to the subject an immune checkpoint inhibitor when Mohania and Prakash et al., already it was known to treat cancer in a human subject comprising administering to the subject *Lactobacillus* in combination with other therapies that also treat cancer. One of ordinary skill in the art would have a reasonable expectation of success by combining both components because the prior art teach combination therapy was well known to produce beneficial by suppressing or reducing PD-1 expression.

Additionally, *KSR International Co. v. Teleflex Inc.,* 127 S. Ct. 1727, 1741 (2007), discloses combining prior art elements according to known methods to yield predictable

results, thus the combination is obvious unless its application is beyond that person's skill. *KSR International Co. v. Teleflex Inc.,* 127 S. Ct. 1727, 1741 (2007) also discloses that "The combination of familiar element according to known methods is likely to be obvious when it does no more than yield predictable results". It is well known to take a method of treating cancer when each ingredient is well-known to treat cancer, and where there is no change in the respective function of immune check point inhibitor or the *Lactobacillus*; thus the combination would have yielded a reasonable expectation or success along with predictable results to one of ordinary skill in the art at the time of the invention. Therefore, it would have been obvious to a person of ordinary skill in the art to combine prior art elements according to known methods that is ready for improvement to yield predictable results. The claimed invention is *prima facie* obvious in view of the teachings of the prior art, absent any convincing evidence to the contrary.

Claim Rejections - 35 USC § 103

3. Claims 40-49 are rejected under 35 U.S.C. 103 as being unpatentable over Korman et al., Mohania et al., and Prakash et al., as applied to claims 20-39 above, and further in view of Duncan et al., (US Patent publication 2007/0258953 published Nov. 2007).

The claims are drawn to a method of treating cancer in a human subject comprising co-administering to the subject an immune checkpoint inhibitor and a bacterial formulation comprising bacteria of the genus *Rikenella, Alistipes, Marinilabilia,* or *Anaerostipes.*

Korman et al., Mohania et al., and Prakash et al., have been discussed above as teaching a method of treating cancer in a human subject comprising co-administering to the subject an immune checkpoint inhibitor and a bacterial formulation comprising a bacterium; but the bacteria is not of the genus *Rikenella, Alistipes, Marinilabilia,* or *Anaerostipes.*

Duncan et al., teach a prophylactic method to reduce the incidence or severity of colorectal cancer in mammals caused in part by high lactic acid and low butyric acid concentrations, which method comprises the administration of a therapeutically effective dose of at least one strain of live lactic acid utilising bacteria selected from the group consisting of *Anaerostipes caccae* strain [claim 17]. Duncan et al., teach a prophylactic method to reduce the incidence or severity of colorectal cancer or colitis in mammals caused in part by high lactic acid and low butyric acid concentrations, which method comprises the administration of a therapeutically effective dose of at least one above identified strains of live lactic acid utilising bacteria and/or butyric acid producing bacteria mentioned above or of *Anaerostipes caccae* [para. 0031]. The use of live *Anaerostipes caccae* or at least one of the above mentioned lactic acid utilising bacteria as a medicament. Advantageously the strain chosen may produce butyric acid as its sole or predominant fermentation product from lactate [para. 0032]. Another aspect taught by Duncan et al., is where at least one lactate-utilising strain of bacteria, *Anaerostipes caccae* are used in combination with lactic acid producing bacteria including those such as *Lactobacillus* spp. or other additives or growth enhancing supplement currently used as probiotics [para.0033]. The combination of strains would potentially enhance the health-promoting benefits of the lactic acid bacterium by

converting its fermentation products (lactic acid alone or lactic acid plus acetic acid) into butyrate. Indeed it is possible that certain health-promoting properties currently ascribed to lactic acid bacteria might actually be due to stimulation of other species such as lactate-consumers in vivo, particularly where probiotic approaches (see below) are used to boost native populations in the gut. Furthermore the presence of the lactic acid producing bacteria in a combined inoculum could help to protect the lactate consumer against oxygen prior to ingestion [para. 0034]. For the use, prevention or treatment of conditions described herein, the bacteria or prebiotic(s) or symbiotic(s) are preferentially delivered to the site of action in the gastro-intestinal tract by oral or rectal administration in any appropriate formulae or carrier or excipient or diluent or stabiliser. Such modes of delivery may be of any formulation included but not limited to solid formulations such as tablets or capsules; liquid solutions such as yoghurts or drinks or suspensions. Ideally, the delivery mechanism delivers the bacteria or prebiotic or symbiotic without harm through the acid environment of the stomach and through the rumen to the site of action within the gastro-intestinal tract [para. 0038]. Example 5 teach the total viable count for *Anaerostipes caccae* was at 2.4 x 10^8 CFU/ml.

Therefore, it would have been *prima facie* obvious at the time of applicants' invention to incorporate Duncan's *Anaerostipes caccae* bacteria to treat cancer to the method of Korman, Mohania and Prakash et al's using lactic-acid bacteria to treat cancer. Korman, and Mohania already teach the modulation of PD-1 to treat colorectal cancer; while Mohania, Prakash and Duncan all teach using lactic acid bacteria such as *Lactobacillus* and/or *Anaerostipes* to treat colorectal cancer. One of ordinary skill in the art would have a reasonable expectation of success by combining the components

because the prior art teach combination therapy was well known to produce beneficial by suppressing or reducing PD-1 expression.

Additionally, *KSR International Co. v. Teleflex Inc.,* 127 S. Ct. 1727, 1741 (2007), discloses combining prior art elements according to known methods to yield predictable results, thus the combination is obvious unless its application is beyond that person's skill. *KSR International Co. v. Teleflex Inc.,* 127 S. Ct. 1727, 1741 (2007) also discloses that "The combination of familiar element according to known methods is likely to be obvious when it does no more than yield predictable results". It is well known to take a method of treating cancer when each ingredient is well-known to treat cancer, and where there is no change in the respective function of immune check point inhibitor, or the *Anaerostipes*; thus the combination would have yielded a reasonable expectation or success along with predictable results to one of ordinary skill in the art at the time of the invention. Therefore, it would have been obvious to a person of ordinary skill in the art to combine prior art elements according to known methods that is ready for improvement to yield predictable results. The claimed invention is *prima facie* obvious in view of the teachings of the prior art, absent any convincing evidence to the contrary.

Pertinent Art

4. The prior art made of record and not relied upon is considered pertinent to applicant's disclosure. Sharon et al., (Chin. J.Cancer. 2014. 33(9):434-444). Sharon et al., teach immunology-based therapy is rapidly developing into an effective treatment option for a surprising range of cancers (abstract). Sharon et al., learned over the last decade that powerful immunologic effector cells may be blocked by inhibitory regulatory

pathways controlled by specific molecules often called “immune checkpoints” (page 434). These checkpoints serve to control or turn off the immune response when it is no longer needed to prevent tissue injury and autoimmunity. Cancer cells have learned or evolved to use these mechanisms to evade immune control and elimination. The development of a new therapeutic class of drugs that inhibit these inhibitory pathways has recently emerged as a potent strategy in oncology. Three sets of agents have emerged in clinical trials exploiting this strategy. These agents are antibody-based therapies targeting cytotoxic T-lymphocyte antigen 4 (CTLA4), programmed cell death 1 (PD-1), and programmed cell death ligand 1 (PD-L1)(page 434). See Table 1 for additional immune checkpoint proteins and their inhibitors, such as B7H3, Lag3, and KIR (page 435). Sharon et al., teach CTLA4 inhibition, human cancers and the inhibitor being an antibody (page 436). These inhibitors of immune inhibition have demonstrated extensive activity as single agents and in combinations. Clinical responses have been seen in melanoma, renal cell carcinoma, non-small cell lung cancer, urothelial, head and neck, ovarian cancer and various lymphomas (page 434). Sharon et al., teach PD-1 and PDL1 targeting agents (pages 439-440). Sharon et al., a method of treating cancer in a human subject comprising administering to the subject an immune checkpoint inhibitor. Bristol Myers Squibb PD-1 Immune Checkpoint Inhibitor Nivolumab Showed Antitumor Activity In Previously Treated And Chemotherapy-Naïve Patients In Phase 1b Non-Small Cell Lung Cancer Trials (Published May 14, 2014.

Conclusion

5. No claims allowed.

6. Any inquiry concerning this communication or earlier communications from the examiner should be directed to JA-NA A HINES whose telephone number is (571)272-0859. The examiner can normally be reached Monday thru Thursday.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor Gary Nickol, can be reached on 571-272-0835. The fax phone number for the organization where this application or proceeding is assigned is 571-273-8300.

Information regarding the status of an application may be obtained from the Patent Application Information Retrieval (PAIR) system. Status information for published applications may be obtained from either Private PAIR or Public PAIR. Status information for unpublished applications is available through Private PAIR only. For more information about the PAIR system, see http://pair-direct.uspto.gov. Should you have questions on access to the Private PAIR system, contact the Electronic Business Center (EBC) at 866-217-9197 (toll-free).

/JANA A HINES/
Primary Examiner, Art Unit 1645

Search Notes	Application/Control No.	Applicant(s)/Patent Under Reexamination
	15/718,735	Gajewski et al.
	Examiner	Art Unit
	JA-NA A HINES	1645

CPC - Searched*		
Symbol	**Date**	**Examiner**
A61K39/02; A61P1/00; A61P1/02; A61P1/04; A61P1/12; A61P1/16; A61P3/04 ; A61P3/10; A61P5/00; A61P7/02; A61P7/06; A61P9/00; A61P9/10; A61P11/ 00; A61P11/06; A61P13/12; A61P15/00; A61P17/00 ; A61P17/02;	11/09/2017	jah
A61K35/74; C12N1/20; C12P7/52	01/03/2018	jah

CPC Combination Sets - Searched*		
Symbol	**Date**	**Examiner**

US Classification - Searched*			
Class	**Subclass**	**Date**	**Examiner**
424	234.1	11/09/2017	jah
435	252.1		
424	93.4	01/03/2018	jah
435	252.1		

* See search history printout included with this form or the SEARCH NOTES box below to determine the scope of the search.

Search Notes		
Search Notes	**Date**	**Examiner**
searched inventors, applications, patents. Commercial database search of claim text	11/09/2017	jah
Search based upon claim amendments	01/03/2018	jah

Interference Search			
US Class/CPC Symbol	**US Subclass/CPC Group**	**Date**	**Examiner**

/JANA A HINES/ Primary Examiner, Art Unit 1645	

Search Notes	Application/Control No.	Applicant(s)/Patent Under Reexamination
	15/718,735	Gajewski et al.
	Examiner	Art Unit
	JA-NA A HINES	1645

/JANA A HINES/ Primary Examiner, Art Unit 1645	

Notice of References Cited	Application/Control No. 15/718,735	Applicant(s)/Patent Under Reexamination Gajewski et al.	
	Examiner JA-NA A HINES	Art Unit 1645	Page 1 of 1

U.S. PATENT DOCUMENTS

*		Document Number Country Code-Number-Kind Code	Date MM-YYYY	Name	CPC Classification	US Classification
*	A	US-20090217401-A1	08-2009	Korman; Alan J.	C07K16/2818	800/18
*	B	US-20100028449-A1	02-2010	Prakash; Satya	A23C9/1232	424/490
*	C	US-20070258953-A1	11-2007	Duncan; Sylvia Helen	A23C9/12	424/93.4
	D					
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FOREIGN PATENT DOCUMENTS

*		Document Number Country Code-Number-Kind Code	Date MM-YYYY	Country	Name	CPC Classification
	N					
	O					
	P					
	Q					
	R					
	S					
	T					

NON-PATENT DOCUMENTS

*		Include as applicable: Author, Title Date, Publisher, Edition or Volume, Pertinent Pages)
	U	Mohania et al., (Acta. BioMed. 2013. 84:102-109)
	V	
	W	
	X	

*A copy of this reference is not being furnished with this Office action. (See MPEP § 707.05(a).)
Dates in MM-YYYY format are publication dates. Classifications may be US or foreign.

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APPLICATION NUMBER	FILING or 371(c) DATE	GRP ART UNIT	FIL FEE REC'D	ATTY.DOCKET.NO	TOT CLAIMS	IND CLAIMS
15/718,735	09/28/2017	1645	1200	UCHI-34458/US-4/CON	30	1

CONFIRMATION NO. 5538

UPDATED FILING RECEIPT

72960
Casimir Jones, S.C.
2275 DEMING WAY, SUITE 310
MIDDLETON, WI 53562

OC000000098112215

Date Mailed: 12/18/2017

Receipt is acknowledged of this non-provisional patent application. The application will be taken up for examination in due course. Applicant will be notified as to the results of the examination. Any correspondence concerning the application must include the following identification information: the U.S. APPLICATION NUMBER, FILING DATE, NAME OF APPLICANT, and TITLE OF INVENTION. Fees transmitted by check or draft are subject to collection. Please verify the accuracy of the data presented on this receipt. **If an error is noted on this Filing Receipt, please submit a written request for a Filing Receipt Correction. Please provide a copy of this Filing Receipt with the changes noted thereon. If you received a "Notice to File Missing Parts" for this application, please submit any corrections to this Filing Receipt with your reply to the Notice. When the USPTO processes the reply to the Notice, the USPTO will generate another Filing Receipt incorporating the requested corrections**

Inventor(s)

Thomas F. Gajewski, Chicago, IL;
Ayelet Sivan, Chicago, IL;
Leticia Corrales, Chicago, IL;

Applicant(s)

The University of Chicago, Chicago, IL;

Power of Attorney: The patent practitioners associated with Customer Number 72960

Domestic Priority data as claimed by applicant

This application is a CON of 15/170,284 06/01/2016 PAT 9855302
which claims benefit of 62/169,112 06/01/2015
and claims benefit of 62/248,741 10/30/2015

Foreign Applications for which priority is claimed (You may be eligible to benefit from the **Patent Prosecution Highway** program at the USPTO. Please see http://www.uspto.gov for more information.) - None.
Foreign application information must be provided in an Application Data Sheet in order to constitute a claim to foreign priority. See 37 CFR 1.55 and 1.76.

Permission to Access Application via Priority Document Exchange: Yes

Permission to Access Search Results: Yes

Applicant may provide or rescind an authorization for access using Form PTO/SB/39 or Form PTO/SB/69 as appropriate.

If Required, Foreign Filing License Granted: 10/11/2017

The country code and number of your priority application, to be used for filing abroad under the Paris Convention, is **US 15/718,735**

Projected Publication Date: 01/18/2018

Non-Publication Request: No

Early Publication Request: No
**** SMALL ENTITY ****
Title

TREATMENT OF CANCER BY MANIPULATION OF COMMENSAL MICROFLORA

Preliminary Class

424

Statement under 37 CFR 1.55 or 1.78 for AIA (First Inventor to File) Transition Applications: No

PROTECTING YOUR INVENTION OUTSIDE THE UNITED STATES

Since the rights granted by a U.S. patent extend only throughout the territory of the United States and have no effect in a foreign country, an inventor who wishes patent protection in another country must apply for a patent in a specific country or in regional patent offices. Applicants may wish to consider the filing of an international application under the Patent Cooperation Treaty (PCT). An international (PCT) application generally has the same effect as a regular national patent application in each PCT-member country. The PCT process **simplifies** the filing of patent applications on the same invention in member countries, but **does not result** in a grant of "an international patent" and does not eliminate the need of applicants to file additional documents and fees in countries where patent protection is desired.

Almost every country has its own patent law, and a person desiring a patent in a particular country must make an application for patent in that country in accordance with its particular laws. Since the laws of many countries differ in various respects from the patent law of the United States, applicants are advised to seek guidance from specific foreign countries to ensure that patent rights are not lost prematurely.

Applicants also are advised that in the case of inventions made in the United States, the Director of the USPTO must issue a license before applicants can apply for a patent in a foreign country. The filing of a U.S. patent application serves as a request for a foreign filing license. The application's filing receipt contains further information and guidance as to the status of applicant's license for foreign filing.

Applicants may wish to consult the USPTO booklet, "General Information Concerning Patents" (specifically, the section entitled "Treaties and Foreign Patents") for more information on timeframes and deadlines for filing foreign patent applications. The guide is available either by contacting the USPTO Contact Center at 800-786-9199, or it can be viewed on the USPTO website at http://www.uspto.gov/web/offices/pac/doc/general/index.html.

For information on preventing theft of your intellectual property (patents, trademarks and copyrights), you may wish to consult the U.S. Government website, http://www.stopfakes.gov. Part of a Department of Commerce initiative, this website includes self-help "toolkits" giving innovators guidance on how to protect intellectual property in specific countries such as China, Korea and Mexico. For questions regarding patent enforcement issues, applicants may call the U.S. Government hotline at 1-866-999-HALT (1-866-999-4258).

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Title 35, United States Code, Section 184

Title 37, Code of Federal Regulations, 5.11 & 5.15

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APPLICATION NUMBER	FILING OR 371(C) DATE	FIRST NAMED APPLICANT	ATTY. DOCKET NO./TITLE
15/718,735	09/28/2017	Thomas F. Gajewski	UCHI-34458/US-4/CON

CONFIRMATION NO. 5538

72960
Casimir Jones, S.C.
2275 DEMING WAY, SUITE 310
MIDDLETON, WI 53562

INFORMAL NOTICE

OC000000096112216

Date Mailed: 12/18/2017

INFORMATIONAL NOTICE TO APPLICANT

Applicant is notified that the above-identified application contains the deficiencies noted below. No period for reply is set forth in this notice for correction of these deficiencies. However, if a deficiency relates to the inventor's oath or declaration, the applicant must file an oath or declaration in compliance with 37 CFR 1.63, or a substitute statement in compliance with 37 CFR 1.64, executed by or with respect to each actual inventor no later than the expiration of the time period set in the "Notice of Allowability" to avoid abandonment. See 37 CFR 1.53(f).

The item(s) indicated below are also required and should be submitted with any reply to this notice to avoid further processing delays.

- A properly executed inventor's oath or declaration has not been received for the following inventor(s):
  Thomas F. Gajewski
  Ayelet Sivan
  Leticia Corrales

Questions about the contents of this notice and the requirements it sets forth should be directed to the Office of Data Management, Application Assistance Unit, at **(571) 272-4000** or **(571) 272-4200** or **1-888-786-0101**.

/yteferra/

PATENT APPLICATION FEE DETERMINATION RECORD Substitute for Form PTO-875	Application or Docket Number 15/718,735

APPLICATION AS FILED - PART I

	(Column 1)	(Column 2)	SMALL ENTITY		OR	OTHER THAN SMALL ENTITY	
FOR	NUMBER FILED	NUMBER EXTRA	RATE($)	FEE($)		RATE($)	FEE($)
BASIC FEE (37 CFR 1.16(a), (b), or (c))	N/A	N/A	N/A	70		N/A	
SEARCH FEE (37 CFR 1.16(k), (i), or (m))	N/A	N/A	N/A	300		N/A	
EXAMINATION FEE (37 CFR 1.16(o), (p), or (q))	N/A	N/A	N/A	360		N/A	
TOTAL CLAIMS (37 CFR 1.16(i))	30 minus 20 =	* 10	x 40 =	400	OR		
INDEPENDENT CLAIMS (37 CFR 1.16(h))	1 minus 3 =	*	x 210 =	0.00			
APPLICATION SIZE FEE (37 CFR 1.16(s))	If the specification and drawings exceed 100 sheets of paper, the application size fee due is $310 ($155 for small entity) for each additional 50 sheets or fraction thereof. See 35 U.S.C. 41(a)(1)(G) and 37 CFR 1.16(s).			0.00			
MULTIPLE DEPENDENT CLAIM PRESENT (37 CFR 1.16(j))				0.00			
* If the difference in column 1 is less than zero, enter "0" in column 2.			TOTAL	1130		TOTAL	

APPLICATION AS AMENDED - PART II

		(Column 1)		(Column 2)	(Column 3)	SMALL ENTITY		OR	OTHER THAN SMALL ENTITY	
		CLAIMS REMAINING AFTER AMENDMENT		HIGHEST NUMBER PREVIOUSLY PAID FOR	PRESENT EXTRA	RATE($)	ADDITIONAL FEE($)		RATE($)	ADDITIONAL FEE($)
AMENDMENT A	Total (37 CFR 1.16(i))	*	Minus	**	=	x =		OR	x =	
	Independent (37 CFR 1.16(h))	*	Minus	***	=	x =		OR	x =	
	Application Size Fee (37 CFR 1.16(s))									
	FIRST PRESENTATION OF MULTIPLE DEPENDENT CLAIM (37 CFR 1.16(j))							OR		
						TOTAL ADD'L FEE		OR	TOTAL ADD'L FEE	

		(Column 1)		(Column 2)	(Column 3)					
		CLAIMS REMAINING AFTER AMENDMENT		HIGHEST NUMBER PREVIOUSLY PAID FOR	PRESENT EXTRA	RATE($)	ADDITIONAL FEE($)		RATE($)	ADDITIONAL FEE($)
AMENDMENT B	Total (37 CFR 1.16(i))	*	Minus	**	=	x =		OR	x =	
	Independent (37 CFR 1.16(h))	*	Minus	***	=	x =		OR	x =	
	Application Size Fee (37 CFR 1.16(s))									
	FIRST PRESENTATION OF MULTIPLE DEPENDENT CLAIM (37 CFR 1.16(j))							OR		
						TOTAL ADD'L FEE		OR	TOTAL ADD'L FEE	

* If the entry in column 1 is less than the entry in column 2, write "0" in column 3.
** If the "Highest Number Previously Paid For" IN THIS SPACE is less than 20, enter "20".
*** If the "Highest Number Previously Paid For" IN THIS SPACE is less than 3, enter "3".
The "Highest Number Previously Paid For" (Total or Independent) is the highest found in the appropriate box in column 1.

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant:	University of Chicago	Confirmation No.:	5538
Serial No.:	15/718,735	Art Unit:	N/A
Filed:	28-Sep-2017	Examiner:	N/A
Title:	**TREATMENT OF CANCER BY MANIPULATION OF COMMENSAL MICROFLORA**		

RESPONSE TO NOTICE TO FILE MISSING PARTS MAILED OCTOBER 13, 2017

VIA EFS-WEB
COMMISSIONER FOR PATENTS
P.O. BOX 1450
ALEXANDRIA, VA 22313-1450

Sir or Madam:

This communication is responsive the Notice to File Missing Parts mailed October 13, 2017. Applicant requests that the amendments provided herein be entered under 37 C.F.R. 1.312.

The Commissioner is authorized by this paper to charge any fees during the entire pendency of this application, including fees due under 37 C.F.R. §§ 1.16 and 1.17 that may be required, including any required extension of time fees, or credit any overpayment to Deposit Account 50-4302, referencing Attorney Docket No.: UCHI-34458/US-4/CON. This paragraph is intended to be a CONSTRUCTIVE PETITION FOR EXTENSION OF TIME in accordance with 37 C.F.R. § 1.136(a)(3).

AMENDMENTS TO THE CLAIMS

This listing of the claims will replace all prior listings and versions of claims in the application:

1-19. (Cancelled)

20. (New) A method of treating cancer in a human subject comprising co-administering to the subject an immune checkpoint inhibitor and a bacterial formulation comprising bacteria of the genus *Adlercreutzia, Oscillopira, Mollicutes, Butyrivibrio, Bacteroides, Clostridium, Fusobacterium, Eubacterium, Ruminococcus, Peptococcus, Peptostreptococcus, Rikenella, Alistipes, Marinilabilia, Anaerostipes, Escherichia,* or *Lactobacillus.*

21. (New) The method of claim 20, wherein at least 50% of the bacteria in the bacterial formulation are of the genus *Adlercreutzia, Oscillopira, Mollicutes, Butyrivibrio, Bacteroides, Clostridium, Fusobacterium, Eubacterium, Ruminococcus, Peptococcus, Peptostreptococcus, Rikenella, Alistipes, Marinilabilia, Anaerostipes, Escherichia,* or *Lactobacillus.*

22. (New) The method of claim 20, wherein at least 90% of the bacteria in the bacterial formulation are of the genus *Adlercreutzia, Oscillopira, Mollicutes, Butyrivibrio, Bacteroides, Clostridium, Fusobacterium, Eubacterium, Ruminococcus, Peptococcus, Peptostreptococcus, Rikenella, Alistipes, Marinilabilia, Anaerostipes, Escherichia,* or *Lactobacillus.*

23. (New) The method of claim 20, wherein the bacteria are of the genus *Lactobacillus.*

24. (New) The method of claim 23, wherein the bacteria are of the species *Lactobacillus acidophilus, Lactobacillus casei, Lactobacillus kefiri, Lactobacillus bifidus, Lactobacillus brevis, Lactobacillus helveticus, Lactobacillus paracasei , Lactobacillus rhamnosus, Lactobacillus salivarius, Lactobacillus curvatus, Lactobacillus bulgaricus, Lactobacillus sakei, Lactobacillus reuteri, Lactobacillus fermentum, Lactobacillus farciminis, Lactobacillus lac tis,*

Lactobacillus delbrueckii, Lactobacillus plantarum, Lactobacillus paraplantarum, Lactobacillus crispatus, Lactobacillus gasseri, Lactobacillus johnsonii or *Lactobacillus jensenii.*

25. (New) The method of claim 20, wherein the bacterial formulation is administered by oral administration or rectal administration.

26. (New) The method of claim 25, wherein the bacterial formulation is administered by oral administration.

27. (New) The method of claim 20, wherein the bacterial formulation comprises at least $5x10^6$ CFU of bacteria of the genus *Adlercreutzia, Oscillopira, Mollicutes, Butyrivibrio, Bacteroides, Clostridium, Fusobacterium, Eubacterium, Ruminococcus, Peptococcus, Peptostreptococcus, Rikenella, Alistipes, Marinilabilia, Anaerostipes, Escherichia,* or *Lactobacillus.*

28. (New) The method of claim 20, wherein the bacterial formulation is administered to the subject in two or more doses.

29. (New) The method of claim 28, wherein the administration of the two or more doses are separated by at least 1 week.

30. (New) The method of claim 20, further comprising administering to the subject an antibiotic prior to the administration of the bacterial formulation.

31. (New) The method of claim 29, wherein the antibiotic is administered to the subject at least 1 day before the bacterial formulation is administered to the subject.

32. (New) The method of claim 20, wherein the immune checkpoint inhibitor is a protein or polypeptide that binds to an immune checkpoint protein.

33. (New) The method of claim 32, wherein the immune checkpoint protein is CTLA4, PD-1, PD-L1, PD-L2, A2AR, B7-H3, B7-H4, BTLA, KIR, LAG3, TIM-3 or VISTA.

34. (New) The method of claim 33, wherein the immune checkpoint protein is PD-1 or PD-L1.

35. (New) The method of claim 20, wherein the immune checkpoint inhibitor is an antibody or antigen binding fragment thereof that binds to an immune checkpoint protein.

36. (New) The method of claim 35, wherein the immune checkpoint protein is CTLA4, PD-1, PD-L1, PD-L2, A2AR, B7-H3, B7-H4, BTLA, KIR, LAG3, TIM-3 or VISTA.

37. (New) The method of claim 36, wherein the immune checkpoint protein is PD-1 or PD-L1.

38. (New) The method of claim 20, wherein the immune checkpoint inhibitor is nivolumab, pembrolizumab, pidilizumab, AMP-224, AMP-514, STI-A1110, TSR-042, RG-7446, BMS-936559, BMS-936558, MK-3475, CT Ol l, MPDL3280A, MEDI-4736, MSB-0020718C, AUR-012 and STI-A1010.

39. (New) The method of claim 20, wherein the immune checkpoint inhibitor is administered by intravenous injection, intramuscular injection, intratumoral injection or subcutaneous injection.

40. (New) The method of claim 20, wherein the bacteria are of the genus *Rikenella, Alistipes, Marinilabilia* or *Anaerostipes.*

41. (New) The method of claim 40, wherein the bacterial formulation comprises at least $5x10^6$ CFU of bacteria of the genus *Rikenella, Alistipes, Marinilabilia* or *Anaerostipes,* and wherein at least 50% of the bacteria in the bacterial formulation are of the genus *Rikenella, Alistipes, Marinilabilia or Anaerostipes.*

42. (New) The method of claim 41, wherein at least 90% of the bacteria in the bacterial formulation are of the genus *Rikenella, Alistipes, Marinilabilia or Anaerostipes.*

43. (New) The method of claim 40, wherein the bacterial formulation is administered by oral administration or rectal administration.

44. (New) The method of claim 43, wherein the bacterial formulation is administered by oral administration.

45. (New) The method of claim 40, wherein the bacterial formulation is administered to the subject in two or more doses.

46. (New) The method of claim 40, further comprising administering to the subject an antibiotic before the bacterial formulation is administered to the subject.

47. (New) The method of claim 40, wherein the immune checkpoint inhibitor is an antibody or antigen binding fragment thereof that binds to CTLA4, PD-1, PD-L1, PD-L2, A2AR, B7-H3, B7-H4, BTLA, KIR, LAG3, TIM-3 or VISTA.

48. (New) The method of claim 40, wherein the immune checkpoint inhibitor is an antibody or antigen binding fragment thereof that binds to PD-1 or PD-L1.

49. (New) The method of claim 40, wherein the immune checkpoint inhibitor is nivolumab, pembrolizumab, pidilizumab, AMP-224, AMP-514, STI-A1110, TSR-042, RG-7446, BMS-936559, BMS-936558, MK-3475, CT Ol l, MPDL3280A, MEDI-4736, MSB-0020718C, AUR-012 and STI-A1010.

REMARKS

Following entry of this amendment, claims 1-19 are cancelled and new claims 20-49 are provided. Claims 1-19 have been canceled without prejudice or disclaimer. Applicants expressly reserve the right to pursue the subject matter of those claims in the future. Support for new claims 20-49 is located throughout the application .These amendments do not contain new matter.

Fees

The Notice indicates that the small entity surcharge is due. Applicants submit $70 for the surcharge. The claims as amended contain a total of 30 claims. Applicants submit $400 for 10 claims in excess of 20. The Commissioner is hereby authorized to charge said fees to Attorney Deposit Account 504302 referencing Docket Number UCHI-34458/US-4/CON

Please charge any additional fees required for entry of this Amendment and Response, or credit any overpayment, to Deposit Account No. 50-4302.

CONCLUSION

Applicant respectfully submits that the remarks herein overcome the Office's rejections and place the claims in condition for allowance. If the Examiner wishes to discuss this case, Applicants encourage the Examiner to call the undersigned at 608-662-1277 at the Examiner's convenience.

Respectfully submitted,

Date: December 13, 2017

/David W. Staple/

David W. Staple
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Tel.: 608-662-1277
Fax.: 608-662-1276

Electronic Patent Application Fee Transmittal

Application Number:	15718735
Filing Date:	28-Sep-2017
Title of Invention:	TREATMENT OF CANCER BY MANIPULATION OF COMMENSAL MICROFLORA
First Named Inventor/Applicant Name:	Thomas F. Gajewski
Filer:	David William Staple/Lisa M. DAy
Attorney Docket Number:	UCHI-34458/US-4/CON

Filed as Small Entity

Filing Fees for Utility under 35 USC 111(a)

Description	Fee Code	Quantity	Amount	Sub-Total in USD($)
Basic Filing:				
Pages:				
Claims:				
CLAIMS IN EXCESS OF 20	2202	10	40	400
Miscellaneous-Filing:				
LATE FILING FEE FOR OATH OR DECLARATION	2051	1	70	70
Petition:				
Patent-Appeals-and-Interference:				

Description	Fee Code	Quantity	Amount	Sub-Total in USD($)
Post-Allowance-and-Post-Issuance:				
Extension-of-Time:				
Miscellaneous:				
		Total in USD ($)		**470**

Electronic Acknowledgement Receipt

EFS ID:	31217557
Application Number:	15718735
International Application Number:	
Confirmation Number:	5538
Title of Invention:	TREATMENT OF CANCER BY MANIPULATION OF COMMENSAL MICROFLORA
First Named Inventor/Applicant Name:	Thomas F. Gajewski
Customer Number:	72960
Filer:	David William Staple/Lisa M. DAy
Filer Authorized By:	David William Staple
Attorney Docket Number:	UCHI-34458/US-4/CON
Receipt Date:	13-DEC-2017
Filing Date:	28-SEP-2017
Time Stamp:	15:49:52
Application Type:	Utility under 35 USC 111(a)

Payment information:

Submitted with Payment	yes
Payment Type	CARD
Payment was successfully received in RAM	$470
RAM confirmation Number	121417INTEFSW15505500
Deposit Account	
Authorized User	

The Director of the USPTO is hereby authorized to charge indicated fees and credit any overpayment as follows:

File Listing:

Document Number	Document Description	File Name	File Size(Bytes)/ Message Digest	Multi Part /.zip	Pages (if appl.)
1		34458US4CON_RNTMP_12-13-17.pdf	109193 35c7d5a1f84f53e575e40f0dbf4dc4787e35abf8	yes	7

	Multipart Description/PDF files in .zip description		
	Document Description	**Start**	**End**
	Applicant Response to Pre-Exam Formalities Notice	1	1
	Claims	2	6
	Applicant Arguments/Remarks Made in an Amendment	7	7

Warnings:					
Information:					
2	Fee Worksheet (SB06)	fee-info.pdf	32092 fd86f6d3ccbf82725c9cc49155454a7c859833cb	no	2
Warnings:					
Information:					
		Total Files Size (in bytes):	141285		

This Acknowledgement Receipt evidences receipt on the noted date by the USPTO of the indicated documents, characterized by the applicant, and including page counts, where applicable. It serves as evidence of receipt similar to a Post Card, as described in MPEP 503.

New Applications Under 35 U.S.C. 111

If a new application is being filed and the application includes the necessary components for a filing date (see 37 CFR 1.53(b)-(d) and MPEP 506), a Filing Receipt (37 CFR 1.54) will be issued in due course and the date shown on this Acknowledgement Receipt will establish the filing date of the application.

National Stage of an International Application under 35 U.S.C. 371

If a timely submission to enter the national stage of an international application is compliant with the conditions of 35 U.S.C. 371 and other applicable requirements a Form PCT/DO/EO/903 indicating acceptance of the application as a national stage submission under 35 U.S.C. 371 will be issued in addition to the Filing Receipt, in due course.

New International Application Filed with the USPTO as a Receiving Office

If a new international application is being filed and the international application includes the necessary components for an international filing date (see PCT Article 11 and MPEP 1810), a Notification of the International Application Number and of the International Filing Date (Form PCT/RO/105) will be issued in due course, subject to prescriptions concerning national security, and the date shown on this Acknowledgement Receipt will establish the international filing date of the application.

UNITED STATES PATENT AND TRADEMARK OFFICE

UNITED STATES DEPARTMENT OF COMMERCE
United States Patent and Trademark Office
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P.O. Box 1450
Alexandria, Virginia 22313-1450
www.uspto.gov

APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
15/718,735	09/28/2017	Thomas F. Gajewski	UCHI-34458/US-4/CON	5538

72960 7590 11/27/2017

Casimir Jones, S.C.
2275 DEMING WAY, SUITE 310
MIDDLETON, WI 53562

EXAMINER
NICKOL, GARY B

ART UNIT	PAPER NUMBER
1645	

NOTIFICATION DATE	DELIVERY MODE
11/27/2017	ELECTRONIC

Please find below and/or attached an Office communication concerning this application or proceeding.

The time period for reply, if any, is set in the attached communication.

Notice of the Office communication was sent electronically on above-indicated "Notification Date" to the following e-mail address(es):

docketing@casimirjones.com
pto.correspondence@casimirjones.com

UNITED STATES DEPARTMENT OF COMMERCE
U.S. Patent and Trademark Office
Address : COMMISSIONER FOR PATENTS
P.O. Box 1450
Alexandria, Virginia 22313-1450

APPLICATION NO./ CONTROL NO.	FILING DATE	FIRST NAMED INVENTOR / PATENT IN REEXAMINATION	ATTORNEY DOCKET NO.
15/718,735	28 September, 2017	GAJEWSKI ET AL.	UCHI-34458/US-4/CON

Casimir Jones, S.C.
2275 DEMING WAY, SUITE 310
MIDDLETON, WI 53562

EXAMINER	
GARY NICKOL	
ART UNIT	PAPER
1645	20171121

DATE MAILED:

Please find below and/or attached an Office communication concerning this application or proceeding.

Commissioner for Patents

The non-final rejection mailed 11/15/2017 is vacated. This application is being returned to pre-examination so that applicants can respond to the notice of missing parts mailed 10/13/2017.

/GARY NICKOL/ Supervisory Patent Examiner, Art Unit 1645	Gary B. Nickol SPE Art Unit: 1645

PTO-90C (Rev.04-03)

Notice of References Cited	Application/Control No. 15/718,735	Applicant(s)/Patent Under Reexamination Gajewski et al.	
	Examiner JA-NA A HINES	Art Unit 1645	Page 1 of 1

U.S. PATENT DOCUMENTS

*		Document Number Country Code-Number-Kind Code	Date MM-YYYY	Name	CPC Classification	US Classification
*	A	US-20100028449-A1	02-2010	Prakash; Satya	A23C9/1232	424/490
*	B	US-20090217401-A1	08-2009	Korman; Alan J.	C07K16/2818	800/18
	C					
	D					
	E					
	F					
	G					
	H					
	I					
	J					
	K					
	L					
	M					

FOREIGN PATENT DOCUMENTS

*		Document Number Country Code-Number-Kind Code	Date MM-YYYY	Country	Name	CPC Classification
	N					
	O					
	P					
	Q					
	R					
	S					
	T					

NON-PATENT DOCUMENTS

*		Include as applicable: Author, Title Date, Publisher, Edition or Volume, Pertinent Pages)
	U	Mohania et al. Acta BioMed. 2013. Vol. 84. pages 102-109. (Year: 2013)
	V	
	W	
	X	

*A copy of this reference is not being furnished with this Office action. (See MPEP § 707.05(a).)
Dates in MM-YYYY format are publication dates. Classifications may be US or foreign.

UNITED STATES PATENT AND TRADEMARK OFFICE

UNITED STATES DEPARTMENT OF COMMERCE
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APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
15/718,735	09/28/2017	Thomas F. Gajewski	UCHI-34458/US-4/CON	5538

72960 7590 11/15/2017

Casimir Jones, S.C.
2275 DEMING WAY, SUITE 310
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EXAMINER
HINES, JANA A

ART UNIT	PAPER NUMBER
1645	

NOTIFICATION DATE	DELIVERY MODE
11/15/2017	ELECTRONIC

Please find below and/or attached an Office communication concerning this application or proceeding.

The time period for reply, if any, is set in the attached communication.

Notice of the Office communication was sent electronically on above-indicated "Notification Date" to the following e-mail address(es):

docketing@casimirjones.com
pto.correspondence@casimirjones.com

Office Action Summary	Application No. 15/718,735	Applicant(s) Gajewski et al.	
	Examiner JA-NA A HINES	Art Unit 1645	AIA Status No

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE 3 MONTHS FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133). Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

1)☑ Responsive to communication(s) filed on 09/28/17 .

☐ A declaration(s)/affidavit(s) under **37 CFR 1.130(b)** was/were filed on _____ .

2a)☐ This action is **FINAL.** 2b) ☑ This action is non-final.

3)☐ An election was made by the applicant in response to a restriction requirement set forth during the interview on _____; the restriction requirement and election have been incorporated into this action.

4)☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims*

5)☑ Claim(s) 1-19 is/are pending in the application.

5a) Of the above claim(s) _____ is/are withdrawn from consideration.

6)☐ Claim(s) _____ is/are allowed.

7)☑ Claim(s) 1-19 is/are rejected.

8)☐ Claim(s) _____ is/are objected to.

9)☐ Claim(s) _____ are subject to restriction and/or election requirement.

* If any claims have been determined allowable, you may be eligible to benefit from the **Patent Prosecution Highway** program at a participating intellectual property office for the corresponding application. For more information, please see http://www.uspto.gov/patents/init_events/pph/index.jsp or send an inquiry to **PPHfeedback@uspto.gov.**

Application Papers

10)☐ The specification is objected to by the Examiner.

11)☐ The drawing(s) filed on _____ is/are: a)☐ accepted or b)☐ objected to by the Examiner.

Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).

Replacement drawing sheet(s) including the correction is required if the drawing(s) is objected to. See 37 CFR 1.121(d).

Priority under 35 U.S.C. § 119

12)☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

Certified copies:

a)☐ All b)☐ Some** c)☐ None of the:

1.☐ Certified copies of the priority documents have been received.

2.☐ Certified copies of the priority documents have been received in Application No. _____ .

3.☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

** See the attached detailed Office action for a list of the certified copies not received.

Attachment(s)

1) ☑ Notice of References Cited (PTO-892)

2) ☐ Information Disclosure Statement(s) (PTO/SB/08a and/or PTO/SB/08b) Paper No(s)/Mail Date _____ .

3) ☐ Interview Summary (PTO-413) Paper No(s)/Mail Date _____ .

4) ☐ Other: _____ .

DETAILED CORRESPONDENCE

Notice of Pre-AIA or AIA Status

The present application is being examined under the pre-AIA first to invent provisions.

Claim Status

1. Claims filed September 28, 2017 have been entered. Claims 1-19 are under consideration in this Office Action.

Claim Rejections - 35 USC § 103

In the event the determination of the status of the application as subject to AIA 35 U.S.C. 102 and 103 (or as subject to pre-AIA 35 U.S.C. 102 and 103) is incorrect, any correction of the statutory basis for the rejection will not be considered a new ground of rejection if the prior art relied upon, and the rationale supporting the rejection, would be the same under either status.

The following is a quotation of pre-AIA 35 U.S.C. 103(a) which forms the basis for all obviousness rejections set forth in this Office action:

> (a) A patent may not be obtained though the invention is not identically disclosed or described as set forth in section 102, if the differences between the subject matter sought to be patented and the prior art are such that the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art to which said subject matter pertains. Patentability shall not be negatived by the manner in which the invention was made.

The factual inquiries set forth in *Graham v. John Deere Co.*, 383 U.S. 1, 148 USPQ 459 (1966), that are applied for establishing a background for determining obviousness under pre-AIA 35 U.S.C. 103(a) are summarized as follows:

1. Determining the scope and contents of the prior art.

2. Ascertaining the differences between the prior art and the claims at issue.

3. Resolving the level of ordinary skill in the pertinent art.

4. Considering objective evidence present in the application indicating obviousness or nonobviousness.

2. Claims 1-19 are rejected under pre-AIA 35 U.S.C. 103(a) as being unpatentable over Korman et al., (Us Patent Publication 20090217401 published Aug. 2009] in view of Mohania et al., (Acta. BioMed. 2013. 84:102-109) and Prakash et al., (US 2010/0028449 published Feb, 2010).

The claims are drawn to a method of treating cancer in a human subject comprising administering to the subject an immune checkpoint inhibitor and a bacterial formulation comprising bacteria of the genera *Bifidobacterium*.

Korman et al., teach methods for treating cancer, using anti-PD-1 antibodies. The methods provide for using a combination immunotherapy, such as the combination of anti-CTLA-4 and anti-PD-1 antibodies, to treat hyperproliferative disease, such as cancer [abstract]. Korman et al., teach the use of anti-PD-1 antibodies and the use of combination immunotherapy, including the combination of anti-CTLA-4 and anti-PD-1 antibodies, to treat cancer [para. 0001]. The method of inhibiting growth of tumor cells in a subject, comprising administering to a subject a therapeutically effective amount of an anti-PD-1 antibody, or antigen-binding portion thereof [para. 0133]. The "subject" includes any human or nonhuman animal [page 232]. Korman et al., commercially available anti-PD-1 antibodies. Compositions comprising an antibody, or antigen-binding portion thereof, or immunoconjugate or bispecific molecule of the invention, and

a pharmaceutically acceptable carrier, are also provided [para. 0130]. The antibodies of the invention exhibit one or more desirable functional properties, such as high affinity binding to PD-1, lack of cross-reactivity to other CD28 family members, the ability to stimulate T cell proliferation, IFN-γ and/or IL-2 secretion in mixed lymphocyte reactions, the ability to inhibit binding of one or more PD-1 ligands (e.g., PD-L1 and/or PD-L2), the ability to cross-react with monkey PD-1, the ability to stimulate antigen-specific memory responses, the ability to stimulate antibody responses and/or the ability to inhibit growth of tumor cells in vivo [para. 202]. Dosage regimens are adjusted to provide the optimum desired response (e.g., a therapeutic response). For example, a single bolus may be administered, several divided doses may be administered over time or the dose may be proportionally reduced or increased as indicated by the exigencies of the therapeutic situation [para. 450]. An exemplary treatment regime entails administration once per week, once every two weeks, once every three weeks, once every four weeks, once a month, once every 3 months or once every three to 6 months [para 451]. Preferred routes of administration for antibodies of the invention include intravenous, intramuscular, intradermal, intraperitoneal, subcutaneous, spinal or other parenteral routes of administration, for example by injection [para. 458].

Mohania et al., teach modulation of expression of Programmed Death-1 by administration of probiotic Dahi in DMH-induced colorectal carcinogenesis in rats. Interaction of probiotic bacteria with the host immune system elicits beneficial immune modulating effects. Although, there are many published studies on interaction of probiotics with immune system focusing on activation of immune system by bacterial cell wall through the engagement of Toll-like receptor family, very few studies have

focused on molecules involved in the T-cell activation, and not much work has been executed to study the correlation of probiotics and programmed death-1 in colorectal carcinogenesis in animal models. Hence, the present study was carried out to assess the effect of probiotic Dahi on expression of programmed death (PD-1) in colorectum of 1, 2-dimethylhydrazine treated rats [abstract]. Mohania et al., prepared probiotic fermented milk namely probiotic Dahi (LaLp Dahi or LaBb Dahi) along with mixed Dahi cultures of lactococci for the delivery of probiotic strains that can provide protection against colorectal cancer in animal models [page 106, col. 1]. The Dahi preparation LaBb contained *Lactobacillus acidophilus* LaVK2 and *Bifidobacterium bifidum* BbVK3 and the isolates had probiotic attributes tested through *in vitro* tests as per FAO/WHO guidelines [page 103, col. 2]. The final product contained, *L. acidophilus,* 2-20x10^8 cfu/g and *B. bifidus*, 2- 20x10^8cfu/g [page 103, col. 2]. PD-1 expression was observed in colorectal tissues of normal and DMH-treated rats. An increase in PD-1 expression upon DMH treatment was observed and its reversion by two different preparations of the probiotic Dahi [abstract]. Mohania et al., feed rats with probiotic Dahi treatment decreased the expression of PD-1 in DMH-induced colorectal mucosa. Combined treatment with probiotic Dahi was significantly more effective in reducing the expression of PD-1 [abstract]. PD-1 is expressed independently of carcinogen administration in normal colonic mucosa and may play a role in immune response modulation in DMH-induced colorectal carcinogenesis. It was concluded that PD-1 is expressed independent of carcinogen administration and is upregulated by DMH administration in colorectal carcinogenesis. The findings of this study indicate that consumption of probiotic Dahi (LaBb Dahi) decreased expression of PD-1antigen [page

107, col. 2]. Mohania et al., teach that probiotic Dahi can be used as an effective chemopreventive agent in the management of colorectal cancer [abstract].

Prakash et al., teach method of treating a patient is suffering from a cancer disease or disorder including colorectal cancer, breast cancer, prostate cancer, lung cancer, [para. 0018]. The oral formulation [para. 0012] contains bacteria such as *Bifidobacterium* bacteria; i.e., *Bifidobacterium infantis, Bifidobacterium breve, Bifidobacterium longum,* or *Bifidobacterium bifidum* [para. 0011]. The bacteria may be live and the bacteria may be present in a range from 10^9 to 10^{12} colony forming units (CFU) [para. 0031-0032]. The subject or patient may be a mammal, optionally a human [para.0027]. It is estimated that a decrease of at least 60-70 percent in breast, colorectal, and prostate cancers and 40-50 percent in lung cancer would occur when a diet is complied with (according to the anti-cancer diet guidelines) which includes probiotic yogurt products. In order to be labeled probiotic, yogurt must contain a cell load of at least 107 cfu/g at the time of manufacture [para. 005]. Therefore Prakash et al., teach a method of treating cancer in a human comprising administering a bacterial formulation comprising Bifidobacterium as instantly claimed.

Therefore, it would have been *prima facie* obvious at the time of applicants' invention to incorporate Mohania and Prakash et al's Bifidobacterium to Korman's method for treating cancer in a human subject comprising administering to the subject an immune checkpoint inhibitor when Mohania and Prakash et al., already it was known to treat cancer in a human subject comprising administering to the subject Bifidobacterium in combination with other therapies that also treat cancer. One

of ordinary skill in the art would have a reasonable expectation of success by combining both components because the prior art combination therapy was well known to produce beneficial by suppressing or reducing PD-1 expression.

Additionally, *KSR International Co. v. Teleflex Inc.,* 127 S. Ct. 1727, 1741 (2007), discloses combining prior art elements according to known methods to yield predictable results, thus the combination is obvious unless its application is beyond that person's skill. *KSR International Co. v. Teleflex Inc.,* 127 S. Ct. 1727, 1741 (2007) also discloses that "The combination of familiar element according to known methods is likely to be obvious when it does no more than yield predictable results". It is well known to take a method of treating cancer when each ingredient is well-known to treat cancer, and where there is no change in the respective function of immune check point inhibitor or the *Bifidobacterium*; thus the combination would have yielded a reasonable expectation or success along with predictable results to one of ordinary skill in the art at the time of the invention. Therefore, it would have been obvious to a person of ordinary skill in the art to combine prior art elements according to known methods that is ready for improvement to yield predictable results. The claimed invention is *prima facie* obvious in view of the teachings of the prior art, absent any convincing evidence to the contrary.

Double Patenting

3. The nonstatutory double patenting rejection is based on a judicially created doctrine grounded in public policy (a policy reflected in the statute) so as to prevent the unjustified or improper timewise extension of the “right to exclude” granted by a patent

and to prevent possible harassment by multiple assignees. A nonstatutory double patenting rejection is appropriate where the conflicting claims are not identical, but at least one examined application claim is not patentably distinct from the reference claim(s) because the examined application claim is either anticipated by, or would have been obvious over, the reference claim(s). See, e.g., *In re Berg*, 140 F.3d 1428, 46 USPQ2d 1226 (Fed. Cir. 1998); *In re Goodman*, 11 F.3d 1046, 29 USPQ2d 2010 (Fed. Cir. 1993); *In re Longi*, 759 F.2d 887, 225 USPQ 645 (Fed. Cir. 1985); *In re Van Ornum*, 686 F.2d 937, 214 USPQ 761 (CCPA 1982); *In re Vogel*, 422 F.2d 438, 164 USPQ 619 (CCPA 1970); *In re Thorington*, 418 F.2d 528, 163 USPQ 644 (CCPA 1969).

A timely filed terminal disclaimer in compliance with 37 CFR 1.321I or 1.321(d) may be used to overcome an actual or provisional rejection based on nonstatutory double patenting provided the reference application or patent either is shown to be commonly owned with the examined application, or claims an invention made as a result of activities undertaken within the scope of a joint research agreement. See MPEP § 717.02 for applications subject to examination under the first inventor to file provisions of the AIA as explained in MPEP § 2159. See MPEP §§ 706.02(l)(1) – 706.02(l)(3) for applications not subject to examination under the first inventor to file provisions of the AIA. A terminal disclaimer must be signed in compliance with 37 CFR 1.321(b).

The USPTO Internet website contains terminal disclaimer forms which may be used. Please visit www.uspto.gov/patent/patents-forms. The filing date of the application in which the form is filed determines what form (e.g., PTO/SB/25, PTO/SB/26, PTO/AIA/25, or PTO/AIA/26) should be used. A web-based eTerminal Disclaimer may

be filled out completely online using web-screens. An eTerminal Disclaimer that meets all requirements is auto-processed and approved immediately upon submission. For more information about eTerminal Disclaimers, refer to www.uspto.gov/patents/process/file/efs/guidance/eTD-info-I.jsp.

4. Claims 1-19 are provisionally rejected on the ground of nonstatutory double patenting as being unpatentable over claims 1-19 of copending Application No. 15/170,284 (reference application). Although the claims at issue are not identical, they are not patentably distinct from each other because the instant claims recite claims are drawn to a method of treating cancer in a human subject comprising administering to the subject an immune checkpoint inhibitor and a bacterial formulation comprising bacteria of the genera *Bifidobacterium*. While the claims of 15/170,284 the claims are drawn to a method of treating cancer in a human subject comprising co-administering to the subject an immune checkpoint inhibitor and a bacterial formulation comprising bacteria of the genera *Bifidobacterium*.

This is a provisional nonstatutory double patenting rejection because the patentably indistinct claims have not in fact been patented.

Pertinent Art

5. The prior art made of record and not relied upon is considered pertinent to applicant's disclosure. Sharon et al., (Chin. J.Cancer. 2014. 33(9):434-444). Sharon et al., teach immunology-based therapy is rapidly developing into an effective treatment option for a surprising range of cancers (abstract). Sharon et al., learned over the last

decade that powerful immunologic effector cells may be blocked by inhibitory regulatory pathways controlled by specific molecules often called "immune checkpoints" (page 434). These checkpoints serve to control or turn off the immune response when it is no longer needed to prevent tissue injury and autoimmunity. Cancer cells have learned or evolved to use these mechanisms to evade immune control and elimination. The development of a new therapeutic class of drugs that inhibit these inhibitory pathways has recently emerged as a potent strategy in oncology. Three sets of agents have emerged in clinical trials exploiting this strategy. These agents are antibody-based therapies targeting cytotoxic T-lymphocyte antigen 4 (CTLA4), programmed cell death 1 (PD-1), and programmed cell death ligand 1 (PD-L1)(page 434). See Table 1 for additional immune checkpoint proteins and their inhibitors, such as B7H3, Lag3, and KIR (page 435). Sharon et al., teach CTLA4 inhibition, human cancers and the inhibitor being an antibody (page 436). These inhibitors of immune inhibition have demonstrated extensive activity as single agents and in combinations. Clinical responses have been seen in melanoma, renal cell carcinoma, non-small cell lung cancer, urothelial, head and neck, ovarian cancer and various lymphomas (page 434). Sharon et al., teach PD-1 and PDL1 targeting agents (pages 439-440). Sharon et al., a method of treating cancer in a human subject comprising administering to the subject an immune checkpoint inhibitor. O'Mahoney et al., (US Patent Pub. 2012/0276143 published Nov. 2012) teach a variety of administration protocols for *Bifidobacterium* strain for use in the prophylaxis and/or treatment of cancer [para. 016].

Conclusion

6. No claims allowed.

7. Any inquiry concerning this communication or earlier communications from the examiner should be directed to JA-NA A HINES whose telephone number is (571)272-0859. The examiner can normally be reached Monday thru Thursday.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor Gary Nickol, can be reached on 571-272-0835. The fax phone number for the organization where this application or proceeding is assigned is 571-273-8300.

Information regarding the status of an application may be obtained from the Patent Application Information Retrieval (PAIR) system. Status information for published applications may be obtained from either Private PAIR or Public PAIR. Status information for unpublished applications is available through Private PAIR only. For more information about the PAIR system, see http://pair-direct.uspto.gov. Should you have questions on access to the Private PAIR system, contact the Electronic Business Center (EBC) at 866-217-9197 (toll-free).

/JANA A HINES/
Primary Examiner, Art Unit 1645

Search Notes	Application/Control No.	Applicant(s)/Patent Under Reexamination
	15/718,735	Gajewski et al.
	Examiner	Art Unit
	JA-NA A HINES	1645

CPC - Searched*		
Symbol	Date	Examiner
A61K39/02; A61P1/00; A61P1/02; A61P1/04; A61P1/12; A61P1/16; A61P3/04 ; A61P3/10; A61P5/00; A61P7/02; A61P7/06; A61P9/00; A61P9/10; A61P11/ 00; A61P11/06; A61P13/12; A61P15/00; A61P17/00; A61P17/02;	11/09/2017	jah

CPC Combination Sets - Searched*		
Symbol	Date	Examiner

US Classification - Searched*			
Class	Subclass	Date	Examiner
424	234.1	11/09/2017	jah
435	252.1		

* See search history printout included with this form or the SEARCH NOTES box below to determine the scope of the search.

Search Notes		
Search Notes	Date	Examiner
searched inventors, applications, patents. Commercial database search of claim text	11/09/2017	jah

Interference Search			
US Class/CPC Symbol	US Subclass/CPC Group	Date	Examiner

/JANA A HINES/ Primary Examiner, Art Unit 1645	

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United States Patent and Trademark Office
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APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
15/718,735	09/28/2017	Thomas F. Gajewski	UCHI-34458/US-4/CON	5538

72960 7590 10/31/2017

Casimir Jones, S.C.
2275 DEMING WAY, SUITE 310
MIDDLETON, WI 53562

EXAMINER	

ART UNIT	PAPER NUMBER
1643	

NOTIFICATION DATE	DELIVERY MODE
10/31/2017	ELECTRONIC

Please find below and/or attached an Office communication concerning this application or proceeding.

The time period for reply, if any, is set in the attached communication.

Notice of the Office communication was sent electronically on above-indicated "Notification Date" to the following e-mail address(es):

docketing@casimirjones.com
pto.correspondence@casimirjones.com

UNITED STATES PATENT AND TRADEMARK OFFICE

Commissioner for Patents
United States Patent and Trademark Office
P.O. Box 1450
Alexandria, VA 22313-1450
www.uspto.gov

Doc Code:
TRACK1.GRANT

Decision Granting Request for Prioritized Examination (Track I or After RCE)	Application No.: 15/718,735

1. THE REQUEST FILED September 28, 2017 IS **GRANTED.**

The above-identified application has met the requirements for prioritized examination
- A. ☒ for an original nonprovisional application (Track I).
- B. ☐ for an application undergoing continued examination (RCE).

2. **The above-identified application will undergo prioritized examination.** The application will be accorded special status throughout its entire course of prosecution until one of the following occurs:

- A. filing a **petition for extension of time** to extend the time period for filing a reply;
- B. filing an **amendment to amend the application to contain more than four independent claims, more than thirty total claims**, or a multiple dependent claim;
- C. filing a **request for continued examination**;
- D. filing a notice of appeal;
- E. filing a request for suspension of action;
- F. mailing of a notice of allowance;
- G. mailing of a final Office action;
- H. completion of examination as defined in 37 CFR 41.102; or
- I. abandonment of the application.

Telephone inquiries with regard to this decision should be directed to JoAnne Burke at 571-272-4584. In his/her absence, calls may be directed to Brian Brown, 571-272-5338.

/JoAnne Burke/
[*Signature*]

Paralegal Specialist, Office of Petitions
(Title)

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APPLICATION NUMBER	FILING OR 371(C) DATE	FIRST NAMED APPLICANT	ATTY. DOCKET NO./TITLE
15/718,735	09/28/2017	Thomas F. Gajewski	UCHI-34458/US-4/CON

CONFIRMATION NO. 5538

72960
Casimir Jones, S.C.
2275 DEMING WAY, SUITE 310
MIDDLETON, WI 53562

FORMALITIES LETTER

OC000000094685702

Date Mailed: 10/13/2017

NOTICE TO FILE MISSING PARTS OF NONPROVISIONAL APPLICATION

FILED UNDER 37 CFR 1.53(b)

Filing Date Granted

Items Required To Avoid Abandonment:

An application number and filing date have been accorded to this application. The item(s) indicated below, however, are missing.

Applicant is given **TWO MONTHS** from the date of this Notice within which to file all required items below to avoid abandonment. Extensions of time may be obtained by filing a petition accompanied by the extension fee under the provisions of 37 CFR 1.136(a).

- Surcharge as set forth in 37 CFR 1.16(f) must be submitted.
  The surcharge is due for any one of:
  - late submission of the basic filing fee, search fee, or examination fee,
  - late submission of inventor's oath or declaration,
  - filing an application that does not contain at least one claim on filing, or
  - submission of an application filed by reference to a previously filed application.

SUMMARY OF FEES DUE:

The fee(s) required within **TWO MONTHS** from the date of this Notice to avoid abandonment is/are itemized below. Small entity discount is in effect. If applicant is qualified for micro entity status, an acceptable Certification of Micro Entity Status must be submitted to establish micro entity status. (See 37 CFR 1.29 and forms PTO/SB/15A and 15B.)

- $ **70** surcharge.
- $(**0**) previous unapplied payment amount.
- $ **70** TOTAL FEE BALANCE DUE.

Items Required To Avoid Processing Delays:

Applicant is notified that the above-identified application contains the deficiencies noted below. No period for reply is set forth in this notice for correction of these deficiencies. However, if a deficiency relates to the inventor's oath or declaration, the applicant must file an oath or declaration in compliance with 37 CFR 1.63, or a substitute statement in compliance with 37 CFR 1.64, executed by or with respect to each actual inventor no later than the expiration of the time period set in the "Notice of Allowability" to avoid abandonment. See 37 CFR 1.53(f).

- A properly executed inventor's oath or declaration has not been received for the following inventor(s):

Thomas F. Gajewski
Ayelet Sivan
Leticia Corrales

Replies must be received in the USPTO within the set time period or must include a proper Certificate of Mailing or Transmission under 37 CFR 1.8 with a mailing or transmission date within the set time period. For more information and a suggested format, see Form PTO/SB/92 and MPEP 512.

Replies should be mailed to:

Mail Stop Missing Parts
Commissioner for Patents
P.O. Box 1450
Alexandria VA 22313-1450

Registered users of EFS-Web may alternatively submit their reply to this notice via EFS-Web, including a copy of this Notice and selecting the document description "Applicant response to Pre-Exam Formalities Notice". https://sportal.uspto.gov/authenticate/AuthenticateUserLocalEPF.html

For more information about EFS-Web please call the USPTO Electronic Business Center at 1-866-217-9197 or visit our website at http://www.uspto.gov/ebc.

If you are not using EFS-Web to submit your reply, you must include a copy of this notice.

Questions about the contents of this notice and the requirements it sets forth should be directed to the Office of Data Management, Application Assistance Unit, at **(571) 272-4000** or **(571) 272-4200** or **1-888-786-0101**.

/aabranyos/

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APPLICATION NUMBER	FILING or 371(c) DATE	GRP ART UNIT	FIL FEE REC'D	ATTY.DOCKET.NO	TOT CLAIMS	IND CLAIMS
15/718,735	09/28/2017	1653	730	UCHI-34458/US-4/CON	19	1

CONFIRMATION NO. 5538

72960
Casimir Jones, S.C.
2275 DEMING WAY, SUITE 310
MIDDLETON, WI 53562

FILING RECEIPT

OC000000094685701

Date Mailed: 10/13/2017

Receipt is acknowledged of this non-provisional patent application. The application will be taken up for examination in due course. Applicant will be notified as to the results of the examination. Any correspondence concerning the application must include the following identification information: the U.S. APPLICATION NUMBER, FILING DATE, NAME OF APPLICANT, and TITLE OF INVENTION. Fees transmitted by check or draft are subject to collection. Please verify the accuracy of the data presented on this receipt. **If an error is noted on this Filing Receipt, please submit a written request for a Filing Receipt Correction. Please provide a copy of this Filing Receipt with the changes noted thereon. If you received a "Notice to File Missing Parts" for this application, please submit any corrections to this Filing Receipt with your reply to the Notice. When the USPTO processes the reply to the Notice, the USPTO will generate another Filing Receipt incorporating the requested corrections**

Inventor(s)

Thomas F. Gajewski, Chicago, IL;
Ayelet Sivan, Chicago, IL;
Leticia Corrales, Chicago, IL;

Applicant(s)

The University of Chicago, Chicago, IL;

Power of Attorney: The patent practitioners associated with Customer Number 72960

Domestic Priority data as claimed by applicant

This application is a CON of 15/170,284 06/01/2016
which claims benefit of 62/169,112 06/01/2015
and claims benefit of 62/248,741 10/30/2015

Foreign Applications for which priority is claimed (You may be eligible to benefit from the **Patent Prosecution Highway** program at the USPTO. Please see http://www.uspto.gov for more information.) - None.
Foreign application information must be provided in an Application Data Sheet in order to constitute a claim to foreign priority. See 37 CFR 1.55 and 1.76.

Permission to Access Application via Priority Document Exchange: Yes

Permission to Access Search Results: Yes

Applicant may provide or rescind an authorization for access using Form PTO/SB/39 or Form PTO/SB/69 as appropriate.

If Required, Foreign Filing License Granted: 10/11/2017

The country code and number of your priority application, to be used for filing abroad under the Paris Convention, is **US 15/718,735**

Projected Publication Date: 01/18/2018

Non-Publication Request: No

Early Publication Request: No
**** SMALL ENTITY ****
Title

TREATMENT OF CANCER BY MANIPULATION OF COMMENSAL MICROFLORA

Preliminary Class

424

Statement under 37 CFR 1.55 or 1.78 for AIA (First Inventor to File) Transition Applications: No

PROTECTING YOUR INVENTION OUTSIDE THE UNITED STATES

Since the rights granted by a U.S. patent extend only throughout the territory of the United States and have no effect in a foreign country, an inventor who wishes patent protection in another country must apply for a patent in a specific country or in regional patent offices. Applicants may wish to consider the filing of an international application under the Patent Cooperation Treaty (PCT). An international (PCT) application generally has the same effect as a regular national patent application in each PCT-member country. The PCT process **simplifies** the filing of patent applications on the same invention in member countries, but **does not result** in a grant of "an international patent" and does not eliminate the need of applicants to file additional documents and fees in countries where patent protection is desired.

Almost every country has its own patent law, and a person desiring a patent in a particular country must make an application for patent in that country in accordance with its particular laws. Since the laws of many countries differ in various respects from the patent law of the United States, applicants are advised to seek guidance from specific foreign countries to ensure that patent rights are not lost prematurely.

Applicants also are advised that in the case of inventions made in the United States, the Director of the USPTO must issue a license before applicants can apply for a patent in a foreign country. The filing of a U.S. patent application serves as a request for a foreign filing license. The application's filing receipt contains further information and guidance as to the status of applicant's license for foreign filing.

Applicants may wish to consult the USPTO booklet, "General Information Concerning Patents" (specifically, the section entitled "Treaties and Foreign Patents") for more information on timeframes and deadlines for filing foreign patent applications. The guide is available either by contacting the USPTO Contact Center at 800-786-9199, or it can be viewed on the USPTO website at http://www.uspto.gov/web/offices/pac/doc/general/index.html.

For information on preventing theft of your intellectual property (patents, trademarks and copyrights), you may wish to consult the U.S. Government website, http://www.stopfakes.gov. Part of a Department of Commerce initiative, this website includes self-help "toolkits" giving innovators guidance on how to protect intellectual property in specific countries such as China, Korea and Mexico. For questions regarding patent enforcement issues, applicants may call the U.S. Government hotline at 1-866-999-HALT (1-866-999-4258).

LICENSE FOR FOREIGN FILING UNDER

Title 35, United States Code, Section 184

Title 37, Code of Federal Regulations, 5.11 & 5.15

GRANTED

The applicant has been granted a license under 35 U.S.C. 184, if the phrase "IF REQUIRED, FOREIGN FILING LICENSE GRANTED" followed by a date appears on this form. Such licenses are issued in all applications where the conditions for issuance of a license have been met, regardless of whether or not a license may be required as set forth in 37 CFR 5.15. The scope and limitations of this license are set forth in 37 CFR 5.15(a) unless an earlier license has been issued under 37 CFR 5.15(b). The license is subject to revocation upon written notification. The date indicated is the effective date of the license, unless an earlier license of similar scope has been granted under 37 CFR 5.13 or 5.14.

This license is to be retained by the licensee and may be used at any time on or after the effective date thereof unless it is revoked. This license is automatically transferred to any related applications(s) filed under 37 CFR 1.53(d). This license is not retroactive.

The grant of a license does not in any way lessen the responsibility of a licensee for the security of the subject matter as imposed by any Government contract or the provisions of existing laws relating to espionage and the national security or the export of technical data. Licensees should apprise themselves of current regulations especially with respect to certain countries, of other agencies, particularly the Office of Defense Trade Controls, Department of State (with respect to Arms, Munitions and Implements of War (22 CFR 121-128)); the Bureau of Industry and Security, Department of Commerce (15 CFR parts 730-774); the Office of Foreign AssetsControl, Department of Treasury (31 CFR Parts 500+) and the Department of Energy.

NOT GRANTED

No license under 35 U.S.C. 184 has been granted at this time, if the phrase "IF REQUIRED, FOREIGN FILING LICENSE GRANTED" DOES NOT appear on this form. Applicant may still petition for a license under 37 CFR 5.12, if a license is desired before the expiration of 6 months from the filing date of the application. If 6 months has lapsed from the filing date of this application and the licensee has not received any indication of a secrecy order under 35 U.S.C. 181, the licensee may foreign file the application pursuant to 37 CFR 5.15(b).

PATENT APPLICATION FEE DETERMINATION RECORD Substitute for Form PTO-875	Application or Docket Number 15/718,735

APPLICATION AS FILED - PART I

	(Column 1)	(Column 2)	SMALL ENTITY		OR	OTHER THAN SMALL ENTITY	
FOR	NUMBER FILED	NUMBER EXTRA	RATE($)	FEE($)		RATE($)	FEE($)
BASIC FEE (37 CFR 1.16(a), (b), or (c))	N/A	N/A	N/A	70		N/A	
SEARCH FEE (37 CFR 1.16(k), (i), or (m))	N/A	N/A	N/A	300		N/A	
EXAMINATION FEE (37 CFR 1.16(o), (p), or (q))	N/A	N/A	N/A	360		N/A	
TOTAL CLAIMS (37 CFR 1.16(i))	19 minus 20 =	*	x 40 =	0.00	OR		
INDEPENDENT CLAIMS (37 CFR 1.16(h))	1 minus 3 =	*	x 210 =	0.00			
APPLICATION SIZE FEE (37 CFR 1.16(s))	If the specification and drawings exceed 100 sheets of paper, the application size fee due is $310 ($155 for small entity) for each additional 50 sheets or fraction thereof. See 35 U.S.C. 41(a)(1)(G) and 37 CFR 1.16(s).			0.00			
MULTIPLE DEPENDENT CLAIM PRESENT (37 CFR 1.16(j))				0.00			
* If the difference in column 1 is less than zero, enter "0" in column 2.			TOTAL	730		TOTAL	

APPLICATION AS AMENDED - PART II

		(Column 1)		(Column 2)	(Column 3)	SMALL ENTITY		OR	OTHER THAN SMALL ENTITY	
		CLAIMS REMAINING AFTER AMENDMENT		HIGHEST NUMBER PREVIOUSLY PAID FOR	PRESENT EXTRA	RATE($)	ADDITIONAL FEE($)		RATE($)	ADDITIONAL FEE($)
AMENDMENT A	Total (37 CFR 1.16(i))	*	Minus	**	=	x =		OR	x =	
	Independent (37 CFR 1.16(h))	*	Minus	***	=	x =		OR	x =	
	Application Size Fee (37 CFR 1.16(s))									
	FIRST PRESENTATION OF MULTIPLE DEPENDENT CLAIM (37 CFR 1.16(j))							OR		
						TOTAL ADD'L FEE		OR	TOTAL ADD'L FEE	

		(Column 1)		(Column 2)	(Column 3)					
		CLAIMS REMAINING AFTER AMENDMENT		HIGHEST NUMBER PREVIOUSLY PAID FOR	PRESENT EXTRA	RATE($)	ADDITIONAL FEE($)		RATE($)	ADDITIONAL FEE($)
AMENDMENT B	Total (37 CFR 1.16(i))	*	Minus	**	=	x =		OR	x =	
	Independent (37 CFR 1.16(h))	*	Minus	***	=	x =		OR	x =	
	Application Size Fee (37 CFR 1.16(s))									
	FIRST PRESENTATION OF MULTIPLE DEPENDENT CLAIM (37 CFR 1.16(j))							OR		
						TOTAL ADD'L FEE		OR	TOTAL ADD'L FEE	

* If the entry in column 1 is less than the entry in column 2, write "0" in column 3.
** If the "Highest Number Previously Paid For" IN THIS SPACE is less than 20, enter "20".
*** If the "Highest Number Previously Paid For" IN THIS SPACE is less than 3, enter "3".
The "Highest Number Previously Paid For" (Total or Independent) is the highest found in the appropriate box in column 1.

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Form Revision Date: August 26, 2013

Doc Code: TRACK1.REQ
Document Description: TrackOne Request

PTO/AIA/424 (04-14)

CERTIFICATION AND REQUEST FOR PRIORITIZED EXAMINATION UNDER 37 CFR 1.102(e) (Page 1 of 1)

First Named Inventor:	Gajewski	Nonprovisional Application Number (if known):	
Title of Invention:	TREATMENT OF CANCER BY MANIPULATION OF COMMENSAL MICROFLORA		

APPLICANT HEREBY CERTIFIES THE FOLLOWING AND REQUESTS PRIORITIZED EXAMINATION FOR THE ABOVE-IDENTIFIED APPLICATION.

1. The processing fee set forth in 37 CFR 1.17(i)(1) and the prioritized examination fee set forth in 37 CFR 1.17(c) have been filed with the request. The publication fee requirement is met because that fee, set forth in 37 CFR 1.18(d), is currently $0. The basic filing fee, search fee, and examination fee are filed with the request or have been already been paid. I understand that any required excess claims fees or application size fee must be paid for the application.

2. I understand that the application may not contain, or be amended to contain, more than four independent claims, more than thirty total claims, or any multiple dependent claims, and that any request for an extension of time will cause an outstanding Track I request to be dismissed.

3. The applicable box is checked below:

I. ☑ Original Application (Track One) - Prioritized Examination under § 1.102(e)(1)

i. (a) The application is an original nonprovisional utility application filed under 35 U.S.C. 111(a). This certification and request is being filed with the utility application via EFS-Web.

---OR---

(b) The application is an original nonprovisional plant application filed under 35 U.S.C. 111(a). This certification and request is being filed with the plant application in paper.

ii. An executed inventor's oath or declaration under 37 CFR 1.63 or 37 CFR 1.64 for each inventor, **or** the application data sheet meeting the conditions specified in 37 CFR 1.53(f)(3)(i) is filed with the application.

II. ☐ Request for Continued Examination - Prioritized Examination under § 1.102(e)(2)

i. A request for continued examination has been filed with, or prior to, this form.
ii. If the application is a utility application, this certification and request is being filed via EFS-Web.
iii. The application is an original nonprovisional utility application filed under 35 U.S.C. 111(a), or is a national stage entry under 35 U.S.C. 371.
iv. This certification and request is being filed prior to the mailing of a first Office action responsive to the request for continued examination.
v. No prior request for continued examination has been granted prioritized examination status under 37 CFR 1.102(e)(2).

Signature /David W. Staple/	Date 2017-09-28
Name (Print/Typed) David W. Staple	Practitioner Registration Number 65903

Note: *This form must be signed in accordance with 37 CFR 1.33. See 37 CFR 1.4(d) for signature requirements and certifications. Submit multiple forms if more than one signature is required.**

☑ *Total of 1 forms are submitted.

Privacy Act Statement

The **Privacy Act of 1974 (P.L. 93-579)** requires that you be given certain information in connection with your submission of the attached form related to a patent application or patent. Accordingly, pursuant to the requirements of the Act, please be advised that: (1) the general authority for the collection of this information is 35 U.S.C. 2(b)(2); (2) furnishing of the information solicited is voluntary; and (3) the principal purpose for which the information is used by the U.S. Patent and Trademark Office is to process and/or examine your submission related to a patent application or patent. If you do not furnish the requested information, the U.S. Patent and Trademark Office may not be able to process and/or examine your submission, which may result in termination of proceedings or abandonment of the application or expiration of the patent.

The information provided by you in this form will be subject to the following routine uses:

1. The information on this form will be treated confidentially to the extent allowed under the Freedom of Information Act (5 U.S.C. 552) and the Privacy Act (5 U.S.C 552a). Records from this system of records may be disclosed to the Department of Justice to determine whether disclosure of these records is required by the Freedom of Information Act.
2. A record from this system of records may be disclosed, as a routine use, in the course of presenting evidence to a court, magistrate, or administrative tribunal, including disclosures to opposing counsel in the course of settlement negotiations.
3. A record in this system of records may be disclosed, as a routine use, to a Member of Congress submitting a request involving an individual, to whom the record pertains, when the individual has requested assistance from the Member with respect to the subject matter of the record.
4. A record in this system of records may be disclosed, as a routine use, to a contractor of the Agency having need for the information in order to perform a contract. Recipients of information shall be required to comply with the requirements of the Privacy Act of 1974, as amended, pursuant to 5 U.S.C. 552a(m).
5. A record related to an International Application filed under the Patent Cooperation Treaty in this system of records may be disclosed, as a routine use, to the International Bureau of the World Intellectual Property Organization, pursuant to the Patent Cooperation Treaty.
6. A record in this system of records may be disclosed, as a routine use, to another federal agency for purposes of National Security review (35 U.S.C. 181) and for review pursuant to the Atomic Energy Act (42 U.S.C. 218(c)).
7. A record from this system of records may be disclosed, as a routine use, to the Administrator, General Services, or his/her designee, during an inspection of records conducted by GSA as part of that agency's responsibility to recommend improvements in records management practices and programs, under authority of 44 U.S.C. 2904 and 2906. Such disclosure shall be made in accordance with the GSA regulations governing inspection of records for this purpose, and any other relevant (*i.e.*, GSA or Commerce) directive. Such disclosure shall not be used to make determinations about individuals.
8. A record from this system of records may be disclosed, as a routine use, to the public after either publication of the application pursuant to 35 U.S.C. 122(b) or issuance of a patent pursuant to 35 U.S.C. 151. Further, a record may be disclosed, subject to the limitations of 37 CFR 1.14, as a routine use, to the public if the record was filed in an application which became abandoned or in which the proceedings were terminated and which application is referenced by either a published application, an application open to public inspection or an issued patent.
9. A record from this system of records may be disclosed, as a routine use, to a Federal, State, or local law enforcement agency, if the USPTO becomes aware of a violation or potential violation of law or regulation.

FIG. 1A

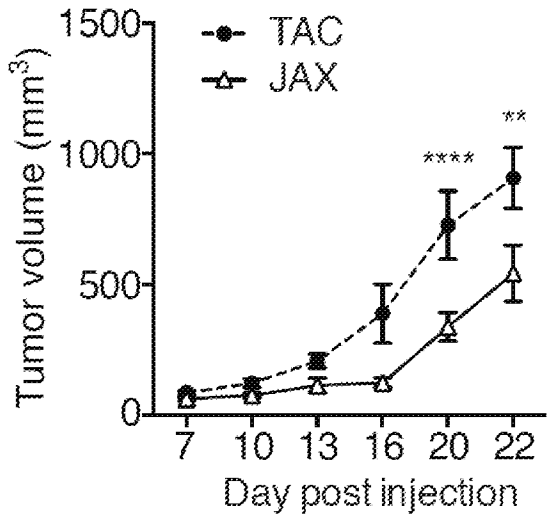


FIG. 1B

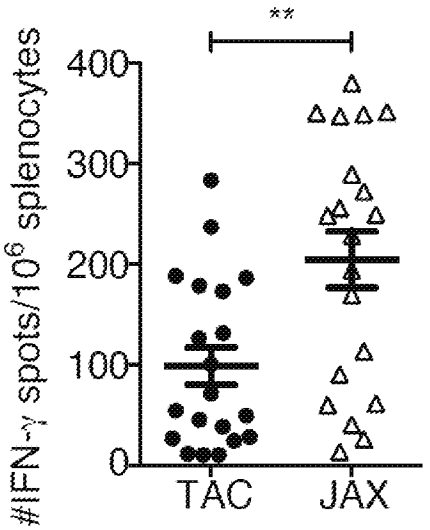


FIG. 1C

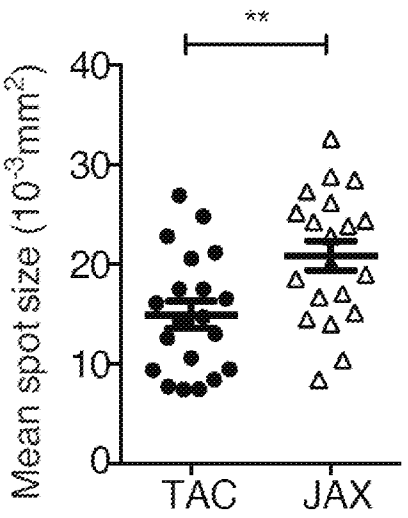


FIG. 1D

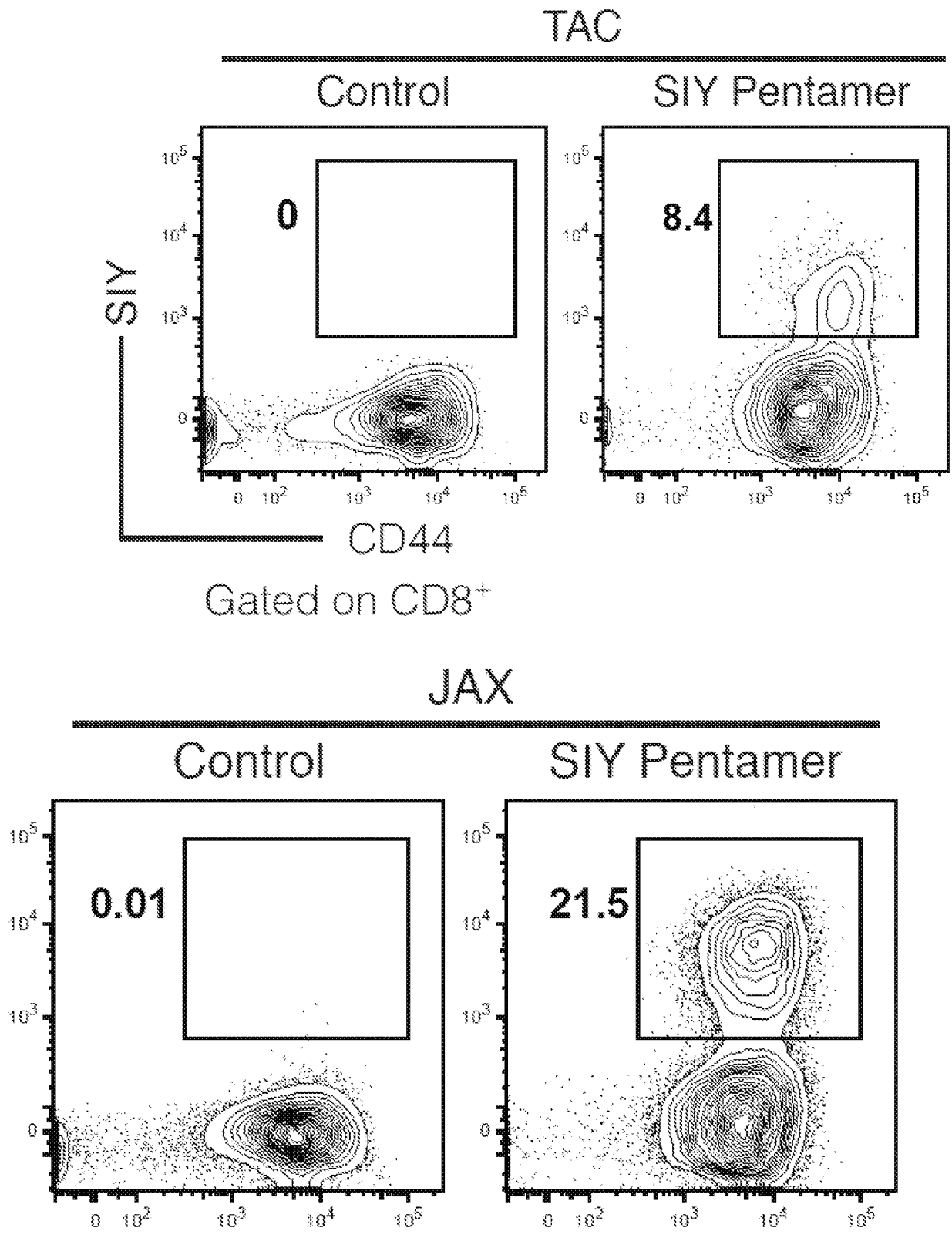
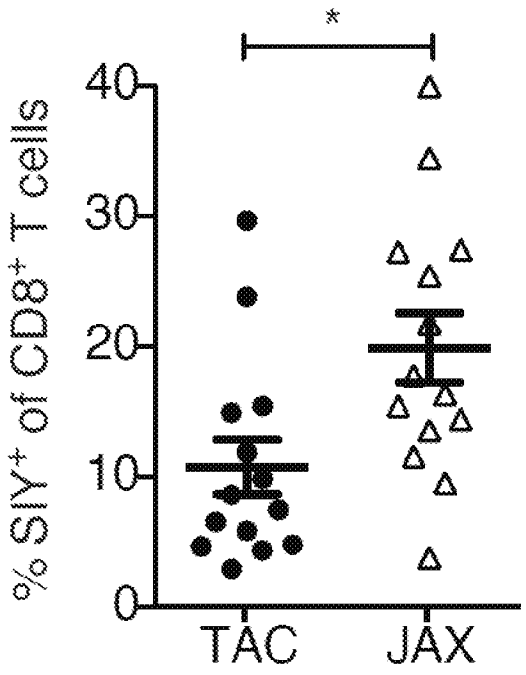

TAC
Control
SIY Pentamer
0
8.4
SIY
CD44
Gated on CD8+
JAX
Control
SIY Pentamer
0.01
21.5

FIG. 1D



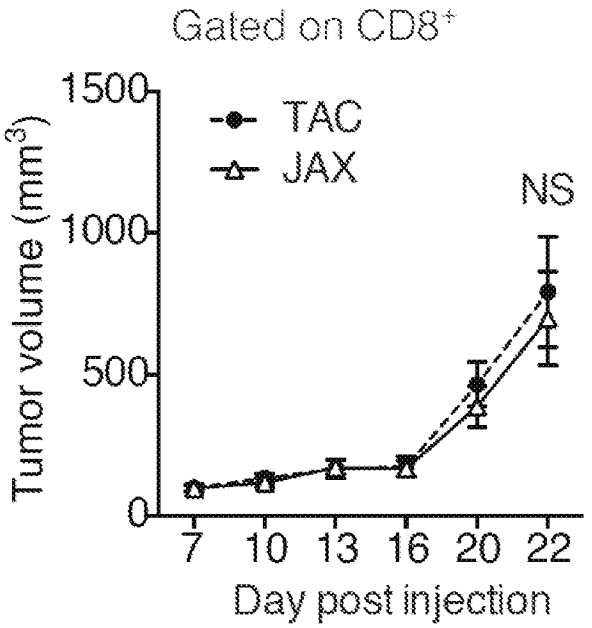


FIG. 1E

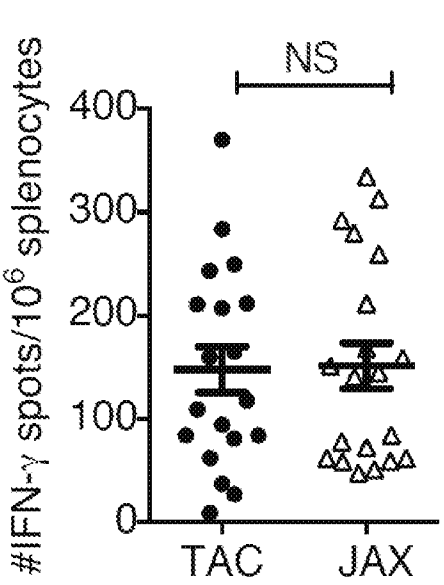


FIG. 1F

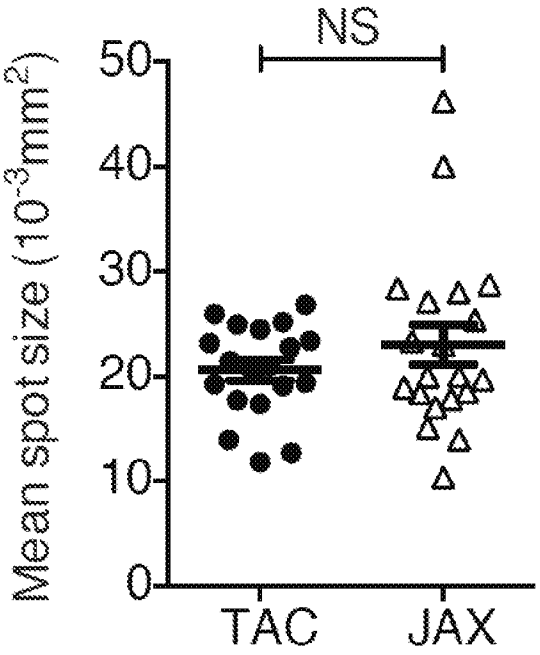


FIG. 1G

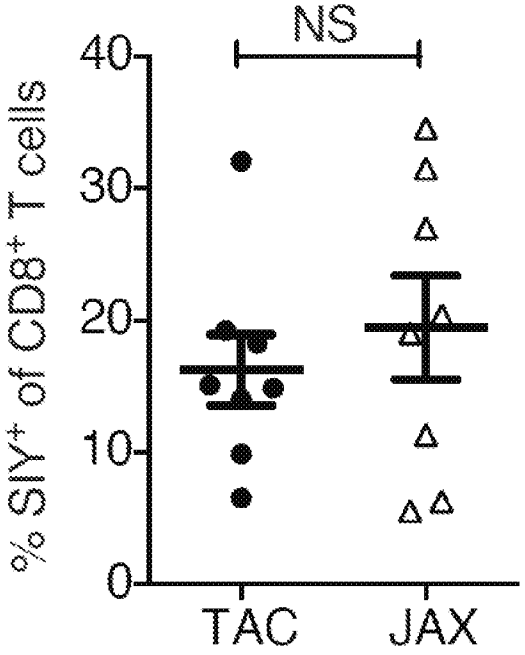


FIG. 1H

FIG. 2A

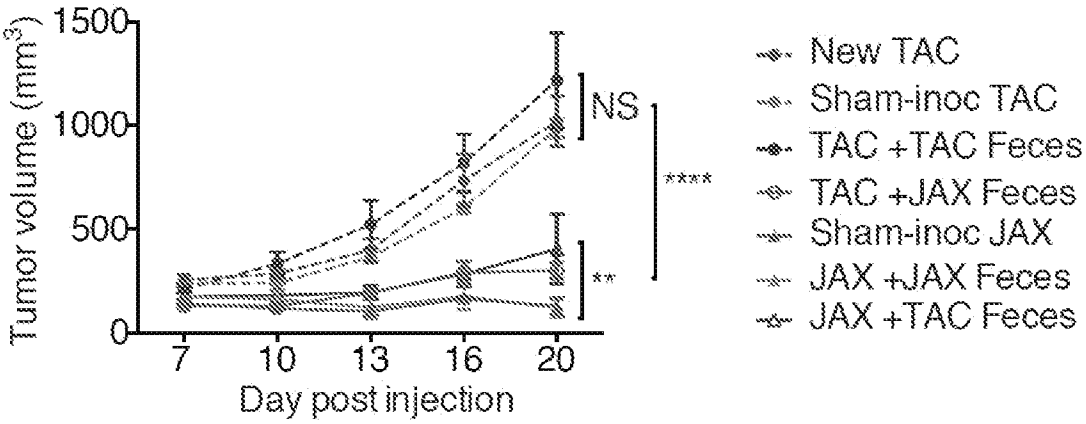
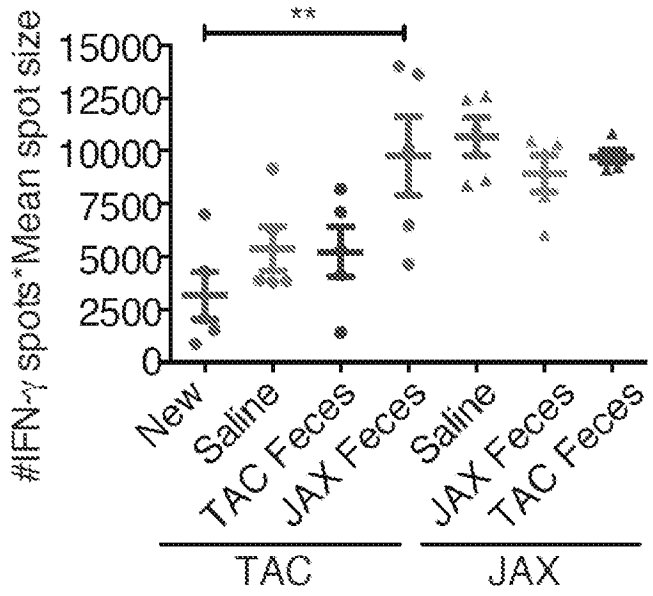

Tumor volume (mm3)
1500
1000
500
0
7
10
13
16
20
Day post injection
NS
****
**
New TAC
Sham-inoc TAC
TAC +TAC Feces
TAC +JAX Feces
Sham-inoc JAX
JAX +JAX Feces
JAX +TAC Feces

FIG. 2B


#IFN-γ spots*Mean spot size
15000
12500
10000
7500
5000
2500
0
**
New
Saline
TAC Feces
JAX Feces
Saline
JAX Feces
TAC Feces
TAC
JAX

SIY pentamer
Control
Gated on CD8+
SHAM
TAC feces
JAX feces
CD44
0.04
7.03
0
7.45
0.13
27.5

FIG. 2C

% SIY+ of CD8+ T cells
5
15
25
35
45
55
65
New
Saline
TAC Feces
JAX Feces
Saline
JAX Feces
TAC Feces
TAC
JAX
**

FIG. 2C (cont.)

FIG. 2D

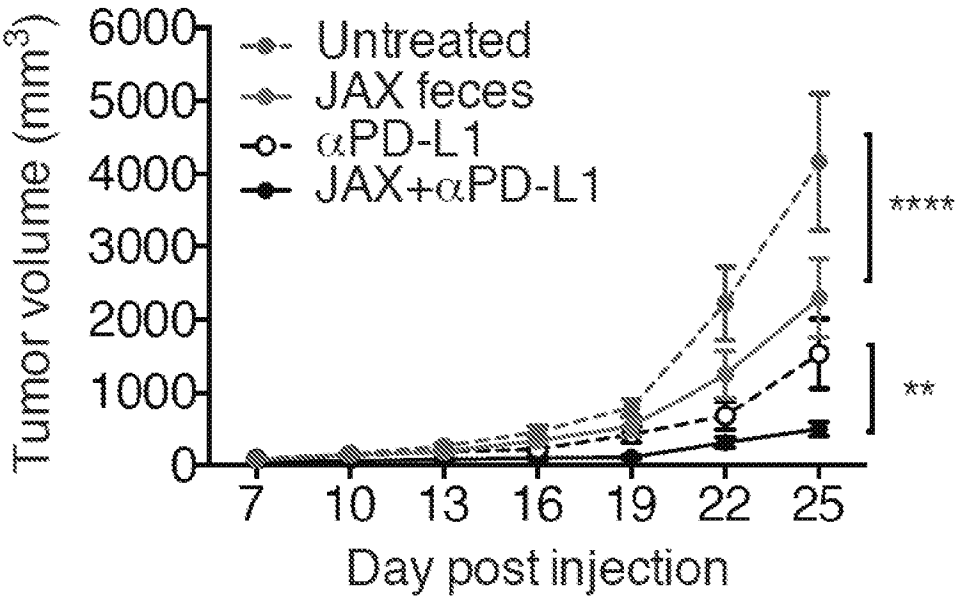

Tumor volume (mm3)
6000
5000
4000
3000
2000
1000
0
Untreated
JAX feces
αPD-L1
JAX+αPD-L1
****
**
7
10
13
16
19
22
25
Day post injection

FIG. 2E

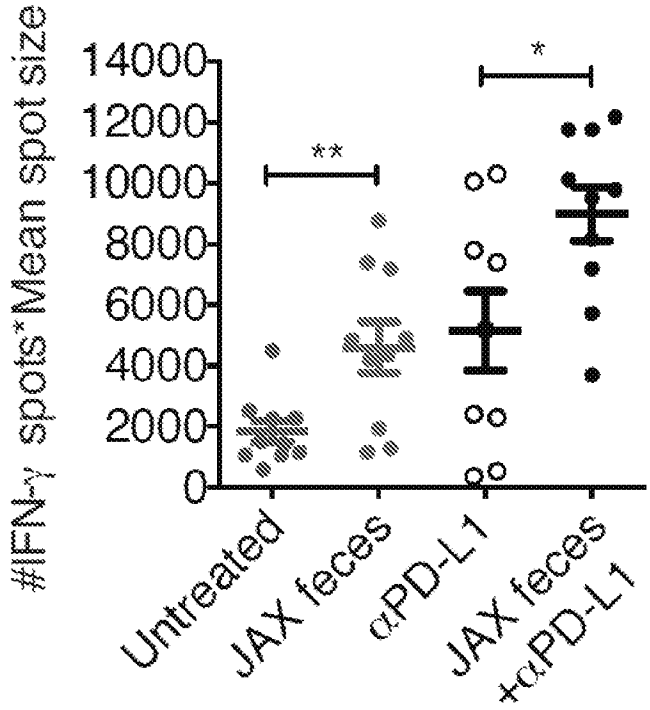

#IFN-γ spots*Mean spot size
14000
12000
10000
8000
6000
4000
2000
0
**
*
Untreated
JAX feces
αPD-L1
JAX feces
+αPD-L1

FIG. 2F

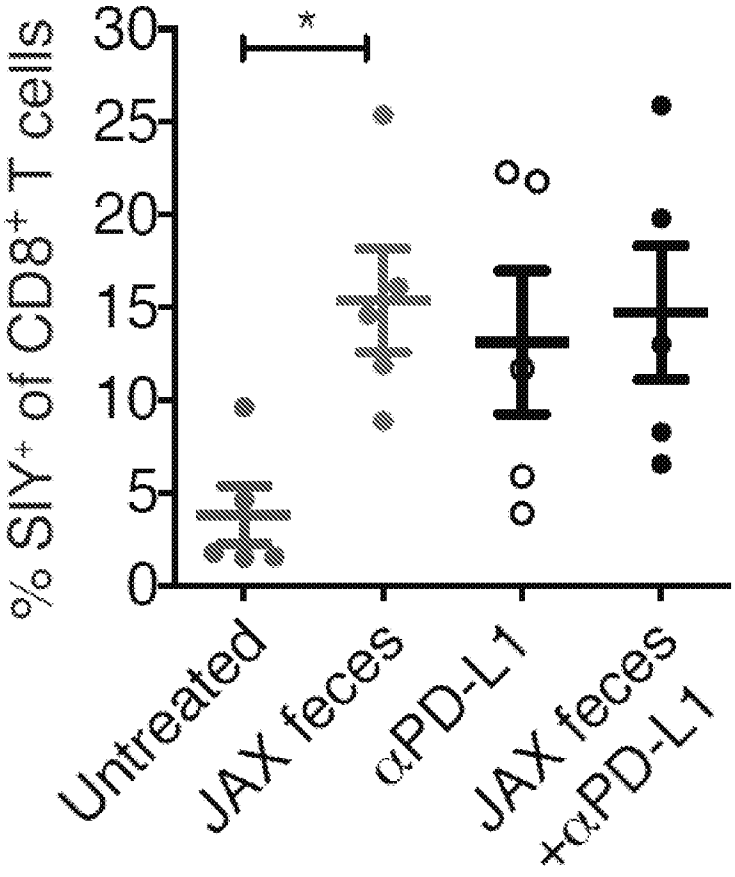

% SIY+ of CD8+ T cells
30
25
20
15
10
5
0
*
Untreated
JAX feces
αPD-L1
JAX feces
+αPD-L1

FIG. 2G

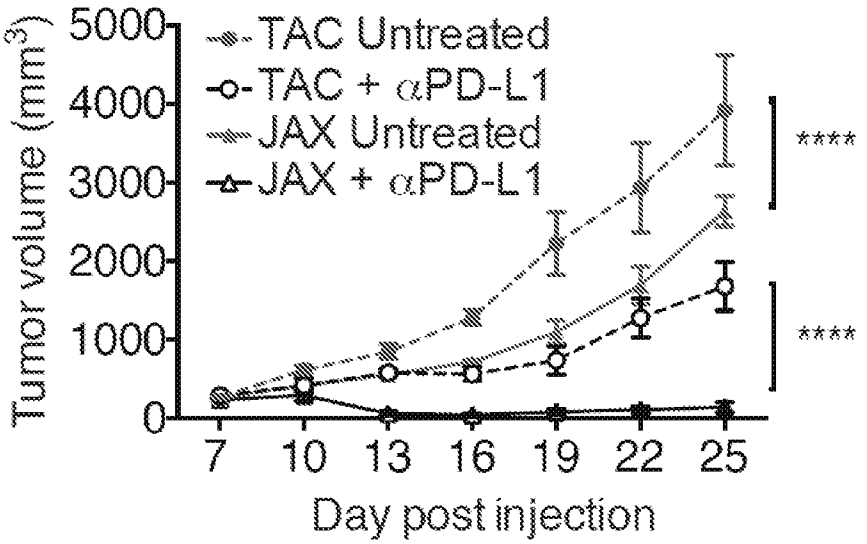

Tumor volume (mm3)
5000
4000
3000
2000
1000
0
TAC Untreated
TAC + αPD-L1
JAX Untreated
JAX + αPD-L1
****
****
7
10
13
16
19
22
25
Day post injection

FIG. 3A

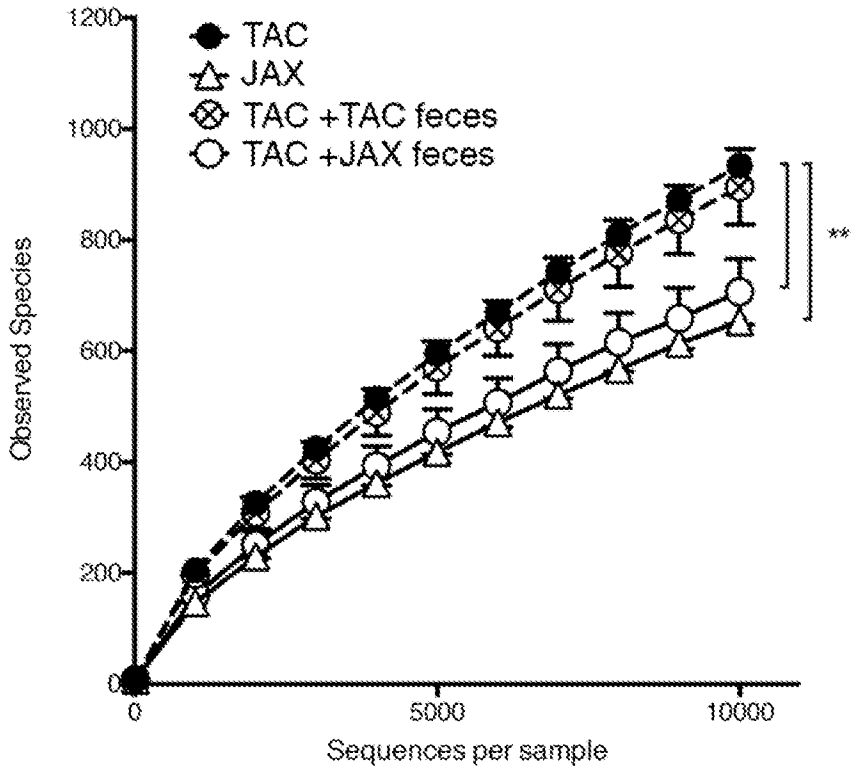


FIG. 3B

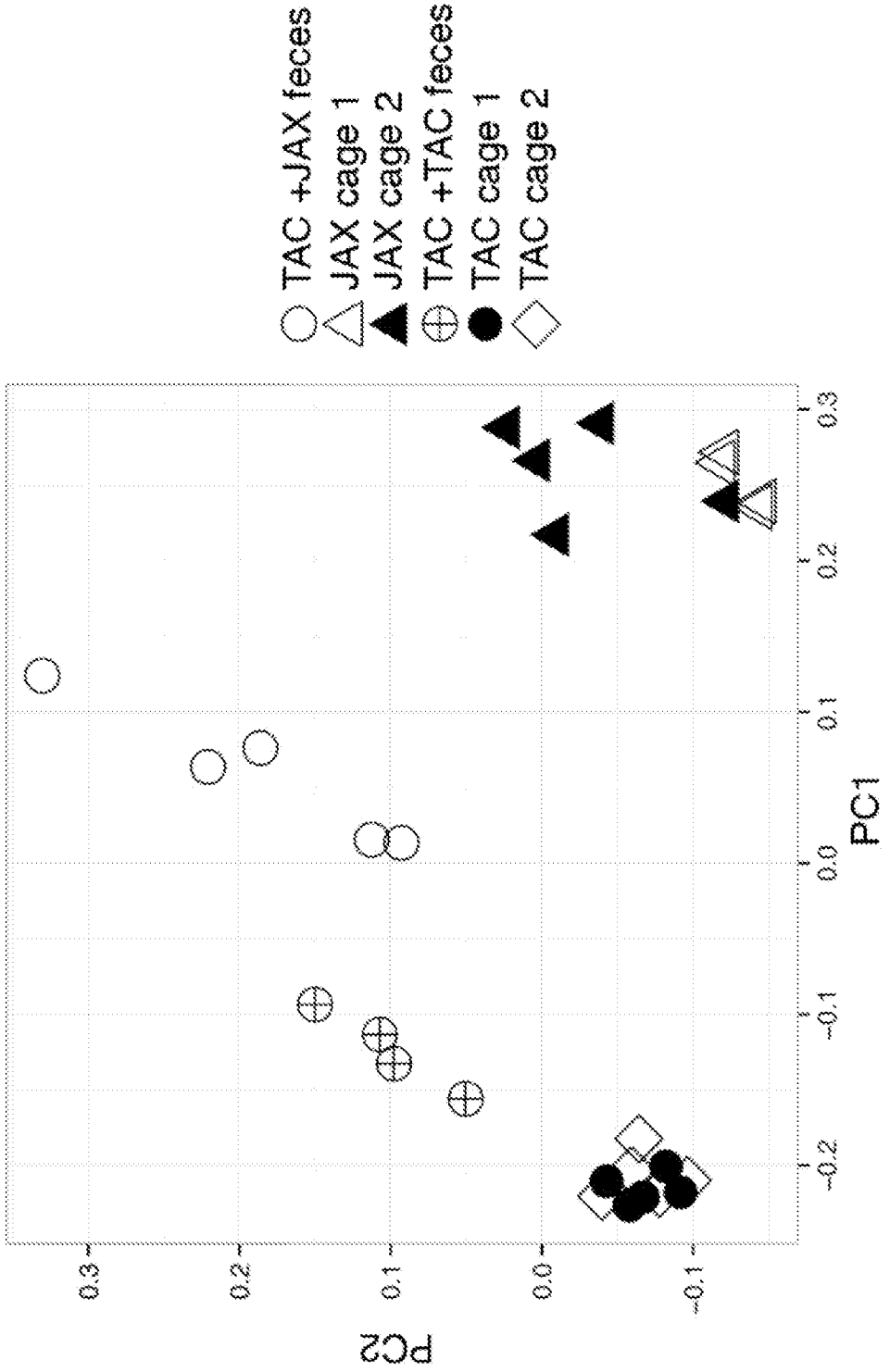

TAC +JAX feces
JAX cage 1
JAX cage 2
TAC +TAC feces
TAC cage 1
TAC cage 2
PC2
PC1
0.3
0.2
0.1
0.0
−0.1
−0.2
−0.1
0.0
0.1
0.2
0.3

FIG. 3C

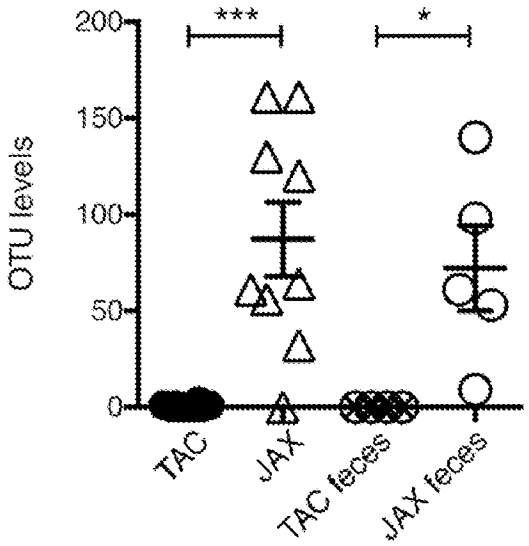


FIG. 3D

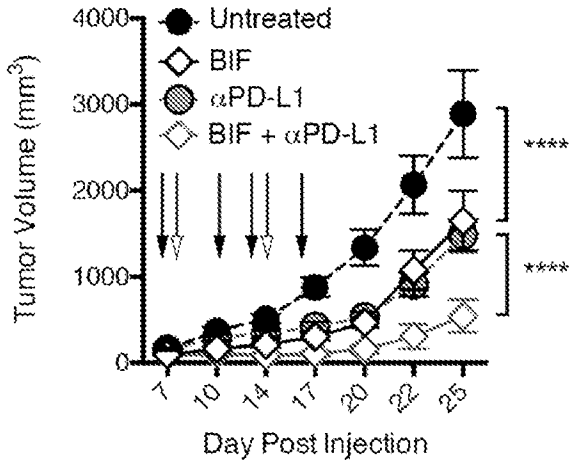


FIG. 3E

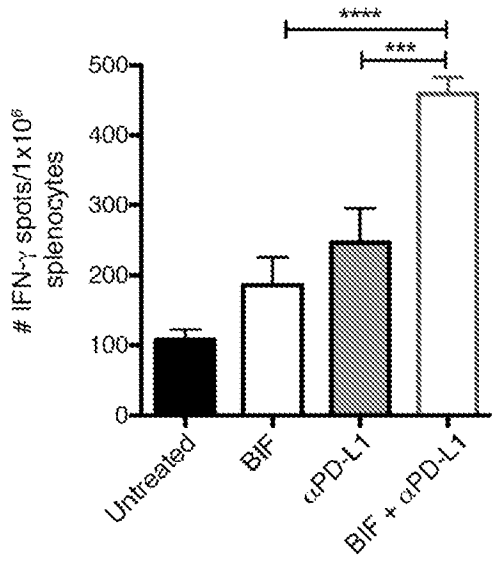


FIG. 3F

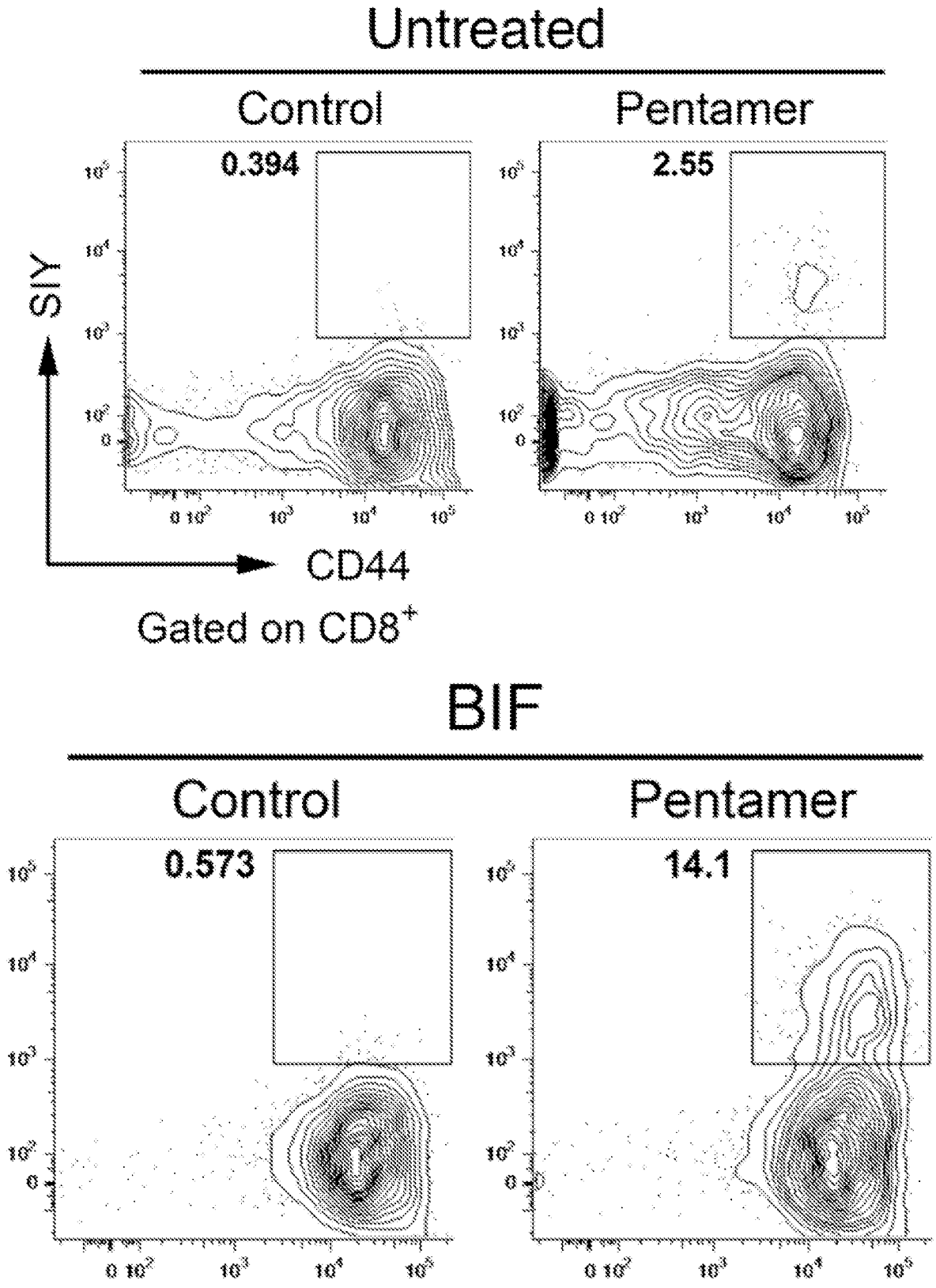

Untreated
Control
Pentamer
0.394
2.55
SIY
CD44
Gated on CD8+
BIF
Control
Pentamer
0.573
14.1

FIG. 3F (cont.)

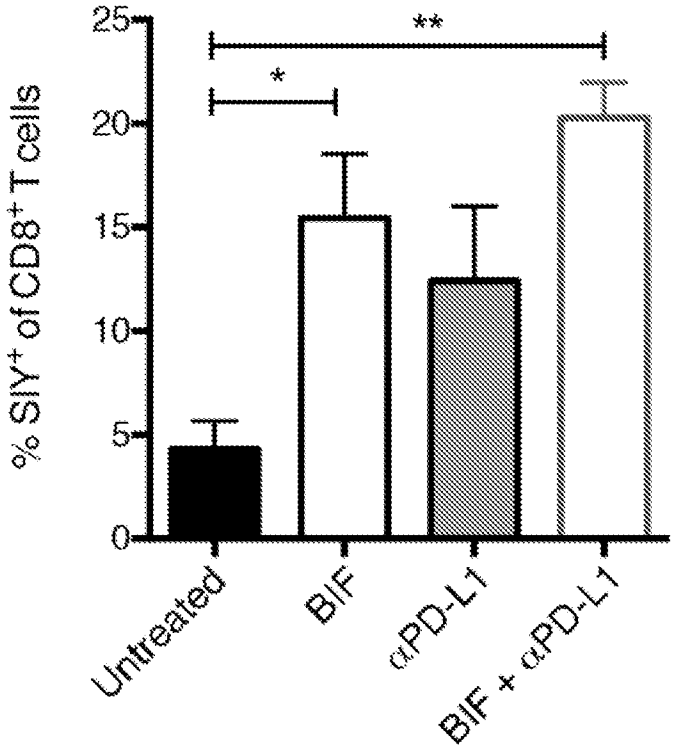


FIG. 3G

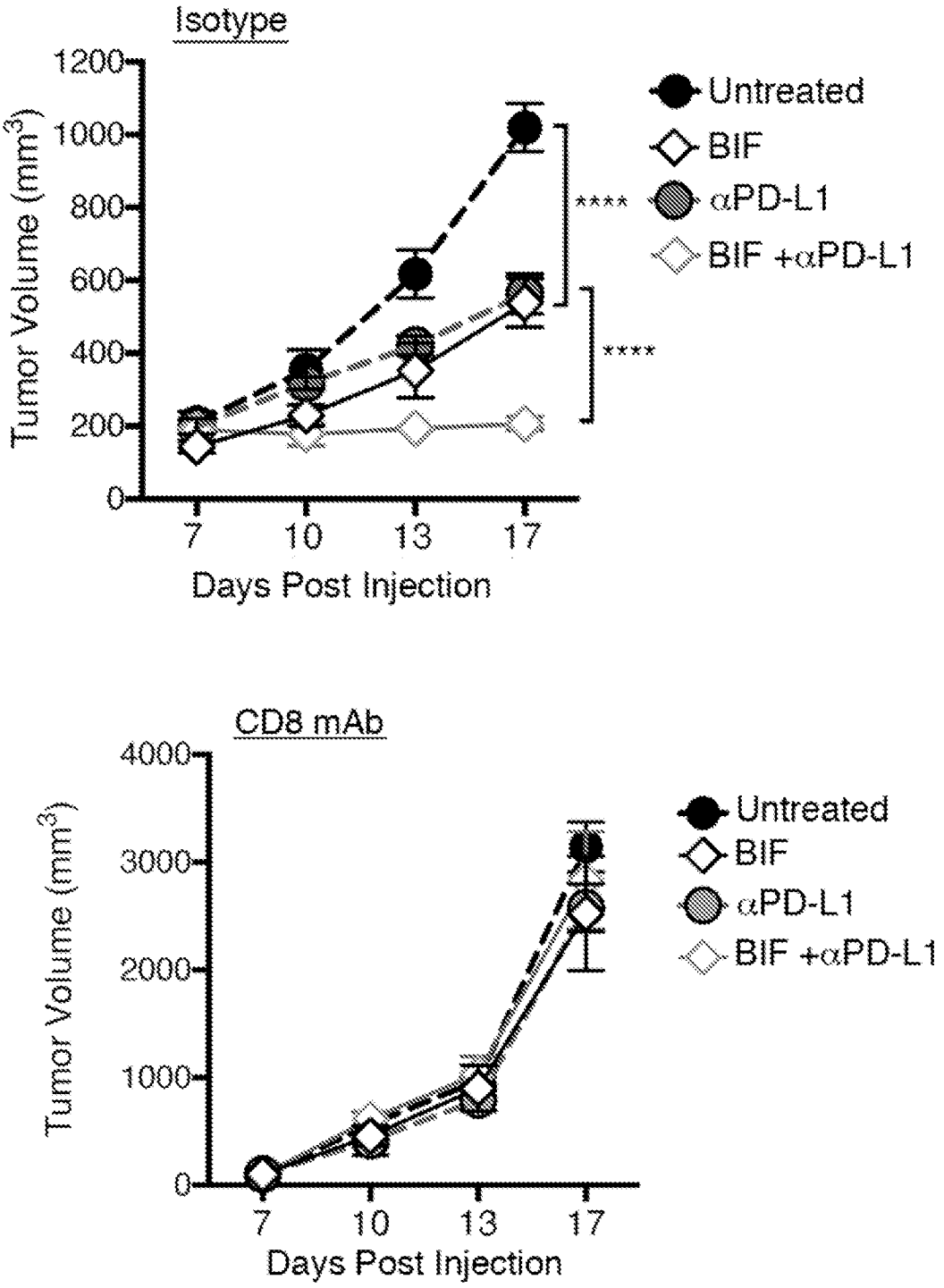

Isotype
Tumor Volume (mm3)
1200
1000
800
600
400
200
0
7
10
13
17
Days Post Injection
****
****
Untreated
BIF
αPD-L1
BIF +αPD-L1
CD8 mAb
Tumor Volume (mm3)
4000
3000
2000
1000
0
7
10
13
17
Days Post Injection
Untreated
BIF
αPD-L1
BIF +αPD-L1

FIG. 4A

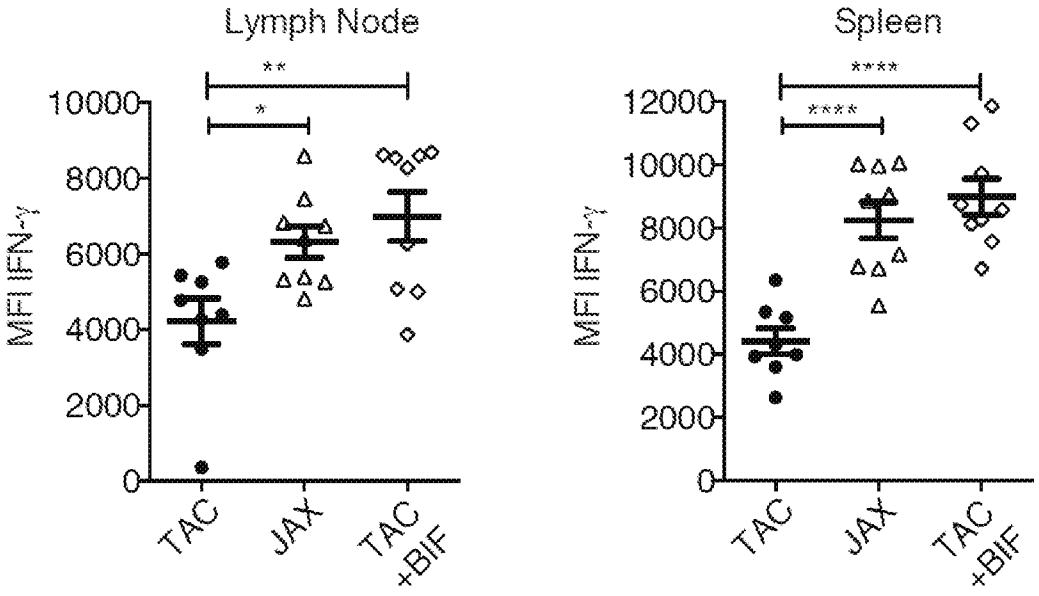

Lymph Node
Spleen
MFI IFN-γ
10000
8000
6000
4000
2000
0
12000
**
*
****
TAC
JAX
TAC +BIF

FIG. 4B

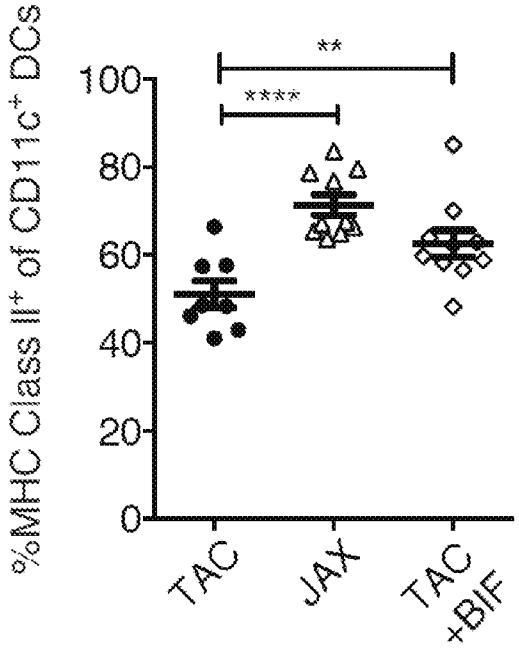

%MHC Class II+ of CD11c+ DCs
100
80
60
40
20
0
**
****
TAC
JAX
TAC +BIF

FIG. 4C

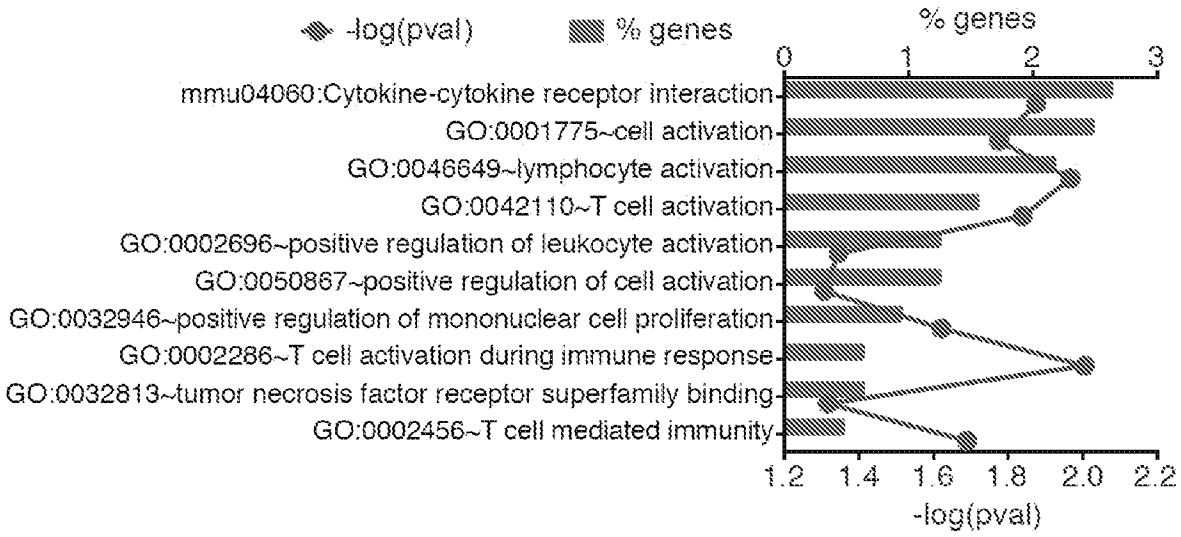

-log(pval)
% genes
% genes
0
1
2
3
mmu04060:Cytokine-cytokine receptor interaction
GO:0001775~cell activation
GO:0046649~lymphocyte activation
GO:0042110~T cell activation
GO:0002696~positive regulation of leukocyte activation
GO:0050867~positive regulation of cell activation
GO:0032946~positive regulation of mononuclear cell proliferation
GO:0002286~T cell activation during immune response
GO:0032813~tumor necrosis factor receptor superfamily binding
GO:0002456~T cell mediated immunity
1.2
1.4
1.6
1.8
2.0
2.2
-log(pval)

FIG. 4E

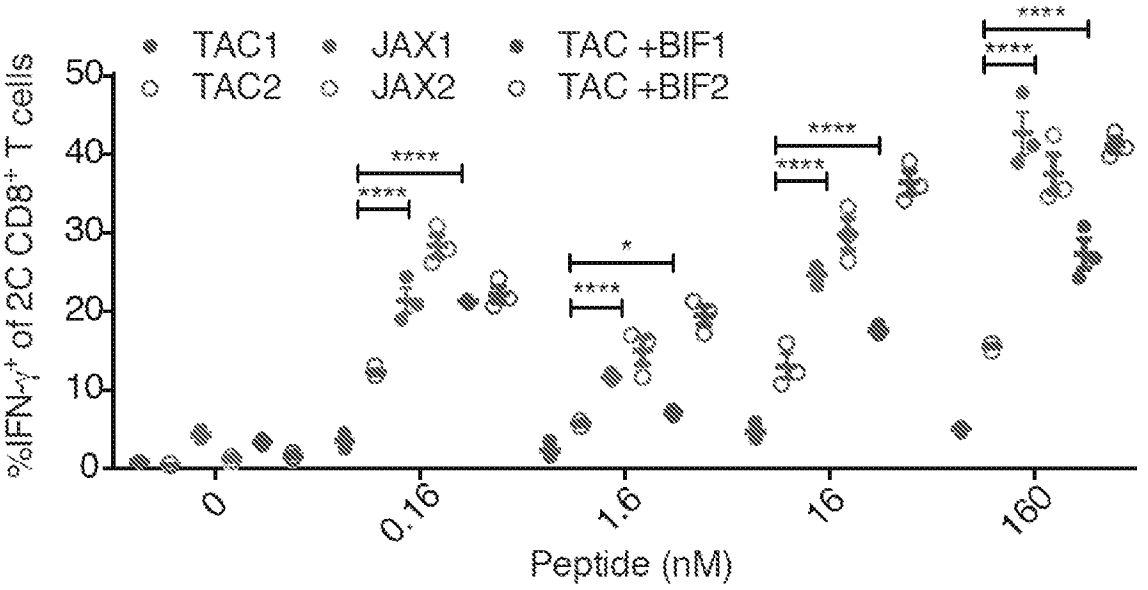
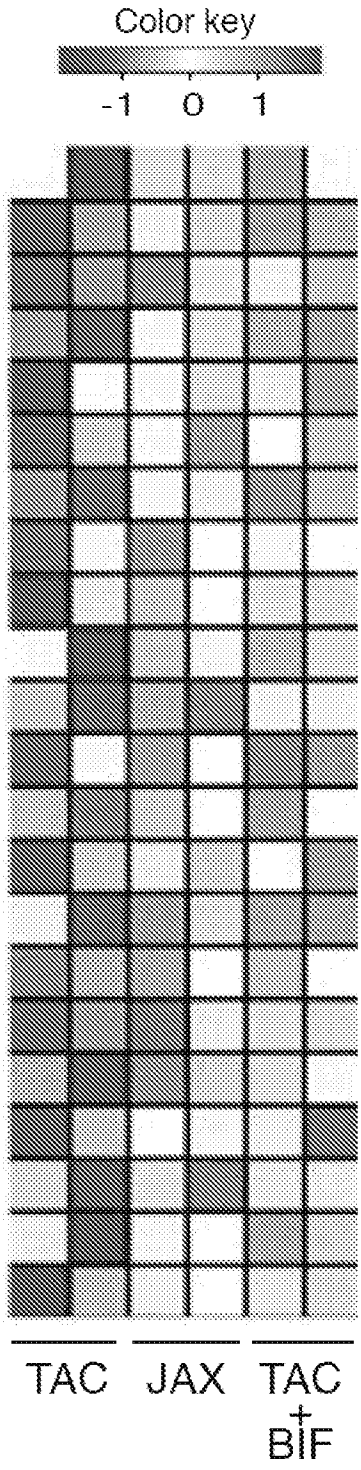

TAC1
TAC2
JAX1
JAX2
TAC +BIF1
TAC +BIF2
%IFN-γ+ of 2C CD8+ T cells
50
40
30
20
10
0
****
****
*
****
****
****
****
****
0
0.16
1.6
16
160
Peptide (nM)

FIG. 4D



Gene	JAX/TAC	BIF/TAC
H2m2	2.18	6.99
Cd40	1.58	2.11
Cd70	9.67	4.33
Icam1	1.57	2.00
Tnfrsf18	1.51	1.68
Tnfsf14	2.05	1.70
Relb	1.79	2.54
Ifngr2	3.10	1.71
Tapbp	42.40	14.97
Rab27a	2.55	2.96
Slc11a1	3.46	1.79
Cxcl9	2.64	1.53
Cx3cl1	3.96	6.36
Cxcr4	2.20	2.41
Irf1	1.55	2.44
Ifnar2	2.70	2.33
Oas2	1.68	1.51
Ifi35	1.92	1.57
Ifitm1	2.82	5.00
Azi2	7.71	3.04
Ikbke	1.60	2.03
Ulk1	5.78	3.51

FIG. 5A

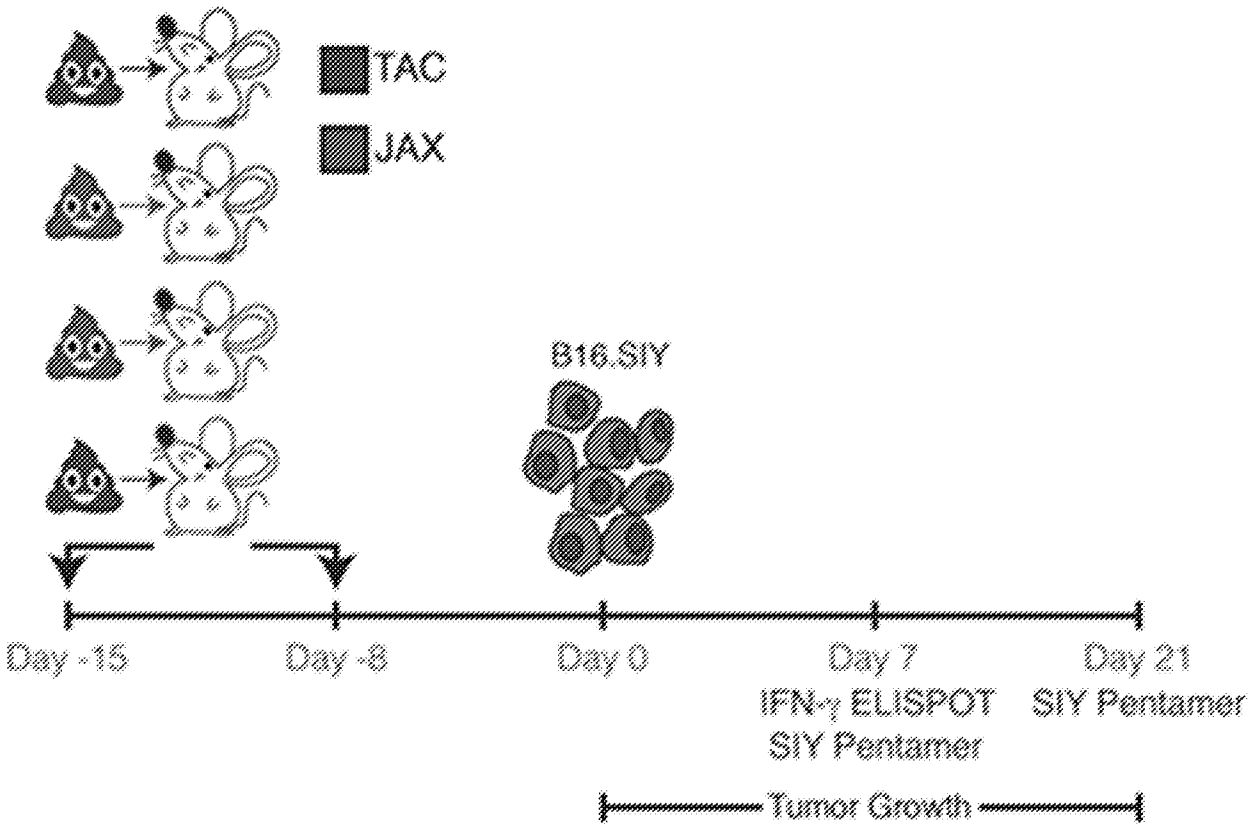

TAC
JAX
B16.SIY
Day -15
Day -8
Day 0
Day 7
Day 21
IFN-γ ELISPOT
SIY Pentamer
SIY Pentamer
Tumor Growth

FIG. 5B

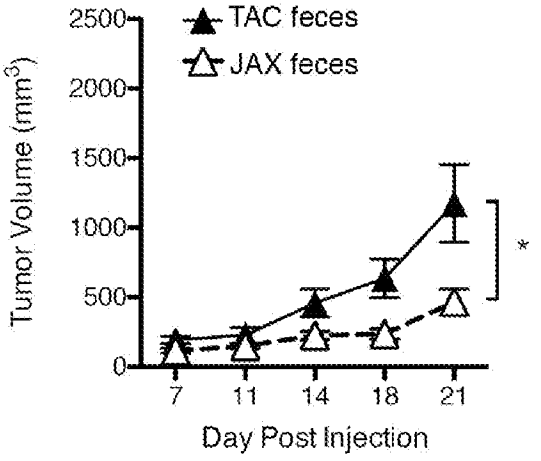


FIG. 5C

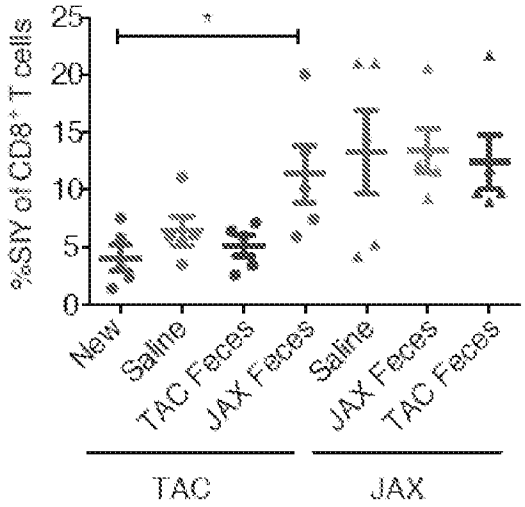


FIG. 5D

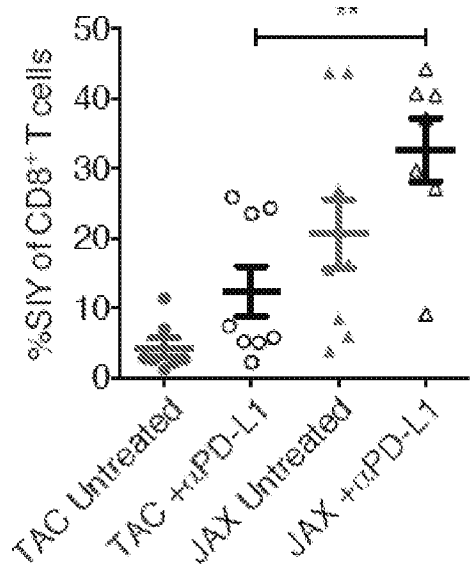


FIG. 6A

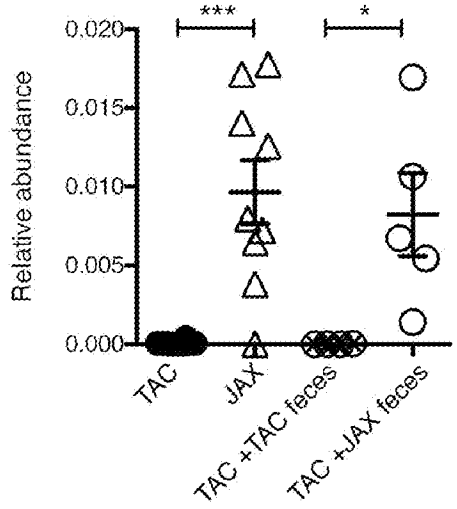


FIG. 6B

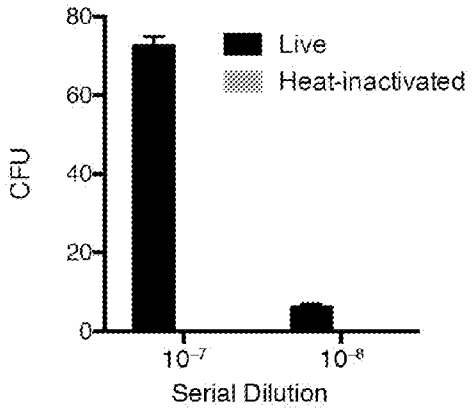


FIG. 6C

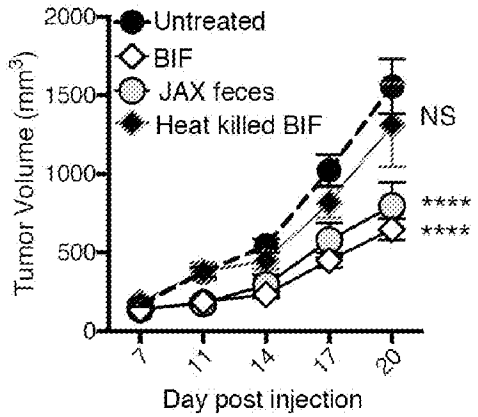


FIG. 6D

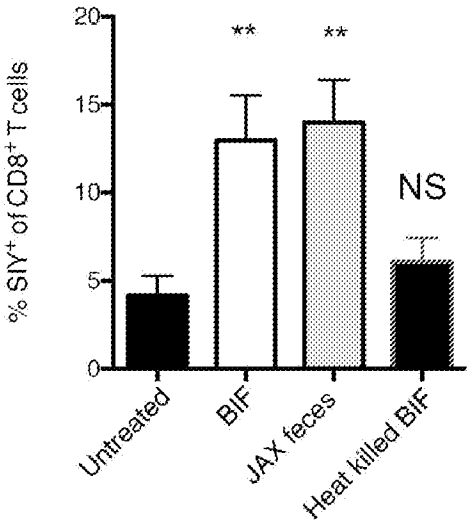


FIG. 6E

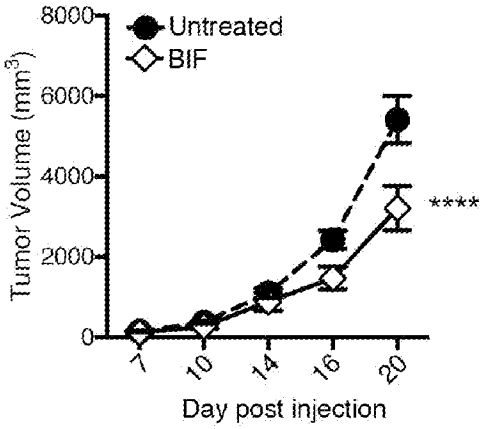


FIG. 6F

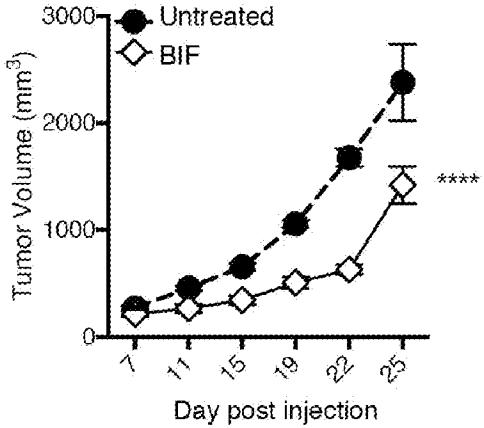


FIG. 6G

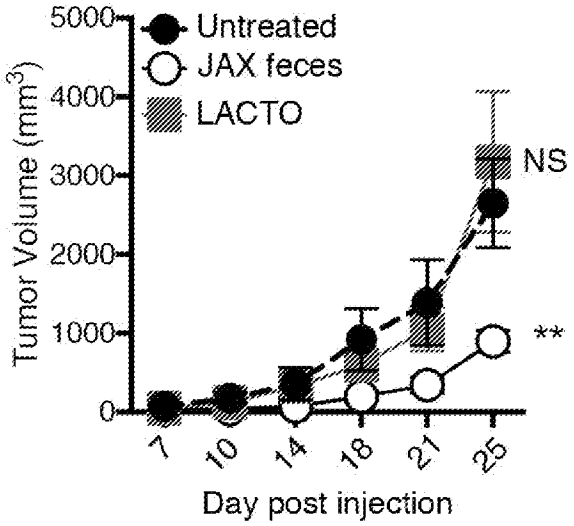

Untreated
JAX feces
LACTO
Tumor Volume (mm3)
5000
4000
3000
2000
1000
0
NS
**
7
10
14
18
21
25
Day post injection

FIG. 6H

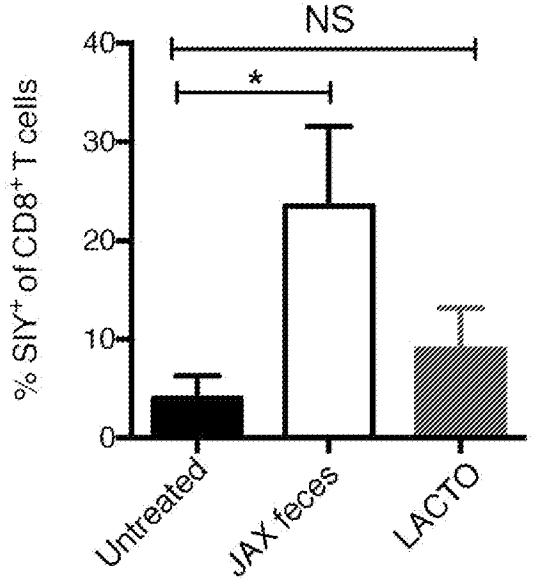
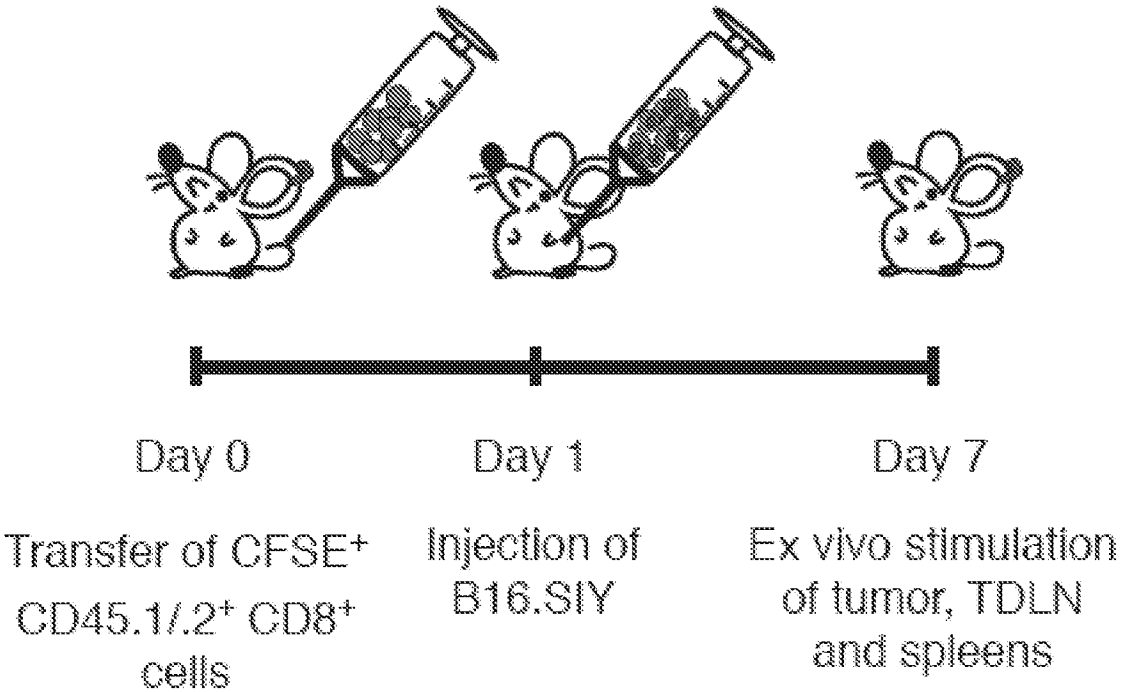

NS
*
% SIY+ of CD8+ T cells
40
30
20
10
0
Untreated
JAX feces
LACTO

FIG. 7A


Day 0
Day 1
Day 7
Transfer of CFSE+ CD45.1/.2+ CD8+ cells
Injection of B16.SIY
Ex vivo stimulation of tumor, TDLN and spleens

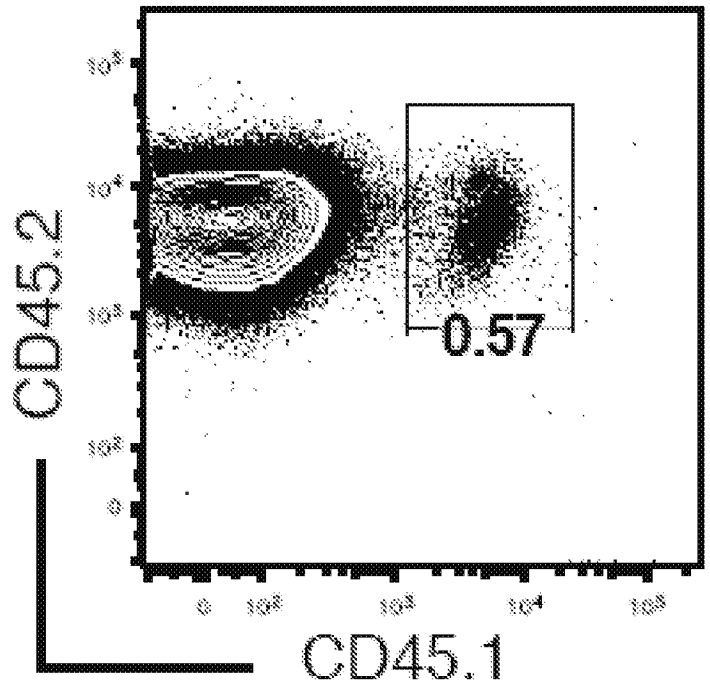

CD45.2
CD45.1
0.57

FIG. 7B

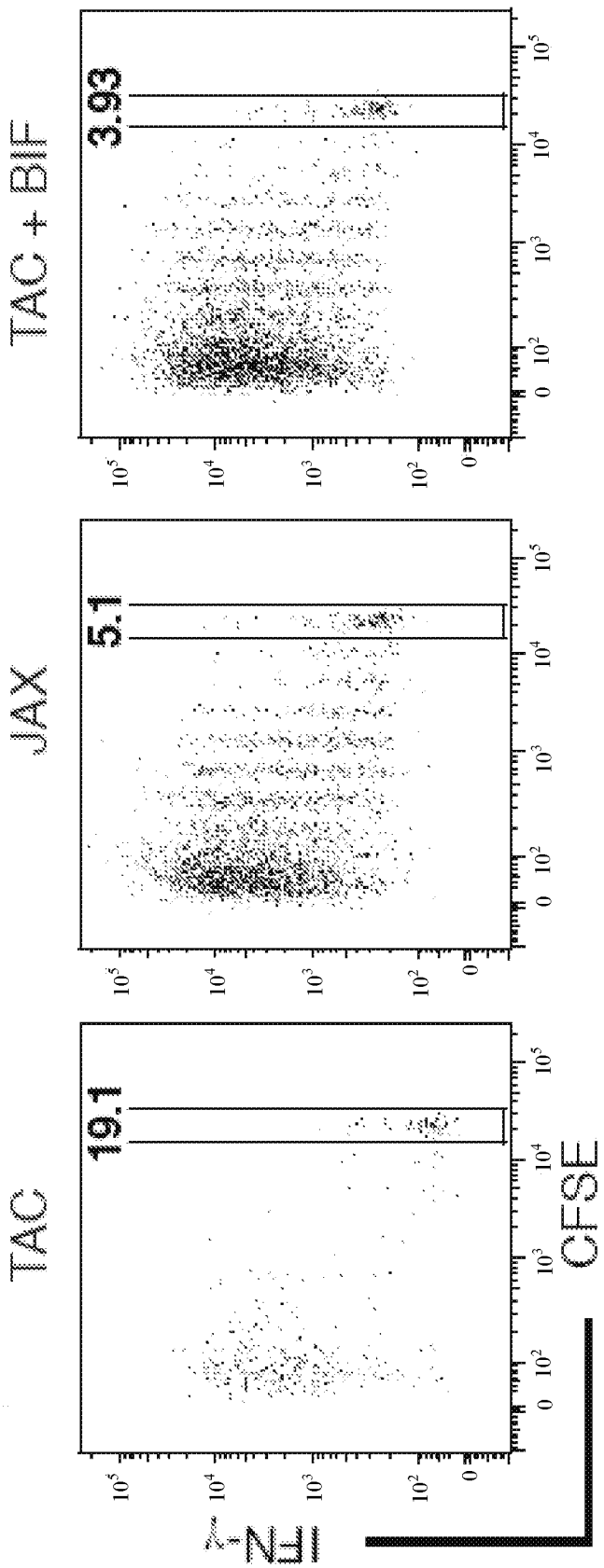


FIG. 7B (cont.)

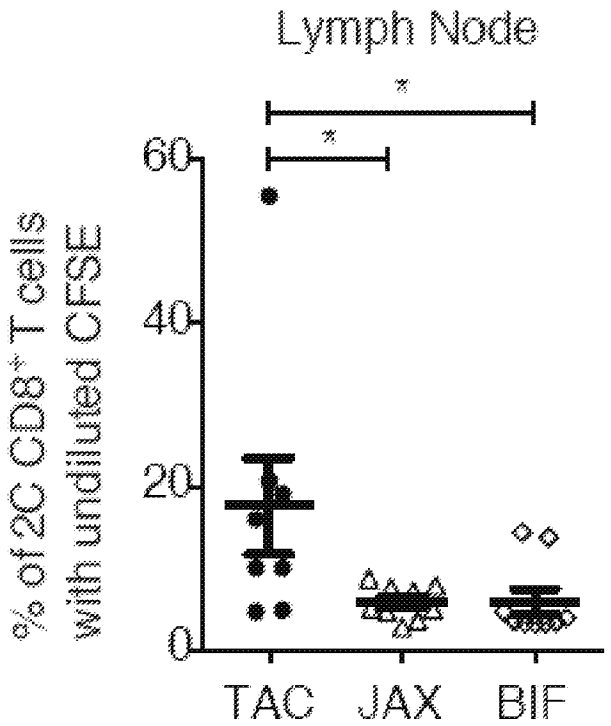

Lymph Node
*
*
60
40
20
0
% of 2C CD8+ T cells with undiluted CFSE
TAC
JAX
BIF

FIG. 8A

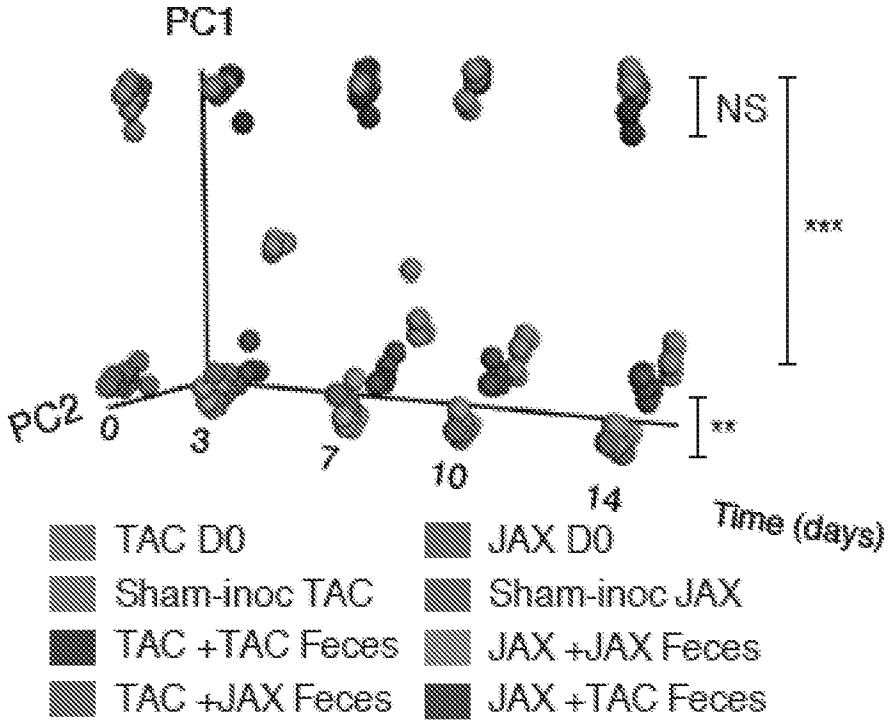

PC1
PC2
NS
***
**
0
3
7
10
14
Time (days)
TAC D0
Sham-inoc TAC
TAC +TAC Feces
TAC +JAX Feces
JAX D0
Sham-inoc JAX
JAX +JAX Feces
JAX +TAC Feces

FIG. 8B

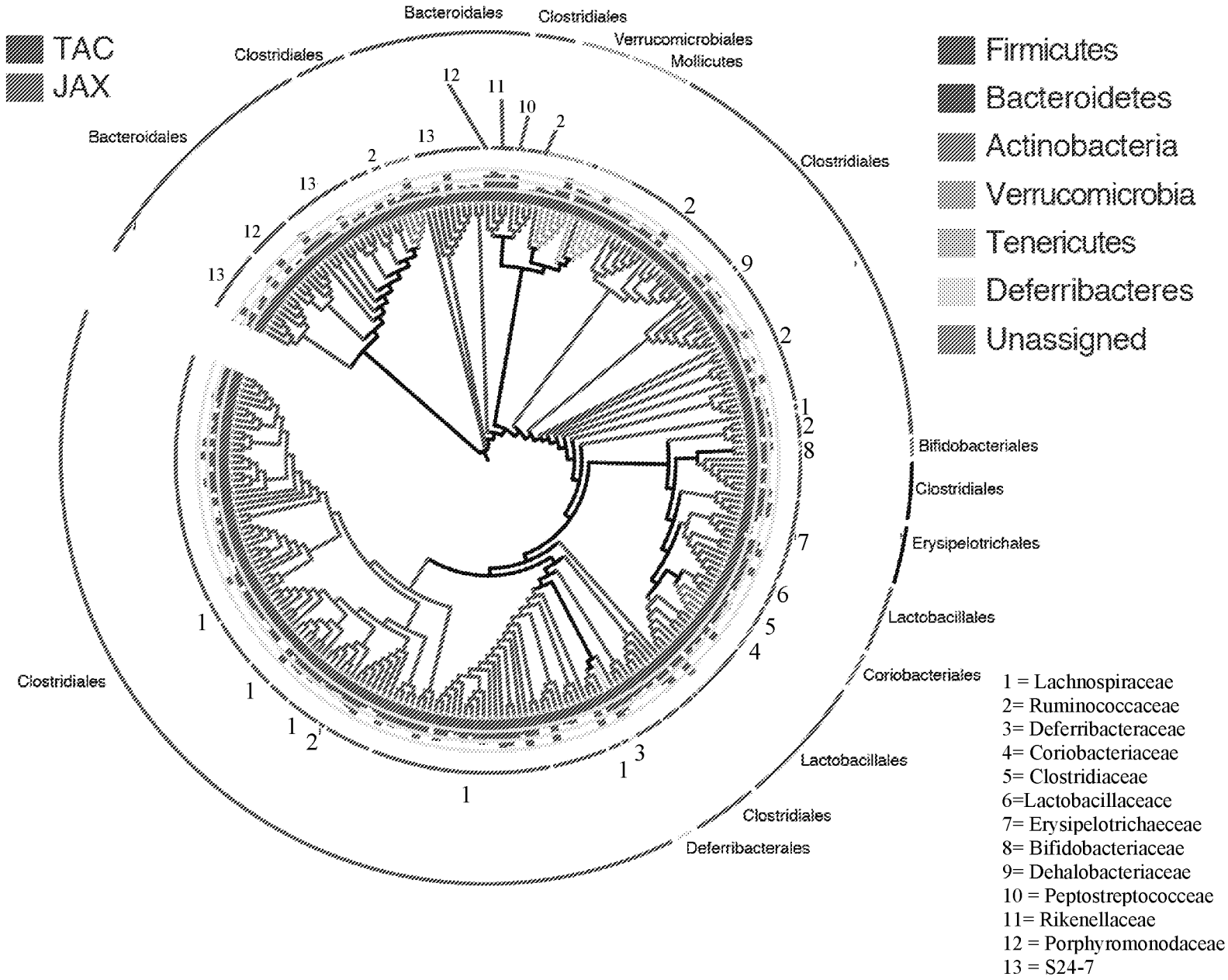

TAC
JAX
Bacteroidales
Clostridiales
Verrucomicrobiales
Mollicutes
Clostridiales
Bacteroidales
Clostridiales
Clostridiales
Bifidobacteriales
Clostridiales
Erysipelotrichales
Lactobacillales
Coriobacteriales
Lactobacillales
Clostridiales
Deferribacterales
Firmicutes
Bacteroidetes
Actinobacteria
Verrucomicrobia
Tenericutes
Deferribacteres
Unassigned
1 = Lachnospiraceae
2= Ruminococcaceae
3= Deferribacteraceae
4= Coriobacteriaceae
5= Clostridiaceae
6=Lactobacillaceace
7= Erysipelotrichaeceae
8= Bifidobacteriaceae
9= Dehalobacteriaceae
10 = Peptostreptococceae
11= Rikenellaceae
12 = Porphyromonodaceae
13 = S24-7

FIG. 8C

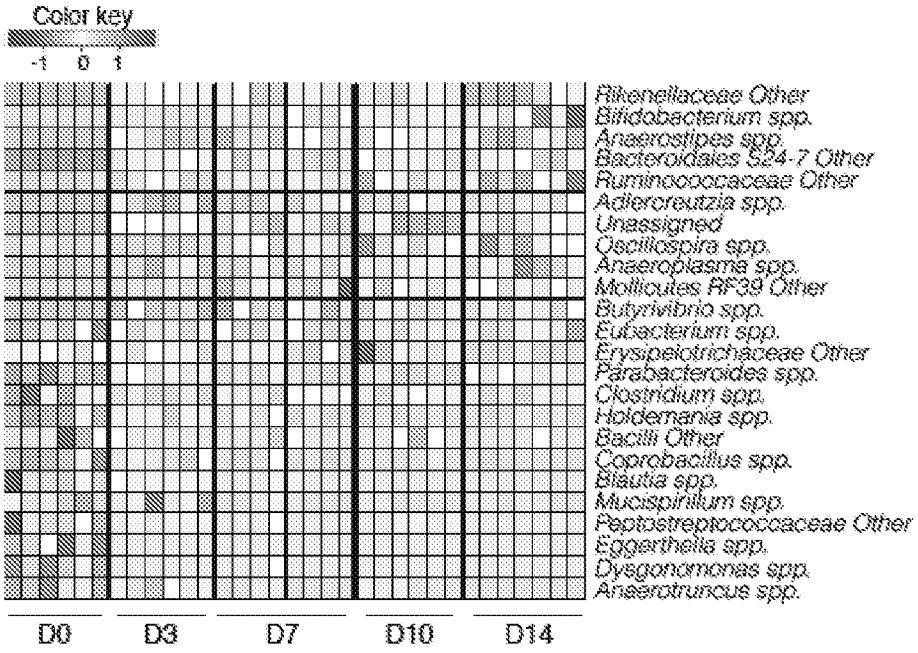

Color key
-1 0 1
Rikenellaceae Other
Bifidobacterium spp.
Anaerostipes spp.
Bacteroidales S24-7 Other
Ruminococcaceae Other
Adlercreutzia spp.
Unassigned
Oscillospira spp.
Anaeroplasma spp.
Mollicutes RF39 Other
Butyrivibrio spp.
Eubacterium spp.
Erysipelotrichaceae Other
Parabacteroides spp.
Clostridium spp.
Holdemania spp.
Bacilli Other
Coprobacillus spp.
Blautia spp.
Mucispirillum spp.
Peptostreptococcaceae Other
Eggerthella spp.
Dysgonomonas spp.
Anaerotruncus spp.
D0
D3
D7
D10
D14

FIG. 8D

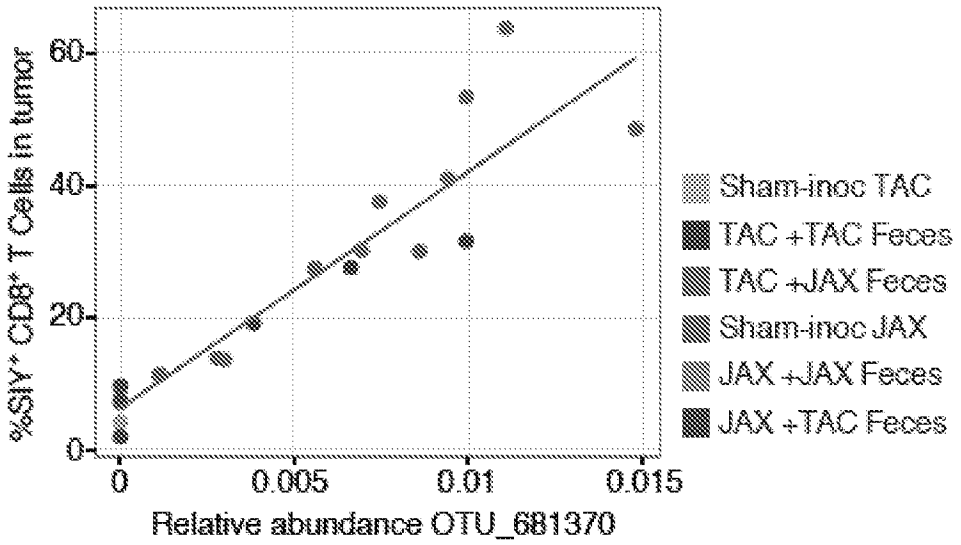

%SIY+ CD8+ T Cells in tumor
60
40
20
0
0
0.005
0.01
0.015
Relative abundance OTU_681370
Sham-inoc TAC
TAC +TAC Feces
TAC +JAX Feces
Sham-inoc JAX
JAX +JAX Feces
JAX +TAC Feces

FIG. 8E

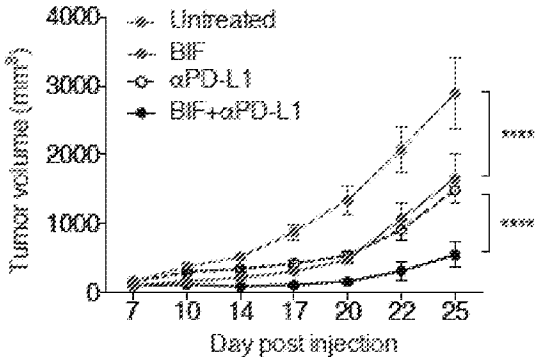


FIG. 8F

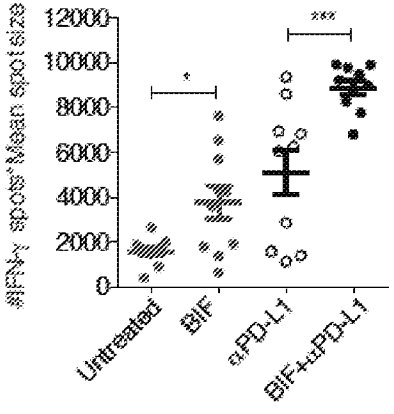


FIG. 8G

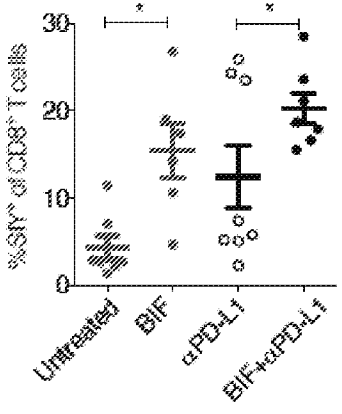


FIG. 9A

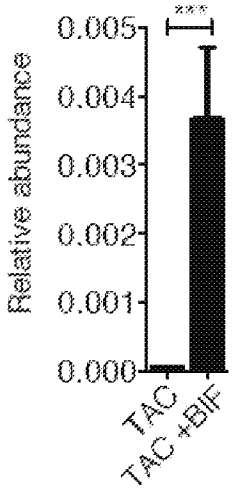


FIG. 9B

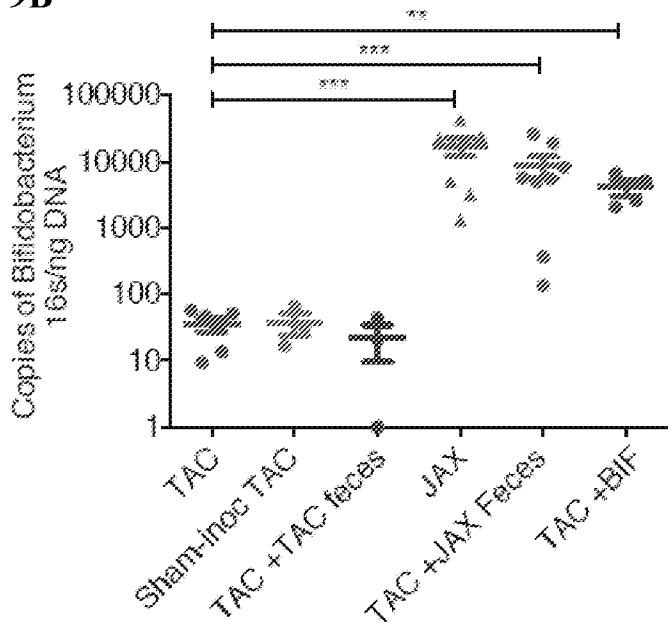


FIG. 9D

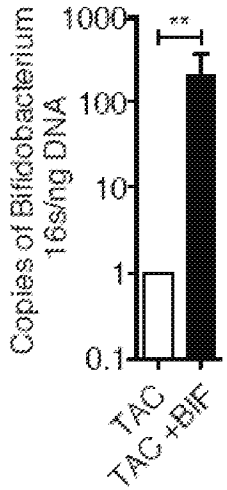


FIG. 9C

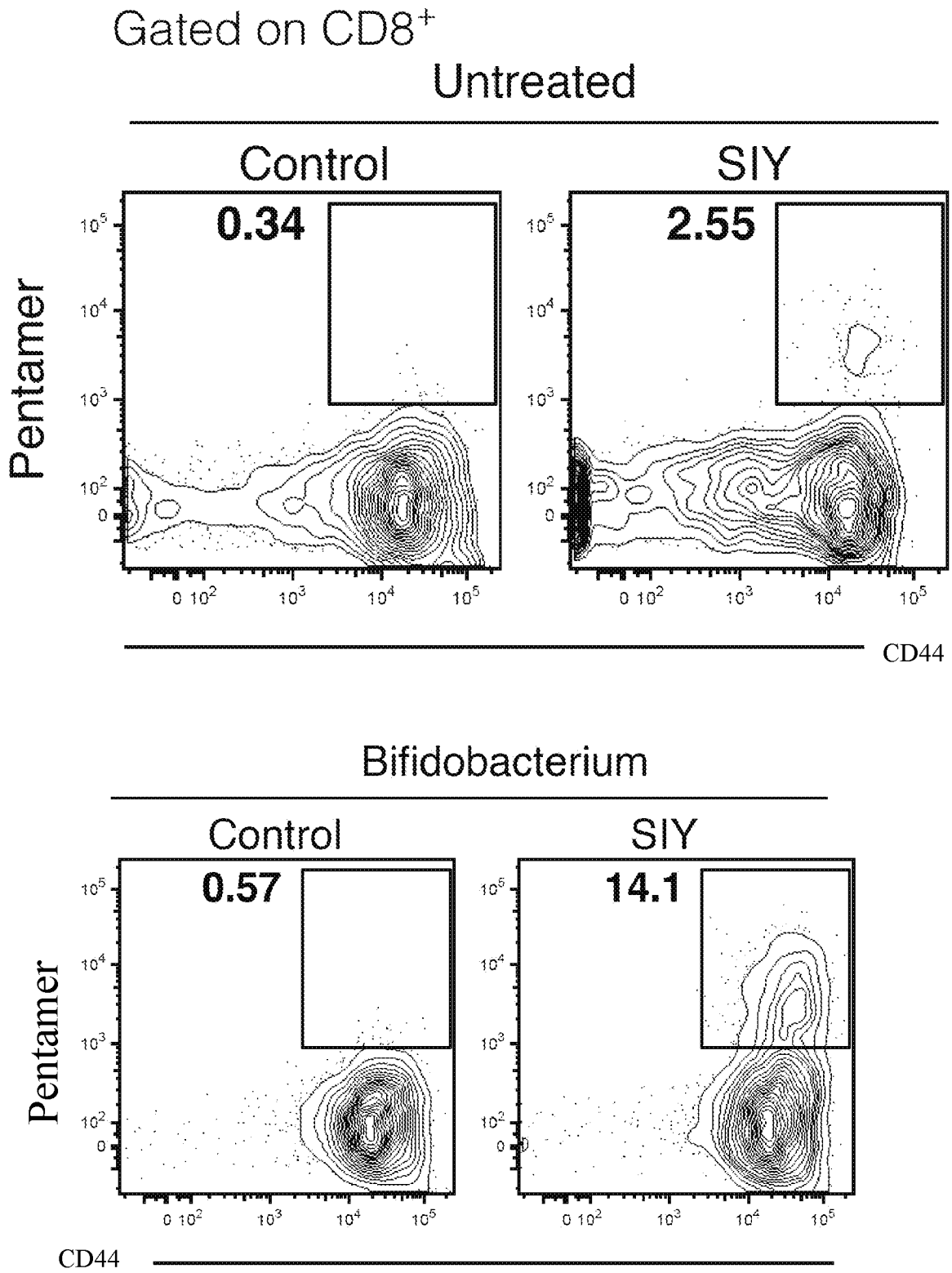

Gated on CD8+
Untreated
Control
SIY
0.34
2.55
Pentamer
CD44
Bifidobacterium
Control
SIY
0.57
14.1
Pentamer
CD44

FIG. 9E

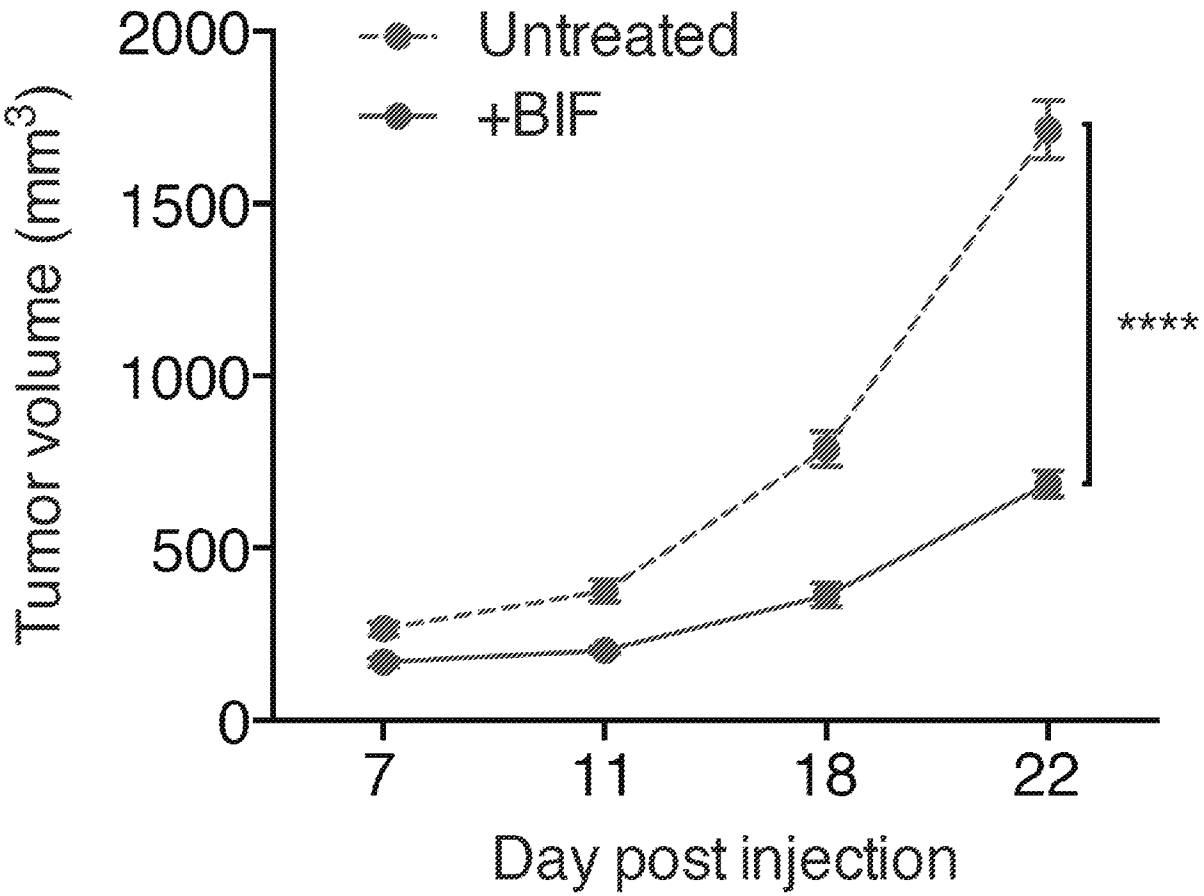

2000
1500
1000
500
0
Tumor volume (mm3)
Untreated
+BIF
****
7
11
18
22
Day post injection

FIG. 10A

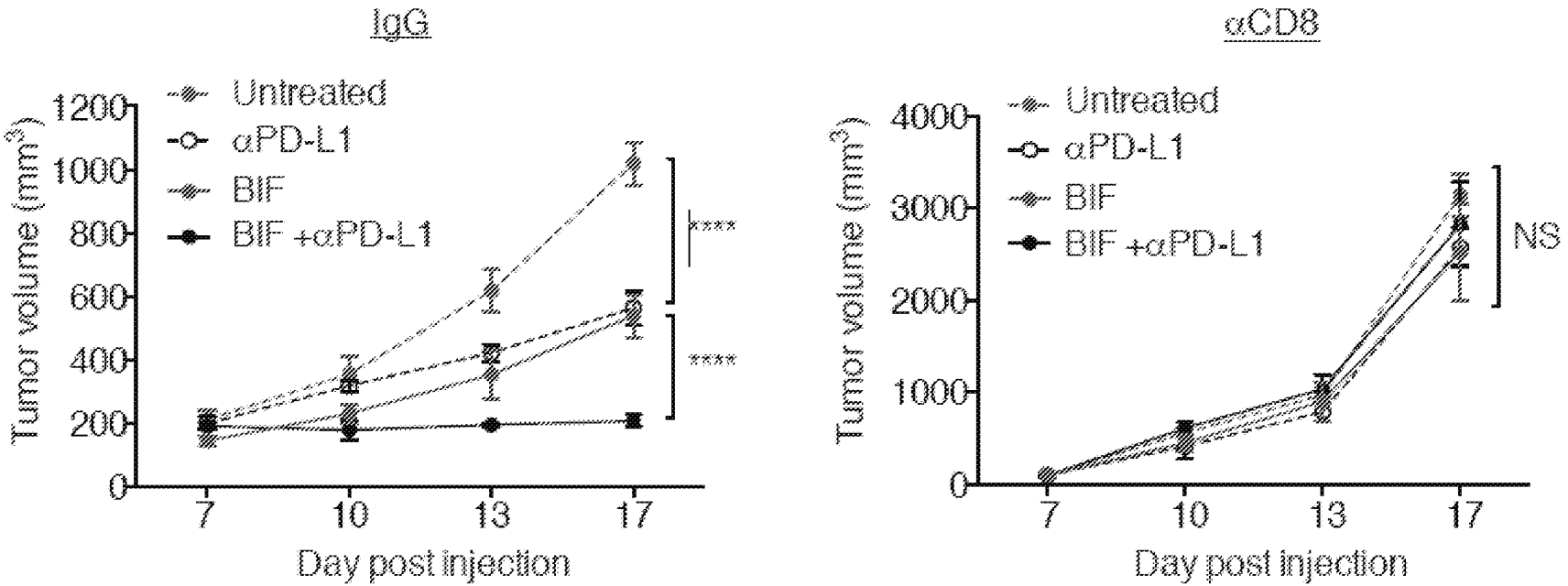

IgG
Untreated
αPD-L1
BIF
BIF +αPD-L1
Tumor volume (mm3)
1200
1000
800
600
400
200
0
7
10
13
17
Day post injection
****
****
αCD8
Untreated
αPD-L1
BIF
BIF +αPD-L1
Tumor volume (mm3)
4000
3000
2000
1000
0
7
10
13
17
Day post injection
NS

FIG. 10B

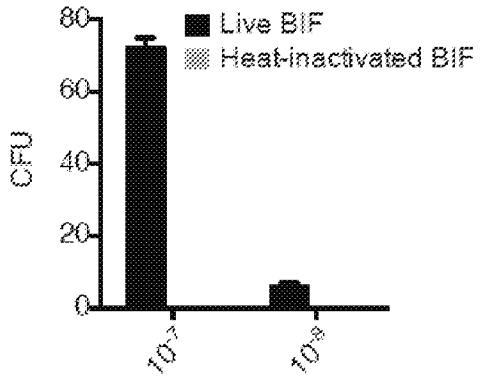


FIG. 10C

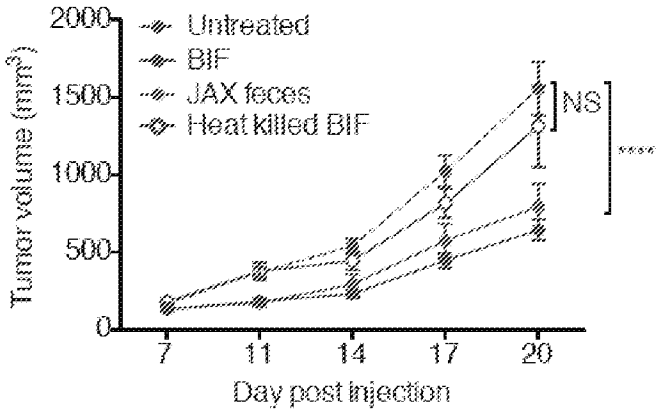


FIG. 10D

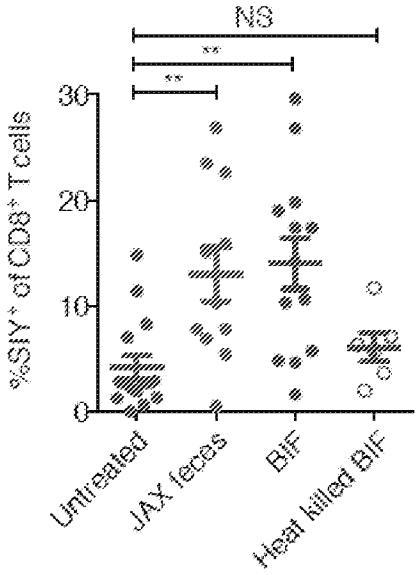


FIG. 11A

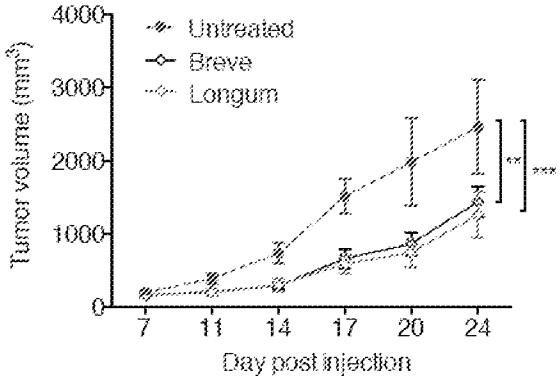


FIG. 11B

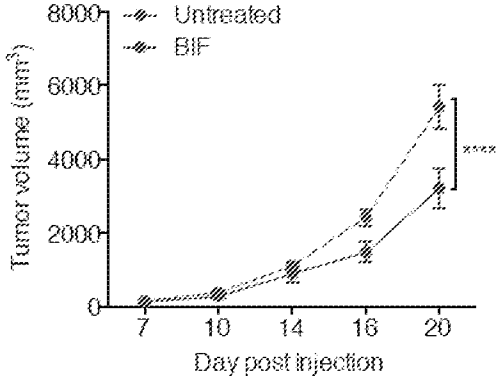


FIG. 11C

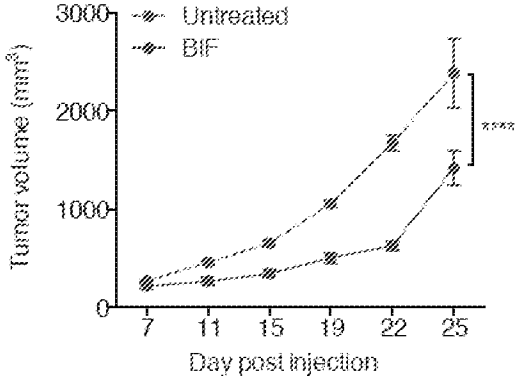


FIG. 11D

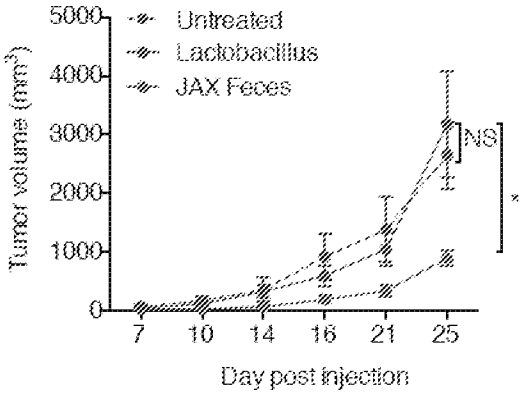


FIG. 11E

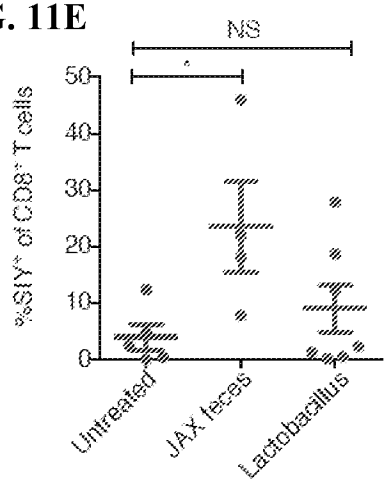


FIG. 12A

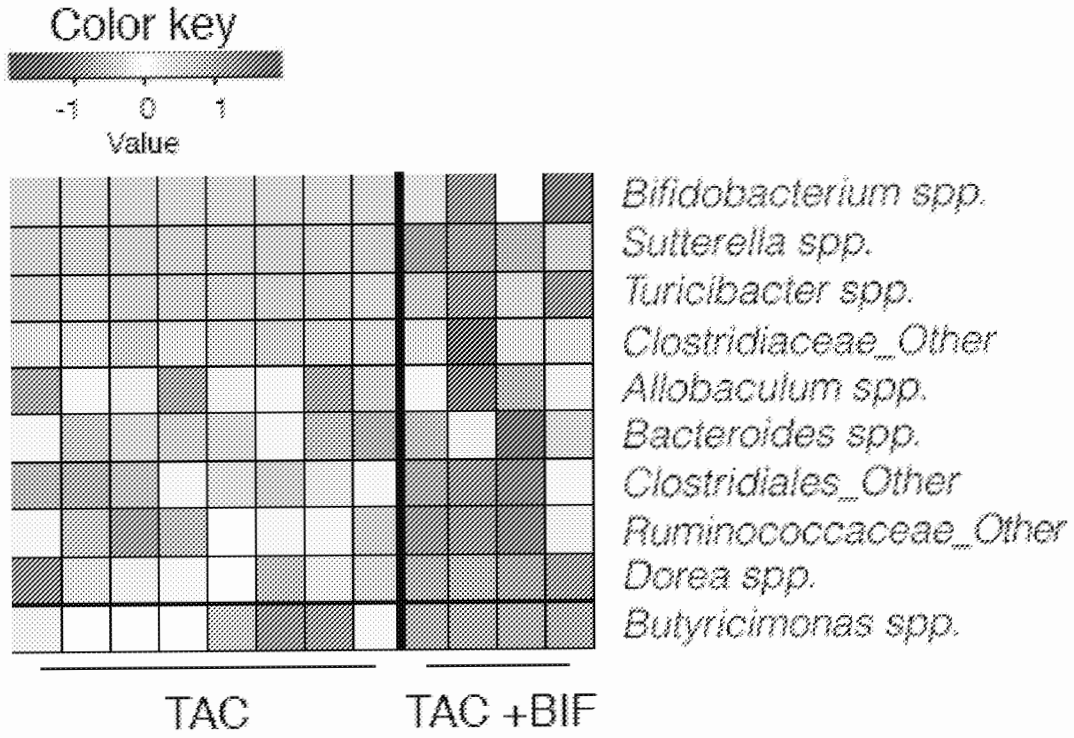
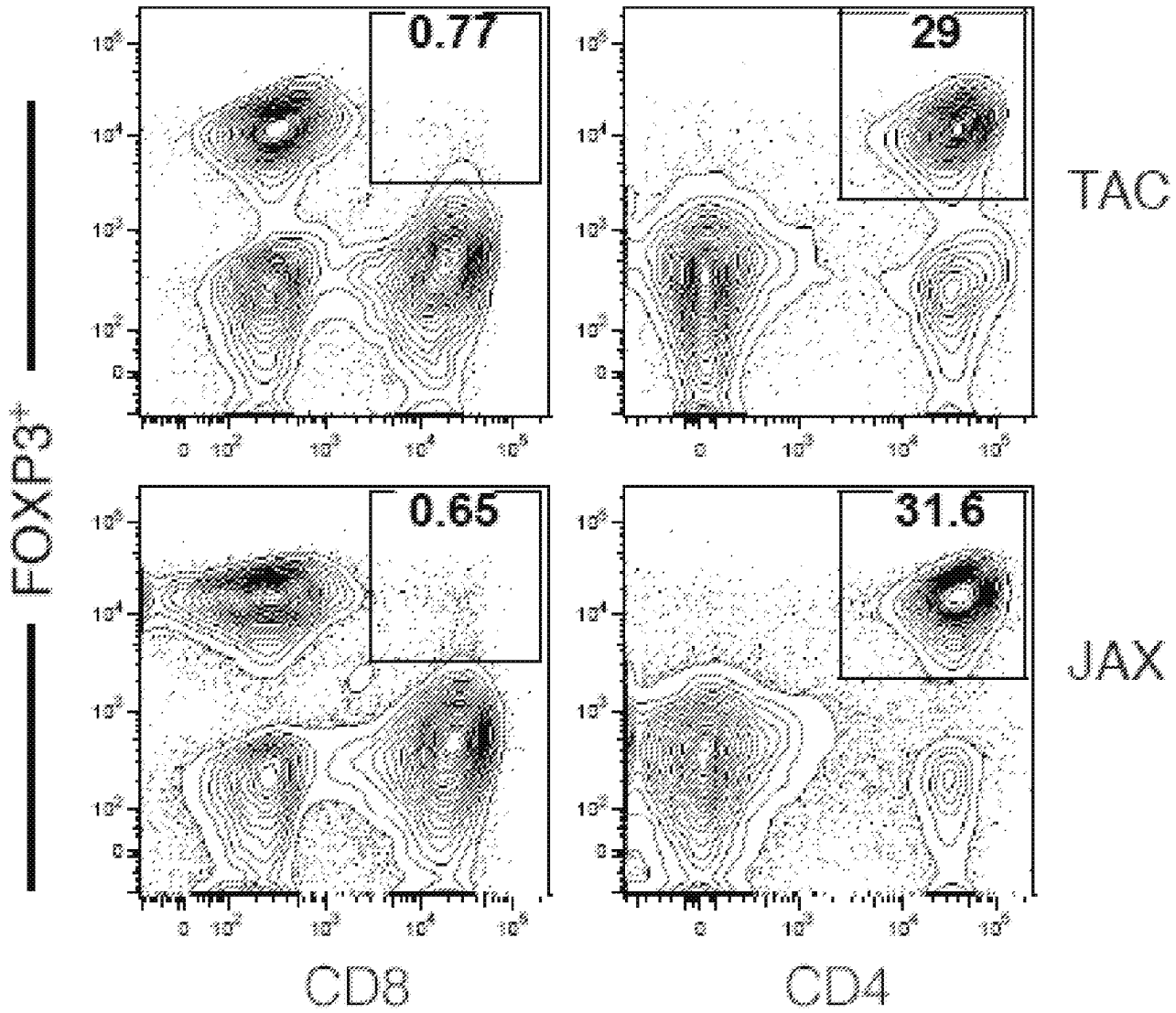
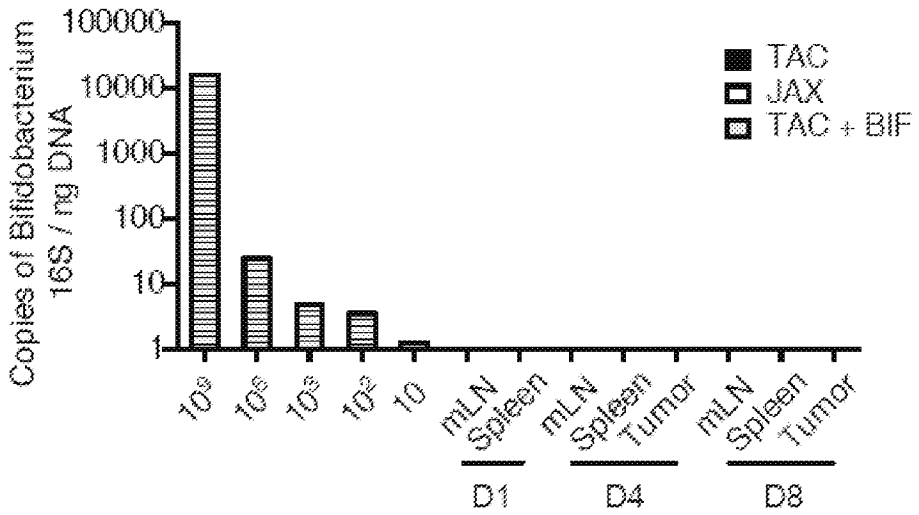


FIG. 12B




Copies of Bifidobacterium 16S / ng DNA
100000
10000
1000
100
10
1
TAC
JAX
TAC + BIF
10^9
10^6
10^3
10^2
10
mLN
Spleen
mLN
Spleen
Tumor
mLN
Spleen
Tumor
D1
D4
D8

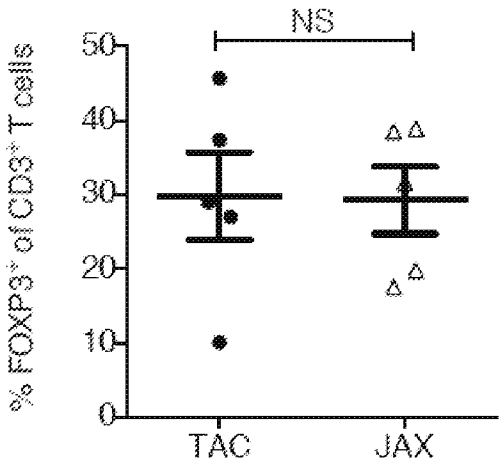

% FOXP3+ of CD3+ T cells
NS
50
40
30
20
10
0
TAC
JAX

FIG. 12C

FIG. 13A

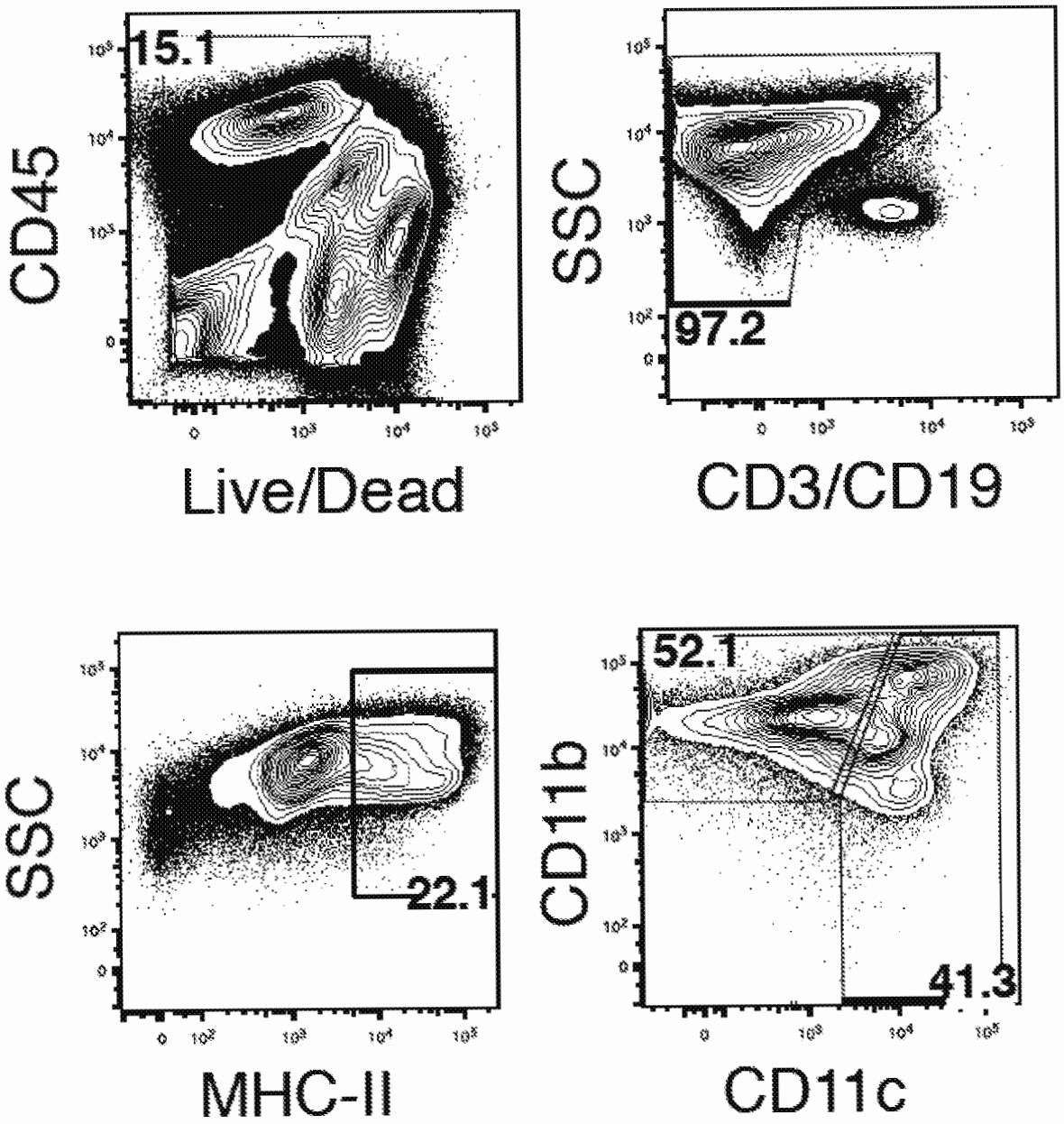


FIG. 13B

fat cell differentiation
blood vessel development
protein modification by small protein conjugation
regulation of apoptotic process
regulation of transcription, DNA-templated
Ras protein signal transduction
cell activation
intracellular signal transduction
T cell mediated immunity
lymphocyte activation
small GTPase mediated signal transduction
cellular macromolecule catabolism
macromolecule catabolism
cofactor biosynthesis
cofactor metabolism
vesicle-mediated transport
negative regulation of cellular component organization
establishment of vesicle localization
intracellular transport
positive regulation of protein transport
cell cycle process
cell cycle
regulation of cell proliferation
meiotic cell cycle
mitotic cell cycle
nuclear division
organelle fission

FIG. 13C

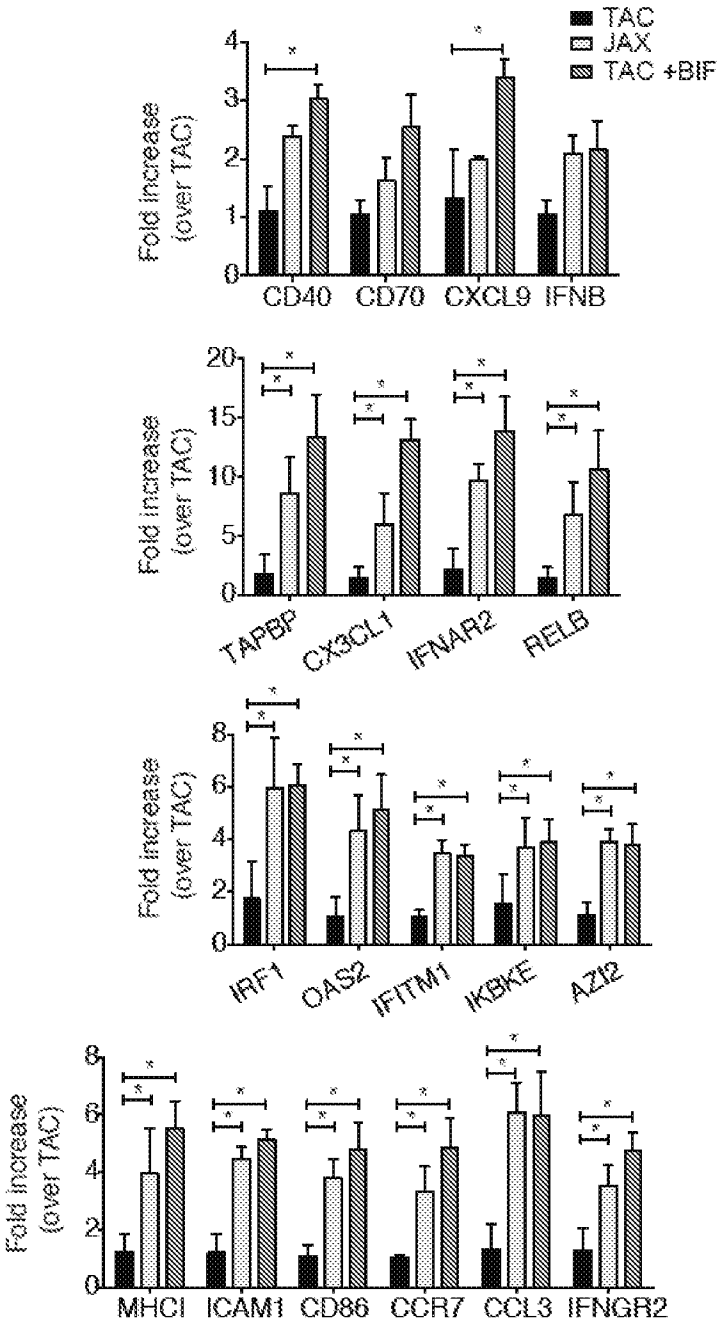

TAC
JAX
TAC +BIF
Fold increase (over TAC)
CD40
CD70
CXCL9
IFNB
TAPBP
CX3CL1
IFNAR2
RELB
IRF1
OAS2
IFITM1
IKBKE
AZI2
MHCI
ICAM1
CD86
CCR7
CCL3
IFNGR2

FIG. 14A

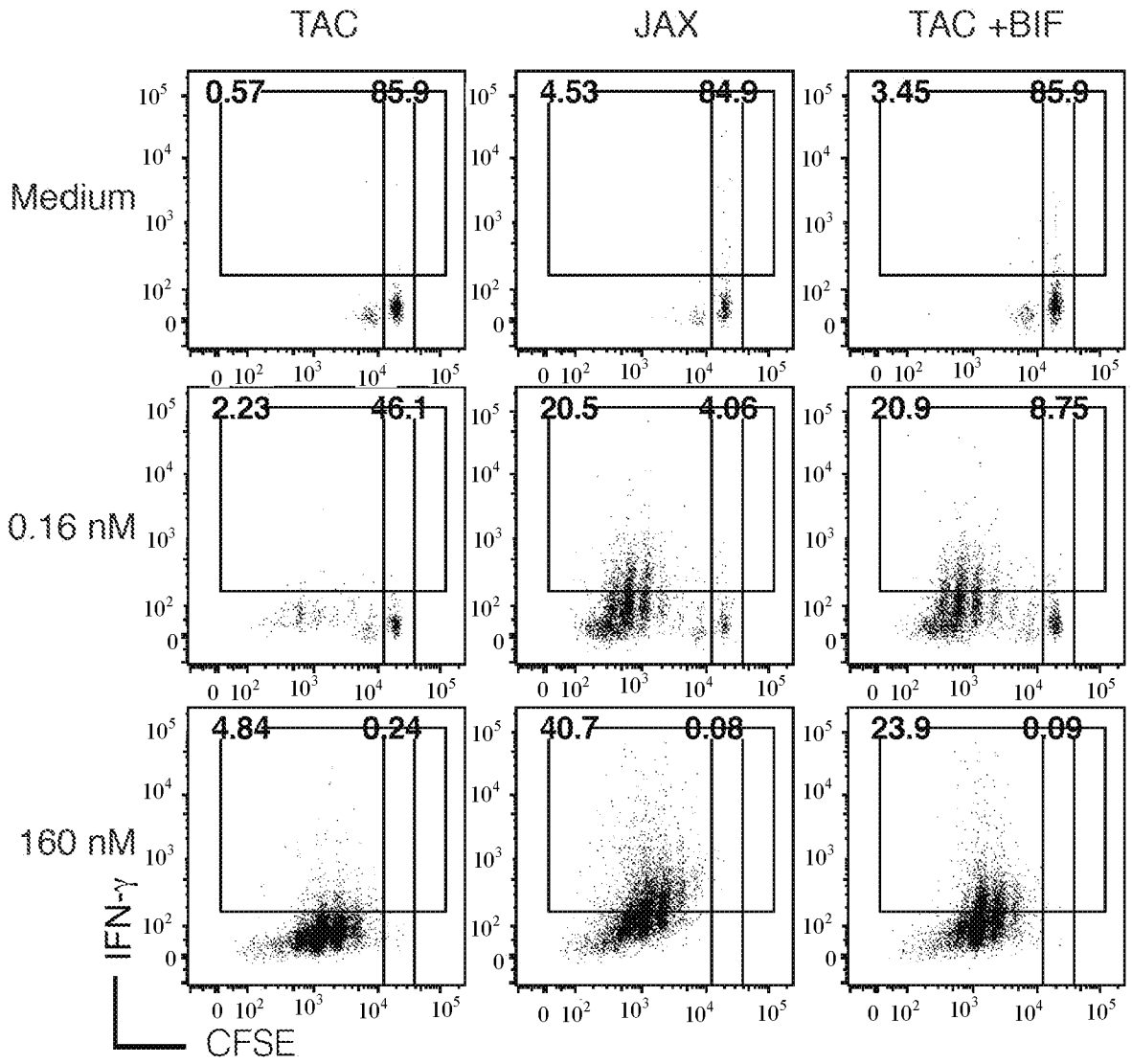

TAC
JAX
TAC +BIF
Medium
0.16 nM
160 nM
IFN-γ
CFSE
0.57
85.9
4.53
84.9
3.45
85.9
2.23
46.1
20.5
4.06
20.9
8.75
4.84
0.24
40.7
0.08
23.9
0.09

FIG. 14B

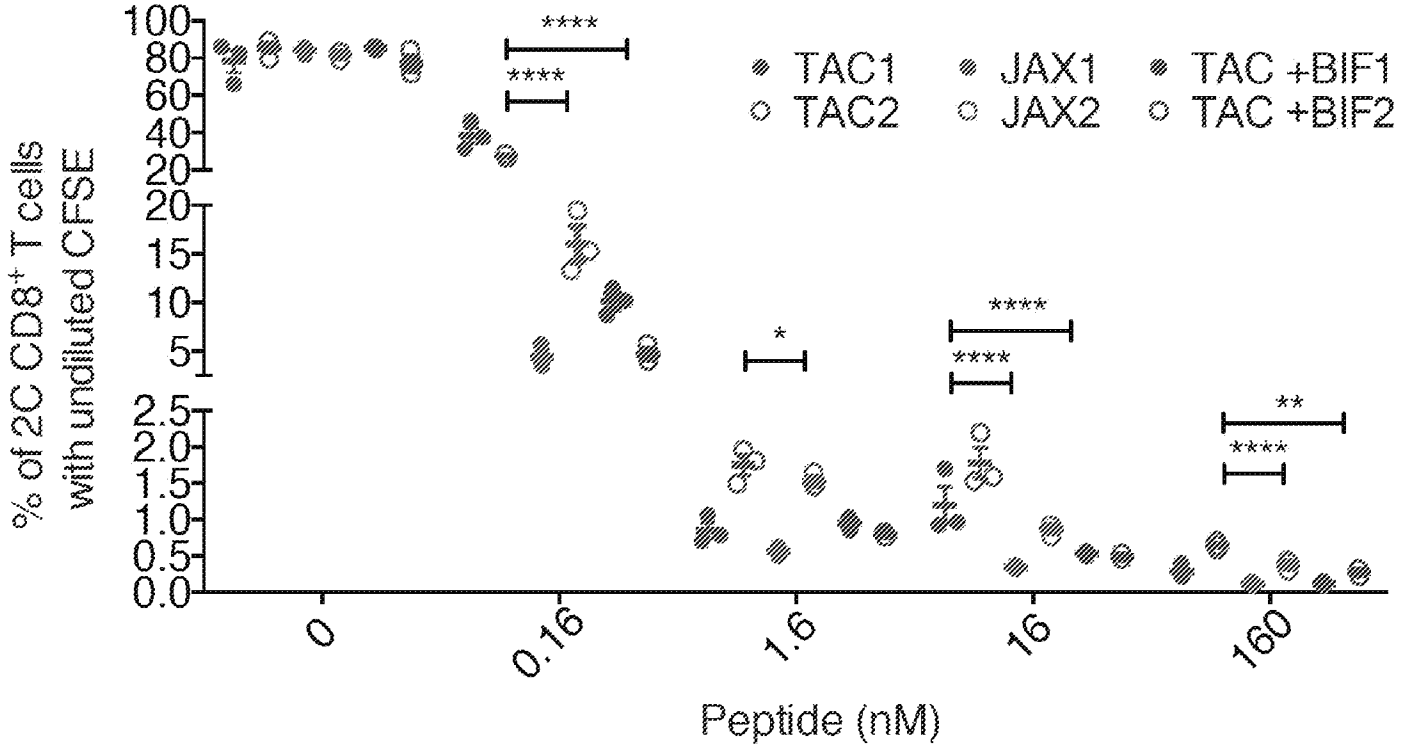
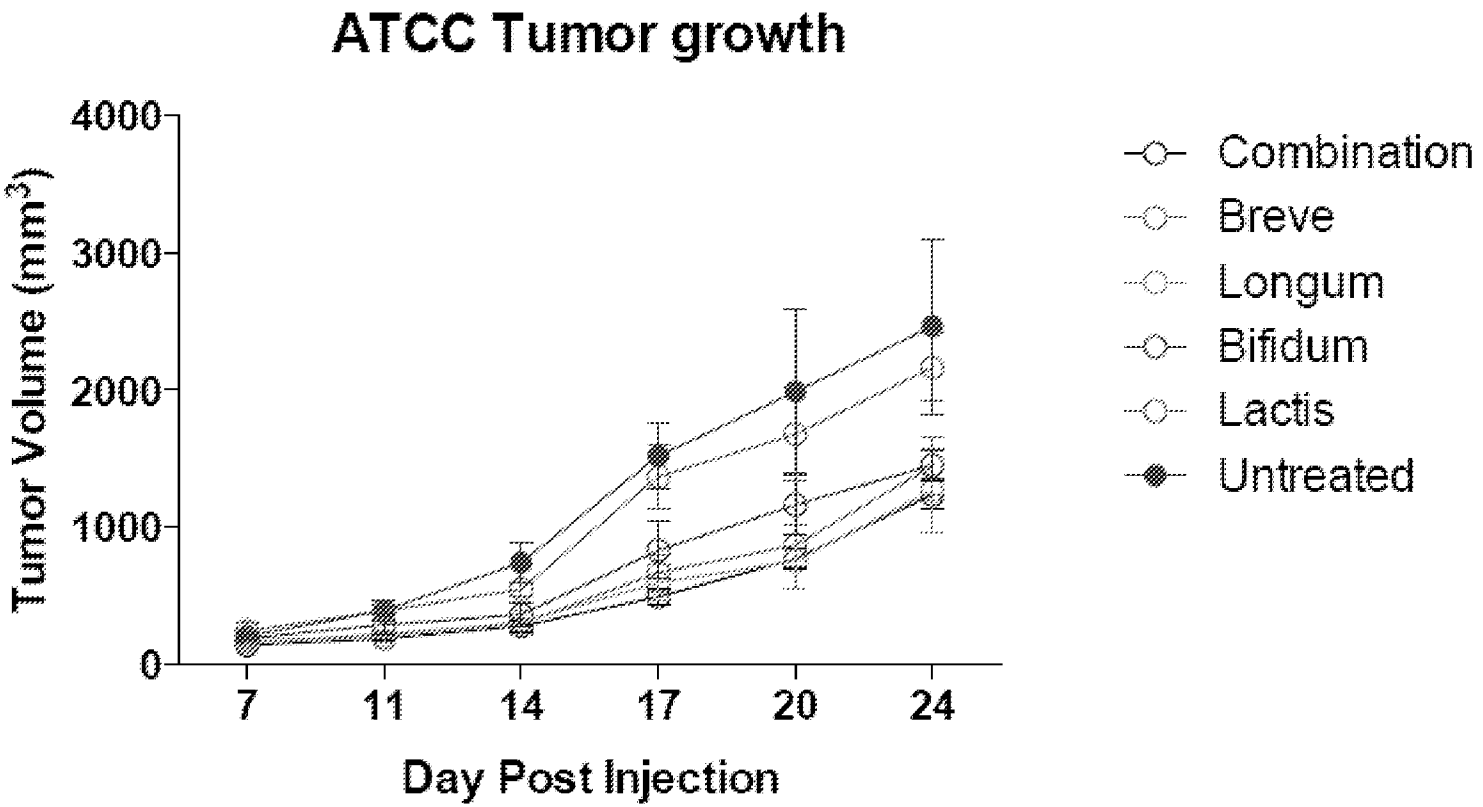

% of 2C CD8+ T cells with undiluted CFSE
100
80
60
40
20
20
15
10
5
2.5
2.0
1.5
1.0
0.5
0.0
****
****
*
****
****
**
****
TAC1
TAC2
JAX1
JAX2
TAC +BIF1
TAC +BIF2
0
0.16
1.6
16
160
Peptide (nM)

FIG. 15


ATCC Tumor growth
Tumor Volume (mm3)
4000
3000
2000
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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant:	University of Chicago	Confirmation No.:	TBD
Serial No.:	TBD	Art Unit:	TBD
Filed:	Herewith	Examiner:	TBD
Title:	**TREATMENT OF CANCER BY MANIPULATION OF COMMENSAL MICROFLORA**		

INFORMATION DISCLOSURE STATEMENT TRANSMITTAL LETTER

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The citations listed in the attached IDS Form SB08A may be material to the examination of the above-identified application, and are therefore submitted in compliance with the duty of disclosure defined in 37 C.F.R. §§ 1.56 and 1.97.

Applicants wish to bring to the Examiner's attention that we are not providing copies of US Patents or published US patent applications as instructed under 37 CFR 1.98(a)(2). Copies of any cited foreign publications and non-patent literature are not provided herewith as they are available in the Image File Wrapper of parent U.S. Patent Application 15/170,284, filed June 1, 2016. The Examiner is requested to make these citations of official record in this application.

This Information Disclosure Statement under 37 C.F.R. §§ 1.56 and 1.97 is not to be construed as a representation that a search has been made, that additional information material to the examination of this application does not exist, or that any one or more of these citations constitutes prior art.

Dated: September 28, 2017

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(608) 662-1277

INFORMATION DISCLOSURE STATEMENT BY APPLICANT (Use as many sheets as necessary)				Complete if Known	
				Application Number	
				Filing Date	
				First Named Inventor	Gajewski
				Art Unit	
				Examiner Name	
Sheet	1	of	6	Attorney Docket Number	UCHI-34458/US-4/CON

U.S. PATENTS					
Examiner Initials*	Cite No.[1]	Document Number Number-Kind Code[2] *(if known)*	Issue or Publication Date YYYY-MM-DD	Name of Patentee or Applicant of Cited Document	Pages, Columns, Lines, Where Relevant Passages or Relevant Figures Appear
		4,816,567	1989-03-28	CABILLY et al.	
		4,946,778	1990-08-07	LADNER et al.	
		5,260,203	1993-11-09	LADNER et al.	
		7,195,906	2007-03-27	COLLINS et al.	
		8,449,878	2013-05-28	YONAK et al.	

U.S. PUBLISHED PATENT APPLICATIONS					
Examiner Initials*	Cite No.[3]	Document Number Number-Kind Code[4] *(if known)*	Issue or Publication Date YYYY-MM-DD	Name of Patentee or Applicant of Cited Document	Pages, Columns, Lines, Where Relevant Passages or Relevant Figures Appear
		20120276143	2012-11-01	O'MAHONY et al.	
		20160354416	2016-12-08	GAJEWSKI et al.	

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FOREIGN PATENT DOCUMENTS						
Examiner Initials*	Cite No.[1]	Foreign Patent Document Country Code[5] Number[6] Kind Code[7] *(if known)*	Publication Date YYYY-MM-DD	Name of Patentee or Applicant of Cited Document	Pages, Columns, Lines, Where Relevant Passages or Relevant Figures Appear	Translation[8]
		EP 2876167	2015-05-27	INSTITUT GUSTAVE ROUSSY		
		WO 1988/01649	1988-03-10	GENEX CORPORATION		
		WO 2011/068810	2011-06-09	SHIRE HUMAN GENETIC THERAPIES		
		WO 2014/145958	2014-09-18	SERES HEALTH, CIN.		
		WO 2015/061372	2015-04-30	HEMOSHEAR, LLC		

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				Examiner Name	
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NONPATENT LITERATURE DOCUMENTS			
Examiner Initials*	Cite No.[1]	Include name of the author (in CAPITAL LETTERS), title of the article (when appropriate), title of the item (book, magazine, journal, serial, symposium, catalog, etc.), date, page(s), volume-issue number(s), publisher, city and-or country where published.	Translation[6]
		ABT et al. Commensal bacteria calibrate the activation threshold of innate antiviral immunity. Immunity. 2012 Jul 27;37(1):158-70	
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		HARLOW, et al. Antibodies: A Laboratory Manual Ch. 6, (Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.) 1988	

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Sheet	3	of	6	Attorney Docket Number	UCHI-34458/US-4/CON

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				Art Unit	
				Examiner Name	
Sheet	4	of	6	Attorney Docket Number	UCHI-34458/US-4/CON

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Examiner Signature		Date Considered	

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				Art Unit	
				Examiner Name	
Sheet	5	of	6	*Attorney Docket Number*	UCHI-34458/US-4/CON

NONPATENT LITERATURE DOCUMENTS			
		International Search Report and Written Opinion for PCT/US2016/035228, mailed August 30, 2016, 15 pages	

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				Examiner Name	
Sheet	6	of	6	*Attorney Docket Number*	UCHI-34458/US-4/CON

CERTIFICATION STATEMENT

Please see 37 CFR 1.97 and 1.98 to make the appropriate selection(s):

☐ That each item of information contained in the information disclosure statement was first cited in any communication from a foreign patent office in a counterpart foreign application not more than three months prior to the filing of the information disclosure statement. See 37 CFR 1.97(e)(1).

OR

☐ That no item of information contained in the information disclosure statement was cited in a communication from a foreign patent office in a counterpart foreign application, and, to the knowledge of the person signing the certification after making reasonable inquiry, no item of information contained in the information disclosure statement was known to any individual designated in 37 CFR 1.56(c) more than three months prior to the filing of the information disclosure statement. See 37 CFR 1.97(e)(2).

☐ See attached certification statement.

☐ The fee set forth in 37 CFR 1.17(p) has been submitted herewith.

☒ A certification statement is not submitted herewith.

SIGNATURE

A signature of the applicant or representative is required in accordance with CFR 1.33, 10.18. Please see CFR 1.4(d) for the form of the signature.

Signature	/David W. Staple/	Date (YYYY-MM-DD)	2017-09-28
Name/Print	David W. Staple	Registration Number	65903

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Application Number	Filing Date
15/170,284	01-Jun-2016

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I am the Applicant (if the Applicant is a juristic entity, list the Applicant name in the box):

THE UNIVERSITY OF CHICAGO

☐ Inventor or Joint Inventor (title not required below)

☐ Legal Representative of a Deceased or Legally Incapacitated Inventor (title not required below)

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SIGNATURE of Applicant for Patent

The undersigned (whose title is supplied below) is authorized to act on behalf of the applicant (e.g., where the applicant is a juristic entity).

Signature		Date (Optional)	
Name	ALAN THOMAS		
Title	ASSO. VP AND DIRECTOR, UCHICAGO TECH		

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TREATMENT OF CANCER BY MANIPULATION OF COMMENSAL MICROFLORA

CROSS-REFERENCE TO RELATED APPLICATIONS

The present application is a continuation of U.S. Patent Application 15/170,284, filed June 1, 2016, which claims the priority benefit of U.S. Provisional Patent Application 62/169,112, filed June 1, 2015, and U.S. Provisional Patent Application 62/248,741, filed October 30, 2015, each of which is incorporated by reference in its entirety.

FIELD

Provided herein are methods of treatment and/or prevention of cancer by manipulation of commensal microflora. In particular, the amount, identity, presence, and/or ratio of microflora (e.g., gut microflora) in a subject is manipulated to facilitate one or more co-treatments.

BACKGROUND

Harnessing the host immune system constitutes a promising approach for the treatment of cancer because of its potential to specifically target tumor cells while limiting harm to normal tissue, with durability of benefit associated with immunologic memory. Enthusiasm has been fueled by recent clinical success, particularly with antibodies that block immune inhibitory pathways, specifically CTLA-4 and the PD-1/PD-L1 axis (Hodi et al. The New England journal of medicine 363, 711-723 (2010).; Hamid et al. The New England journal of medicine 369, 134-144 (2013).; herein incorporated by reference in their entireties). Early data have indicated that clinical responses to these immunotherapies are more frequent in patients who show evidence of an endogenous T cell response ongoing in the tumor microenvironment at baseline (Tumeh et al. Nature 515, 568-571 (2014).; Spranger et al. Science translational medicine 5, 200ra116 (2013).; Ji et al. Cancer immunology, immunotherapy : CII 61, 1019-1031 (2012).; Gajewski et al. Cancer journal 16, 399-403 (2010).; herein incorporated by reference in their entireties). Despite the functional and clinical importance of this T cell-inflamed tumor microenvironment, the mechanisms that govern the presence or absence of this phenotype have not been well understood. Theoretical sources of inter-patient heterogeneity include germline genetic

differences at the level of the host, variability in patterns of somatic alterations in tumor cells, and environmental differences with the potential to impact on systemic immunity.

SUMMARY

Provided herein are methods of treatment and/or prevention of cancer by manipulation of commensal microflora. In particular, the amount, identity, presence, and/or ratio of microflora (e.g., gut microflora) in a subject is manipulated to facilitate one or more co-treatments.

In some embodiments, provided herein are methods of treating or preventing cancer in a subject, comprising modulating levels of one or more commensal microbes within the subject to: (A) enhance an immune response by the subject, (B) inhibit the growth or spread of the cancer, (C) inhibit immune evasion by the cancer, and/or (D) enhance the efficacy of a therapeutic. In some embodiments, the levels of one or more commensal microbes are modulated within the gut of the subject. In some embodiments, modulating the levels of one or more commensal microbes comprises increasing and/or decreasing levels of one or more bacterial selected from the genera *Adlercreutzia, Oscillopira, Mollicutes, Butyrivibrio, Bacteroides, Clostridium, Fusobacterium, Eubacterium, Ruminococcus, Peptococcus, Peptostreptococcus, Bifidobacterium, Rikenella, Alistipes, Marinilabilia, Anaerostipes, Escherichia*, and/or *Lactobacillus.*

In some embodiments, modulating the levels of one or more commensal microbes comprises administering a beneficial microbes to the subject. In some embodiments, the beneficial microbes are bacteria. In some embodiments, the bacteria are selected from the genera *Adlercreutzia, Oscillopira, Mollicutes, Butyrivibrio, Bacteroides, Clostridium, Fusobacterium, Eubacterium, Ruminococcus, Peptococcus, Peptostreptococcus, Bifidobacterium, Rikenella, Alistipes, Marinilabilia, Anaerostipes, Escherichia*, and/or *Lactobacillus.* In some embodiments, the bacteria are *Bifidobacterium*. In some embodiments, the *Bifidobacterium* include bacteria selected from the group consisting of *Bifidobacterium lactis, Bifidobacterium bifidium, Bifidobacterium longum, Bifidobacterium animalis, Bifidobacterium breve, Bifidobacterium infantis, Bifidobacterium catenulatum, Bifidobacterium pseudocatenulatum, Bifidobacterium adolescentis,* and *Bifidobacterium angulatum.* In some embodiments, the beneficial microbes are administered as a probiotic composition or via microflora transplant from a donor.

In some embodiments, modulating the levels of one or more commensal microbes comprises administering one or more antimicrobials. In some embodiments, the antimicrobial

kills detrimental microbes. In some embodiments, the antimicrobial is an antibiotic. In some embodiments, methods further comprise administration of beneficial microbes to the subject.

In some embodiments, methods further comprise administering to the subject a cancer therapy. In some embodiments, wherein the modulating levels of one or more commensal microbes within the subject enhances an immune response by the subject and/or inhibits immune evasion by the cancer, and the cancer therapy is an immunotherapy. In some embodiments, the immunotherapy comprises administration of anti-CTLA-4 antibodies and/or anti-PD-L1 or anti-PD-1 antibodies. In some embodiments, wherein the modulating levels of one or more commensal microbes within the subject enhance the efficacy of a therapeutic, and the cancer therapy is said therapeutic. In some embodiments, the therapeutic comprises a chemotherapeutic. In some embodiments, methods further comprise testing the subject for immune evasion by the cancer. In some embodiments, methods further comprise surgical, radiation, and/or chemotherapeutic cancer intervention.

In some embodiments, provided herein are kits or compositions comprising a beneficial commensal microbe and a cancer therapeutic, said compositions or components of said kits formulated for therapeutic delivery to a subject.

In some embodiments, provided herein are beneficial commensal microbes for use as a medicament in the treatment of cancer and/or inhibition of immune evasion.

In some embodiments, provided herein are methods of treating or preventing cancer in a subject comprising administering to the subject bacterial formulation comprising bacteria of the genera *Bifidobacterium*, *Rikenella, Alistipes, Marinilabilia*, or *Anaerostipes*. In some embodiments, at least 50% of the bacteria in the bacterial formulation are of the genera Bifidobacterium, *Rikenella, Alistipes, Marinilabilia*, or *Anaerostipes*. In some embodiments, at least 90% of the bacteria in the bacterial formulation are of the genera Bifidobacterium, *Rikenella, Alistipes, Marinilabilia*, or *Anaerostipes*. In some embodiments, the bacterial formulation comprise bacteria of the genus Bifidobacterium. In some embodiments, at least 50% of the bacteria in the bacterial formulation are of the genus Bifidobacterium. In some embodiments, at least 90% of the bacteria in the bacterial formulation are of the genus Bifidobacterium.

In some embodiments, the bacteria of genus Bifidobacterium are selected from the group consisting of *Bifidobacterium lactis, Bifidobacterium bifidium, Bifidobacterium longum,*

Bifidobacterium animalis, Bifidobacterium breve, Bifidobacterium infantis, Bifidobacterium catenulatum, Bifidobacterium pseudocatenulatum, Bifidobacterium adolescentis, Bifidobacterium angulatum, Bifidobacterium asteroides, Bifidobacterium boum, Bifidobacterium choerinum, Bifidobacterium coryneforme, Bifidobacterium cuniculi, Bifidobacterium denticolens, Bifidobacterium dentium, Bifidobacterium gallicum, Bifidobacterium gallinarum, Bifidobacterium indicum, Bifidobacterium inopinatum, Bifidobacterium magnum, Bifidobacterium merycicum, Bifidobacterium minimum, Bifidobacterium pseudolongum, Bifidobacterium pullorum, Bifidobacterium psychraerophilum, Bifidobacterium ruminantium, Bifidobacterium saeculare, Bifidobacterium scardovii, Bifidobacterium simiae, Bifidobacterium subtile, Bifidobacterium therammcidophilum, Bifidobacterium thermophilum, Bifidobacterium tsurumiense, Bifidobacterium urinalis, Bifidobacterium sp.

In some embodiments, the cancer is cancer is selected from the group consisting of acute nonlymphocytic leukemia, chronic lymphocytic leukemia, acute granulocytic leukemia, chronic granulocytic leukemia, acute promyelocytic leukemia, adult T-cell leukemia, aleukemic leukemia, a leukocythemic leukemia, basophilic leukemia, blast cell leukemia, bovine leukemia, chronic myelocytic leukemia, leukemia cutis, embryonal leukemia, eosinophilic leukemia, Gross' leukemia, Rieder cell leukemia, Schilling's leukemia, stem cell leukemia, subleukemic leukemia, undifferentiated cell leukemia, hairy-cell leukemia, hemoblastic leukemia, hemocytoblastic leukemia, histiocytic leukemia, stem cell leukemia, acute monocytic leukemia, leukopenic leukemia, lymphatic leukemia, lymphoblastic leukemia, lymphocytic leukemia, lymphogenous leukemia, lymphoid leukemia, lymphosarcoma cell leukemia, mast cell leukemia, megakaryocytic leukemia, micromyeloblastic leukemia, monocytic leukemia, myeloblastic leukemia, myelocytic leukemia, myeloid granulocytic leukemia, myelomonocytic leukemia, Naegeli leukemia, plasma cell leukemia, plasmacytic leukemia, promyelocytic leukemia, acinar carcinoma, acinous carcinoma, adenocystic carcinoma, adenoid cystic carcinoma, carcinoma adenomatosum, carcinoma of adrenal cortex, alveolar carcinoma, alveolar cell carcinoma, basal cell carcinoma, carcinoma basocellulare, basaloid carcinoma, basosquamous cell carcinoma, bronchioalveolar carcinoma, bronchiolar carcinoma, bronchogenic carcinoma, cerebriform carcinoma, cholangiocellular carcinoma, chorionic carcinoma, colloid carcinoma, comedo carcinoma, corpus carcinoma, cribriform carcinoma, carcinoma en cuirasse, carcinoma cutaneum, cylindrical carcinoma, cylindrical cell carcinoma, duct carcinoma, carcinoma durum,

embryonal carcinoma, encephaloid carcinoma, epiennoid carcinoma, carcinoma epitheliale adenoides, exophytic carcinoma, carcinoma ex ulcere, carcinoma fibrosum, gelatiniform carcinoma, gelatinous carcinoma, giant cell carcinoma, signet-ring cell carcinoma, carcinoma simplex, small-cell carcinoma, solanoid carcinoma, spheroidal cell carcinoma, spindle cell carcinoma, carcinoma spongiosum, squamous carcinoma, squamous cell carcinoma, string carcinoma, carcinoma telangiectaticum, carcinoma telangiectodes, transitional cell carcinoma, carcinoma tuberosum, tuberous carcinoma, verrucous carcinoma, carcinoma villosum, carcinoma gigantocellulare, glandular carcinoma, granulosa cell carcinoma, hair-matrix carcinoma, hematoid carcinoma, hepatocellular carcinoma, Hurthle cell carcinoma, hyaline carcinoma, hypernephroid carcinoma, infantile embryonal carcinoma, carcinoma in situ, intraepidermal carcinoma, intraepithelial carcinoma, Krompecher's carcinoma, Kulchitzky-cell carcinoma, large-cell carcinoma, lenticular carcinoma, carcinoma lenticulare, lipomatous carcinoma, lymphoepithelial carcinoma, carcinoma medullare, medullary carcinoma, melanotic carcinoma, carcinoma molle, mucinous carcinoma, carcinoma muciparum, carcinoma mucocellulare, mucoepidermoid carcinoma, carcinoma mucosum, mucous carcinoma, carcinoma myxomatodes, naspharyngeal carcinoma, oat cell carcinoma, carcinoma ossificans, osteoid carcinoma, papillary carcinoma, periportal carcinoma, preinvasive carcinoma, prickle cell carcinoma, pultaceous carcinoma, renal cell carcinoma of kidney, reserve cell carcinoma, carcinoma sarcomatodes, schneiderian carcinoma, scirrhous carcinoma, carcinoma scroti, chondrosarcoma, fibrosarcoma, lymphosarcoma, melanosarcoma, myxosarcoma, osteosarcoma, endometrial sarcoma, stromal sarcoma, Ewing' s sarcoma, fascial sarcoma, fibroblastic sarcoma, giant cell sarcoma, Abemethy's sarcoma, adipose sarcoma, liposarcoma, alveolar soft part sarcoma, ameloblastic sarcoma, botryoid sarcoma, chloroma sarcoma, chorio carcinoma, embryonal sarcoma, Wilms' tumor sarcoma, granulocytic sarcoma, Hodgkin's sarcoma, idiopathic multiple pigmented hemorrhagic sarcoma, immunoblastic sarcoma of B cells, lymphoma, immunoblastic sarcoma of T-cells, Jensen's sarcoma, Kaposi's sarcoma, Kupffer cell sarcoma, angiosarcoma, leukosarcoma, malignant mesenchymoma sarcoma, parosteal sarcoma, reticulocytic sarcoma, Rous sarcoma, serocystic sarcoma, synovial sarcoma, telangiectaltic sarcoma, Hodgkin's Disease, Non-Hodgkin's Lymphoma, multiple myeloma, neuroblastoma, breast cancer, ovarian cancer, lung cancer, rhabdomyosarcoma, primary thrombocytosis, primary macroglobulinemia, small-cell lung tumors, primary brain tumors, stomach cancer, colon cancer, malignant pancreatic

insulanoma, malignant carcinoid, premalignant skin lesions, testicular cancer, lymphomas, thyroid cancer, neuroblastoma, esophageal cancer, genitourinary tract cancer, malignant hypercalcemia, cervical cancer, endometrial cancer, adrenal cortical cancer, Harding-Passey melanoma, juvenile melanoma, lentigo maligna melanoma, malignant melanoma, acral-lentiginous melanoma, amelanotic melanoma, benign juvenile melanoma, Cloudman's melanoma, S91 melanoma, nodular melanoma subungal melanoma, and superficial spreading melanoma.

In some embodiments, the subject is human. In some embodiments, the bacterial formulation is administered by oral administration, rectal administration, topical administration, inhalation or injection. In some embodiments, the bacterial formulation is a food product. In some embodiments, the bacterial formulation comprises at least about $5x10^6$ CFU of bacteria. In some embodiments, the bacterial formulation is administered to the subject in two or more doses. In some embodiments, the administration of at least two of the two or more doses are separated by at least 1 day. In some embodiments, the administration of at least two of the two or more doses are separated by at least 1 week.

In some embodiments, methods further comprise administering to the subject an antibiotic. In some embodiments, the antibiotic is administered to the subject before the bacterial formulation. In some embodiments, the antibiotic is administered to the subject at least 1 day before the bacterial formulation is administered to the subject.

In some embodiments, methods further comprise administering to the subject an immune checkpoint inhibitor. In some embodiments, the immune checkpoint inhibitor is a protein or polypeptide that specifically binds to an immune checkpoint protein. In some embodiments, the immune checkpoint protein is selected from the group consisting of CTLA4, PD-1, PD-L1, PD-L2, A2AR, B7-H3, B7-H4, BTLA, KIR, LAG3, TIM-3 or VISTA. In some embodiments, the polypeptide or protein is an antibody or antigen-binding fragment thereof. In some embodiments, the immune checkpoint inhibitor is an interfering nucleic acid molecule. In some embodiments, the interfering nucleic acid molecule is an siRNA molecule, an shRNA molecule or an antisense RNA molecule. In some embodiments, the immune checkpoint inhibitor is selected from the group consisting of nivolumab, pembrolizumab, pidilizumab, AMP-224, AMP-514, STI-A1110, TSR-042, RG-7446, BMS-936559, BMS-936558, MK-3475, CT Ol l, MPDL3280A, MEDI-4736, MSB-0020718C, AUR-012 and STI-A1010. In some

embodiments, the immune checkpoint inhibitor is administered before the bacterial formulation. In some embodiments, the immune checkpoint inhibitor is administered at least one day before the bacterial formulation. In some embodiments, the immune checkpoint is administered at about the same time as the bacterial formulation. In some embodiments, the immune checkpoint inhibitor is administered on the same day as the bacterial formulation. In some embodiments, the immune checkpoint inhibitor is administered after the bacterial formulation. In some embodiments, the immune checkpoint inhibitor is administered at least one day after the bacterial formulation. In some embodiments, the immune checkpoint inhibitor is administered by injection. In some embodiments, the injection is an intravenous, intramuscular, intratumoral or subcutaneous injection.

In some embodiments, provided herein are methods of treating cancer in a human subject comprising administering to the subject an immune checkpoint inhibitor and a bacterial formulation comprising bacteria of the genera *Bifidobacterium*. In some embodiments, at least 50% (e.g., 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 99%, or more, or ranges therebetween) of the bacteria in the bacterial formulation are of the genera *Bifidobacterium*. In some embodiments, at least 90% (e.g., 90%, 95%, 99%, 99.9%, 99.99%, or more or ranges therebetween) of the bacteria in the bacterial formulation are of the genera *Bifidobacterium*. In some embodiments, the bacteria of the genus *Bifidobacterium* comprise bacteria of the species *Bifidobacterium lactis, Bifidobacterium bifidium, Bifidobacterium longum, Bifidobacterium animalis, Bifidobacterium breve, Bifidobacterium infantis, Bifidobacterium catenulatum, Bifidobacterium pseudocatenulatum, Bifidobacterium adolescentis, Bifidobacterium angulatum, Bifidobacterium asteroides, Bifidobacterium boum, Bifidobacterium choerinum, Bifidobacterium coryneforme, Bifidobacterium cuniculi, Bifidobacterium denticolens, Bifidobacterium dentium, Bifidobacterium gallicum, Bifidobacterium gallinarum, Bifidobacterium indicum, Bifidobacterium inopinatum, Bifidobacterium magnum, Bifidobacterium merycicum, Bifidobacterium minimum, Bifidobacterium pseudolongum, Bifidobacterium pullorum, Bifidobacterium psychraerophilum, Bifidobacterium ruminantium, Bifidobacterium saeculare, Bifidobacterium scardovii, Bifidobacterium simiae, Bifidobacterium subtile, Bifidobacterium therammcidophilum, Bifidobacterium thermophilum, Bifidobacterium tsurumiense, Bifidobacterium urinalis* or *Bifidobacterium sp.* In some embodiments, the bacterial formulation is administered by oral administration or rectal administration. In some embodiments, the

bacterial formulation is administered by oral administration. In some embodiments, the bacterial formulation comprises at least $5x10^6$ CFU (e.g., $5x10^6$ CFU, $1x10^7$ CFU, $2x10^7$ CFU, $5x10^7$ CFU, $1x10^8$ CFU, $2x10^8$ CFU, $5x10^8$ CFU, $1x10^9$ CFU, $2x10^9$ CFU, $5x10^9$ CFU, $1x10^{10}$ CFU, $2x10^{10}$ CFU, $5x10^{10}$ CFU, $1x10^{11}$ CFU, $2x10^{11}$ CFU, $5x10^{11}$ CFU, $1x10^{12}$ CFU, $2x10^{12}$ CFU, $5x10^{12}$ CFU, or more or ranges therebetween) of bacteria of the genera *Bifidobacterium*. In some embodiments, the bacterial formulation is administered to the subject in two or more doses (e.g., 2, 3, 4, 5, 6, 7, 8, 9, 10, or more, or ranges therebetween). In some embodiments, the administration of doses are separated by at least 1 week. In some embodiments, methods further comprise administering to the subject an antibiotic prior to the administration of the bacterial formulation. In some embodiments, the antibiotic is administered to the subject at least 1 day before the bacterial formulation is administered to the subject. In some embodiments, the immune checkpoint inhibitor is a protein or polypeptide that binds to an immune checkpoint protein. In some embodiments, the immune checkpoint inhibitor is an antibody or antigen binding fragment thereof that binds to an immune checkpoint protein. In some embodiments, the immune checkpoint protein is CTLA4, PD-1, PD-L1, PD-L2, A2AR, B7-H3, B7-H4, BTLA, KIR, LAG3, TIM-3 or VISTA. In some embodiments, the immune checkpoint protein is PD-1 or PD-L1. In some embodiments, the immune checkpoint inhibitor is nivolumab, pembrolizumab, pidilizumab, AMP-224, AMP-514, STI-A1110, TSR-042, RG-7446, BMS-936559, BMS-936558, MK-3475, CT Ol l, MPDL3280A, MEDI-4736, MSB-0020718C, AUR-012 and STI-A1010. In some embodiments, the immune checkpoint inhibitor is administered by intravenous injection, intramuscular injection, intratumoral injection or subcutaneous injection.

In some embodiments, provided herein are methods of treating cancer in a human subject comprising administering to the subject a bacterial formulation comprising at least $5x10^6$ CFU (e.g., $5x10^6$ CFU, $1x10^7$ CFU, $2x10^7$ CFU, $5x10^7$ CFU, $1x10^8$ CFU, $2x10^8$ CFU, $5x10^8$ CFU, $1x10^9$ CFU, $2x10^9$ CFU, $5x10^9$ CFU, $1x10^{10}$ CFU, $2x10^{10}$ CFU, $5x10^{10}$ CFU, $1x10^{11}$ CFU, $2x10^{11}$ CFU, $5x10^{11}$ CFU, $1x10^{12}$ CFU, $2x10^{12}$ CFU, $5x10^{12}$ CFU, or more or ranges therebetween) of bacteria of the genera *Bifidobacterium,* wherein at least 50% (e.g., 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 99%, or more, or ranges therebetween) of the bacteria in the bacterial formulation are of the genera *Bifidobacterium*. In some embodiments, at least 90% (e.g., 90%, 95%, 99%, 99.9%, 99.99%, or more or ranges therebetween) of the bacteria in the bacterial formulation are of the genera *Bifidobacterium*. In some embodiments,

the bacteria of the genus *Bifidobacterium* comprise bacteria of the species *Bifidobacterium lactis, Bifidobacterium bifidium, Bifidobacterium longum, Bifidobacterium animalis, Bifidobacterium breve, Bifidobacterium infantis, Bifidobacterium catenulatum, Bifidobacterium pseudocatenulatum, Bifidobacterium adolescentis, Bifidobacterium angulatum, Bifidobacterium asteroides, Bifidobacterium boum, Bifidobacterium choerinum, Bifidobacterium coryneforme, Bifidobacterium cuniculi, Bifidobacterium denticolens, Bifidobacterium dentium, Bifidobacterium gallicum, Bifidobacterium gallinarum, Bifidobacterium indicum, Bifidobacterium inopinatum, Bifidobacterium magnum, Bifidobacterium merycicum, Bifidobacterium minimum, Bifidobacterium pseudolongum, Bifidobacterium pullorum, Bifidobacterium psychraerophilum, Bifidobacterium ruminantium, Bifidobacterium saeculare, Bifidobacterium scardovii, Bifidobacterium simiae, Bifidobacterium subtile, Bifidobacterium therammcidophilum, Bifidobacterium thermophilum, Bifidobacterium tsurumiense, Bifidobacterium urinalis* or *Bifidobacterium sp.* In some embodiments, the bacterial formulation is administered by oral administration or rectal administration. In some embodiments, the bacterial formulation is administered by oral administration. In some embodiments, the bacterial formulation is administered to the subject in two or more doses (e.g., 2, 3, 4, 5, 6, 7, 8, 9, 10, or more, or ranges therebetween). In some embodiments, methods further comprise administering to the subject an antibiotic before the bacterial formulation is administered to the subject. In some embodiments, methods further comprise administering to the subject an immune checkpoint inhibitor. In some embodiments, the immune checkpoint inhibitor is an antibody or antigen binding fragment thereof that binds to CTLA4, PD-1, PD-L1, PD-L2, A2AR, B7-H3, B7-H4, BTLA, KIR, LAG3, TIM-3 or VISTA. In some embodiments, the immune checkpoint inhibitor is an antibody or antigen binding fragment thereof that binds to PD-1 or PD-L1. In some embodiments, the immune checkpoint inhibitor is nivolumab, pembrolizumab, pidilizumab, AMP-224, AMP-514, STI-A1110, TSR-042, RG-7446, BMS-936559, BMS-936558, MK-3475, CT Ol l, MPDL3280A, MEDI-4736, MSB-0020718C, AUR-012 and STI-A1010.

BRIEF DESCRIPTION OF THE DRAWINGS

Fig. 1A-H. Differences in melanoma outgrowth and tumor-specific immune responses between C57BL/6 JAX and TAC mice are eliminated upon cohousing. (A) B16.SIY tumor

growth kinetics in newly arrived JAX and TAC mice. (B) IFN-γ ELISPOT in tumor-bearing JAX and TAC mice 7 days following tumor inoculation. (C) Mean size of IFN-γ spots (10^{-3} mm^2). (D) Percentage of SIY^+ T cells of total $CD8^+$ T cells within the tumor of JAX and TAC mice as determined by flow cytometry 21 days post-tumor inoculation. Representative plots (left), quantification (right). (E) B16.SIY tumor growth kinetics in JAX and TAC mice cohoused for 3 weeks prior to tumor inoculation. (F) Number of IFN-γ spots/10^6 splenocytes in tumor-bearing JAX and TAC mice cohoused for 3 weeks prior to tumor inoculation. (G) Mean size of IFN-γ spots (10^{-3} mm^2). (H) Percentage of SIY^+ T cells of total $CD8^+$ T cells within the tumor of JAX and TAC mice cohoused for 3 weeks prior to tumor inoculation.

Fig. 2A-G. Oral administration of JAX fecal material to TAC mice enhances spontaneous anti-tumor immunity and response to αPD-L1 mAb therapy. (A) B16.SIY tumor growth in newly arrived TAC mice, TAC and JAX mice orally gavaged with PBS, TAC or JAX fecal material prior to tumor implantation. (B) Number of IFN-γ spots x mean spot size (10^{-3} mm^2), determined by ELISPOT 7 days following tumor inoculation. (C) Percentage of SIY^+ $CD8^+$ T cells within the tumor of TAC and JAX mice treated as in (A), 21 days post-tumor inoculation. Representative plots (left), quantification (right). (D) B16.SIY tumor growth in TAC mice, untreated or treated with JAX fecal material 7 and 14 days post tumor implantation, αPD-L1 mAb 7, 10, 13 and 16 days post tumor implantation, or both regimens. (E) IFN-γ ELISPOT assessed 5 days after start of treatment. (F) Percentage of tumor-infiltrating SIY^+ $CD8^+$ T cells, determined by flow cytometry 14 days after start of treatment. (G) B16.SIY tumor growth kinetics in TAC and JAX mice, untreated or treated with αPD-L1 mAb 7, 10, 13 and 16 days post tumor implantation.

Fig. 3A-G. Direct administration of *Bifidobacterium* to TAC recipients with established tumors improves tumor-specific immunity and response to αPD-L1 mAb therapy. (A-C) Bacterial species diversity (A), principal coordinate analysis plot of bacterial β-diversity (B) and operational taxonomic unit (OTU) levels of top *Bifidobacterium* taxon (C) in fecal material obtained from JAX, TAC, TAC-fed TAC and JAX-fed TAC mice. Comparisons in A-C were performed using 9-10 replicates from each vendor and 4-5 replicates from each treatment. (D) B16.SIY tumor growth kinetics in TAC mice, untreated or treated with *Bifidobacterium* 7 and 14 days post tumor implantation (white arrows), αPD-L1 mAb 7, 10, 13 and 16 days post tumor

implantation (black arrows) or both regimens. (E) IFN-γ ELISPOT assessed 5 days after start of treatment. (F) Percentage of tumor-infiltrating SIY^+ $CD8^+$ T cells, determined by flow cytometry 14 days following start of treatment. Representative plots (left), quantification of data combined from 2 independent experiments (right). (G) B16.SIY tumor growth for isotype-treated (left) or CD8-depleted (right) groups as in D.

Fig. 4A-E. Dendritic cells isolated from JAX and *Bifidobacterium*-fed TAC mice show increased expression of genes associated with antitumor immunity and heightened capability for T cell activation (A) Quantification of IFN-γ MFI (mean fluorescence intensity) of 2C $CD8^+$ T cells in the tumor-draining lymph node (left) and spleen (right) of TAC, JAX, *Bifidobacterium*-fed TAC mice on day 7 post adoptive transfer. (B) Percentage of MHC Class IIhi DCs in tumors isolated from TAC, JAX, and *Bifidobacterium*-fed TAC mice 40 hours post tumor implantation as assessed by flow cytometry. (C) Enriched biological pathways and functions found within the subset of elevated genes in JAX and *Bifidobacterium*-treated TAC-derived DCs relative to untreated TAC DCs isolated from tumors 40hrs post tumor inoculation, as assessed by DAVID pathway analysis. Bars indicate the percent of genes in a pathway upregulated in DCs isolated from JAX and *Bifidobacterium*-fed TAC mice. Line indicates p-values calculated by Fisher's exact test. (D) Heat map of key antitumor immunity genes in DCs isolated from JAX, *Bifidobacterium*-treated TAC or untreated TAC mice. Mean fold-change for each gene transcript is shown on the right. (E) Quantification of IFN-γ^+ 2C TCR Tg $CD8^+$ T cells stimulated in vitro with DCs purified from peripheral lymphoid tissues of naïve TAC, JAX, and *Bifidobacterium*-treated TAC mice in the presence of different concentrations of SIY peptide.

Fig. 5A-D. (A) Schematic of prophylactic fecal transfer: fecal pellets collected from JAX and TAC mice upon arrival in our facility were resuspended in PBS, homogenized and the supernatant was introduced by oral gavage into either JAX or TAC recipients as shown, once a week for two weeks prior to B16.SIY tumor inoculation. (B) B16.SIY tumor growth in JAX mice orally gavaged with TAC or JAX fecal material once weekly for two weeks prior to tumor implantation. (C) Percentage of SIY^+ T cells of total $CD8^+$ T cells within the tumor of groups as in Figure 2A, determined by flow cytometry 7 days post-tumor inoculation. (D) Percentage of SIY^+ T cells of total $CD8^+$ T cells within the tumor of JAX and TAC mice, untreated or treated with αPD-L1 mAb, as determined by flow cytometry 21 days post-tumor inoculation.

Fig. 6A-H. (A) Relative abundance of all taxa combined belonging to the *Bifidobacterium* genus in fecal material obtained from TAC, JAX, TAC-fed TAC and JAX-fed TAC mice. Comparisons were performed using 9-10 replicates from each vendor and 4-5 replicates from each treatment. (B) Number of colony forming units (CFU) of live and heat inactivated bifidobacteria, plated in RCM agar following serial dilution in reduced PBS and incubated in an anaerobic chamber for 72 hours. (C) B16.SIY tumor growth kinetics in TAC mice, untreated or treated with live *Bifidobacterium*, heat inactivated *Bifidobacterium* or JAX fecal material 7 and 14 days post tumor implantation. (D) Percentage of tumor-infiltrating SIY^+ T cells of total $CD8^+$ T cells for treatment groups as in C, determined by flow cytometry 14 days after start of treatment. C-D show data combined from 2-4 independent experiments, 5 mice per group. (E) B16.F10 tumor growth kinetics in TAC mice, untreated or treated with *Bifidobacterium* 7 and 14 days post tumor implantation. (F) MB49 tumor growth kinetics in TAC mice, untreated or treated with *Bifidobacterium* 7 and 14 days post tumor implantation. (G) B16.SIY tumor growth kinetics in TAC mice, untreated or treated with *Lactobacillus murinus* or JAX fecal material 7 and 14 days post tumor implantation. (H) Percentage of tumor-infiltrating SIY^+ T cells of total $CD8^+$ T cells for treatment groups as in G, determined by flow cytometry 18 days after start of treatment.

Fig. 7A-B. (A) Schematic of *in vivo* 2C proliferation assays. CD8+ T cells were isolated from the spleen and lymph node of naïve 2C TCR Tg CD45.1+/.2+mice, labeled with CFSE and injected i.v. into CD45.2+ C57BL/6 mice derived from either TAC, JAX or *Bifidobacterium*-treated TAC mice. 24 hours later, mice were inoculated with 1x106 B16.SIY melanoma cells s.c. Spleen and tumor-draining lymph node were harvested and restimulated ex-vivo with SIY peptide. Intracellular IFN-γ production and CFSE dilution were assessed in gated CD45.1+/.2+ 2C T cells by flow cytometry; TDLN=tumordraining lymph node. (B) Representative CFSE dilution assessed in gated CD45.1+/.2+ 2C T cells by flow cytometry (left) and quantification (right).

Fig. 8A-G. Direct administration of *Bifidobacterium* to TAC recipients with established tumors improves tumor-specific immunity and response to αPD-L1 mAb therapy. (A) Principal coordinate analysis plot of bacterial β-diversity over time in groups treated as in Figure 2A. (B) Phylogenetic analysis of taxa that are of significantly different abundance in newly arrived JAX vs TAC mice FDR<0.05 (non-parametric *t* test); bars represent log-transformed fold changes,

inner circle=log10(10); middle circle=log10(100); outer circle=log10(1000). (C) Heatmap demonstrating relative abundance over time of significantly altered genus-level taxa in JAX-fed TAC mice FDR<0.05 (non-parametric *t* test); columns depict individual mice; each timepoint shows mice from two separate cages, 3-4 mice per cage. (D) Correlation plot of relative abundance of *Bifidobacterium* OTU_681370 in fecal material obtained from groups as in (A) 14 days post arrival and frequency of SIY^+ $CD8^+$ T cells in tumor; p=1.4x10-5, FDR=0.0002, R2=0.86 (univariate regression). (E) B16.SIY tumor growth kinetics in TAC mice, untreated or treated with *Bifidobacterium* 7 and 14 days post tumor implantation, αPD-L1 mAb 7, 10, 13 and 16 days post tumor implantation, or both regimens. (F) IFN-γ ELISPOT assessed 5 days after start of treatment. (G) Percentage of tumor-infiltrating SIY^+ $CD8^+$ T cells, determined by flow cytometry 14 days following start of treatment.

Fig. 9A-E. (A) Relative abundance of *Bifidobacterium* OTU_681370 in fecal material obtained from TAC mice 7 days following inoculation with commercial *Bifidobacterium* species. (B) *Bifidobacterium* levels in fecal material obtained from groups as shown, assessed by qPCR using genus-specific primers. (C) Representative plots showing percentage of SIY^+ T cells of total $CD8^+$ T cells within the tumor of untreated and *Bifidobacterium*-treated TAC mice, as assessed by flow cytometry 14 days following start of treatment. (D) *Bifidobacterium* levels in TAC mice 3 weeks post *Bifidobacterium* administration, assessed by qPCR. (E) B16.SIY tumor growth in TAC mice, untreated or inoculated with *Bifidobacterium* 6 weeks prior to tumor implantation.

Fig. 10A-D. (A) B16.SIY tumor growth for isotype-treated (left) or CD8-depleted (right) groups as in Figure 3E. (B) Number of colony forming units (CFU) of live and heat inactivated bifidobacteria, plated in RCM agar following serial dilution in reduced PBS and incubated in an anaerobic chamber for 72 hours. Bars represent 2 replicate plates of each dilution. (C) B16.SIY tumor growth kinetics in TAC mice, untreated or treated with live *Bifidobacterium*, heat inactivated *Bifidobacterium* or JAX fecal material 7 and 14 days post tumor implantation. (D) Percentage of tumor-infiltrating SIY^+ T cells of total $CD8^+$ T cells for treatment groups as in (C), determined by flow cytometry 14 days after start of treatment.

Fig. 11A-E. (A) B16.SIY tumor growth kinetics in TAC mice, untreated or treated with ATCCderived *B. breve* or *B. longum.* (B) B16.F10 tumor growth kinetics in TAC mice, untreated or treated with *Bifidobacterium* 7 and 14 days post tumor implantation. (C) MB49

tumor growth kinetics in TAC mice, untreated or treated with *Bifidobacterium* 7 and 14 days post tumor implantation. (D) B16.SIY tumor growth kinetics in TAC mice, untreated or treated with *Lactobacillus murinus* or JAX fecal material 7 and 14 days post tumor implantation. (E) Percentage of tumor-infiltrating SIY^+ T cells of total $CD8^+$ T cells for treatment groups as in (D), determined by flow cytometry 18 days after start of treatment.

Fig. 12A-C. (A) Heatmap demonstrating relative abundance of significantly altered genus-level taxa in *Bifidobacterium*-fed TAC mice FDR<0.05 (non-parametric *t*-test); columns depict individual mice; *n = 4-8* mice per group. (B) Frequency of CD4+ FOXP3+ T cells in tumors isolated from JAX and TAC mice 21 days post tumor inoculation, assessed by flow cytometry; representative plot (top), quantification (bottom). (C) Evaluation of translocation of *Bifidobacterium* into mesenteric lymph nodes (mLN), spleen and tumor of TAC, JAX and *Bifidobacterium*-inoculated mice, assessed by qPCR.

Fig. 13A-C. (A) Representative plots depicting the strategy for isolation of DCs from tumors in JAX, TAC and *Bifidobacterium*-treated TAC mice: live CD45+CD3-CD19-MHCIIhiCD11c+ dendritic cells were sorted as shown. (B) All enriched biological pathways and functions found within the subset of elevated genes (fold change $\geq$ 1.5) in JAX and *Bifidobacterium*-treated TAC-derived DCs relative to untreated TAC DCs isolated from tumors 40hrs post inoculation, as assessed by DAVID pathway analysis. (C) qPCR validation of genes identified by microarray gene expression profiling as in (B).

Fig. 14A-B. (A) Representative flow plots of CFSE dilution and IFN-γ production in 2C CD8+ T cells stimulated in vitro with DCs purified from naive TAC, JAX and *Bifidobacterium*-treated TAC mice in the presence of different concentrations of SIY peptide as shown. (B) Percentage of 2C CD8+ T cells with undiluted CFSE, stimulated in vitro with DCs purified from naive TAC, JAX and *Bifidobacterium*-treated TAC mice in the presence of different concentrations of SIY peptide as shown.

Fig. 15. B16.SIY tumor growth in TAC mice, untreated or treated individually with ATCC 15700 *B. breve,* ATCC BAA-999 *B. longum,* ATCC 27536 *B. Lactis* or ATCC 15696 *B. Bifidum,* or treated with all four strains combined.

DEFINITIONS

As used herein, the term "microbe" refers to cellular microorganisms including bacteria, fungi, and archaea, and encompasses both individual organisms and populations comprising any number of the organisms.

As used herein, the term "microflora" refers to an assemblage of microorganisms localized to a distinct environment. Microflora may include, for example, populations of various bacteria, fungi, and/or archaea that inhabit a particular environment. For example, "gut microflora," "vaginal microbiota," and "oral microflora" are an assemblage of one or more species of microorganisms that are localized to, or found in, the gut, vagina, or mouth, respectively. "Normal microflora" refers to a population of microorganisms that localize in a particular environment in a normal, non-pathological state (e.g., a sample of gut microflora from a subject without cancer). "Pathologic microflora" refers to a population of various microorganisms that localize in a particular environment in pathological state and differs from normal microflora in terms of identify, absolute amount, or relative amount of the various microbes.

As used herein, the term "commensal microbe" refers to a microorganism that is non-pathogenic to a host and is part of the normal microflora of the host.

As used herein, the term "co-administration" refers to the administration of at least two agents (e.g., commensal microflora and a cancer therapy) or therapies to a subject. In some embodiments, the co-administration of two or more agents/therapies is concurrent. In other embodiments, the co-administration of two or more agents/therapies is sequential (e.g., a first agent/therapy is administered prior to a second agent/therapy).

As used herein, the term "beneficial microbe" refers to a microbe (e.g., bacterium) strain or species that inhibits the growth of cancer/tumor cells and/or facilitates treatment of cancer/tumor cells (e.g., inhibits immune evasion). Beneficial microbes may function by, for example, creating an anti-cancer/anti-tumor environment, microenvironment and/or metabolome, and/or by creating an environment, microenvironment and/or metabolome that inhibits immune evasion or other mechanisms by which cancer cells resist therapy.

As used herein, the term "detrimental microbe" refers to a microbe (e.g., bacterium) strain or species that facilitates the growth of cancer/tumor cells and/or prevents or reduces the effectiveness of treatment of cancer/tumor cells (e.g., inhibits immune evasion). Detrimental microbes may function by, for example, creating an environment, microenvironment and/or

metabolome that facilitates immune evasion or other mechanisms by which cancer cells resist therapy and/or enhance cancer/tumor growth.

As used herein, the term "pharmaceutical agent" refers to a compound, macromolecule, or other chemical/non-biological entity that is administered to a subject to elicit a desired biological response. A pharmaceutical agent may be a "drug" or another entity which is biologically active in a human being or other mammal, locally and/or systemically. Examples of drugs are disclosed in the Merck Index and the Physicians Desk Reference, the entire disclosures of which are incorporated by reference herein for all purposes.

As used herein, the terms "microbial agent," "commensal microbial agent," and "probiotic" refer to compositions comprising a microbe or population of multiple different microbes for administration to a subject.

As used herein, the term "antimicrobial agent" is used to describe a therapeutic compound or bioactive agent which treats a microbial infection, for example, an infection caused by a bacteria, virus, protozoa or fungus. The antimicrobial agent may be an antibiotic, an antifungal agent, an antiviral or an antiprotozoal or antiparasitic agent (which may also be used to treat multicellular parasites).

As used herein, the terms "antibiotic" and "antibacterial agent" refer to a chemical agent which is active against bacteria. In common usage, an antibiotic is a substance or compound (also called chemotherapeutic agent) that kills or inhibits the growth of bacteria. Anti-bacterial antibiotics can be categorized based on their target specificity: "narrow-spectrum" antibiotics target particular types of bacteria, such as Gram-negative or Gram-positive bacteria, while broad-spectrum antibiotics affect a wide range of bacteria. Antibiotics which target the bacterial cell wall (e.g., penicillins, cephalosporins, cephems), or cell membrane (e.g., polymixins), or interfere with essential bacterial enzymes (e.g., quinolones, sulfonamides) usually are bactericidal in nature. Those which target protein synthesis such as the aminoglycosides, macrolides and tetracyclines are usually bacteriostatic. Three newer classes of antibiotics include: cyclic lipopeptides (e.g., daptomycin), glycylcyclines (e.g., tigecycline), and oxazolidinones (e.g., linezolid). Tigecycline is a broad-spectrum antibiotic, while the two others are useful for Gram-positive infections.

As used herein, the term "antiviral agent" refers to a chemical agent which is used to treat a viral infection. Antiviral drugs are a class of medication used specifically for treating viral

infections, specific antivirals are useful for treating infection by specific viruses. Antivirals typically only inhibit virus development.

As used herein, the term "antifungal agent" refers to a therapeutic compound or bioactive agent which may be used to treat a fungal infection in a patient. An antifungal drug is a medication used to treat fungal infections such as athlete's foot, ringworm, candidiasis (thrush), serious systemic infections such as cryptococcal meningitis, and related fungal infections. Antifungal agents include, for example, polyene antifungals, imidazole, triazole and thiazole antifungals, allylamines, echinocandins, griseofulvin, flycystosine, undecylenic acid, among others.

As used herein, the term "antiparasitic agent" refers to a therapeutic compound or bioactive agent that is used to treat parasitic diseases including nematodes, cestodes, trematodes, infectious protozoa, and amoebas. Exemplary antiparasitic agents include: antinematodes (e.g., mebendazole, pyrantel pamoate, thiabendazole, diethycarbazine), anticestodes (e.g., niclosamide, praziquantel), antitrematodes (e.g., praziquantel), antiamoebics (e.g., rifampin and amphotericin B), antiprotozoals (e.g., melarsoprol, eflornithine, metronidazole and tinidazole), among others.

As used herein, the term "pharmaceutical formulation" refers to at least one pharmaceutical agent and/or microbial agent in combination with one or more additional components that assist in rendering the agent(s) suitable for achieving the desired effect upon administration to a subject. The pharmaceutical formulation may include one or more additives, for example pharmaceutically acceptable excipients, carriers, penetration enhancers, coatings, stabilizers, buffers or other materials physically associated with the pharmaceutical/microbial agent to enhance the administration, release (e.g., timing of release), deliverability, bioavailability, effectiveness, etc. of the dosage form. The formulation may be, for example, a liquid, a suspension, a solid, a nanoparticle, emulsion, micelle, ointment, gel, emulsion, coating, etc. A pharmaceutical formulation may contain a single agent or multiple agents (e.g., microbial agent and pharmaceutical agent).

As used herein, the term "subject" broadly refers to any animal, including but not limited to, human and non-human animals (e.g., dogs, cats, cows, horses, sheep, poultry, fish, crustaceans, etc.). As used herein, the term "patient" typically refers to a subject that is being treated for a disease or condition (e.g., cancer, solid tumor cancer, non-T cell-infiltrated tumor cancer, etc.).

As used herein, an "immune response" refers to the action of a cell of the immune system (e.g., T lymphocytes, B lymphocytes, natural killer (NK) cells, macrophages, eosinophils, mast cells, dendritic cells, neutrophils, etc.) and soluble macromolecules produced by any of these cells or the liver (including Abs, cytokines, and complement) that results in selective targeting, binding to, damage to, destruction of, and/or elimination from a subject of invading pathogens, cells or tissues infected with pathogens, or cancerous or other abnormal cells.

As used herein, the term "immunoregulator" refers to an agent or a signaling pathway (or a component thereof) that regulates an immune response. "Regulating," "modifying" or "modulating" an immune response refers to any alteration of the immune system or in the activity of such cell. Such regulation includes stimulation or suppression of the immune system which may be manifested by an increase or decrease in the number of various cell types, an increase or decrease in the activity of these cells, or any other changes which can occur within the immune system. Both inhibitory and stimulatory immunoregulators have been identified, some of which may have enhanced function in a cancer microenvironment.

As used herein, the term "immune evasion" refers to inhibition of a subject's immune system or a component thereof (e.g., endogenous T cell response) by a cancer or tumor cell in order to maximize or allow continued growth or spread of the cancer/tumor.

As used herein, the term "immunotherapy" refers to the treatment or prevention of a disease or condition (e.g., cancer) by a method comprising inducing, enhancing, suppressing or otherwise modifying an immune response.

As used herein, "potentiating an endogenous immune response" means increasing the effectiveness or potency of an existing immune response in a subject. This increase in effectiveness and potency may be achieved, for example, by overcoming mechanisms that suppress the endogenous host immune response or by stimulating mechanisms that enhance the endogenous host immune response.

As used herein, the term "antibody" refers to a whole antibody molecule or a fragment thereof (e.g., fragments such as Fab, Fab', and F(ab')2), it may be a polyclonal or monoclonal antibody, a chimeric antibody, a humanized antibody, a human antibody, etc.

A native antibody typically has a tetrameric structure. A tetramer typically comprises two identical pairs of polypeptide chains, each pair having one light chain (in certain embodiments, about 25 kDa) and one heavy chain (in certain embodiments, about 50-70 kDa). In a native

antibody, a heavy chain comprises a variable region, VH, and three constant regions, CH1, CH2, and CH3. The VH domain is at the amino-terminus of the heavy chain, and the CH3 domain is at the carboxy-terminus. In a native antibody, a light chain comprises a variable region, VL, and a constant region, CL. The variable region of the light chain is at the amino-terminus of the light chain. In a native antibody, the variable regions of each light/heavy chain pair typically form the antigen binding site. The constant regions are typically responsible for effector function.

In a native antibody, the variable regions typically exhibit the same general structure in which relatively conserved framework regions (FRs) are joined by three hypervariable regions, also called complementarity determining regions (CDRs). The CDRs from the two chains of each pair typically are aligned by the framework regions, which may enable binding to a specific epitope. From N-terminus to C-terminus, both light and heavy chain variable regions typically comprise the domains FR1, CDR1, FR2, CDR2, FR3, CDR3 and FR4. The CDRs on the heavy chain are referred to as H1, H2, and H3, while the CDRs on the light chain are referred to as L1, L2, and L3. Typically, CDR3 is the greatest source of molecular diversity within the antigen-binding site. H3, for example, in certain instances, can be as short as two amino acid residues or greater than 26. The assignment of amino acids to each domain is typically in accordance with the definitions of Kabat et al. (1991) Sequences of Proteins of Immunological Interest (National Institutes of Health, Publication No. 91-3242, vols. 1-3, Bethesda, Md.); Chothia, C., and Lesk, A. M. (1987) J. Mol. Biol. 196:901-917; or Chothia, C. et al. Nature 342:878-883 (1989). In the present application, the term "CDR" refers to a CDR from either the light or heavy chain, unless otherwise specified.

As used herein, the term "heavy chain" refers to a polypeptide comprising sufficient heavy chain variable region sequence to confer antigen specificity either alone or in combination with a light chain.

As used herein, the term "light chain" refers to a polypeptide comprising sufficient light chain variable region sequence to confer antigen specificity either alone or in combination with a heavy chain.

As used herein, when an antibody or other entity "specifically recognizes" or "specifically binds" an antigen or epitope, it preferentially recognizes the antigen in a complex mixture of proteins and/or macromolecules, and binds the antigen or epitope with affinity which is substantially higher than to other entities not displaying the antigen or epitope. In this regard,

"affinity which is substantially higher" means affinity that is high enough to enable detection of an antigen or epitope which is distinguished from entities using a desired assay or measurement apparatus. Typically, it means binding affinity having a binding constant (Ka) of at least 10^7 M^{-1} (e.g., $>10^7$ M^{-1}, $>10^8$ M^{-1}, $>10^9$ M^{-1}, $>10^{10}$ M^{-1}, $>10^{11}$ M^{-1}, $>10^{12}$ M^{-1}, $>10^{13}$ M^{-1}, etc.). In certain such embodiments, an antibody is capable of binding different antigens so long as the different antigens comprise that particular epitope. In certain instances, for example, homologous proteins from different species may comprise the same epitope.

As used herein, the term "monoclonal antibody" refers to an antibody which is a member of a substantially homogeneous population of antibodies that specifically bind to the same epitope. In certain embodiments, a monoclonal antibody is secreted by a hybridoma. In certain such embodiments, a hybridoma is produced according to certain methods known to those skilled in the art. See, e.g., Kohler and Milstein (1975) Nature 256: 495-499; herein incorporated by reference in its entirety. In certain embodiments, a monoclonal antibody is produced using recombinant DNA methods (see, e.g., U.S. Pat. No. 4,816,567). In certain embodiments, a monoclonal antibody refers to an antibody fragment isolated from a phage display library. See, e.g., Clackson et al. (1991) Nature 352: 624-628; and Marks et al. (1991) J. Mol. Biol. 222: 581-597; herein incorporated by reference in their entireties. The modifying word "monoclonal" indicates properties of antibodies obtained from a substantially-homogeneous population of antibodies, and does not limit a method of producing antibodies to a specific method. For various other monoclonal antibody production techniques, see, e.g., Harlow and Lane (1988) Antibodies: A Laboratory Manual (Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.); herein incorporated by reference in its entirety.

As used herein, the term "antibody fragment" refers to a portion of a full-length antibody, including at least a portion antigen binding region or a variable region. Antibody fragments include, but are not limited to, Fab, Fab', F(ab')2, Fv, scFv, Fd, diabodies, and other antibody fragments that retain at least a portion of the variable region of an intact antibody. See, e.g., Hudson et al. (2003) Nat. Med. 9:129-134; herein incorporated by reference in its entirety. In certain embodiments, antibody fragments are produced by enzymatic or chemical cleavage of intact antibodies (e.g., papain digestion and pepsin digestion of antibody) produced by recombinant DNA techniques, or chemical polypeptide synthesis.

For example, a "Fab" fragment comprises one light chain and the CH1 and variable region of one heavy chain. The heavy chain of a Fab molecule cannot form a disulfide bond with another heavy chain molecule. A "Fab'" fragment comprises one light chain and one heavy chain that comprises additional constant region, extending between the CH1 and CH2 domains. An interchain disulfide bond can be formed between two heavy chains of a Fab' fragment to form a "F(ab')2" molecule.

An "Fv" fragment comprises the variable regions from both the heavy and light chains, but lacks the constant regions. A single-chain Fv (scFv) fragment comprises heavy and light chain variable regions connected by a flexible linker to form a single polypeptide chain with an antigen-binding region. Exemplary single chain antibodies are discussed in detail in WO 88/01649 and U.S. Pat. Nos. 4,946,778 and 5,260,203; herein incorporated by reference in their entireties. In certain instances, a single variable region (e.g., a heavy chain variable region or a light chain variable region) may have the ability to recognize and bind antigen.

Other antibody fragments will be understood by skilled artisans.

As used herein, the term "chimeric antibody" refers to an antibody made up of components from at least two different sources. In certain embodiments, a chimeric antibody comprises a portion of an antibody derived from a first species fused to another molecule, e.g., a portion of an antibody derived from a second species. In certain such embodiments, a chimeric antibody comprises a portion of an antibody derived from a non-human animal fused to a portion of an antibody derived from a human. In certain such embodiments, a chimeric antibody comprises all or a portion of a variable region of an antibody derived from a non-human animal fused to a constant region of an antibody derived from a human.

A "humanized" antibody refers to a non-human antibody that has been modified so that it more closely matches (in amino acid sequence) a human antibody. A humanized antibody is thus a type of chimeric antibody. In certain embodiments, amino acid residues outside of the antigen binding residues of the variable region of the non-human antibody are modified. In certain embodiments, a humanized antibody is constructed by replacing all or a portion of a complementarity determining region (CDR) of a human antibody with all or a portion of a CDR from another antibody, such as a non-human antibody, having the desired antigen binding specificity. In certain embodiments, a humanized antibody comprises variable regions in which all or substantially all of the CDRs correspond to CDRs of a non-human antibody and all or

substantially all of the framework regions (FRs) correspond to FRs of a human antibody. In certain such embodiments, a humanized antibody further comprises a constant region (Fc) of a human antibody.

The term "effective dose" or "effective amount" refers to an amount of an agent, e.g., an antibody, that results in the reduction of symptoms in a patient or results in a desired biological outcome. In certain embodiments, an effective dose or effective amount is sufficient to treat or reduce symptoms of a disease or condition.

DETAILED DESCRIPTION

Provided herein are methods of treatment and/or prevention of cancer by manipulation of commensal microflora. In particular, the amount, identity, presence, and/or ratio of microflora (e.g., gut microflora) in a subject is manipulated to facilitate one or more co-treatments.

T cell infiltration of solid tumors is associated with favorable patient outcomes, yet the mechanisms underlying variable endogenous immune responses between individuals are not well understood. Experiments were conducted during development of embodiments described herein to examine potential effects of microbial composition on spontaneous anti-tumor immunity. B16 melanoma growth was compared in C57BL/6 mice having distinct commensal microbiota. The two populations of mice showed robust versus weak spontaneous anti-tumor immunity. This phenotypic difference was eliminated upon cohousing or following fecal transfer. 16S rRNA sequencing identified *Bifidobacterium* as associated with the anti-tumor effects. Oral administration of *Bifidobacterium* alone or in combination with systemic αPD-L1 in tumor-bearing mice markedly improved tumor control in a $CD8^+$ T cell-dependent manner. Mechanistically, the effect was mediated by augmented dendritic cell function leading to more robust antigen-specific $CD8^+$ T cell priming and markedly increased accumulation of activated T cells in the tumor microenvironment. These data, for example, demonstrate advantages manipulating commensal microbes as a cancer therapeutic.

In some embodiments, the effectiveness of an endogenous immune response, immunotherapy, chemotherapeutic, or other treatment (e.g., surgery, radiation, etc.) in the treatment or prevention of reoccurrence of cancer and/or tumor is dependent upon conditions within the subject (e.g., the tumor microenvironment). In particular, the identity or characteristics (e.g., concentration or level) of the microflora within a subject affects the

effectiveness of cancer treatments (e.g., generally or specific treatments) and/or the effectiveness of the subject's own immune response to cancer.

In some embodiments, the presence or increased level of one or more microbes (e.g., one or more types of bacteria) in a subject potentiates cancer/tumor growth, spread (e.g., malignancy), and/or evasion of treatment/immune response. In some embodiments, the presence or increased level of one or more microbes (e.g., one or more types of bacteria) in a subject inhibits treatment (e.g., immunotherapy, chemotherapy, etc.) and/or the subject's endogenous immune response to cancer and/or tumor cells. In some embodiments, the absence and/or decreased level of one or more microbes (e.g., one or more types of bacteria) in a subject potentiates cancer/tumor growth, spread, and/or evasion of treatment/immune response. In some embodiments, the absence or decreased level of one or more microbes (e.g., one or more types of bacteria) in a subject inhibits treatment (e.g., immunotherapy, chemotherapy, etc.) and/or the subject's endogenous immune response to cancer and/or tumor cells.

In some embodiments, the presence or increased level of one or more microbes (e.g., one or more types of bacteria) in a subject discourages cancer/tumor growth, spread, and/or evasion of treatment/immune response. In some embodiments, the presence or increased level of one or more microbes (e.g., one or more types of bacteria) in a subject facilitates treatment (e.g., immunotherapy, chemotherapy, etc.) and/or the subject's endogenous immune response to cancer and/or tumor cells. In some embodiments, the absence and/or decreased level of one or more microbes (e.g., one or more types of bacteria) in a subject discourages cancer/tumor growth, spread, and/or evasion of treatment/immune response. In some embodiments, the absence or decreased level of one or more microbes (e.g., one or more types of bacteria) in a subject facilitates treatment (e.g., immunotherapy, chemotherapy, etc.) and/or the subject's endogenous immune response to cancer and/or tumor cells.

In some embodiments, the presence of beneficial microbes (e.g., microbes that facilitate cancer treatment) in a subject creates an environment or microenvironment (e.g., metabolome) that is conducive to the treatment of cancer and/or inhibits cancer/tumor growth. In some embodiments, the presence of detrimental microbes (e.g., microbes that facilitate cancer/tumor growth and/or prevent treatment) in a subject creates an environment or microenvironment (e.g., metabolome) that is conducive to the treatment of cancer and/or inhibits cancer/tumor growth.

Experiments conducted during development of embodiments described herein demonstrate that modulation of levels and/or identity of the microflora in a subject facilitates treatment of cancer/tumor within the subject, enhances the endogenous immune response, decreases immune evasion or other inhibitory mechanisms to treatment of endogenous immune response, and/or improves cancer outcomes for the subject. Modulation of microflora levels and/or identity may comprise encouraging or facilitating growth of one or more types of beneficial microbes (e.g., microbes that facilitate cancer treatment), discouraging or inhibiting growth of one or more types of detrimental microbes (e.g., microbes that facilitate cancer/tumor growth and/or prevent treatment), administering one or more types of beneficial microbes (e.g., microbes that facilitate cancer treatment) to the subject, and/or combinations thereof. Embodiments within the scope herein are not limited by the mechanisms for introducing one or more microbes (e.g., fecal transplant, probiotic administration, etc.), encouraging growth of beneficial microbes (e.g., administering agents that skew the environment within the subject toward growth conditions for the beneficial microbes), discouraging or inhibiting growth of detrimental microbes (e.g., administering agents that skew the environment within the subject away from growth conditions for the detrimental microbes, administration of antimicrobial(s), etc.), and combinations thereof.

In some embodiments, methods are provided for the treatment or prevention of cancer by the manipulation of the presence, amount, or relative ratio of commensal microflora (e.g., gut microflora). In some embodiments, the presence, amount, or relative ratio of particular bacteria, fungi, and/or archaea within a subject is manipulated. For example, in some embodiments, the presence, amount, or relative ratio of one or more bacteria from the phyla *Firmicutes*, *Bacteroidetes*, *Actinobacteria*, and/or *Proteobacteria* are manipulated. In some embodiments, the presence, amount, or relative ratio of one or more bacteria belonging to the genera *Bacteroides*, *Clostridium*, *Fusobacterium*, *Eubacterium*, *Ruminococcus*, *Peptococcus*, *Peptostreptococcus*, *Bifidobacterium*, *Rikenella, Alistipes, Marinilabilia, Anaerostipes, Escherichia*, and/or *Lactobacillus* are manipulated. In some embodiments, the presence, amount, or relative ratio of one or more fungi belonging to the genus *Candida*, *Saccharomyces*, *Aspergillus*, and/or *Penicillium* are manipulated.

In some embodiments, the presence and/or levels of one or more commensal microbes are manipulated in a subject suffering from cancer, at heightened risk of cancer, and/or receiving

treatment for cancer. Exemplary commensal microbes include *Lactococcus* (e.g., *Lactococcus cremoris* and *Lactococcus lactis*), *Lactobacillus* (e.g., *Lactobacillus acidophilus, Lactobacillus casei, Lactobacillus kefiri, Lactobacillus bifidus, Lactobacillus brevis, Lactobacillus helveticus, Lactobacillus paracasei , Lactobacillus rhamnosus, Lactobacillus salivarius, Lactobacillus curvatus, Lactobacillus bulgaricus, Lactobacillus sakei, Lactobacillus reuteri, Lactobacillus fermentum, Lactobacillus farciminis, Lactobacillus lactis, Lactobacillus delbrueckii, Lactobacillus plantarum, Lactobacillus paraplantarum, Lactobacillus crispatus, Lactobacillus gasseri, Lactobacillus johnsonii* and *Lactobacillus jensenii*), *Leuconostoc, Carnobacterium, Enterococcus, Propionibacteium, Pediococcus, Bifidobacterium* (e.g., *Bifidobacterium lactis, Bifidobacterium bifidium, Bifidobacterium longum, Bifidobacterium animalis, Bifidobacterium breve, Bifidobacterium infantis, Bifidobacterium catenulatum, Bifidobacterium pseudocatenulatum, Bifidobacterium adolescentis, Bifidobacteriumangulatum*, etc.), Streptococcus (e.g., Stre*ptococcus thermophiles, Streptococcus salivarius, Streptococcus oralis, Streptococcus uberis, Streptococcus rattus*, etc.); *Escherichia coli, Bacillus coagulans, Bacillus lansii,* Yeast (e.g., Saccharomyces cerevisiae, Saccharomyces boulardii, etc.); and combinations thereof.

In some embodiments, one or more species, genera, and/or types of microbes are administered and/or the growth thereof is facilitated. In some embodiments, the growth of one or more species, genera, and/or types of microbes is inhibited. In some embodiments, one or more species, genera, and/or types of microbes are administered and/or the growth thereof is facilitated; and the growth of one or more other species, genera, and/or types of microbes is inhibited.

In some embodiments, the level or presence of one or more beneficial microbes (e.g., microbes that inhibit cancer/tumor growth or spread, enhance cancer/tumor treatment, etc.) is modulated by the administration of such microbes to a subject.

In some embodiments, microflora-modulation utilizes prepared probiotic compositions for administration to/by a subject. Probiotic compositions comprise one or more beneficial microbes (e.g., bacteria) formulated such that administration of the probiotic (e.g., orally, rectally, by inhalation, etc.) results in population of the subject by the beneficial microbes.

In some embodiments, probiotic compositions comprise cultured microbes that are combined and/or formulated for administration to a subject. In some embodiments, probiotics

contain microbes of known genera, species, etc. and/or at known concentrations (cfus). Probiotic compositions may be in the form of a pharmaceutical-type composition (e.g., capsule, tables, liquid, aerosol, etc.) or in the form of a food supplement.

In some embodiments, probiotic microbes (e.g., bacteria) are formulated in a pharmaceutically acceptable composition for delivery to a subject. In some embodiments, probiotics are formulated with a pharmaceutically acceptable carrier suitable for a solid or semi-solid formulation. In some embodiments, probiotic microbes are formulated with a pharmaceutically acceptable carrier suitable for a liquid or gel formulation. Probiotic formulations may be formulated for enteral delivery, e.g., oral delivery, or delivery as a suppository, but can also be formulated for parenteral delivery, e.g., vaginal delivery, inhalational delivery (e.g., oral delivery, nasal delivery, and intrapulmonary delivery), and the like.

The probiotic compositions that find use in embodiments described herein may be formulated in a wide variety of oral administration dosage forms, with one or more pharmaceutically acceptable carriers. The pharmaceutically acceptable carriers can be either solid or liquid. Solid form preparations include powders, tablets, pills, capsules, cachets, suppositories, and dispersible granules. A solid carrier can be one or more substances which may also act as diluents, flavoring agents, solubilizers, lubricants, suspending agents, binders, preservatives, tablet disintegrating agents, or an encapsulating material. In powders, the carrier is a finely divided solid which is a mixture with the probiotic microbes. In tablets, the microbes are mixed with the carrier having the necessary binding capacity in suitable proportions and compacted in the shape and size desired. Suitable carriers are magnesium carbonate, magnesium stearate, talc, sugar, lactose, pectin, dextrin, starch, gelatin, tragacanth, methylcellulose, sodium carboxymethylcellulose, a low melting wax, cocoa butter, and the like. Other forms suitable for oral administration include liquid form preparations such as emulsions, syrups, elixirs, aqueous solutions, aqueous suspensions, or solid form preparations which are intended to be converted shortly before use to liquid form preparations. Aqueous suspensions can be prepared by dispersing the probiotic microbes in water with viscous material, such as natural or synthetic gums, resins, methylcellulose, sodium carboxymethylcellulose, and other well-known suspending agents.

The probiotic compositions (e.g., microbes (e.g., bacteria)) may be formulated for administration as suppositories. A low melting wax, such as a mixture of fatty acid glycerides or cocoa butter is first melted and the probiotic microbes are dispersed homogeneously, for example, by stirring. The molten homogeneous mixture is then poured into conveniently sized molds, allowed to cool, and to solidify.

The probiotic compositions (e.g., microbes (e.g., bacteria)) may be formulated for vaginal administration. Pessaries, tampons, creams, gels, pastes, foams or sprays, may contain agents in addition to the bacteria, such carriers, known in the art to be appropriate.

In some embodiments, probiotic compositions (e.g., microbes (e.g., bacteria)) may be formulated for delivery by inhalation. As used herein, the term "aerosol" is used in its conventional sense as referring to very fine liquid or solid particles carries by a propellant gas under pressure to a site of therapeutic application. The term "liquid formulation for delivery to respiratory tissue" and the like, as used herein, describe compositions comprising probiotic microbes with a pharmaceutically acceptable carrier in flowable liquid form. Such formulations, when used for delivery to a respiratory tissue, are generally solutions, e.g. aqueous solutions, ethanolic solutions, aqueous/ethanolic solutions, saline solutions and colloidal suspensions.

Rather than pharmaceutical-type formulation, probiotic compositions may be formulated as food additive and/or food product and incorporated into a variety of foods and beverages. Suitable foods and beverages include, but are not limited to, yogurts, ice creams, cheeses, baked products such as bread, biscuits and cakes, dairy and dairy substitute foods, soy-based food products, grain-based food products, starch-based food products, confectionery products, edible oil compositions, spreads, breakfast cereals, infant formulas, juices, power drinks, and the like.

In some embodiments, a probiotic composition is administered over a dosing time period (e.g., < 1 minute, <1 hour, <2 hours, <4 hours, <6 hours, <12 hours, <24 hours, etc.) in an amount that is sufficient to provide a desired therapeutic benefit (e.g., as a single dose, in combination with other doses, in combination with a co-administered therapeutic, etc.) In some embodiments, the dose of the probiotic composition administered for the dosing time period is concentration of from about 10 to about 1 x 10^{14} colony forming units (cfu) of the commensal microbial agent(s) (e.g., 10 cfu, 100 cfu, 10^{13} cfu, 10^{13} cfu, 10^{13} cfu, 10^{13} cfu, 10^{13} cfu, 10^{13} cfu, 10^{13} cfu, 10^{13} cfu, 10^{13} cfu, 10^{13} cfu, 10^{13} cfu, or any suitable ranges therein (e.g., from

about 10^2 cfu to about 10^{13} cfu, about 1 x 10^4 to about 1 x 10^{11} cfu, about 1 x 10^6 to about 1 x 10^9 cfu, about 1 x 10^{10} to about 1 x 10^{12} cf, etc.), etc.).

In some embodiments, the microbial make-up of a probiotic composition consists or consists essentially of one or more beneficial microbes (e.g., bacteria). In some embodiments, the microbial make-up of a probiotic composition consists or consists essentially of 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25, 30, 40, 50, 60, 70, 80, 90, 100, or any ranges therein (e.g., 1-4, 5-10, 8-20, etc.) strains and/or species of microbes. In some embodiments, fewer than 50 microbial strains (e.g., 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25, 30, 40, 50, or any ranges therein (e.g., 1-4, 5-10, 8-20, etc.) are at least 50% (e.g., 50%, 60%, 70%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 99.5%. 99.9%, 99.99%) of the microbial population (e.g., by mass, by cfu, etc.) of a probiotic composition. For example, in some embodiments, a single species or strain of *Bifidobacterium* is at least 95% of the microbial population, as measured by colony forming units, of a particular probiotic composition. As another example, in some embodiments, a single species or strain of *Bifidobacterium* is at least 40% and bacteria from the genus *Lactobacillus* are at least 50% of the microbial population, as measured by mass, of a particular probiotic composition. These examples are not limiting.

In some embodiments, microflora in a subject (e.g., a subject suffering from cancer, a subject with microflora that promotes cancer growth, a subject with microflora that promotes evasion of cancer treatment (e.g., by immunotherapy), etc.) are modulated by transplantation of microbiota from a subject with favorable characteristics (e.g., a subject without cancer, a subject with microflora that inhibits cancer growth, a subject with microflora that promotes treatment of cancer (e.g., by immunotherapy), etc.) into the recipient subject.

In some embodiments, donor microflora are obtained sampling microflora from the desired region of the donor subject body (e.g., colon, oral cavity, vagina, etc.). In particular embodiments, fecal material (e.g., 100 g – 500 g) is obtained from a donor. The material may be administered to a recipient subject with or without subsequent preparation steps (e.g., diluting, mixing, oxygenating, filtering, supplementing (e.g., with prebiotics, with growth media, etc.), testing (e.g., for pathogens or detrimental microbes), etc.). The donor microflora (e.g., fecal material) may be administered without preservation (e.g., administered within 12 hours (e.g., <6 hours, <4 hours, <2 hours, <1 hour, etc.)) or may be preserved (e.g., frozen, freeze dried, etc.),

for example, to allow for delay (e.g., 1 day, 2, days, 1 week, 1 month, or more) before delivery to the subject.

In some embodiments, donor microflora are proceed to remove one or more components. For example, parasitic of detrimental microbes may be removed or killed. Contaminants within the donor sample may be removed. In some embodiments, donor microflora is enriched for one or more specific microbes (e.g., 2-fold, 3-fold, 4 fold, 10-fold, 20-fold, or more enrichment). In some embodiments, donor microflora is enriched such that at least 1% of the microbes in the population are the desired beneficial microbes (e.g., 1%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%, 10%, 15%, 20%, 25%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, or more). In some embodiments, donor microflora are doped with one or more cultured beneficial microbes.

In particular embodiments, transplanted microflora may be administered to the recipient subject by any suitable delivery mechanism, including but not limited to enema, colonoscope, nasogastric or nasoduodenal tube, lavage or irrigation, or orally (e.g., in the form of a capsule).

In some embodiments, a commensal microbial agent or population of microbial agents is administered (e.g., via probiotic composition or microflora transplant) over a dosing time period (e.g., < 1 minute, <1 hour, <2 hours, <4 hours, <6 hours, <12 hours, <24 hours, etc.) in an amount that is sufficient to provide a desired therapeutic benefit (e.g., as a single dose, in combination with other doses, in combination with a co-administered therapeutic, etc.) In some embodiments, the dose of commensal microbial agent(s) administered for the dosing time period is concentration of from about 10 to about 1 x 10^{14} colony forming units (cfu) of the commensal microbial agent(s) (e.g., 10 cfu, 100 cfu, 10^{13} cfu, 10^{13} cfu, 10^{13} cfu, 10^{13} cfu, 10^{13} cfu, 10^{13} cfu, 10^{13} cfu, 10^{13} cfu, 10^{13} cfu, 10^{13} cfu, 10^{13} cfu, 10^{13} cfu, or any suitable ranges therein (e.g., from about 10^{2} cfu to about 10^{13} cfu, about 1 x 10^{4} to about 1 x 10^{11} cfu, about 1 x 10^{6} to about 1 x 10^{9} cfu, about 1 x 10^{10} to about 1 x 10^{12} cf, etc.), etc.).

The dose can be administered in a single unit dose administered at any time during a day. Alternatively the loading dose can be administered in two or more doses administered at a single time of day or at two or more separate times of day.

Over the course of multiple dosing periods, the dose can be tapered from an initial dose to a higher dose (or increased from an initial dose to a higher dose), on predetermined timing or by the when the subject and/or clinician based on the results of the treatment. The appropriate

dosage amount will vary by, for example, an individual subject's age, weight, condition or disease, severity of disease, etc.

By way of non-limiting example (both in terms of identify of the microbe as well as dose), in some embodiments, one or more strains of *Bifidobacterium* are administered via 3 capsules daily, each capsule containing 1×10^9 cfu of *Bifidobacterium*. Alternatively, in other embodiments one or more strains of *Bifidobacterium* are administered at a dosage of one capsule daily containing 1×10^{12} cfu of bacteria. Any other dosages (e.g., cfu), doses (e.g., times per day, week, etc.), and identity of the microbe(s) (e.g., within the ranges described herein) are within the scope herein.

In some embodiments, microbes for probiotic compositions are obtained from culture. In some embodiments, strains of beneficial microbes are genetically engineered to enhance one or more of production (e.g., at scale), formulation, delivery, or the biological effect of the microbe. In some embodiments, microbes are engineered to express a detectable marker that allows tracking of the microbes within a subject, or confirmation that the microbe has integrated into a subjects microflora. In some embodiments, microbes are engineered to express a cancer therapeutic (e.g., chemotherapeutic, immunotherapeutic, antibodies, etc.), anti-inflammatory agent, of other drug.

In some embodiments, one or more prebiotics are administered to a subject as an independent treatment (e.g., to increase the level of a beneficial microbe) or in conjunction with other treatments described herein. Prebiotics are agents that increase the in vivo growth rate or activity of commensal microbes, such as *Lactobacillus* and/or *Bifidobacterium*. In some embodiments, prebiotics are soluble fiber sources. In some embodiments, when prebiotics are administered (e.g., fed) to a subject they are not digested or are not fully digested by the subject's digestive enzymes, but rather support the intestinal health of the subject and provide an energy source for the beneficial microbes and enhance the growth thereof. Prebiotics include, for example, naturally occurring lecithins and/or oleic acid, and are described, for example in U.S. Pat. No. 8,449,878 which is herein incorporated by reference in its entirety.

In some embodiments, the level or presence of one or more detrimental microbes (e.g., microbes that facilitate cancer/tumor growth or spread, inhibit cancer/tumor treatment, etc.) is modulated, for example, by the administration of one or more antimicrobial agents to a subject or

modulation of conditions within the subject to disfavor growth of the detrimental microbes. In some embodiments, antimicrobial agents are administered.

In some embodiments, the antimicrobial agent is an antibiotic. Exemplary antibiotics that may find use in some embodiments include, but are not limited to: amikacin, gentamicin, kanamycin, neomycin, netilmicin, streptomycin, tobramycin, paromycin, geldanamycin, herbimycin, loracarbef, ertapenem, doripenem, imipenem, meropenem, cefaclor, cefamandole, cefotoxin, cefprozil, cefuroxime, cefixime, cefdinir, cefditoren, cefpodoxime, ceftazidime, ceftibuten, ceftizoxime, ceftriaxone, cefepime, ceftobirprole, vancomycin, azithromycin, clarithromycin, dirithromycin, erythromycin, roxithromycin, troleandomycin, telithromycin, spectinomycin, aztreonam, amoxicillin, ampicillin, azociling, carbenicillin, cloxacillin, dicloxacillin, flucloxacillin, mezlocillin, meticillin, nafcillin, oxacillin, peperacillin, ticarcillin, bacitracin, colistin, polymyxin B, ciprofloxacin, clavulanic acid, enoxacin, gatifloxacin, levofloxacin, lomefloxacin, moxifloxacin, nonfloxacin, ofloxacin, trovafloxacin, grepafloxacin, sparfloxacin, AL-15469A, AL-38905, OP-145, afenide, prontosil, sulfacetamide, sulfamethiazole, sulfanamide, sulfasalazine, sulfisoxazole, trimethoprim, cotrimoxazole, demeclocycline, doxycycline, minocycline, oxytetracycline, tetraycline, linezolid, arsogebanubem chloramphenicol, clindamycin, lincomycin, ethambutol, fosfomycin, fusidic acid, furazolidone, isoniazid, linezolid, metronidazole, mupirocin, nitrofurantoin, rifampicin, thamphenicol, tinidazole, amoxicillin+clavulanic acid, Maximin H5, Dermcidin, Cecropins, andropin, moricin, ceratotoxin, melittin, Magainin, dermaseptin, bombinin, brevinin-l,esculentins and buforin II, CAP 18, LL37 , abaecin, apidaecins, prophenin, indolicidin, brevinins, protegrin, tachyplesins, defensins, drosomycin, alamethicin, pexiganan or MSI-78, MSI-843, MSI-594, polyphemusin, colicin, pyocin, klebicin, subtilin, epidermin, herbicolacin, brevicin, halocin , agrocin, alveicin, carnocin, curvaticin, divercin ,enterocin, enterolysin, erwiniocin, glycinecin, lactococin, lacticin, leucoccin, mesentericin, pediocin, plantaricin, sakacin, sulfolobicin, vibriocin, warnerinand, nisin, or a salt or cocrystal, or prodrug or solvate thereof, or a combination thereof.

In some embodiments, the antimicrobial is an antifungal agent. Exemplary antifungals that may find use in some embodiments include, but are not limited to: amrolfine, utenafine, naftifine, terbinafine, flucytosine, fluconazole, itraconazole, ketoconazole, posaconazole, ravuconazole, voriconazole, clotrimazole, econazole, miconazole, oxiconazole, sulconazole,

terconazole, tioconazole, nikkomycin Z, caspofungin, micafungin, anidulafungin, amphotericin B, liposomal nystastin, pimaricin, griseofulvin, ciclopirox olamine, haloprogin, tolnaftate, undecylenate, clioquinol, and combinations thereof.

In some embodiments, the antimicrobial is an antiparasitic. Exemplary antiparasitics that may find use in some embodiments include, but are not limited to:amitraz, amoscanate, avermectin, carbadox, diethylcarbamizine, dimetridazole, diminazene, ivermectin, macrofilaricide, malathion, mitaban, oxamniquine, permethrin, praziquantel, prantel pamoate, selamectin, sodium stibogluconate, thiabendazole, and combinations thereof.

In some embodiments, methods and compositions for reduction of detrimental microbe levels are co-administered (e.g., serially, concurrently, etc.) with methods and compositions for increasing beneficial microbe levels. In some embodiments, by reducing overall microbe levels or by reducing the levels of specific microbes (e.g., detrimental microbes, high population microbes, etc.), the population of beneficial microbes can more effectively be modulated (e.g., increased).

In some embodiments, in order to develop a microflora population within a subject that facilitates cancer treatment or inhibits cancer growth/spread, antimicrobial agents are first administered to eliminate or reduce the microflora within the subject, and then the microflora population is reestablished using the methods and compositions described herein (e.g., administration of beneficial microbes). In some embodiments, antimicrobials (e.g., antibiotics) that reduce the microbe (e.g., bacteria) population generally are employed. In some embodiments, antimicrobials that target detrimental microbes preferentially are employed.

In some embodiments, modulating the microflora composition is sufficient on its own to allow the endogenous immune system of a subject to respond to the presence of cancer cells and or tumor growth. However, in other embodiments, microflora composition is manipulated along with one or more other cancer therapies. In some embodiments, manipulation of the microflora composition (e.g., identity and/or level) treats cancer by a mechanism independent of one or more additional cancer treatments. In other embodiments, modulation of microflora composition facilitates (e.g., increases the effectiveness of) the cancer treatment. In some embodiments, one or more cancer treatments enhance the effectiveness of the modulation of microflora composition. Embodiments herein are not limited by the types of cancer treatments (e.g., surgery, radiation, immunotherapy, chemotherapeutic, etc.) unless specifically noted.

In some embodiments, immunotherapeutic cancer treatment encompasses blockade of immune-inhibitory receptors, for example using monoclonal antibodies (mAbs) against CTLA-4 and PD-1/PD-L1 (Wolchok, J. D. et al. The New England Journal of Medicine 369, 122-133 (2013).; Topalian, S. L. et al. Journal of clinical oncology 32, 1020-1030 (2014).; Topalian, S. L. et al. The New England journal of medicine 366, 2443-2454 (2012).; Hodi, F. S. et al. The New England journal of medicine 363, 711-723 (2010).; herein incorporated by reference in their entireties).

In some embodiments, the immunotherapy includes the administration of an immune checkpoint inhibitor. Immune Checkpoint inhibition broadly refers to inhibiting the checkpoints that cancer cells can produce to prevent or downregulate an immune response. Examples of immune checkpoint proteins include, but are not limited to, CTLA4, PD-1, PD-L1, PD-L2, A2AR, B7-H3, B7-H4, BTLA, KIR, LAG3, TIM-3 or VISTA. Immune checkpoint inhibitors can be antibodies or antigen binding fragments thereof that bind to and inhibit an immune checkpoint protein. Examples of immune checkpoint inhibitors include, but are not limited to, nivolumab, pembrolizumab, pidilizumab, AMP-224, AMP-514, STI-A1110, TSR-042, RG-7446, BMS-936559, BMS-936558, MK-3475, CT Ol l, MPDL3280A, MEDI-4736, MSB-0020718C, AUR-012 and STI-A1010. In some embodiments, the immune checkpoint inhibitor may be administered via injection (e.g., intravenously, intratumorally, subcutaneously, or into lymph nodes), but may also be administered orally, topically, or via aerosol.

In some embodiments, the compositions for and/or methods of modulating microflora in a subject overcome immune invasion of cancer cells, tumor, tumor microenvironment, etc. In some embodiments, one or more additional cancer immunotherapies are employed (e.g., concurrently or serially) to make use of the induced immune-responsiveness treated cells/tumor. Suitable immunotherapies may include, but are not limited to: cell-based therapies (e.g., dendritic cell or T cell therapy, etc.), monoclonal antibody (mAb) therapy (e.g., naked mAbs, conjugated mAbs), cytokine therapy (e.g., interferons, interleukins, etc.), adjuvant treatment (e.g., polysaccharide-K), etc.

Examples of antibodies that may find use in the compositions and methods disclosed herein, particularly for use in immunotherapies (but not so limited) include, but are not limited, to antibodies such as trastuzumab (anti-HER2/neu antibody); Pertuzumab (anti-HER2 mAb); cetuximab (chimeric monoclonal antibody to epidermal growth factor receptor EGFR);

panitumumab (anti-EGFR antibody); nimotuzumab (anti-EGFR antibody); Zalutumumab (anti-EGFR mAb); Necitumumab (anti-EGFR mAb); MDX-210 (humanized anti-HER-2 bispecific antibody); MDX-210 (humanized anti-HER-2 bispecific antibody); MDX-447 (humanized anti-EGF receptor bispecific antibody); Rituximab (chimeric murine/human anti-CD20 mAb); Obinutuzumab (anti-CD20 mAb); Ofatumumab (anti-CD20 mAb); Tositumumab-1131 (anti-CD20 mAb); Ibritumomab tiuxetan (anti-CD20 mAb); Bevacizumab (anti-VEGF mAb); Ramucirumab (anti-VEGFR2 mAb); Ranibizumab (anti-VEGF mAb); Aflibercept (extracellular domains of VEGFR1 and VEGFR2 fused to IgG1 Fc); AMG386 (angiopoietin-1 and -2 binding peptide fused to IgG1 Fc); Dalotuzumab (anti-IGF-1R mAb); Gemtuzumab ozogamicin (anti-CD33 mAb); Alemtuzumab (anti-Campath-1/CD52 mAb); Brentuximab vedotin (anti-CD30 mAb): Catumaxomab (bispecific mAb that targets epithelial cell adhesion molecule and CD3); Naptumomab (anti-5T4 mAb); Girentuximab (anti-Carbonic anhydrase ix); or Farletuzumab (anti-folate receptor). Other examples include antibodies such as Panorex™ (17-1A) (murine monoclonal antibody); Panorex (@(17-1A)) (chimeric murine monoclonal antibody); BEC2 (ami-idiotypic mAb, mimics the GD epitope) (with BCG); Oncolym (Lym-1 monoclonal antibody); SMART M195 Ab, humanized 13′ 1 LYM-1 (Oncolym). Ovarex (B43.13, anti-idiotypic mouse mAb); 3622W94 mAb that binds to EGP40 (17-1A) pancarcinoma antigen on adenocarcinomas; Zenapax (SMART Anti-Tac (IL-2 receptor); SMART M195 Ab, humanized Ab, humanized); NovoMAb-G2 (pancarcinoma specific Ab); TNT (chimeric mAb to histone antigens); TNT (chimeric mAb to histone antigens); Gliomab-H (Monoclonals—Humanized Abs); GNI-250 Mab; EMD-72000 (chimeric-EGF antagonist); LymphoCide (humanized IL.L.2 antibody); and MDX-260 bispecific, targets GD-2, ANA Ab, SMART IDIO Ab, SMART ABL 364 Ab, or ImmuRAIT-CEA.

In some embodiments, an immunotherapy, utilized as a co-therapy with the microflora modulation described herein, directly or indirectly targets one of more of: a regulatory T cell, myeloid suppressor cell, or dendritic cell. In another aspect, an immunotherapy specifically targets one of the following molecules: CD4; CD25 (IL-2α receptor; IL-2αR); cytotoxic T-lymphocyte antigen-4 (CTLA-4; CD152); Interleukin-10 (IL-10); Transforming growth factor-beta receptor (TGF-βR); Transforming growth factor-beta (TGF-β); Programmed Death-1 (PD-1); Programmed death-1 ligand (PD-L1 or PD-L2); Receptor activator of nuclear factor-κB (RANK); Receptor activator of nuclear factor-κB (RANK) ligand (RANKL); LAG-3;

glucocorticoid-induced tumor necrosis factor receptor family-related gene (GITR; TNFRSF18); or Interleukin-4 receptor (IL-4R). In some embodiments, the immunotherapy acts as an agonist that increases the function of the targeted molecule. In other embodiments, the immunotherapy is an antagonist that inhibits the function of the targeted molecule.

In some embodiments, an immunotherapy, utilized as a co-therapy with the microflora modulation described herein, directly or indirectly targets one of more of a specific cytokine, cytokine receptor, co-stimulatory molecule, co-inhibitory molecule, or immunomodulatory receptor that modulates the immune system. In another aspect, one of the following molecules are targeted by co-treatment with microflora modulation: tumor necrosis factor (TNF) superfamily; tumor necrosis factor-α (TNF-α); tumor necrosis factor receptor (TNFR) superfamily; Interleukin-12 (IL-12); IL-12 receptor; 4-1BB (CD137); 4-1BB ligand (4-1BBL; CD137L); OX40 (CD134; TNR4); OX40 ligand (OX40L; CD40; CD40 ligand (CD40L); CTLA-4; Programmed death-1 (PD-1); PD-1 ligand I (PD-L1: B7-H1); or PD-1 ligand 2 (PD-L2; B7-DC); B7 family; B7-1 (CD80); B7-2 (CD86); B7-H3; B7-H4; GITR/AITR: GITRL/AITRL; BTLA; CD70; CD27; LIGHT; HVEM: Toll-like receptor (TLR) (TLR 1, 2, 3, 4, 5, 6, 7, 8, 9, 10).

In some embodiments, the compositions for and/or methods of modulating microflora in a subject sensitize the cancer cells and/or tumor to treatment by one or more chemotherapeutic agents. In some embodiments, one or more chemotherapies are employed in addition to microflora modulation (e.g., concurrently or serially) to make use of the induced chemotherapeutic sensitivity. In other embodiments, one or more chemotherapeutics are provided as co-therapies with microflora modulation, with or without (known) synergism between the microflora modulation and the chemotherapy.

In some embodiments, exemplary anticancer agents suitable for use in compositions and methods described herein (e.g., co-administered with a β-catenin inhibitor) include, but are not limited to: 1) alkaloids, including microtubule inhibitors (e.g., vincristine, vinblastine, and vindesine, etc.), microtubule stabilizers (e.g., paclitaxel (Taxol), and docetaxel, etc.), and chromatin function inhibitors, including topoisomerase inhibitors, such as epipodophyllotoxins (e.g., etoposide (VP-16), and teniposide (VM-26), etc.), and agents that target topoisomerase I (e.g., camptothecin and isirinotecan (CPT-11), etc.); 2) covalent DNA-binding agents (alkylating agents), including nitrogen mustards (e.g., mechlorethamine, chlorambucil, cyclophosphamide,

ifosphamide, and busulfan (MYLERAN), etc.), nitrosoureas (e.g., carmustine, lomustine, and semustine, etc.), and other alkylating agents (e.g., dacarbazine, hydroxymethylmelamine, thiotepa, and mitomycin, etc.); 3) noncovalent DNA-binding agents (antitumor antibiotics), including nucleic acid inhibitors (e.g., dactinomycin (actinomycin D), etc.), anthracyclines (e.g., daunorubicin (daunomycin, and cerubidine), doxorubicin (adriamycin), and idarubicin (idamycin), etc.), anthracenediones (e.g., anthracycline analogues, such as mitoxantrone, etc.), bleomycins (BLENOXANE), etc., and plicamycin (mithramycin), etc.; 4) antimetabolites, including antifolates (e.g., methotrexate, FOLEX, and MEXATE, etc.), purine antimetabolites (e.g., 6-mercaptopurine (6-MP, PURINETHOL), 6-thioguanine (6-TG), azathioprine, acyclovir, ganciclovir, chlorodeoxyadenosine, 2-chlorodeoxyadenosine (CdA), and 2'-deoxycoformycin (pentostatin), etc.), pyrimidine antagonists (e.g., fluoropyrimidines (e.g., 5-fluorouracil (ADRUCIL), 5-fluorodeoxyuridine (FdUrd) (floxuridine)) etc.), and cytosine arabinosides (e.g., CYTOSAR (ara-C) and fludarabine, etc.); 5) enzymes, including L-asparaginase, and hydroxyurea, etc.; 6) hormones, including glucocorticoids, antiestrogens (e.g., tamoxifen, etc.), nonsteroidal antiandrogens (e.g., flutamide, etc.), and aromatase inhibitors (e.g., anastrozole (ARIMIDEX), etc.); 7) platinum compounds (e.g., cisplatin and carboplatin, etc.); 8) monoclonal antibodies (e.g., conjugated with anticancer drugs, toxins, and/or radionuclides, etc.; neutralizing antibodies; etc.); 9) biological response modifiers (e.g., interferons (e.g., IFN-.alpha., etc.) and interleukins (e.g., IL-2, etc.), etc.); 10) adoptive immunotherapy; 11) hematopoietic growth factors; 12) agents that induce tumor cell differentiation (e.g., all-trans-retinoic acid, etc.); 13) gene therapy techniques; 14) antisense therapy techniques; 15) tumor vaccines; 16) therapies directed against tumor metastases (e.g., batimastat, etc.); 17) angiogenesis inhibitors; 18) proteosome inhibitors (e.g., VELCADE); 19) inhibitors of acetylation and/or methylation (e.g., HDAC inhibitors); 20) modulators of NF kappa B; 21) inhibitors of cell cycle regulation (e.g., CDK inhibitors); and 22) modulators of p53 protein function.

In some embodiments, compositions and methods herein comprise multiple modes for the treatment and/or prevention of cancer. In some embodiments, beneficial microbes are provided/administered (e.g., by a probiotic composition, fecal transplant, etc.) with prebiotics and/or other agents that facilitate the growth of the beneficial microbes. In some embodiments, beneficial microbes are provided/administered (e.g., by a probiotic composition, fecal transplant, etc.) with antimicrobial(s) (e.g., antibiotics) directed to kill or inhibit the growth of detrimental

microbes. In some embodiments, prebiotics and/or other agents that facilitate the growth of the beneficial microbes are provided/administered with antimicrobial(s) (e.g., antibiotics) directed to kill or inhibit the growth of detrimental microbes. In some embodiments, beneficial microbes, prebiotics and/or other agents that facilitate the growth of the beneficial microbes, and an antimicrobial(s) (e.g., antibiotics) directed to kill or inhibit the growth of detrimental microbes are all co-administered.

In some embodiments, the co-administered agents are formulated into a single dose and/or composition. In some embodiments, the co-administered agents are in separate doses and/or compositions. In some embodiments in which separate doses and/or compositions are administered, the doses and/or compositions are administered simultaneously, consecutively, or spaced over a time span (e.g., <30 minutes, 1 hour, 2 hours, 4 hours, 6 hours, 12 hours, 1 day, 2 days, 3 days, 4 days, 5 days, 6 days, 1 week, or more, or any suitable ranges therebetween).

In some embodiments, beneficial microbes, prebiotics and/or other agents that facilitate the growth of the beneficial microbes, antimicrobial(s) (e.g., antibiotics) directed to kill or inhibit the growth of detrimental microbes, or any of the above mentioned combinations thereof are administered with a treatment for cancer. In embodiments, in which the modulation of microflora itself provides treatment for cancer, suitable co-treatments include immunotherapy, chemotherapy, surgery (e.g., tumor removal), radiation, etc. In other embodiments, in which the modulation of microflora sensitizes a subject or the tumor microenvironment to a particular cancer therapy (e.g., an immunotherapy, a chemotherapy, etc.), the particular cancer therapy is administered (e.g., optionally in addition to one or more other cancer therapies to which the subject is not directly sensitized to by the modulation).

In some embodiments, microflora modulation is provided as a co-therapy (e.g., chemotherapy, immunotherapy, etc.) with one or more additional therapies that target and/or bind to specific cancer or tumor cell markers. Such markers may be selected from the group including but not limited to, epidermal growth factor receptor (EGFR, EGFR1, ErbB-1, HER1). ErbB-2 (HER2/neu), ErbB-3/HER3, ErbB-4/HER4, EGFR ligand family; insulin-like growth factor receptor (IGFR) family, IGF-binding proteins (IGFBPs), IGFR ligand family (IGF-1R); platelet derived growth factor receptor (PDGFR) family, PDGFR ligand family; fibroblast growth factor receptor (FGFR) family, FGFR ligand family, vascular endothelial growth factor receptor (VEGFR) family, VEGF family; HGF receptor family: TRK receptor family; ephrin

(EPH) receptor family: AXL receptor family; leukocyte tyrosine kinase (LTK) receptor family; TIE receptor family, angiopoietin 1, 2; receptor tyrosine kinase-like orphan receptor (ROR) receptor family; discoidin domain receptor (DDR) family; RET receptor family; KLG receptor family; RYK receptor family; MuSK receptor family; Transforming growth factor alpha (TGF-α), TGF-α receptor; Transforming growth factor-beta (TGF-β), TGF-β receptor; Interleukin β receptor alpha2 chain (IL13Ralpha2), Interleukin-6 (IL-6), 1L-6 receptor, interleukin-4, IL-4 receptor, Cytokine receptors, Class I (hematopoietin family) and Class II (interferon/1L-10 family) receptors, tumor necrosis factor (TNF) family, TNF-α, tumor necrosis factor (TNF) receptor superfamily (TNTRSF), death receptor family, TRAIL-receptor; cancer-testis (CT) antigens, lineage-specific antigens, differentiation antigens, alpha-actinin-4, ARTC1, breakpoint cluster region-Abelson (Bcr-abl) fusion products, B-RAF, caspase-5 (CASP-5), caspase-8 (CASP-8), beta-catenin (CTNNB1), cell division cycle 27 (CDC27), cyclin-dependent kinase 4 (CDK4), CDKN2A, COA-1, dek-can fusion protein, EFTUD-2, Elongation factor 2 (ELF2), Ets variant gene 6/acute myeloid leukemia 1 gene ETS (ETC6-AML1) fusion protein, fibronectin (FN), GPNMB, low density lipid receptor/GDP-L fucose: beta-Dgalactose 2-alpha-Lfucosyltraosferase (LDLR/FUT) fusion protein, HLA-A2, MLA-A11, heat shock protein 70-2 mutated (HSP70-2M), KIAA0205, MART2, melanoma ubiquitous mutated 1, 2, 3 (MUM-1, 2, 3), prostatic acid phosphatase (PAP), neo-PAP, Myosin class 1, NFYC, OGT, OS-9, pml-RARalpha fusion protein, PRDX5, PTPRK, K-ras (KRAS2), N-ras (NRAS), HRAS, RBAF600, SIRT12, SNRPD1, SYT-SSX1 or -SSX2 fusion protein, Triosephosphate Isomerase, BAGE, BAGE-1, BAGE-2, 3, 4, 5, GAGE-1, 2, 3, 4, 5, 6, 7, 8, GnT-V (aberrant N-acetyl glucosaminyl transferase V, MGAT5), HERV-K MEL, KK-LC, KM-HN-1, LAGE, LAGE-1, CTL-recognized antigen on melanoma (CAMEL), MAGE-A1 (MAGE-1). MAGE-A2, MAGE-A3, MAGE-A4, MAGE-AS, MAGE-A6, MAGE-A8, MAGE-A9, MAGE-A10. MAGE-All, MAGE-A12, MAGE-3, MAGE-B1, MAGE-B2, MAGE-B5. MAGE-B6, MAGE-C1, MAGE-C2, mucin 1 (MUC1), MART-1/Melan-A (MLANA), gp100, gp100/Pme117 (S1LV), tyrosinase (TYR), TRP-1, HAGE, NA-88, NY-ESO-1, NY-ESO-1/LAGE-2, SAGE, Sp17. SSX-1, 2, 3, 4, TRP2-1NT2, carcino-embryonic antigen (CEA), Kallikrein 4, mammaglobin-A, OA1, prostate specific antigen (PSA), prostate specific membrane antigen, TRP-1/, 75. TRP-2 adipophilin, interferon inducible protein absent in melanorna 2 (AIM-2). BING-4, CPSF, cyclin D1, epithelial cell adhesion molecule (Ep-CAM), EpbA3, fibroblast growth factor-5 (FGF-5), glycoprotein 250

(gp250intestinal carboxyl esterase (iCE), alpha-feto protein (AFP), M-CSF, mdm-2, MUCI, p53 (TP53), PBF, PRAME, PSMA, RAGE-1, RNF43, RU2AS, SOX10, STEAP1, survivin (BIRCS), human telomerase reverse transcriptase (hTERT), telomerase, Wilms' tumor gene (WT1), SYCP1, BRDT, SPANX, XAGE, ADAM2, PAGE-5, LIP1, CTAGE-1, CSAGE, MMA1, CAGE, BORIS, HOM-TES-85, AF15q14, HCA66I, LDHC, MORC, SGY-1, SPO11, TPX1, NY-SAR-35, FTHLI7, NXF2 TDRD1, TEX 15, FATE, TPTE, immunoglobulin idiotypes, Bence-Jones protein, estrogen receptors (ER), androgen receptors (AR), CD40, CD30, CD20, CD19, CD33, CD4, CD25, CD3, cancer antigen 72-4 (CA 72-4), cancer antigen 15-3 (CA 15-3), cancer antigen 27-29 (CA 27-29), cancer antigen 125 (CA 125), cancer antigen 19-9 (CA 19-9), beta-human chorionic gonadotropin, 1-2 microglobulin, squamous cell carcinoma antigen, neuron-specific enolase, heat shock protein gp96. GM2, sargramostim, CTLA-4, 707 alanine proline (707-AP), adenocarcinoma antigen recognized by T cells 4 (ART-4), carcinoembryogenic antigen peptide-1 (CAP-1), calcium-activated chloride channel-2 (CLCA2), cyclophilin B (Cyp-B), human signet ring tumor-2 (HST-2), etc.

Non-limiting examples of cancers that may be treated with the compositions and methods described herein include, but are not limited to: cancer cells from the bladder, blood, bone, bone marrow, brain, breast, colon, esophagus, gastrointestine, gum, head, kidney, liver, lung, nasopharynx, neck, ovary, prostate, skin, stomach, testis, tongue, or uterus. In addition, the cancer may specifically be of the following histological type, though it is not limited to these: neoplasm, malignant; carcinoma; carcinoma, undifferentiated; giant and spindle cell carcinoma; small cell carcinoma; papillary carcinoma; squamous cell carcinoma; lymphoepithelial carcinoma; basal cell carcinoma; pilomatrix carcinoma; transitional cell carcinoma; papillary transitional cell carcinoma; adenocarcinoma; gastrinoma, malignant; cholangiocarcinoma; hepatocellular carcinoma; combined hepatocellular carcinoma and cholangiocarcinoma; trabecular adenocarcinoma; adenoid cystic carcinoma; adenocarcinoma in adenomatous polyp; adenocarcinoma, familial polyposis coli; solid carcinoma; carcinoid tumor, malignant; branchiolo-alveolar adenocarcinoma; papillary adenocarcinoma; chromophobe carcinoma; acidophil carcinoma; oxyphilic adenocarcinoma; basophil carcinoma; clear cell adenocarcinoma; granular cell carcinoma; follicular adenocarcinoma; papillary and follicular adenocarcinoma; nonencapsulating sclerosing carcinoma; adrenal cortical carcinoma; endometroid carcinoma; skin appendage carcinoma; apocrine adenocarcinoma; sebaceous adenocarcinoma; ceruminous

adenocarcinoma; mucoepidermoid carcinoma; cystadenocarcinoma; papillary cystadenocarcinoma; papillary serous cystadenocarcinoma; mucinous cystadenocarcinoma; mucinous adenocarcinoma; signet ring cell carcinoma; infiltrating duct carcinoma; medullary carcinoma; lobular carcinoma; inflammatory carcinoma; paget's disease, mammary; acinar cell carcinoma; adenosquamous carcinoma; adenocarcinoma w/squamous metaplasia; thymoma, malignant; ovarian stromal tumor, malignant; thecoma, malignant; granulosa cell tumor, malignant; and roblastoma, malignant; sertoli cell carcinoma; leydig cell tumor, malignant; lipid cell tumor, malignant; paraganglioma, malignant; extra-mammary paraganglioma, malignant; pheochromocytoma; glomangiosarcoma; malignant melanoma; amelanotic melanoma; superficial spreading melanoma; malig melanoma in giant pigmented nevus; epithelioid cell melanoma; blue nevus, malignant; sarcoma; fibrosarcoma; fibrous histiocytoma, malignant; myxosarcoma; liposarcoma; leiomyosarcoma; rhabdomyosarcoma; embryonal rhabdomyosarcoma; alveolar rhabdomyosarcoma; stromal sarcoma; mixed tumor, malignant; mullerian mixed tumor; nephroblastoma; hepatoblastoma; carcinosarcoma; mesenchymoma, malignant; brenner tumor, malignant; phyllodes tumor, malignant; synovial sarcoma; mesothelioma, malignant; dysgerminoma; embryonal carcinoma; teratoma, malignant; struma ovarii, malignant; choriocarcinoma; mesonephroma, malignant; hemangiosarcoma; hemangioendothelioma, malignant; kaposi's sarcoma; hemangiopericytoma, malignant; lymphangiosarcoma; osteosarcoma; juxtacortical osteosarcoma; chondrosarcoma; chondroblastoma, malignant; mesenchymal chondrosarcoma; giant cell tumor of bone; ewing's sarcoma; odontogenic tumor, malignant; ameloblastic odontosarcoma; ameloblastoma, malignant; ameloblastic fibrosarcoma; pinealoma, malignant; chordoma; glioma, malignant; ependymoma; astrocytoma; protoplasmic astrocytoma; fibrillary astrocytoma; astroblastoma; glioblastoma; oligodendroglioma; oligodendroblastoma; primitive neuroectodermal; cerebellar sarcoma; ganglioneuroblastoma; neuroblastoma; retinoblastoma; olfactory neurogenic tumor; meningioma, malignant; neurofibrosarcoma; neurilemmoma, malignant; granular cell tumor, malignant; malignant lymphoma; Hodgkin's disease; Hodgkin's lymphoma; paragranuloma; malignant lymphoma, small lymphocytic; malignant lymphoma, large cell, diffuse; malignant lymphoma, follicular; mycosis fungoides; other specified non-Hodgkin's lymphomas; malignant histiocytosis; multiple myeloma; mast cell sarcoma; immunoproliferative small intestinal disease; leukemia; lymphoid leukemia; plasma cell leukemia; erythroleukemia; lymphosarcoma

cell leukemia; myeloid leukemia; basophilic leukemia; eosinophilic leukemia; monocytic leukemia; mast cell leukemia; megakaryoblastic leukemia; myeloid sarcoma; and hairy cell leukemia. In some embodiments, the cancer is a melanoma (e.g., metastatic malignant melanoma), renal cancer (e.g. clear cell carcinoma), prostate cancer (e.g. hormone refractory prostate adenocarcinoma), pancreatic cancer (e.g., adenocarcinoma), breast cancer, colon cancer, gallbladder cancer, lung cancer (e.g. non-small cell lung cancer), esophageal cancer, squamous cell carcinoma of the head and neck, liver cancer, ovarian cancer, cervical cancer, thyroid cancer, glioblastoma, glioma, leukemia, lymphoma, and other neoplastic malignancies. In some embodiments, the cancer is a solid tumor cancer.

In some embodiments, the methods provided herein relate to the treatment and/or prevention of a leukemia. The term "leukemia" is meant broadly progressive, malignant diseases of the hematopoietic organs/systems and is generally characterized by a distorted proliferation and development of leukocytes and their precursors in the blood and bone marrow. Non-limiting examples of leukemia diseases include, acute nonlymphocytic leukemia, chronic lymphocytic leukemia, acute granulocytic leukemia, chronic granulocytic leukemia, acute promyelocytic leukemia, adult T-cell leukemia, aleukemic leukemia, a leukocythemic leukemia, basophilic leukemia, blast cell leukemia, bovine leukemia, chronic myelocytic leukemia, leukemia cutis, embryonal leukemia, eosinophilic leukemia, Gross' leukemia, Rieder cell leukemia, Schilling's leukemia, stem cell leukemia, subleukemic leukemia, undifferentiated cell leukemia, hairy-cell leukemia, hemoblastic leukemia, hemocytoblastic leukemia, histiocytic leukemia, stem cell leukemia, acute monocytic leukemia, leukopenic leukemia, lymphatic leukemia, lymphoblastic leukemia, lymphocytic leukemia, lymphogenous leukemia, lymphoid leukemia, lymphosarcoma cell leukemia, mast cell leukemia, megakaryocytic leukemia, micromyeloblastic leukemia, monocytic leukemia, myeloblastic leukemia, myelocytic leukemia, myeloid granulocytic leukemia, myelomonocytic leukemia, Naegeli leukemia, plasma cell leukemia, plasmacytic leukemia, and promyelocytic leukemia.

In some embodiments, the methods provided herein relate to the treatment and/or prevention of a carcinoma. The term "carcinoma" refers to a malignant growth made up of epithelial cells tending to infiltrate the surrounding tissues, and/or resist physiological and non-physiological cell death signals and gives rise to metastases. Non-limiting exemplary types of carcinomas include, acinar carcinoma, acinous carcinoma, adenocystic carcinoma, adenoid cystic

carcinoma, carcinoma adenomatosum, carcinoma of adrenal cortex, alveolar carcinoma, alveolar cell carcinoma, basal cell carcinoma, carcinoma basocellulare, basaloid carcinoma, basosquamous cell carcinoma, bronchioalveolar carcinoma, bronchiolar carcinoma, bronchogenic carcinoma, cerebriform carcinoma, cholangiocellular carcinoma, chorionic carcinoma, colloid carcinoma, comedo carcinoma, corpus carcinoma, cribriform carcinoma, carcinoma en cuirasse, carcinoma cutaneum, cylindrical carcinoma, cylindrical cell carcinoma, duct carcinoma, carcinoma durum, embryonal carcinoma, encephaloid carcinoma, epiennoid carcinoma, carcinoma epitheliale adenoides, exophytic carcinoma, carcinoma ex ulcere, carcinoma fibrosum, gelatiniform carcinoma, gelatinous carcinoma, giant cell carcinoma, signet-ring cell carcinoma, carcinoma simplex, small-cell carcinoma, solanoid carcinoma, spheroidal cell carcinoma, spindle cell carcinoma, carcinoma spongiosum, squamous carcinoma, squamous cell carcinoma, string carcinoma, carcinoma telangiectaticum, carcinoma telangiectodes, transitional cell carcinoma, carcinoma tuberosum, tuberous carcinoma, verrucous carcinoma, carcinoma villosum, carcinoma gigantocellulare, glandular carcinoma, granulosa cell carcinoma, hair-matrix carcinoma, hematoid carcinoma, hepatocellular carcinoma, Hurthle cell carcinoma, hyaline carcinoma, hypernephroid carcinoma, infantile embryonal carcinoma, carcinoma in situ, intraepidermal carcinoma, intraepithelial carcinoma, Krompecher's carcinoma, Kulchitzky-cell carcinoma, large-cell carcinoma, lenticular carcinoma, carcinoma lenticulare, lipomatous carcinoma, lymphoepithelial carcinoma, carcinoma medullare, medullary carcinoma, melanotic carcinoma, carcinoma molle, mucinous carcinoma, carcinoma muciparum, carcinoma mucocellulare, mucoepidermoid carcinoma, carcinoma mucosum, mucous carcinoma, carcinoma myxomatodes, naspharyngeal carcinoma, oat cell carcinoma, carcinoma ossificans, osteoid carcinoma, papillary carcinoma, periportal carcinoma, preinvasive carcinoma, prickle cell carcinoma, pultaceous carcinoma, renal cell carcinoma of kidney, reserve cell carcinoma, carcinoma sarcomatodes, schneiderian carcinoma, scirrhous carcinoma, and carcinoma scroti.

In some embodiments, the methods provided herein relate to the treatment and/or prevention of a sarcoma. The term "sarcoma" generally refers to a tumor which is made up of a substance like the embryonic connective tissue and is generally composed of closely packed cells embedded in a fibrillar, heterogeneous, or homogeneous substance. Sarcomas include, but are not limited to, chondrosarcoma, fibrosarcoma, lymphosarcoma, melanosarcoma, myxosarcoma, osteosarcoma, endometrial sarcoma, stromal sarcoma, Ewing' s sarcoma, fascial sarcoma,

fibroblastic sarcoma, giant cell sarcoma, Abemethy's sarcoma, adipose sarcoma, liposarcoma, alveolar soft part sarcoma, ameloblastic sarcoma, botryoid sarcoma, chloroma sarcoma, chorio carcinoma, embryonal sarcoma, Wilms' tumor sarcoma, granulocytic sarcoma, Hodgkin's sarcoma, idiopathic multiple pigmented hemorrhagic sarcoma, immunoblastic sarcoma of B cells, lymphoma, immunoblastic sarcoma of T-cells, Jensen's sarcoma, Kaposi's sarcoma, Kupffer cell sarcoma, angiosarcoma, leukosarcoma, malignant mesenchymoma sarcoma, parosteal sarcoma, reticulocytic sarcoma, Rous sarcoma, serocystic sarcoma, synovial sarcoma, and telangiectaltic sarcoma.

Additional exemplary neoplasias that can be treated and/or prevented using the methods described herein include Hodgkin's Disease, Non-Hodgkin's Lymphoma, multiple myeloma, neuroblastoma, breast cancer, ovarian cancer, lung cancer, rhabdomyosarcoma, primary thrombocytosis, primary macroglobulinemia, small-cell lung tumors, primary brain tumors, stomach cancer, colon cancer, malignant pancreatic insulanoma, malignant carcinoid, premalignant skin lesions, testicular cancer, lymphomas, thyroid cancer, neuroblastoma, esophageal cancer, genitourinary tract cancer, malignant hypercalcemia, cervical cancer, endometrial cancer, and adrenal cortical cancer.

In some embodiments, the cancer treated and/or prevented is a melanoma. The term "melanoma" is taken to mean a tumor arising from the melanocytic system of the skin and other organs. Non-limiting examples of melanomas are Harding-Passey melanoma, juvenile melanoma, lentigo maligna melanoma, malignant melanoma, acral-lentiginous melanoma, amelanotic melanoma, benign juvenile melanoma, Cloudman's melanoma, S91 melanoma, nodular melanoma subungal melanoma, and superficial spreading melanoma.

Particular categories of tumors that can be treated and/or prevented using methods described herein include lymphoproliferative disorders, breast cancer, ovarian cancer, prostate cancer, cervical cancer, endometrial cancer, bone cancer, liver cancer, stomach cancer, colon cancer, pancreatic cancer, cancer of the thyroid, head and neck cancer, cancer of the central nervous system, cancer of the peripheral nervous system, skin cancer, kidney cancer, as well as metastases of all the above. Particular types of tumors include hepatocellular carcinoma, hepatoma, hepatoblastoma, rhabdomyosarcoma, esophageal carcinoma, thyroid carcinoma, ganglioblastoma, fibrosarcoma, myxosarcoma, liposarcoma, chondrosarcoma, osteogenic sarcoma, chordoma, angiosarcoma, endotheliosarcoma, Ewing's tumor, leimyosarcoma,

rhabdotheliosarcoma, invasive ductal carcinoma, papillary adenocarcinoma, melanoma, pulmonary squamous cell carcinoma, basal cell carcinoma, adenocarcinoma (well differentiated, moderately differentiated, poorly differentiated or undifferentiated), bronchioloalveolar carcinoma, renal cell carcinoma, hypernephroma, hypernephroid adenocarcinoma, bile duct carcinoma, choriocarcinoma, seminoma, embryonal carcinoma, Wilms' tumor, testicular tumor, lung carcinoma including small cell, non-small and large cell lung carcinoma, bladder carcinoma, glioma, astrocyoma, medulloblastoma, craniopharyngioma, ependymoma, pinealoma, retinoblastoma, neuroblastoma, colon carcinoma, rectal carcinoma, hematopoietic malignancies including all types of leukemia and lymphoma including: acute myelogenous leukemia, acute myelocytic leukemia, acute lymphocytic leukemia, chronic myelogenous leukemia, chronic lymphocytic leukemia, mast cell leukemia, multiple myeloma, myeloid lymphoma, Hodgkin' s lymphoma, non-Hodgkin' s lymphoma.

Cancers prevented and/or treated in certain embodiments also include precancerous lesions, e.g. actinic keratosis (solar keratosis), moles (dysplastic nevi), acitinic chelitis (farmer's lip), cutaneous horns, Barrett's esophagus, atrophic gastritis, dyskeratosis congenita, sideropenic dysphagia, lichen planus, oral submucous fibrosis, actinic (solar) elastosis and cervical dysplasia.

Cancers prevented and/or treated in some embodimentsinclude non-cancerous or benign tumors, e.g. of endodermal, ectodermal or mesenchymal origin, including, but not limited to cholangioma, colonic polyp, adenoma, papilloma, cystadenoma, liver cell adenoma, hydatidiform mole, renal tubular adenoma, squamous cell papilloma, gastric polyp, hemangioma, osteoma, chondroma, lipoma, fibroma, lymphangioma, leiomyoma, rhabdomyoma, astrocytoma, nevus, meningioma, and ganglioneuroma.

Some embodiments described herein are particularly useful for the treatment of tumors that do not otherwise respond to immunotherapeutic approaches. In some embodiments, such tumors are non-responsive (or have a reduced response) to T cells (e.g., prevent infiltration of one or more T cell types (e.g., $CD8^+$ T cells) or antigen presenting cells (e.g., dendritic cells (e.g., $CD103^+$DCs, etc.), etc.). In some embodiments, compositions and methods described herein find use in the treatment of cancers in which T cells are not appropriately primed against tumor-associated antigens.

In some embodiments, methods are provided for testing sample (e.g., cell, tissue, population of cells, tumor, blood, urine, saliva, etc.) from a subject for one or more biomarkers

of cancer, immune evasion, cancer promoting microenvironment, malignancy-promoting microenvironment, etc. Such biomarkers may comprise nucleic acids, small molecules, proteins, peptides, etc., and may be detected using any suitable assay of technique. In some embodiments, provided herein are DNA-, RNA-, small molecule, and/or protein-based diagnostic methods that either directly or indirectly detect the biomarkers of the evasion of immune response or immunotherapy by cancer cells or tumors. The present invention also provides compositions, reagents, and kits for such diagnostic purposes.

In some embodiments, biomarkers are detected at the nucleic acid (e.g., RNA) level. For example, the presence or amount of biomarker nucleic acid (e.g.,mRNA) in a sample is determined (e.g., to determine the presence or level of biomarker expression). Biomarker nucleic acid (e.g., RNA, amplified cDNA, etc.) may be detected/quantified using a variety of nucleic acid techniques known to those of ordinary skill in the art, including but not limited to nucleic acid sequencing, nucleic acid hybridization, nucleic acid amplification (e.g., by PCR, RT-PCR, qPCR, etc.), micorarray, Southern and Northern blotting, sequencing, etc. Non-amplified or amplified nucleic acids can be detected by any conventional means. For example, in some embodiments, nucleic acids are detected by hybridization with a detectably labeled probe and measurement of the resulting hybrids. Nucleic acid detection reagents may be labeled (e.g., fluorescently) or unlabeled, and may by free in solution or immobilized (e.g., on a bead, well, surface, chip, etc.).

In some embodiments, biomarkers are detected at the protein level. For example, the presence or amount of biomarker protein in a sample is determined (e.g., to determine the presence or level of biomarker expression or localization). In some embodiments, reagents are provided for the detection and/or quantification of biomarker proteins. Suitable reagents include primary antibodies (e.g., that bind to the biomarkers), secondary antibodies (e.g., that bind primary antibodies), antibody fragments, aptamers, etc. Protein detection reagents may be labeled (e.g., fluorescently) or unlabeled, and may by free in solution or immobilized (e.g., on a bead, well, surface, chip, etc.).

In some embodiments, kits are provided comprising, for example, the probiotic or microflora transplant compositions described herein. Kits may further comprise instructions, cancer treatments, other probiotics, agents to enhance integration of microbes into the subject's microflora, etc.

EXPERIMENTAL

Example 1

Materials and methods:

Animals and tumor model: C57BL/6 mice were obtained from Jackson laboratory or Taconic farms. 6–8-week-old female mice were used. The C57BL/6-derived melanoma cell line B16.F10.SIY (referred to herein as B16.SIY) was generated (Blank et al. Cancer research 64, 1140-1145 (2004).; herein incorporated by reference in its entirety). For tumor growth experiments, mice were injected subcutaneously with 1×10^6 B16.SIY tumor cells. Tumor size was measured twice a week until endpoint and tumor volume was determined as length × width^2 × 0.5. For B16 parental tumor model experiments, mice were injected subcutaneously with 1×10^6 B16.F10 tumor cells. For bladder cancer model experiments, mice were injected subcutaneously with $2x10^6$ MB49 cells. All experimental animal procedures were approved by the University of Chicago Animal Care and Use Committee (IACUC).

IFN-γ ELISPOT and SIY Pentamer analyses: Elispot plates (*Millipore, MAIP S4510*) were coated with purified αIFN-γ (*BD*) overnight at 4 °C. Plates were blocked with 10%FBS in DMEM for 2 hours at room temperature. Whole splenocytes were plated at 10^6 cells per well and stimulated with SIY peptide overnight at 37°C. Spots were developed using the BD mouse IFN-γ kit (*Cat. No. 552569*), and the number of spots was measured using an Immunospot Series 3 Analyzer and analyzed using ImmunoSpot software (Cellular Technology). For pentamer staining, cells were labeled with PE-MHC class I pentamer (*Proimmune*) consisting of murine H-2K^b complexed to SIYRYYGL (SIY) peptide or to control SIINFEKL peptide, and stained with CD3-AX700 (*Ebioscience, 17A2*), CD8-PacBlue (*Biolegend, 53-6.7*), CD4-APC (*Pharmingen, RM4-5*), CD62L-PECy7 (*Ebioscience, MEL-14*), CD44-FITC (*BD, IM7*) and Fixable Viability-ef780 (*Ebioscience*). Stained cells were analyzed using an LSR II cytometer with FACSDiva software (BD). Data analysis was conducted with FlowJo software (Tree Star).

Fecal transfers and αPD-L1 mAb immunotherapy: Fecal pellets from JAX and TAC-derived mice were collected upon arrival in our facility and each fecal pellet was resuspended in 1 ml of phosphate-buffered saline (PBS). The supernatant from each fecal pellet was used for oral gavage of two recipient mice, 100μl per gavage. For prophylactic fecal transfer experiments

mice were gavaged with JAX or TAC fecal suspensions once a week for two weeks prior to tumor inoculation. For therapeutic fecal transfer experiments, mice were gavaged on days 7 and 14 post tumor implantation. For combination therapy experiments, mice were additionally injected intraperitoneally with 100μg αPD-L1 mAb (*BioXCell*) in 100μl PBS on days 7, 10, 13 and 16 post-tumor implantation.

Microbial DNA analysis: Bacterial DNA was extracted from murine fecal pellets using PowerSoil®-htp 96 Well Soil DNA Isolation Kit (MoBio cat.# 12955-4). The V4-V5 region of the 16S rRNA encoding gene was amplified (earthmicrobiome.org/emp-standard-protocols/; Earth Microbiome Project, 2011) and sequenced at the High-Throughput Genome Analysis Core at Argonne National Laboratory. Quantitative Insights Into Microbial Ecology (QIIME) was used to trim and classify sequences (Caporaso et al. Bioinformatics 26, 266-267 (2010).; herein incorporated by reference in its entirety); specifically, the open reference OTU picking protocol was used at 97% sequence identity against the Greengenes database (05/13 release)(McDonald et al. The ISME journal 6, 610-618 (2012).;herein incorporated by reference in its entirety). PYNAST was used to align sequences (Caporaso et al. Nat Meth 7, 335-336 (2010).; herein incorporated by reference in its entirety) and RDP Classifier was used for taxonomic assignment (Wang et al. Appl Environ Microbiol 73, 5261-5267 (2007).; herein incorporated by reference in its entirety). Community structure was compared using weighted and unweighted UniFrac distances (Lozupone et al. Appl Environ Microbiol 71, 8228-8235 (2005).; herein incorporated by reference in its entirety). G-test was performed to determine differences in bacterial taxa occurrence between fecal communities. Prinicipal Coordinate Analysis (PCoA) ordination were generated to visually compare beta diversity and Analysis of Similarity (ANOSIM) test statistics were performed to statistically compare within- to between-group similarity in QIIME.

Bacterial administration and heat inactivation: A cocktail of lyophilized *Bifidobacterium* species (*B. bifidum, B. longum, B. lactis and B. breve*, Seeking Health) were resuspended in PBS at $5x10^9$ CFU/ml. Each mouse was given 200μl of *Bifidobacterium* ($1x10^9$ CFU/mouse) by oral gavage 7 and 14 days following tumor inoculation. Heat inactivation was performed by boiling rehydrated bifidobacteria at 100°C for 2 hours. Heat-treated and live bifidobacteria were serially diluted in reduced PBS and plated on reduced clostridial medium (RCM) agar in anaerobic conditions. Plates were subsequently incubated in an anaerobic chamber for three days to test

efficacy of killing. *Lactobacillus murinus* was cultured in MRS broth overnight, then washed and resuspended in PBS at $5x10^{10}$ CFU/ml. Each mouse was orally gavaged with 100µl of bacterial suspension ($5x10^9$ CFU/mouse) 7 and 14 days following tumor inoculation.

CFSE-labeled 2C CD8$^+$ T cell adoptive transfer: CD8$^+$ T cells were isolated from the spleen and lymph node of naïve CD45.1/.2$^+$ 2C TCR Tg mice using the MACS CD8 T cell Isolation Kit (*Miltenyi, Cat No. 130-095-236*), labeled with 2.5 mM CFSE and injected i.v. into CD45.2$^+$ C57BL/6 mice derived from either JAX or TAC. 24 hours later, mice were inoculated with $1x10^6$ B16.SIY melanoma cells s.c. Seven days post-adoptive T cell transfer, spleen and tumor-draining lymph node were harvested and restimulated ex-vivo with SIY peptide in the presence of brefeldin A. Samples were stained with Fixable Viability-ef780 (*Ebioscience*), CD45.1-PerCPCy5.5 (*Ebioscience, E20*), CD45.2-APC (*Ebioscience, 104*), CD3-AX700 (*Ebioscience, 17A2*), CD8-BV711 (*Biolegend, 53-6.7*), CD4-BV605 (*Biolegend, RM4-5*) and IFN-γ-PE (*BD, XMG1.2*). Intracellular IFN-γ production and CFSE dilution were assessed in gated CD45.1/.2$^+$ 2C CD8$^+$ T cells by flow cytometry.

Dendritic cell sorting and gene expression profiling: TAC mice were gavaged with *Bifidobacterium* once a week for two weeks. *Bifidobacterium*-fed mice, newly arrived JAX mice, and newly arrived TAC mice were inoculated subcutaneously in both flanks with $5x10^6$ DRAQ5-labeled B16.SIY tumor cells. 40hrs following tumor implantation, whole tumors including infiltrating immune cells were digested in collagenase (*Worthington*) and filtered into single cell suspensions. Samples from 5 mice in each group were pooled and subsequently stained with Fixable Viability-ef506 (*Ebioscience*), CD45-AF488 (*Bioloegend, 30-F11*), CD3-ef450 (*Ebioscience, 145-2C11*), CD19-PB (*Ebioscience, 1D3*), I-A/I-E-PECy7 (*Biolegend, M5/114.15.2*), CD11c-PE (*Ebioscience, N418*) and CD11b-PerCpCy5.5 (*BD, M1/70*). Live CD45$^+$CD3$^-$CD19$^-$MHCII^{hi}CD11c$^+$ dendritic cells were sorted directly into RLT Buffer (*Qiagen*) using FACSAriaIII (*BD*) and stored immediately on dry ice. Total RNA was isolated using RNeasy® Micro kit (*Qiagen*). RNA was submitted to the Functional Genomics Facility at the University of Chicago for gene expression profiling. RNA integrity and concentration were assessed using an Agilent Bioanalyzer 2100, and all RNA samples used for microarray analysis had an RNA Integrity Number > 9.0. Total RNA was processed into biotinylated cRNA using the Epicentre TargetAmp™ 2-Round Biotin-aRNA Amplification Kit 3.0 (*TAB2R71024*). The

cRNA was hybridized to Illumina MouseRef8v2 arrays using Illumina provided protocols and scanned using an Illumina HiScan. Quantile normalized and background subtracted values were subsequently analyzed using R. Genes whose expression value was under 10 were removed from the analysis. Mean fold-change in gene transcript levels between JAX samples relative to TAC, and BIF samples relative to TAC were calculated, and genes whose fold change was over 1.5 in both comparisons (761 gene transcripts) were inputted into *The **D**atabase for **A**nnotation, **V**isualization and **I**ntegrated **D**iscovery (**DAVID**)* v6.7 for pathway analysis. Genes found to be significantly enriched ($p<0.05$) for immune function were then plotted in a heatmap using R software.

Statistical analysis: Tumor growth curves were analyzed using two-way ANOVA, with either Sidak's multiple comparisons posttest for comparison of two groups, or Tukey's multiple comparisons posttest for comparison of more than two groups. For comparisons other than tumor growth, Mann Whitney's non-parametric T-test was used when comparing two groups and one-way ANOVA with Tukey's multiple comparisons posttest was used when comparing more than two groups. $P<0.05$ was considered statistically significant and denoted as follows: $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$, $^{****}p < 0.0001$. Statistical analysis was performed using GraphPad PRISM.

Example 2

Results

Experiments were conducted during development of embodiments of the present invention to test whether differences in the specific composition of the normal microbiota influence the immune response to a growing tumor in vivo. Subcutaneous B16.SIY melanoma growth was observed in genetically similar C57BL/6 mice derived from two different mouse facilities, Jackson Laboratory (JAX) and Taconic Farms (TAC), which have been shown to differ in their commensal microbes (Ivanov et al. Cell 139, 485-498 (2009).; herein incorporated by reference in its entirety). It was found that JAX and TAC mice exhibited significant differences in B16.SIY melanoma growth rate, with tumors growing more aggressively in TAC mice (Figure 1A). To evaluate whether this difference was immune-mediated, tumor antigen-specific T cell responses, as well as T cell accumulation in the tumor microenvironment, were assessed. In fact, tumor-specific T cell responses were significantly higher in JAX mice (Figure 1B and 1C), and

markedly increased numbers of tumor-infiltrating T cells were observed (Figure 1D). To begin to address whether this difference could be mediated by commensal microbiota, JAX and TAC mice were co-housed for 3 weeks prior to tumor implantation. It was found that co-housing ablated the differences in tumor growth (Figure 1E) and immune responses (Figure 1F-H) between the two mouse populations, arguing for an environmental influence. Notably, TAC mice appeared to acquire the JAX phenotype upon cohousing, indicating that JAX mice might be colonized by commensal microbes that dominantly facilitate improved anti-tumor immunity.

To directly test the role of commensal bacteria in regulating anti-tumor immunity, JAX fecal suspensions or control TAC fecal suspensions were transferred into TAC recipients by oral gavage prior to tumor implantation Figure 5A). Strikingly, it was found that prophylactic transfer of JAX fecal material into TAC recipients was sufficient to delay tumor growth (Figure 2A) and enhance induction and infiltration of tumor-specific $CD8^+$ T cells (Figure 2B-C and 5B), supporting a microbe- or microbial product-derived effect. Reciprocal transfer of TAC fecal material into JAX recipients resulted in only a minimal increase in tumor growth rate and did not significantly alter anti-tumor T cell responses (Figure 2A-C and Figure 5B).

To test whether manipulation of the microbial community could be effective as a therapy, we administered JAX fecal material alone or in combination with antibodies targeting PD-L1 (αPD-L1) to TAC mice bearing established tumors. Transfer of JAX fecal material alone resulted in significantly slower tumor growth (Figure 2D), accompanied by increased tumor-specific T cell responses (Figure 2E) and infiltration of antigen-specific T cells into the tumor (Figure 2F), to the same degree as treatment with systemic αPD-L1 mAb. Combination treatment with both JAX fecal transfer and αPDL1 mAb improved tumor control (Figure 2D) and circulating tumor antigen-specific T cell responses (Figure 2E), while there was little additive effect on accumulation of activated T cells within the tumor microenvironment (Figure 2F). Consistent with these results, αPD-L1 therapy alone was significantly more efficacious in JAX mice compared to TAC mice (Figure 2G), which paralleled improved anti-tumor T cell responses (Figure 5C). These data indicate that the commensal microbial composition can influence spontaneous anti-tumor immunity as well as response to immunotherapy with αPD-L1 mAb.

To identify specific bacteria associated with protective anti-tumor immune responses, the fecal bacterial content in mice obtained from TAC mice, JAX mice, and JAX-fed and TAC-fed TAC mice we compared using the 16S ribosomal RNA (rRNA) miSeq Illumina platform.

Overall, 933.9 ± 55.2 taxa were identified in TAC mice and 653.4 ± 60 taxa were identified in JAX mice, demonstrating decreased species diversity in mice obtained from JAX. TAC mice that were orally administered JAX fecal material showed decreased taxa diversity (706.6 ± 117.9, p=0.006) similar to JAX mice, whereas TAC mice that were administered TAC fecal material did not show altered diversity (895.7 ± 118, p=1, Figure 3A). Principal coordinate analysis revealed that fecal samples analyzed from TAC mice that received JAX fecal material co-clustered separately from samples from control TAC mice and were more similar to samples obtained from JAX mice (Figure 3B, and became similar to samples obtained from sham and JAX feces-inoculated JAX mice (Figure 8A). In contrast, TAC-inoculated TAC mice did not change in community diversity relative to sham-inoculated TAC mice (p=0.4, ANOSIM). Analysis of similarity confirmed that TAC mice fed with JAX fecal material were more similar to each other than to TAC mice that were given TAC fecal material (p=0.008) or mice obtained from TAC (p=0.002). Reciprocal transfer of TAC fecal material into JAX hosts resulted in a statistically significant change in community diversity (p=0.003, ANOSIM), yet the distance of the microbial shift was smaller (Figure 8A).

Comparative analysis of specific bacterial taxa showed that 97 taxa were significantly more abundant in JAX mice relative to TAC mice (FDR<0.05) (Figure 8B), and 51 taxa were significantly increased in JAX-fed TAC mice relative to TAC-fed TAC mice (p<0.05). Only 32 taxa overlapped between these two comparisons, such that they were of greater abundance in both JAX mice and JAX-fed TAC mice. A significant association was observed for *Bifidobacterium*, which showed a positive association with anti-tumor T cell responses and increased in relative abundance over 400-fold in JAX-fed TAC mice (Figure 8C). Members belonging to several of these groups were similarly altered in JAX-fed TAC mice relative to sham- or TAC-inoculated TAC mice (Figure 8C). These included several unidentified taxa from the family S24-7 of the order *Bacteroidales*, one unassigned taxon, and four taxa with genus-level identifications, all of which are anaerobic gram-positive bacteria. Of these, the two most significant differentially abundant taxa belong to the *Bifidobacterium* genus, with the top *Bifidobacterium* taxon being over 200-fold more abundant in JAX relative to TAC (p=0.001), and similarly abundant in JAX-fed mice but not detected at all in TAC-fed TAC mice (p=0.01) (Figure 3C). Comparison of relative abundance of all taxa combined belonging to the *Bifidobacterium* genus yielded similar results (Figure 6A). Given that interactions between

bifidobacteria and the host immune system have been described previously (Lopez et al. International journal of food microbiology 138, 157-165 (2010).; Ménard et al. Applied and Environmental Microbiology 74, 660-666 (2008).; Dong et al. Early human development 86, 51-58 (2010).; herein incorporated by reference in their entireties), it was contemplated that members of this genus represent one source of the beneficial anti-tumor immune effects observed in JAX mice.

At the sequence level, *Bifidobacterium* operational taxonomic unit OTU_681370 showed the largest increase in relative abundance in JAX-fed TAC mice and the strongest association with anti-tumor T cell responses across all permutations (Figure 8D). This bacterium was further identified as most similar to *B. breve*, *B. longum* and *B. adolescentis* (99% identity). To test whether *Bifidobacterium* spp. may be sufficient to augment protective immunity against tumors, a commercially available cocktail of *Bifidobacterium* species was obtained, which included *B. breve* and *B. longum* and administered this by oral gavage, alone or in combination with αPD-L1, to TAC 7 recipients bearing established tumors. Analysis of fecal bacterial content revealed that the most significant change in response to *Bifidobacterium* inoculation occurred in the *Bifidobacterium* genus ($p=0.0009$, FDR=0.015, non-parametric t-test), with a 120-fold increase in OTU_681370 (Figure 9A), indicating that the commercial inoculum contained bacteria that were at least 97% identical to the taxon identified in JAX and JAX-fed TAC mice. An increase in *Bifidobacterium* could also be detected by quantitative PCR (Figure 9B).

Bifidobacterium-treated mice displayed significantly improved tumor control in comparison to non-*Bifidobacterium* treated counterparts (Figure 8E), which was accompanied by robust induction of tumor-specific T cells in the periphery (Figure 8F) and increased accumulation of antigen-specific CD8+ T cells within the tumor (Figure 8G and Figure 9C). These effects were durable for several weeks (Figure 9D-E).

The therapeutic effect of *Bifidobacterium* feeding was abrogated in CD8-depleted mice (Figure 10A), suggesting that the mechanism was not direct but rather through host anti-tumor T cell responses. Heat inactivation of the bacteria prior to oral administration also abrogated the therapeutic effect on tumor growth and reduced tumor-specific T cell responses to baseline (Figure 10B-D), suggesting that the anti-tumor effect requires live bacteria. As an alternative strategy, the therapeutic effect of *B. breve* and *B. longum* strains obtained from the ATCC was tested, which also showed significantly improved tumor control (Figure 11A). Administration of

Bifidobacterium to TAC mice inoculated with B16 parental tumor cells or MB49 bladder cancer cells also resulted in delayed tumor outgrowth (Figure 11, B and C respectively). Oral administration of *Lactobacillus murinus* to TAC mice, which was not among the overrepresented taxa in JAX-fed mice, had no effect on tumor growth (Figure 11D) or on tumor-specific T cell responses (Figure 11E), suggesting that modulation of anti-tumor immunity depends on the specific bacteria administered. Collectively, these data point to *Bifidobacterium* as a positive regulator of anti-tumor immunity in vivo.

Upon inoculation with *Bifidobacterium*, a small set of species were altered in parallel with *Bifidobacterium* (ANOSIM, p=0.003, Figure 12A), however, they largely did not resemble the changes observed with JAX-feces administration. Although reductions were observed (~2-10 fold) in members of the order *Clostridiales* as well as in butyrate-producing species upon *Bifidobacterium* inoculation, which could point to an inhibitory effect on the regulatory T cell compartment, no difference was observed in the frequency of $CD4^+$ $Foxp3^+$ T cells in tumors isolated from JAX and TAC mice (Figure 12B). Thus, it is unlikely that *Bifidobacterium* is acting primarily through modulation of the abundance of other bacteria.

It was next assessed whether translocation of *Bifidobacterium* was occurring into the mesenteric lymph nodes, spleen or tumor, however no *Bifidobacterium* was detected in any of the organs isolated from *Bifidobacterium*-gavaged tumor-bearing mice (Figure 12C). It was thus concluded that the observed systemic immunological effects are occurring independently of bacterial translocation.

To test whether *Bifidobacterium spp* may be sufficient to augment protective immunity against tumors, we administered a combination of four *Bifidobacterium* species was administered by oral gavage, alone or in combination with αPD-L1, to TAC recipients bearing 7-day established tumors. *Bifidobacterium*-treated mice displayed significantly improved tumor control in comparison to non-*Bifidobacterium* treated counterparts (Figure 3D), which was accompanied by robust induction of tumor-specific T cells in the periphery (Figure 3E) and markedly increased accumulation of antigen-specific $CD8^+$ T cells within the tumor (Figure 3F). This therapeutic effect was completely abrogated in CD8-depleted mice (Figure 3G), arguing that the mechanism was not direct but rather through host anti-tumor T cell responses. Heat inactivation of the bacteria prior to oral administration also abrogated the therapeutic effect on tumor growth and reduced tumor-specific T cell responses to baseline (Figure 6B-D), indicating

that the anti-tumor effect requires live bacteria. Administration of *Bifidobacterium* to TAC mice inoculated with B16 parental tumor cells or MB49 bladder cancer cells also resulted in delayed tumor outgrowth (Figure 6E-F). Oral administration of *Lactobacillus murinus* to TAC mice, which was not among the overrepresented taxa in JAX or JAX-fed mice, had no effect on tumor growth (Figure 6G) nor on tumor-specific T cell responses (Figure 6H), indicating that modulation of commensal bacterial communities through introduction of new bacteria in itself does not induce immunity to tumors, but rather immunity depends on the specific bacteria administered. Collectively, data identify *Bifidobacterium* as a positive regulator of anti-tumor immunity in vivo.

To interrogate mechanisms underlying the observed differences in T cell responses between JAX and TAC mice, we transferred CFSE-labeled SIY-specific 2C TCR Tg T cells into tumor-bearing mice and tested their proliferation and acquisition of IFN-γ production ex vivo (Figure 7A). $CD8^+$ SIY-specific 2C TCR Tg T cells exposed to tumors in JAX mice exhibited similar expansion in the tumor-draining lymph node as compared to their counterparts in TAC mice (Figure 7B). However, they produced markedly greater IFN-γ in both the tumor draining lymph node and the spleen of JAX tumor-bearing mice (Figure 4A and 4B), suggesting that signals upstream of T cells in the JAX environment enhanced acquisition of T cell effector function. These data indicated an improvement in immune responses upstream of T cells, at the level of host dendritic cells (DCs). Genome-wide transcriptional profiling of early tumor-infiltrating DCs isolated from JAX, TAC and *Bifidobacterium*-treated TAC mice was employed (Figure 13A). In total, there were 761 gene transcripts upregulated by ≥1.5-fold in both JAX and *Bifidobacterium*-treated TAC-derived DCs relative to DCs from untreated TAC mice (Figure 4C). Pathway analysis identified cytokine-cytokine receptor interaction, T cell activation, and positive regulation of mononuclear cell proliferation as significantly enriched pathways among upregulated genes (Figure 4C and Figure 13B). Many of these genes have been shown to be critical for anti-tumor responses including those involved in $CD8^+$ T cell activation and costimulation (*H2-m2*(MHC-I), *Cd40*, *Cd70*, *Icam1)*) (Mackey et al. Journal of immunology (Baltimore, Md. : 1950) 161, 2094-2098 (1998).; Scholer et al. Immunity 28, 258-270 (2008).; Bak et al. Journal of immunology (Baltimore, Md. : 1950) 189, 1708-1716 (2012).; herein incorporated by reference in their entireties), DC maturation (*Relb, Ifngr2*) (Pan et al. Immunology letters 94, 141-151 (2004).; Pettit et al. Journal of immunology (Baltimore, Md. :

1950) 159, 3681-3691 (1997).; herein incorporated by reference in their entireties), antigen processing and cross presentation (*Tapbp*, *Rab27a*, *Slc11a1*) (Compeer et al. Frontiers in Immunology 3, (2012).; Jancic et al. Nature cell biology 9, 367-378 (2007).; Stober et al. Infection and Immunity 75, 5059-5067 (2007).; herein incorporated by reference in their entireties), chemokine–mediated recruitment of immune cells to the tumor microenvironment (*Cxcl9*, *Cx3cl1*, *Cxcr4*) (Kabashima et al. The American Journal of Pathology 171, 1249-1257 (2007).; Nukiwa et al. European journal of immunology 36, 1019-1027 (2006).; Zhang et al. New England Journal of Medicine 348, 203-213 (2003).; herein incorporated by reference in their entireties) and type I interferon signaling (*Irf1*, *Ifnar2*, *Oas2*, *Ifi35*, *Ifitm1*) (Fuertes et al. The Journal of experimental medicine 208, 2005-2016 (2011).; Woo et al. Immunity 41, 830-842 10.; herein incorporated by reference in their entireties) (Figure 4D). Expression of these genes was also strongly induced in murine bone marrow-derived DCs stimulated with *Bifidobacterium in vitro*. Taken together, these data indicate that commensal bacteria-derived (e.g., *Bifidobacterium*-derived) signals modulate the activation of innate antigen-presenting cells, which in turn support improved activation of tumor antigen-specific $CD8^+$ T cells.

To test whether functional differences in DCs isolated from TAC, JAX and *Bifidobacterium*-treated TAC mice could be sufficient to explain the differences in T cell priming observed in vivo, DCs were purified from lymphoid tissues of naïve TAC, JAX, and *Bifidobacterium*-treated TAC mice and tested their ability to induce CFSE-labeled $CD8^+$ SIY^- specific 2C TCR Tg T cell proliferation and acquisition of IFN-γ production *in vitro*. DCs purified from JAX and *Bifidobacterium*-treated TAC mice induced 2C T cell proliferation at lower antigen concentration compared to DCs purified from naïve TAC mice (Figure 14, A and B). Furthermore, at all antigen concentrations, JAX-derived DCs elicited elevated levels of T cell IFN-γ production (Figure 4E and Figure 14A). Similar effects were observed upon oral administration of *Bifidobacterium* to TAC mice prior to DC isolation (Figure 4E and Figure 14A). Taken together, these data indicate that commensal *Bifidobacterium*-derived signals modulate the activation of DCs in the steady state, which in turn supports improved effector function of tumor-specific $CD8^+$ T cells.

Experiments conducted during development of embodiments herein demonstrate an unexpected role for commensal microflora (e.g., *Bifidobacterium*) in enhancing anti-tumor immunity. These data support the idea that one source of inter-subject heterogeneity with regard

to spontaneous anti-tumor immunity and therapeutic effects of antibodies targeting the PD-1/PD-L1 axis may be the specific composition of gut microbes, which can be manipulated for therapeutic benefit.

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CLAIMS

We claim:

1. A method of treating cancer in a human subject comprising administering to the subject an immune checkpoint inhibitor and a bacterial formulation comprising bacteria of the genera *Bifidobacterium*.

2. The method of claim 1, wherein at least 50% of the bacteria in the bacterial formulation are of the genera *Bifidobacterium*.

3. The method of claim 1, wherein at least 90% of the bacteria in the bacterial formulation are of the genera *Bifidobacterium*.

4. The method of claim 1, wherein the bacteria of the genus *Bifidobacterium* comprise bacteria of the species *Bifidobacterium lactis, Bifidobacterium bifidium, Bifidobacterium longum, Bifidobacterium animalis, Bifidobacterium breve, Bifidobacterium infantis, Bifidobacterium catenulatum, Bifidobacterium pseudocatenulatum, Bifidobacterium adolescentis, Bifidobacterium angulatum, Bifidobacterium asteroides, Bifidobacterium boum, Bifidobacterium choerinum, Bifidobacterium coryneforme, Bifidobacterium cuniculi, Bifidobacterium denticolens, Bifidobacterium dentium, Bifidobacterium gallicum, Bifidobacterium gallinarum, Bifidobacterium indicum, Bifidobacterium inopinatum, Bifidobacterium magnum, Bifidobacterium merycicum, Bifidobacterium minimum, Bifidobacterium pseudolongum, Bifidobacterium pullorum, Bifidobacterium psychraerophilum, Bifidobacterium ruminantium, Bifidobacterium saeculare, Bifidobacterium scardovii, Bifidobacterium simiae, Bifidobacterium subtile, Bifidobacterium therammcidophilum, Bifidobacterium thermophilum, Bifidobacterium tsurumiense, Bifidobacterium urinalis* or *Bifidobacterium sp.*.

5. The method of claim 1, wherein the bacterial formulation is administered by oral administration or rectal administration.

6. The method of claim 5, wherein the bacterial formulation is administered by oral administration.

7. The method of claim 1, wherein the bacterial formulation comprises at least $5x10^6$ CFU of bacteria of the genera *Bifidobacterium*.

8. The method of claim 1, wherein the bacterial formulation is administered to the subject in two or more doses.

9. The method of claim 9, wherein the administration of the two or more doses are separated by at least 1 week.

10. The method of claim 1, further comprising administering to the subject an antibiotic prior to the administration of the bacterial formulation.

11. The method of claim 10, wherein the antibiotic is administered to the subject at least 1 day before the bacterial formulation is administered to the subject.

12. The method of claim 1, wherein the immune checkpoint inhibitor is a protein or polypeptide that binds to an immune checkpoint protein.

13. The method of claim 12, wherein the immune checkpoint protein is CTLA4, PD-1, PD-L1, PD-L2, A2AR, B7-H3, B7-H4, BTLA, KIR, LAG3, TIM-3 or VISTA.

14. The method of claim 13, wherein the immune checkpoint protein is PD-1 or PD-L1.

15. The method of claim 1, wherein the immune checkpoint inhibitor is an antibody or antigen binding fragment thereof that binds to an immune checkpoint protein.

16. The method of claim 15, wherein the immune checkpoint protein is CTLA4, PD-1, PD-L1, PD-L2, A2AR, B7-H3, B7-H4, BTLA, KIR, LAG3, TIM-3 or VISTA.

17. The method of claim 16, wherein the immune checkpoint protein is PD-1 or PD-L1.

18. The method of claim 1, wherein the immune checkpoint inhibitor is nivolumab, pembrolizumab, pidilizumab, AMP-224, AMP-514, STI-A1110, TSR-042, RG-7446, BMS-936559, BMS-936558, MK-3475, CT Ol l, MPDL3280A, MEDI-4736, MSB-0020718C, AUR-012 and STI-A1010.

19. The method of claim 1, wherein the immune checkpoint inhibitor is administered by intravenous injection, intramuscular injection, intratumoral injection or subcutaneous injection.

ABSTRACT

Provided herein are methods of treatment and/or prevention of cancer by manipulation of commensal microflora. In particular, the amount, identity, presence, and/or ratio of microflora (e.g., gut microflora) in a subject is manipulated to facilitate one or more co-treatments.

PTO/AIA/14 (11-15)
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U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE

Application Data Sheet 37 CFR 1.76	Attorney Docket Number	UCHI-34458/US-4/CON
	Application Number	
Title of Invention	TREATMENT OF CANCER BY MANIPULATION OF COMMENSAL MICROFLORA	

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Attorney Docket Number	UCHI-34458/US-4/CON	Small Entity Status Claimed	☒
Application Type	Nonprovisional		
Subject Matter	Utility		
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	Continuation of	15170284	2016-06-01
Prior Application Status	Pending		Remove
Application Number	Continuity Type	Prior Application Number	Filing or 371(c) Date (YYYY-MM-DD)
15170284	Claims benefit of provisional	62169112	2015-06-01
Prior Application Status	Expired		Remove
Application Number	Continuity Type	Prior Application Number	Filing or 371(c) Date (YYYY-MM-DD)
15170284	Claims benefit of provisional	62248741	2015-10-30

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Statement under 37 CFR 1.55 or 1.78 for AIA (First Inventor to File) Transition Applications

☐ This application (1) claims priority to or the benefit of an application filed before March 16, 2013 and (2) also contains, or contained at any time, a claim to a claimed invention that has an effective filing date on or after March 16, 2013.
NOTE: By providing this statement under 37 CFR 1.55 or 1.78, this application, with a filing date on or after March 16, 2013, will be examined under the first inventor to file provisions of the AIA.

PTO/AIA/14 (11-15)
Approved for use through 04/30/2017. OMB 0651-0032
U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE

Application Data Sheet 37 CFR 1.76	Attorney Docket Number	UCHI-34458/US-4/CON
	Application Number	
Title of Invention	TREATMENT OF CANCER BY MANIPULATION OF COMMENSAL MICROFLORA	

Authorization or Opt-Out of Authorization to Permit Access:

When this Application Data Sheet is properly signed and filed with the application, applicant has provided written authority to permit a participating foreign intellectual property (IP) office access to the instant application-as-filed (see paragraph A in subsection 1 below) and the European Patent Office (EPO) access to any search results from the instant application (see paragraph B in subsection 1 below).

Should applicant choose not to provide an authorization identified in subsection 1 below, applicant **must opt-out** of the authorization by checking the corresponding box A or B or both in subsection 2 below.

NOTE: This section of the Application Data Sheet is **ONLY** reviewed and processed with the **INITIAL** filing of an application. After the initial filing of an application, an Application Data Sheet cannot be used to provide or rescind authorization for access by a foreign IP office(s). Instead, Form PTO/SB/39 or PTO/SB/69 must be used as appropriate.

1. Authorization to Permit Access by a Foreign Intellectual Property Office(s)

A. Priority Document Exchange (PDX) - Unless box A in subsection 2 (opt-out of authorization) is checked, the undersigned hereby **grants the USPTO authority** to provide the European Patent Office (EPO), the Japan Patent Office (JPO), the Korean Intellectual Property Office (KIPO), the State Intellectual Property Office of the People's Republic of China (SIPO), the World Intellectual Property Organization (WIPO), and any other foreign intellectual property office participating with the USPTO in a bilateral or multilateral priority document exchange agreement in which a foreign application claiming priority to the instant patent application is filed, access to: (1) the instant patent application-as-filed and its related bibliographic data, (2) any foreign or domestic application to which priority or benefit is claimed by the instant application and its related bibliographic data, and (3) the date of filing of this Authorization. See 37 CFR 1.14(h)(1).

B. Search Results from U.S. Application to EPO - Unless box B in subsection 2 (opt-out of authorization) is checked, the undersigned hereby **grants the USPTO authority** to provide the EPO access to the bibliographic data and search results from the instant patent application when a European patent application claiming priority to the instant patent application is filed. See 37 CFR 1.14(h)(2).

The applicant is reminded that the EPO's Rule 141(1) EPC (European Patent Convention) requires applicants to submit a copy of search results from the instant application without delay in a European patent application that claims priority to the instant application.

2. Opt-Out of Authorizations to Permit Access by a Foreign Intellectual Property Office(s)

☐ A. Applicant **DOES NOT** authorize the USPTO to permit a participating foreign IP office access to the instant application-as-filed. If this box is checked, the USPTO will not be providing a participating foreign IP office with any documents and information identified in subsection 1A above.

☐ B. Applicant **DOES NOT** authorize the USPTO to transmit to the EPO any search results from the instant patent application. If this box is checked, the USPTO will not be providing the EPO with search results from the instant application.

NOTE: Once the application has published or is otherwise publicly available, the USPTO may provide access to the application in accordance with 37 CFR 1.14.

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Title of Invention	TREATMENT OF CANCER BY MANIPULATION OF COMMENSAL MICROFLORA	

Applicant Information:

Providing assignment information in this section does not substitute for compliance with any requirement of part 3 of Title 37 of CFR to have an assignment recorded by the Office.

Applicant 1 — Remove

If the applicant is the inventor (or the remaining joint inventor or inventors under 37 CFR 1.45), this section should not be completed. The information to be provided in this section is the name and address of the legal representative who is the applicant under 37 CFR 1.43; or the name and address of the assignee, person to whom the inventor is under an obligation to assign the invention, or person who otherwise shows sufficient proprietary interest in the matter who is the applicant under 37 CFR 1.46. If the applicant is an applicant under 37 CFR 1.46 (assignee, person to whom the inventor is obligated to assign, or person who otherwise shows sufficient proprietary interest) together with one or more joint inventors, then the joint inventor or inventors who are also the applicant should be identified in this section.

Clear

● Assignee	Legal Representative under 35 U.S.C. 117	Joint Inventor
Person to whom the inventor is obligated to assign.	Person who shows sufficient proprietary interest	

If applicant is the legal representative, indicate the authority to file the patent application, the inventor is:

Name of the Deceased or Legally Incapacitated Inventor:

If the Applicant is an Organization check here. ☒

Organization Name	The University of Chicago		
Mailing Address Information For Applicant:			
Address 1	5801 S. Ellis Avenue		
Address 2			
City	Chicago	**State/Province**	IL
Country	US	Postal Code	60637
Phone Number		Fax Number	
Email Address			

Additional Applicant Data may be generated within this form by selecting the Add button. Add

Assignee Information including Non-Applicant Assignee Information:

Providing assignment information in this section does not substitute for compliance with any requirement of part 3 of Title 37 of CFR to have an assignment recorded by the Office.

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	Application Number	
Title of Invention	TREATMENT OF CANCER BY MANIPULATION OF COMMENSAL MICROFLORA	

Assignee 1

Complete this section if assignee information, including non-applicant assignee information, is desired to be included on the patent application publication. An assignee-applicant identified in the "Applicant Information" section will appear on the patent application publication as an applicant. For an assignee-applicant, complete this section only if identification as an assignee is also desired on the patent application publication.

Remove

If the Assignee or Non-Applicant Assignee is an Organization check here. ☐

Prefix	**Given Name**	Middle Name	**Family Name**	Suffix

Mailing Address Information For Assignee including Non-Applicant Assignee:

Address 1			
Address 2			
City		**State/Province**	
Country i		Postal Code	
Phone Number		Fax Number	
Email Address			

Additional Assignee or Non-Applicant Assignee Data may be generated within this form by selecting the Add button. Add

Signature:

Remove

NOTE: This Application Data Sheet must be signed in accordance with 37 CFR 1.33(b). **However, if this Application Data Sheet is submitted with the INITIAL filing of the application and either box A or B is not checked in subsection 2 of the "Authorization or Opt-Out of Authorization to Permit Access" section, then this form must also be signed in accordance with 37 CFR 1.14(c).**

This Application Data Sheet **must** be signed by a patent practitioner if one or more of the applicants is a **juristic entity** (e.g., corporation or association). If the applicant is two or more joint inventors, this form must be signed by a patent practitioner, **all** joint inventors who are the applicant, or one or more joint inventor-applicants who have been given power of attorney (e.g., see USPTO Form PTO/AIA/81) on behalf of **all** joint inventor-applicants.

See 37 CFR 1.4(d) for the manner of making signatures and certifications.

Signature	/David W. Staple/			Date (YYYY-MM-DD)	2017-09-28
First Name	David W.	Last Name	Staple	Registration Number	65903

Additional Signature may be generated within this form by selecting the Add button. Add

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Privacy Act Statement

The Privacy Act of 1974 (P.L. 93-579) requires that you be given certain information in connection with your submission of the attached form related to a patent application or patent. Accordingly, pursuant to the requirements of the Act, please be advised that: (1) the general authority for the collection of this information is 35 U.S.C. 2(b)(2); (2) furnishing of the information solicited is voluntary; and (3) the principal purpose for which the information is used by the U.S. Patent and Trademark Office is to process and/or examine your submission related to a patent application or patent. If you do not furnish the requested information, the U.S. Patent and Trademark Office may not be able to process and/or examine your submission, which may result in termination of proceedings or abandonment of the application or expiration of the patent.

The information provided by you in this form will be subject to the following routine uses:

1 The information on this form will be treated confidentially to the extent allowed under the Freedom of Information Act (5 U.S.C. 552) and the Privacy Act (5 U.S.C. 552a). Records from this system of records may be disclosed to the Department of Justice to determine whether the Freedom of Information Act requires disclosure of these records.

2. A record from this system of records may be disclosed, as a routine use, in the course of presenting evidence to a court, magistrate, or administrative tribunal, including disclosures to opposing counsel in the course of settlement negotiations.

3 A record in this system of records may be disclosed, as a routine use, to a Member of Congress submitting a request involving an individual, to whom the record pertains, when the individual has requested assistance from the Member with respect to the subject matter of the record.

4. A record in this system of records may be disclosed, as a routine use, to a contractor of the Agency having need for the information in order to perform a contract. Recipients of information shall be required to comply with the requirements of the Privacy Act of 1974, as amended, pursuant to 5 U.S.C. 552a(m).

5. A record related to an International Application filed under the Patent Cooperation Treaty in this system of records may be disclosed, as a routine use, to the International Bureau of the World Intellectual Property Organization, pursuant to the Patent CooperationTreaty.

6. A record in this system of records may be disclosed, as a routine use, to another federal agency for purposes of National Security review (35 U.S.C. 181) and for review pursuant to the Atomic Energy Act (42 U.S.C. 218(c)).

7. A record from this system of records may be disclosed, as a routine use, to the Administrator, General Services, or his/her designee, during an inspection of records conducted by GSA as part of that agency's responsibility to recommend improvements in records management practices and programs, under authority of 44 U.S.C. 2904 and 2906. Such disclosure shall be made in accordance with the GSA regulations governing inspection of records for this purpose, and any other relevant (i.e., GSA or Commerce) directive. Such disclosure shall not be used to make determinations about individuals.

8. A record from this system of records may be disclosed, as a routine use, to the public after either publication of the application pursuant to 35 U.S.C. 122(b) or issuance of a patent pursuant to 35 U.S.C. 151. Further, a record may be disclosed, subject to the limitations of 37 CFR 1.14, as a routine use, to the public if the record was filed in an application which became abandoned or in which the proceedings were terminated and which application is referenced by either a published application, an application open to public inspections or an issued patent.

9. A record from this system of records may be disclosed, as a routine use, to a Federal, State, or local law enforcement agency, if the USPTO becomes aware of a violation or potential violation of law or regulation.

Electronic Patent Application Fee Transmittal

Application Number:	
Filing Date:	
Title of Invention:	TREATMENT OF CANCER BY MANIPULATION OF COMMENSAL MICROFLORA
First Named Inventor/Applicant Name:	Thomas F. Gajewski
Filer:	David William Staple/Stephanie Filandrinos
Attorney Docket Number:	UCHI-34458/US-4/CON

Filed as Small Entity

Filing Fees for Track I Prioritized Examination - Nonprovisional Application under 35 USC 111(a)

Description	Fee Code	Quantity	Amount	Sub-Total in USD($)
Basic Filing:				
UTILITY FILING FEE (ELECTRONIC FILING)	4011	1	70	70
UTILITY SEARCH FEE	2111	1	300	300
UTILITY EXAMINATION FEE	2311	1	360	360
REQUEST FOR PRIORITIZED EXAMINATION	2817	1	2000	2000
Pages:				
Claims:				
Miscellaneous-Filing:				

Description	Fee Code	Quantity	Amount	Sub-Total in USD($)
PUBL. FEE- EARLY, VOLUNTARY, OR NORMAL	1504	1	0	0
PROCESSING FEE, EXCEPT PROV. APPLS.	2830	1	70	70

Petition:	
Patent-Appeals-and-Interference:	
Post-Allowance-and-Post-Issuance:	
Extension-of-Time:	
Miscellaneous:	
Total in USD ($)	**2800**

Electronic Acknowledgement Receipt

EFS ID:	30503648
Application Number:	15718735
International Application Number:	
Confirmation Number:	5538
Title of Invention:	TREATMENT OF CANCER BY MANIPULATION OF COMMENSAL MICROFLORA
First Named Inventor/Applicant Name:	Thomas F. Gajewski
Customer Number:	72960
Filer:	David William Staple/Stephanie Filandrinos
Filer Authorized By:	David William Staple
Attorney Docket Number:	UCHI-34458/US-4/CON
Receipt Date:	28-SEP-2017
Filing Date:	
Time Stamp:	15:40:37
Application Type:	Utility under 35 USC 111(a)

Payment information:

Submitted with Payment	yes
Payment Type	CARD
Payment was successfully received in RAM	$2800
RAM confirmation Number	092917INTEFSW15412600
Deposit Account	
Authorized User	

The Director of the USPTO is hereby authorized to charge indicated fees and credit any overpayment as follows:

File Listing:

Document Number	Document Description	File Name	File Size(Bytes)/ Message Digest	Multi Part /.zip	Pages (if appl.)
1	TrackOne Request	34458US4CON_TRACK1.pdf	129935 993fe5fb2ad55427217d7b42a3896bea59628597	no	2
Warnings:					
Information:					
2	Drawings-other than black and white line drawings	34458US4CON_FIGURES.pdf	4229426 8e1543b79bb5ed55820f77868d1ff3c1461053fe	no	46
Warnings:					
Information:					
3	Transmittal Letter	34458US3ORD_IDS_Transmittal_Letter.pdf	74185 2ede47b7fa97b8541a99dbee0eed773491a9c006	no	1
Warnings:					
Information:					
4	Information Disclosure Statement (IDS) Form (SB08)	34458US4CON_IDS_SB08.pdf	178240 a9c4ea6c301ba53307e442c890ee5a503c72e26d	no	7
Warnings:					
Information:					
This is not an USPTO supplied IDS fillable form					
5	Power of Attorney	34458US4CON_UCHI_EXE_POA.pdf	87265 fb70a30270370389d8532afd11d0bfe0571f5811	no	1
Warnings:					
Information:					
6		34458US4CON_Application.pdf	399980 7e3a3bd91c08b0bd494add65918b3c79586dff1d	yes	60

	Multipart Description/PDF files in .zip description		
	Document Description	**Start**	**End**
	Specification	1	57
	Claims	58	59
	Abstract	60	60

Warnings:					
Information:					
7	Application Data Sheet	34458US4CON_ADS.pdf	1793411 ad9126244b58a57e78a948c40664b5584156c667	no	9
Warnings:					
Information:					
8	Fee Worksheet (SB06)	fee-info.pdf	40450 80a82dabd57b6c5154835e1339640148e6d65dee	no	2
Warnings:					
Information:					
		Total Files Size (in bytes):	6932892		

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New Applications Under 35 U.S.C. 111

If a new application is being filed and the application includes the necessary components for a filing date (see 37 CFR 1.53(b)-(d) and MPEP 506), a Filing Receipt (37 CFR 1.54) will be issued in due course and the date shown on this Acknowledgement Receipt will establish the filing date of the application.

National Stage of an International Application under 35 U.S.C. 371

If a timely submission to enter the national stage of an international application is compliant with the conditions of 35 U.S.C. 371 and other applicable requirements a Form PCT/DO/EO/903 indicating acceptance of the application as a national stage submission under 35 U.S.C. 371 will be issued in addition to the Filing Receipt, in due course.

New International Application Filed with the USPTO as a Receiving Office

If a new international application is being filed and the international application includes the necessary components for an international filing date (see PCT Article 11 and MPEP 1810), a Notification of the International Application Number and of the International Filing Date (Form PCT/RO/105) will be issued in due course, subject to prescriptions concerning national security, and the date shown on this Acknowledgement Receipt will establish the international filing date of the application.