IMMUNOGENIC ANTI-INFLAMMATORY COMPOSITIONS

FIELD OF THE INVENTION

[0001] In various aspects, the invention relates to immunological therapies for treating a condition characterized by inflammation. In alternative embodiments, the invention provides methods of formulating antigenic compositions for treating inflammatory conditions.

BACKGROUND OF THE INVENTION

[0002] More than one in three people in the developed nations are diagnosed with cancer. More than one in four die from it. Therapies for cancer have primarily relied upon treatments such as surgery, chemotherapy, and radiation. These approaches however, while beneficial for some types and stages of cancer, have proved to be of limited efficacy in many common types and stages of cancers. For example, surgical treatment of a tumor requires complete removal of cancerous tissue to prevent reoccurrence. Similarly, radiation therapy requires complete destruction of cancerous cells. This is difficult since, in theory, a single malignant cell can proliferate sufficiently to cause reoccurrence of the cancer. Also, both surgical treatment and radiation therapy are directed to localized areas of cancer, and are relatively ineffective when the cancer metastasizes. Often surgery or radiation or both are used in combination with systemic approaches such as chemotherapy. Chemotherapy however has the problem of non-selectivity with the concomitant problem of deleterious side effects, as well as the possibility of the cancer cells developing resistance to the drugs.

[0003] The inherent shortcomings of chemotherapy have led to disparate efforts to recruit various aspects of the immune system to treat cancers. A subset of this work relates to immunization with microbial vaccines. Although this approach has a relatively long history, as discussed in more detail below, the field is a very confused mixture of

sometimes intriguing successes mixed with many failures that together have failed to produce a cohesive therapeutic approach amenable to widespread clinical adoption.

[0004] Alternative approaches for the treatment of cancers have included therapies that involve augmentation of immune system function such as cytokine therapy (for *e.g.,* recombinant interleukin 2 and gamma interferon for kidney cancers), dendritic cell therapy, autologous tumor vaccine therapy, genetically-altered vaccine therapy, lymphocyte therapy, and microbial vaccine therapies, the latter being thought to engage the host system in a non-specific manner. Microbial vaccines have been used to vaccinate subjects against pathogens that are associated with cancer, such as the human papillomavirus. Immunostimulatory microbial vaccines that are not targeted to cancer-causing organisms, *i.e.,* non-specific immunostimulatory vaccines, such as pyrogenic vaccines, have a long clinical history that includes reports of successes and failures in treating a variety of cancers. For example, Coley's vaccine (a combination of *Streptococcus pyogenes* and *Serratia marcescens*) has been reported to be helpful for the treatment of sarcomas, and lymphomas (see, for *e.g.,* Nauts HC, Fowler GAA, Bogato FH. A review of the influence of bacterial infection and of bacterial products [Coley's toxins] on malignant tumors in man. Acta Med Scand 1953; 145 [Suppl. 276]:5-103). Clinical trials have reportedly demonstrated the benefit of Coley's vaccine treatment for lymphoma and melanoma (see, for *e.g.,* Kempin S, Cirrincone C, Myers J et al: Combined modality therapy of advanced nodular lymphomas: the role of nonspecific immunotherapy [MBV] as an important determinant of response and survival. Proc Am Soc Clin Oncol 1983;24:56; Kolmel KF, Vehmeyer K. Treatment of advanced malignant melanoma by a pyrogenic bacterial lysate: a pilot study. Onkologie 1991;14:411-17).

[0005] It has been suggested that the effectiveness of some non-specific bacterial cancer vaccines is attributable to particular bacterial components or products, such as bacterial DNA or endotoxin (LPS), or because they induce the expression of particular factors, such as tumor necrosis factor (TNF) or interleukin-12. A correspondingly broad

range of physiological mechanisms have been ascribed to such treatments, ranging from generalized effects of fever to anti-angiogenic mechanisms. In accordance with these various principles, a wide variety of microbial vaccines have been tested as general immune stimulants for the treatment of cancer. While most have shown negative results, a few have shown some intruiguing positive results in certain contexts, as discussed below.

[0006] Intradermal BCG (*Mycobacterium bovis*) vaccine treatment has been reported to be effective for the treatment of stomach cancer (see, for *e.g.*, Ochiai T, Sato J, Hayashi R, et al: Postoperative adjuvant immunotherapy of gastric cancer with BCG-cell wall endoskeleton. Three- to six-year follow-up of a randomized clinical trial. Cancer Immunol Immunother 1983; 14:167-171) and colon cancer (Smith RE, Colangelo L, Wieand HS, Begovic M, Wolmark N. Randomized trial of adjuvant therapy in colon carcinoma: 10-Year results of NSABP protocol C-01. J. NCI 2004;96[15]:1128-32; Uyl-de Groot CA, Vermorken JB, Hanna MG, Verboon P, Groot MT, Bonsel GJ, Meijer CJ, Pinedo HM. Immunotherapy with autologous tumor cell-BCG vaccine in patients with colon cancer: a prospective study of medical and economic benefits Vaccine 2005; 23[17-18]:2379-87).

[0007] *Mycobacterium w* vaccine therapy, in combination with chemotherapy and radiation, was found to significantly improve quality of life and response to treatment in patients with lung cancer (see for *e.g.*, Sur P, Dastidar A. Role of *Mycobacterium w* as adjuvant treatment of lung cancer [non-small cell lung cancer]. J Indian Med Assoc 2003 Feb;101[2]:118-120). Similarly, *Mycobacterium vaccae* vaccine therapy was found to improve quality of life (see, for *e.g.*, O'Brien M, Anderson H, Kaukel E, et al. SRL172 [killed *Mycobacterium vaccae*] in addition to standard chemotherapy improves quality of life without affecting survival, in patients with advanced non-small-cell lung cancer: phase III results. Ann Oncol 2004 Jun;15[6];906-14) and symptom control (Harper-Wynne C, Sumpter K, Ryan C, et al. Addition of SRL 172 to standard

chemotherapy in small cell lung cancer [SCLC] improves symptom control. Lung Cancer 2005 Feb;47[2]:289-90) in lung cancer patients.

[0008] *Corynebacterium parvum* vaccine was linked with a trend towards improved survival for the treatment of melanoma (see, for *e.g.*, Balch CM, Smalley RV, Bartolucci AA, et al. A randomized prospective trial of adjuvant *C. parvum* immunotherapy in 260 patients with clinically localized melanoma [stage I]. Cancer 1982 Mar 15;49[6]:1079-84).

[0009] Intradermal *Streptococcus pyogenes* vaccine therapy was found to be effective for the treatment of stomach cancer (see, for e.g., Hanaue H, Kim DY, Machimura T, et al. Hemolytic streptococcus preparation OK-432; beneficial adjuvant therapy in recurrent gastric carcinoma. Tokai J Exp Clin Med 1987 Nov;12[4]:209-14).

[0010] *Nocardia rubra* vaccine was found to be effective for the treatment of lung cancer (see, for *e.g.*, Yasumoto K, Yamamura Y. Randomized clinical trial of non-specific immunotherapy with cell-wall skeleton of *Nocardia rubra*. Biomed Pharmacother 1984;38[1]:48-54; Ogura T. Immunotherapy of respectable lung cancer using Nocardia rubra cell wall skeleton. Gan To Kagaku Ryoho 1983 Feb;10[2 Pt 2]:366-72) and linked to a trend to improved survival for the treatment acute myelogenous leukemia (Ohno R, Nakamura H, Kodera Y, et al. Randomized controlled study of chemoimmunotherapy of acute myelogenous leukemia [AML] in adults with *Nocardia rubra* cell-wall skeleton and irradiated allogeneic AML cells. Cancer 1986 Apr 15;57[8]:1483-8).

[0011] *Lactobacillus casei* vaccine treatment combined with radiation was found to more effective for the treatment of cervical cancer than radiation alone. (see, for *e.g.*, Okawa T, Kita M, Arai T, et al. Phase II randomized clinical trial of LC9018 concurrently used with radiation in the treatment of carcinoma of the uterine cervix. Its effect on tumor reduction and histology. Cancer 1989 Nov 1;64[9]:1769-76)

[0012] *Pseudomonas aeruginosa* vaccine treatment was found to increase the effectiveness of chemotherapy in the treatment of lymphoma and lung cancer (see, for *e.g.,* Li Z, Hao D, Zhang H, Ren L, et al. A clinical study on PA_MSHA vaccine used for adjuvant therapy of lymphoma and lung cancer. Hua Xi Yi Ke Da Xue Xue Bao 2000 Sep;31[3]:334-7).

[0013] Childhood vaccination with the smallpox vaccine (*i.e.,* Vaccinia virus vaccine) was found to be associated with a decreased risk of melanoma later in life (see, for *e.g.,* Pfahlberg A, Kolmel KF, Grange JM. et al. Inverse association between melanoma and previous vaccinations against tuberculosis and smallpox: results of the FEBIM study. J Invest Dermatol 2002[119]:570-575) as well as decreased mortality in those patients who did develop melanoma (see, for *e.g.,* Kolmel KF, Grange JM, Krone B, et al. Prior immunization of patients with malignant melanoma with vaccinia or BCG is associated with better survival. European Organization for Research and Treatment of Cancer cohort study on 542 patients. Eur J Cancer 41[2005]:118-125).

[0014] Treatment with rabies virus vaccine was found to result in temporary remission in 8 of 30 patients with melanoma (see, for *e.g.,* Higgins G, Pack G. Virus therapy in the treatment of tumors. Bull Hosp Joint Dis 1951;12:379-382; Pack G. Note on the experimental use of rabies vaccine for melanomatosis. Arch Dermatol 1950;62:694-695).

[0015] In spite of substantial efforts to engage the immune system to combat cancers using non-specific immunostimulatory microbial vaccines, the vast majority of these efforts have failed and there is little clinical or research evidence of widespread success in improving the survival of cancer patient populations. While it has been recognized that immunostimulatory microbial vaccine approaches have promise, it has also been recognized that significant challenges characterize the field (see, for *e.g.,* Ralf Kleef, Mary Ann Richardson, Nancy Russell, Cristina Ramirez. "Endotoxin and

Exotoxin Induced Tumor Regression with Special Reference to Coley Toxins: A Survey of the Literature and Possible Immunological Mechanisms." Report to the National Cancer Institute Office of Alternative and Complementary Medicine August 1997; DL Mager. "Bacteria and Cancer: Cause, Coincidence or Cure? A Review."Journal of Translational Medicine 28 March 2006 4[14]:doi:10.1186/1479-5876-4-14).

[0016] Inflammatory bowel disease (IBD) is a name frequently given to a group of inflammatory conditions of the colon and small intestine, generally characterized by similar symptoms and indeterminate etiology. Major sub-types of IBD are recognized clinically as Crohn's disease and ulcerative colitis. In addition to Crohn's disease and ulcerative colitis, IBD may also include conditions recognized as any one of the following: collagenous colitis, lymphocytic colitis, ischaemic colitis, diversion colitis, Behçet's syndrome or indeterminate colitis. The difference between these conditions relate primarily to the location and nature of the inflammatory changes in the gastrointestinal tract (GIT). Crohn's disease, for example, is generally recognized as potentially affecting any part of the gastrointestinal tract, from mouth to anus, with a majority of the cases marked by relapsing and remitting granulomatous inflammation of the alimentary tract in the terminal ileum and colon. Ulcerative colitis, in contrast, is generally considered to be restricted to the colon and the rectum. The various regions of the gastrointestinal tract in which these inflammatory conditions may exhibit symptoms include: the bowel or intestine, including: the small intestine (which has three parts: the duodenum, the jejunum, and the ileum); the large intestine (which has three parts: the cecum, the colon, which includes the ascending colon, transverse colon, descending colon and sigmoid flexure; and the rectum); and, the anus.

[0017] The understanding of inflammatory bowel diseases is evolving, but is as yet incomplete in many respects (see, for *e.g.*, Baumgart DC, Carding SR (2007) "Inflammatory bowel disease: cause and immunobiology" *The Lancet* 369 (9573): 1627–40; Baumgart DC, Sandborn WJ (2007) "Inflammatory bowel disease: clinical aspects and established and evolving therapies" *The Lancet* 369 (9573): 1641–57;

Xavier RJ, Podolsky DK (2007) "Unravelling the pathogenesis of inflammatory bowel disease" *Nature* 448 (7152): 427–34; J. H. Cho (2008) "The genetics and immunopathogenesis of inflammatory bowel disease" *Nature Reviews Immunology* 8, 458-466).

[0018] Anti-inflammatory drugs and immune system suppressants may be used in the treatment of IBD, such as sulfasalazine (Azulfidine™), mesalamine (Asacol™, Rowasa™), corticosteroids (e.g. prednisone), azathioprine (Imuran™), mercaptopurine (Purinethol™), infliximab (Remicade™), adalimumab (Humira™), certolizumab pegol (Cimzia™), methotrexate (Rheumatrex™), cyclosporine (Gengraf™, Neoral™, Sandimmune™) or natalizumab (Tysabri™).

[0019] Alternative treatments for IBD have been suggested, including the use of various biological agents, or treatments that purportedly adjust natural intestinal flora, sometimes called probiotic treatments (US 2007/0258953; US 2008/0003207; WO 2007/076534; WO 2007/136719; WO 2010/099824). It has for example been reported that IBD may be treated with a deliberate infestation of parasitic worms, for example by consumption of the live ova of the Trichuris suis helminth (Summers et al. (2003) "Trichuris suis seems to be safe and possibly effective in the treatment of inflammatory bowel disease". Am. J. Gastroenterol. 98 (9): 2034–41; Büning et al., (2008) "Helminths as governors of inflammatory bowel disease" Gut 57:1182-1183; Weinstock and Elliott (2009) "Helminths and the IBD hygiene hypothesis" Inflamm Bowel Dis. 2009 Jan;15(1):128-33).

SUMMARY OF THE DISCLOSURE

[0020] In one aspect a method of formulating an anti-inflammatory composition for treating a condition characterized by inflammation in a specific organ or tissue is provided. The method involves selecting at least one pathogen that is pathogenic in the specific organ or tissue; producing an antigenic composition comprising antigenic determinants that together are specific for the pathogen; and formulating the antigenic

composition for administration as an anti-inflammatory composition capable of eliciting an anti-inflammatory response in the specific organ or tissue, wherein the condition characterized by inflammation is not a cancer.

[0021] The method may further involve a diagnostic step of identifying the specific organ or tissue within which the inflammation is symptomatic prior to producing the antigenic composition. Optionally, a tumor or proliferative condition may be situated in the specific organ or tissue.

[0022] Optionally, the antigenic composition may be formulated for subcutaneous injection or intradermal injection. Optionally, the the antigenic composition may be formulated for injection to produce a localized skin immune response at a site of administration. Optionally, the method detailed herein is provided such that when the specific tissue or organ is X, the pathogen is selected from the group consisting of Y. More specifically, the following combinations are considered within the scope of this method:

when X is:	Y is one or more of:
hematological, skin, soft tissue, lung/trachea/bronchi, peritoneum, meninges, bile duct, gallbladder, kidney, bladder, ureter	Achromobacter spp.
skin, soft tissue, lung/trachea/bronchi, mediastinum, brain, spinal cord, hematological, meninges	Actinomadura spp.
small bowel, colon, hematological, peritoneum	Aerobacter spp.

hematological, heart, bone, kidney, bladder, ureter, meninges	Aerococcus spp.
Lung/trachea/bronchi	Alcaligenes spp.
meninges, hematological, liver, spleen, bone, lung/trachea/bronchi	Anaplasma spp.
lung/trachea/bronchi, lymph nodes pulmonary hilum, mediastinum, meninges, skin, nasopharynx, tonsil, oral, small bowel, colon, hematological	*Bacillus anthracis*
colon, eye, hematological	*Bacillus cereus*
hematological, bone, meninges, brain, heart, lung/trachea/bronchi, mediastinum, skin, soft tissue, colon, stomach, small bowel, eye	other Bacillus spp.
lung/trachea/bronchi, meninges, hematological	Balneatrix spp.
skin, hematological, liver, muscle, lymph nodes	*Bartonella bacilliformis*
brain, spinal cord, hematological, skin, liver, bone, pleura, lung/trachea/bronchi, mediastinum, axillary and inguinal lymph nodes, eye	*Bartonella henselae*
Skin, hematological, liver, spleen	*Bartonella quintana*

skin, soft tissue, meninges, hematological, lung/trachea/bronchi	*Bergeyella zoohelcum*
lung/trachea/bronchi, hematological	*Bordetella holmesii*
nasopharynx, tonsil, lung/trachea/bronchi	*Bordetella parapertussis*
nasopharynx, tonsil, lung/trachea/bronchi	*Bordetella pertussis*
meninges, brain, spinal cord, skin, eye, hematological, inguinal/axillary/cervical lymph nodes, muscle, liver, spleen, nasopharynx, lung/trachea/bronchi, testes	*Borrelia burgdorferi*
brain, spinal cord, hematological, small bowel, liver, spleen, salivary glands, lung/trachea/bronchi, lymph nodes, eye, skin	*Borrelia recurrentis*
peritoneum, hematological, skin, soft tissue	Brevundimonas spp.
lung/trachea/bronchi, lymph nodes pulmonary hilum, meninges, brain, spinal cord, lymph nodes, mediastinum, bone, eye, small bowel, colon, liver, biliary tract, kidney, ureter, bladder, hematological, skin, testes, spleen, prostate	Brucella spp.

hematological, meninges, lung/trachea/bronchi	*Burkholderia gladioli*
lung/trachea/bronchi, skin, soft tissue, liver, spleen, muscle, lymph nodes pulmonary hilum, mediastinal lymph nodes, mediastinum, head and neck lymph nodes, hematological	*Burkholderia mallei*
lung/trachea/bronchi, skin, kidney, bladder, ureter, soft tissue, bone, brain, spinal cord, muscle, hematological, prostate, kidney, ureter, meninges	*Burkholderia pseudomallei*
skin, penis, vulva, soft tissue, vagina, cervix, bone, hematological, inguinal lymph nodes	*Calymmatobacterium granulomatis*
small bowel, colon	*Campylobacter coli*
lung/trachea/bronchi, small bowel, colon, meninges, brain, peritoneum, bone, gallbladder, ovaries, hematological, heart, kidney, bladder, ureter	*Campylobacter fetus*
colon, hematological, gallbladder, pancreas, bladder, bone, meninges	*Campylobacter jejuni*
small bowel, colon	*Campylobacter sputorum*
skin, soft tissue, meninges, hematological, bone, lung/trachea/bronchi, eye	*Capnoctyophaga canimorsus*

skin, soft tissue, meninges, hematological, bone, lung/trachea/bronchi, eye	*Capnoctyophaga cynodegmi*
hematological, eye, skin, soft tissue	*CDC groups EF-4a and EF-4b*
lung/trachea/bronchi, lymph nodes pulmonary hilum, liver, brain, meninges, skin, thyroid, pancreas, hematological	*Chlamydia pneumoniae*
lung/trachea/bronchi, lymph nodes pulmonary hilum, mediastinum, liver, brain, meninges, hematological, skin, thyroid, pancreas	*Chlamydia psittaci*
inguinal lymph nodes, penis, vulva, vagina, cervix, uterus, ovaries and adnexae, peritoneum, prostate, eye	*Chlamydia trachomatis*
laryngx, trachea/bronchi, hematological	*Chlamydophila pneumoniae*
hematological, liver, spleen, lung/trachea/bronchi, kidney, bladder, ureter, eye/orbit, bone, brain, meninges, spinal cord	*Chromobacterium violaceum*
Lung/trachea/bronchi	*Chlamydophila psittaci*
meninges, lung/trachea/bronchi, hematological	*Chryseobacterium spp.*
small bowel, colon, stomach, skin, soft tissue, hematological	*Clostridium bifermentans*
colon, small bowel, skin	*Clostridium botulinum*

colon	*Clostridium difficile*
small bowel, colon, stomach, skin, soft tissue, hematological	*Clostridium indolis*
small bowel, colon, stomach, skin, soft tissue, hematological	*Clostridium mangenolii*
small bowel, colon, stomach, skin, soft tissue, hematological, heart	*Clostridium perfringens*
small bowel, colon, stomach, skin, soft tissue, hematological	*Clostridium sordellii*
small bowel, colon, stomach, skin, soft tissue, hematological	*Clostridium sporogenes*
small bowel, colon, stomach, skin, soft tissue, hematological	*Clostridium subterminale*
Skin, soft tissue	*Clostridium tetani*
hematological, peritoneum, eye	Comamonas spp.
neck/axillary/inguinal/mediastinal lymph nodes, lymph nodes pulmonary hilum, lung/trachea/bronchi, mediastinum	*Corynebacterium pseudotuberculosis*
lung/bronchi/trachea, brain, spinal cord, liver, bone	*Coxiella burnetii*
skin, soft tissue, liver, meninges, small bowel, colon, bone, uterus, ovaries	*Edwarsiella tarda*
meninges, brain, spinal cord, hematological, bone, liver, kidney, spleen, lymph nodes	Ehrlichia spp.

skin, hematological, bone, brain, peritoneum	*Erysipelothrix rhusiopathiae*
nasopharynx, oral, tonsil, lung/trachea/bronchi, skin, axillary/head and neck/inguinal lymph nodes, hematological, eye, small bowel	*Francisella tularensis*
Skin, soft tissue, hematological	Fusobacterium spp.
skin, soft tissue, lung/trachea/bronchi, mediastinum, brain, spinal cord, hematological, meninges, eye	Gordonia spp.
skin, inguinal lymph nodes, penis, vulva, vagina	*Haemophilus ducreyi*
stomach	*Helicobacter pylori*
lung/trachea/bronchi, lymph nodes pulmonary hilum, hematological, brain, spinal cord, muscle, pancreas	Legionella spp.
lung/trachea/bronchi, pancreas, meninges, brain, spinal cord, skin, lymph nodes, eye, hematological, nasopharynx, oral, tonsil, kidney, liver, spleen	Leptospirosis spp.
hematological, brain, meninges, spinal cord, small bowel, colon	*Listeria monocytogenes*
hematological, peritoneum, skin, soft tissue, bone	Methylobacterium spp.

lung/bronchi/trachea, lymph nodes pulmonary hilum, prostate, pancreas, spleen, skin, neck lymph nodes, esophagus, bone, hematological	*Mycobacterium avium*
colon, small bowel	*Mycobacterium bovis*
lung/bronchi/trachea, lymph nodes pulmonary hilum, prostate, bone	*Mycobacterium kansasii*
Skin, soft tissues, testes, eye	*Mycobacterium leprae*
Skin, soft tissue, bone	*Mycobacterium marinum*
head and neck lymph nodes	*Mycobacterium scrofulaceum*
lung/bronchi/trachea, lymph nodes pulmonary hilum, prostate, peritoneum, pancreas, spleen, lymph nodes, small bowel, meninges, brain, spinal cord, kidney, ureter, bladder, muscle, esophagus, colon, testes, eye, ovaries, cervix, vagina, uterus, mediastinum, larynx, skin, hematological, pleura	*Mycobacterium tuberculosis*
Skin, soft tissue	*Mycobacterium ulcerans*
lung/bronchi/trachea, lymph nodes pulmonary hilum, skin, soft tissues, bone, head and neck lymph nodes	other Mycobacterium spp.
kidney, bladder, ureter, skin, soft tissue, hematological	Myroides spp.

nasopharyx, oral, tonsil, prostate, penis, vagina, cervix, uterus, ovary/adnexae, peritoneum, skin, muscle, bone, liver, hematological, head and neck and inguinal and intra-abdominal lymph nodes, anus	*Neisseria gonorrhoeae*
hematological, bone, lymph nodes, liver, spleen	*Neorickettsia sennetsu*
lung/bronchi/trachea, pancreas, meninges, spinal cord, brain, skin, soft tissue, eye, bone, kidney, heart, hematological	Nocardia spp.
meninges, brain, spinal cord, hematological, skin, inguinal and axillary lymph nodes, spleen, lung/bronchi/trachea	*Orientia tsutsugamushi*
lung/trachea/bronchi, hematological	Pandoraea spp.
Skin, soft tissue, hematological	*Pasteurella canis*
Skin, soft tissue, hematological	*Pasteurella dagmatis*
Skin, soft tissue, hematological	*Pasteurella stomatis*
hematological, liver, colon	Pediococcus spp.
Skin	*Pityrosporum ovale*
small bowel, colon, hematological, meninges, bone, gall bladder, skin, soft tissue	*Plesiomonas shigelloides*

lung/trachea/bronchi, hemaotogical, skin, soft tissue, bone, meninges, brain, eye, kidney, bladder, ureter, heart	*Pseudomonas aeruginosa*
skin, soft tissue, lung/trachea/bronchi, mediastinum, hematological	other Pseudomonas spp.
hematological, meninges, bone	Ralstonia spp.
hematological, peritoneum, eye, kidney, bladder, ureter	Rhizobium spp.
lung/trachea/bronchi, hematological, brain, skin, lymph nodes, bone, mediastinum, liver, spleen, soft tissue, spinal cord, meninges	Rhodococcus spp.
Skin	*Rickettsia akari*
lung/bronchi/trachea, lymph nodes pulmonary hilum, meninges, brain, spinal cord, hematolofical, skin, kidney, liver, spleen, pancreas	*Rickettsia conorii*
Skin, brain, spinal cord	*Rickettsia felis*
meninges, brain, spincal cord, hematological, lung/bronchi/trachea, skin, spleen	*Rickettsia prowazekii*

lung/bronchi/trachea, lymph nodes pulmonary hilum, meninges, brain, spinal cord, hematological, muscle, small bowel, liver, skin	*Rickettsia rickettsiae*
skin, head and neck lymph nodes	*Rickettsia slovaca*
meninges, hematological, liver, kidney, brain, lung/bronchi/trachea, spleen	*Rickettsia typhi*
hematological, peritoneum, skin, soft tissue, bladder, kidney, ureter	Roseomonas spp.
lung/bronchi/trachea, pancreas, spleen, intra-abdominal lymph nodes, stomach, small bowel, colon, meninges, skin, muscle, bone, hematological, heart	Salmonella spp.
skin, soft tissue, eye, bone, hematological, peritoneum	Shewanella spp.
colon	*Shigella boydii*
colon	*Shigella dysenteriae*
colon	*Shigella flexneri*
colon	*Shigella sonnei*
brain, meninges, spinal cord, eye, skin, soft tissue	Sphingobacterium spp.
hematological, meninges, peritoneum, skin, soft tissue, kidney, bladder, ureter	Sphingomonas spp.

skin, axillary/inguinal/neck lymph nodes, hematological, liver, spleen	*Spirillum minus*
colon	other Spirillum spp.
meninges, hematological, peritoneum, lung/trachea/bronchi, eye, kidney, bladder, ureter, skin, soft tissue	*Stenotrophomonas maltophilia*
skin, bone, hematological, lung/trachea/bronchi, meninges, brain, liver, spleen	*Streptobacillus moniliformis*
Skin, hematological, soft tissue	*Streptococcus iniae*
small bowel, nasopharynx, bone, meninges, hematological, head and neck lymph nodes	*Streptococcus zooepidemicus*
skin, soft tissue, lung/trachea/bronchi, mediastinum, brain, spinal cord, hematological, meninges	Streptomices spp.
nasopharynx, tonsil, oral, meninges, brain, spinal cord, penis, vulva, vagina, anus, cervix, eye, hematological, inguinal and head and neck lymph nodes	*Treponema pallidum*
brain, spinal cord, hematological, small bowel, colon, heart, lung/trachea/bronchi, eye	*Tropheryma whipplei*

skin, soft tissue, lung/trachea/bronchi, mediastinum, brain, spinal cord, hematological, meninges	Tsukamurella spp.
colon, small bowel	*Vibrio cholerae*
hematological, meninges	*Vibrio cincinnatiensis*
Skin, soft tissue	*Vibrio damsela*
small bowel, colon	*Vibrio fluvialis*
small bowel, colon	*Vibrio furnissii*
small bowel, colon, skin, soft tissue	*Vibrio hollisae*
hematological	*Vibrio metschnikovii*
colon, small bowel	*Vibrio parahaemolyticus*
Soft tissue, blood, skin	*Vibrio vulnificus*
nasopharynx, tonsil, small bowel, intra-abdominal lymph nodes, colon, muscle, lung/trachea/bronchi, liver, spleen, hematological	*Yersinia enterocolitica*
lung/trachea/bronchi, lymph nodes pulmonary hilum, inguinal/axillary/neck lymph nodes, oral, tonsil, hematological, skin	*Yersinia pestis*
small bowel, colon, abdominal lymph nodes	*Yersinia pseudotuberculosis*

[0023] Further, the method detailed herein is provided such that when the specific tissue or organ is X, the pathogen is selected from the group consisting of Y. More specifically, the following combinations are considered within the scope of this method:

when X is:	Y is one or more of:
skin, hematological, lymph nodes, brain, spinal cord, eye, heart	Vaccinia
skin, hematological, lymph nodes, brain	Variola (smallpox)
skin, hematological, head and neck lymph nodes, brain, eye, lung/trachea/bronchi, pulmonary hilar lymph nodes, mediastinum, nasopharynx	Monkeypox
skin, hematological, lymph nodes	Cowpox
Skin	Parapoxviruses
Skin	Molluscum contagiosum
skin, hematological, axillary and inguinal lymph nodes	Tanapox
nasopharynx, oral, tonsil, hematological, lung/bronchi/trachea, pancreas, meninges, brain, spinal cord, inguinal and head/neck lymph nodes, penis, vulva, perineum, esophagus, liver, eye, skin, rectum, tonsil, mediastinum, anus, vagina, cervix	Herpes Simplex virus (1 and 2)

nasopharynx, sinus, lung/trachea/bronchi, pulmonary hilar lymph nodes, hematological, pancreas, meninges, brain, spinal cord, esophagus, liver, eye, skin, heart, mediastinum	Varicella-zoster
nasopharynx, lymph nodes, tonsil, hematological, lung/trachea/bronchi, pancreas, abdomincal lymph nodes, brain, spinal cord, esophagus, small bowel, colon/rectum, eye, liver, heart, skin, mediastinum, esophagus	Cytomegalovirus
nasopharynx, tonsil, oral, lymph nodes, hematological, lung, abdomincal lymph nodes, brain, spinal cord, muscles, esophagus, liver, heart, skin, spleen, kidney, muscle, heart, lung/trachea/bronchi, pulmonary hilar lymph nodes, mediastinum	Epstein-Barr virus
skin, hematological, lung/trachea/bronchi, pulmonary hilar lymph nodes, brain, meninges, liver	Human herpesvirus 6
Skin, brain, liver	Human herpesvirus 7

nasopharynx, tonsil, hematological, skin, spleen, head and neck lymph nodes	Human herpesvirus 8
brain, spinal cord, skin, hematological, lymph nodes	Simian herpes B virus
nasopharynx, oral, larynx, trachea, bronchi, lung, lymph nodes, meninges, brain, spinal cord, small bowel, colon, liver, intra-abdominal lymph nodes, mediastinum, bladder, sinus, hematological, ureter, kidney, bladder, thyroid, heart	Adenovirus
kidney	BK virus
skin, anus, penis, vulva, cervix, vagina, oral	Human papillomavirus
Liver, pancreas, hematological	Hepatitis B virus
Liver	Hepatitis D virus
skin, hematological, nasopharynx, bone, kidney, heart, liver, brain, meninges	Parvovirus B19
nasopharynx, small bowel, colon, oral, sinus, lymph nodes, skin, lung/trachea/bronchi, meninges, brain, spinal cord, liver	Orthoreoviruses
brain, muscle, hematological,	Orbiviruses
hematological, skin, muscle, oral, spleen, lymph nodes, meninges, brain	Coltiviruses

small bowel, colon, liver, hematological, pancreas, nasopharynx, billiary tract, meninges, brain	Rotaviruses
brain, spinal cord, small bowel, colon, hematological, skin, bone	Alphaviruses
skin, hematological, head and neck lymph nodes, spleen, nasopharynx, bone, brain, tonsil, bronchi, liver, heart	Rubella
hematological, liver, lung/trachea/bronchi, kidney, adrenal gland, spleen, lymph nodes, stomach, kidney	Yellow fever virus
hematological, lymph nodes, skin, spleen, muscle, liver, brain, nasopharynx	Dengue fever virus
brain, hematological, spinal cord	Japanese encephalitis virus
brain, hematological, spinal cord, muscle, lymph nodes, liver, spleen, pancreas, meninges	West Nile encephalitis virus
brain, hematological, spinal cord, meninges, muscle, nasopharynx	St. Louis encephalitis virus
brain, hematological, spinal cord, muscle, meninges	Tick-borne encephalitis virus

hematological, brain, meninges, bone, muscles, skin, lymph nodes	other Flaviviruses
hematological, liver	Hepatitis C virus
Liver	Hepatitis G virus
nasopharynx, sinus, oral, tonsil, larynx, lung/trachea/bronchi, pulmonary hilar lymph nodes, small bowel, colon, tonsil, hematological	Coronaviruses
small bowel, colon, hematological	Toroviruses
nasopharynx, sinus, tonsil, oral, larynx, lung/trachea/bronchi, pulmonary hilar lymph nodes, meninges, hematological, mediastinum	Parainfluenza viruses
salivary glands, pancreas, brain, spinal cord, liver, testes, hematological, meninges, ovaries, bone, heart, kidney, thyroid, prostate, breast	Mumps virus
nasopharynx, tonsil, sinus, lung/trachea/bronchi, pulmonary hilar lymph nodes, mediastinum, hematological, oral, pleura	Respiratory syncytial virus

nasopharynx, lung/trachea/bronchi, pulmonary hilar lymph nodes, tonsil, sinus, mediastinum, hematological, oral, pleura, larynx, eye, skin, small bowel, colon	Human metapneumovirus
nasopharynx, sinus, hematological, lung/trachea/bronchi, pulmonary hilar lymph nodes, intra-abdominal lymph nodes, meninges, brain, spinal cord, liver, spleen, lymph nodes, skin, thymus, eye, oral, heart	Rubeola
brain, meninges, lung/trachea/bronchi, kidney, hematological, muscle,	Hendra virus
brain, meninges, spleen, lymph nodes, thymus, lung/trachea/bronchi, kidneys, brain, spinal cord, meninges, hematological	Nipah virus
hematological, muscle, oral, tonsil, nasopharyngeal, lymph nodes, small bowel, colon	Vesicular stomatitis virus
skin, meninges, brain, spinal cord, oral, nasopharynx, salivary glands, hematological	Rabies virus
brain, spinal cord	Lyssaviruses

nasopharynx, laryngx, lung/trachea/bronchi, pulmonary hilar lymph nodes, meninges, muscle, hematological, mediastinum, muscle, sinus, tonsil, oral, eye, pleura, brain, spinal cord, salivary glands, thyroid, heart	Influenza virus
hematological, brain, meninges	California encephalitis virus
hematological, kidney, eye, skin, oral, muscle, lung/trachea/bronchi	Hantaviruses
brain, hematological, muscle, meninges, spinal cord	other Bunyaviruses
hematological, muscle, lymph nodes, skin, brain, meninges, testes, bone	Lymphocytic choriomeningitis virus
nasopharynx, brain, spinal cord, lung/trachea/bronchi, pulmonary hilar lymph nodes, mediastinum, muscle, testes, eye, heart,	Lassa virus
brain, meninges, hematological, muscle, eye, skin, lymph nodes, nasopharynx, small bowel, colon	Machupo virus
brain, meninges, hematological, muscle, eye, skin, lymph nodes, nasopharynx, small bowel, colon	Junin virus

hematological, skin, lymph nodes, muscle, eye, bone, lung, pulmonary hilar lymph nodes, spinal cord, brain	Human T-Cell Lymphotropic viruses
nasopharynx, lung/trachea/bronchi, pulmonary hilar lymph nodes, small bowel, neck and intra-abdominal lymph nodes, colon, hematological, liver, spleen, skin, brain, spinal cord, meninges, heart	Poliovirus
nasopharynx, larynx, oral, tonsil, lung/trachea/bronchi, pulmonary hilar lymph nodes, mediastinum, pancreas, muscle, brain, meninges, small bowel, neck and intra-abdominal lymph nodes, colon, hematological, spleen, skin, eye, sinus, liver, testes, bone, pleura, salivary glands, heart	Coxsackieviruses

nasopharynx, oral, tonsil, lung/trachea/bronchi, pulmonary hilar lymph nodes, muscle, brain, meninges, small bowel, neck and intra-abdominal lymph nodes, colon, hematological, mediastinum, spleen, skin, eye, sinus, liver, pancreas, testes, bone, salivary glands, heart	Echoviruses
lung/trachea/bronchi, pulmonary hilar lymph nodes, meninges, brain, skin, heart	other Enteroviruses
small bowel, colon, hematological, liver, spleen, brain, spinal cord, gallbladder, pancreas, kidney	Hepatitis A virus
nasopharynx, sinus, oral, tonsil, larynx, lung/trachea/bronchi, pulmonary hilar lymph nodes	Rhinoviruses
small bowel, colon	Noroviruses and other Caliciviruses
small bowel, colon	Astroviruses
small bowel, colon	Picobirnaviruses
liver, small bowel, colon, hematological	Hepatitis E virus

[0024] Optionally, the antigenic composition may be formulated for repeated subcutaneous or intradermal administration. Optionally, the antigenic composition may

be formulated for administration by a route that is not enteric. Optionally, the pathogen detailed herein is a bacteria, a virus, a protozoa, a fungus or a helminth. Further, the method may involve killing the pathogen to formulate the antigenic composition as a whole killed pathogen composition. Further, the pathogen may be a member of a species that is endogenous flora of the specific organ or tissue. Further, the pathogen may be an exogenous species.

[0025] In another aspect, a method of treating an individual for a condition characterized by inflammation in a specific organ or tissue is provided. The method involves administering to the individual an anti-inflammatory composition comprising antigenic determinants. The antigenic determinants are selected or formulated so that together they are specific for at least one pathogen that is pathogenic in the specific organ or tissue. Optionally, the anti-inflammatory composition may be administered at an administration site in successive doses given at a dosage interval of between one hour and one month, over a dosage duration of at least two weeks.

[0026] In another aspect, use of an anti-inflammatory composition for treating an individual for a condition characterized by inflammation in a specific organ or tissue is disclosed. The anti-inflammatory composition contains antigenic determinants selected or formulated so that together they are specific for at least one microbial pathogen that is pathogenic in the specific organ or tissue.

[0027] In another aspect, use of an anti-inflammatory composition to formulate a medicament for treating an individual for a condition characterized by inflammation in a specific organ or tissue is disclosed. The anti-inflammatory composition contains antigenic determinants selected or formulated so that together they are specific for at least one microbial pathogen that is pathogenic in the specific organ or tissue.

[0028] Optionally, the uses disclosed herein include those in which the individual has a cancer situated in the organ or tissue. Further, and optinally, the anti-

inflammatory composition may be administered at an administration site in successive doses given at a dosage interval of between one hour and one month, over a duration of at least two weeks.

[0028a] In an aspect, there is provided the use of an anti-inflammatory composition to formulate a medicament for treating an individual for an inflammatory bowel disease (IBD) characterized by inflammation in a region of the gastrointestinal tract (GIT), wherein the anti-inflammatory composition comprises antigenic determinants selected or formulated so that together they are specific for at least one microbial pathogen that is pathogenic in the region of the GIT, and wherein:

the region is a small bowel region, and the pathogen is selected from the group consisting of *Escherichia coli, Clostridium difficile, Bacteroides fragilis, Bacteroides vulgatus, Bacteroides thetaiotaomicron, Clostridium perfringens, Salmonella enteriditis, Yersinia enterocolitica, Shigella flexneri,* adenoviruses, astroviruses, caliciviruses, noroviruses, rotaviruses, and cytomegalovirus; or,

the region is a colon or rectum region, and the pathogen is selected from the group consisting of *Escherichia coli, Clostridium difficile, Bacteroides fragilis, Bacteroides vulgatus, Bacteroides thetaiotaomicron, Clostridium perfringens, Salmonella enteriditis, Yersinia enterocolitica, Shigella flexneri,* adenoviruses, astroviruses, caliciviruses, noroviruses, rotaviruses, and cytomegalovirus; or,

the region is an anus region, and the pathogen is selected from the group consisting of E. *coli, Streptococcus pyogenes, Bacteroides* spp., *Fusobacterium* spp., anaerobic streptococci, *Clostridium* spp., *Enterobacter* spp., *Pseudomonas aeruginosa, Treponema pallidum,* and herpes simplex virus; or,

the region is an oral region, and the pathogen is selected from the group consisting of *Prevotella melaninogenicus, anaerobic streptococci, viridans streptococci, Actinomyces* spp., *Peptostreptococcus* spp., *Bacteroides* spp., herpes simplex virus, coxsackieviruses, and Epstein-Barr virus; or,

the region is a stomach region, and the pathogen is selected from the group consisting of *Streptococcus pyogenes, Helicobacter pylori,* cytomegalovirus, herpes simplex virus, Epstein-Barr virus, rotaviruses, noroviruses, and adenoviruses.

[0029] In one aspect, a method of comparing immune responses is provided. The method involves administering to an animal having an organ or tissue a medicament having an antigenic composition having antigenic determinants selected or formulated so that together the antigenic determinants are specific for at least one microbial pathogen that is pathogenic in the organ or tissue, extracting a quantifiable immune sample from the organ or tissue, measuring a characteristic of the immune response in the organ or tissue in the quantifiable immune sample following the administration of the medicament, and, comparing the characteristic of the immune response in the quantifiable immune sample to a corresponding characteristic of the immune response in a reference immune sample obtained from a corresponding organ or tissue. Optionally, the reference immune sample may be obtained from the corresponding organ or tissue in the animal prior to the step of administering the medicament. Optionally, the reference immune sample may be obtained from the corresponding organ or tissue in a second animal. Optionally, the animal may have a cancer situated in the organ or tissue.

[0030] Comparing the characteristic of the immune response may involve comparing, in the quantifiable and reference immune samples, an indication of the numbers of any one or more of the following cells: inflammatory monocytes, macrophages, CD11b+ Gr-1+ cells, dendritic cells, CD11c+ MHC class II+ cells, CD4+ T cells, CD8+ T cells, or NK cells. Optionally, the macrophages may include any one or more of the following: M1-like macrophages or M2-like macrophages. Further, comparing the characteristic of the immune response may involve comparing a shift in an activation state of macrophages. Optionally, the macrophages may shift from being M2-like macrophages to being M1-like macrophages. Further and optionally, the macrophages may shift from being M1-like macrophages to being M2-like macrophages.

[0031] Optionally, comparing the characteristic of the immune response may involve identifying, in the quantifiable and reference immune samples, cellular markers on any one or more of the following cells: inflammatory monocytes, macrophages, CD11b+ Gr-1+ cells, dendritic cells, CD11c+ MHC class II+ cells, CD4+ T cells, CD8+ T cells, or NK cells. The macrophages may include any one or more of the following: M1-like macrophages or M2-like macrophages.

[0032] Optionally, comparing the characteristic of the immune response may involve identifying, in the quantifiable and reference immune samples, cytokines produced by any one or more of the following cells: inflammatory monocytes, macrophages, CD11b+ Gr-1+ cells, dendritic cells, CD11c+ MHC class II+ cells, CD4+ T cells, CD8+ T cells, or NK cells. As detailed herein, the macrophages may include any one or more of the following: M1-like macrophages or M2-like macrophages. Optionally, the cytokines are produced as a result of a shift in an activation state of the macrophages. Optionally, the macrophages shift from being M2-like macrophages to being M1-like macrophages. Further and optionally, the macrophages shift from being M1-like macrophages to being M2-like macrophages.

[0033] Optionally, comparing the characteristic of the immune response may involve identifying, in the quantifiable and reference immune samples, differential gene expression produced by any one or more of the following cells: inflammatory monocytes, macrophages, CD11b+ Gr-1+ cells, dendritic cells, CD11c+ MHC class II+ cells, CD4+ T cells, CD8+ T cells, or NK cells. The macrophages may include any one or more of the following: M1-like macrophages or M2-like macrophages. Optionally, the differential gene expression is produced as a result of a shift in an activation state of the macrophages. Optionally, macrophages may shift from being M2-like macrophages to being M1-like macrophages. Further and optionally, the macrophages shift from being M1-like macrophages to being M2-like macrophages.

[0034] Optionally, the medicament may be administered at an administration site in successive doses given at a dosage interval of between one hour and one month, over a dosage duration of at least one week. Optionally, the medicament may be administered intradermally or subcutaneously. Optionally, the medicament may be administered in a dose so that each dose is effective to cause a visible localized inflammatory immune response at the administration site. Optionally, the medicament may be administered so that visible localized inflammation at the administration site occurs within 1 to 48 hours. Further and optionally, the animal may be a mammal. Optionally, the animal may be a human or a mouse.

[0035] In another aspect, a method of selecting a therapeutic preparation suitable for treating an individual for a cancer in a specific organ or tissue is provided. The method involves providing an animal having a cancer situated in a specific organ or tissue, providing a test preparation having one or more antigenic determinants of a microbial pathogen which is pathogenic in the corresponding specific organ or tissue in a healthy individual, measuring a characterisic of the immune response in a reference immune sample obtained from the organ or tissue of the animal, administering the test preparation to the animal, measuring a characteristic of the immune response in a quantifiable immune sample obtained from a corresponding organ or tissue of the animal, comparing the characteristic of the immune response in the in the reference and quabtifiable immune samples, and treating an enhanced characterisic of the immune response in the quantifiable immune sample compared to the reference immune sample as an indication of the suitability of the test preparation as a therapeutic preparation. Optionally, the animal is sacrificed before the quantifiable immune sample has been obtained.

[0036] Optionally, comparing the characteristic of the immune response may involve comparing, in the quantifiable and reference immune samples, an indication of the numbers of any one or more of the following cells: inflammatory monocytes, macrophages, CD11b+ Gr-1+ cells, dendritic cells, CD11c+ MHC class II+ cells, CD4+

T cells, CD8+ T cells, or NK cells. Optionally, the macrophages may include any one or more of the following: M1-like macrophages or M2-like macrophages. Optionally, comparing the characteristic of the immune response may involve comparing a shift in an activation state of macrophages. Optionally, the macrophages may shift from being M2-like macrophages to being M1-like macrophages. Further and optionally, the macrophages may shift from being M1-like macrophages to being M2-like macrophages.

[0037] Optionally, comparing the characteristic of the immune response may involve identifying, in the quantifiable and reference immune samples, cellular markers on any one or more of the following cells: inflammatory monocytes, macrophages, CD11b+ Gr-1+ cells, dendritic cells, CD11c+ MHC class II+ cells, CD4+ T cells, CD8+ T cells, or NK cells. Optionally, the macrophages may include any one or more of the following: M1-like macrophages or M2-like macrophages.

[0038] Optionally, comparing the characteristic of the immune response may involve identifying, in the quantifiable and reference immune samples, cytokines produced by any one or more of the following cells: inflammatory monocytes, macrophages, CD11b+ Gr-1+ cells, dendritic cells, CD11c+ MHC class II+ cells, CD4+ T cells, CD8+ T cells, or NK cells. The macrophages may include any one or more of the following: M1-like macrophages or M2-like macrophages. Optionally, the cytokines are produced as a result of a shift in an activate state of the macrophages. Optionally, the macrophages may shift from being M2-like macrophages to being M1-like macrophages. Further, the macrophages may shift from being M1-like macrophages to being M2-like macrophages.

[0039] Further and optionally, comparing the characteristic of the immune response may involve identifying, in the quantifiable and reference immune samples, differential gene expression produced by any one or more of the following cells: inflammatory monocytes, macrophages, CD11b+ Gr-1+ cells, dendritic cells, CD11c+ MHC class II+

cells, CD4+ T cells, CD8+ T cells, or NK cells. Optionally, the macrophages may include any one or more of the following: M1-like macrophages or M2-like macrophages. Optionally, the differential gene expression may be produced as a result of a shift in an activation state of the macrophages. Optionally, the macrophages may shift from being M2-like macrophages to being M1-like macrophages. Further and optionally, the macrophages may shift from being M1-like macrophages to being M2-like macrophages.

[0040] In another aspect, a method of selectively targeting an immune response to a cancerous tissue or an organ in a human subject is provided. The method involves administering to the subject a medicament having an effective amount of a microbial pathogen antigenic composition, wherein the microbial pathogen may be pathogenic in the specific cancerous organ or tissue of the subject and the antigenic composition comprises antigenic determinants that together are specific for the microbial pathogen. Optionally, the antigenic composition may include a whole killed bacterial cell composition. Optionally, the medicament may be administered to the subject in an amount and for a time that is effective to up-regulate an immune response in the cancerous organ or tissue of the subject. Optionally, the method may further involve measuring a characteristic of the immune response. The method also includes treatment of pre-cancerous lesions including, but not limited to, actinic keratosis, cervical dysplasia, and colonic adenomas.

[0041] In another aspect, a method for treating a human subject for a cancer situated in a tissue or an organ is provided. The method involves administering to the subject a medicament having an effective amount of a microbial pathogen antigenic composition comprising a whole killed bacterial cell composition, wherein the microbial pathogen is pathogenic in the specific organ or tissue of the subject within which the cancer is situated. The medicament may be administered to the subject in an amount and for a time that is effective to modulate an immune response. Optionally, the modulation of the immune response may involve a shift in the activation state of macrophages.

Optionally, the modulation of the immune response may involve shifting from a M2-like macrophage response to a M1-like macrophage response. The modulation of the immune response may involve a shift from M1-like macrophages to M2-like macrophages, as those terms are defined herein. Optionally and without limitation, the method may further involve measuring a characteristic of the immune response.

[0042] Optionally, comparing the characteristic of the immune response may involve comparing, in the quantifiable and reference immune samples, an indication of the numbers of any one or more of the following cells: inflammatory monocytes, macrophages, CD11b+ Gr-1+ cells, dendritic cells, CD11c+ MHC class II+ cells, CD4+ T cells, CD8+ T cells, or NK cells. Optionally, the macrophages may include any one or more of the following: M1-like macrophages or M2-like macrophages. Optionally, comparing the characteristic of the immune response may involve comparing a shift in an activation state of macrophages. Further and optionally, the macrophages may shift from being M2-like macrophages to being M1-like macrophages. Optionally, the macrophages may shift from being M1-like macrophages to being M2-like macrophages.

[0043] Further and without limitation, comparing the characteristic of the immune response may involve identifying, in the quantifiable and reference immune samples, cellular markers on any one or more of the following cells: inflammatory monocytes, macrophages, CD11b+ Gr-1+ cells, dendritic cells, CD11c+ MHC class II+ cells, CD4+ T cells, CD8+ T cells, or NK cells. The macrophages may include any one or more of the following: M1-like macrophages or M2-like macrophages. Optionally, comparing the characteristic of the immune response may involve identifying, in the quantifiable and reference immune samples, cytokines produced by any one or more of the following cells: inflammatory monocytes, macrophages, CD11b+ Gr-1+ cells, dendritic cells, CD11c+ MHC class II+ cells, CD4+ T cells, CD8+ T cells, or NK cells. Optionally, the macrophages may include any one or more of the following: M1-like macrophages or M2-like macrophages. Further, cytokines may be produced as a result of a shift in an

activation state of the macrophages. The macrophages may shift from being M2-like macrophages to being M1-like macrophages. Optionally, the macrophages may shift from being M1-like macrophages to being M2-like macrophages.

[0044] Further and optionally, comparing the characteristic of the immune response may involve identifying, in the quantifiable and reference immune samples, differential gene expression produced by any one or more of the following cells: inflammatory monocytes, macrophages, CD11b+ Gr-1+ cells, dendritic cells, CD11c+ MHC class II+ cells, CD4+ T cells, CD8+ T cells, or NK cells. The macrophages may include any one or more of the following: M1-like macrophages or M2-like macrophages. Optionally, the differential gene expression may be produced as a result of a shift in an activation state of the macrophages. Further and optionally, the macrophages may shift from being M2-like macrophages to being M1-like macrophages. The macrophages may shift from being M1-like macrophages to being M2-like macrophages.

[0045] In another aspect, a method of monitoring efficacy of a treatment regime in an individual being treated for a cancer in a specific organ or tissue is provided. The method involves measuring a characteristic of an immune response in a post-treatment immune sample obtained from the specific organ or tissue after the individual has been subject to the treatment regime for a period of time, wherein the presence of a characteristic of the immune response which is greater in magnitude than would be expected had the individual not been subject to the treatment regime, is indicative of the efficacy of the treatment regime; and the treatment regime involves administering a preparation comprising one or more antigenic determinants of a microbial pathogen which is pathogenic in the corresponding specific organ or tissue in a healthy subject.

[0046] The method detailed herein may further involve measuring the characteristic of the immune response in a pre-treatment reference sample, wherein the pre-treatment reference sample was obtained from the specific organ or tissue before, at the same time as or after commencement of the treatment regime, but prior to obtaining the post-

treatment immune sample, and comparing the characteristic of the immune response in the pre-treatment and post-treatment samples, wherein an increase in the magnitude of the immune response in the post-treatment immune sample compared to the pre-treatment reference sample is indicative of the efficacy of the treatment regime. Optionally, measuring the characteristic of the immune response may involve determining an indication of the number of inflammatory monocytes in a sample of the organ or tissue. Optionally, measuring the characteristic of the immune response may involve determining an indication of the number of macrophages in a sample of the organ or tissue. The macrophages may include any one or more of the following: M1-like macrophages or M2-like macrophages.

[0047] Optionally, measuring the characteristic of the immune response may involve determining an indication of the number of CD11b+ Gr-1+ cells in a sample of the organ or tissue or determining an indication of the number of dendritic cells in a sample of the organ or tissue. Further and optionally, measuring the characteristic of the immune response may involve determining an indication of the number of CD11c+ MHC class II+ cells in a sample of the organ or tissue or determining an indication of the number of CD4+ T cells in a sample of the organ or tissue or determining an indication of the number of CD8+ T cells in a sample of the organ or tissue.

[0048] Optionally, measuring the magnitude of the immune response may involve determining an indication of the number of NK cells in a sample of the organ or tissue. Further and optionally, comparing the characteristic of the immune response may involve identifying, in the reference and immune samples, cellular markers on any one or more of the following cells: inflammatory monocytes, macrophages, CD11b+ Gr-1+ cells, dendritic cells, CD11c+ MHC class II+ cells, CD4+ T cells, CD8+ T cells, or NK cells. Optionally, the macrophages may include any one or more of the following: M1-like macrophages or M2-like macrophages.

[0049] Further and optionally, comparing the characteristic of the immune response may involve identifying, in the reference and immune samples, cytokines produced by any one or more of the following cells: inflammatory monocytes, macrophages, CD11b+ Gr-1+ cells, dendritic cells, CD11c+ MHC class II+ cells, CD4+ T cells, CD8+ T cells, or NK cells. The macrophages may include any one or more of the following: M1-like macrophages or M2-like macrophages. Optionally, the cytokines may be produced as a result of a shift in an activation state of the macrophages. The macrophages may shift from being M2-like macrophages to being M1-like macrophages. Further and optionally, the macrophages may shift from being M1-like macrophages to being M2-like macrophages.

[0050] Optionally, comparing the characteristic of the immune response may involve identifying, in the reference and immune samples, differential gene expression produced by any one or more of the following cells: inflammatory monocytes, macrophages, CD11b+ Gr-1+ cells, dendritic cells, CD11c+ MHC class II+ cells, CD4+ T cells, CD8+ T cells, or NK cells. The macrophages may include any one or more of the following: M1-like macrophages or M2-like macrophages. The differential gene expression may be produced as a result of a shift in an activation state of the macrophages. The macrophages may shift from being M2-like macrophages to being M1-like macrophages. Optionally, the macrophages may shift from being M1-like macrophages to being M2-like macrophages.

[0051] As detailed herein in another aspect, the disclosure provides methods for formulating an immunogenic composition for treating a cancer situated in a specific organ or tissue in a mammal, such as human patient. The method may include selecting at least one microbial pathogen that is naturally pathogenic in the organ or tissue of the mammal within which the cancer is situated. An antigenic composition may be produced that includes antigenic determinants that together are specific for or characteristic of the microbial pathogen.

[0052] A diagnostic step may be used to identify the specific organ or tissue within which the cancer is situated, prior to producing the antigenic composition targeted to the site of the cancer. The site of the cancer may be a primary site, or a secondary site of metastasis. The antigenic composition may be sufficiently specific that it would be capable of eliciting an immune response in the mammal specific to the microbial pathogen. The antigenic composition may be a bacterial composition, for example derived from a bacterial species or species that are endogenous to the flora of the patient or from an exogenous species or species. In alternative embodiments, the antigenic composition may be derived from a virus or viruses. Accordingly, the microbial pathogen from which the antigenic composition is derived may be a virus. The microbial pathogen may be killed. In alternative embodiments, the microbial pathogen may be live or attenuated. Immunogenic compositions of the disclosure may also be formulated or administered with anti-inflammatory modalities, such as an NSAID. The site of administration may be at a site distant from the site of the cancer, for example in an organ or tissue that is not the organ or tissue within which the cancer is situated, for example the skin or subcutaneous tissue.

[0053] The antigenic composition may for example be formulated for subcutaneous injection, intradermal injection or oral administration. In embodiments for subcutaneous or intradermal injection, the dosing or formulation of the antigenic composition may be adjusted in order to produce a localized immune reaction visible in the skin at the site of administration, for example an area of inflammation from 2mm to 100mm in diameter appearing, for example, 2 - 48 hours after administration and lasting, for example, 2 - 72 hours or longer. The antigenic composition may be formulated for repeated subcutaneous or intradermal administration, for example at alternating successive sites.

[0054] In some embodiments, the disclosure involves methods of treating a mammal for a cancer situated in a tissue or an organ. In alternative embodiments, the treatment may anticipate the development of the cancer in the tissue, for example if the site of a

primary tumour suggests the likelihood of metastasis to a particular tissue or organ, then the patient may be prophylactically treated to prevent or ameliorate metastasis to that tissue or organ. The method may include administering to the subject an effective amount of an antigenic composition comprising antigenic determinants that together are specific for at least one microbial pathogen. An aspect of the disclosure involves the use of a microbial pathogen that is pathogenic in the specific organ or tissue of the mammal within which the cancer is situated. The antigenic composition may be administered, for example by subcutaneous or intradermal injection at an administration site, in successive doses given at a dosage interval, for example of between one hour and one month, over a dosage duration, for example of at least 1 week, 2 weeks, 2 months, 6 months, 1, 2, 3, 4, or 5 years or longer. Each injection dose may for example be metered so that it is effective to cause visible localized inflammation at the administration site, appearing, for example, 1 – 48 hours after injection.

[0055] In another aspect, methods are provided for treating cancers of a specific organ or tissue in a subject by administering one or more antigens of one or more microbial pathogens, such as bacterial or viral species that are pathogenic in the specific organ or tissue.

[0056] In alternative embodiments, the pathogenic microbial species may be capable of causing infection naturally, (*i.e.*, without human intervention) in the specific organ or tissue in a healthy subject, or may have caused an infection in the specific organ or tissue in a healthy subject. In alternative embodiments, the antigen may be administered by administering a whole microbial species. In alternative embodiments, the method may, for example, include administering at least two or more microbial species, or administering at least three or more microbial species, and the microbes may be bacteria or viruses. In alternative embodiments, the method may further include administering a supplement or an adjuvant. An aspect of the disclosure involves

administering antigenic compositions so as to elicit an immune response in said subject.

[0057] In alternative embodiments, the microbial pathogen in the antigenic composition may be killed, and thus rendered non-infectious. In some embodiments, the antigenic composition is administered at a site distant from the cancer site, and in selected embodiments of this kind, methods of the disclosure may be carried out so that they do not produce infection at the cancer site.

[0058] As detailed herein, various aspects of the disclosure involve treating cancers. In this context, treatment may be carried out so as to provide a variety of outcomes. For example, treatment may: provoke an immune reaction that is effective to inhibit or ameliorate the growth or proliferation of a cancer; inhibit the growth or proliferation of cancer cells or tumors; cause remission of a cancer; improve quality of life; reduce the risk of recurrence of a cancer; inhibit metastasis of a cancer; or, improve patient survival rates in a patient population. In this context, extending the life expectancy of a patient, or patient population, means to increase the number of patients who survive for a given period of time following a particular diagnosis. In some embodiments, treatment may be of patients who have not responded to other treatments, such as patients for whom a chemotherapy or surgery has not been an effective treatment. Treatment in alternative embodiments may for example be before or after onset of cancer. For example prophylactic treatment may be undertaken, for example of patients diagnosed as being at risk of a particular cancer. For example a patient having a genetic or lifestyle predisposition to cancer of a certain tissue or organ may be treated with an immunogenic composition comprising antigenic determinants of a pathogen that is pathogenic in that organ or tissue. Similarly, the prophylactic treatment of metastasis may be undertaken, so that patients having a primary cancer with a propensity to metastasize to a particular tissue or organ may be treated with an immunogenic composition comprising antigenic determinants of a pathogen that is pathogenic in that organ or tissue.

[0059] In another aspect, a method of treating a cancer situated in a lung in a subject is provided. The method involves administering to the subject an effective amount of an antigen of one or more microbial species that is pathogenic in the lung and administering to the subject an effective amount of a platinum-containing chemotherapeutic agent. The microbial species may be a viral pathogen or a bacterial pathogen or a fungal pathogen.

[0060] The viral pathogen may be, without limitation: influenza, adenovirus, respiratory syncytial virus, or parainfluenza. The bacterial pathogen may be, without limitation: *Streptococcus pneumoniae, Moraxella catarrhalis, Mycoplasma pneumoniae, Klebsiella pneumoniae, Haemophilus influenza, Staphylococcus aureus, Chlamydia pneumoniae*, or *Legionella pneumophila*. The fungal pathogen may be, without limitation: *Aspergillus fumigatus, Blastomyces sp., Coccidiodes immitis, Coccidiodes posadasii, Cryptococcus neoformans, Cryptococcus gattii, Fusarium sp., Histoplasma capsulatum, Paecilomyces sp., Paracoccidiodes brasiliensis, Penicillium marneffei, Pneumocystis jiroveci, Pseudallescheria boydii, Scedosporium apiospermum, Rhizopus sp., Mucor sp., Absidia sp., Cunninghamella sp., Scedosporium prolificans, Stachybotrys chartarum, Trichoderma longibrachiatium,* or *Trichosporon sp.* Further, the platinum-containing chemotherapeutic agent may be, without limitation: Cisplatin, carboplatin or oxaliplatin.

[0061] In another aspect, use of an effective amount of an antigen of one or more microbial species that is pathogenic in the lung is provided to formulate a medicament for use with a platinum-containing chemotherapeutic agent for treating lung cancer in a subject. In another aspect, use of an effective amount of an antigen of one or more microbial species that is pathogenic in the lung is provided for use with a platinum-containing chemotherapeutic agent for treating lung cancer in a subject. The microbial species may be a viral pathogen or a bacterial pathogen or a fungal pathogen.

[0062] The viral pathogen may be, without limitation: influenza, adenovirus, respiratory syncytial virus, or parainfluenza. The bacterial pathogen may be, without limitation: *Streptococcus pneumoniae, Moraxella catarrhalis, Mycoplasma pneumoniae, Klebsiella pneumoniae, Haemophilus influenza, Staphylococcus aureus, Chlamydia pneumoniae*, or *Legionella pneumophila*. The fungal pathogen may be, without limitation: *Aspergillus fumigatus, Blastomyces sp., Coccidiodes immitis, Coccidiodes posadasii, Cryptococcus neoformans, Cryptococcus gattii, Fusarium sp., Histoplasma capsulatum, Paecilomyces sp., Paracoccidiodes brasiliensis, Penicillium marneffei, Pneumocystis jiroveci, Pseudallescheria boydii, Scedosporium apiospermum, Rhizopus sp., Mucor sp., Absidia sp., Cunninghamella sp., Scedosporium prolificans, Stachybotrys chartarum, Trichoderma longibrachiatium,* or *Trichosporon sp.* Further, the platinum-containing chemotherapeutic agent may be, without limitation: Cisplatin, carboplatin or oxaliplatin.

[0063] In another aspect, an effective amount of an antigen of one or more microbial species that is pathogenic in the lung is provided to formulate a medicament for use with a platinum-containing chemotherapeutic agent for treating lung cancer in a subject. In another aspect, an effective amount of an antigen of one or more microbial species that is pathogenic in the lung is provided for use with a platinum-containing chemotherapeutic agent for treating lung cancer in a subject. The microbial species may be a viral pathogen or a bacterial pathogen or a fungal pathogen.

[0064] The viral pathogen may be, without limitation: influenza, adenovirus, respiratory syncytial virus, or parainfluenza. The bacterial pathogen may be, without limitation: *Streptococcus pneumoniae, Moraxella catarrhalis, Mycoplasma pneumoniae, Klebsiella pneumoniae, Haemophilus influenza, Staphylococcus aureus, Chlamydia pneumoniae*, or *Legionella pneumophila*. The fungal pathogen may be, without limitation: *Aspergillus fumigatus, Blastomyces sp., Coccidiodes immitis, Coccidiodes posadasii, Cryptococcus neoformans, Cryptococcus gattii, Fusarium sp., Histoplasma capsulatum, Paecilomyces sp., Paracoccidiodes brasiliensis, Penicillium marneffei, Pneumocystis*

jiroveci, Pseudallescheria boydii, Scedosporium apiospermum, Rhizopus sp., Mucor sp., Absidia sp., Cunninghamella sp., Scedosporium prolificans, Stachybotrys chartarum, Trichoderma longibrachiatium, or *Trichosporon sp.* Further, the platinum-containing chemotherapeutic agent may be, without limitation: Cisplatin, carboplatin or oxaliplatin.

[0065] In another aspect, a kit is provided. The kit includes an antigen of one or more microbial species that is pathogenic in the lung, a platinum-containing chemotherapeutic agent; and instructions for providing the antigen and the platinum-containing chemotherapeutic agent to a subject in need thereof. The microbial species may be a viral pathogen or a bacterial pathogen or a fungal pathogen.

[0066] The viral pathogen may be, without limiting the foregoing: influenza, adenovirus, respiratory syncytial virus, or parainfluenza. The bacterial pathogen may be, without limitation: *Streptococcus pneumoniae, Moraxella catarrhalis, Mycoplasma pneumoniae, Klebsiella pneumoniae, Haemophilus influenza, Staphylococcus aureus, Chlamydia pneumoniae*, or *Legionella pneumophila*. The fungal pathogen may be, without limitation: *Aspergillus fumigatus*, *Blastomyces sp., Coccidiodes immitis, Coccidiodes posadasii, Cryptococcus neoformans, Cryptococcus gattii, Fusarium sp., Histoplasma capsulatum, Paecilomyces sp., Paracoccidiodes brasiliensis, Penicillium marneffei, Pneumocystis jiroveci, Pseudallescheria boydii, Scedosporium apiospermum, Rhizopus sp., Mucor sp., Absidia sp., Cunninghamella sp., Scedosporium prolificans, Stachybotrys chartarum, Trichoderma longibrachiatium,* or *Trichosporon sp.* Further, the platinum-containing chemotherapeutic agent may be, without limitation: Cisplatin, carboplatin or oxaliplatin.

[0067] In various aspects, the disclosure provides methods for formulating and using an immunogenic composition for treating an IBD. The IBD may for example be an IBD that is symptomatic in one or more regions of the GIT of a human patient, such as Crohn's disease, ulcerative colitis, collagenous colitis, lymphocytic colitis, ischaemic

colitis, diversion colitis, Behçet's syndrome or indeterminate colitis. The treatment of the patient may involve a diagnostic step of identifying the region of the GIT within which the IBD is symptomatic. The formulation may comprise an antigenic composition comprising antigenic determinants that together are specific for at least one pathogen, such as a bacteria, a virus, a protozoa or a helminth, that is pathogenic in the effected region of the GIT. The formulation may be prepared for administration as an immunogenic composition capable of eliciting an immune reaction to treat the IBD. The composition may for example be formulated for a route that is not enteric, such as subcutaneous injection or intradermal injection, for example to produce a localized skin immune response, such as an inflammatory response, at a site of administration.

BRIEF DESCRIPTION OF THE DRAWINGS

[0068] **Figure 1** shows a survival curve for a cumulative series of patients diagnosed with stage 3B or 4 inoperable lung cancer (all patients), comparing patients treated with MRV, patients not treated with the MRV, and a standard SEER survival curve.

[0069] **Figure 2** shows a survival curve for a cumulative series of patients diagnosed with stage 3B or 4 inoperable lung cancer (patients treated for at least 2 months with MRV), comparing patients treated with MRV, patients not treated with the MRV, and a standard SEER survival curve.

[0070] **Figure 3** shows a survival curve for a cumulative series of patients diagnosed with stage 3B or 4 lung cancer, illustrating the benefits of treatment with the MRV composition of the invention, comparing patients treated with MRV, patients not treated with the MRV, and a standard SEER survival curve.

[0071] **Figure 4** shows a survival curve for a cumulative series of patients diagnosed with stage 3B or 4 lung cancer, illustrating the effect of treatments for at least 2 months,

comparing patients treated with MRV, patients not treated with the MRV, and a standard SEER survival curve.

[0072] **Figure 5** shows a survival curve for a cumulative series of patients diagnosed with stage 3B or 4 lung cancer, illustrating the effect of treatments for at least 6 months duration, comparing patients treated with MRV, patients not treated with the MRV, and a standard SEER survival curve.

[0073] **Figure 6** shows a survival curve for a cumulative series of 52 breast cancer patients with metastases to bone and/or lung, comparing patients treated with MRV, patients not treated with the MRV, and a standard SEER survival curve.

[0074] **Figure 7** is a comparison of survival of a cumulative series of metastatic prostate cancer patients who had surgery or radiation to destroy their prostate gland (and thus, the primary tumour) and who had detectable cancer limited to bone metastases, comparing patients treated with MRV, patients not treated with the MRV, and a standard SEER survival curve.

[0075] **Figure 8** shows a survival curve for a cumulative series of patients initially diagnosed with Stage 4 colorectal cancer, comparing patients treated with PVF, patients treated with MRV, patients not treated with an antigenic composition and a standard SEER survival curve.

[0076] **Figure 9** shows a survival curve for a cumulative series of patients initially diagnosed with Stage 4 Colorectal Cancer, with date from patients receiving treatment within 3 months of diagnosis, comparing patients treated with PVF, patients treated with MRV, patients not treated with an antigenic composition and a standard SEER survival curve.

[0077] **Figure 10** shows a survival curve for a cumulative series of stage 3B lung cancer patients who were treated with an oral antigen therapy, Respivax, compared to patients who did not use an antigenic composition.

[0078] **Figure 11** shows a survival curve for a cumulative series of patients diagnosed with stage 3B lung cancer, illustrating the benefits of treatment with the MRV composition of the invention, comparing patients treated with MRV, patients not treated with the MRV, and a standard SEER survival curve.

[0079] **Figure 12** shows a survival curve measured from date of first visit for a cumulative series of patients diagnosed with stage 3B lung cancer, illustrating the benefits of treatment with the MRV composition of the invention, comparing patients treated with MRV, patients not treated with the MRV, and a standard SEER survival curve.

[0080] **Figure 13** shows a survival curve for a cumulative series of patients diagnosed with stage 3B lung cancer whose first visit was within 3 months of diagnosis, illustrating the benefits of early treatment with the MRV composition of the invention, comparing patients treated with MRV and patients not treated with the MRV.

[0081] **Figure 14** shows a photograph of lungs from mice treated (row 1) and not-treated (row 2) with *K. pneumoniae* cells-only vaccine following challenge with Lewis lung carcinoma cells, as described in Example 4A herein. The bottom row (not-numbered) depicts lungs from mice that were not exposed to the mouse model of lung cancer.

[0082] **Figure 15** shows the average tumor volume of mice treated [AB1 – AB6] and not-treated [AB-7] with bacterial vaccines following challenge with B16 melanoma cells, as described in Example 4B herein.

[0083] **Figure 16** shows a survival curve of colon cancer model mouse groups treated with or without a variety of bacterial vaccines, as described in Example 4C herein.

[0084] **Figure 17** shows the number of inflammatory monocytes and dendritic cells in the draining lymph node, lungs, and spleen following treatment with either a *K. pneumoniae* antigenic composition or PBS, as described in Example 5A herein.

[0085] **Figure 18** shows the total number of monocytes and dendritic cells in the lung, peritoneum and spleen of mice following treatment with either a *K. pneumoniae* antigenic composition, an *E. coli* antigenic composition, or PBS, as described in Example 5B herein.

[0086] **Figure 19** shows the total number of CD4+ T cells, CD8+ T cells, and NK cells from mice treated with either a *K. pneumoniae* antigenic composition, an *E. coli* antigenic composition, or PBS, as described in Example 5B herein.

[0087] **Figure 20** shows the total number of (A) inflammatory monocytes and (B) CD4+ T cells, CD8+ T cells, and NK cells on either of day 9 or 16 from mice treated with either heat-inactivated a *K. pneumoniae* antigenic composition, a phenol-inactivated *K. pneumoniae* antigenic composition, or PBS, as described in Example 5C herein.

[0088] **Figure 21** shows the total number of (A) inflammatory monocytes and dendritic cells and (B) CD4+ T cells, CD8+ T cells, and NK cells from mice treated with either a heat-inactivated *K. pneumoniae* antigenic composition, a phenol-inactivated *K. pneumoniae* antigenic composition, or PBS, as described in Example 5D herein.

[0089] **Figure 22** shows the total number of tumour nodules in mice treated with either PBS, or different dosages of *K. pneumoniae* antigenic composition as described herein.

[0090] **Figure 23** shows a photograph of lungs from mice treated with either PBS, or different dosages of *K. pneumoniae* antigenic composition as described herein.

[0091] **Figure 24** shows the total number of tumour nodules in mice treated with either PBS, or different dosages of *K. pneumoniae* antigenic composition as described herein.

[0092] **Figure 25** shows the total number of tumour nodules in mice treated with either PBS, or dosages of *K. pneumoniae* antigenic composition in combination (or not) with cisplatin as described herein.

[0093] **Figure 26** shows a survival curve for mice injected with Lewis Lung carcinoma cells and treated with either (i) vehicle control; (ii) Cisplatin; (iii) antigenic composition or (iv) antigenic composition and Cisplatin.

[0094] **Figure 27** shows the percentage of CD11b+ myeloid cells in the blood on day 13 of the relevant experiment detailed herein.

[0095] **Figure 28** shows (left panel) the frequency of CD11b+ NK1.1- cells from lungs of mice treated with various dosages of *K. pneumoniae* antigenic compositions or with PBS control or (right panel) the frequency of CD11b+ NK1.1+ cells from lungs of mice treated with various dosages of *K. pneumoniae* antigenic compositions or with PBS control.

[0096] **Figure 29** shows the amounts of numerous cytokines detected (in pg/g) from lung tissue derived from mice treated with various dosages of *K. pneumoniae* antigenic compositions or with PBS control.

[0097] **Figure 30** shows the amounts of numerous cytokines detected (in pg/ml) from BAL fluid from mice treated with various dosages of *K. pneumoniae* antigenic compositions or with PBS control.

[0098] **Figure 31** shows the relative gene expression ratios of NOS2 to Arg1 from mice treated with various dosages of *K. pneumoniae* antigenic compositions or with PBS control.

[0099] **Figure 32** shows the relative frequency of CD206 expression in lung macrophages from mice treated with various dosages of *K. pneumoniae* antigenic compositions or with PBS control.

[00100] **Figure 33** shows the relative frequency of F4/80 expression in lung macrophages from mice treated with various dosages of *K. pneumoniae* antigenic compositions or with PBS control.

[00101] **Figure 34** shows the relative frequency of CD11b+ Gr-1+ cells detected from the colons of mice treated with either *K. pneumoniae* or *E. coli* antigenic compositions or with PBS control.

[00102] **Figure 35** shows the relative frequency of CD11b+ Gr-1+ cells detected from the lungs of mice treated with either *K. pneumoniae* or *E. coli* antigenic compositions or with PBS control.

[00103] **Figure 36** shows the relative frequency of CD11b+ cells that were M1-like as isolated from subQ 4T1 tumours (left panel) and the relative frequency of CD11b+ cells that were M2-like as isolated from subQ 4T1 tumours (right panel).

[00104] **Figure 37** shows the tumour volume (mm^3) from mice treated with either indomethacin and PBS; or indomethacin and a *S. aureus* antigenic composition; or EtOH and PBS; or EtOH and a *S. aureus* antigenic composition [left panel]. The right panel of this Figure also shows the relative frequency and makeup of CD11b+ cells in the tumours at day 11 of the relevant experiment detailed herein.

[00105] **Figure 38** shows the relative frequency and makeup of CD11b+ cells in the tumours at day 22 of the relevant experiment detailed herein.

[00106] **Figure 39** shows the tumour volume in cohorts of animals treated with Indomethacin, Indomethacin + antigenic compositions, vehicle alone, or antigenic compositions alone.

[00107] **Figure 40** shows the percentage of CD11b+ cells in the tumours of animals treated with Indomethacin, Indomethacin + antigenic compositions, vehicle alone, or antigenic compositions alone (Day 11 data).

[00108] **Figure 41** shows the percentage of CD11b+ cells in the tumours of animals treated with Indomethacin, Indomethacin + antigenic compositions, vehicle alone, or antigenic compositions alone (Day 22 data).

[00109] **Figure 42** shows the percentage of CD11b+ CD94+ cells in the tumours of animals treated with Indomethacin, Indomethacin + antigenic compositions, vehicle alone, or antigenic compositions alone (Day 22 data).

[00110] **Figure 43** shows the relative expression of (A) Fizz1 and (B) Ym1 in excised tumors as described herein.

[00111] **Figure 44** shows the relative expression of (A) Arg1 and (B) Fizz1 from tumors and spleens [(C) and (D)] as described herein.

[00112] **Figure 45** shows the relative expression of Nos2 and Ym1 from tumors and spleens as described herein.

[00113] **Figure 46** shows the amount of IFN-γ produced in the lungs of mice with and without tumours and with and without antigenic composition treatment as described herein.

[00114] **Figure 47** shows the amount of IL-10 produced from bone marrow macrophages cultured overnight with *K. pneumoniae* antigenic composition, LPS, and media alone.

[00115] **Figure 48** shows the relative expression of IL-10 produced in the tumours of mice under conditions described herein.

DETAILED DESCRIPTION OF THE INVENTION

[00116] In various aspects, an embodiment of the invention relates to the surprising discovery that administration, for example at a site distant from the cancer, of microbial pathogens, such as killed microbial pathogens, that are pathogenic in a particular tissue or organ is effective in treating cancer situated in that specific tissue or organ. Accordingly, the invention provides antigenic compositions derived from these microbial pathogens, including whole killed bacterial or viral species, or components thereof, for the treatment of cancer, and methods for using the same.

[00117] Based on observations from treating patients, it was found that administering compositions of killed bacteria which included many of the bacterial species that commonly cause lung infection was surprisingly and unexpectedly effective in improving the clinical course of cancer of the lung. Similarly, it was found that administering compositions including killed *Staphylococcus aureus*, one of the most common causes of bone, breast, skin, perineal and lymph node infection and septicemia was surprising and unexpectedly effective in improving the clinical course of cancer of the bone, breast, skin, perineum, and lymphoma (cancer of the lymph glands) and multiple myeloma (a type of hematological cancer). Similarly, it was surprisingly

and unexpectedly found that administering a composition including *Escherichia coli*, which is a common cause of colon, kidney, peritoneal, liver, abdominal, pancreatic and ovarian infection, was effective in improving the clinical course of cancer in the colon, kidney, peritoneum, liver, abdominal lymph nodes, pancreas and ovary.

[00118] These results indicate that a composition including antigens of pathogenic microbial species that cause infection in a particular tissue or organ will be an effective formulation for treating a cancer in that tissue or organ. For example, cancer in the lung is effectively treated with a microbial composition including one or more pathogenic species that commonly cause lung infection, while cancer in the colon is effectively treated with a composition including pathogenic microbial species that commonly cause colon infections.

[00119] Antigenic compositions of the invention may be produced that include antigenic determinants that together are specific for or characteristic of a microbial pathogen. In this context, by "specific", it is meant that the antigenic determinants are sufficiently characteristic of the pathogen that they could be used to raise an immune response, such as an adaptive immune response, against the pathogen in the patient, if the antigenic determinants were to be administered in an appropriate manner to have that effect. It will be recognized that the antigenic determinants need not be so specific that they are characteristic of only one particular strain or species of pathogen, since even a specific immune response against a particular pathogen may be cross reactive with other closely related organisms that are also naturally pathogenic in the tissue or organ in which the cancer is situated and that the antigenic composition is formulated or selected to target.

[00120] In some embodiments, the compositions of pathogenic microbes may be used for treating primary cancer sites and/or sites of metastasis. Thus, for example, the microbial compositions may be used for the treatment of a cancer at a particular site, regardless of whether the cancer is a primary cancer or a metastasis. The composition

may be directed to the treatment of each cancer site, or may be a combined composition for both the primary cancer and the metastatic site(s). For example, to treat kidney cancer that has metastasized to the lung and bone, three different compositions, including one or more species that are known to be kidney pathogens, one or more species that are known to be lung pathogens and one or more species that are known to be bone pathogens, or a combined composition thereof may be used. In some embodiments, the compositions may be administered in different locations at the same time or at different times.

[00121] For example, for lung cancer with metastasis to the bone, in alternative embodiments, both a microbial composition including one or more bacterial species (or viruses) which commonly cause lung infection and a microbial composition including one or more bacterial species (or viruses) which commonly cause bone infection may be used. Similarly, for colon cancer with metastasis to the lungs, both a pathogenic bacterial (or viral) composition including one or more bacterial species (or viruses) which commonly cause colon infection and a microbial composition including one or more bacterial species (or viruses) which commonly cause lung infection may be used; for prostate cancer with metastasis to the bones, both a pathogenic bacterial (or viral) composit)on includlng one or more bacterial species (or viruses) which commonly cause prostate infection and a pathogenic bacterial (or viral) composition including one or more bacterial species (or viruses that commonly cause bone infection may be used.

[00122] The following list provides some non-limiting examples of primary cancers and their common sites for secondary spread (metastases):

Primary cancer	Common sites for metastases
prostate	bone, lungs
breast	bone, lungs, skin, liver, brain
lung	bone, brain, liver, lungs
colon	liver, lungs, bone, brain

kidney	lungs, bone, brain
pancreas	liver, lungs
melanoma	lungs, skin, liver, brain
uterus	lungs, bones, ovaries
ovary	liver, lung
bladder	bone, lung, liver
head and neck	bone, lungs
sarcoma	lungs, brain
stomach	liver
cervix	bone, lungs
testes	lungs
thyroid	bone, lungs

[00123] In some embodiments, the antigenic compositions may be used for treating or preventing cancers at primary sites or for treating or preventing metastasis. For example, in long-term smokers, an antigenic composition specific for cancer of the lung (for example including antigenic determinants of one or more bacterial species or viruses which commonly cause lung infection) may be used to appropriately stimulate the immune system to defend against the development of cancer within the lung tissue. As another example, an antigenic composition specific for cancer of the breast (for example including antigenic determinants of one or more bacterial species which commonly cause breast infection) could be used to prevent breast cancer in women with a strong family history of breast cancer or a genetic predisposition. In alternative embodiments, an antigenic composition including one or more bacterial species which commonly cause bone infection may be used to prevent or treat bone metastases in a patient with prostate cancer. In further alternative embodiments, an antigenic composition including one or more bacterial species or viruses which commonly cause lung infection may be used to prevent or treat lung metastases in a patient with malignant melanoma.

[00124] Various alternative embodiments and examples of the invention are described herein. These embodiments and examples are illustrative and should not be construed as limiting the scope of the invention.

Cancers

[00125] Most cancers fall within three broad histological classifications: carcinomas, which are the predominant cancers and are cancers of epithelial cells or cells covering the external or internal surfaces of organs, glands, or other body structures (for *e.g.*, skin, uterus, lung, braast, prostate, stomach, bowel), and which tend to metastasize; carcomas, which are derived from connective or supportive tissue (for *e.g.*, bone, cartilage, tendons, ligaments, fat, muscle); and hematologic tumors, which are derived from bone marrow and lymphatic tissue. Carcinomas may be adenocarcinomas (which generally develop in organs or glands capable of secretion, such as breast, lung, colon, prostate or bladder) or may be squamous cell carcinomas (which originate in the squamous epithelium and generally develop in most areas of the body). Sarcomas may be osteosarcomas or osteogenic sarcomas (bone), chondrosarcomas (cartilage), leiomyosarcomas (smooth muscle), rhabdomyosarcomas (skeletal muscle), mesothelial sarcomas or mesotheliomas (membranous lining of body cavities), fibrosarcomas (fibrous tissue), angiosarcomas or hemangioendotheliomas (blood vessels), liposarcomas (adipose tissue), gliomas or astrocytomas (neurogenic connective tissue found in the brain), myxosarcomas (primitive embryonic connective tissue), or mesenchymous or mixed mesodermal tumors (mixed connective tissue types). Hematologic tumors may be myelomas, which originate in the plasma cells of bone marrow; leukemias which may be "liquid cancers" and are cancers of the bone marrow and may be myelogenous or granulocytic leukemia (myeloid and granulocytic white blood cells), lymphatic, lymphocytic, or lymphoblastic leukemias (lymphoid and lymphocytic blood cells) or polycythemia vera or erythremia (various blood cell products, but with red cells predominating); or lymphomas, which may be solid tumors and which develop in the glands or nodes of the lymphatic system, and which may be Hodgkin or Non-Hodgkin lymphomas. In addition, mixed type cancers, such as

adenosquamous carcinomas, mixed mesodermal tumors, carcinosarcomas, or teratocarcinomas also exist.

[00126] Cancers named based on primary site may be correlated with histological classifications. For example, lung cancers are generally small cell lung cancers or non-small cell lung cancers, which may be squamous cell carcinoma, adenocarcinoma, or large cell carcinoma; skin cancers are generally basal cell cancers, squamous cell cancers, or melanomas. Lymphomas may arise in the lymph nodes associated with the head, neck and chest, as well as in the abdominal lymph nodes or in the axillary or inguinal lymph nodes. Identification and classification of types and stages of cancers may be performed by using for example information provided by the Surveillance, Epidemiology, and End Results (SEER) Program of the National Cancer Institute, which is an authoritative source of information on cancer incidence and survival in the United States and is recognized around the world. The SEER Program currently collects and publishes cancer incidence and survival data from 14 population-based cancer registries and three supplemental registries covering approximately 26 percent of the US population. The program routinely collects data on patient demographics, primary tumor site, morphology, stage at diagnosis, first course of treatment, and follow-up for vital status, and is the only comprehensive source of population-based information in the United States that includes stage of cancer at the time of diagnosis and survival rates within each stage. Information on more than 3 million in situ and invasive cancer cases is included in the SEER database, and approximately 170,000 new cases are added each year within the SEER coverage areas. The incidence and survival data of the SEER Program may be used to access standard survival for a particular cancer site and stage. For example, to ensure an optimal comparison group, specific criteria may be selected from the database, including date of diagnosis and exact stage (for example, in the case of the lung cancer example herein, the years were selected to match the time-frame of the retrospective review, and stage 3B and 4 lung cancer were selected; and in the case of the colon cancer example herein, the

years were also selected to match the time-frame of the retrospective review, and the stage 4 colon cancer was selected).

[00127] Cancers may also be named based on the organ in which they originate *i.e.,* the "primary site," for example, cancer of the breast, brain, lung, liver, skin, prostate, testicle, bladder, colon and rectum, cervix, uterus, *etc.* This naming persists even if the cancer metastasizes to another part of the body that is different from the primary site. With the present invention, treatment is directed to the site of the cancer, not type of cancer, so that a cancer of any type that is situated in the lung, for example, would be treated on the basis of this localization in the lung.

[00128] A "cancer" or "neoplasm" is any unwanted growth of cells serving no physiological function. In general, a cancer cell has been released from its normal cell division control, *i.e.,* a cell whose growth is not regulated by the ordinary biochemical and physical influences in the cellular environment. Thus, "cancer" is a general term for diseases characterized by abnormal uncontrolled cell growth. In most cases, a cancer cell proliferates to form clonal cells that are malignant. The lump or cell mass, "neoplasm" or "tumor," is generally capable of invading and destroying surrounding normal tissues. By "malignancy", as used herein, is meant as an abnormal growth of any cell type or tissue that has a deleterious effect in tha organism having the abnormal growth. The term "malignancy" or "cancer" includes cell growths that are technically benign but which carry the risk of becoming malignant. Cancer cells may spread from their original site to other parts of the body through the lymphatic system or blood stream in a process known as "metastasis." Many cancers are refractory to treatment and prove fatal. Examples of cancers or neoplasms include, without limitation, transformed and immortalized cells, tumors, carcinomas, in various organs and tissues as described herein or known to those of skill in the art.

[00129] A "cell" is the basic structural and functional unit of a living organism. In higher organisms, *e.g.,* animals, cells having similar structure and function generally

aggregate into "tissues" that perform particular functions. Thus, a tissue includes a collection of similar cells and surrounding intercellular substances, *e.g.*, epithelial tissue, connective tissue, muscle, nerve. An "organ" is a fully differentiated structural and functional unit in a higher organism that may be composed of different types of tissues and is specialized for some particular function, *e.g.*, kidney, heart, brain, liver, *etc.* Accordingly, by "specific organ, tissue, or cell" is meant herein to include any particular organ, and to include the cells and tissues found in that organ.

[00130] "Pathogenic" agents are agents, such as microbes, such as bacteria or viruses, which are known to cause infection in a host in nature, and in this sense, "pathogenic" is used in the context of the present invention to mean "naturally pathogenic". Although a wide variety of microbes may be capable of causing infection under artificial conditions, such as artificial innoculations of a microbe into a tissue, the range of microbes that naturally cause infection is necessarily limited, and well established by medical practice.

[00131] An "infection" is the state or condition in which the body or a part of it is invaded by a pathogenic agent (e.g., a microbe, such as a bacterium) which, under favorable conditions, multiplies and produces effects that are injurious (Taber's Cyclopedic Medical Dictionary, 14th Ed., C.L. Thomas, Ed., F.A. Davis Company, PA, USA). An infection may not always be apparent clinically and may result in only localized cellular injury. Infections may remain subclinical, and temporary if the body's defensive mechanisms are effective. Infections may spread locally to become clinically apparent as an acute, a subacute, or a chronic clinical infection or disease state. A local infection may also become systemic when the pathogenic agent gains access to the lymphatic or vascular system (On-Line Medical Dictionary, http://cancerweb.ncl.ac.uk/omd/). Infection is usually accompanied by inflammation, but inflammation may occur without infection.

[00132] "Inflammation" is the characteristic tissue reaction to injury (marked by swelling, redness, heat, and pain), and includes the successive changes that occur in living tissue when it is injured. Infection and inflammation are different conditions, although one may arise from the other (Taber's Cyclopedic Medical Dictionary, *supra*). Accordingly, inflammation may occur without infection and infection may occur without inflammation (although inflammation typically results from infection by pathogenic bacteria or viruses). Inflammation is characterized by the following symptoms: redness (rubor), heat (calor), swelling (tumor), pain (dolor). Localized visible inflammation on the skin may be apparent from a combination of these symptoms, particularly redness at a site of administration.

[00133] Various subjects may be treated in accordance with alternative aspects of the invention. As used herein, a "subject" is an animal, for *e.g,* a mammal, to whom the specific pathogenic bacteria, bacterial antigens, viruses, viral antigens or compositions thereof of the invention may be administered. Accordingly, a subject may be a patient, *e.g.,* a human, suffering from a cancer, or suspected of having a cancer, or at risk for developing a cancer. A subject may also be an experimental animal, *e.g.,* an animal model of a cancer, as is described in Example 5. In some embodiments, the terms "subject" and "patient" may be used interchangeably, and may include a human, a non-human mammal, a non-human primate, a rat, mouse, dog, *etc.* A healthy subject may be a human who is not suffering from a cancer or suspected of having a cancer, or who is not suffering from a chronic disorder or condition. A "healthy subject" may also be a subject who is not immunocompromised. By immunocompromised is meant any condition in which the immune system functions in an abnormal or incomplete manner. Immunocompromisation may be due to disease, certain medications, or conditions present at birth. Immunocompromised subjects may be found more frequently among infants, the elderly, and individuals undergoing extensive drug or radiation therapy.

[00134] An "immune response" includes, but is not limited to, one or more of the following responses in a mammal: induction or activation of antibodies, neutrophils,

monocytes, macrophages (including both M1-like macrophages and M2-like macrophages as described herein), B cells, T cells (including helper T cells, natural killer cells, cytotoxic T cells, γδ T cells), such as induction or activation by the antigen(s) in a composition or vaccine, following administration of the composition or vaccine. An immune response to a composition or vaccine thus generally includes the development in the host animal of a cellular and/or antibody-mediated response to the composition or vaccine of interest. In some embodiments, the immune response is such that it will also result in slowing or stopping the progression of a cancer in the animal. An immune response includes both cellular immune responses and humoral immune responses as understood by those persons skilled in the art.

Bacteria and Bacterial Colonizations and Infections

[00135] Most animals are colonized to some degree by other organisms, such as bacteria, which generally exist in symbiotic or commensal relationships with the host animal. Thus, many species of normally harmless bacteria are found in healthy animals, and are usually localized to the surface of specific organs and tissues. Often, these bacteria aid in the normal functioning of the body. For example, in humans, symbiotic *Escherichia coli* bacteria may be found in the intestine, where they promote immunity and reduce the risk of infection with more virulent pathogens.

[00136] Bacteria that are generally harmless, such as *Escherichia coli*, can cause infection in healthy subjects, with results ranging from mild to severe infection to death. Whether or not a bacterium is pathogenic (*i.e.*, causes infection) depends to some extent on factors such as the route of entry and access to specific host cells, tissues, or organs; the intrinsic virulence of the bacterium; the amount of the bacteria present at the site of potential infection; or the health of the host animal. Thus, bacteria that are normally harmless can become pathogenic given favorable conditions for infection, and even the most virulent bacterium requires specific circumstances to cause infection. Accordingly, microbial species that are members of the normal flora can be pathogens when they move beyond their normal ecological role in the endogenous flora. For

example, endogenous species can cause infection outside of their ecological niche in regions of anatomical proximity, for example by contiguous spread. When this occurs, these normally harmless endogenous bacteria are considered pathogenic.

[00137] Specific bacterial species and viruses are known to cause infections in specific cells, tissues, or organs in otherwise healthy subjects. Examples of bacteria and viruses that commonly cause infections in specific organs and tissues of the body are listed below; it will be understood that these examples are not intended to be limiting and that a skilled person would be able to readily recognize and identify infectious or pathogenic bacteria that cause infections, or commonly cause infections, in various organs and tissues in healthy adults (and recognize the relative frequency of infection with each bacterial species) based on the knowledge in the field as represented, for example, by the following publications: Manual of Clinical Microbiology 8th Edition, Patrick Murray, Ed., 2003, ASM Press American Society for Microbiology, Washington DC, USA; Mandell, Douglas, and Bennett's Principles and Practice of Infectious Diseases 5th Edition, G. L. Mandell, J.E. Bennett, R. Dolin, Eds., 2000, Churchill Livingstone, Philadelphia, PA, USA, all of which are incorporated by reference herein.

[00138] Infections of the skin are commonly caused by the following bacterial species: *Staphylococcus aureus*, Beta hemolytic streptococci group A, B, C or G, *Corynebacterium diptheriae, Corynebacterium ulcerans*, or *Pseudomonas aeruginosa*; or viral pathogens: rubeola, rubella, varicella-zoster, echoviruses, coxsackieviruses, adenovirus, vaccinia, herpes simplex, or parvo B19.

[00139] Infections of the soft tissue (*e.g.*, fat and muscle) are commonly caused by the following bacterial species: *Streptococcus pyogenes, Staphylococcus aureus*, *Clostridium perfringens*, or other Clostridium spp.; or viral pathogens: influenza, or coxsackieviruses.

[00140] Infections of the breast are commonly caused by the following bacterial species: *Staphylococcus aureus*, or *Streptococcus pyogenes*.

[00141] Infections of the lymph nodes of the head and neck are commonly caused by the following bacterial species: Staphylococcus *aureus, or Streptococcus pyogenes;* or viral pathogens: Epstein-Barr, cytomegalovirus, adenovirus, measles, rubella, herpes simplex, coxsackieviruses, or varicella-zoster.

[00142] Infections of the lymph nodes of the arm/axillae are commonly caused by the following bacterial species: Staphylococcus *aureus, or Streptococcus pyogenes*; or viral pathogens: measles, rubella, Epstein-Barr, cytomegalovirus, adenovirus, or varicella-zoster.

[00143] Infections of the lymph nodes of the mediastinum are commonly caused by the following bacterial species: viridans streptococci, Peptococcus spp., Peptostreptococcus spp., Bacteroides spp., Fusobacterium spp., or Mycobacterium *tuberculosis*; or viral pathogens: measles, rubella, Epstein-Barr, cytomegalovirus, varicella-zoster, or adenovirus.

[00144] Infections of the pulmonary hilar lymph nodes are commonly caused by the following bacterial species: *Streptococcus pneumoniae, Moraxella catarrhalis, Mycoplasma pneumoniae, Klebsiella pneumoniae, Haemophilus influenza, Chlamydophila pneumoniae, Bordetella pertussis or Mycobacterium tuberculosis*; or viral pathogens: influenza, adenovirus, rhinovirus, coronavirus, parainfluenza, respiratory syncytial virus, human metapneumovirus, or coxsackievirus.

[00145] Infections of the intra-abdominal lymph nodes are commonly caused by the following bacterial species: *Yersinia enterocolitica, Yersinia pseudotuberculosis,* Salmonella spp., *Streptococcus pyogenes, Escherichia coli, Staphylococcus aureus,* or

Mycobacterium tuberculosis; or viral pathogens: measles, rubella, Epstein-Barr, cytomegalovirus, varicella-zoster, adenovirus, influenza, or coxsackieviruses.

[00146] Infections of the lymph nodes of the leg/inguinal region are commonly caused by the following bacterial species: *Staphylococcus aureus*, or *Streptococcus pyogenes*; or viral pathogens: measles, rubella, Epstein-Barr, cytomegalovirus, or herpes simplex.

[00147] Infections of the blood (*i.e.*, septicemia) are commonly caused by the following bacterial species: *Staphylococcus aureus, Streptococcus pyogenes,* coagulase-negative staphylococci, Enterococcus spp., *Escherichia coli,* Klebsiella spp., Enterobacter spp., Proteus spp., *Pseudomonas aeruginosa, Bacteroides fragilis, Streptococcus pneumoniae*, or group B streptococci; or viral pathogens: rubeola, rubella, varicella-zoster, echoviruses, coxsackieviruses, adenovirus, Epstein-Barr, herpes simplex, or cytomegalovirus.

[00148] Infections of the bone are commonly caused by the following bacterial species: *Staphylococcus aureus*, coagulase-negative staphylococci, *Streptococcus pyogenes, Streptococcus pneumoniae, Streptococcus agalactiae,* other streptococci spp., *Escherichia coli,* Pseudomonas spp., Enterobacter spp., Proteus spp., or Serratia spp.; or viral pathogens: parvovirus B19, rubella, or hepatitis B.

[00149] Infections of the meninges are commonly caused by the following bacterial species: *Haemophilus influenzae, Neisseria meningitidis, Streptococcus pneumoniae, Streptococcus agalactiae,* or *Listeria monocytogenes*; or viral pathogens: echoviruses, coxsackieviruses, other enteroviruses, or mumps.

[00150] Infections of the brain are commonly caused by the following bacterial species: Streptococcus spp. (including *S. anginosus, S. constellatus, S. intermedius*), *Staphylococcus aureus*, Bacteroides spp., Prevotella spp., Proteus spp., *Escherichia*

coli, Klebsiella spp., Pseudomonas spp., Enterobacter spp., or *Borrelia burgdorferi*; or viral pathogens: coxsackieviruses, echoviruses, poliovirus, other enteroviruses, mumps, herpes simplex, varicella-zoster, flaviviruses, or bunyaviruses.

[00151] Infections of the spinal cord are commonly caused by the following bacterial species: *Haemophilus influenzae, Neisseria meningitidis, Streptococcus pneumoniae, Streptococcus agalactiae, Listeria monocytogenes,* or *Borrelia burgdorferi*; or viral pathogens: coxsackieviruses, echoviruses, poliovirus, other enteroviruses, mumps, herpes simplex, varicella-zoster, flaviviruses, or bunyaviruses.

[00152] Infections of the eye/orbit are commonly caused by the following bacterial species: *Staphylococcus aureus, Streptococcus pyogenes, Streptococcus pneumoniae, Streptococcus milleri, Escherichia coli, Bacillus cereus, Chlamydia trachomatis, Haemophilus influenza,* Pseudomonas spp., Klebsiella spp., or *Treponema pallidum*; or viral pathogens: adenoviruses, herpes simplex, varicella-zoster, or cytomegalovirus.

[00153] Infections of the salivary glands are commonly caused by the following bacterial species: *Staphylococcus aureus*, viridans streptococci (e.g., *Streptococcus salivarius, Streptococcus sanguis, Streptococcus mutans*), Peptostreptococcus spp., or Bacteroides spp., or other oral anaerobes; or viral pathogens: mumps, influenza, enteroviruses, or rabies.

[00154] Infections of the mouth are commonly caused by the following bacterial species: *Prevotella melaninogenicus*, anaerobic streptococci, viridans streptococci, Actinomyces spp., Peptostreptococcus spp., or Bacteroides spp., or other oral anaerobes; or viral pathogens: herpes simplex, coxsackieviruses, or Epstein-Barr.

[00155] Infections of the tonsils are commonly caused by the following bacterial species: *Streptococcus pyogenes,* or Group C or G B-hemolytic streptococci; or viral

pathogens: rhinoviruses, influenza, coronavirus, adenovirus, parainfluenza, respiratory syncytial virus, or herpes simplex.

[00156] Infections of the sinuses are commonly caused by the following bacterial species: *Streptococcus pneumoniae, Haemophilus influenza, Moraxella catarrhalis*, α-streptococci, anaerobic bacteria (*e.g.,* Prevotella spp.), or *Staphylococcus aureus*; or viral pathogens: rhinoviruses, influenza, adenovirus, or parainfluenza.

[00157] Infections of the nasopharynx are commonly caused by the following bacterial species: *Streptococcus pyogenes*, or Group C or G B-hemolytic streptococci; or viral pathogens: rhinoviruses, influenza, coronavirus, adenovirus, parainfluenza, respiratory syncytial virus, or herpes simplex.

[00158] Infections of the thyroid are commonly caused by the following bacterial species: *Staphylococcus aureus, Streptococcus pyogenes, or Streptococcus pneumoniae*; or viral pathogens: mumps, or influenza.

[00159] Infections of the larynx are commonly caused by the following bacterial species: *Mycoplasma pneumoniae, Chlamydophila pneumoniae,* or *Streptococcus pyogenes*; or viral pathogens: rhinovirus, influenza, parainfluenza, adenovirus, corona virus, or human metapneumovirus.

[00160] Infections of the trachea are commonly caused by the following bacterial species: *Mycoplasma pneumoniae*; or viral pathogens: parainfluenza, influenza, respiratory syncytial virus, or adenovirus.

[00161] Infections of the bronchi are commonly caused by the following bacterial species: *Mycoplasma pneumoniae, Chlamydophila pneumoniae, Bordetella pertussis, Streptococcus pneumoniae,* or *Haemophilus influenzae*; or viral pathogens: influenza,

adenovirus, rhinovirus, coronavirus, parainfluenza, respiratory syncytial virus, human metapneumovirus, or coxsackievirus.

[00162] Infections of the lung are commonly caused by the following bacterial species: *Streptococcus pneumoniae, Moraxella catarrhalis, Mycoplasma pneumoniae, Klebsiella pneumoniae,* or *Haemophilus influenza*; or viral pathogens: influenza, adenovirus, respiratory syncytial virus, or parainfluenza.

[00163] Infections of the pleura are commonly caused by the following bacterial species: *Staphylococcus aureus, Streptococcus pyogenes, Streptococcus pneumoniae, Haemophilus influenzae, Bacteroides fragilis*, Prevotella spp., *Fusobacterium nucleatum*, peptostreptococcus spp., or *Mycobacterium tuberculosis*; or viral pathogens: influenza, adenovirus, respiratory syncytial virus, or parainfluenza.

[00164] Infections of the mediastinum are commonly caused by the following bacterial species: viridans streptococci, Peptococcus spp., Peptostreptococcus spp., Bacteroides spp., Fusobacterium spp., or *Mycobacterium tuberculosis*; or viral pathogens: measles, rubella, Epstein-Barr, or cytomegalovirus.

[00165] Infections of the heart are commonly caused by the following bacterial species: Streptococcus spp. (including *S. mitior, S. bovis, S. sanguis, S. mutans, S. anginosus*), Enterococcus spp., Staphylococcus spp., *Corynebacterium diptheriae, Clostridium perfringens, Neisseria meningitidis*, or Salmonella spp.; or viral pathogens: enteroviruses, coxsackieviruses, echoviruses, poliovirus, adenovirus, mumps, rubeola, or influenza.

[00166] Infections of the esophagus are commonly caused by the following bacterial species: Actinomyces spp., *Mycobacterium avium, Mycobacterium tuberculosis*, or Streptococcus spp.; or viral pathogens: cytomegalovirus, herpes simplex, or varicella-zoster.

[00167] Infections of the stomach are commonly caused by the following bacterial species: *Streptococcus pyogenes or Helicobacter pylori*; or viral pathogens: cytomegalovirus, herpes simplex, Epstein-Barr, rotaviruses, noroviruses, or adenoviruses.

[00168] Infections of the small bowel are commonly caused by the following bacterial species: *Escherichia coli, Clostridium difficile, Bacteroides fragilis, Bacteroides vulgatus, Bacteroides thetaiotaomicron, Clostridium perfringens, Salmonella enteriditis, Yersinia enterocolitica,* or *Shigella flexneri*; or viral pathogens: adenoviruses, astroviruses, caliciviruses, noroviruses, rotaviruses, or cytomegalovirus.

[00169] Infections of the colon/rectum are commonly caused by the following bacterial species: *Escherichia coli, Clostridium difficile, Bacteroides fragilis, Bacteroides vulgatus, Bacteroides thetaiotaomicron, Clostridium perfringens, Salmonella enteriditis, Yersinia enterocolitica,* or *Shigella flexneri*; or viral pathogens: adenoviruses, astroviruses, caliciviruses, noroviruses, rotaviruses, or cytomegalovirus.

[00170] Infections of the anus are commonly caused by the following bacterial species: *Streptococcus pyogenes*, Bacteroides spp., Fusobacterium spp., anaerobic streptococci, Clostridium spp., *Escherichia coli*, Enterobacter spp., *Pseudomonas aeruginosa*, or *Treponema pallidum*; or viral pathogens: herpes simplex.

[00171] Infections of the perineum are commonly caused by the following bacterial species: *Escherichia coli*, Klebsiella spp., Enterococcus spp., Bacteroides spp., Fusobacterium spp., Clostridium spp., *Pseudomonas aeruginosa*, anaerobic streptococci, Clostridium spp., or Enterobacter spp.; or viral pathogens: herpes simplex.

[00172] Infections of the liver are commonly caused by the following bacterial species: *Escherichia coli*, Klebsiella spp., Streptococcus (anginosus group), Enterococcus, spp. other viridans streptococci, or Bacteroides spp.; or viral pathogens: hepatitis A, Epstein-Barr, herpes simplex, mumps, rubella, rubeola, varicella-zoster, coxsackieviruses, or adenovirus.

[00173] Infections of the gallbladder are commonly caused by the following bacterial species: *Escherichia coli*, Klebsiella spp., Enterobacter spp., enterococci, Bacteroides spp., Fusobacterium spp., Clostridium spp., *Salmonella enteriditis, Yersinia enterocolitica,* or *Shigella flexneri.*

[00174] Infections of the biliary tract are commonly caused by the following bacterial species: *Escherichia coli*, Klebsiella spp., Enterobacter spp., enterococci, Bacteroides spp., Fusobacterium spp., Clostridium spp., *Salmonella enteriditis, Yersinia enterocolitica,* or *Shigella flexneri*; or viral pathogens: hepatitis A, Epstein-Barr, herpes simplex, mumps, rubella, rubeola, varicella-zoster, cocsackieviruses, or adenovirus.

[00175] Infections of the pancreas are commonly caused by the following bacterial species: *Escherichia coli,* Klebsiella spp., Enterococcus spp., Pseudomonas spp., Staphylococcal spp., Mycoplasma spp., *Salmonella typhi,* Leptospirosis spp., or Legionella spp.; or viral pathogens: mumps, coxsackievirus, hepatitis B, cytomegalovirus, herpes simplex 2, or varicella-zoster.

[00176] Infections of the spleen are commonly caused by the following bacterial species: Streptococcus spp., Staphylococcus spp., Salmonella spp., Pseudomonas spp., *Escherichia coli,* or Enterococcus spp.; or viral pathogens: Epstein-Barr, cytomegalovirus, adenovirus, measles, rubella, coxsackieviruses, or varicella-zoster.

[00177] Infections of the adrenal gland are commonly caused by the following bacterial species: Streptococcus spp., Staphylococcus spp., Salmonella spp., Pseudomonas spp., *Escherichia coli*, or Enterococcus spp.; or viral pathogens: varicella-zoster.

[00178] Infections of the kidney are commonly caused by the following bacterial species: *Escherichia coli, Proteus mirabilis, Proteus vulgatus*, Providentia spp., Morganella spp., *Enterococcus faecalis,* or *Pseudomonas aeruginosa*; or viral pathogens: BK virus, or mumps.

[00179] Infections of the ureter are commonly caused by the following bacterial species: *Escherichia coli, Proteus mirabilis, Proteus vulgatus*, Providentia spp., Morganella spp., or Enterococcus spp.

[00180] Infections of the bladder are commonly caused by the following bacterial species: *Escherichia coli, Proteus mirabilis, Proteus vulgatus,* Providentia spp., Morganella spp., *Enterococcus faecalis,* or *Corynebacterium jekeum*; or viral pathogens: adenovirus, or cytomegalovirus.

[00181] Infections of the peritoneum are commonly caused by the following bacterial species: *Staphylococcus aureus, Streptococcus pyogenes, Streptococcus pneumonia, Escherichia coli,* Klebsiella spp., Proteus spp., enterococci, *Bacteroides fragilis, Prevotella melaninogenica,* Peptococcus spp., Peptostreptococcus spp., Fusobacterium spp., or Clostridium spp.

[00182] Infections of the retroperitoneal area are commonly caused by the following bacterial species: *Escherichia coli*, or *Staphylococcus aureus*.

[00183] Infections of the prostate are commonly caused by the following bacterial species: *Escherichia coli,* Klebsiella spp., Enterobacter spp., *Proteus mirabilis,*

enterococci spp., Pseudomonas spp., Corynebacterium spp., or *Neisseria gonorrhoeae*; or viral pathogens: herpes simplex.

[00184] Infections of the testicle are commonly caused by the following bacterial species: *Escherichia coli, Klebsiella pneumoniae, Pseudomonas aeruginosa,* Staphylococcus spp., Streptococcus spp., or *Salmonella enteriditis*; or viral pathogens: mumps, coxsackievirus, or lymphocytic choriomeningitis virus.

[00185] Infections of the penis are commonly caused by the following bacterial species: *Staphylococcus aureus, Streptococcus pyogenes, Neisseria gonorrhoeae,* or *Treponema pallidum*; or viral pathogens: herpes simplex.

[00186] Infections of the ovary/adnexae are commonly caused by the following bacterial species: *Neisseria gonorrhoeae, Chlamydia trachomatis, Gardenerella vaginalis,* Prevotella spp., Bacteroides spp., Peptococcus spp. Streptococcus spp., or *Escherichia coli.*

[00187] Infections of the uterus are commonly caused by the following bacterial species: *Neisseria gonorrhoeae, Chlamydia trachomatis, Gardenerella vaginalis,* Prevotella spp., Bacteroides spp., Peptococcus spp., Streptococcus spp., or *Escherichia coli.*

[00188] Infections of the cervix are commonly caused by the following bacterial species: *Neisseria gonorrhoeae, Chlamydia trachomatis,* or *Treponema pallidum;* or viral pathogens: herpes simplex.

[00189] Infections of the vagina are commonly caused by the following bacterial species: *Gardenerella vaginalis,* Prevotella spp., Bacteroides spp., peptococci spp., *Escherichia coli, Neisseria gonorrhoeae, Chlamydia Trachomatis,* or *Treponema pallidum*; or viral pathogens: herpes simplex.

[00190] Infections of the vulva are commonly caused by the following bacterial species: *Staphylococcus aureus, Streptococcus pyogenes,* or *Treponema pallidum;* or viral pathogens: herpes simplex.

Bacterial Strains/Viral Subtypes

[00191] It will be understood by a skilled person in the art that bacterial species are classified operationally as collections of similar strains (which generally refers to groups of presumed common ancestry with identifiable physiological but usually not morphological distinctions, and which may be identified using serological techniques against bacterial surface antigens). Thus, each bacterial species (*e.g.*, Streptococcus pneumoniae) has numerous strains (or serotypes), which may differ in their ability to cause infection or differ in their ability to cause infection in a particular organ/site. For example, although there are at least 90 serotypes of *Streptococcus pneumoniae*, serotypes 1, 3, 4, 7, 8, and 12 are most frequently responsible for pneumococcal disease in humans.

[00192] As a second example, certain strains of *Escherichia coli*, referred to as extraintestinal pathogenic *E. coli* (ExPEC), are more likely to cause urinary tract infection or other extraintestinal infections such as neonatal meningitis, whereas other strains, including enterotoxigenic *E. coli* (ETEC), enteropathogenic *E. coli* (EPEC), enterohemorrhagic *E. coli* (EHEC), Shiga toxin-producing *E. coli* (STEC), enteroaggregative *E. coli* (EAEC), enteroinvasive *E. coli* (EIEC) and diffuse adhering *E. coli* (DAEC) are more likely to cause gastrointestinal infection/diarrhea. Even among the sub-category of ExPEC strains, specific virulence factors (*e.g.*, production of type-1 fimbriae) enable certain strains to be more capable of causing infection of the bladder, while other virulence factors (*e.g.*, production of P fimbriae) enable other strains to be more capable of causing infection in the kidneys. In accordance with the present invention, an ExPEC strain(s) that is more likely to cause infection in the bladder may be chosen for a formulation to target bladder cancer, whereas an ExPEC strain(s) that

is more likely to cause infection in the kidney may be chosen for a formulation to target kidney cancer. Likewise, one or more of an ETEC, EPEC, EHEC, STEC, EAEC, EIEC or DAEC strains of *E. coli* (*i.e.,* strains that cause colon infection), may be chosen for a formulation to treat colon cancer.

[00193] Similarly, there may be numerous subtypes of specific viruses. For example, there are three types of influenza viruses, influenza A, influenza B and influenza C, which differ in epidemiology, host range and clinical characteristics. For example, influenza A is more likely to be associated with viral lung infection, whereas influenza B is more likely to be associated with myositis (*i.e.,* muscle infection). Furthermore, each of these three types of influenza virus have numerous subtypes, which also may differ in epidemiology, host range and clinical characteristics. In accordance with the present invention, one may choose an influenza A subtype most commonly associated with lung infection to target lung cancer, whereas one may choose an influenza B strain most commonly associated with myositis to treat cancer of the muscle/soft tissues.

[00194] It is understood that a clinical microbiologist skilled in the art would therefore be able to select, based on the present disclosure and the body of art relating to bacterial strains for each species of bacteria (and viral subtypes for each type of virus), the strains of a particular bacterial species (or subtype of a particular virus) to target a specific organ or tissue.

Bacterial Compositions, Dosages, and Administration

[00195] The compositions of the invention include antigens of pathogenic microbial (bacterial or viral) species that are pathogenic in a specific tissue or organ. The compositions may include whole bacterial species, or may include extracts or preparations of the pathogenic bacterial species of the invention, such as cell wall or cell membrane extracts, or whole cells, or exotoxins, or whole cells and exotoxins. The

compositions may also include one or more isolated antigens from one or more of the pathogenic bacterial species of the invention; in some embodiments, such compositions may be useful in situations where it may be necessary to precisely administer a specific dose of a particular antigen, or may be useful if administering a whole bacterial species or components thereof (*e.g.*, toxins) may be harmful. Pathogenic bacterial species may be available commercially (from, for example, ATCC (Manassas, VA, USA), or may be clinical isolates from subjects having a bacterial infection of a tissue or organ (*e.g.*, pneumonia).

[00196] The microbial compositions of the invention can be provided alone or in combination with other compounds (for example, nucleic acid molecules, small molecules, peptides, or peptide analogues), in the presence of a liposome, an adjuvant, or any pharmaceutically acceptable carrier, in a form suitable for administration to mammals, for example, humans. As used herein "pharmaceutically acceptable carrier" or "excipient" includes any and all solvents, dispersion media, coatings, antibacterial and antifungal agents, isotonic and absorption delaying agents, and the like that are physiologically compatible. The carrier can be suitable for any appropriate form of administration, including subcutaneous, intradermal, intravenous, parenteral, intraperitoneal, intramuscular, sublingual, inhalational, intratumoral or oral administration. Pharmaceutically acceptable carriers include sterile aqueous solutions or dispersions and sterile powders for the extemporaneous preparation of sterile injectable solutions or dispersion. The use of such media and agents for pharmaceutically active substances is well known in the art. Except insofar as any conventional media or agent is incompatible with the active compound (*i.e.*, the specific bacteria, bacterial antigens, or compositions thereof of the invention), use thereof in the pharmaceutical compositions of the invention is contemplated. Supplementary active compounds can also be incorporated into the compositions.

[00197] If desired, treatment with bacterial antigens according to the invention may be combined with more traditional and existing therapies for cancer, such as

chemotherapy, radiation therapy, surgery, etc., or with any other therapy intended to stimulate the immune system, reduce inflammation or otherwise benefit the subject, such as nutrients, vitamins and supplements. For example, vitamin A, vitamin D, vitamin E, vitamin C, vitamin B complex, selenium, zinc, co-enzyme Q10, beta carotene, fish oil, curcumin, green tea, bromelain, resveratrol, ground flaxseed, garlic, lycopene, milk thistle, melatonin, other antioxidants, cimetidine, indomethacin, or COX-2 Inhibitors (*e.g.*, Celebrex™ [celecoxib] or Vioxx™ [rofecoxib]) may be also be administered to the subject.

[00198] Conventional pharmaceutical practice may be employed to provide suitable formulations or compositions to administer the compounds to subjects suffering from a cancer. Any appropriate route of administration may be employed, for example, parenteral, intravenous, intradermal, subcutaneous, intramuscular, intracranial, intraorbital, ophthalmic, intraventricular, intracapsular, intraspinal, intrathecal, intracisternal, intraperitoneal, intranasal, inhalational, aerosol, topical, intratumoral, sublingual or oral administration. Therapeutic formulations may be in the form of liquid solutions or suspensions; for oral administration, formulations may be in the form of tablets or capsules; for intranasal formulations, in the form of powders, nasal drops, or aerosols; and for sublingual formulations, in the form of drops, aerosols or tablets.

[00199] Methods well known in the art for making formulations are found in, for example, "Remington's Pharmaceutical Sciences" (20th edition), ed. A. Gennaro, 2000, Mack Publishing Company, Easton, PA. Formulations for parenteral administration may, for example, contain excipients, sterile water, or saline, polyalkylene glycols such as polyethylene glycol, oils of vegetable origin, or hydrogenated napthalenes. Biocompatible, biodegradable lactide polymer, lactide/glycolide copolymer, or polyoxyethylene-polyoxypropylene copolymers may be used to control the release of the compounds. Other potentially useful parenteral delivery systems for include ethylene-vinyl acetate copolymer particles, osmotic pumps, implantable infusion

systems, and liposomes. Formulations for inhalation may contain excipients, for example, lactose, or may be aqueous solutions containing, for example, polyoxyethylene-9-lauryl ether, glycocholate and deoxycholate, or may be oily solutions for administration in the form of nasal drops, or as a gel. For therapeutic or prophylactic compositions, the pathogenic bacterial species are administered to an individual in an amount effective to stop or slow progression or metastasis of the cancer, or to increase survival of the subject (relative to, for example, prognoses derived from the SEER database) depending on the disorder.

[00200] An "effective amount" of a pathogenic microbial species or antigen thereof according to the invention includes a therapeutically effective amount or a prophylactically effective amount. A "therapeutically effective amount" refers to an amount effective, at dosages and for periods of time necessary, to achieve the desired therapeutic result, such as reduction or elimination of the cancer cells or tumors, prevention of carcinogenic processes, slowing the growth of the tumour, or an increase in survival time beyond that which is expected using for example the SEER database. A therapeutically effective amount of a pathogenic microbial (bacterial or viral) species or antigen(s) thereof may vary according to factors such as the disease state, age, sex, and weight of the individual, and the ability of the compound to elicit a desired response in the individual. Dosage regimens may be adjusted to provide the optimum therapeutic response. A therapeutically effective amount may also be one in which any toxic or detrimental effects of the pathogenic bacterial species or virus or antigen thereof are outweighed by the therapeutically beneficial effects. A "prophylactically effective amount" refers to an amount effective, at dosages and for periods of time necessary, to achieve the desired prophylactic result, such as prevention of cancer, prevention of metastasis, slowing the growth of the tumour, reduction or elimination of the cancer cells, tissues, organs, or tumors, or an increase in survival time beyond that which is expected using for example the SEER database. Typically, a prophylactic dose is used in subjects prior to or at an earlier stage of cancer, so that a prophylactically effective amount may be less than a therapeutically effective amount.

[00201] For administration by subcutaneous or intradermal injection, an exemplary range for therapeutically or prophylactically effective amounts of one or more pathogenic bacterial species may be about 1 million to 100,000 million organisms per ml, or may be 100 million to 7000 million organisms per ml, or may be 500 million to 6000 million organisms per ml, or may be 1000 million to 5000 million organisms per ml, or may be 2000 million to 4000 million organisms per ml, or any integer within these ranges. The total concentration of bacteria per ml may range from 1 million to 100,000 million organisms per ml, or may be 50 million to 7000 million organisms per ml, or may be 100 million to 6000 million organisms per ml, or may be 500 million to 5000 million organisms per ml, or may be 1000 million to 4000 million organisms per ml, or any integer within these ranges. The range for therapeutically or prophylactically effective amounts of antigens of a pathogenic bacterial species may be any integer from 0.1 nM-0.1 M, 0.1 nM-0.05M, 0.05 nM-15μM or 0.01 nM-10μM.

[00202] It is to be noted that dosage concentrations and ranges may vary with the severity of the condition to be alleviated, or may vary with the subject's immune response. In general, the goal is to achieve an adequate immune response. For administration by subcutaneous or intradermal infection, the extent of an immune response may be determined, for example, by size of delayed local immune skin reaction at the site of injection (*e.g.,* from 0.25 inch to 4 inch diameter). The dose required to achieve an appropriate immune response may vary depending on the individual (and their immune system) and the response desired. Standardized dosages may also be used. In the context of subcutaneous or intradermal administration, if the goal is to achieve a 2 inch local skin reaction, the total bacterial composition dose may, for example, range from 2 million bacteria (*e.g.,* 0.001 ml of a vaccine with a concentration of 2,000 million organisms per ml) to more than 20,000 million bacteria (*e.g.,* 1 ml of a vaccine with a concentration of 20,000 million organisms per ml). The concentrations of individual bacterial species or antigens thereof within a composition may also be considered. For example, if the concentration of one particular pathogenic

bacterial species, cell size of that species or antigenic load thereof is much higher relative to the other pathogenic bacterial species in the vaccine, then the local immune skin reaction of an individual may be likely due to its response to this specific bacterial species. In some embodiments, the immune system of an individual may respond more strongly to one bacterial species within a composition than another, depending for example on past history of exposure to infection by a particular species, so the dosage or composition may be adjusted accordingly for that individual. However, in some embodiments detailed herein, an immune response will not be monitored by way of a skin reaction. For example, in some mouse models utilized herein, the effective treatment of such animals with antigenic compositions may not result in corresponding skin reactions. A person skilled in the art will understand that there are alternate ways in which an immune response can be monitored beside relying on the presence or absence of a skin reaction.

[00203] For any particular subject, the timing and dose of treatments may be adjusted over time (*e.g.*, timing may be daily, every other day, weekly, monthly) according to the individual need and the professional judgement of the person administering or supervising the administration of the compositions. For example, in the context of subcutaneous or intradermal administration, the compositions may be administered every second day. An initial dose of approximately 0.05 ml may be administered subcutaneously, followed by increases from 0.01-0.02 ml every second day until an adequate skin reaction is achieved at the injection site (for example, a 1 inch to 2 inch diameter delayed reaction of visible redness at the injection site). Once this adequate immune reaction is achieved, this dosing is continued as a maintenance dose. The maintenance dose may be adjusted from time to time to achieve the desired visible skin reaction (inflammation) at the injection site. Dosing may be for a dosage duration, for example of at least 1 week, 2 weeks, 2 months, 6 months, 1, 2, 3, 4, or 5 years or longer.

[00204] Oral dosages may for example range from 10 million to 1,000,000 million organisms per dose, comprising antigenic determinants of one or more species. Oral dosages may be given, for example, from 4 times per day, daily or weekly. Dosing may be for a dosage duration, for example of at least 1 week, 2 weeks, 2 months, 6 months, 1, 2, 3, 4, or 5 years or longer.

[00205] In some embodiments, the invention may include antigenic compositions administered sublingually or by inhalation, or administered to one or more epithelial tissues (*i.e.*, skin by intradermal or subcutaneous injection; lung epithelium by inhalation; gastrointestinal mucosa by oral ingestion; mouth mucosa by sublingual administration) simultaneously or sequentially. Accordingly, in some embodiments the antigenic compositions of the invention are administered so as to provoke an immune response in an epithelial tissue. In some embodiments, one or more epithelial routes of administration may be combined with one or more additional routes of administration, such as intratumoral, intramuscular or intravenous administration.

[00206] In various aspects of the invention, the antigenic compositions that are administered to a patient may be characterized as having an antigenic signature, *i.e.*, a combination of antigens or epitopes that are sufficiently specific that the antigenic composition is capable of eliciting an immune response that is specific to a particular pathogen, such as an adaptive immune response. A surprising and unexpected aspect of the invention is that the non-adaptive or non-specific activation of the immune response that is mediated by these specific antigenic compositions is effective to treat cancers situated in the tissues in which the particular pathogen is pathogenic.

[00207] Routes of administration and dosage ranges set forth herein are exemplary only and do not limit the route of administration and dosage ranges that may be selected by medical practitioners. The amount of active compound (*e.g.*, pathogenic bacterial species or viruses or antigens thereof) in the composition may vary according to factors such as the disease state, age, sex, and weight of the

individual. Dosage regimens may be adjusted to provide the optimum 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. It may be advantageous to formulate parenteral compositions in dosage unit form for ease of administration and uniformity of dosage.

[00208] In the case of antigenic formulations (analogous to a vaccine), an immunogenically effective amount of a compound of the invention can be provided, alone or in combination with other compounds, with an immunological adjuvant. The compound may also be linked with a carrier molecule, such as bovine serum albumin or keyhole limpet hemocyanin to enhance immunogenicity. An antigenic composition ("vaccine") is a composition that includes materials that elicit a desired immune response. An antigenic composition may select, activate or expand, without limitation: memory B, T cells, neutrophils, monocytes or macrophages of the immune system to, for example, reduce or eliminate the growth or proliferation of cancerous cells or tissue. In some embodiments, the specific pathogenic microbe, virus, viral antigens, bacteria, bacterial antigens, or compositions thereof of the invention are capable of eliciting the desired immune response in the absence of any other agent, and may therefore be considered to be an antigenic composition. In some embodiments, an antigenic composition includes a suitable carrier, such as an adjuvant, which is an agent that acts in a non-specific manner to increase the immune response to a specific antigen, or to a group of antigens, enabling the reduction of the quantity of antigen in any given vaccine dose, or the reduction of the frequency of dosage required to generate the desired immune response. A bacterial antigenic composition may include live or dead bacteria capable of inducing an immune response against antigenic determinants normally associated with the bacteria. In some embodiments, an antigenic composition may include live bacteria that are of less virulent strains (attenuated), and therefore cause a less severe infection. In some embodiments the antigenic composition may

include live, attenuated or dead viruses capable of inducing an immune response against antigenic determinants normally associated with the virus.

[00209] An antigenic composition comprising killed bacteria for administration by injection may be made as follows. The bacteria may be grown in suitable media, and washed with physiological salt solution. The bacteria may then be centrifuged, resuspended in saline solution, and killed with heat. The suspensions may be standardized by direct microscopic count, mixed in required amounts, and stored in appropriate containers, which may be tested for safety, shelf life, and sterility in an approved manner. In addition to the pathogenic bacterial species and/or antigens thereof, a killed bacterial vaccine suitable for administration to humans may include 0.4% phenol preservative and/or 0.9% sodium chloride. The bacterial vaccine may also include trace amounts of brain heart infusion (beef), peptones, yeast extract, agar, sheep blood, dextrose, sodium phosphate and/or other media components.

[00210] In some embodiments, the bacterial vaccine may be used in tablet or capsule form or drops for oral ingestion, as an aerosol for inhalation, or as drops, aerosol or tablet form for sublingual administration.

[00211] In antigenic compositions comprising bacteria, the concentrations of specific bacterial species in compositions for subcutaneous or intradermal injection may be about 1 million to 100,000 million organisms per ml, or may be 100 million to 7000 million organisms per ml, or may be 500 million to 6000 million organisms per ml, or may be 1000 million to 5000 million organisms per ml, or may be 2000 million to 4000 million organisms per ml, or any integer within these ranges. The total concentration of bacteria per ml may range from 1 million to 100,000 million organisms per ml, or may be 50 million to 7000 million organisms per ml, or may be 100 million to 6000 million organisms per ml, or may be 500 million to 5000 million organisms per ml, or may be 1000 million to 4000 million organisms per ml, or any integer within these ranges.

[00212] In some embodiments, a selected killed bacterial vaccine for cancer of the lung tissue would include the common bacterial lung pathogens, and may for example be:

	bacteria per ml
Streptococcus pneumoniae	600 million
Haemophilus influenzae	400 million
Moraxella catarrhalis	400 million
Mycoplasma pneumoniae	300 million
Klebsiella pneumoniae	300 million
total:	2,000 million

or alternatively:

	bacteria per ml
Streptococcus pneumoniae	600 million
Haemophilus influenzae	300 million
Moraxella catarrhalis	300 million
Mycoplasma pneumoniae	400 million
Klebsiella pneumoniae	400 million
total:	2,000 million

In some selected embodiments, a selected killed bacterial vaccine for cancer of the lung tissue would include only more common bacterial lung pathogens, and may for example be:

	bacteria per ml
Streptococcus pneumoniae	800 million
Haemophilus influenzae	600 million

Moraxella catarrhalis		600 million
	total:	2,000 million

or alternatively:

		bacteria per ml
Streptococcus pneumoniae		800 million
Mycoplasma pneumoniae		600 million
Klebsiella pneumoniae		600 million
	total:	2,000 million

[00213] In further selected embodiments, a selected killed bacterial vaccine for cancer of the lung tissue would include only the most common bacterial lung pathogen, and may be:

		bacteria per ml
Streptococcus pneumoniae		2,000 million
	total:	2,000 million

or

		bacteria per ml
Klebsiella pneumoniae		2,000 million
	total:	2,000 million

or

		bacteria per ml
Mycoplasma pneumoniae		2,000 million
	total:	2,000 million

[00214] In some embodiments, an antigenic microbial composition for treating cancer at a particular site (*e.g.*, cancer of the lung tissue) may include pathogenic microbes that commonly, more commonly, or most commonly cause infection in that tissue or organ (*e.g.*, infection in the lung tissue *i.e.*, pneumonia).

[00215] In general, the pathogenic bacterial species and antigens thereof of the invention should be used without causing substantial toxicity. Toxicity of the compounds of the invention can be determined using standard techniques, for example, by testing in cell cultures or experimental animals and determining the therapeutic index, *i.e.*, the ratio between the LD50 (the dose lethal to 50% of the population) and the LD100 (the dose lethal to 100% of the population).

[00216] In some aspects, the invention involves the use of an anti-inflammatory in conjunction with vaccinations. In these embodiments, a wide variety of anti-inflammatory treatments may be employed, including effective amounts of non-steroidal anti-inflammatory drugs (NSAIDs), including but not limited to: diclofenac potassium, diclofenac sodium, etodolac, indomethicin, ketorolac tromethamine, sulindac, tometin sodium, celecoxib, meloxicam, valdecoxib, floctafenine, mefenamic acid, nabumetone, meloxicam, piroxicam, tenoxicam, fenoprofen calcium, flubiprofen, ibuprofen, ketoprofen, naproxen, naproxen sodium, oxaprozin, tiaprofenic acid, acetylsalicylic acid, diflunisal, choline magnesium trisalicylate, choline salicylate, triethanolamine salicylate, COX1 inhibitors, COX2 inhibitors (*e.g.*, Vioxx™, and Celebrex™). A variety of herbs and natural health products may also be used to provide anti-inflammatory treatment, including but not limited to: green tea, fish oil, vitamin D, antioxidant vitamins and minerals (*e.g.*, B carotene, vitamin A, vitamin C, vitamin D, vitamin E, co-enzyme Q10, selenium, *etc.*), resveratrol, turmeric, bromelain, boswellia, feverfew, quercetin, ginger, rosemary, oregano, cayenne, clove, nutmeg, willowbark. Alternative anti-inflammatory modalities may also include lifestyle modifications, such as: exercise, weight loss, smoking cessation, stress reduction, seeking social support, treatment of depression, stress management, abdominal breath work and dietary change (such as

adopting a mediterranean diet, a low glycemic diet, eating non-charred foods, including foods having omega-3 fatty acids).

[00217] As detailed herein and in an aspect of the invention, a method of comparing immune responses is provided. The method involves administering to an animal having an organ or tissue a medicament having an antigenic composition, as defined herein. The antigenic composition may have antigenic determinants selected or formulated so that together the antigenic determinants are specific for at least one microbial pathogen that is pathogenic in the organ or tissue, extracting a quantifiable immune sample from the organ or tissue, measuring a characteristic of the immune response in the organ or tissue in the quantifiable immune sample following the administration of the medicament, and, comparing the characteristic of the immune response in the quantifiable immune sample to a corresponding characteristic of the immune response in a reference immune sample obtained from a corresponding organ or tissue. As used herein, an immune sample would contain sufficient biological material to determine a characteristic of an immune response. As used herein, a "characteristic" of an immune response can include, without limitation, the particular number of a particular immune cell type (*e.g.*, macrophage), or a particular cellular marker (*e.g.*, upregulation of an integrin) or gene product (*e.g.*, a cytokine). The foregoing is provided as an example and is non-limiting.

[00218] Optionally, the reference immune sample may be obtained from the corresponding organ or tissue in the animal prior to the step of administering the medicament. In another aspect, the reference immune sample may be obtained from the corresponding organ or tissue in a second animal such that it is specifically contemplated that at least two animals (*i.e.*, an animal from which a reference immune sample is obtained and a second animal from which a quantifiable immune sample) could be used in the methods described herein. Optionally, the animal may have a cancer situated in the organ or tissue.

[00219] Comparing the characteristic of the immune response may involve comparing, in the quantifiable and reference immune samples, an indication of the numbers of any one or more of the following cells as these cells are known to those skilled in the art: inflammatory monocytes, macrophages, CD11b+ Gr-1+ cells, dendritic cells, CD11c+ MHC class II+ cells, CD4+ T cells, CD8+ T cells, or NK cells. Optionally, the macrophages may include any one or more of the following: M1-like macrophages or M2-like macrophages.

[00220] Those persons skilled in the art will appreciate that macrophages can be defined as either "M1-like macrophages" or "M2-like macrophages". For example, M1-like macrophages are generally understood by those persons skilled in the art to promote a Th1 CD4+ T cell-mediated response (see, for *e.g.*, Biswas and Mantovani (2010), *Nature Immunology* 10:889-96). Moreover, M1-like macrophages are generally understood to have efficient antigen presentation capacity, and to be proficient at killing intracellular pathogens (for *e.g.*, viruses). Moreover, M1-like macrophages are generally understood to be proficient, at least as compared with M2-like macrophages, in playing an immunological role in tumour destruction. Those skilled in the art will appreciate that there are numerous biological markers which can be employed to differentiate between M1-like macrophages and M2-like macrophages. For example, and as detailed herein, the expression of Nos2 is generally understood to correlate with an M1-like macrophage as compared with an M2-like macrophage (see, for *e.g.*, Laskin *et al.* (2010) *Annual Rev. Pharmacol. Toxicol.* 51: 267-288). Further, and for example, M1-like macrophages are generally understood to produce IL-12 and to be effectively activated by IFN-γ through the IFN- γR (Biswas and Mantovain, *supra*).

[00221] In contrast to M1-like macrophages, those persons skilled in the art will generally understand that M2-like macrophages promote a Th2 CD4+ T cell-mediated response (see, generally: Biswas and Mantovani (2010), *Nature Immunology* 10:889-96). Moreover, M2-like macrophages are generally understood to be effective and encapsulating and clearing extracellular parasites *etc.* Further, and in comparison to

M1-like macrophages, M2-like macrophages are generally understood by those persons skilled in the art as playing a more significant role in immunoregulation both with respect to T_{reg} and B cells (Biswas and Mantovain, *supra*).. Those persons skilled in the art will appreciate that there are numerous biological markers which can be employed to differentiate between M2-like macrophages and M1-like macrophages. For example, and as described herein, a diminished expression of Nos2 will generally be understood to correlate with M2-like macrophages as compared with higher expression being generally found in M1-like macrophages. Further, and as detailed in experiments herein, the expression of CD206 is generally understood as correlating with M2-like macrophages (see, for *e.g.,* Choi *et al.* (2010) *Gastroenterology* 138(7) 2399-409). Further, and as detailed in experiments herein, the expression of F4/80 is generally understood to correlate with M2-like macrophages. Further, and for example, M2-like macrophages are generally undersood to be effectively activated by IL-4 or by IL-13 through IL-4Rα (Biswas and Mantovain, *supra*).

[00222] Further, comparing the characteristic of the immune response may involve comparing a shift in an activation state of macrophages. The shift in the activation state of macrophages may optionally be characterized as a shift from M2-like macrophages to M1-like macrophages or vice versa. Those persons skilled in the art will appreciate that there are numerous biological markers that can be employed to monitor the activation of macrophages. As detailed herein, those skilled in the art will appreciate that defining a macrophage as being activated towards either a M1-like phenotype or a M2-like phenotype can be accomplished by chosing markers that are known to associate with either of the respective phenotypes described herein. Diseases which have been associated with M1 and M2 macrophages include at least the following: atherosclerosis (see, for *e.g.,* Hirata *et al.* (2011) *J. Am. Coll. Cardiol.* 58(3): 248-255), allergic asthma (see, for *e.g.,* Moreira and Hogaboam (2011) *J. Interferon. Cytokine Res.* 31(6): 485-91), autoimmune prostatitis (see, for *e.g.,* Zhang and Schluesener (2011) *Prostate*), colitis (see, for *e.g.,* Waddell *et al.* (2011) *J. Immunol.* 186(10): 5993-6003), COPD (see, for *e.g.,* Kunz *et al.* (2011) *Respir. Res.*

22: 34), glomerulonephritis (see, for *e.g.*, Fujita *et al.* (2010) *Am. J. Pathol.* 177(3): 1143-54), inflammatory bowel disease (see, for *e.g.*, Wendelsdorf *et al.* (2010) *J. Theor. Biol.* 264(4): 1225-39), chronic lung inflammation (see, for *e.g.*, Redente *et al.* (2010) *J. Leukoc. Biol.* 88(1): 159-68), steatohepatitis (see, for *e.g.*, Rensen *et al.* (2009) *Am. J. Pathol.* 175(4): 1473-82), pancreatitis (see, for *e.g.*, Gea-Sorli and Closa (2009) *BMC Immunol.* 31: 42), mycocarditis (see, for *e.g.*, Li *et al.* (2009) *Circ. Res.* 105(4): 353-64), liver fibrosis (see, for *e.g.*, Heymann *et al.* (2009) *Inflamm. Allergy Drug Targets* 8(4): 307-18), cystic fibrosis (see, for *e.g.*, Meyer *et al.* (2009) *Am. J. Respir. Cell Mol. Biol.* 41(5): 590-602), inflammatory renal disease (see, for *e.g.*, Wang *et al.* (2007) *Kidney Int.* 72(3): 290-299), and silicosis (see, for *e.g.*, Misson *et al.* (2004) *J. Leukoc. Biol.* 76(5): 926-232).

[00223] Optionally, comparing the characteristic of the immune response may involve identifying, in the quantifiable and reference immune samples, cellular markers on any one or more of the following cells as they are commonly understood to those persons skilled in the art: inflammatory monocytes, macrophages, CD11b+ Gr-1+ cells, dendritic cells, CD11c+ MHC class II+ cells, CD4+ T cells, CD8+ T cells, or NK cells. The macrophages may include any one or more of the following: M1-like macrophages or M2-like macrophages. A person skilled in the art will appreciate that there are numerous cell markers (both extracellular and intracellular) that can be selected which can identify an immune response. For example, as described herein, the marker CD206 is generally understood as correlating with M2-like macrophages (see, for *e.g.*, Choi *et al.* (2010) *Gastroenterology* 138(7) 2399-409).

[00224] Optionally, comparing the characteristic of the immune response may involve identifying, in the quantifiable and reference immune samples, cytokines produced by any one or more of the following cells as they are commonly understood to those persons skilled in the art: inflammatory monocytes, macrophages, CD11b+ Gr-1+ cells, dendritic cells, CD11c+ MHC class II+ cells, CD4+ T cells, CD8+ T cells, or NK cells. Those persons skilled in the art will appreciate that cytokines refer to small

cell-signalling protein molecules and that there are numerous cytokines known in the art. For example, cytokines have been grouped into type 1 and type 2 classifications based on their role in immunological responses. Common type 1 cytokines include IFN-γ and TGF-β. Common type 2 cytokines include, but are not limited to IL-4 and IL-13. Cytokines can be detected by numerous methodologies known to those persons skilled in the art. For example, and as detailed herein, ELISA experiments were utilized to determine cytokine production from lung tissue (see, for *e.g.*, Figure 27).

[00225] As detailed herein, the macrophages may include any one or more of the following: M1-like macrophages or M2-like macrophages as has been defined herein. Optionally, the cytokines are produced as a result of a shift in an activation state of the macrophages. Optionally, the macrophages shift from being M2-like macrophages to being M1-like macrophages. Further and optionally, the macrophages shift from being M1-like macrophages to being M2-like macrophages.

[00226] Optionally, comparing the characteristic of the immune response may involve identifying, in the quantifiable and reference immune samples, differential gene expression produced by any one or more of the following cells as they are commonly understood to those persons skilled in the art: inflammatory monocytes, macrophages, CD11b+ Gr-1+ cells, dendritic cells, CD11c+ MHC class II+ cells, CD4+ T cells, CD8+ T cells, or NK cells. The macrophages may include any one or more of the following: M1-like macrophages or M2-like macrophages. The term "differential gene expression" is understood to mean an appreciable difference between the expression of a particular gene of interest from at least two experimental conditions. For example, if under a first experimental condition a particular gene has a defined expression level as defined by gene expression methods used by those persons skilled in the art and if under a second experimental condition the same gene has an appreciable difference in its expression level, then there is differential expression of the gene of interest. Those persons skilled in the art will understand that there are numerous methodologies with which to detect differential gene expression. For example, commercially available

quantitative PCR techniques can be used as detailed herein with respect to determining the relative Nos2/Arg1 ratios (see, for *e.g.,* Figure 29). Optionally, the differential gene expression is produced as a result of a shift in an activation state of the macrophages. Optionally, macrophages may shift from being M2-like macrophages to being M1-like macrophages as those terms have been defined herein.

[00227] In another embodiment, the medicament may be administered at an administration site in successive doses given at a dosage interval of between one hour and one month, over a dosage duration of at least one week. Optionally, the medicament may be administered intradermally or subcutaneously. Optionally, the medicament may be administered in a dose so that each dose is effective to cause a visible localized inflammatory immune response at the administration site. Optionally, the medicament may be administered so that visible localized inflammation at the administration site occurs within 1 to 48 hours. However, a visable localized inflammatory immune response may not always be present in all circumstances despite an immune response being initiated. Those skilled in the art will appreciate that there are other methods by which the mounting of an immune response can be monitored. For example, the profile (and relative change in characterization) of immune cells from a subject undergoing an immune reaction can be compared with those from a subject that is not undergoing an immune reaction.

[00228] Further and optionally with respect to the methods disclosed herein, the animal may be a mammal. Optionally, the animal may be a human or a mouse. The foregoing are provided as examples and are not meant to be limiting.

[00229] In another aspect, a method of selecting a therapeutic preparation suitable for treating an individual for a cancer in a specific organ or tissue is provided. The method involves providing an animal having a cancer situated in a specific organ or tissue, providing a test preparation having one or more antigenic determinants of a microbial pathogen which is pathogenic in the corresponding specific organ or tissue in

a healthy individual, measuring a characterisic of the immune response in a reference immune sample obtained from the organ or tissue of the animal, administering the test preparation to the animal, measuring a characteristic of the immune response in a quantifiable immune sample obtained from a corresponding organ or tissue of the animal, comparing the characteristic of the immune response in the in the reference and quabtifiable immune samples, and treating an enhanced characterisic of the immune response in the quantifiable immune sample compared to the reference immune sample as an indication of the suitability of the test preparation as a therapeutic preparation. Optionally, the animal is sacrificed before the quantifiable immune sample has been obtained.

[00230] Optionally, comparing the characteristic of the immune response may involve comparing, in the quantifiable and reference immune samples, an indication of the numbers of any one or more of the following cells as they are commonly understood to those persons skilled in the art: inflammatory monocytes, macrophages, CD11b+ Gr-1+ cells, dendritic cells, CD11c+ MHC class II+ cells, CD4+ T cells, CD8+ T cells, or NK cells. Optionally, the macrophages may include any one or more of the following: M1-like macrophages or M2-like macrophages as those terms have been defined herein. Optionally, comparing the characteristic of the immune response may involve comparing a shift in an activation state of macrophages. Optionally, the macrophages may shift from being M2-like macrophages to being M1-like macrophages. Further and optionally, the macrophages may shift from being M1-like macrophages to being M2-like macrophages.

[00231] Optionally, comparing the characteristic of the immune response may involve identifying, in the quantifiable and reference immune samples, cellular markers on any one or more of the following cells as they are commonly understood to those persons skilled in the art: inflammatory monocytes, macrophages, CD11b+ Gr-1+ cells, dendritic cells, CD11c+ MHC class II+ cells, CD4+ T cells, CD8+ T cells, or NK

cells. Optionally, the macrophages may include any one or more of the following: M1-like macrophages or M2-like macrophages as those terms have been defined herein.

[00232] Optionally, comparing the characteristic of the immune response may involve identifying, in the quantifiable and reference immune samples, cytokines produced by any one or more of the following cells: inflammatory monocytes, macrophages, CD11b+ Gr-1+ cells, dendritic cells, CD11c+ MHC class II+ cells, CD4+ T cells, CD8+ T cells, or NK cells. The macrophages may include any one or more of the following: M1-like macrophages or M2-like macrophages as those terms have been defined herein. Optionally, the cytokines are produced as a result of a shift in an activate state of the macrophages. Optionally, the macrophages may shift from being M2-like macrophages to being M1-like macrophages.

[00233] Further and optionally, comparing the characteristic of the immune response may involve identifying, in the quantifiable and reference immune samples, differential gene expression produced by any one or more of the following cells: inflammatory monocytes, macrophages, CD11b+ Gr-1+ cells, dendritic cells, CD11c+ MHC class II+ cells, CD4+ T cells, CD8+ T cells, or NK cells. Optionally, the macrophages may include any one or more of the following: M1-like macrophages or M2-like macrophages as those terms have been defined herein. Optionally, the differential gene expression may be produced as a result of a shift in an activation state of the macrophages. Optionally, the macrophages may shift from being M2-like macrophages to being M1-like macrophages. Further and optionally, the macrophages may shift from being M1-like macrophages to being M2-like macrophages.

[00234] In another aspect, a method of selectively targeting an immune response to a cancerous tissue or an organ in a human subject is provided. The method involves administering to the subject a medicament having an effective amount of a microbial pathogen antigenic composition, wherein the microbial pathogen may be pathogenic in the specific cancerous organ or tissue of the subject and the antigenic composition

comprises antigenic determinants that together are specific for the microbial pathogen. Optionally, the antigenic composition may include a whole killed bacterial cell composition. Optionally, the medicament may be administered to the subject in an amount and for a time that is effective to up-regulate an immune response in the cancerous organ or tissue of the subject. Optionally, the method may further involve measuring a characteristic of the immune response.

[00235] In another aspect, a method for treating a human subject for a cancer situated in a tissue or an organ is provided. The method involves administering to the subject a medicament having an effective amount of a microbial pathogen antigenic composition comprising a whole killed bacterial cell composition, wherein the microbial pathogen is pathogenic in the specific organ or tissue of the subject within which the cancer is situated. The medicament may be administered to the subject in an amount and for a time that is effective to modulate an immune response. Optionally, the modulation of the immune response may involve a shift in the activation state of macrophages. Optionally, the modulation of the immune response may involve shifting from a M2-like macrophage response to a M1-like macrophage response. The modulation of the immune responses may involve shifting from a M1-like macrophage response to a M2-like macrophage response. Optionally, the method may further involve measuring a characteristic of the immune response.

[00236] Optionally, comparing the characteristic of the immune response may involve comparing, in the quantifiable and reference immune samples, an indication of the numbers of any one or more of the following cells as they are commonly understood to those persons skilled in the art: inflammatory monocytes, macrophages, CD11b+ Gr-1+ cells, dendritic cells, CD11c+ MHC class II+ cells, CD4+ T cells, CD8+ T cells, or NK cells. Optionally, the macrophages may include any one or more of the following: M1-like macrophages or M2-like macrophages as those terms have been defined herein. Optionally, comparing the characteristic of the immune response may involve comparing a shift in an activation state of macrophages. Further and optionally,

the macrophages may shift from being M2-like macrophages to being M1-like macrophages. Optionally, the macrophages may shift from being M1-like macrophages to being M2-like macrophages.

[00237] Further and optionally, comparing the characteristic of the immune response may involve identifying, in the quantifiable and reference immune samples, cellular markers on any one or more of the following cells as they are commonly understood to those persons skilled in the art: inflammatory monocytes, macrophages, CD11b+ Gr-1+ cells, dendritic cells, CD11c+ MHC class II+ cells, CD4+ T cells, CD8+ T cells, or NK cells. The macrophages may include any one or more of the following: M1-like macrophages or M2-like macrophages as those terms have been defined herein. Optionally, comparing the characteristic of the immune response may involve identifying, in the quantifiable and reference immune samples, cytokines produced by any one or more of the following cells as they are commonly understood to those persons skilled in the art: inflammatory monocytes, macrophages, CD11b+ Gr-1+ cells, dendritic cells, CD11c+ MHC class II+ cells, CD4+ T cells, CD8+ T cells, or NK cells. Optionally, the macrophages may include any one or more of the following: M1-like macrophages or M2-like macrophages. Further, cytokines may be produced as a result of a shift in an activation state of the macrophages. The macrophages may shift from being M2-like macrophages to being M1-like macrophages. Optionally, the macrophages may shift from being M1-like macrophages to being M2-like macrophages.

[00238] Further and optionally, comparing the characteristic of the immune response may involve identifying, in the quantifiable and reference immune samples, differential gene expression produced by any one or more of the following cells as they are commonly understood to those persons skilled in the art: inflammatory monocytes, macrophages, CD11b+ Gr-1+ cells, dendritic cells, CD11c+ MHC class II+ cells, CD4+ T cells, CD8+ T cells, or NK cells. The macrophages may include any one or more of the following: M1-like macrophages or M2-like macrophages. Optionally, the differential

gene expression may be produced as a result of a shift in an activation state of the macrophages. Further and optionally, the macrophages may shift from being M2-like macrophages to being M1-like macrophages. The macrophages may shift from being M1-like macrophages to being M2-like macrophages.

[00239] In another aspect, a method of monitoring efficacy of a treatment regime in an individual being treated for a cancer in a specific organ or tissue is provided. The method involves measuring a characteristic of an immune response in a post-treatment immune sample obtained from the specific organ or tissue after the individual has been subject to the treatment regime for a period of time, wherein the presence of a characteristic of the immune response which is greater in magnitude than would be expected had the individual not been subject to the treatment regime, is indicative of the efficacy of the treatment regime; and the treatment regime involves administering a preparation comprising one or more antigenic determinants of a microbial pathogen which is pathogenic in the corresponding specific organ or tissue in a healthy subject.

[00240] The method detailed herein may further involve measuring the characteristic of the immune response in a pre-treatment reference sample, wherein the pre-treatment reference sample was obtained from the specific organ or tissue before, at the same time as or after commencement of the treatment regime, but prior to obtaining the post-treatment immune sample, and comparing the characteristic of the immune response in the pre-treatment and post-treatment samples, wherein an increase in the magnitude of the immune response in the post-treatment immune sample compared to the pre-treatment reference sample is indicative of the efficacy of the treatment regime. Optionally, measuring the characteristic of the immune response may involve determining an indication of the number of inflammatory monocytes in a sample of the organ or tissue. Optionally, measuring the characteristic of the immune response may involve determining an indication of the number of macrophages in a sample of the organ or tissue. The macrophages may include any one or more of the following: M1-like macrophages or M2-like macrophages.

[00241] Optionally, measuring the characteristic of the immune response may involve determining an indication of the number of CD11b+ Gr-1+ cells in a sample of the organ or tissue or determining an indication of the number of dendritic cells in a sample of the organ or tissue. Further and optionally, measuring the characteristic of the immune response may involve determining an indication of the number of CD11c+ MHC class II+ cells in a sample of the organ or tissue or determining an indication of the number of CD4+ T cells in a sample of the organ or tissue or determining an indication of the number of CD8+ T cells in a sample of the organ or tissue.

[00242] Optionally, measuring the magnitude of the immune response may involve determining an indication of the number of NK cells in a sample of the organ or tissue. Further and optionally, comparing the characteristic of the immune response may involve identifying, in the reference and immune samples, cellular markers on any one or more of the following cells as they are commonly understood to those persons skilled in the art: inflammatory monocytes, macrophages, CD11b+ Gr-1+ cells, dendritic cells, CD11c+ MHC class II+ cells, CD4+ T cells, CD8+ T cells, or NK cells. Optionally, the macrophages may include any one or more of the following: M1-like macrophages or M2-like macrophages.

[00243] Further and optionally, comparing the characteristic of the immune response may involve identifying, in the reference and immune samples, cytokines produced by any one or more of the following cells as they are commonly understood to those persons skilled in the art: inflammatory monocytes, macrophages, CD11b+ Gr-1+ cells, dendritic cells, CD11c+ MHC class II+ cells, CD4+ T cells, CD8+ T cells, or NK cells. The macrophages may include any one or more of the following: M1-like macrophages or M2-like macrophages. Optionally, the cytokines may be produced as a result of a shift in an activation state of the macrophages. The macrophages may shift from being M2-like macrophages to being M1-like macrophages. Further and

optionally, the macrophages may shift from being M1-like macrophages to being M2-like macrophages.

[00244] Optionally, comparing the characteristic of the immune response may involve identifying, in the reference and immune samples, differential gene expression produced by any one or more of the following cells as they are commonly understood to those persons skilled in the art: inflammatory monocytes, macrophages, CD11b+ Gr-1+ cells, dendritic cells, CD11c+ MHC class II+ cells, CD4+ T cells, CD8+ T cells, or NK cells. The macrophages may include any one or more of the following: M1-like macrophages or M2-like macrophages. The differential gene expression may be produced as a result of a shift in an activation state of the macrophages. The macrophages may shift from being M2-like macrophages to being M1-like macrophages. Optionally, the macrophages may shift from being M1-like macrophages to being M2-like macrophages.

[00245] In various aspects, embodiments of the invention relate to a method of treating a cancer situated in a lung in a subject. The method involves administering to the subject an effective amount of an antigen of one or more microbial species that is pathogenic in the lung and administering to the subject an effective amount of a platinum-containing chemotherapeutic agent. The microbial species may be a viral pathogen or a bacterial pathogen or a fungal pathogen.

[00246] As used herein, the phrase "treating a cancer" may include, without limitation, reducing the lung tumour burden in a subject or increasing life expectancy in a subject that has lung cancer. It will be understood that there are numerous biological read-outs that can be used by a person skilled in the art to determine whether a cancer is being treated or not.

[00247] The viral pathogen utilized herein may be, without limitation: influenza, adenovirus, respiratory syncytial virus, parainfluenza, monkeypox, herpes simplex virus

(1 and 2), Varicella zoster, cytomegalovirus, Epstein-Barr virus, coronavirus, human metapneumovirus, Hendra virus, Nipah virus, Hanatvirus, Lassa virus, human T-cell lymphotrophic virus, cocksackievirus, echovirus, enterovirus, or rhinovirus, or any virus that is pathogenic in the lung.

[00248] The bacterial pathogen utilized herein may be, without limitation: *Streptococcus pneumoniae, Moraxella catarrhalis, Mycoplasma pneumoniae, Klebsiella pneumoniae, Haemophilus influenza, Staphylococcus aureus, Chlamydia pneumoniae, Legionella pneumophila*, or *Bordatella pertussis* or any bacterium that is pathogenic in the lung.

[00249] The fungal pathogen utilized herein may be, without limitation: *Aspergillus fumigatus, Blastomyces sp., Coccidiodes immitis, Coccidiodes posadasii, Cryptococcus neoformans, Cryptococcus gattii, Fusarium sp., Histoplasma capsulatum, Paecilomyces sp., Paracoccidiodes brasiliensis, Penicillium marneffei, Pneumocystis jiroveci, Pseudallescheria boydii, Scedosporium apiospermum, Rhizopus sp., Mucor sp., Absidia sp., Cunninghamella sp., Scedosporium prolificans, Stachybotrys chartarum, Trichoderma longibrachiatium, Trichosporon sp.*, or any fungus that is pathogenic in the lung.

[00250] As used herein, the term "platinum-containing chemotherapeutic agent" includes, but is not limited to: Cisplatin, carboaplatin or ormaplatin, oxaliplatin, DWA2114R ((-)-(R)-2aminomethylpyrrolidine (1,1-cyclobutane dicarboxylato) platinum), zeniplatin, enloplatin, lobaplatin, CI-973 (SP-43(R)-l,l-cyclobutane-dicarboxylato(2-)-(2-methyl-l,4-65 butanediamine-N,N')platinum), 254-S nedaplatin, JM-216 (bis-acetato-ammine-dichloro-cyclohexylamineplatinum(IV)), (see: Weiss, R.B. and Christian, M.C., "New Cisplatin Analogue in Development," *Drugs*. 46:(03) 360-377 (1993)); CPA)2Pt[DOLYM] and (DACH)Pt[DOLYM] cisplatin (Choi et al., *Arch. Pharmacal Res.* 22(2):151-156, 1999); 254-S cisplatin analogue (Koga *et al.*, *Neurol. Res.* 18(3):244-247,1996); cis-l,4-diaminocyclohexane cisplatin analogues (Shamsuddin *et al.*, *J. Inorg.*

Biochem. 61(4): 291-301, 1996); MeOH cisplatin (Shamsuddin *et al., Inorg. Chem.* 36(25):59695971, 1997); CI-973 cisplatin analogue (Yang etal. *Int. J. Oncol.* 5(3):597-602, 1994); cis-diaminedichloroplatinum (II) and its analogues cis-l,l-cyclobutanedicarbosylato(2R)2-methyl-l,4-butanediamineplatinum(II) and cis-diammine (glycolato)platinum (Claycamp & Zimbrick, *J. Inorg. Biochem.* 26(4):257-67, 1986; Fan *et al. Cancer Res.* 48(11): 3135-9, 1988; Heiger-Bernays et al. *Biochemistry* 29(36): 8461-6, 1990; Kikkawa *et al., J. Exp. Clin. Cancer Res.* 12(4):233-40, 1993; Murray *et al. Biochemistry* 31(47):11812-17,1992; Takahashi *et al., Cancer Chemother. Pharmacol.* 33(I):31-5, 1993), gem-diphosphonate cisplatin analogues (FR 2683529), cisplatin analogues containing a tethered dansyl group (Hartwig *et al., J. Am. Chem. Soc.* 114(21):8292-3, 1992), aminoalkylaminoanthraquinone-derived cisplatin analogues (Kitov *et al., Eur. J. Med. Chem.* 23(4):381-3, 1988), spiroplatin, carboplatin, iproplatin and JM40 platinum analogues (Schroyen *et al., Eur. J. Cancer Clin. Oncol.* 24(8): 1309-12, 1988), and JM8 and JM9 cisplatin analogues (Harstrick *et al., Int. J. Androl.* 10(1); 139-45, 1987).

[00251] In another aspect, use of an effective amount of an antigen of one or more microbial species that is pathogenic in the lung is provided to formulate a medicament for use with a platinum-containing chemotherapeutic agent for treating lung cancer in a subject. In another aspect, use of an effective amount of an antigen of one or more microbial species that is pathogenic in the lung is provided for use with a platinum-containing chemotherapeutic agent, as detailed herein, for treating lung cancer in a subject. The microbial species may be a viral pathogen or a bacterial pathogen or a fungal pathogen, as detailed herein.

[00252] In another aspect, an effective amount of an antigen of one or more microbial species that is pathogenic in the lung is provided to formulate a medicament for use with a platinum-containing chemotherapeutic agent for treating lung cancer in a subject. In another aspect, an effective amount of an antigen of one or more microbial species that is pathogenic in the lung is provided for use with a platinum-containing

chemotherapeutic agent, as detailed herein, for treating lung cancer in a subject. The microbial species may be a viral pathogen or a bacterial pathogen or a fungal pathogen, as detailed herein. The platinum-containing chemotherapeutic agent may be, without limitation: Cisplatin, carboplatin or oxaliplatin.

[00253] In another aspect, a kit is provided. The kit includes an antigen of one or more microbial species that is pathogenic in the lung, a platinum-containing chemotherapeutic agent; and instructions for providing the antigen and the platinum-containing chemotherapeutic agent to a subject in need thereof. The microbial species may be a viral pathogen or a bacterial pathogen or a fungal pathogen, as detailed herein.

[00254] In various aspects, embodiments of the invention relate to compositions comprising components of organisms that may cause infections of the gastrointestinal tract, so that the organism may be characterized as a pathogen. However, an organism that is in some cases pathogenic may not always cause disease. Most animals are colonized to some degree by other organisms, such as bacteria, which generally exist in symbiotic or commensal relationships with the host animal. Thus, many species of normally harmless bacteria are found in healthy animals, and are usually localized to the surface of specific organs and tissues. Often, these bacteria aid in the normal functioning of the body. For example, in humans, symbiotic *Escherichia coli* bacteria may be found in the intestine, where they promote immunity and reduce the risk of infection with more virulent pathogens.

[00255] Bacteria that are generally harmless, such as *Escherichia coli*, can cause infection in healthy subjects, with results ranging from mild to severe infection to death. Whether or not an organism, such as a bacterium, is pathogenic (i.e., causes infection) depends to some extent on factors such as the route of entry and access to specific host cells, tissues, or organs; the intrinsic virulence of the bacterium; the amount of the bacteria present at the site of potential infection; or the health of the host animal. Thus,

organisms that are normally harmless can become pathogenic given favorable conditions for infection, and even virulent organisms may require specific circumstances to cause infection. Accordingly, organisms that are members of the normal flora can be pathogens when they move beyond their normal ecological role in the endogenous flora. For example, endogenous species can cause infection outside of their ecological niche in regions of anatomical proximity, for example by contiguous spread. When this occurs, and in the context of the present invention, these normally harmless endogenous organisms are considered pathogenic.

[00256] Specific organisms, such as bacterial species, viruses, worms, and protazoa are known to cause infections in specific regions of the GIT in otherwise healthy subjects. Examples of organisms that commonly cause infections in specific regions of the GIT are listed below; it will be understood that these examples are not intended to be limiting and that a skilled person would be able to readily recognize and identify infectious or pathogenic organisms that cause infections, or commonly cause infections, in various regions of the GIT in healthy adults, based for example on knowledge about particular patient populations, as represented for example by the following publications: Manual of Clinical Microbiology 8th Edition, Patrick Murray, Ed., 2003, ASM Press American Society for Microbiology, Washington DC, USA; Mandell, Douglas, and Bennett's Principles and Practice of Infectious Diseases 5th Edition, G. L. Mandell, J.E. Bennett, R. Dolin, Eds., 2000, Churchill Livingstone, Philadelphia, PA, USA, all of which are incorporated by reference herein.

[00257] Infections of the mouth are commonly caused by the following bacterial species: Prevotella melaninogenicus, anaerobic streptococci, viridans streptococci, Actinomyces spp., Peptostreptococcus spp., or Bacteroides spp., or other oral anaerobes; or viral pathogens: herpes simplex, coxsackieviruses, or Epstein-Barr.

[00258] Infections of the esophagus are commonly caused by the following bacterial species: Actinomyces spp., Mycobacterium avium, Mycobacterium

tuberculosis, or Streptococcus spp.; or viral pathogens: cytomegalovirus, herpes simplex, or varicella-zoster.

[00259] Infections of the stomach are commonly caused by the following bacterial species: Streptococcus pyogenes or Helicobacter pylori; or viral pathogens: cytomegalovirus, herpes simplex, Epstein-Barr, rotaviruses, noroviruses, or adenoviruses.

[00260] Infections of the small bowel are commonly caused by the following bacterial species: Escherichia coli, Clostridium difficile, Bacteroides fragilis, Bacteroides vulgatus, Bacteroides thetaiotaomicron, Clostridium perfringens, Salmonella enteriditis, Yersinia enterocolitica, or Shigella flexneri; or viral pathogens: adenoviruses, astroviruses, caliciviruses, noroviruses, rotaviruses, or cytomegalovirus.

[00261] Infections of the colon/rectum are commonly caused by the following bacterial species: Escherichia coli, Clostridium difficile, Bacteroides fragilis, Bacteroides vulgatus, Bacteroides thetaiotaomicron, Clostridium perfringens, Salmonella enteriditis, Yersinia enterocolitica, or Shigella flexneri; or viral pathogens: adenoviruses, astroviruses, caliciviruses, noroviruses, rotaviruses, or cytomegalovirus.

[00262] Infections of the anus are commonly caused by the following bacterial species: Streptococcus pyogenes, Bacteroides spp., Fusobacterium spp., anaerobic streptococci, Clostridium spp., Escherichia coli, Enterobacter spp., Pseudomonas aeruginosa, or Treponema pallidum; or viral pathogens: herpes simplex.

[00263] Organisms such as bacteria are often classified operationally as collections of similar strains (which generally refer to groups of presumed common ancestry with identifiable physiological but usually not morphological distinctions, and which may be identified using serological techniques against bacterial surface antigens). Thus, each bacterial species (e.g., Escherichia coli) has numerous strains

(or serotypes), which may differ in their ability to cause infection or differ in their ability to cause infection in a particular organ/site. Certain strains of Escherichia coli are more likely to cause gastrointestinal infection/diarrhea, including enterotoxigenic E. coli (ETEC), enteropathogenic E. coli (EPEC), enterohemorrhagic E. coli (EHEC), Shiga toxin-producing E. coli (STEC), enteroaggregative E. coli (EAEC), enteroinvasive E. coli (EIEC) and diffuse adhering E. coli (DAEC). In accordance with the present invention, one or more of an ETEC, EPEC, EHEC, STEC, EAEC, EIEC or DAEC strains of E.coli (i.e., strains that cause colon infection), may be chosen for a formulation to treat and IBD.

[00264] Similarly, there may be numerous subtypes of specific viruses, worms, or protozoa, which are associated with disease in a particular population, and are therefore amenable for use in the present invention.

[00265] The compositions of the invention include antigens of organisms that are pathogenic in a specific region of the GIT. The compositions may include the components of whole organisms, whole cells or whole virions, or may include extracts or preparations of the organisms, such as cell wall or cell membrane extracts, or exotoxins. The compositions may also include one or more isolated antigens from these organisms. Pathogenic organisms may be available commercially (for example from the American Type Culture Collection, Manassas, VA, USA), or may be clinical isolates from subjects having an infection.

[00266] The compositions of the invention derived from pathogens can be provided alone or in combination with other compounds (for example, nucleic acid molecules, small molecules, peptides, or peptide analogues), in the presence of a liposome, an adjuvant, or any pharmaceutically acceptable carrier, in a form suitable for administration to mammals, for example, humans. As used herein "pharmaceutically acceptable carrier" or "excipient" includes any and all solvents, dispersion media, coatings, antibacterial and antifungal agents, isotonic and absorption delaying agents,

and the like that are physiologically compatible. The carrier can be suitable for any appropriate form of administration, including subcutaneous, intradermal, intravenous, parenteral, intraperitoneal, intramuscular, sublingual, inhalational, intratumoral or oral administration. Pharmaceutically acceptable carriers include sterile aqueous solutions or dispersions and sterile powders for the extemporaneous preparation of sterile injectable solutions or dispersion. The use of such media and agents for pharmaceutically active substances is well known in the art. Except insofar as any conventional media or agent is incompatible with the active compound (i.e., the specific bacteria, bacterial antigens, or compositions thereof of the invention), use thereof in the pharmaceutical compositions of the invention is contemplated. Supplementary active compounds can also be incorporated into the compositions.

[00267] Methods well known in the art for making formulations are found in, for example, "Remington's Pharmaceutical Sciences" (20th edition), ed. A. Gennaro, 2000, Mack Publishing Company, Easton, PA. Formulations for parenteral administration may, for example, contain excipients, sterile water, or saline, polyalkylene glycols such as polyethylene glycol, oils of vegetable origin, or hydrogenated napthalenes. Biocompatible, biodegradable lactide polymer, lactide/glycolide copolymer, or polyoxyethylene-polyoxypropylene copolymers may be used to control the release of the compounds. Other potentially useful parenteral delivery systems for include ethylene-vinyl acetate copolymer particles, osmotic pumps, implantable infusion systems, and liposomes. Formulations for inhalation may contain excipients, for example, lactose, or may be aqueous solutions containing, for example, polyoxyethylene-9-lauryl ether, glycocholate and deoxycholate, or may be oily solutions for administration in the form of nasal drops, or as a gel. For therapeutic or prophylactic compositions, the formulations may be administered to an individual in an amount effective to stop or slow progression of the IBD.

[00268] An "effective amount" of a pathogenic species or antigen thereof according to the invention includes a therapeutically effective amount or a

prophylactically effective amount. A "therapeutically effective amount" refers to an amount effective, at dosages and for periods of time necessary, to achieve the desired therapeutic result, such as reduction or elimination of symptoms of IBD. A therapeutically effective amount of a pathogenic species or antigen(s) thereof may vary according to factors such as the disease state, age, sex, and weight of the individual, and the ability of the compound to elicit a desired response in the individual. Dosage regimens may be adjusted to provide the optimum therapeutic response. A therapeutically effective amount may also be one in which any toxic or detrimental effects of the pathogenic species or antigen thereof are outweighed by the therapeutically beneficial effects. A "prophylactically effective amount" refers to an amount effective, at dosages and for periods of time necessary, to achieve the desired prophylactic result, such as prevention of IBD. Typically, a prophylactic dose is used in subjects prior to or at an earlier stage of IBD, so that a prophylactically effective amount may be less than a therapeutically effective amount.

[00269] For administration by subcutaneous or intradermal injection, an exemplary range for therapeutically or prophylactically effective amounts of one or more pathogenic bacterial species may be about 1 million to 100,000 million organisms per ml, or may be 100 million to 7000 million organisms per ml, or may be 500 million to 6000 million organisms per ml, or may be 1000 million to 5000 million organisms per ml, or may be 2000 million to 4000 million organisms per ml, or any integer within these ranges. The total concentration of bacteria per ml may range from 1 million to 100,000 million organisms per ml, or may be 50 million to 7000 million organisms per ml, or may be 100 million to 6000 million organisms per ml, or may be 500 million to 5000 million organisms per ml, or may be 1000 million to 4000 million organisms per ml, or any integer within these ranges. The range for therapeutically or prophylactically effective amounts of antigens of a pathogenic bacterial species may be any integer from 0.1 nM-0.1 M, 0.1 nM-0.05M, 0.05 nM-15μM or 0.01 nM-10μM.

[00270] It is to be noted that dosage concentrations and ranges may vary with the severity of the condition to be alleviated, or may vary with the subject's immune response. In general, the goal is to achieve an adequate immune response. For administration by subcutaneous or intradermal infection, the extent of an immune response may be determined, for example, by size of delayed local immune skin reaction at the site of injection (e.g., from 0.25 inch to 4 inch diameter). The dose required to achieve an appropriate immune response may vary depending on the individual (and their immune system) and the response desired. Standardized dosages may also be used.

[00271] In the context of subcutaneous or intradermal administration, if the goal is to achieve a 2 inch local skin reaction, using a bacterial composition, the total dose may, for example, range from 2 million bacteria (e.g., 0.001 ml of a vaccine with a concentration of 2,000 million organisms per ml) to more than 20,000 million bacteria (e.g., 1 ml of a vaccine with a concentration of 20,000 million organisms per ml). The concentrations of individual bacterial species or antigens thereof within a composition may also be considered. For example, if the concentration of one particular pathogenic bacterial species, cell size of that species or antigenic load thereof is much higher relative to the other pathogenic bacterial species in the vaccine, then the local immune skin reaction of an individual may be likely due to its response to this specific bacterial species. In some embodiments, the immune system of an individual may respond more strongly to one bacterial species within a composition than another, depending for example on past history of exposure to infection by a particular species, so the dosage or composition may be adjusted accordingly for that individual.

[00272] For any particular subject, the timing and dose of treatments may be adjusted over time (e.g., timing may be daily, every other day, weekly, monthly) according to the individual need and the professional judgement of the person administering or supervising the administration of the compositions. For example, in the context of subcutaneous or intradermal administration, the compositions may be

administered every second day. An initial dose of approximately 0.05 ml may be administered subcutaneously, followed by increases from 0.01-0.02 ml every second day until an adequate skin reaction is achieved at the injection site (for example, a 1 inch to 2 inch diameter delayed reaction of visible redness at the injection site). Once this adequate immune reaction is achieved, this dosing is continued as a maintenance dose. The maintenance dose may be adjusted from time to time to achieve the desired visible skin reaction (inflammation) at the injection site. Dosing may be for a dosage duration, for example of at least 2 weeks, 2 months, 6 months, 1, 2, 3, 4, or 5 years or longer.

[00273] In some embodiments, the invention may include antigenic compositions administered to one or more epithelial tissues by a non-enteric route. For example: to skin by intradermal or subcutaneous injection; to lung epithelium by inhalation. Accordingly, in some embodiments the antigenic compositions of the invention are administered so as to provoke an immune response in a non-enteric tissue, such as an epithelial tissue. In some embodiments, one or more non-enteric routes of administration may be combined with one or more additional routes of administration, such as intratumoral, intramuscular or intravenous administration.

[00274] In various aspects of the invention, the antigenic compositions that are administered to a patient may be characterized as having an antigenic signature, i.e., a combination of antigens or epitopes, that is sufficiently specific that the antigenic composition is capable of eliciting an immune response that is specific to a particular pathogen, such as an adaptive immune response.

[00275] The amount of active compound (e.g., bacterial species, viruses, protozoa or helminths, or antigens thereof) in compositions of the invention may vary according to factors such as the disease state, age, sex, and weight of the individual. Dosage regimens may be adjusted to provide the optimum 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. It may be advantageous to formulate parenteral compositions in dosage unit form for ease of administration and uniformity of dosage.

[00276] In the case of antigenic formulations (analogous to a vaccine), an immunogenically effective amount of a compound of the invention can be provided, alone or in combination with other compounds, with an immunological adjuvant. The compound may also be linked with a carrier molecule, such as bovine serum albumin or keyhole limpet hemocyanin to enhance immunogenicity. An antigenic composition ("vaccine") is a composition that includes materials that elicit a desired immune response. An antigenic composition may select, activate or expand memory B, T cells, neutrophils, monocytes or macrophages of the immune system to, for example, reduce or eliminate the symptoms of the IBD. In some embodiments, the specific pathogenic microbe, virus, viral antigens, bacteria, bacterial antigens, or compositions thereof of the invention are capable of eliciting the desired immune response in the absence of any other agent, and may therefore be considered to be an antigenic composition. In some embodiments, an antigenic composition includes a suitable carrier, such as an adjuvant, which is an agent that acts in a non-specific manner to increase the immune response to a specific antigen, or to a group of antigens, enabling the reduction of the quantity of antigen in any given vaccine dose, or the reduction of the frequency of dosage required to generate the desired immune response. A bacterial antigenic composition may include live or dead bacteria capable of inducing an immune response against antigenic determinants normally associated with the bacteria. In some embodiments, an antigenic composition may include live bacteria that are of less virulent strains (attenuated), and therefore cause a less severe infection. In some embodiments the antigenic composition may include live, attenuated or dead viruses capable of inducing an immune response against antigenic determinants normally associated with the virus.

[00277] An antigenic composition comprising killed organisms for administration by injection may be made as follows. The organism may be grown in suitable media, and washed with physiological salt solution. The organism may then be centrifuged, resuspended in saline solution, and killed with heat. The suspensions may be standardized by direct microscopic count, mixed in required amounts, and stored in appropriate containers, which may be tested for safety, shelf life, and sterility in an approved manner. In addition to the organism and/or antigens thereof, a killed preparation suitable for administration to humans may include phenol preservative (for example 0.4%) and/or sodium chloride (for example on the order of 0.9%). The composition may also for example include trace amounts of brain heart infusion (beef), peptones, yeast extract, agar, sheep blood, dextrose, sodium phosphate and/or other media components.

[00278] In some embodiments, the antigenic composition may be used as an aerosol for inhalation.

[00279] In general, the compositions of the invention should be used without causing substantial toxicity. Toxicity of the compounds of the invention can be determined using standard techniques, for example, by testing in cell cultures or experimental animals and determining the therapeutic index, i.e., the ratio between the LD50 (the dose lethal to 50% of the population) and the LD100 (the dose lethal to 100% of the population).

[00280] In some embodiments, bacteria that are members of the endogenous flora of a particular region of the GIT may be used to formulate antigenic compositions of the invention. The rows of Table 1 list a number of bacterial species, together with the biological regions in which each species may form a part of the endogenous flora. For example, Abiotrophia spp. are typically members of the endogenous flora of the mouth.

Table 1: Human Bacterial Normal Flora (Endogenous Bacterial Human Pathogens)

Bacterial species	Mouth	Stomach	Duodenum/ Jejunum	Ileum	Colon
CFL /mL	10^5	10^2	10^5	10^8	10^11
Abiotrophia spp	+				
Acholeplasma laidlawii	+				
Acidaminococcus fermentans	+		+	+	+
Acinetobacter spp.	+		+	+	+
Actinobacillus spp.	+				
Actinobaculum spp.	+		+	+	+
Actinomyces spp.	+		+	+	+
Aeromonas spp.			+	+	+
Anaerorhabdus furcosus				+	+
Anaerococcus hydrogenalis				+	+
Anaerococcus lactolyticus				+	+
Anaerococcus prevotii				+	+
Atopobium spp.	+		+	+	+
Bacillus spp.				+	+

Bacteroides caccae				+	+
Bacteroides distasonis				+	+
Bacteroides eggerthii				+	+
Bacteroides fragilis				+	+
Bacteroides merdae				+	+
Bacteroides ovatus				+	+
Bacteroides splanchnicus				+	+
Bacteroides thetaiotaomicron				+	+
Bacteroides vulgatus				+	+
Bifidobacterium adolescentis			+	+	+
Bifidobacterium bifidum			+	+	+
Bifidobacterium breve			+	+	+
Bifidobacterium catenulatum			+	+	+
Bifidobacterium dentium	+		+	+	+
Bifidobacterium longum			+	+	+

Bilophila wadsworthia	+		+	+	+
Burkholderia cepacia			+	+	+
Butyrivibrio fibrisolvens			+	+	+
Campylobacter concisus			+	+	+
Campylobacter curvus			+	+	+
Campylobacter gracilis			+	+	+
Campylobacter jejuni			+	+	+
Campylobacter rectus			+	+	+
Campylobacter showae	+		+	+	+
Campylobacter sputorum	+				
Capnocytophaga granulosum	+				
Capnocytophaga gingivalis	+				
Campylobacter haemolytica	+				
Capnocytophaga ochracea	+		+	+	+
Capnocytophaga sputigena	+				

Cardiobacterium hominis	+				
Cedecea spp					+
Centipeda periodontii	+				
Citrobacter freundii			+	+	+
Citrobacter koseri			+	+	+
Clostridium spp.			+	+	+
Corynebacterium accolens	+				
Corynebacterium afermentans	+				
Desulfomonas pigra			+	+	+
Dysgonomonas spp.			+	+	+
Eikenella corrodens	+		+	+	+
Enterobacter aerogenes			+	+	+
Enterobacter cloacae			+	+	+
Enterobacter gergoviae			+	+	+
Enterobacter sakazakii			+	+	+
Enterobacter taylorae			+	+	+

Enterococcus spp.			+	+	+
Escherichia coli			+	+	+
Escherichia fergusonii			+	+	+
Escherichia hermannii			+	+	+
Escherichia vulneris			+	+	+
Eubacterium spp.	+		+	+	+
Ewingella americana	+				
Finegoldia magnus			+	+	+
Fusobacterium alocis	+				
Fusobacterium gonidiaformans			+	+	+
Fusobacterium mortiferum			+	+	+
Fusobacterium naviforme			+	+	+
Fusobacterium necrophorum	+		+	+	+
Fusobacterium nucleatum	+				
Fusobacterium sulci	+				

Fusobacterium russii			+	+	+
Fusobacterium varium			+	+	+
Gardnerella vaginalis			+	+	+
Gemella haemolysans	+				
Gemella morbillorum	+		+	+	+
Globicatella spp.	+				+
Granulicatella spp.	+				
Haemophilus spp.	+				
Hafnia alvei			+	+	+
Helcococcus kunzii					
Helicobacter spp.			+	+	+
Kingella spp.	+				
Klebsiella spp.	+		+	+	+
Lactobacillus acidophilus	+	+	+	+	+
Lactobacillus breve	+				
Lactobacillus casei	+				
Lactobacillus fermentum	+	+	+	+	+

Lactobacillus reuteri		+	+	+	+
Lactobacillus salivarius	+	+	+	+	+
Leclercia adecarboxylata			+	+	+
Leminorella spp.			+	+	+
Leptotrichia buccalis	+				
Megasphaera elsdenii			+	+	+
Micrococcus luteus	+				
Micrococcus lylae	+				
Micromonas micros	+				
Mitsuokella multiacidus			+	+	+
Mobiluncus curisii			+	+	+
Mobiluncus mulieris			+	+	+
Moellerella wisconsensis			+	+	+
Moraxella catarrhalis	+				
other Moraxella spp.	+				

Morganella morganii			+	+	+
Mycoplasma buccale	+				
Mycoplasma fermentans	+				
Mycoplasma hominis	+				
Mycoplasma lipophilum	+				
Mycoplasma orale	+				
Mycoplasma pneumoniae	+				
Mycoplasma salivarium	+				
Pantoea agglomerans			+	+	+
Pasteurella multocida	+				
Pediococcus spp.	+				+
Peptoniphilus asaccharolyticus			+	+	+
Peptostreptococcus anaerobus	+		+	+	+
Peptostreptococcus productus			+	+	+
Porphyromonas asaccharolytica	+		+	+	+

Porphyromonas catoniae	+		+		
Porphyromonas endodontalis	+		+		
Porphyromonas gingivalis	+		+		
Prevotella buccae	+		+		
Prevotella buccalis	+		+		
Prevotella corporis	+		+		
Prevotella dentalis	+		+		
Prevotella denticola	+		+		
Prevotella enoeca	+		+		
Prevotella heparinolytica	+		+		
Prevotella intermedia	+		+		
Prevotella loescheii	+		+		
Prevotella melaninogenica	+		+		
Prevotella nigrescens	+		+		
Prevotella oralis	+		+		
Prevotella oris	+		+		

Prevotella oulorum	+		+		
Prevotella tannerae	+		+		
Prevotella veroralis	+		+		
Prevotella zoogleoformans	+		+		
Propionibacterium propionicum	+				
Proteus mirabilis				+	+
Proteus penneri				+	+
Proteus vulgaris				+	+
Providencia rettgeri				+	+
Providencia stuartii			+	+	+
Pseudomonas aeruginosa			+	+	+
Retortamonas intestinalis			+	+	+
Rothia dentocariosa	+				
Rothia mucilaginosa	+				
Ruminococcus productus			+	+	+
Selenomonas spp.	+				

Serratia liquefaciens				+	+
Serratia marcescens				+	+
Serratia odorifera				+	+
Staphylococcus aureus	+				
Staphylococcus epidermidis	+				
Streptococcus agalactiae			+	+	+
Streptococcus anginosus	+		+	+	+
Streptococcus bovis			+	+	+
Streptococcus constellatus	+		+	+	+
Streptococcus criceti	+				
Streptococcus crista	+				
Streptococcus equisimilis	+				
Streptococcus gordonii	+				
Streptococcus intermedius	+			+	+
Streptococcus mitis	+	+			

Streptococcus mutans	+				
Streptococcus oralis	+				
Streptococcus parasanguis	+				
Streptococcus pyogenes	+	+			
Streptococcus salivarius	+	+			
Streptococcus sanguis	+	+			
Streptococcus sobrinus	+				
Streptococcus vestibularis	+				
Group C + G Streptococci	+				+
Succinivibrio dextrinosolvens			+	+	+
Sutterella spp.	+			+	+
Suttonella indologenes	+				
Tissierella praeacuta			+	+	+
Treponema denticola	+				
Treponema maltophilum	+				

Treponema socranskii	+				
Treponema vincentii	+				
Ureaplasma urealyticum	+				
Veillonella spp.	+		+	+	+

[00281] Endogenous microbial flora, such as bacteria, have access to tissues for pathogenesis either through contiguous spread or bacteremic spread. Under favorable conditions, all endogenous organisms can become pathogenic and invade locally and spread by contiguous spread to adjacent tissues and organs. Endogenous bacterial flora of the skin, mouth and colon are the species that are understood to also be amenable to bacteremic spread. Bacteria that are members of a particular endogenous flora domain may therefore cause infection in tissues or organs to which these bacteria may spread. Accordingly, one aspect of the invention involves the use of endogenous microbial pathogens to treat an IBD having symptoms localized to a region of the GIT in which the endogenous bacteria may spread to cause infection. The columns of Table 2 list domains for endogenous flora. The rows of Table 2 list regions of the GIT within which IBD may be situated. Accordingly, one aspect of the invention involves the use of endogenous microbial pathogens to formulate antigenic compositions, or the selection of existing formulations having the pathogens, for treating an IBD situated in the region of the GIT to which the pathogen may spread to cause an infection. Accordingly, in alternative embodiments, an IBD that is symptomatic in the region listed in the first column of Table 2 may be treated with antigenic compositions comprising antigenic determinants that are specific for microbial pathogens that are members of the endogenous flora of one or more of the endogenous flora domains listed in the first row of Table 2 and indicated with an X or a check mark in the appropriate row.

Table 2: Tissue/Organ Pathogenicity of Endogenous Flora

Tissue/ organ site	Mouth	Stomach	Duo-denum/ Jejunum	Ileum	Colon
Oral	x				
Tonsil	x				
Nasopha-rynx/Sinus	x				
Esophagus		x			
Stomach		x			
Small bowel			x	x	
Colon/ Rectum					x
Anus					x

[00282] In accordance with the combined information in Tables 1 and 2, IBD manifest in a particular region of the GIT set out in column 1 of Table 2 may be treated with antigenic compositions comprising antigenic determinants of the corresponding bacterial species of Table 1, so that the column headings in Table 2 are in effect replaced with the bacterial species of Table 1.

[00283] In some embodiments, pathogens for use in the invention may be exogenous bacterial pathogens. For example, the organisms listed in Table 3 may be used as microbial pathogens to formulate antigenic compositions, or antigenic compositions having those pathogens may selected, for use to treat an IBD situated in the region of the GIT listed with the relevant organism in Table 3. In some embodiments, antigenic determinants of both endogenous and exogenous bacterial species targeted to a specific tissue or organ may be used in combination. For example, an antigenic composition derived from, or specific for, Clostridium difficile, may be used to treat a IBD situated in the colon.

Table 3 Exogenous Bacterial Human Pathogens, and their Sites of Infection in the GIT

Bacterial Species	Region of the GIT
Aerobacter spp.	small bowel, colon,
Bacillus anthracis	oral, small bowel, colon, hematological
Bacillus cereus	colon,
other Bacillus spp.	colon, stomach, small bowel
Brucella spp.	small bowel, colon
Campylobacter coli	small bowel, colon
Campylobacter jejuni	colon
Campylobacter sputorum	small bowel, colon
Clostridium bifermentans	small bowel, colon, stomach
Clostridium botulinum	colon, small bowel
Clostridium difficile	colon
Clostridium indolis	small bowel, colon, stomach,
Clostridium mangenolii	small bowel, colon, stomach
Clostridium perfringens	small bowel, colon, stomach
Clostridium sordellii	small bowel, colon, stomach
Clostridium sporogenes	small bowel, colon, stomach
Clostridium subterminale	small bowel, colon, stomach
Edwarsiella tarda	small bowel, colon

Francisella tularensis	small bowel
Helicobacter pylori	stomach
Leptospirosis spp.	oral
Listeria monocytogenes	small bowel, colon
Mycobacterium bovis	colon, small bowel
Mycobacterium tuberculosis	small bowel, colon
Pediococcus spp.	colon
Plesiomonas shigelloides	small bowel, colon
Rickettsia rickettsiae	small bowel
Salmonella spp.	stomach, small bowel, colon
Shigella boydii	colon
Shigella dysenteriae	colon
Shigella flexneri	colon
Shigella sonnei	colon
other Spirillum spp.	colon
Streptococcus zooepidemicus	small bowel
Treponema pallidum	oral, anus
Tropheryma whipplei	small bowel, colon
Vibrio cholerae Vibrio fluvialis	colon, small bowel small bowel, colon

Vibrio furnissii	small bowel, colon
Vibrio hollisae Vibrio parahaemolyticus	small bowel, colon colon, small bowel
Yersinia enterocolitica	small bowel, colon
Yersinia pseudotuberculosis	small bowel, colon

[00284] In some embodiments, pathogens for use in the invention may be viral pathogens. Table 4 provides an exemplary list of viral pathogens together with the tissue and organ sites for which each viral species is reportedly a pathogen. Accordingly, one aspect of the invention involves utilizing immunogenic compositions that are specific for the named viruses to treat an IBD situated in the region of the GIT that is identified adjacent to the name of the virus in Table 4.

Table 4 Viral Human Pathogens and Their Sites of Infection

Virus	Region of the GIT
Herpes Simplex virus (1 and 2)	rectum, anus
Cytomegalovirus	small bowel, colon/rectum
Epstein-Barr virus	oral
Adenovirus	oral, small bowel, colon
Human papillomavirus	anus, oral
Orthoreoviruses	small bowel, colon, oral
Coltiviruses	oral
Rotaviruses	small bowel, colon
Alphaviruses	small bowel, colon,

Coronaviruses	oral, small bowel, colon
Toroviruses	small bowel, colon
Parainfluenza viruses	oral
Respiratory syncytial virus	oral
Human metapneumovirus	oral, small bowel, colon
Vesicular stomatitis virus	oral, small bowel, colon
Rabies virus	oral
Influenza virus	oral
Hantaviruses	oral
Machupo virus	small bowel, colon
Junin virus	small bowel, colon
Poliovirus	small bowel, colon
Coxsackieviruses	small bowel, colon
Echoviruses	oral, small bowel, colon
Hepatitis A virus	small bowel, colon
Rhinoviruses	oral
Noroviruses and other Caliciviruses	small bowel, colon
Astroviruses	small bowel, colon
Picobirnaviruses	small bowel, colon
Hepatitis E virus	small bowel, colon

[00285] The cumulative information in Tables 1 through 4 provides an extensive identification of pathogens that may be used in the formulation of antigenic compositions of the invention, together with an identification of the region of the GIT in which these organisms are pathogenic, and accordingly identifies the region of the GIT

in which an IBD is situated that may be treated with an antigenic formulation of the invention.

[00286] In some embodiments, the pathogen selected for use in antigenic compositions of the invention may be one that is a common cause of acute infection in the region of the GIT in which the IBD to be treated is situated. Table 5 identifies bacterial and viral pathogens of this kind, together with the region of the GIT in which they commonly cause infection. Accordingly, in selected embodiments, an IBD residing in a region of the GIT identified in the first column of Table 5 may be treated with an antigenic composition that comprises antigenic determinants for one or more of the pathogenic organisms listed in the second column of Table 5.

Table 5: Common causes of acute infection (bacteria and viruses) for selected regions of the GIT

Selected regions of the GIT	Common Bacterial or Viral Pathogens
Oral	Prevotella melaninogenicus, anaerobic streptococci, viridans streptococci, Actinomyces spp., Peptostreptococcus spp., Bacteroides spp., and other oral anaerobes
	herpes simplex, coxsackieviruses, Epstein-Barr
Stomach	Streptococcus pyogenes, Helicobacter pylori
	cytomegalovirus, herpes simplex, Epstein-Barr, rotaviruses, noroviruses, adenoviruses
Small bowel	Escherichia coli, Clostridium difficile, Bacteroides fragilis, Bacteroides vulgatus, Bacteroides thetaiotaomicron, Clostridium perfringens, Salmonella enteriditis, Yersinia enterocolitica, Shigella flexneri
	adenoviruses, astroviruses, caliciviruses, noroviruses, rotaviruses, cytomegalovirus

Colon/Rectum	Escherichia coli, Clostridium difficile, Bacteroides fragilis, Bacteroides vulgatus, Bacteroides thetaiotaomicron, Clostridium perfringens, Salmonella enteriditis, Yersinia enterocolitica, Shigella flexneri
	adenoviruses, astroviruses, caliciviruses, noroviruses, rotaviruses, cytomegalovirus
Anus	Streptococcus pyogenes, Bacteroides spp., Fusobacterium spp., anaerobic streptococci, Clostridium spp., E. coli, Enterobacter spp., Pseudomonas aeruginosa, Treponema pallidum
	herpes simplex

[00287] The specific organisms which commonly cause infection in a specific region of the GIT may vary by geographical location. Table 5 is thus not an exhaustive list of common pathogens for all geographic locations and population groups. It is understood that a clinical microbiologist skilled in the art could determine the common pathogenic species in a particular geographic area or population group for a specific region of the GIT in accordance with the invention.

[00288] Humans are hosts to a wide range of gastrointestinal parasites, including various protozoa and helminths, which for purposes of the present invention constitute pathogens of the GIT (Schafer, T.W., Skopic, A. Parasites of the small intestine. Curr Gastroenterol Reports 2006;8:312-20; Jernigan, J., Guerrant, R.L., Pearson, R.D. Parasitic infections of the small intestine. Gut 1994;35:289-93; Sleisenger & Fordtran's Gastrointestinal and liver disease. 8th ed. 2006; Garcia, L.S. Diagnostic medical parasitology. 5th ed. 2007). Compositions of the invention may accordingly include antigenic components of various protozoa, including for example: *Giardia lamblia, Cryptosporidium parvum, Cryptosporidium hominus, Isospora belli,* Sarcocystis species, Coccidian like bodies (Cyclospora species), *Enterocytozoon bieneusi,*

Entamoeba histolytica, Entamoeba dispar, Entamoeba coli, Entamoeba hartmanni, Endolimax nana, Iodamoeba bütschlii, Dientameoba fragilis, Blastocystis hominus, Cyclospora cayetanensis, Microsporidia, *Trypanosoma cruzi, Chilomastix mesnili, Pentatrichomonas hominis, Balantidium coli.* Similarly, compositions of the invention may include antigenic components of various helminths, including for example: Cestodes (tapeworms), *Taenia saginata, Taenia solium,* Diphyllobothrium species, *Hymenolepis nana, Hymenolepis diminuta, Dipylidium caninum,* Nematodes (round worms), *Ascaris lumbricoides, Strongyloides stercoralis, Necator americanus, Ancylostoma duodenale, Ancylostoma caninum, Tichuris trichiura, Capillaria philippinensis,* Trichostrongylus species, Trichinella species, *Necator americanus,* Anisakis and related species, *Angiostrongylus costaricensis, Enterobius vermicularis,* Trematodes (flukes), *Fasciolopsis buski,* Heterophyes speicies, Echinostoma species, *Clonorchis sinensis,* Opisthorchis species, Fasciola species, *Metagonimus yokogawi, Schistosoma mansoni, Schistosoma japonicum, Schistosoma mekongi, Schistosoma intercalatum,* Echinostoma species and Paragonimus species.

[00289] In selected embodiments, the invention involves diagnostic steps to assess a patient's previous exposure to an organism. For example, the diagnostic steps may include taking a medical history of exposure to selected pathogens, and/or evaluating a patient's immune response to a selected pathogen. For example, a serology test may be conducted to detect antibodies to selected pathogens in a patient's sera. In connection with this aspect of the invention, antigenic determinants of a selected pathogen may be chosen for use in an immunogenic composition on a selected patient based on a diagnostic indication that the patient has had one or more prior exposure(s) to the pathogen, for example by virtue of the presence of antibodies to antigenic determinants of that pathogen in the patient's sera.

[00290] In further selected embodiments, the invention involves diagnostic steps to assess a patient's immunological response to treatment with a selected immunogenic composition. For example, the diagnostic steps may include evaluating a

patient's immune response to the antigenic determinants of that immunogenic composition, for example using a serological test to detect antibodies to those antigenic determinants. In connection with this aspect of the invention a treatment with a selected immunogenic composition may be continued if the evaluation indicates that there is an active immunological response to the antigenic determinants of that composition, and the vaccine treatment may be discontinued, and an alternative treatment with a different immunogenic composition may be initiated, if the evaluation indicates that there is not a sufficiently active immunological response to the antigenic determinants of the immunogenic composition.

[00291] Although various embodiments of the invention are disclosed herein, many adaptations and modifications may be made within the scope of the invention in accordance with the common general knowledge of those skilled in this art. Such modifications include the substitution of known equivalents for any aspect of the invention in order to achieve the same result in substantially the same way. Numeric ranges are inclusive of the numbers defining the range. The word "comprising" is used herein as an open-ended term, substantially equivalent to the phrase "including, but not limited to", and the word "comprises" has a corresponding meaning. As used herein, the singular forms "a", "an" and "the" include plural referents unless the context clearly dictates otherwise. Thus, for example, reference to "a thing" includes more than one such thing. Citation of references herein is not an admission that such references are prior art to the present invention. Any priority document(s) and all publications, including but not limited to patents and patent applications, cited in this specification are incorporated herein by reference as if each individual publication were specifically and individually indicated to be incorporated by reference herein and as though fully set forth herein. The invention includes all embodiments and variations substantially as hereinbefore described and with reference to the examples and drawings.

[00292] In some embodiments, the invention excludes steps that involve medical or surgical treatment.

[00293] The following examples illustrate embodiments of the invention.

EXAMPLE 1: Clinical Studies

Bacterial Compositions

[00294] Five killed bacterial compositions have been used to treat a wide variety of cancer types and stages in blinded studies, as follows:

1. The Bayer Corporation MRV™ "Bayer MRV" (Hollister-Steir Laboratories, Spokane, WA, U.S.A.), containing the following bacterial species:

Organisms per ml	
Staphylococcus aureus	1200 million
viridans and non-hemolytic Streptococci	200 million
Streptococcus pneumoniae	150 million
Moraxella (Neisseria) catarrhalis	150 million
Klebsiella pneumoniae	150 million
Haemophilus influenzae	150 million

[00295] This vaccine was produced for the following indications: rhinitis, infectious asthma, chronic sinusitis, nasal polyposis and chronic serous otitis media. Cancer treatment was not indicated as an intended use for this vaccine. The vaccine also included the following ingredients: 0.4% phenol, 0.9% NaCl, trace amounts of brain heart infusion (beef), peptones, yeast extract, agar, sheep blood, dextrose, and sodium phosphates.

2. Stallergenes MRV "Stallergenes MRV" (Laboratories des Stallergenes, S.A., Fresnes, France), containing the following:

Organisms per ml

Staphylococcus aureus	600 million
Staphylococcus albus	600 million
non-hemolytic Streptococci	200 million
Streptococcus pneumoniae	150 million
Moraxella (Neisseria) catarrhalis	150 million
Klebsiella pneumoniae	150 million
Haemophilus influenzae	150 million

[00296] This vaccine was produced for the same indications as the MRV vaccine i.e., recurrent respiratory tract infections, and listed cancer as a contraindication.

[00297] As set out below, surprisingly, these MRV vaccines, which contain many common lung pathogens, were found to be effective for the treatment of lung cancer.

3. Polyvaccinum Forte (PVF; Biomed S.A., Krakow, Poland), containing the following:

Organisms per ml	
Staphylococcus aureus	500 million
Staphylococcus epidermidis	500 million
Escherichia coli	200 million
Corynebacterium pseudodiphtheriticum	200 million
Streptococcus pyogenes	100 million
Streptococcus salivarius (viridans Streptococci)	100 million
Streptococcus pneumoniae	100 million
Moraxella (Neisseria) catarrhalis	100 million
Klebsiella pneumoniae	100 million
Haemophilus influenzae	100 million

[00298] This vaccine was produced for chronic and recurrent inflammatory conditions of the upper and lower respiratory tract and genitourinary tract, including rhinopharyngitis, recurrent laryngitis, tracheitis, bronchitis, otitis media, chronic and recurrent neuralgia of trigeminal and occipital nerve, ischialgia, brachial plexitis, intercostals neuralgia, chronic cystoureteritis, vaginitis, adnexitis, and endometrium inflammation. Cancer treatment was not indicated as an intended use for this vaccine.

[00299] Of note, although the total concentration of bacteria in PVF is identical to that of the MRVs (Bayer and Stallergenes), patients typically demonstrated a visible inflammatory immune response to subcutaneous injection of the PVF composition at a much smaller dose than the usual dose required to achieve a similar skin response with the MRV composition, indicating that the immune reaction was likely occurring to one of the novel components in the Polyvaccinum Forte vaccine, such as E. coli. As set out below, surprisingly, PVF, which contains *E. coli* a common pathogen of the colon, abdomen, kidney, ovaries, peritoneum, liver and pancreas, has been found to be effective in the treatment of cancers in the colon, abdominal lymph nodes, kidney, ovary, peritoneum, liver and pancreas.

4. Staphage Lysate (Delmont Laboratories Inc., Swarthmore, PA, USA), containing the following:

Staphylococcus aureus

[00300] As set out below, surprisingly, Staphage Lysate, which contains *Staphylococcus aureus* a common pathogen of the breast and bone, was found to be effective in the treatment of cancer in the breast and bone.

Administration of MRV, Staphage Lysate and PVF

[00301] The bacterial compositions (vaccines) were a suspension of killed bacterial cells and therefore, the suspensions were gently shaken prior to use to ensure uniform distribution prior to withdrawing dose from vial, and administered subcutaneously three times a week on Mondays, Wednesdays, and Fridays. Patients were advised to continue treatment for at least 6 months. The dose of vaccine required was determined by the adequacy of the immune reaction to the vaccine. Beginning with a very small dose (0.05cc), the dose was gradually increased (by 0.01-0.02cc each time) until an adequate immune reaction was achieved. This delayed local reaction at the injection site appeared within 2 – 48 hours after injection and lasted for up to 72 hours or longer. The goal was to achieve a one to two inch diameter round patch of pinkness/redness at the injection site, indicating adequate immune stimulation. Once this reaction was achieved, the dose was maintained at the level required to achieve this reaction. If the reaction was significantly less than two inches (*e.g.*, half an inch) the dose was increased, if it was significantly more than two inches (*e.g.*, three inches), the dose was decreased. This local immune reaction generally occurs within the first 24 hours after the injection. Patients were asked to check for this reaction and, if present, to measure or mark it. The maintenance dose required to achieve an adequate immune reaction varies considerably, depending on the individual's immune response - as little as 0.001cc for some people, as much as 2cc for others. The vaccine must be stored in a refrigerator (2° to 8°C). The usual site for injection is the upper arms, the thighs or the abdomen. The exact site of each injection was varied so that it was not given in sites in which pinkness/redness was still present. A known contraindication to the vaccines is hypersensitivity to any component of the vaccine.

[00302] A fifth vaccine, a polymicrobial oral vaccine, was used in alternative aspects of the invention, as follows:

5. Respivax, produced by BB-NCIPD Ltd (Bulgaria). This oral vaccine contained the following freeze-dried killed bacterial species:

	Organisms per mg
Streptococcus pneumoniae	25 million
Neisseria catarrhalis	25 million
Streptococcus pyogenes	25 million
Haemophilus influenzae	25 million
Staphylococcus aureus	25 million
Klebsiella pneumoniae	25 million

Administration of Respivax

[00303] The Respivax oral vaccine was produced for the treatment for chronic respiratory infection, and contains many of the most common respiratory tract pathogens, including many of the most common causes of lung infection. Patients were treated with a dose of one 50 mg tablet per day, providing the equivalent of 1.25×10^9 cells of each species per dose. Patients were prescribed the above dose for a continuous period of at least 6 months.

[00304] As set out below, surprisingly, Respivax oral vaccine, which contains many common lung pathogens, was found to be effective for the treatment of cancer of the lung.

EXAMPLE 1A: Cancer of the Lung

[00305] This section relates to primary cancer in the lung, or metastases to the lung, treated with microbial pathogens of the lung, such as endogenous respiratory bacteria flora.

[00306] Patients qualified for the lung cancer study if they were initially diagnosed with stage 3B or 4-lung (inoperable) cancer. Lung cancer staging was performed using standard methods as for example described in AJCC: Cancer Staging Handbook (sixth edition) 2002; Springer-Verlag New York: Editors: Fredrick Greene, David Page and

Irvin Fleming, or in International Union Against Cancer: TNM Classification of Malignant Tumors (sixth edition) 2002; Wiley-Liss Geneva Switzerland: Editors: L.H. Sobin and C.H. Wittekind. For example, lung cancers may be classified as follows:

TNM lung clinical and pathological classification

T	**PRIMARY TUMOUR**
TX	Primary tumour cannot be assessed, *or* tumour proven by the presence of malignant cells in sputum *or* bronchial washings but not visualized by imaging or bronchoscopy
Tis	Carcinoma in situ
T0	No evidence of primary tumour
T1	Tumour 3 cm or less in greatest dimension, surrounded by lung or visceral pleura, without bronchoscopic evidence of invasion more proximal than the lobar bronchus (*ie,* not in the main bronchus)
T2	Tumour with *any* of the following features of size or extent: More than 3 cm in greatest dimension Involves main bronchus, 2 cm or more distal to the carina Invades visceral pleura Associated with atelectasis or obstructive pneumonitis that extends to the hilar region but does not involve the entire lung
T3	Tumour of any size that directly invades any of the following: chest wall (including superior sulcus tumours), diaphragm, mediastinal pleura, parietal pericardium; *or* tumour in the main bronchus less than 2 cm distal to the carina but without involvement of the carina; *or* associated atelectasis or obstructive pneumonitis of the entire lung
T4	Tumour of any size that invades any of the following: mediastinum, heart, great vessels, trachea, esophagus, vertebral body, carina; *or* tumour with a malignant pleural or pericardial effusion; *or* with separate tumour nodule(s) within the ipsilateral primary-tumour lobe of the lung

N REGIONAL LYMPH NODES

NX Regional lymph nodes cannot be assessed

N0 No regional lymph node metastasis

N1 Metastasis in ipsilateral peribronchial and/or ipsilateral hilar lymph nodes and intrapulmonary nodes, including involvement by direct extension

N2 Metastasis in ipsilateral mediastinal and/or subcarinal lymph node(s)

N3 Metastasis in contralateral mediastinal, contralateral hilar, ipsilateral or contralateral scalene, or supraclavicular lymph node(s)

M DISTANT METASTASIS

MX Distant metastasis cannot be assessed

M0 No distant metastasis

M1 Distant metastasis; includes separate tumour nodule(s) in the non-primary-tumour lobe (ipsilateral or contralateral)

Stage Grouping of TNM Subsets:

Occult carcinoma	TX	N0	M0
Stage 0	Tis	N0	M0
Stage IA	T1	N0	M0
Stage IB	T2	N0	M0
Stage IIA	T1	N1	M0
Stage IIB	T2	N1	M0
	T3	N0	M0
Stage IIIA	T3	N1	M0
	T1	N2	M0
	T2	N2	M0
	T3	N2	M0
Stage IIIB	Any T	N3	M0
	T4	Any N	M0

Stage IV	Any T	Any N	M1

[00307] Charts with diagnostic codes 162.9 (lung cancer) and 197 (metastatic cancer) were collected manually and electronically. Information was collected on these patients, such as date of diagnosis, date of death, and cancer stage. Charts for patients were reviewed to confirm the date of diagnosis and cancer stage. Patients were excluded from the analysis for the following reasons: 1) wrong stage; 2) missing data; 3) no chart, or; 4) chart did not reach in time for the data analysis. 20 patients were excluded from the study because their charts have not arrived yet or there was insufficient information, of which 6 were MRV users. The study group includes 108 patients in total: 50 who took the MRV vaccine and 58 who did not take the MRV vaccine.

[00308] Comparison of survival of patients initially diagnosed with stage 3B and 4 lung cancer who took MRV with patients who didn't take MRV and with SEER standard survival data for patients initially diagnosed with stage 3B and 4 lung cancer (Figure 1) was as follows:

	SEER	non-MRV	MRV
median survival:	5 months	10.5 months	12.5 months
survival at 1 year:	25%	45%	58%
survival at 3 years:	5%	3%	20%
survival at 5 years:	3%	0%	10%

[00309] A comparison of survival (as above), including only those patients who took MRV for at least 2 months (Figure 2) is as follows:

median survival:	16.5 months
survival at 1 year:	70%

survival at 3 years: 27%
survival at 5 years: 15%

[00310] Median survival and survival at 1 year, 3 years and 5 years, was substantially better in the group that was treated with MRV (containing bacteria which commonly cause lung infection), evidence of the effectiveness of this vaccine for the treatment of lung cancer. Patients who were treated with the MRV vaccine for more than 2 months had higher survival rates, further evidence of the effectiveness of this vaccine for the treatment of lung cancer.

[00311] An alternative analysis was conducted on data that included a patient population to whom the MRV composition was not available, to address a perceived potential for bias caused by sicker patients being more likely to choose the novel treatment (with MRV) and healthier patients being potentially less likely to submit to the use of the antigenic compositions of the invention. Comparison of survival of MRV patients to whom the MRV composition was available (designated "Lung 1") to survival of non-MRV patients to whom the MRV composition was not available (designated "Lung 2") removes some of this selection bias, providing a clearer and more accurate illustration of the benefit of MRV treatment, as illustrated in Figure 3.

[00312] In some embodiments, particularly striking clinical benefits have been obtained with antigenic bacterial compositions used in repeated frequent injections (*i.e.*, three times per week) for a prolonged period of time - such as at least 2, 3, 4, 5, 6 or 12 months, or 2, 3, 4 or 5 years (in the context of advanced cancer such as inoperable lung cancer, the longer periods may be most beneficial). Treatments of this kind may be carried out so as to provide sustained, prolonged immune stimulation. When the above analysis is restricted to patients who were treated with MRV for a minimum of 2 months, the survival advantage of MRV treatment is even more clearly illustrated Figure 4.

[00313] As illustrated in Figure 4, one-year survival of stage 3B or 4 lung cancer patients treated with MRV for at least two months was 70%, compared to just 48% for the non-MRV Lung 2 group and 23% for the SEER database group. 3-year survival of the MRV group was more than 4 times that of both the non-MRV patients and the SEER registry. None of the non-MRV group in the Lung 2 study survived for 5 years, whereas 15% of patients treated with MRV for a minimum two-month period were still alive 5 years after diagnosis. In the context of an illness such as inoperable lung cancer that is considered terminal and has a usual 5-year survival rate of only 3% (SEER registry), the above results are extremely encouraging and surprising.

[00314] When the analysis of patient data is restricted to patients who were treated with MRV for at least 6 months, the survival curve is truly remarkable, as illustrated in Figure 5. More than 60% of patients were alive at 3 years, more than 10 times the survival in both the non-MRV group and the SEER registry. 36% (5 of 14 patients) of patients who were treated with MRV for at least 6 months were alive 5 years after diagnosis, compared with only 3% in the SEER database and 0% in the non-MRV group. These remarkable results, in the context of a cancer diagnosis that is considered terminal, are extremely promising and surprising. Accordingly, in some embodiments, cancers, such as advanced cancers, such as inoperable lung cancer, may be treated over a dosing duration of at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11 or 12 months, 2 years, 3 years, 4 years, 5 years, or indefinitely.

[00315] Restricting analysis to those patients who were treated with MRV for a minimum period of time (*e.g.*, 6 months) introduces a bias in favour of the MRV group, since MRV patients who survived for less than that period of time are excluded from the group (including those who died before they could complete the 6 months of treatment). A detailed statistical analysis of this bias, with compensatory exclusion of short-term survivors in both the non-MRV and SEER groups, demonstrates that this bias played a

very minor role in the truly remarkable survival advantage of patients who were treated with the MRV for at least 6 months.

[00316] In stage 3B lung cancer, cancer is confined to the lungs, and thus, a targeted anti-cancer treatment response may be stimulated, in accordance with various aspects of the invention, by a vaccine, such as MRV, comprised of lung pathogens. In stage 4 lung cancer, the cancer has metastasized to distant organs not amenable to targeted stimulation by lung pathogens in accordance with methods of the invention. Thus, in accordance with some embodiments, patients with stage 3B lung cancer may be selected for treatment with MRV vaccine, since all of the cancer is confined to the lungs and thus, will be targeted by the MRV vaccine. When the analysis of patient data is restricted to patients with stage 3B lung cancer, comparison of survival curves even more clearly illustrates the benefit of MRV treatment. As illustrated in Figure 11, one-year survival of stage 3B lung cancer patients treated with MRV was 76%, compared to just 53% for the non-MRV Lung 2 group and 23% for the SEER database group. 3-year survival of the MRV group was 3 times that the non-MRV patients and more than 6 times the SEER registry. None of the non-MRV group survived for 5 years, whereas 14% of stage 3B patients treated with MRV were still alive 5 years after diagnosis. In the context of an illness such as inoperable stage 3B lung cancer that is considered terminal and has a usual 5-year survival rate of only 5% (SEER registry), the above results are extremely encouraging and surprising.

[00317] As some patients did not have their first visit for many months or even a year or two after diagnosis, their inclusion in the survival curves skews the curve towards longer survival. In order to determine whether this bias influenced the difference in survival curves, survival was analysed from date of first visit which excludes this bias, as illustrated in Figure 12. Comparison of survival curves of stage 3B lung cancer patients in Figure 12 demonstrates an even greater survival benefit for MRV treatment than illustrated in Figure 11, indicating that the benefit of MRV treatment was partially masked in Figure 11. As illustrated in Figure 12, 1-year survival

(from date of first visit) of stage 3B lung cancer patients treated with MRV was 57%, compared to only 21% for stage 3B patients not treated with MRV. While no stage 3B lung cancer patients not treated with MRV survived for 3 years, 3-year survival of stage 3B patients treated with MRV was 33% and 5-year survival was 14%, a remarkable and unexpected result.

[00318] When analysis was restricted to stage 3B lung cancer patients whose first visit was within 3 months of diagnosis, the benefits of early treatment with MRV are clearly illustrated. As illustrated in Figure 13, while all stage 3B lung cancer patients who had their first visit within 3 months of diagnosis died within 1 year of diagnosis, 70% of stage 3B lung cancer patients treated with MRV within 3 months of diagnosis survived for 1 year, 40% survived 3 years and 20% survived 5 years, a truly remarkable survival benefit for early MRV treatment.

[00319] One aspect of the invention involves the treatment of primary lung cancers or metastasis to the lung with antigenic compositions that comprise antigenic determinants of microbial pathogens that are known to be lung pathogens, such as exogenous lung pathogens or pathogens that are members of the endogenous flora of the respiratory system. For example, antigenic determinants of the endogenous bacterial respiratory flora species that most commonly cause infection in the lung (see Table 5) may be used to treat primary and metastatic cancers situated in the lung: *Streptococcus pneumoniae, Moraxella catarrhalis, Mycoplasma pneumoniae, Klebsiella pneumoniae, Haemophilus influenza.* Similarly, common viral lung pathogens from Table 5 may be selected for use in some embodiments. Alternatively, a more exhaustive list of endogenous lung pathogens may be selected from Table 1, based on the pathogenicity information provided in Table 2. In further alternative embodiments, viral lung pathogens listed in Table 4 may be used. And in further alternative embodiments, exogenous bacterial lung pathogens from Table 3 may be used in formulating antigenic compositions of the invention, i.e. selected from the group consisting of: Achromobacter spp., Actinomadura spp., Alcaligenes spp., Anaplasma

spp., *Bacillus anthracis*, other Bacillus spp., Balneatrix spp., *Bartonella henselae, Bergeyella zoohelcum, Bordetella holmesii, Bordetella parapertussis, Bordetella pertussis, Borrelia burgdorferi, Borrelia recurrentis, Brucella spp., Burkholderia gladioli, Burkholderia mallei, Burkholderia pseudomallei, Campylobacter fetus, Capnoctyophaga canimorsus, Capnoctyophaga cynodegmi, Chlamydia pneumoniae, Chlamydia psittaci, Chlamydophila pneumoniae, Chromobacterium violaceum, Chlamydophila psittaci,* Chryseobacterium spp., *Corynebacterium pseudotuberculosis, Coxiella burnetii, Francisella tularensis,* Gordonia spp., Legionella spp., Leptospirosis spp., *Mycobacterium avium, Mycobacterium kansasii, Mycobacterium tuberculosis,* other Mycobacterium spp., Nocardia spp., *Orientia tsutsugamushi,* Pandoraea spp., *Pseudomonas aeruginosa,* other Pseudomonas spp., Rhodococcus spp., *Rickettsia conorii, Rickettsia prowazekii, Rickettsia rickettsiae, Rickettsia typhi.*

[00320] For example, since the MRV compositions contain many of the most common lung pathogens, these vaccines may be used to treat primary lung cancer or lung metastases, as illustrated in the cumulative data presented here, and in a number of the case reports. In accordance with the foregoing results, one aspect of the invention involves the treatment of primary lung cancer and metastasis to the lung with antigenic compositions that comprise antigenic determinants of microbial pathogens that are known to be pathogenic in the lung, such as exogenous lung pathogens or pathogens that are members of the endogenous flora of the respiratory tract. In selected embodiments, antigenic determinants of the common lung pathogens may be used to treat primary and metastatic cancers situated in the lung, for example, antigenic determinants from one or more of the following bacterial species or viral types: *Streptococcus pneumoniae, Moraxella catarrhalis, Mycoplasma pneumoniae, Klebsiella pneumoniae, Haemophilus influenza*, influenza virus, adenovirus, respiratory syncytial virus, and parainfluenza. In further selected embodiments, antigenic determinants of *Streptococcus pneumoniae,* the most common cause of bacterial lung infection, may be used alone or with other of the most common pathogens of the lung to treat cancer of the lung.

[00321] Primary lung cancer may also arise from bronchial tissue and therefore, in some embodiments, antigenic compositions that comprise antigenic determinants of microbial pathogens that are known to cause bronchial infection may be used to treat patients with cancer situated in the bronchial tissue, including, for example, the following common causes of bronchial infection: *Mycoplasma pneumoniae, Chlamydophila pneumoniae, Bordetella pertussis, Streptococcus pneumoniae, Haemophilus influenzae*, influenza virus, adenovirus, rhinovirus, coronavirus, parainfluenza, respiratory syncytial virus, human metapneumovirus, or coxsackievirus. Lung cancer (or lung metastases) that is located in both lung and bronchial tissue may be treated with antigenic compositions that comprise antigenic determinants of microbial pathogens that are known to cause both lung and bronchial infection (for example, *Streptococcus pneumoniae, Haemophilus influenza* and *Mycoplasma pneumoniae* are all common lung and bronchial pathogens) or alternatively, with antigenic compositions that comprise antigenic determinants of microbial pathogens that are known to cause lung infection and antigenic determinants of microbial pathogens that are known to cause bronchial infection.

EXAMPLE 1B: Breast Cancer with Metastasis to the Bone or Lung

[00322] The most common cause of both breast infection and bone infection is *Staphylococcus aureus*. Accordingly, in one aspect of the invention, an antigenic composition comprising antigenic determinants of *S. aureus* may be used to treat breast cancer with metastases to the bone. The remarkable case of Patient R (PtR), treated with a *Staphylococcus aureus* vaccine, set out below in the Case Reports, illustrates the efficacy of this approach to treating breast cancer with bone metastases. As illustrated in Figure 6, in a cumulative series of 52 patients, survival of breast cancer patients with metastases to bone and/or lung treated with MRV (n=19), which contains *Staphylococcus aureus*, was better than the survival of patients not treated with the MRV vaccine (n=33):

	% survival MRV patients	%survival non-MRV patients
10 months	95%	76%
20 months	74%	61%
5 years	26%	18%

[00323] In accordance with the foregoing results, one aspect of the invention involves the treatment of primary cancer in the breast or metastasis to the breast with antigenic compositions that comprise antigenic determinants of microbes that are known to cause breast infection, and treatment of primary cancer of the bone or metastasis to the bone with antigenic compositions that comprise antigenic determinants of bacterial species or viruses that are known to cause bone infection. In selected embodiments, a vaccine comprising antigenic determinants of *Staphylococcus aureus*, the most common cause of both breast and bone infection, may be used alone or in combination with other of the most common pathogens of the breast to treat cancer in the breast, or alone or in combination with other of the most common pathogens of the bone to treat cancer in the bone.

EXAMPLE 1C: Metastases to the Bone

[00324] One of the most common sites for metastases in patients with prostate cancer is bone. In one aspect of the invention, the MRV composition, which contains antigenic determinants of *S. aureus*, the most common cause of bone infection, may be used for the treatment of metastases to the bone, for example in patients who have, or who have had, a primary prostate cancer. The graph of Figure 7 is a comparison of survival of a cumulative series of metastatic prostate cancer patients who had surgery or radiation to destroy their prostate gland (and thus, the primary tumour) and who had detectable cancer limited to bone metastases. As illustrated, the survival of patients treated with MRV (n=4) is substantially better than that of patients not treated with MRV (n=7):

	% survival MRV patients	%survival non-MRV patients
2 years	100 %	57 %
3 years	75 %	43 %
5 years	50 %	0 %

[00325] In accordance with the foregoing results, one aspect of the invention involves the treatment of primary bone cancers or metastases to the bone with antigenic compositions that comprise antigenic determinants of microbial pathogens that are known to cause bone infection, such as exogenous bone pathogens or pathogens that are members of the endogenous flora of the skin, mouth or colon. For example, in selected embodiments, antigenic determinants of one or more of the following microbial species from the list of common bone pathogens may be used to treat primary and metastatic cancers situated in the bone: *Staphylococcus aureus,* coagulase-negative staphylococci, *Streptococcus pyogenes, Streptococcus pneumoniae, Streptococcus agalactiae,* other streptococci spp., *Escherichia coli,* Pseudomonas spp., Enterobacter spp., Proteus spp., Serratia spp., parvovirus B19, rubella, hepatitis B. In further selected embodiments, *Staphylococcus aureus*, the most common cause of bone infection, may be used alone or with other of the most common pathogens of the bone to treat cancer of the bone.

EXAMPLE 1D: Cancer Situated in the Colon

[00326] Treatment with the PVF composition has been shown to improve the survival of colon cancer patients (see Figure 8), as illustrated by a comparison of the following four colon cancer patient groups:

- Stage 4 colon cancer patients who were treated with MRV.
- Stage 4 colon cancer patients who were not treated with a vaccine.
- Stage 4 colon cancer patients who were treated with PVF vaccine.
- Stage 4 colon cancer patients from the SEER (Surveillance, Epidemiology and End Results) database.

[00327] This example illustrates that patients with colon cancer treated with PVF, which contains *E. coli* the most common cause of bacterial infection of the colon, have substantially improved survival.

[00328] Patients qualified for the first two groups of this study if they presented with stage 4 colon cancer. Patients were excluded from this analysis for the following reasons:

- incorrect diagnosis
- incorrect stage
- missing essential data (*e.g.*, date of death)
- no chart
- chart did not reach us in time for the data analysis.

[00329] The patient group included a total of 136 stage 4 colon cancer patients: 15 who took the PVF vaccine, 56 who took the MRV vaccine, and 65 who did not take a vaccine. Results are illustrated in Figure 8, as follows:

SEER	no vaccine	MRV	PVF	
median survival:	8.4 mo.	15.1 mo.	15.0 mo.	33.6 mo.
at 10 months	45 %	69 %	71 %	100 %
at 20 months	24 %	42 %	36 %	67 %
at 30 months	14 %	29 %	23 %	52 %
at 5 years	5 %	6 %	7 %	10 %

[00330] The median survival of patients with stage 4 colon cancer treated with PVF (which contains *E. coli*, one of the most common colonic pathogens) was more than double that of patients treated with MRV (which does not contain colonic pathogens) or patients not treated with a vaccine, and four times that of the SEER

registry. All 15 patients treated with PVF were still alive 10 months after diagnosis, compared to only 71% for the MRV group, 69% for the no-vaccine group and only 45% for the SEER registry. Survival at 30 months for the PVF group was double that of both the MRV group and the no-vaccine group and almost 4 times that of the SEER registry.

[00331] The wilcoxon test shows a statistically significant survival difference between patients treated with PVF vaccine and both the MRV group ($p = 0.0246$) and the no vaccine group ($p = 0.0433$). This is remarkable considering the small size of the PVF group ($n = 15$), indicative of substantial therapeutic effect. As evidenced by these results, the PVF composition, which contains *E. coli* the most common cause of bacterial infection of the colon, is an effective treatment for colon cancer.

[00332] Survival of those patients who presented for immunological treatment in accordance with the invention within 3 months of diagnosis (*i.e.*, excluding those patients who were long-term survivors before presenting for treatment) has also been analyzed. The results of this analysis are presented in Figure 9. As illustrated, the 'MRV' and 'No Vaccine' survival curves in Figure 9 are shifted substantially to the left (indicating that a selection bias towards 'long-term' survivors may have artifactually shifted these curves to the right in Figure 8), whereas, remarkably, the PVF curve in Figure 9 is actually further to the right than the curve in Figure 8, indicating that the benefit of earlier treatment with PVF (i.e., within 3 months of diagnosis) more than outweighed any long-term survivor bias excluded in Figure 9. This analysis provides compelling evidence that the benefit of PVF treatment for stage 4 colon cancer may be even greater than that illustrated in Figure 8, and that the earlier the treatment with the compositions of the invention is begun following diagnosis, the greater the benefit.

[00333] In accordance with the foregoing results, one aspect of the invention involves the treatment of colon cancers with antigenic compositions that comprise antigenic determinants of microbial pathogens that are known to be colon pathogens, such as pathogens that are members of the endogenous flora of the colon or

exogenous colonic pathogens. For example, antigenic determinants of the following microbial species may be used to treat primary and metastatic cancers situated in the colon: *Escherichia coli, Clostridium difficile, Bacteroides fragilis, Bacteroides vulgatus, Bacteroides thetaiotaomicron, Clostridium perfringens, Salmonella enteriditis, Yersinia enterocolitica, Shigella flexneri; adenoviruses, astroviruses, caliciviruses, noroviruses, rotaviruses, or cytomegalovirus.* For example, cancers situated in the colon may be treated with the PVF composition, which contains *E. coli,* or alternative formulations that include only antigenic determinants of colonic pathogens. In selected embodiments, antigenic determinants of E. coli, the most common bacterial cause of colon infection, may be used alone or with antigenic determinants of other common pathogens of the colon to treat cancer of the colon.

EXAMPLE 1E: Use of Respivax, an Oral Vaccine to Treat Lung Cancer

[00334] Oral Respivax vaccine was administered as described above, with a dose of one 50 mg tablet per day, providing the equivalent of 1.25×10^9 cells of each species per dose. Patients were advised to continue the above dose for at least 6 months.

[00335] As illustrated in Figure 10, survival of stage 3B lung cancer patients who were treated with the oral Respivax antigens was substantially better than patients who were not treated with the antigenic composition. Median survival was 37 months for the patients treated with Respivax, compared to only 20 months for those patients not treated with an antigenic composition vaccine. 40% of patients treated with Respivax were alive 5 years after diagnosis, whereas none of the untreated patients survived for more than 2 years.

[00336] In accordance with the foregoing results, one aspect of the invention involves the treatment of primary of the lung or metastases to the lung with oral administration of antigenic compositions that comprise antigenic determinants of microbial pathogens that commonly cause lung infection.

EXAMPLE 2: Case Reports

[00337] These case reports are indicative of the patients that make up the patient populations reflected in the foregoing cumulative studies, as well as illustrating additional aspects of the invention. In particular, the individual case reports of Patients A – N are illustrative of surprising results in some patients being treated with anti-inflammatories, while the case reports of Patients O – AA are representative of the general patient population that includes many examples of vaccine treatment that was effective in the absence of anti-inflammatory therapy.

MRV for Cancer of the Lung with and without Anti-inflammatories

[00338] Patient A (PtA): In September year 0, PtA developed right upper chest pain with an associated wheeze. These symptoms persisted and in January, year 1, she had a chest x-ray that revealed a large 7 cm x 8 cm mass in the apex of the right lung. A fine needle aspiration was positive for non-small cell lung cancer. On January 27, year 1, an MRI showed invasion of the subclavian arteries, making surgical resection impossible and thus, PtA was diagnosed with stage 3B inoperable terminal lung cancer. She underwent a short course of palliative radiation and declined chemotherapy. She was told that she had terminal cancer with a 3 to 6 months life expectancy.

[00339] On April 29, year 1, PtA began therapy with MRV vaccine three times per week. On that same date she also began treatment with the non-steroidal anti-inflammatory agent (NSAID) indomethicin 50 mg four times per day and a regime of antioxidant supplements and vitamin D. 18 months later, by October, year 2, the tumour had markedly reduced in size to 3 cm in diameter and, by May 19, year 5, four years after starting treatment with the combined regime of MRV vaccine, indomethicin, antioxidants vitamins and vitamin D, only residual scarring remained. PtA continued treatment with this combination of MRV vaccine and adjuvant anti-inflammatory therapies for more than 4 years until the end of May, year 5 at which time there was no evidence of residual cancer, in spite of a diagnosis of terminal inoperable lung cancer

more than 4 years previously. More than 14 years since diagnosis with terminal lung cancer, PtA continues to feel well with no evidence of residual cancer.

[00340] In accordance with the foregoing results, one aspect of the invention involves the treatment of cancers in the lung with administration of antigenic compositions that comprise antigenic determinants of microbial pathogens that commonly cause lung infection.

[00341] In accordance with the forgoing results, another aspect of the invention involves the administration of the immunogenic compositions repeatedly relatively frequently over a relatively long period of time.

[00342] The concomitant use of anti-inflammatory agents, such as antioxidants, vitamin D and indomethicin, in conjunction with targeted MRV therapy, was associated with substantially improved survival, which was greater than that of otherwise similar cases, in which these adjuvant anti-inflammatory modalities were not used in conjunction with the compositions of the invention. For example, Patient B, an otherwise similar case in which anti-inflammatories were not administered, was diagnosed with inoperable stage 3B non-small cell lung cancer, which was fatal within 3 months of diagnosis. These cases provide evidence of a synergistic effect between the antigenic compositions of the invention and anti-inflammatory treatments.

[00343] In accordance with the foregoing results, one aspect of the invention involves the treatment of cancers with both the administration of antigenic compositions that comprise antigenic determinants of microbial pathogens that are pathogenic to the organ or tissue targeted, as well as adjuvant anti-inflammatory treatments, for synergistic effect.

MRV for Cancer of the Lung with and without Anti-inflammatories

[00344] Patient C (PtC): In the spring of year 0, PtC began having pain in his right upper chest area. This pain persisted and on October 5, year 0 he had a chest x-ray that revealed a large 12 cm x 11 cm mass occupying virtually the entire right upper lobe. A fine needle aspiration was positive for poorly differentiated non-small cell lung cancer. Exploratory thoracotomy was performed on December 7, year 0, which revealed tumour invasion of the chest wall and superior vena cava and therefore, PtC's tumour was inoperable (*i.e.*, stage 3B). PtC underwent a short course of palliative radiation and declined chemotherapy. He was told that he had terminal cancer with a 3 to 6 months life expectancy. By January 27, year 1, the rapidly growing tumour had increased in size to 14 cm x 11.5 cm.

[00345] On February 9, year 1, PtC began treatment with indomethicin 50 mg four times per day, antioxidant vitamins, and vitamin D. Three weeks later, on March 1, year 1, PtC began treatment with MRV vaccine three times per week. By June, year 1, PtC was feeling well and was running 8 km 3-4 times per week. On June 4, year 1, a chest x-ray revealed that the tumour had reduced in size to 11 cm diameter. PtC continued to feel very well, leading a full and active life with return to full employment and continued full physical activity. PtC continued treatment with a combination of the MRV vaccine and adjuvant anti-inflammatory therapies (indomethicin, antioxidants and vitamin D) for more than 16 months until July 24, year 2, at which time indomethicin treatment was discontinued (as a result of decreased kidney function, a known potential side-effect of long-term indomethicin use). 6 months later, in December, year 2, after 22 months of targeted vaccine therapy, MRV treatment was discontinued (since MRV was no longer available past that date). PtC continued to feel well until June, year 6, at which time he was diagnosed with a recurrence of cancer in both lungs, which lead to his death on May 26, year 7, more than 6.5 years after he was diagnosed with terminal lung cancer and told he had 3-6 months to live.

[00346] In this case, the use of adjuvant anti-inflammatory agents, including antioxidants, vitamin D and indomethicin, used in conjunction with targeted MRV

therapy for more than 16 months, was associated with substantially improved survival in the face of a diagnosis that is usually fatal within 1 year, which was greater than that of an otherwise similar case, Patient D, in which these adjuvant anti-inflammatory modalities were not used in conjunction with the compositions of the invention, and an inoperable lung cancer was fatal within 8 months of diagnosis. These cases provide evidence of a synergistic effect between the antigenic compositions of the invention and anti-inflammatory treatments.

PVF for Cancer of the Colon with Metastases to the Liver and Lung, and without Anti-inflammatories

[00347] Patient E (PtE): PtE had a surgical resection of colon cancer on June 17, year 0, followed by chemotherapy. On August 15, year 0, he was diagnosed stage 4 cancer with metastases to the liver and lungs, a diagnosis with a very poor prognosis. On October 20, year 0, PtE began treatment with an antioxidant and vitamin D regime and, on Dec 10, year 0, he began treatment with the PVF composition three times per week, which he has continued in combination with the antioxidants and vitamin D. In September, year 1, he began treatment with Celebrex™ 100 mg twice per day. In spite of a very poor initial prognosis, PtE was still and feeling well at last contact, more than 3 years after diagnosis with terminal metastatic colon cancer.

[00348] In accordance with the foregoing results, one aspect of the invention involves the treatment of cancers of the colon, liver and lung with administration of antigenic compositions that comprise antigenic determinants of microbial pathogens that are known to be pathogenic in the colon, liver and lung.

[00349] In contrast to PtE, of the 15 patients diagnosed with stage 4 colon cancer and treated with PVF, the patient with the shortest survival, Patient F, was not treated with anti-inflammatories. These cases provide compelling evidence that anti-inflammatory modalities (*i.e.*, Celebrex™, anti-oxidants and vitamin D) taken in conjunction with targeted PVF therapy has a synergistic effect, contributing to PtE's

prolonged survival, which was greater than that of otherwise similar cases in which these adjuvant anti-inflammatory modalities were not used in conjunction with the compositions of the invention.

PVF for Cancer of the Colon with Metastases to Lung, with Anti-inflammatories

[00350] Patient G (PtG): PtG developed rectal bleeding in May, year 0, and was diagnosed with colon cancer. He underwent surgery, chemotherapy and radiation, but developed metastases to his lungs (stage 4 cancer) on 16 August, year 1, a terminal diagnosis with a poor prognosis. He had begun a regime of antioxidant vitamins and vitamin D in June, year 0, and, on September 23, year 1, he began taking the NSAID Celebrex 100 mg twice per day. In March, year 3, he began PVF vaccine three times per week, which he continued till April, year 4 at which time he developed brain metastases, which lead to his death on June 2, year 4, almost 3 years after a diagnosis of stage 4 terminal colon cancer. PtG lived substantially longer than would normally be expected with a diagnosis of stage 4 colon cancer with metastases to the lungs. In this context, the invention provides for the use of anti-inflammatory modalities in conjunction with immunogenic compositions, such as PVF, for synergistic effect.

[00351] Patient H (PtH): PtH was diagnosed with colon cancer with metastases to the liver and lungs on February 13, year 0. On January 11, year 1, he was prescribed an antioxidant and vitamin D regime. However, in March, year 1, he entered a chemotherapy research study and discontinued these supplements at that time at the request of the study coordinators. He was not treated with any NSAIDs. On May 12, year 1, he began treatment with PVF, which he took three times per week until his death just 2.5 months later. When contrasted to similar cases that involved the use of anti-infammatories, this case illustrates that, if adjuvant anti-inflammatory modalities are not given concomitantly with the targeted antigenic activation therapy, there is a lack of a synergistic effect that would otherwise occur with concomitant use of adjuvant anti-inflammatory modalities.

[00352] In summary, in cases of stage 4 colon cancer treated with targeted PVF vaccine therapy, the use of adjuvant anti-inflammatory agents, including antioxidants, vitamin D and Celebrex, used in conjunction with targeted antigenic activation therapy, was associated with substantially improved survival, much greater than that of the two cases in which these adjuvant anti-inflammatory modalities were not used in conjunction with the vaccine, providing evidence suggestive of a synergistic effect.

PVF with and without Anti-Inflammatories for Cancer of the Pancreas with Metastases to the Lungs, Liver and Abdominal Lymph Nodes

[00353] Patient I (PtI): PtI was diagnosed with pancreatic cancer in August, year 1, at which time he had surgery to remove his pancreas (i.e., Whipple's procedure). However, in July year 2, he developed metastases to the lungs bilaterally and in Feb year 4 he developed recurrence of cancer in the pancreatic area with abdominal and liver metastases. This is a terminal diagnosis with a very poor prognosis. PtI began a regime of antioxidant vitamins, vitamin D, large doses of turmeric (curcumin), fish oil (9 gm per day), resveratrol and green tea (equivalent of 36 cups per day) on September 27, year 2, all of which are anti-inflammatory modalities, all of which he continued to take. In March year 3, he began treatment with Celebrex 100 mg twice per day, which he took for more than 20 months. PtI began treatment with PVF three times per week in May year 4, which he has continued to use regularly for more than 3 years. PtI survived for 5 years after a diagnosis of terminal metastatic pancreatic cancer, a remarkably prolonged survival in the context of a diagnosis that has an extremely poor prognosis. This case provides evidence that high doses of multiple anti-inflammatory modalities (*i.e.*, Celebrex, antioxidants, vitamin D, turmeric, fish oil, resveratrol, green tea) taken in conjunction with the PVF compositions, resulted in a synergistic effect which has contributed to PtI's remarkable survival for 5 years after developing metastatic pancreatic cancer, a diagnosis that is usually fatal within 6 months.

[00354] In accordance with the foregoing results, one aspect of the invention involves the treatment of cancer of the pancreas, abdominal lymph nodes, liver and

lung with administration of antigenic compositions that comprise antigenic determinants of microbial pathogens that are known to cause infection in the pancreas, abdominal lymph nodes, liver and lungs.

[00355] Patient J (PtJ) had an essentially identical diagnosis to PtI (i.e., pancreatic cancer with metastases to abdominal lymph nodes, lungs and liver). PtJ, who did not take any other anti-inflammatories along with the PVF vaccine except antioxidants and vitamin D, died within 4 months of diagnosis, whereas PtI, who took large doses of numerous other anti-inflammatories modalities (i.e., Celebrex, turmeric, fish oil, resveratrol and green tea) in conjunction with PVF vaccine, survived for 5 years after diagnosis. These cases provide evidence of a synergistic effect of high dose multiple anti-inflammatory modalities and targeted vaccine therapy.

MRV for Cancer of the Breast with Metastases to the Bone

[00356] Patient K (PtK): In March, year 0, PtK developed neck and back pain, which persisted. On July 28, year 0, she was diagnosed with stage 4 breast cancer with metastases to the cervical spine, an incurable diagnosis. She underwent surgery to remove two breast lumps (axillary lymph nodes positive) and palliative radiation to the metastases in her spine. On January 18, year 1, PtK began treatment with doses of antioxidants and vitamin D, as well as the NSAID indomethicin 50 mg four times per day. Three days later, on January 21, year 1, she began treatment with the MRV composition, which contains *Staphylococcus aureus* the most common pathogen of both the breast and bone. Although there was no documentation of the exact length of time that treatment with this combination of MRV/indomethicin/antioxidant/vitamin D was continued, the patient was given sufficient vaccine (20 ml) for approximately 2 years of treatment at the usual dose and frequency (*i.e.*, three times per week) and PtK stated that she completed the recommended treatment course at home. Remarkably, PtK was still alive at last contact, 13 years after diagnosis with stage 4 metastatic breast cancer with metastases to bone.

[00357] In accordance with the foregoing results, one aspect of the invention involves the treatment of cancers of the breast and bone with administration of antigenic compositions that comprise antigenic determinants of microbial pathogens that are known to be pathogenic in the breast and bone infection.

[00358] In contrast to Patient K, Patient L (PtL) was diagnosed with breast cancer with metastases to bone on October 11, year 0. She was not prescribed an NSAID or other anti-inflammatories. PtL began treatment with MRV on February 27, year 1. She died 9 months later on November 4, year 1, just over one year after diagnosis with stage 4 breast cancer with metastases to bone. The contrast between the otherwise similar cases of PtK and PtL illustrates the potential for synergistic treatment with anti-inflammatories and the antigenic compositions of the invention.

MRV with and without Anti-inflammatories for Cancer of the Breast with Metastases to the Bone

[00359] Patient M (PtM): PtM was diagnosed with stage 4 breast cancer with metastases to bone on June 15, year 0. She began on the NSAID Naprosyn 250 mg twice per day on an ongoing basis for pain relief and, in October, year 3, she began doses of antioxidants and vitamin D. Three months later, on January 15, year 4, she began treatment with MRV vaccine (which contains *Staphylococcus aureus*, the most common breast and bone pathogen) in combination with these anti-inflammatory therapies (*i.e.*, Naprosyn, antioxidants and vitamin D). PtM lived for more than 9 years after being first diagnosed with stage 4 metastatic breast cancer with metastases to bone, an unusually long survival considering the usual poor prognosis associated with this diagnosis.

[00360] In accordance with the foregoing results, one aspect of the invention involves the treatment of cancers of the breast and bone with administration of antigenic compositions that comprise antigenic determinants of microbial pathogens that are known to be common causes of breast and bone infection.

[00361] In contrast to PtM, Patient N (PtN): PtN was diagnosed with stage 4 cancer with metastases to bone on April 8, year 0. She began doses of antioxidants and vitamin D on April 24, year 0. However, prior to starting MRV, she was prescribed the blood thinner warfarin, limiting supplementation with vitamin E and vitamin C, two important antioxidants that can lead to potential complications if used in conjunction with warfarin. In addition, NSAIDs could not be prescribed in this case since they are contraindicated with warfarin use. On June 2, year 1 PtN began treatment with MRV. She died 14 months later in August, year 2. In this context, it is possible that the use of targeted vaccine therapy without the synergistic effect of adjuvant anti-inflammatories (*i.e.*, NSAID, vitamin E and therapeutic doses of vitamin C) limited its potential benefit.

[00362] In summary, in the cases of stage 4 breast cancer with metastases to the bone treated with targeted MRV therapy detailed above, the use of adjuvant anti-inflammatory agents in conjunction with MRV was associated with substantially improved survival, much greater than that of the two cases in which these adjuvant anti-inflammatory modalities were not used in conjunction with the vaccine, providing evidence suggestive of a synergistic effect.

MRV for Metastases to the Lungs

[00363] Patient O (PtO) was diagnosed in June, year 0 with kidney cancer with metastases to the lungs bilaterally and to the bone (left femur). This is generally considered to be an incurable terminal diagnosis with a poor prognosis. He began treatment with the MRV on August 10, year 0 and continued regular treatment (three times per week) for 16 months (after which MRV was no longer available). In September, year 0, he began 7 months of treatment with an experimental drug, pegylated interferon alpha-2a. His left femur was 'pinned' due to the risk of fracture as a result of the metastasis but, due to surgical complications, amputation of the left leg below the mid-thigh was required. In September, year 2, his cancerous right kidney

was removed. In October, year 2, a PET scan found no evidence of cancer in the lungs and no further evidence of bone metastases. PtO is alive with no evidence of cancer in his lungs, more than 9 years after a diagnosis of bilateral pulmonary metastases, a remarkable result.

[00364] In accordance with the foregoing results, one aspect of the invention involves the treatment of metastases to the lung with administration of antigenic compositions that comprise antigenic determinants of microbial pathogens that are known to be lung pathogens.

MRV for Metastases to the Bone and Lungs

[00365] Patient P (PtP) was diagnosed with kidney cancer in July, year 0, and underwent excision of this right kidney. In December, year 4, he developed metastases to the bone (femurs bilaterally) and lungs (bilaterally). PtP declined conventional treatment and began treatment with MRV in April, year 5, which he continued regularly, three times per week, for 18 months. PtP's health improved and he returned to normal daily activities. X-rays and imaging of the chest and femurs showed no progression, with stable disease in the lungs and femurs during the 18 months that PtP was on MRV treatment.

[00366] In accordance with the foregoing results, one aspect of the invention involves the treatment of metastases to the lung and bone with administration of antigenic compositions that comprise antigenic determinants of microbial pathogens that are common causes of lung and bone infection.

MRV for Metastases to the Lungs

[00367] Patient Q (PtQ) was diagnosed with colon cancer with probable metastases to the lungs in June, year 0. At that time, the primary colon tumour was fully excised, leaving only several lung metastases. PtQ started treatment with MRV on Dec. 11, year 0 which she continued three times per week for 4 months. On April 19,

year 1, after 6 months treatment with chemotherapy, she had surgery to excise the only visible lung lesion remaining, which was confirmed to be a metastatic lesion. A diagnosis of colon cancer with lung metastases has a poor prognosis, even in the context of chemotherapy followed by surgery to excise visible metastases. In spite of her original poor prognosis, PtQ remains in excellent health, with no evidence of cancer more than 10 years after her initial diagnosis with metastases to the lung and treatment with MRV.

S. aureus Antigens for Breast Cancer with Metastasis to the Bone

[00368] Patient R (PtR): In May, year 0, PtR was diagnosed with breast cancer with metastases to her sternum, femur and cervical spine, an incurable cancer with a poor prognosis. She was treated with radiation and Tamoxefen. In May, year 4, she developed an additional area of metastasis in her lumbar spine and she began on treatment with Megace. In November, year 4, she began treatment with a vaccine (Staphage Lystate vaccine) containing only *Staphylococcus aureus*, the most common cause of infection of both the breast and bone and thus, a selected formulation for the treatment of breast and bone cancer. She continued regular therapy with this vaccine for 5 years. In spite of a diagnosis of metastatic breast cancer with multiple bone metastases, PtR survived for more than 17 years, a remarkable survival in the context of incurable metastatic breast cancer and a testament to the promise of targeted vaccine therapy for the treatment of breast cancer.

[00369] In accordance with the foregoing results, one aspect of the invention involves the treatment of cancers of the breast and bone with administration of antigenic compositions that comprise antigenic determinants of microbial pathogens that are known to be the most common cause of the breast and bone infection.

[00370] This embodiment illustates that a formulation that includes antigenic determinants of only the most frequently pathogenic organism or organisms for a tissue may provide particular advantages, as is also illustrated in the mouse model data set

out below. In keeping with this, we have found enhanced effectiveness of Respivax as opposed to MRV in treating cancers situated in the lung, reflecting the fact that the Respivax formulation is somewhat more optimal because it includes higher relative concentrations of the pathogenic species which most commonly cause lung infection (*i.e.*, 67% of the bacterial cell count of Respivax is comprised of species that most commonly cause lung infection, whereas only 30% of the MRV vaccines are comprised of species that most commonly cause lung infection).

[00371] In accordance with the foregoing results, one aspect of the invention involves formulating the antigenic compositions such that antigenic determinants of microbial pathogens that are known to be the common causes of infection are given preferential priority in the proportions of the formulation, with the most common cause of infection receiving the greatest preferential priority. For example, the proportion of antigenic determinants that are derived from pathogens that are known to be a common cause of infection may be 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 95% or 100%.

[00372] Accordingly, in some embodiments, the invention provides antigenic compositions in which a threshold proportion of antigenic determinants selected in accordance with the invention are used, relative to any other antigenic determinants in the composition. For example, antigenic compositions may have greater than X% of the antigenic determinants therein derived from pathogenic (or commonly pathogenic, or most commonly pathogenic) species, where X may for example be 10, 20, 30, 40, 50, 60, 70, 80, 90, 95 or 100 (or any integer value between 10 and 100). For example, at least X% of the antigenic determinants in the antigenic composition may be specific for microbial pathogens that are pathogenic (or commonly pathogenic, or most commonly pathogenic) in the specific organ or tissue of the patient within which the cancer is situated. Using an alternative measure, of the total number of microbial pathogens in the antigenic composition, at least X% may be selected to be microbial pathogens that are pathogenic (or commonly pathogenic, or most commonly pathogenic) in the specific

organ or tissue of the patient within which the cancer is situated. In some embodiments, the antigenic composition may accordingly consist essentially of antigenic determinants of one or more microbial pathogens that are each pathogenic in the specific organ or tissue within which the cancer is situated. In selected embodiments, the antigenic composition may consist essentially or entirely of antigenic determinants of microbial pathogens that are commonly pathogenic in the specific organ or tissue of the patients with which the cancer is situated. In further selected embodiments, the antigenic antigenic composition may consist essentially or entirely of antigenic determinants of a microbial pathogen (or pathogens) that are most commonly pathogenic in the specific organ or tissue of the patients with which the cancer is situated.

[00373] In the context of various aspects of the invention, organisms are characterized by the frequency with which they are pathogenic. In this context, the invention also relates to the frequency with which endogenous flora are pathogenic. For clarity, in this regard, the characterizations herein of the frequency of pathogenicity, such as the designation "commonly pathogenic" relate, in general, to the proportion of infections in a particular organ or tissue that are commonly attributed to a particular organism, and not to the frequency with which microbial colonization of a tissue is converted to a pathogenic infection. In North America, the majority of human infections are understood to be caused by endogenous organisms, even though these organisms commonly are present as part of the endogenous flora without causing infection. For example, while *S. pneumonia* is a common cause of lung infection (*i.e.*, pneumonia) in humans (and thus, is designated “commonly pathogenic” in the lung), it is nevertheless also true that *S. pneumoniae* is commonly present as part of the endogenous flora of the respiratory tract without causing infection and thus, in the nature of an endogenous colonization, is not normally pathogenic.

MRV for Multiple Myeloma

[00374] Patient S (PtS) was diagnosed with multiple myeloma (stage 3A) in the fall of year 0, with multiple lesions on bone scan, including skull, humeri and pelvis. He was treated with standard chemotherapy (melphalan and prednisone) for 6 months. However, in December year 3, he developed a pathological fracture of his right femur as a result of his disease, which required pinning and local radiation. On April 28, year 4, PtS began treatment with MRV, which contains *Staphylococcus aureus* a common cause of septicemia, which he continued for more than 13 years until this vaccine was no longer available in December year 17. Remarkably, PtS is still alive almost 25 years after being diagnosed with multiple myeloma, a truly extraordinary outcome considering his 'terminal' diagnosis.

[00375] In accordance with the foregoing results, one aspect of the invention involves the treatment of hematological cancers with administration of antigenic compositions that comprise antigenic determinants of microbial pathogens that are known to cause septicemia.

[00376] In accordance with the forgoing results, and as illustrated in other patient case reports detailed herein, another aspect of the invention involves the administration of the immunogenic compositions repeatedly relatively frequently over a relatively long period of time, as described elsewhere herein.

PVF for Colon Cancer with Metastases of the Liver and Abdominal Lymph Nodes

[00377] Patient T (PtT) was diagnosed with colon cancer and was treated with excision of the primary tumour (and subsequent chemotherapy) in September year 0. Ten months later, she developed a liver metastasis, which was surgically excised in July year 1. PtT remained well until June year 7, when she was diagnosed with recurrent disease – an inoperable mass of abdominal lymph nodes in close proximity to the aorta and spine, obstructing her left ureter, requiring insertion of a nephrostomy tube. PtT was considered terminal and treated with palliative radiation in October year 7. She began treatment with PVF on November 17, year 7, which she continued every

second day. PtT survived for almost 4 years after being diagnosed with terminal recurrent metastatic colon cancer.

[00378] In accordance with the foregoing results, one aspect of the invention involves the treatment of cancer in abdominal lymph nodes with administration of antigenic compositions that comprise antigenic determinants of microbial pathogens that are known to cause infection in abdominal lymph nodes.

MRV for Metastasis to the Skin and Perineum

[00379] Patient U (PtU) was diagnosed with colon cancer and was treated with excision of the primary tumour in November year 0. He was diagnosed with stage 4 cancer in July year 2 with metastases to the perineum (*i.e.*, peri-anal/genital soft tissue area) and skin. He had further surgery to remove as much of the cancer as possible in the perineum (cancer extended past surgical margins) with follow-up radiation and chemotherapy. The only known cancer sites remaining were in the skin and perineum. PtU started treatment with MRV, which contains *Staphylococcus aureus* a common cause of skin and perineal infection, on May 25, year 3, which he continued three times per week for 5 months. In spite of his original poor prognosis, PtU is in excellent health more than 10 years after his diagnosis with stage 4 cancer with metastases to the perineum and skin.

[00380] In accordance with the foregoing results, one aspect of the invention involves the treatment of cancer of the skin and perineum with administration of antigenic compositions that comprise antigenic determinants of microbial pathogens that are known to be common causes infection in the skin and perineum.

PVF for Metastases to the Peritoneum

[00381] Patient V (PtV) was diagnosed with breast cancer in May, year 0, at which time she had a masectomy with adjuvant chemotherapy. In January, year 12, she developed abdominal pain and ascites and was diagnosed with peritoneal

metastases, a diagnosis with a poor prognosis. On August 5, year 12, PtV began treatment with PVF, which contains *E. coli* a common cause of peritoneal infection, which she continued regularly for 1 year. Her tumour markers and ascites decreased and, in August year 13, after one year of PVF treatment, she had abdominal surgery for an unrelated medical condition, at which time the surgeon could not find any evidence of the previous peritoneal cancer. PtV discontinued use of the vaccine. PtV was still alive at last contact, 3 years and 9 months after being diagnosed with terminal peritoneal metastases.

[00382] In accordance with the foregoing results, one aspect of the invention involves the treatment of peritoneal metastases with administration of antigenic compositions that comprise antigenic determinants of microbial pathogens that are known to cause peritoneal infection.

PVF for Ovarian and Pelvic Cancer

[00383] Patient W (PtW) was diagnosed with stage 3B poorly differentiated ovarian cancer in the fall of year 0. She had surgery in November year 0, with removal of the left ovary, but the cancer could not be completely excised and thus, she was at extreme risk for recurrence. She had a full course of post-operative chemotherapy. However, in year 2 her tumour markers began to rise and in January year 3 she was diagnosed with a recurrence in her right ovary area. She had surgery to remove this right ovarian mass in February year 3, but again the cancer could not be completely excised and she had follow-up chemotherapy. However, once again in December year 3 she developed a further recurrence in the pelvic area and retroperitoneal lymphadenopathy. She began treatment with PVF vaccine, which contains *E. coli* a cause of ovarian and pelvic infection, on Jan 5, year 4, which she continued for 6 months. Her tumour markers, which had risen to 2600, fell to the 300 range. PtW was alive and feeling very well at last contact, 2 years and 9 months after being diagnosed with recurrent ovarian cancer. Of note is the fall in her tumour markers following PVF treatment.

[00384] In accordance with the foregoing results, one aspect of the invention involves the treatment of ovarian and pelvic cancer with administration of antigenic compositions that comprise antigenic determinants of microbial pathogens that are known to cause infection in the ovary and pelvic areas.

MRV for Follicular Non-Hodgkin's lymphoma

[00385] Patient Y (PtY): was diagnosed with stage 4A Follicular Non-Hodgkin's lymphoma, with extensive marked lymphadenopathy (*i.e.*, enlarged lymph glands). He declined all conventional treatment. PtY began treatment with the MRV composition, which contains many of the pathogens which commonly cause infection of the lymph nodes of the head and neck, axillae, mediastinum and inguinal areas. In addition, he began treatment with a multiple vitamin/supplement regime, healthful diet and other immune enhancement treatments. He continued regular use of this vaccine for more than 3 years, at which time his lymph glands had begun to greatly reduce in size and he was feeling well. This resolution of lymphadenopathy continued, and imaging showed almost complete resolution of previous extensive lymphadenopathy. PtY was feeling well and there was no lymphadenopathy palpable: a clearly remarkable recovery. Five years after his initial diagnosis with Stage 4A Follicular Non-Hodgkin's lymphoma, PtY had no evidence of recurrence and was leading an active and healthy life. Treatment with MRV vaccine resulted in complete remission of his stage 4A follicular non-Hodgkins' lymphoma.

[00386] In accordance with the foregoing results, one aspect of the invention involves the treatment of lymphoma with administration of antigenic compositions that comprise antigenic determinants of microbial pathogens that are known to be common causes of lymph node infection in the region the lymphoma is located.

PVF for Colon Cancer with Metastases to the Liver and Kidneys

[00387] Patient Z (PtZ) was diagnosed with metastatic spread of previously treated colon cancer, with a metastasis to the liver and probable other metastases to both kidneys. The liver metastasis was excised. The prognosis for this stage (*i.e.*, stage 4) of colon cancer is poor and the benefit of further conventional treatment (*i.e.*, chemotherapy) is limited. PtZ declined chemotherapy initially. Three months after diagnosis with metastatic colon cancer, PtZ began treatment with Polyvaccinum Forte (PVF), which contains *E. coli*, a common cause of infection of the colon, liver and kidneys. In addition PtZ began treatment with a multiple vitamin/supplement regime and healthful diet. He continued regular use of this vaccine and the vitamin and supplement regime, and began chemotherapy. Although the overall course of his disease was slowly progressive, with development of lung metastases and recurrence of liver metastases, 28 months after his initial diagnosis of metastatic disease, his weight was stable and his energy levels were good. Three years (36 months) after diagnosis of stage 4 colon cancer, PtZ was feeling well except for nausea and mild weight loss related to chemotherapy.

[00388] In accordance with the foregoing results, one aspect of the invention involves the treatment of cancer of the colon, liver and kidneys with administration of antigenic compositions that comprise antigenic determinants of microbial pathogens that are known to be pathogenic in the colon, liver and kidneys.

PVF for Colon Cancer with Metastases to the Liver, Porta Hepatic Lymph Nodes and Lung

[00389] Patient AA (PtAA) was diagnosed with metastatic colon cancer with metastases to the liver, portahepatic lymph nodes and lungs. The prognosis for this stage (i.e., stage 4) of colon cancer is very poor (*i.e.*, 'terminal' cancer) and the benefit of conventional treatment (*i.e.*, chemotherapy) is limited. PtAA began chemotherapy, but discontinued treatment approximately 5 months after his diagnosis due to side effects, at which time he began treatment with Polyvaccinum Forte (containing bacterial species which cause infection in the colon, liver, abdominal lymph nodes and lungs)

every second day as well as a multiple vitamin/supplement regime and a healthy diet. PtAA's subsequent CT Scans demonstrated necrotic porta hepatic lymph nodes unchanged in size from the time of his diagnosis and no change in size of the lung metastases, although the two liver metastases grew moderately in size (3.4 cm to 4.5 cm and 1.2 cm to 3.0 cm). In spite of the very poor prognosis, PtAA continued to feel quite well almost one year after a diagnosis of terminal cancer.

[00390] In accordance with the foregoing results, one aspect of the invention involves the treatment of cancer of the colon, liver, abdominal lymph nodes and lungs with administration of antigenic compositions that comprise antigenic determinants of microbial pathogens that are known to be pathogenic in the colon, liver, abdominal lymph nodes and lungs.

EXAMPLE 3: Microbial Pathogens

[00391] In alternative aspects, the invention utilizes microbial antigens, such as bacterial or viral antigens, to formulate antigenic compositions, where the microbial species is selected on the basis of the tissue or organ within which the microbe is known to cause infections. Bacterial resident flora are the most common bacterial pathogens, accounting for the vast majority of bacterial infections in most animals, including humans. Resident flora can for example infect through primary attachment, or attachment and invasion following mucosa damage, resulting for example from vascular, trauma, chemical insult, or damage resulting from primary infection.

[00392] For microbial pathogens, virulence and infection potential is a combination of the ability of the microbe to adhere, to produce enzymes, to survive immunoproducts (complement, antibody) and to survive the microbiocidal activity of macrophage and neutrophils. Some bacteria, including endogenous bacteria, may be sufficiently virulent as to cause monomicrobial infections, while others are more effective with the synergy of polymicrobial infection. In general, it is often not possible to be precise about the specific role of individual microbes within the milieu of mixed

infection. As acute infection may, in some cases, provide more optimal immune stimulation, accordingly, in some embodiments, the invention utilizes microbial species that are involved in acute infection.

[00393] In some embodiments, bacteria that are members of the endogenous flora of a particular region may be used to formulate antigenic compositions of the invention. The rows of Table 6 list a number of bacterial species, together with the biological regions in which each species may form a part of the endogenous flora. For example, *Abiotrophia spp.* are typically members of the endogenous flora of the respiratory tract and the mouth.

Table 6: Human Bacterial Normal Flora (Endogenous Bacterial Human Pathogens)

Bacterial species	Respir-atory	Mouth	Stomach	Duodenum/ Jejunum	Ileum	Colon	GU System	Genital	Skin
CFL /mL		10^5	10^2	10^5	10^8	10^11			
Abiotrophia spp	+	+							
Acholeplasma laidlawii	+	+							
Acidaminococcus fermentans	+	+		+	+	+			
Acinetobacter spp.	+	+		+	+	+	+	+	+
Actinobacillus spp.	+	+							

Actinobaculum spp.	+	+		+	+	+			
Actinomyces spp.	+	+		+	+	+	+	+	
Aerococcus christensenii							+		
Aerococcus viridans									+
Aerococcus urinae							+		
Aeromonas spp.				+	+	+			
Alloiococcus otitis									+
Anaerorhabdus furcosus					+	+			
Anaerococcus hydrogenalis					+	+	+		+
Anaerococcus lactolyticus					+	+	+		
Anaerococcus prevotii					+	+	+		
Arcanobacterium spp.	+								+
Atopobium spp.	+	+		+	+	+			
Bacillus spp.					+	+			+
Bacteroides caccae					+	+			

Bacteroides distasonis					+	+			
Bacteroides eggerthii					+	+			
Bacteroides fragilis					+	+	+	+	
Bacteroides merdae					+	+			
Bacteroides ovatus					+	+			
Bacteroides splanchnicus					+	+			
Bacteroides thetaiotaomicron					+	+			
Bacteroides vulgatus					+	+			
Bifidobacterium adolescentis				+	+	+			
Bifidobacterium bifidum				+	+	+	+	+	
Bifidobacterium breve				+	+	+	+	+	
Bifidobacterium catenulatum				+	+	+	+	+	
Bifidobacterium dentium	+	+		+	+	+	+	+	

Bifidobacterium longum				+	+	+	+	+	
Bilophila wadsworthia	+	+		+	+	+	+	+	
Brevibacterium casei									+
Brevibacterium epidermidis									+
Burkholderia cepacia	+			+	+	+			
Butyrivibrio fibrisolvens				+	+	+			
Campylobacter concisus	+			+	+	+			
Campylobacter curvus	+			+	+	+			
Campylobacter gracilis	+			+	+	+			
Campylobacter jejuni				+	+	+			
Campylobacter rectus				+	+	+			
Campylobacter showae	+	+		+	+	+			
Campylobacter sputorum	+	+							
Capnocytophaga granulosum	+	+							

Capnocytophaga gingivalis	+	+							
Campylobacter haemolytica	+	+							
Capnocytophaga ochracea	+	+		+	+	+	+	+	
Capnocytophaga sputigena	+	+							
Cardiobacterium hominis	+	+							
Cedecea spp						+			
Centipeda periodontii	+	+							
Citrobacter freundii				+	+	+			
Citrobacter koseri				+	+	+			
Clostridium spp.				+	+	+			
Corynebacterium accolens	+	+							+
Corynebacterium afermentans	+	+							+

Corynebacterium amycolatum									+
Corynebacterium auris									+
Corynebacterium diphtheriae	+								+
Corynebacterium durum	+								
Corynebacterium glucuronolyticum							+		
Corynebacterium jeikeium									+
Corynebacterium macginleyi									+
Corynebacterium matruchotii	+								
Corynebacterium minutissimum									+
Corynebacterium propinquum	+								

Corynebacterium pseudodiphtheriticum	+								
Corynebacterium riegelii							+		
Corynebacterium simulans									+
Corynebacterium striatum	+								+
Corynebacterium ulcerans	+								
Corynebacterium urealyticum							+		+
Dermabacter hominis									+
Dermacoccus nishinomiyaensis									+
Desulfomonas pigra				+	+	+			
Dysgonomonas spp.				+	+	+			
Eikenella corrodens	+	+		+	+	+			

Enterobacter aerogenes				+	+	+			
Enterobacter cloacae				+	+	+			
Enterobacter gergoviae				+	+	+			
Enterobacter sakazakii				+	+	+			
Enterobacter taylorae				+	+	+			
Enterococcus spp.				+	+	+			
Escherichia coli				+	+	+	+	+	
Escherichia fergusonii				+	+	+			
Escherichia hermannii				+	+	+			
Escherichia vulneris				+	+	+			
Eubacterium spp.	+	+		+	+	+			
Ewingella americana	+	+							
Finegoldia magnus				+	+	+	+		+
Fusobacterium alocis	+	+							

Fusobacterium gonidiaformans				+	+	+	+	+	
Fusobacterium mortiferum				+	+	+			
Fusobacterium naviforme				+	+	+	+	+	
Fusobacterium necrophorum	+	+		+	+	+			
Fusobacterium nucleatum	+	+							
Fusobacterium sulci	+	+							
Fusobacterium russii				+	+	+			
Fusobacterium varium				+	+	+			
Gardnerella vaginalis				+	+	+	+	+	
Gemella haemolysans	+	+							
Gemella morbillorum	+	+		+	+	+			
Globicatella spp.		+				+			

Granulicatella spp.	+	+							
Haemophilus spp.	+	+						+	
Hafnia alvei				+	+	+			
Helcococcus kunzii									+
Helicobacter spp.				+	+	+			
Kingella spp.	+	+							
Klebsiella spp.	+	+		+	+	+			
Kocuria spp.									+
Kytococcus sedentarius									+
Lactobacillus acidophilus	+	+	+	+	+	+	+	+	
Lactobacillus breve	+	+							
Lactobacillus casei	+	+					+	+	
Lactobacillus cellobiosus							+	+	
Lactobacillus fermentum	+	+	+	+	+	+	+	+	
Lactobacillus reuteri			+	+	+	+			
Lactobacillus salivarius	+	+	+	+	+	+			

Lactococcus spp.							+	+	
Leclercia adecarboxylata				+	+	+			
Leminorella spp.				+	+	+			
Leptotrichia buccalis	+	+					+	+	
Leuconostoc spp.							+	+	
Megasphaera elsdenii				+	+	+			
Micrococcus luteus	+	+							+
Micrococcus lylae	+	+							+
Micromonas micros	+	+							
Mitsuokella multiacidus				+	+	+			
Mobiluncus curisii				+	+	+		+	
Mobiluncus mulieris				+	+	+		+	
Moellerella wisconsensis				+	+	+			
Moraxella catarrhalis	+	+							

other Moraxella spp.	+	+					+		+
Morganella morganii				+	+	+			
Mycoplasma buccale	+	+							
Mycoplasma faucium	+								
Mycoplasma fermentans	+	+					+		
Mycoplasma genitalium	+						+		
Mycoplasma hominis	+	+					+		
Mycoplasma lipophilum	+	+							
Mycoplasma orale	+	+							
Mycoplasma penetrans							+		
Mycoplasma pneumoniae	+	+							
Mycoplasma primatum							+		
Mycoplasma salivarium	+	+							
Mycoplasma spermatophilum							+		

Neisseria cinerea	+								
Neisseria flavescens	+								
Neisseria lactamica	+								
Neisseria meningitidis	+							+	
Neisseria mucosa	+								
Neisseria polysaccharea	+								
Neisseria sicca	+								
Neisseria subflava	+								
Oligella ureolytica							+	+	
Oligella urethralis							+	+	
Pantoea agglomerans				+	+	+			
Pastuerella bettyae							+	+	
Pasteurella multocida	+	+							
Pediococcus spp.		+				+			

Peptococcus niger							+	+	+
Peptoniphilus asaccharolyticus				+	+	+	+	+	+
Peptoniphilus lacrimalis	+								
Peptostreptococcus anaerobus	+	+		+	+	+			
Peptostreptococcus productus				+	+	+			
Peptostreptococcus vaginalis							+	+	+
Porphyromonas asaccharolytica		+		+	+	+	+	+	
Porphyromonas catoniae	+	+		+					
Porphyromonas endodontalis	+	+		+					
Porphyromonas gingivalis	+	+		+					

Prevotella bivia							+	+	
Prevotella buccae	+	+		+					
Prevotella buccalis	+	+		+			+	+	
Prevotella corporis	+	+		+					
Prevotella dentalis	+	+		+					
Prevotella denticola	+	+		+					
Prevotella disiens							+	+	
Prevotella enoeca	+	+		+					
Prevotella heparinolytica	+	+		+					
Prevotella intermedia	+	+		+					
Prevotella loescheii	+	+		+			+	+	
Prevotella melaninogenica	+	+		+			+	+	
Prevotella nigrescens	+	+		+					
Prevotella oralis	+	+		+			+	+	

Prevotella oris	+	+		+					
Prevotella oulorum	+	+		+					
Prevotella tannerae	+	+		+					
Prevotella veroralis	+	+		+			+	+	
Prevotella zoogleoformans	+	+		+					
Propionibacterium acnes									+
Propionibacterium avidum									+
Propionibacterium granulosum									+
Propionibacterium propionicum	+	+							
Propionferax innocuum									+
Proteus mirabilis					+	+	+		
Proteus penneri					+	+	+		
Proteus vulgaris					+	+	+		

Providencia rettgeri					+	+			
Providencia stuartii				+	+	+			
Pseudomonas aeruginosa				+	+	+			
Retortamonas intestinalis				+	+	+			
Rothia dentocariosa	+	+							
Rothia mucilaginosa	+	+							
Ruminococcus productus				+	+	+			
Selenomonas spp.	+	+							
Serratia liquefaciens					+	+			
Serratia marcescens					+	+			
Serratia odorifera					+	+			
Staphylococcus aureus	+	+					+	+	+
Staphylococcus auricularis									+
Staphylococcus capitis									+

Staphylococcus caprae									+
Staphylococcus cohnii									+
Staphylococcus epidermidis	+	+					+	+	+
Staphylococcus haemolyticus									+
Staphylococcus hominis									+
Staphylococcus lugdunensis									+
Staphylococcus pasteuri									+
Staphylococcus saccharolyticus									+
Staphylococcus saprophyticus							+		+
Staphylococcus schleiferia									+
Staphylococcus simulans									+

Staphylococcus xylosus									+
Staphylococcus warneri									+
Streptococcus agalactiae				+	+	+	+	+	
Streptococcus anginosus	+	+		+	+	+	+	+	
Streptococcus bovis				+	+	+			
Streptococcus constellatus	+	+		+	+	+	+	+	
Streptococcus criceti	+	+							
Streptococcus crista	+	+							
Streptococcus equisimilis	+	+							
Streptococcus gordonii	+	+							
Streptococcus intermedius	+	+			+	+	+	+	
Streptococcus mitis	+	+	+						
Streptococcus mutans	+	+							
Streptococcus oralis	+	+							

Streptococcus parasanguis	+	+							
Streptococcus pneumoniae	+								
Streptococcus pyogenes	+	+	+						+
Streptococcus salivarius	+	+	+						
Streptococcus sanguis	+	+	+						
Streptococcus sobrinus	+	+							
Streptococcus vestibularis	+	+							
Group C + G Streptococci		+				+			
Succinivibrio dextrinosolvens				+	+	+			
Sutterella spp.	+	+			+	+	+		
Suttonella indologenes	+	+							
Tissierella praeacuta				+	+	+			
Treponema denticola	+	+							

Treponema maltophilum	+	+							
Treponema minutum							+		
Treponema phagedenis							+		
Treponema refringens							+		
Treponema socranskii	+	+							
Treponema vincentii	+	+							
Turicella otitidis									+
Ureaplasma urealyticum	+	+					+		
Veillonella spp.	+	+		+	+	+			
Weeksella virosa							+	+	

[00394] Endogenous microbial flora, such as bacteria, have access to tissues for pathogenesis either through contiguous spread or bacteremic spread. Under favorable conditions, all endogenous organisms can become pathogenic and invade locally and spread by contiguous spread to adjacent tissues and organs. Endogenous bacterial flora of the skin, mouth and colon are the species that are understood to also be amenable to bacteremic spread. Bacteria that are members of a particular endogenous flora domain may therefore cause infection in tissues or organs to which these bacteria may spread. Accordingly, one aspect of the invention involves the use of endogenous microbial pathogens to treat a cancer of a tissue or organ to which the endogenous

bacteria may spread to cause infection. The columns of Table 7 list 9 domains for endogenous flora, the: skin, respiratory system, genitals, GU system, mouth, stomach, duodenum/jejunum, ileum and colon. The rows of Table 7 list organs or tissues within which cancers may be situated. Accordingly, one aspect of the invention involves the use of endogenous microbial pathogens to formulate antigenic compositions, or the selection of existing formulations having the pathogens, for treating cancers situated in tissues or organs to which the pathogen may spread to cause an infection. Accordingly, in alternative embodiments, tumors situated in the tissues or organs listed in the first column of Table 7 may be treated with antigenic compositions comprising antigenic determinants that are specific for microbial pathogens that are members of the endogenous flora of one or more of the endogenous flora domains listed in the first row of Table 7 and indicated with an X or a check mark in the appropriate row. For example, tumors situated in the prostate may be treated with an antigenic composition having antigenic determinants specific for a microbial pathogen or pathogens endogenous to the GU system and/or genital system. A number of the bacterial species that are endogenous to the endogenous flora domains listed in Table 7 are listed, with the corresponding endogenous flora domains, in Table 6. Accordingly, one aspect of the invention involves the treatment of a cancer situated in a tissue listed in Table 7 with an antigenic composition comprising antigenic determinants of the bacterial species that are listed in Table 6, where the regions of endogenous flora linked to the site of the tumor in Table 7 match the regions of endogenous flora linked to the bacterial species in Table 6.

Table 7: Tissue/Organ Pathogenicity of Endogenous Flora

Tissue/ organ site	Skin	Respi-ratory	Genital	GU System	Mouth	Sto-mach	Duo-denum / Jejunum	Ileum	Colon
Skin	x				x				

Soft tissue (i.e. fat and muscle) (e.g., sarcoma)	x								
Breast	x				x				
Lymph nodes: head and neck	x	x			x				
Lymph nodes: axillae/arm	x				□				□
Lymph nodes: mediastinal		x			□				□
Lymph nodes: pulmonary hilum		x							
Lymph nodes: intra-abdominal				x	□	x	x	x	x
Lymph nodes: inguinal/ leg	x		X		□				□

Hematolog-ical (e.g. leukemias, multiple myeloma)	□				□				□
Bone	x				□				□
Meninges		x			x				
Brain	□				□				□
Spinal cord	□				□				□
Eye/Orbit	x	x	X		x				
Salivary glands					x				
Oral					x				
Tonsil		x			x				
Nasopha-rynx/Sinus		x			x				
Thyroid	□				□				□
Larynx		x			x				
Lung/Trachea/Bronchi		x							
Pleura	□	x			□				□
Mediastinum		x							
Heart	□				□				□
Esophagus						x			
Stomach						x			
Small bowel							x	x	
Colon/ Rectum									x

Anus	x								x
Perineum	x								x
Liver	□				□				□
Gallbladder							x		
Biliary tract							x		
Pancreas							x		
Spleen	□				□				□
Adrenal gland	□				□				□
Kidney	□			x	□				□
Ureter				x					
Bladder	□		x	x					
Peritoneum						x	x	x	x
Retroperito -neal area				x		x	x	x	x
Prostate			x	x					
Testicle			x	x					
Penis	x		x	x					
Ovary/ Adnexae			x	x					x
Uterus			x	x					x
Cervix			x	x					x
Vagina			x						x
Vulva			x						x

* Bacteria have access to tissues/organs either through: Contiguous spread (X) or Bacteremic spread: (□).

[00395] In accordance with the combined information in Tables 6 and 7, cancers located in the tissues or organs set out in column 1 of Table 7 may be treated with antigenic compositions comprising antigenic determinants of the corresponding

bacterial species of Table 6, so that the column headings in Table 7 are in effect replaced with the bacterial species of Table 6.

[00396] In some embodiments, microbial pathogens for use in the invention may be exongenous bacterial pathogens. For example, the organisms listed in Table 8 may be used as microbial pathogens to formulate antigenic compositions, or antigenic compositions having those pathogens may selected, for use to treat cancers situated in the tissues or organs listed with the relevant organism in Table 8. In some embodiments, antigenic determinants of both endogenous and exogenous bacterial species targeted to a specific tissue or organ may be used in combination. For example, an antigenic composition derived from, or specific for, *Clostridium difficile*, may be used to treat a cancer situated in the colon.

Table 8 Exogenous Bacterial Human Pathogens, and their Sites of Infection

bacterial species	tissue/organ sites
Achromobacter spp.	hematological, skin, soft tissue, lung/trachea/bronchi, peritoneum, meninges, bile duct, gallbladder, kidney, bladder, ureter
Actinomadura spp.	skin, soft tissue, lung/trachea/bronchi, mediastinum, brain, spinal cord, hematological, meninges
Aerobacter spp.	small bowel, colon, hematological, peritoneum
Aerococcus spp.	hematological, heart, bone, kidney, bladder, ureter, meninges
Alcaligenes spp.	lung/trachea/bronchi
Anaplasma spp.	meninges, hematological, liver, spleen, bone, lung/trachea/bronchi
Bacillus anthracis	lung/trachea/bronchi, lymph nodes pulmonary hilum, mediastinum, meninges, skin, nasopharynx, tonsil, oral, small bowel, colon, hematological
Bacillus cereus	colon, eye, hematological

other Bacillus spp.	hematological, bone, meninges, brain, heart, lung/trachea/bronchi, mediastinum, skin, soft tissue, colon, stomach, small bowel, eye
Balneatrix spp.	lung/trachea/bronchi, meninges, hematological
Bartonella bacilliformis	skin, hematological, liver, muscle, lymph nodes
Bartonella henselae	brain, spinal cord, hematological, skin, liver, bone, pleura, lung/trachea/bronchi, mediastinum, axillary and inguinal lymph nodes, eye
Bartonella quintana	skin, hematological, liver, spleen
Bergeyella zoohelcum	skin, soft tissue, meninges, hematological, lung/trachea/bronchi
Bordetella holmesii	lung/trachea/bronchi, hematological
Bordetella parapertussis	nasopharynx, tonsil, lung/trachea/bronchi
Bordetella pertussis	nasopharynx, tonsil, lung/trachea/bronchi
Borrelia burgdorferi	meninges, brain, spinal cord, skin, eye, hematological, inguinal/axillary/cervical lymph nodes, muscle, liver, spleen, nasopharynx, lung/trachea/bronchi, testes
Borrelia recurrentis	brain, spinal cord, hematological, small bowel, liver, spleen, salivary glands, lung/trachea/bronchi, lymph nodes, eye, skin
Brevundimonas spp.	peritoneum, hematological, skin, soft tissue

Brucella spp.	lung/trachea/bronchi, lymph nodes pulmonary hilum, meninges, brain, spinal cord, lymph nodes, mediastinum, bone, eye, small bowel, colon, liver, biliary tract, kidney, ureter, bladder, hematological, skin, testes, spleen, prostate
Burkholderia gladioli	hematological, meninges, lung/trachea/bronchi
Burkholderia mallei	lung/trachea/bronchi, skin, soft tissue, liver, spleen, muscle, lymph nodes pulmonary hilum, mediastinal lymph nodes, mediastinum, head and neck lymph nodes, hematological
Burkholderia pseudomallei	lung/trachea/bronchi, skin, kidney, bladder, ureter, soft tissue, bone, brain, spinal cord, muscle, hematological, prostate, kidney, ureter, meninges
Calymmatobacterium granulomatis	skin, penis, vulva, soft tissue, vagina, cervix, bone, hematological, inguinal lymph nodes
Campylobacter coli	small bowel, colon
Campylobacter fetus	lung/trachea/bronchi, small bowel, colon, meninges, brain, peritoneum, bone, gallbladder, ovaries, hematological, heart, kidney, bladder, ureter
Campylobacter jejuni	colon, hematological, gallbladder, pancreas, bladder, bone, meninges
Campylobacter sputorum	small bowel, colon
Capnoctyophaga canimorsus	skin, soft tissue, meninges, hematological, bone, lung/trachea/bronchi, eye
Capnoctyophaga cynodegmi	skin, soft tissue, meninges, hematological, bone, lung/trachea/bronchi, eye

CDC groups EF-4a and EF-4b	hematological, eye, skin, soft tissue
Chlamydia pneumoniae	lung/trachea/bronchi, lymph nodes pulmonary hilum, liver, brain, meninges, skin, thyroid, pancreas, hematological
Chlamydia psittaci	lung/trachea/bronchi, lymph nodes pulmonary hilum, mediastinum, liver, brain, meninges, hematological, skin, thyroid, pancreas
Chlamydia trachomatis	inguinal lymph nodes, penis, vulva, vagina, cervix, uterus, ovaries and adnexae, peritoneum, prostate, eye
Chlamydophila pneumoniae	laryngx, trachea/bronchi, hematological
Chromobacterium violaceum	hematological, liver, spleen, lung/trachea/bronchi, kidney, bladder, ureter, eye/orbit, bone, brain, meninges, spinal cord
Chlamydophila psittaci	lung/trachea/bronchi
Chryseobacterium spp.	meninges, lung/trachea/bronchi, hematological
Clostridium bifermentans	small bowel, colon, stomach, skin, soft tissue, hematological
Clostridium botulinum	colon, small bowel, skin
Clostridium difficile	colon
Clostridium indolis	small bowel, colon, stomach, skin, soft tissue, hematological
Clostridium mangenolii	small bowel, colon, stomach, skin, soft tissue, hematological
Clostridium perfringens	small bowel, colon, stomach, skin, soft tissue, hematological, heart

Clostridium sordellii	small bowel, colon, stomach, skin, soft tissue, hematological
Clostridium sporogenes	small bowel, colon, stomach, skin, soft tissue, hematological
Clostridium subterminale	small bowel, colon, stomach, skin, soft tissue, hematological
Clostridium tetani	skin, soft tissue
Comamonas spp.	hematological, peritoneum, eye
Corynebacterium pseudotuberculosis	neck/axillary/inguinal/mediastinal lymph nodes, lymph nodes pulmonary hilum, lung/trachea/bronchi, mediastinum
Coxiella burnetii	lung/bronchi/trachea, brain, spinal cord, liver, bone
Edwarsiella tarda	skin, soft tissue, liver, meninges, small bowel, colon, bone, uterus, ovaries
Ehrlichia spp.	meninges, brain, spinal cord, hematological, bone, liver, kidney, spleen, lymph nodes
Erysipelothrix rhusiopathiae	skin, hematological, bone, brain, peritoneum
Francisella tularensis	nasopharynx, oral, tonsil, lung/trachea/bronchi, skin, axillary/head and neck/inguinal lymph nodes, hematological, eye, small bowel
Fusobacterium spp.	skin, soft tissue, hematological
Gordonia spp.	skin, soft tissue, lung/trachea/bronchi, mediastinum, brain, spinal cord, hematological, meninges, eye
Haemophilus ducreyi	skin, inguinal lymph nodes, penis, vulva, vagina
Helicobacter pylori	stomach

Legionella spp.	lung/trachea/bronchi, lymph nodes pulmonary hilum, hematological, brain, spinal cord, muscle, pancreas
Leptospirosis spp.	lung/trachea/bronchi, pancreas, meninges, brain, spinal cord, skin, lymph nodes, eye, hematological, nasopharynx, oral, tonsil, kidney, liver, spleen
Listeria monocytogenes	hematological, brain, meninges, spinal cord, small bowel, colon
Methylobacterium spp.	hematological, peritoneum, skin, soft tissue, bone
Mycobacterium avium	lung/bronchi/trachea, lymph nodes pulmonary hilum, prostate, pancreas, spleen, skin, neck lymph nodes, esophagus, bone, hematological
Mycobacterium bovis	colon, small bowel
Mycobacterium kansasii	lung/bronchi/trachea, lymph nodes pulmonary hilum, prostate, bone
Mycobacterium leprae	skin, soft tissues, testes, eye
Mycobacterium marinum	skin, soft tissue, bone
Mycobacterium scrofulaceum	head and neck lymph nodes
Mycobacterium tuberculosis	lung/bronchi/trachea, lymph nodes pulmonary hilum, prostate, peritoneum, pancreas, spleen, lymph nodes, small bowel, meninges, brain, spinal cord, kidney, ureter, bladder, muscle, esophagus, colon, testes, eye, ovaries, cervix, vagina, uterus, mediastinum, larynx, skin, hematological, pleura
Mycobacterium ulcerans	skin, soft tissue

other Mycobacterium spp.	lung/bronchi/trachea, lymph nodes pulmonary hilum, skin, soft tissues, bone, head and neck lymph nodes
Myroides spp.	kidney, bladder, ureter, skin, soft tissue, hematological
Neisseria gonorrhoeae	nasopharyx, oral, tonsil, prostate, penis, vagina, cervix, uterus, ovary/adnexae, peritoneum, skin, muscle, bone, liver, hematological, head and neck and inguinal and intra-abdominal lymph nodes, anus
Neorickettsia sennetsu	hematological, bone, lymph nodes, liver, spleen
Nocardia spp.	lung/bronchi/trachea, pancreas, meninges, spinal cord, brain, skin, soft tissue, eye, bone, kidney, heart, hematological
Orientia tsutsugamushi	meninges, brain, spinal cord, hematological, skin, inguinal and axillary lymph nodes, spleen, lung/bronchi/trachea
Pandoraea spp.	lung/trachea/bronchi, hematological
Pasteurella canis	skin, soft tissue, hematological
Pasteurella dagmatis	skin, soft tissue, hematological
Pasteurella stomatis	skin, soft tissue, hematological
Pediococcus spp.	hematological, liver, colon
Pityrosporum ovale	skin
Plesiomonas shigelloides	small bowel, colon, hematological, meninges, bone, gall bladder, skin, soft tissue
Pseudomonas aeruginosa	lung/trachea/bronchi, hemaotogical, skin, soft tissue, bone, meninges, brain, eye, kidney, bladder, ureter, heart

other Pseudomonas spp.	skin, soft tissue, lung/trachea/bronchi, mediastinum, hematological
Ralstonia spp.	hematological, meninges, bone
Rhizobium spp.	hematological, peritoneum, eye, kidney, bladder, ureter
Rhodococcus spp.	lung/trachea/bronchi, hematological, brain, skin, lymph nodes, bone, mediastinum, liver, spleen, soft tissue, spinal cord, meninges
Rickettsia akari	skin
Rickettsia conorii	lung/bronchi/trachea, lymph nodes pulmonary hilum, meninges, brain, spinal cord, hematolofical, skin, kidney, liver, spleen, pancreas
Rickettsia felis	skin, brain, spinal cord
Rickettsia prowazekii	meninges, brain, spincal cord, hematological, lung/bronchi/trachea, skin, spleen
Rickettsia rickettsiae	lung/bronchi/trachea, lymph nodes pulmonary hilum, meninges, brain, spinal cord, hematological, muscle, small bowel, liver, skin
Rickettsia slovaca	skin, head and neck lymph nodes
Rickettsia typhi	meninges, hematological, liver, kidney, brain, lung/bronchi/trachea, spleen
Roseomonas spp.	hematological, peritoneum, skin, soft tissue, bladder, kidney, ureter
Salmonella spp.	lung/bronchi/trachea, pancreas, spleen, intra-abdominal lymph nodes, stomach, small bowel, colon, meninges, skin, muscle, bone, hematological, heart
Shewanella spp.	skin, soft tissue, eye, bone, hematological, peritoneum
Shigella boydii	colon
Shigella dysenteriae	colon

Shigella flexneri	colon
Shigella sonnei	colon
Sphingobacterium spp.	brain, meninges, spinal cord, eye, skin, soft tissue
Sphingomonas spp.	hematological, meninges, peritoneum, skin, soft tissue, kidney, bladder, ureter
Spirillum minus	skin, axillary/inguinal/neck lymph nodes, hematological, liver, spleen
other Spirillum spp.	colon
Stenotrophomonas maltophilia	meninges, hematological, peritoneum, lung/trachea/bronchi, eye, kidney, bladder, ureter, skin, soft tissue
Streptobacillus moniliformis	skin, bone, hematological, lung/trachea/bronchi, meninges, brain, liver, spleen
Streptococcus iniae	skin, hematological, soft tissue
Streptococcus zooepidemicus	small bowel, nasopharynx, bone, meninges, hematological, head and neck lymph nodes
Streptomices spp.	skin, soft tissue, lung/trachea/bronchi, mediastinum, brain, spinal cord, hematological, meninges
Treponema pallidum	nasopharynx, tonsil, oral, meninges, brain, spinal cord, penis, vulva, vagina, anus, cervix, eye, hematological, inguinal and head and neck lymph nodes
Tropheryma whipplei	brain, spinal cord, hematological, small bowel, colon, heart, lung/trachea/bronchi, eye
Tsukamurella spp.	skin, soft tissue, lung/trachea/bronchi, mediastinum, brain, spinal cord, hematological, meninges
Vibrio cholerae	colon, small bowel

Vibrio cincinnatiensis	hematological, meninges
Vibrio damsela	skin, soft tissue
Vibrio fluvialis	small bowel, colon
Vibrio furnissii	small bowel, colon
Vibrio hollisae	small bowel, colon, skin, soft tissue
Vibrio metschnikovii	hematological
Vibrio parahaemolyticus	colon, small bowel
Vibrio vulnificus	soft tissue, blood, skin
Yersinia enterocolitica	nasopharynx, tonsil, small bowel, intra-abdominal lymph nodes, colon, muscle, lung/trachea/bronchi, liver, spleen, hematological
Yersinia pestis	lung/trachea/bronchi, lymph nodes pulmonary hilum, inguinal/axillary/neck lymph nodes, oral, tonsil, hematological, skin
Yersinia pseudotuberculosis	small bowel, colon, abdominal lymph nodes

[00397] In some embodiments, microbial pathogens for use in the invention may be viral pathogens. Table 9 provides an exemplary list of viral pathogens together with the tissue and organ sites for which each viral species is reportedly a pathogen. Accordingly, one aspect of the invention involves utilizing immunogenic compositions that are specific for the named viruses to treat a cancer situated in the organs or tissues that are identified adjacent to the name of the virus in Table 9. For example, an antigenic composition derived from, or specific for, a vaccinia virus, may be used to

treat a cancer situated in the skin, hematological tissues, lymph nodes, brain, spinal cord, eye or heart.

Table 9: Viral Human Pathogens and Their Sites of Infection

virus	tissue/organ sites
Vaccinia	skin, hematological, lymph nodes, brain, spinal cord, eye, heart
Variola (smallpox)	skin, hematological, lymph nodes, brain
Monkeypox	skin, hematological, head and neck lymph nodes, brain, eye, lung/trachea/bronchi, pulmonary hilar lymph nodes, mediastinum, nasopharynx
Cowpox	skin, hematological, lymph nodes
Parapoxviruses	skin
Molluscum contagiosum	skin
Tanapox	skin, hematological, axillary and inguinal lymph nodes
Herpes Simplex virus (1 and 2)	nasopharynx, oral, tonsil, hematological, lung/bronchi/trachea, pancreas, meninges, brain, spinal cord, inguinal and head/neck lymph nodes, penis, vulva, perineum, esophagus, liver, eye, skin, rectum, tonsil, mediastinum, anus, vagina, cervix
Varicella-zoster	nasopharynx, sinus, lung/trachea/bronchi, pulmonary hilar lymph nodes, hematological, pancreas, meninges, brain, spinal cord, esophagus, liver, eye, skin, heart, mediastinum
Cytomegalovirus	nasopharynx, lymph nodes, tonsil, hematological, lung/trachea/bronchi, pancreas, abdomincal lymph nodes, brain, spinal cord, esophagus, small bowel, colon/rectum, eye, liver, heart, skin, mediastinum, esophagus

Epstein-Barr virus	nasopharynx, tonsil, oral, lymph nodes, hematological, lung, abdomincal lymph nodes, brain, spinal cord, muscles, esophagus, liver, heart, skin, spleen, kidney, muscle, heart, lung/trachea/bronchi, pulmonary hilar lymph nodes, mediastinum
Human herpesvirus 6	skin, hematological, lung/trachea/bronchi, pulmonary hilar lymph nodes, brain, meninges, liver
Human herpesvirus 7	skin, brain, liver
Human herpesvirus 8	nasopharynx, tonsil, hematological, skin, spleen, head and neck lymph nodes
Simian herpes B virus	brain, spinal cord, skin, hematological, lymph nodes
Adenovirus	nasopharynx, oral, larynx, trachea, bronchi, lung, lymph nodes, meninges, brain, spinal cord, small bowel, colon, liver, intra-abdominal lymph nodes, mediastinum, bladder, sinus, hematological, ureter, kidney, bladder, thyroid, heart
BK virus	kidney
Human papillomavirus	skin, anus, penis, vulva, cervix, vagina, oral
Hepatitis B virus	liver, pancreas, hematological
Hepatitis D virus	liver
Parvovirus B19	skin, hematological, nasopharynx, bone, kidney, heart, liver, brain, meninges
Orthoreoviruses	nasopharynx, small bowel, colon, oral, sinus, lymph nodes, skin, lung/trachea/bronchi, meninges, brain, spinal cord, liver
Orbiviruses	brain, muscle, hematological,

Coltiviruses	hematological, skin, muscle, oral, spleen, lymph nodes, meninges, brain
Rotaviruses	small bowel, colon, liver, hematological, pancreas, nasopharynx, billiary tract, meninges, brain
Alphaviruses	brain, spinal cord, small bowel, colon, hematological, skin, bone
Rubella	skin, hematological, head and neck lymph nodes, spleen, nasopharynx, bone, brain, tonsil, bronchi, liver, heart
Yellow fever virus	hematological, liver, lung/trachea/bronchi, kidney, adrenal gland, spleen, lymph nodes, stomach, kidney
Dengue fever virus	hematological, lymph nodes, skin, spleen, muscle, liver, brain, nasopharynx
Japanese encephalitis virus	brain, hematological, spinal cord
West Nile encephalitis virus	brain, hematological, spinal cord, muscle, lymph nodes, liver, spleen, pancreas, meninges
St. Louis encephalitis virus	brain, hematological, spinal cord, meninges, muscle, nasopharynx
Tick-borne encephalitis virus	brain, hematological, spinal cord, muscle, meninges
other Flaviviruses	hematological, brain, meninges, bone, muscles, skin, lymph nodes
Hepatitis C virus	hematological, liver
Hepatitis G virus	liver
Coronaviruses	nasopharynx, sinus, oral, tonsil, larynx, lung/trachea/bronchi, pulmonary hilar lymph nodes, small bowel, colon, tonsil, hematological
Toroviruses	small bowel, colon, hematological

Parainfluenza viruses	nasopharynx, sinus, tonsil, oral, larynx, lung/trachea/bronchi, pulmonary hilar lymph nodes, meninges, hematological, mediastinum
Mumps virus	salivary glands, pancreas, brain, spinal cord, liver, testes, hematological, meninges, ovaries, bone, heart, kidney, thyroid, prostate, breast
Respiratory syncytial virus	nasopharynx, tonsil, sinus, lung/trachea/bronchi, pulmonary hilar lymph nodes, mediastinum, hematological, oral, pleura
Human metapneumovirus	nasopharynx, lung/trachea/bronchi, pulmonary hilar lymph nodes, tonsil, sinus, mediastinum, hematological, oral, pleura, larynx, eye, skin, small bowel, colon
Rubeola	nasopharynx, sinus, hematological, lung/trachea/bronchi, pulmonary hilar lymph nodes, intra-abdominal lymph nodes, meninges, brain, spinal cord, liver, spleen, lymph nodes, skin, thymus, eye, oral, heart
Hendra virus	brain, meninges, lung/trachea/bronchi, kidney, hematological, muscle,
Nipah virus	brain, meninges, spleen, lymph nodes, thymus, lung/trachea/bronchi, kidneys, brain, spinal cord, meninges, hematological
Vesicular stomatitis virus	hematological, muscle, oral, tonsil, nasopharyngeal, lymph nodes, small bowel, colon
Rabies virus	skin, meninges, brain, spinal cord, oral, nasopharynx, salivary glands, hematological
Lyssaviruses	brain, spinal cord
Influenza virus	nasopharynx, laryngx, lung/trachea/bronchi, pulmonary hilar lymph nodes, meninges, muscle, hematological, mediastinum, muscle, sinus, tonsil, oral, eye, pleura, brain, spinal cord, salivary glands, thyroid, heart

California encephalitis virus	hematological, brain, meninges
Hantaviruses	hematological, kidney, eye, skin, oral, muscle, lung/trachea/bronchi
other Bunyaviruses	brain, hematological, muscle, meninges, spinal cord
Lymphocytic choriomeningitis virus	hematological, muscle, lymph nodes, skin, brain, meninges, testes, bone
Lassa virus	nasopharynx, brain, spinal cord, lung/trachea/bronchi, pulmonary hilar lymph nodes, mediastinum, muscle, testes, eye, heart,
Machupo virus	brain, meninges, hematological, muscle, eye, skin, lymph nodes, nasopharynx, small bowel, colon
Junin virus	brain, meninges, hematological, muscle, eye, skin, lymph nodes, nasopharynx, small bowel, colon
Human T-Cell Lymphotropic viruses	hematological, skin, lymph nodes, muscle, eye, bone, lung, pulmonary hilar lymph nodes, spinal cord, brain
Poliovirus	nasopharynx, lung/trachea/bronchi, pulmonary hilar lymph nodes, small bowel, neck and intra-abdominal lymph nodes, colon, hematological, liver, spleen, skin, brain, spinal cord, meninges, heart
Coxsackieviruses	nasopharynx, larynx, oral, tonsil, lung/trachea/bronchi, pulmonary hilar lymph nodes, mediastinum, pancreas, muscle, brain, meninges, small bowel, neck and intra-abdominal lymph nodes, colon, hematological, spleen, skin, eye, sinus, liver, testes, bone, pleura, salivary glands, heart

Echoviruses	nasopharynx, oral, tonsil, lung/trachea/bronchi, pulmonary hilar lymph nodes, muscle, brain, meninges, small bowel, neck and intra-abdominal lymph nodes, colon, hematological, mediastinum, spleen, skin, eye, sinus, liver, pancreas, testes, bone, salivary glands, heart
other Enteroviruses	lung/trachea/bronchi, pulmonary hilar lymph nodes, meninges, brain, skin, heart
Hepatitis A virus	small bowel, colon, hematological, liver, spleen, brain, spinal cord, gallbladder, pancreas, kidney
Rhinoviruses	nasopharynx, sinus, oral, tonsil, larynx, lung/trachea/bronchi, pulmonary hilar lymph nodes
Noroviruses and other Caliciviruses	small bowel, colon
Astroviruses	small bowel, colon
Picobirnaviruses	small bowel, colon
Hepatitis E virus	liver, small bowel, colon, hematological

[00398] The cumulative information in Tables 6 through 9 provides an extensive identification of microbial pathogens that may be used in the formulation of antigenic compositions of the invention, together with an identification of the tissues or organs in which these organisms are pathogenic, and accordingly identifies the tissues or organs in which a cancer is situated that may be treated with the antigenic formulation.

[00399] In some embodiments, the microbial pathogen selected for use in antigenic compositions of the invention may be one that is a common cause of acute infection in the tissue or organ in which the cancer to be treated is situated. Table 10 identifies bacterial and viral pathogens of this kind, together with the tissues and organs in which they commonly cause infection. Accordingly, in selected embodiments, a

cancer residing in a tissue identified in the first column of Table 10 may be treated with an antigenic composition that comprises antigenic determinants for one or more of the pathogenic organisms listed in the second column of Table 10. For example, a cancer residing in the skin may be treated with an antigenic composition comprising antigenic determinants of one or more of the following organisms: *Staphylococcus aureus*, Beta hemolytic streptococci group A, B, C and G, *Corynebacterium diptheriae, Corynebacterium ulcerans, Pseudomonas aeruginosa*, rubeola, rubella, varicella-zoster, echoviruses, coxsackieviruses, adenovirus, vaccinia, herpes simplex, or parvo B19.

Table 10: Common Causes of Acute Infection (Bacterial and Viruses) For Each Tissue/Organ Site

Tissue/organ site	**Common Bacterial or Viral Pathogens of specific tissue/organ site**
Skin	*Staphylococcus aureus*, Beta hemolytic streptococci group A, B, C and G, *Corynebacterium diptheriae, Corynebacterium ulcerans, Pseudomonas aeruginosa*
	rubeola, rubella, varicella-zoster, echoviruses, coxsackieviruses, adenovirus, vaccinia, herpes simplex, parvo B19
Soft tissue (i.e. fat and muscle) (e.g., sarcoma)	*Streptococcus pyogenes, Staphylococcus aureus, Clostridium perfringens*, other Clostridium spp.
	influenza, coxsackieviruses
Breast	*Staphylococcus aureus, Streptococcus pyogenes*
Lymph nodes: head and neck	*Staphylococcus aureus, Streptococcus pyogenes*
	Epstein-Barr, cytomegalovirus, adenovirus, measles, rubella, herpes simplex, coxsackieviruses, varicella-zoster
Lymph nodes:	*Staphylococcus aureus, Streptococcus pyogenes*

axillae/arm	measles, rubella, Epstein-Barr, cytomegalovirus, adenovirus, varicella-zoster
Lymph nodes: mediastinal	viridans streptococci, Peptococcus spp., Peptostreptococcus spp., Bacteroides spp., Fusobacterium spp., *Mycobacterium tuberculosis*
	measles, rubella, Epstein-Barr, cytomegalovirus, varicella-zoster, adenovirus
Lymph nodes: pulmonary hilum	*Streptococcus pneumoniae, Moraxella catarrhalis, Mycoplasma pneumoniae, Klebsiella pneumoniae, Haemophilus influenza, Chlamydophila pneumoniae, Bordetella pertussis, Mycobacterium tuberculosis*
	influenza, adenovirus, rhinovirus, coronavirus, parainfluenza, respiratory syncytial virus, human metapneumovirus, coxsackievirus
Lymph nodes: intra-abdominal	*Yersinia enterocolitica, Yersinia pseudotuberculosis,* Salmonella spp., *Streptococcus pyogenes, Escherichia coli, Staphylococcus aureus, Mycobacterium tuberculosis*
	measles, rubella, Epstein-Barr, cytomegalovirus, varicella-zoster, adenovirus, influenza, coxsackieviruses
Lymph nodes: inguinal/leg	*Staphylococcus aureus, Streptococcus pyogenes*
	measles, rubella, Epstein-Barr, cytomegalovirus, herpes simplex
Hematological (e.g. leukemias, multiple myeloma)	*Staphylococcus aureus, Streptococcus pyogenes,* coagulase-negative staphylococci, Enterococcus spp., *Escherichia coli,* Klebsiella spp., Enterobacter spp., Proteus spp., *Pseudomonas aeruginosa, Bacteroides fragilis, Streptococcus pneumoniae,* group B streptococci
	rubeola, rubella, varicella-zoster, echoviruses, coxsackieviruses, adenovirus, Epstein-Barr, cytomegalovirus, herpes simplex

Bone	*Staphylococcus aureus,* coagulase-negative staphylococci, *Streptococcus pyogenes, Streptococcus pneumoniae, Streptococcus agalactiae,* other streptococci spp., *Escherichia coli,* Pseudomonas spp., Enterobacter spp., Proteus spp., Serratia spp.
	parvovirus B19, rubella, hepatitis B
Meninges	*Haemophilus influenzae, Neisseria meningitidis, Streptococcus pneumoniae, Streptococcus agalactiae, Listeria monocytogenes*
	echoviruses, coxsackieviruses, other enteroviruses, mumps
Brain	Streptococcus spp. (including *S. anginosus, S. constellatus, S. intermedius*), *Staphylococcus aureus,* Bacteroides spp., Prevotella spp., Proteus spp., *Escherichia coli, Klebsiella spp.,* Pseudomonas spp., Enterobacter spp., *Borrelia burgdorferi*
	coxsackieviruses, echoviruses, poliovirus, other enteroviruses, mumps, herpes simplex, varicella-zoster, flaviviruses, bunyaviruses
Spinal cord	*Haemophilus influenzae, Neisseria meningitidis, Streptococcus pneumoniae, Streptococcus agalactiae, Listeria monocytogenes, Borrelia burgdorferi*
	coxsackieviruses, echoviruses, poliovirus, other enteroviruses, mumps, herpes simplex, varicella-zoster, flaviviruses, bunyaviruses
Eye/Orbit	*Staphylococcus aureus, Streptococcus pyogenes, Streptococcus pneumoniae, Streptococcus milleri, Escherichia coli, Bacillus cereus, Chlamydia trachomatis, Haemophilus influenza,* Pseudomonas spp., Klebsiella spp., *Treponema pallidum*

	adenoviruses, herpes simplex, varicella-zoster, cytomegalovirus
Salivary glands	*Staphylococcus aureus,* viridans streptococci (*e.g., Streptococcus salivarius, Streptococcus sanguis, Streptococcus mutans),* Peptostreptococcus spp., Bacteroides spp., and other oral anaerobes
	mumps, influenza, enteroviruses, rabies
Oral	*Prevotella melaninogenicus*, anaerobic streptococci, viridans streptococci, Actinomyces spp., Peptostreptococcus spp., Bacteroides spp., and other oral anaerobes
	herpes simplex, coxsackieviruses, Epstein-Barr
Tonsil	*Streptococcus pyogenes,* Group C and G B-hemolytic streptococci
	rhinoviruses, influenza, coronavirus, adenovirus, parainfluenza, respiratory syncytial virus, herpes simplex
Sinus	*Streptococcus pneumoniae, Haemophilus influenza, Moraxella catarrhalis, α*-streptococci, anaerobic bacteria (e.g., Prevotella spp.), *Staphylococcus aureus*
	rhinoviruses, influenza, adenovirus, parainfluenza
Nasopharynx	*Streptococcus pyogenes,* Group C and G B-hemolytic streptococci
	rhinoviruses, influenza, coronavirus, adenovirus, parainfluenza, respiratory syncytial virus, herpes simplex
Thyroid	*Staphylococcus aureus, Streptococcus pyogenes, Streptococcus pneumoniae*
	mumps, influenza
Larynx	*Mycoplasma pneumoniae, Chlamydophila pneumoniae, Streptococcus pyogenes*

	rhinovirus, influenza, parainfluenza, adenovirus, corona virus, human metapneumovirus
Trachea	*Mycoplasma pneumoniae*
	parainfluenza, influenza, respiratory syncytial virus, adenovirus
Bronchi	*Mycoplasma pneumoniae, Chlamydophila pneumoniae, Bordetella pertussis, Streptococcus pneumoniae, Haemophilus influenzae*
	influenza, adenovirus, rhinovirus, coronavirus, parainfluenza, respiratory syncytial virus, human metapneumovirus, coxsackievirus
Lung	*Streptococcus pneumoniae, Moraxella catarrhalis, Mycoplasma pneumoniae, Klebsiella pneumoniae, Haemophilus influenza*
	influenza, adenovirus, respiratory syncytial virus, parainfluenza
Pleura	*Staphylococcus aureus, Streptococcus pyogenes, Streptococcus pneumoniae, Haemophilus influenzae, Bacteroides fragilis,* Prevotella spp., *Fusobacterium nucleatum,* peptostreptococcus spp., *Mycobacterium tuberculosis*
	influenza, adenovirus, respiratory syncytial virus, parainfluenza
Mediastinum	viridans streptococci, Peptococcus spp., Peptostreptococcus spp., Bacteroides spp., Fusobacterium spp.
	measles, rubella, Epstein-Barr, cytomegalovirus

Heart	Streptococcus spp. (including *S. mitior, S. bovis, S. sanguis, S. mutans, S. anginosus),* Enterococcus spp., Staphylococcus spp., *Corynebacterium diptheriae, Clostridium perfringens, Neisseria meningitidis,* Salmonella spp.
	enteroviruses, coxsackieviruses, echoviruses, poliovirus, adenovirus, mumps, rubeola, influenza
Esophagus	Actinomyces spp., *Mycobacterium avium, Mycobacterium tuberculosis,* Streptococcus spp.
	cytomegalovirus, herpes simplex, varicella-zoster
Stomach	*Streptococcus pyogenes, Helicobacter pylori*
	cytomegalovirus, herpes simplex, Epstein-Barr, rotaviruses, noroviruses, adenoviruses
Small bowel	*Escherichia coli, Clostridium difficile, Bacteroides fragilis, Bacteroides vulgatus, Bacteroides thetaiotaomicron, Clostridium perfringens, Salmonella enteriditis, Yersinia enterocolitica, Shigella flexneri*
	adenoviruses, astroviruses, caliciviruses, noroviruses, rotaviruses, cytomegalovirus
Colon/Rectum	*Escherichia coli, Clostridium difficile, Bacteroides fragilis, Bacteroides vulgatus, Bacteroides thetaiotaomicron, Clostridium perfringens, Salmonella enteriditis, Yersinia enterocolitica, Shigella flexneri*
	adenoviruses, astroviruses, caliciviruses, noroviruses, rotaviruses, cytomegalovirus
Anus	*Streptococcus pyogenes,* Bacteroides spp., Fusobacterium spp., anaerobic streptococci, Clostridium spp., *E. coli,* Enterobacter spp., *Pseudomonas aeruginosa, Treponema pallidum*
	herpes simplex

Perineum	*Escherichia coli,* Klebsiella spp., Enterococcus spp., Bacteroides spp., Fusobacterium spp., Clostridium spp., *Pseudomonas aeruginosa,* anaerobic streptococci, *Clostridium spp., E. coli,* Enterobacter spp.
	herpes simplex
Liver	*Escherichia coli,* Klebsiella spp., *Streptococcus (anginosus* group*),* Enterococcus spp., other viridans streptococci, Bacteroides spp.
	hepatitis A, Epstein-Barr, herpes simplex, mumps, rubella, rubeola, varicella-zoster, coxsackieviruses, adenovirus
Gallbladder	*Escherichia coli,* Klebsiella spp., Enterobacter spp., enterococci, Bacteroides spp., Fusobacterium spp., Clostridium spp., *Salmonella enteriditis, Yersinia enterocolitica, Shigella flexneri*
Biliary tract	*Escherichia coli,* Klebsiella spp., Enterobacter spp., Enterococci spp., Bacteroides spp., Fusobacterium spp., Clostridium spp., *Salmonella enteriditis, Yersinia enterocolitica, Shigella flexneri*
	hepatitis A, Epstein-Barr, herpes simplex, mumps, rubella, rubeola, varicella-zoster, cocsackieviruses, adenovirus
Pancreas	*Escherichia coli,* Klebsiella spp., Enterococcus spp., Pseudomonas spp., Staphylococcal spp., Mycoplasma spp., *Salmonella typhi,* Leptospirosis spp., Legionella spp.
	mumps, coxsackievirus, hepatitis B, cytomegalovirus, herpes simplex 2, varicella-zoster
Spleen	Streptococcus spp., Staphylococcus spp., Salmonella spp., Pseudomonas spp., *Escherichia coli,* Enterococcus spp.
	Epstein-Barr, cytomegalovirus, adenovirus, measles, rubella, coxsackieviruses, varicella-zoster

Adrenal gland	Streptococcus spp., Staphylococcus spp., Salmonella spp., Pseudomonas spp., *Escherichia coli,* Enterococcus spp.
	varicella-zoster
Kidney	*Escherichia coli, Proteus mirabilis, Proteus vulgatus,* Providentia spp., Morganella spp., *Enterococcus faecalis, Pseudomonas aeruginosa*
	BK virus, mumps
Ureter	*Escherichia coli, Proteus mirabilis, Proteus vulgatus,* Providentia spp., Morganella spp., Enterococcus spp.
Bladder	*Escherichia coli, Proteus mirabilis, Proteus vulgatus,* Providentia spp., Morganella spp., *Enterococcus faecalis, Corynebacterium jekeum*
	adenovirus, cytomegalovirus
Peritoneum	*Staphylococcus aureus, Streptococcus pyogenes, Streptococcus pneumonia, Escherichia coli,* Klebsiella spp., Proteus spp., Enterococci spp., *Bacteroides fragilis, Prevotella melaninogenica,* Peptococcus spp., Peptostreptococcus spp., Fusobacterium spp., Clostridium spp.
Retroperitoneal area	*Escherichia coli, Staphylococcus aureus*
Prostate	*Escherichia coli,* Klebsiella spp., Enterobacter spp., *Proteus mirabilis,* Enterococci spp., Pseudomonas spp., Corynebacterium spp., *Neisseria gonorrhoeae*
	herpes simplex
Testicle	*Escherichia coli, Klebsiella pneumoniae, Pseudomonas aeruginosa,* Staphylococcus spp., Streptococcus spp., *Salmonella enteriditis*

	mumps, coxsackievirus, lymphocytic choriomeningitis virus
Penis	*Staphylococcus aureus, Streptococcus pyogenes, Neisseria gonorrhoeae, Treponema pallidum*
	herpes simplex, human papillomavirus
Ovary/Adnexae	*Neisseria gonorrhoeae, Chlamydia trachomatis, Gardenerella vaginalis*, Prevotella spp., Bacteroides spp., Peptococcus spp. Streptococcus spp., *Escherichia coli*
Uterus	*Neisseria gonorrhoeae, Chlamydia trachomatis, Gardenerella vaginalis*, Prevotella spp., Bacteroides spp., Peptococcus spp., Streptococcus spp., *Escherichia coli*
Cervix	*Neisseria gonorrhoeae, Chlamydia trachomatis, Treponema pallidum*
	herpes simplex
Vagina	*Gardenerella vaginalis*, Prevotella spp., Bacteroides spp., peptococci spp., *Escherichia coli, Neisseria gonorrhoeae, Chlamydia Trachomatis, Treponema pallidum,*
	herpes simplex
Vulva	*Staphylococcus aureus, Streptococcus pyogenes, Treponema pallidum*
	herpes simplex

[00400] In selected embodiments, particular microbial pathogens may be suited for treatment of particular cancers, examples of selected embodiments are set out in Table 10. These are exemplary embodiments, and not an exhaustive list of the alternative formulations for use in accordance with the invention.

[00401] The specific microbes which commonly cause infection in a specific tissue or organ may vary by geographical location. For example, *Mycobacterium tuberculosis* is a more common cause of lung infection in some geographical locations and populations than in others and therefore, while *M. tuberculosis* may not be a common lung pathogen in some geographic and population groups it may be a common lung pathogen in others. Table 10 is thus not an exhaustive list of common pathogens for all geographic locations and population groups. It is understood that a clinical microbiologist skilled in the art could determine the common pathogenic species in a particular geographic area or population group for a specific tissue or organ site in accordance with the invention. For veterinary use, there will of course be specific pathogens that are common in selected tissues of selected species, and this may also vary geographically.

[00402] In selected embodiments, the invention involves diagnostic steps to assess a patient's previous exposure to microbial pathogens. For example, the diagnostic steps may include taking a medical history of exposure to selected pathogens, and/or evaluating a patient's immune response to a selected pathogen. For example, a serology test may be conducted to detect antibodies to selected pathogens in a patient's sera. In connection with this aspect of the invention, antigenic determinants of a selected microbial pathogen may be chosen for use in an immunogenic composition on a selected patient based on a diagnostic indication that the patient has had one or more prior exposure(s) to the pathogen, for example by virtue of the presence of antibodies to antigenic determinants of that pathogen in the patient's sera.

[00403] In further selected embodiments, the invention involves diagnostic steps to assess a patient's immunological response to treatment with a selected immunogenic composition. For example, the diagnostic steps may include evaluating a patient's immune response to the antigenic determinants of that immunogenic composition, for example using a serological test to detect antibodies to those antigenic

determinants. In connection with this aspect of the invention a treatment with a selected immunogenic composition may be continued if the evaluation indicates that there is an active immunological response to the antigenic determinants of that composition, and the vaccine treatment may be discontinued, and an alternative treatment with a different immunogenic composition may be initiated, if the evaluation indicates that there is not a sufficiently active immunological response to the antigenic determinants of the immunogenic composition.

[00404] As discussed in the context of Patient R, in selected embodiments, the microbial pathogen selected for use in antigenic compositions of the invention may be one that is the most common cause of acute infection in the tissue or organ in which the cancer to be treated is situated, which may provide particular benefit as illustrated by the case of Patient R. For example, for the treatment of bone cancer, *Staphylococcus aureus* would be the bacterial species selected; for the treatment of cancer in lung tissue, *Streptococcus pneumoniae* would be selected; for the treatment of breast cancer, *Staphylococcus aureus* would be selected; for the treatment of kidney or bladder cancer, Escherichia coli would be selected; and for the treatment of colon cancer, *Escherichia coli* would be the bacterial species selected. It is understood that a clinical microbiologist skilled in the art could determine the most frequently pathogenic species, bacterial or viral, for each specific tissue or organ in accordance with the invention. In selected embodiments, only antigenic determinants of the most common pathogen for the particular tissue or organ could be used to treat cancers of that tissue or organ. In alternative embodiments, antigenic determinants of the most common pathogen for the particular tissue or organ could be used in combination with antigenic determinants of other pathogens that are known to be pathogenic in the of that particular tissue or organ, preferentially selecting from the more common pathogens.

[00405] In some embodiments, the invention provides antigenic compositions in which a threshold proportion of antigenic determinants selected in accordance with the invention are used, relative to any other antigenic determinants in the composition. For

example, antigenic compositions may have greater than X% of the antigenic determinants therein derived from pathogenic (or commonly pathogenic, or most commonly pathogenic) species, where X may for example be 10, 30, 40, 50, 60, 70, 80, 90, 95 or 100 (or any integer value between 10 and 100). For example, at least X% of the antigenic determinants in the antigenic composition may be specific for microbial pathogens that are pathogenic (or commonly pathogenic or most commonly pathogenic) in the specific organ or tissue of the patient within which the cancer is situated. Using an alternative measure, of the total number of microbial pathogens in the antigenic composition, at least X% may be selected to be microbial pathogens that are pathogenic (or commonly pathogenic or most commonly pathogenic) in the specific organ or tissue of the patient within which the cancer is situated. In some embodiments, the antigenic composition may accordingly consist essentially of antigenic determinants of one or more microbial pathogens that are each pathogenic (or commonly pathogenic or most commonly pathogenic) in the specific organ or tissue of the patient within which the cancer is situated. The following data illustrates the surprising effectiveness of these selected formulations:

(1) The use of MRV (which contains many common respiratory tract pathogens and *Staphylococcus aureus*, the most common pathogen of both breast and bone) was found to be helpful for the treatment of breast cancer with metastases to the bone (see Figure 6). However, survival benefit (survival of patients who were treated with MRV compared to those who were not) was modest (*i.e.*, median survival of 31 months for patients treated with the vaccine compared to 26 months for patients not treated with the vaccine). On the other hand, the one patient (Patient R) who was treated with a vaccine specifically targeted for breast cancer and bone cancer (*i.e.*, containing only *Staphylococcus aureus*, by far the most common cause of both breast and bone infection) had a remarkable survival benefit, surviving for more than 17 years. The inclusion, in MRV, of other bacterial species that do not (or far less commonly) cause bone infection and commonly cause infection elsewhere (*i.e.*, respiratory tract) appears

to substantially reduce the benefit of this vaccine for the treatment of cancer of the breast and bone.

(2) The survival of stage 3B lung cancer patients treated with Respivax™ (*i.e.*, median survival of 38 months and 40% 5-year survival) was substantially greater than the survival of stage 3B lung cancer patients treated with MRV (i.e., median survival of 18 months and 14% 5-year survival). Respivax™ contains substantially greater relative concentrations of the bacterial species that commonly cause lung infection than MRV does. 67% of the bacterial cell count of Respivax™ is comprised of bacterial species that are the common causes of lung infection, whereas only 30% of the bacterial cell count of MRV is comprised of bacterial species that are the common causes of lung infection. Thus, the composition having the greater proportion of bacteria that most commonly cause lung infections, Respivax™, is shown to be more effective for the treatment of lung cancer than the MRV formulation.

(3) The survival of stage 4 colon cancer patients treated with MRV (which does not contain any colon pathogens) was poorer than patients not treated with a vaccine. This indicates that treatments that use antigenic determinants that are not derived from microbes that are pathogenic in the organ or tissue in which the cancer is situated may not only be ineffective, but may also be deleterious.

(4) The Murine Studies set out below, and in particular the cancer cell model data involving treatment with *Klebsiella pneumoniae* antigenic determinants alone, compared to treatment with *Klebsiella pneumoniae* antigens in conjunction with additional antigens.

The data herein accordingly provide evidence of an increasing gradation of benefit from pathogenic, to commonly pathogenic, to most commonly pathogenic for the treatment cancer within a specific organ or tissue using targeting antigenic compositions that are derived from microbial pathogens that are pathogenic to that specific organ or tissue.

[00406] In some embodiments, the invention comprises the use of bacterial or viral vaccines that are approved for other purposes (*e.g.,* poliomyelitis vaccine, H. influenza vaccine, meningococcal vaccine, pneumococcal vaccine, influenza vaccine, hepatitis B vaccine, hepatitis A vaccine, diphtheria vaccine, tetanus vaccine, pertussis vaccine, measles vaccine, mumps vaccine, rubella vaccine, varicella vaccine, BCG vaccine, cholera vaccine, Japanese encephalitis vaccine, rabies vaccine, typhoid vaccine, yellow fever vaccine, small pox vaccine, *etc.*) for use as cancer treatments by selecting a vaccine containing a pathogen (or antigenic constituent of a pathogen) that is pathogenic in the specific organ or tissue of the patient within which the cancer is situated by consulting Tables 6-10. For example, a *S. pneumoniae* vaccine, either a whole cell vaccine or a vaccine comprised of one or more antigenic components of *S. pneumoniae* (*e.g.,* pneumococcal polysaccharide-23-valent) could be used to treat cancer at any of the following sites in which S. pneumoniae is listed as a common pathogen in Table 10: pulmonary hilar lymph nodes, hematological cancers, bone, meninges, spinal cord, eye/orbit, sinus, thyroid, bronchi, lungs, pleura or peritoneum. As a further example, a hepatitis B vaccine could be used to treat cancer at any of the following sites in which hepatitis B virus is listed as a pathogen in Table 9, as follows: liver, pancreas, or hematological cancers.

[00407] In some embodiments, selected compositions and methods are specifically excluded from the scope of the invention. For example, the use of the following microbial pathogens in the treatment of the following cancers is excluded from some embodiments, so that the claimed invention may extend to particular embodiments with the exception of one or more of the following:

a) BCG (*Mycobacterium bovis*) for the treatment of stomach cancer and colon cancer, for example by injection;

b) *Mycobacterium w* for the treatment of lung cancer, for example by injection;

c) *Mycobacterium vaccae* for the treatment of non-small-cell lung cancer, for example by injection;

d) *Corynebacterium parvum* for the treatment of melanoma, for example by injection;

e) *Streptococcus pyogenes* for the treatment of stomach cancer, for example by injection;

f) *Nocardia rubra* for the treatment of lung cancer or acute myelogenous leukemia, for example by injection;

g) *Lactobacillus casei* for the treatment of cervical cancer, for example by injection;

h) *Pseudomonas aeruginosa* for the treatment of lymphoma and lung cancer, for example by injection;

i) Vaccinia for the treatment of melanoma, for example by injection;

j) Rabies virus for the treatment of melanoma, for example by injection;

k) A composition consisting of the combined antigens of the following bacterial species for the veterinary (or, alternatively, human) treatment, for example by oral administration, for primary (or, alternatively, metastatic) cancers situated in the lung: *Streptococcus pneumoniae; Neisseria catarrhalis; Streptococcus pyogenes; Haemophilus influenzae; Staphylococcus aureus; Klebsiella pneumoniae.*

l) A composition consisting of the combined antigens of the following bacterial species for the veterinary (or, alternatively, human) treatment, for example by oral administration, for primary (or, alternatively, metastatic) cancers situated in the lung: *Streptococcus pneumoniae; Neisseria catarrhalis; Streptococcus pyogenes; Haemophilus influenzae; Staphylococcus aureus; Klebsiella pneumoniae*; *Klebsiella ozaenae; Streptococcus viridans.*

EXAMPLE 4: Murine Studies

[00408] In the following murine studies, the following common materials were utilized: PBS (Gibco), and mice were 7 week-old female C57BL/6.

Example 4A: Cancer of the Lung

[00409] This section relates to a Lewis lung carcinoma mouse model. The bacterial vaccines used in this experiment were as follows: *Streptococcus pneumoniae* [cells and broth] vaccine (lot #J049-1 [2 x 10^9]; *Klebsiella pneumoniae* [cells and broth]

vaccine (lot #J046-1 [2 x 10^9]; *Staphylococcus aureus* [cells and broth] vaccine (lot #J041-2 [10 x 10^9]; *Escherichia coli* (colon isolate) [cells and broth] vaccine (lot #J047-1 [6 x 10^9]; *Salmonella enterica* [cells and broth] vaccine (lot #J31 [15 x 10^9]; *Klebsiella pneumoniae* [cells only] vaccine (lot #J048-1 [2 x 10^9]; and media only (*Klebsiella pneumoniae* media) (lot #J046-1). Mice were treated in accordance with the experimental groupings defined in Table 11.

Table 11: Experimental Groupings for Lung Cancer Mouse Model

Group	Mice	Lewis Lung i.v. 10e5 cells/ 100 µl Day 0	Vial #	Vaccine	s.c. injection dose schedule Day: -10, -8, -6, -4, -2, +2, +4, +6,+8,+10,+12,+14,+16, etc.
1AB	8	↓	1	*S. pneumoniae* [cells and broth]	200x10e6 CFUs/150µl/mouse
2AB	8		2	*K. pneumoniae* [cells and broth]	150x10e6 CFUs/75µl/mouse
3AB	8		3	*S. aureus* [cells and broth]	1x10e9 CFUs/100µl/mouse
4AB	8		4	*E. coli* (colon) [cells and broth]	0.9x10e9 CFUs/150µl/mouse
5AB	8		5	*S. enterica* [cells and broth]	1.5 x10e9 CFUs/100µl/mouse
6AB	8		1+2	*S.+ K. pneumoniae* [cells and broth]*	*S.* 200x10e6 CFUs/75µl/mouse *K.* 150x10e6 CFUs/75µl/mouse

7AB	8		8	*K. pneumoniae* [cells only]	150x10e6 CFUs/75µl/mouse
8ABC	12		9	Media only (*K. pneumoniae* media) (control)	75µl/mouse
9AB	8	PBS only		none	none

[00410] Specifically, groups of mice were pre-treated sub-cutaneously with bacterial vaccines on days -10, -8, -6, -4, and -2. On day 0, mice were challenged intravenously with a dosage of 10e5 Lewis lung carcinoma cells (Cedarlane lot# 508714). Thereafter, the mice were treated sub-cutaneously with vaccine injections every second day for the duration of the experiment as defined in Table 11. A control group was treated with media only. Animal weights were measured and recorded every 4 days. When mice started showing morbidity the experiment was terminated. Thereafter, all mice were humanely sacrificed and the lungs were surgically removed and weighed. Lungs were placed into the vials with Buoin's fluid and the numbers of lung nodules in each group were counted. These results are illustrated in Table 12. Representative examples of these lungs are depicted in Figure 14.

Table 12: Number of visible lung tumours in mice in each group

Group	Vaccine	Number of visible lung tumours	Average	Median
1	*S. pneumoniae*	13, 1, 7, 1, 20, 38, 50, 35	20.6	16.5
2	*K. pneumoniae*	1, 10, 0, 0, 1, 2, 50	9.1	1
3	*S. aureus*	1, 5, 3, 15, 10, 57, 43, 38	21.5	12.5
4	*E. coli*	4, 3, 0, 9, 16, 3, 42, 40	14.6	6.5
5	*S. enterica*	0, 0, 2, 5, 2, 57, 52	16.8	2
6	*S+K. pneumoniae*	1, 6, 0, 0, 8, 5, 47, 48	14.3	5.5
7	*K. pneumoniae* (cells	0, 3, 0, 0, 0, 1, 46, 49	12.3	0.5

	only)			
8	Control (medium)	2, 3, 12, 25, 39, 26, 62, 39, 78	31.7	26

[00411] The weights of the lungs of mice injected with tumour cells were then compared to the weights of the lungs of mice injected with PBS only, to determine the tumour burden and thus, the therapeutic effect of vaccine treatment. These results are illustrated in Table 13.

Table 13: Average lung weight (mg) and tumour weight inhibition (compared to control) in mice immunized with killed bacterial vaccines in intravenously implanted Lewis lung carcinoma model

Group	Vaccine	Av. Lung weight (mg)	Difference from healthy mice	Tumour Weight Inhibition (%)
1	*S. pneumonia* (cells and broth)	495	318	30
2	*K. pneumonia* (cells and broth)	298	121	73
3	*S. aureus* (cells and broth)	485	308	32
4	*E. coli* (cells and broth)	372	195	57
5	*S. enterica* (cells and broth)	331	154	66
6	*S+K. pneumonia* (cells and broth)	374	197	57
7	*K. pneumoniae* (cells only)	294	117	74
8	Control (media only)	633	456	-
9	Healthy mice	177	-	

Example 4B: Cancer of the Skin

[00412] This section relates to a mouse model of skin cancer. The bacterial vaccines used in this experiment were as follows: *Streptococcus pneumoniae* [cells and broth] vaccine (lot #J049-1 [2 x 10^9]; *Klebsiella pneumoniae* [cells and broth] vaccine (lot #J046-1 [2 x 10^9]; *Staphylococcus aureus* [cells and broth] vaccine (lot #J041-2 [10 x 10^9]; *Escherichia coli* (colon isolate) [cells and broth] vaccine (lot #J047-1 [6 x 10^9]; *Salmonella enterica* [cells and broth] vaccine (lot #J31 [15 x 10^9]; *Staphylococcus aureus* [cells only] vaccine (lot #J041-2 [10 x 10^9]; and media only (*S.aureus* media) (lot #J041-1). Mice were treated in accordance with the experimental groupings defined in Table 14.

Table 14: Experimental Groupings for Skin Cancer Mouse Model

Group	**Mice**	**B16 melanoma s.c. 2x10e5cells/ 0.1 mL Day 0**	**Vaccine**	**s.c. injection dose schedule** Day: -10, -8, -6, -4, -2, +2, +4, +6,+8,+10,+12,+14,+16, etc.
AB1	8	↓	*S. pneumoniae* [cells and broth]	300x10e6 CFUs/150µl/mouse
AB2	8		*K. pneumoniae* [cells and broth]	150x10e6 CFUs/75µl/mouse
AB3	8		*S. aureus* [cells and broth]	1x10e9 CFUs/100 µl/mouse
AB4	8		*E. coli* (colon) [cells and broth]	0.9x10e9 CFUs/150 µl/mouse
AB5	8		*S. enterica* [cells and broth]	1.5 x10e9 CFUs/100 µl/mouse
AB6	8		*S. aureus* [cells only]	1x10e9 CFUs/100 µl/mouse

ABC 7	12		Media only (*S. aureus* media) (control)	100 µl/mouse

[00413] Specifically, groups of mice were pre-treated sub-cutaneously with bacterial vaccines on days -10, -8, -6, -4, and -2. On day 0, mice were challenged intravenously with a dosage of 2 x 10e6 of B16 melanoma cells (lot #3995448; ATCC CRL-6323). Thereafter, the mice were treated sub-cutaneously with vaccine injections every second day for the duration of the experiment as defined in Table 14. A control group was treated with media only. Animal weights were measured and recorded every 4 days. Once tumours were palpable, the tumour diameters were measured every second day using callipers. When mice started showing morbidity, or tumour diameters reached 20 mm in any group, the experiment was terminated. Thereafter, all mice were humanely sacrificed. The average volumes of the tumours present in the groups of mice described herein are illustrated in Figure 15.

Example 4C: Cancer of the Colon

[00414] This section relates to a mouse model of colon cancer. The bacterial vaccines used in this experiment were as follows: *Streptococcus pneumoniae* [cells and broth] vaccine (lot #J049-1 [2 x 10^9]; *Klebsiella pneumoniae* [cells and broth] vaccine (lot #J046-1 [2 x 10^9]; *Staphylococcus aureus* [cells and broth] vaccine (lot #J041-2 [10 x 10^9]; *Escherichia coli* (colon isolate) [cells and broth] vaccine (lot #J047-1 [6 x 10^9]; *Escherichia coli* (prostate isolate) [cells and broth] vaccine (lot #J040-2 [6 x 10^9]; *Salmonella enterica* [cells and broth] vaccine (lot #J31 [15 x 10^9]; and media only (*E. coli* media) (lot #J040-1). Mice were treated in accordance with the experimental groupings defined in Table 15.

Table 15: Experimental Groupings for Colon Cancer Mouse Model

Group	Mice	MC-38 colon i.p.	Vaccine	s.c. injection dose schedule

		2x10e5 Day 0		Day: -10, -8, -6, -4, -2, +2, +4, +6,+8,+10,+12,+14,+16, etc.
1AB	8		*S. pneumoniae* [cells and broth]	300x10e6 CFUs/150µl/mouse
2AB	8		*K. pneumoniae* [cells and broth]	150x10e6 CFUs/75µl/mouse
3AB	8		*S. aureus* [cells and broth]	1x10e9 CFUs/100µl/mouse
4AB	8		*E. coli* (colon) [cells and broth]	0.9x10e9 CFUs/150µl/mouse
5AB	8		*E. coli* (prostate) [cells and broth]	0.9x10e9 CFUs/150µl/mouse
6AB	8		*S. enterica* [cells and broth]	1.5 x10e9 CFUs/100µl/mouse
7AB C	12		Media only (*E. coli* media) (control)	150 µl/mouse

[00415] Specifically, groups of mice were pre-treated sub-cutaneously with bacterial vaccines on days -10, -8, -6, -4, and -2. On day 0, mice were challenged intraperitoneally with a dosage of 2 x 10e5 MC-38 collo adenocarcinoma cells (gift from Dr. Jeff Schlom Lab, NCI). Thereafter, the mice were treated sub-cutaneously with vaccine injections every second day for the duration of the experiment as defined in Table 15. A control group was treated with media only. The mice were observed for the following clinical factors: weight, cold to touch, diarrhea, rapid respiration, closed eyes, decreased movement, piloerection, and convulsions. When a mouse started to show signs of clinical morbidity, the mouse was humanely sacrificed and that day was defined as the day of death. Survival data for this Example is depicted in Figure 16.

[00416] Further, the health versus morbidity/mortality of the mice involved in this Example was calculated on day 29 of the experiment as defined in Table 16.

Table 16: Health v. Morbidity/Mortality Group Score**

Group	Health Status Day 29	Vaccine	Group Score
1	7 dead; 1 tumour + ascites	*S. pneumoniae*	2
2	5 dead; 1 tumour + ascites; 1 tumour only; 1 healthy	*K. pneumoniae*	17
3	4 dead; 2 tumour + ascites; 2 tumour only	*S. aureus*	14
4	3 dead; 3 tumour only; 2 healthy	*E. coli* (colon infection isolate)	35
5	3 dead; 3 tumour only; 2 healthy	*E. coli* (prostate infection isolate)	35
6	3 dead; 5 healthy	*S. enterica*	50
7	12 dead	Control (media only)	0

** healthy = 10 points; tumour only = 5 points; tumour + ascites = 2 points; and dead = 0 points.

Table 17. Summary of survival for each mouse group from colon cancer experiments

Vaccine	Mice / group	MST* (Days)	ILS** (%)	# Cured	P value***
S. pneumoniae	8	27	12	0	0.29
K. pneumoniae	8	36	50	3	0.061
S. aureus	8	30	25	2	0.002
E. coli (colon)	8	35	46	3	0.008
E. coli	8	>78	>100	4	0.001

(prostate)					
S. enterica	8	>78	>100	5	0.003
Control	12	24	0	0	-

*MST – Median survival time

**ILS – Increase in life span over control group

*** comparison to placebo treated control group

Overall Comparison Survival:

Log Rank (Mantel Cox) p = 0.001

Breslow (Generalized Wilcoxon) p = 0.003

Tarone-Ware p = 0.002

Example 4 Summary

Skin Model:

[00417] There is a marked therapeutic advantage for the group treated with *Staph aureus* (cells-only). This study demonstrates the effectiveness of a killed *S. aureus* vaccine for the treatment of skin cancer in a mouse model, consistent with the fact that *S. aureus* is the most common cause of skin infection in mice. The data are consistent with the use of immungenic compositions of the invention to slow or inhibit cancer growth. The *S. aureus* cells-only vaccine (in which media and exotoxins were removed by centrifuging the cells and broth to collect only the cells and then reconstituting with normal saline) was more effective than *S. aureus* cells and broth vaccine (which contains media and exotoxins). This may be because *S. aureus* extoxins inhibit immune function, such as leuocidins that can kill white blood cells. The invention accordingly includes embodiments in which antigenic determinants that are to be used in immunogenic compositions are separated from immunomodulatory compounds produced by a microbial pathogen of interest.

Lung Model:

[00418] The data presented in this Example indicate that there was substantial tumour inhibition with the immunogenic composition derived from *K. pneumoniae*,

consistent with the fact that *K. pnuemoniae* is a common cause of lung infection in mice. In mice (but generally not in humans), *S. enterica* can cause pneumonia, which is consistent with the beneficial effect of this vaccine in the mouse lung model. Unlike humans, for whom *S. pneumoniae* is a common lung pathogen, *S. pneumoniae* is relatively rarely a lung pathogen in mice (although *S. pneumoniae* pneumonia can be induced in mice). *E. coli* and *S. aureus* can uncommonly cause lung infection in mice, which is consistent with their mild benefit illustrated herein. This Example demonstrates that killed *K. pneumoniae* vaccine is remarkablly effective for the treatment of cancer of the lungs in mice, particularly embodiments in which the immunogenic composition includes antigens of only the most commonly pathogenic organism (see Group 2 vs. Group 6).

Colon Model:

[00419] *S. enterica* is one of the most common causes of gastrointestinal and intraperitoneal infection in mice, which is consistent with the beneficial effect illustrated herein in the treatment of gastrointestinal and intraperitonal cancer in mice.

[00420] Of the immunogenic compositions used in this Example (*i.e., S. enterica, E. coli, S. aureus, K. pneumoniae, S. pneumoniae*), *S. enterica* is the most common g.i./abdominal pathogen in mice. *E. coli* is the next most common g.i./abdominal pathogen in mice. *S. aureus* and *K. pneumoniae* can be found as part of the colonic flora and can cause g.i./abdominal infection, although far less commonly. *S. pneumoniae* does not cause g.i./abdominal infection. In accordance with this, the *S. enterica* vaccine is shown to be substantially benefical in this mouse colon tumour model, *E. coli* vaccine is shown to be moderately beneficial, *S. aureus* and *K. pneumoniae* vaccines are shown to be mildly beneficial and *S. pneumoniae* vaccine is shown to be of no benefit. In humans, *Salmonella* species cause g.i. infection and therefore, one would expect *S. enterica* and other *Salmonella* vaccines to be helpful for the treatment of colon cancer in humans.

EXAMPLE 5: Animal Models

Example 5A: Illustrating the influence of a heat inactivated *Klebsiella pneumoniae* antigenic composition on monocyte/macrophage and dendritic cell populations in mice.

[00421] The following methods and materials were utilized in this Example:

[00422] *Mice.* C57BL/6 female mice 7-8 weeks of age were ordered from Harlan Labs (Livermore, CA) for these studies.

[00423] *Antibodies and reagents.* The following antibodies were used in this Example: anti-I-A/I-E FITC (MHC Class II; M5/114.15.2); anti-Gr-1 PE (RB6-8C5); anti-CD11b PerCP-Cy5 (M1/70); anti-CD11c APC (N418); anti-CD4 FITC (GK1.5); anti-NK1.1 PE (PK136); anti-CD8a eFluor780 (53-6.7); anti-CD44 APC (IM7). All antibodies were acquired from eBioscience (San Diego, CA). Liberase TM and DNAse I was acquired from Roche. All media was from HyClone (Fisher).

[00424] *Treatment with antigenic compositions.* Heat killed *K. pneumoniae* with phenol (KO12 [5.0 OD600 units]) was diluted 1/10 in PBS containing 0.4% phenol and 100µl was injected subcutaneously on day 0, 2, 4, and 6 into 4 mice. Control mice (n=5) were injected on day 0, 2, 4, and 6 with PBS.

[00425] *Brochoalveolar lavage.* On day 7 mice were sacrificed and a bronchoalveolar lavage (BAL) was performed by exposing the trachea followed by insertion of a 22G catheter attached to a 1ml syringe. 1ml of PBS was injected into the lungs and removed and placed into a 1.5ml microcentrifuge tube. The lungs were subsequently washed 3 more times with 1ml of PBS and the fluid was pooled. The first wash from each mouse was centrifuged at 400xg and the supernatant was frozen for cytokine analysis. The final 3ml of lavage fluid was centrifuged and the cells were pooled with the cell pellet from the first lavage. The cells were counted and stained with

antibodies specific for MHC class II, Ly6G/C, CD11b, and CD11c. After staining the cells were washed and analyzed on a FACS Calibur flow cytometer.

[00426] *Lung digestion.* After BAL was performed the lungs were placed in 5ml of RPMI containing 417.5µg/ml Liberase TL (Roche) and 200µg/ml DNAse I (Roche). The lungs were then digested at 37°C for 30mins. After digestion the lungs were forced through a 70um cell strainer to create a single cell suspension. The cells were then centrifuged, washed, resuspended in FACS Buffer (PBS with 2%FCS and 5mM EDTA) and counted. After counting the cells were stained and analyzed by FACS using the same antibodies as for the BAL cells.

[00427] *Peritoneal lavage.* 1ml of PBS was injected into the peritoneum of mice using a 1ml syringe attached to a 25G needle after BAL. The abdomen was massaged for 1 minute and 0.5ml of PBS was recovered from the peritoneum using a 1ml pipet. The lavage fluid was put in a 1.5ml centrifuge tube, centrifuged at 400xg for 5mins, and resuspended in FACS buffer prior to staining and FACS analysis.

[00428] *Spleen and lymph node analysis.* The spleen and draining lymph node were removed after BAL and peritoneal lavage and placed in PBS. The spleen was disrupted by mashing through a 70µm cell strainer (Fisher) and the lymph node was disrupted using the rubber end of the plunger from a 1ml syringe. After disruption, the single cell suspension from the spleen and lymph nodes was centrifuged, washed once with FACS Buffer, and resuspended in FACS Buffer prior to counting, staining, and FACS analysis.

[00429] *FACS Analysis.* Cells were stained on ice for 20mins in 96 well plates using 50ul of antibodies diluted in FACS buffer. After 20mins, 100µl of FACs buffer was added to the wells and the plates were centrifuged at 400xg for 5mins. Subsequently the media was removed and the cells were washed 1 more time with FACS buffer. After the final wash the cells were resuspended in 200µl of FACS buffer and the data

was acquired using a FACS Calibur flow cytometer (BD). A minimum of 20,000 live events were collected for all samples except the BAL where a minimum of 5,000 events was collected.

[00430] The following results were obtained in this Example.

[00431] Normal mice without tumours were treated with a *K. pneumoniae* antigenic composition on day 0, 2, 4, and 6. On day 7 the mice were sacrificed and the bronchoalveolar lavage fluid, lung tissue, peritoneal lavage fluid, lymph nodes, and spleen was analyzed for changes in monocyte and macrophages. An increase in the number of acute inflammatory blood monocytes/macrophages, defined by high expression of CD11b and Gr-1 (same marker as Ly6c), and F4/80 in the lymph node draining the site of injection of the *K. pneumoniae* antigenic composition was observed (see: Figure 17A). These acute inflammatory monocytes/macrophages also express very high levels of MHC class II molecules suggesting exposure to bacterial antigens. Importantly, treatment of mice with the *K. pneumoniae* antigenic composition for one week led to a marked increase in the frequency of acute inflammatory monocytes in the bronchoalveolar lavage fluid and in the lungs (*i.e.*, the targeted organ) but not in the spleen or peritoneum of treated mice, suggesting that treatment can induce homing of monocytes specifically to the lungs without affecting other organs (see: Figure 17B). Monocytes can differentiate into dendritic cells (DCs) in the lungs and consistent with our observations of a marked increase in monocyte recruitment it was also observed that there was a marked increase in the frequency of cells displaying markers for mature DCs (see: Figure 17C).

[00432] As illustrated in Figure 17, treatment with a *K. pneumoniae* antigenic composition for 7 days resulted in a marked increase (compared to treatment with placebo = PBS) in both acute inflammatory monocytes and dendritic cells in the lungs of mice. As illustrated in Figure 17, mice were treated with either a *K. pneumoniae* antigenic composition for or PBS on day 0, 2, 4, and 6. On day 7, the mice were

sacrificed and the total number of A) and B) inflammatory monocytes (CD11b+ Gr-1+ cells) and C) dendritic cells (CD11c+ MHC class II+ cells) were determined by flow cytometry in the lung and spleen. The error bars depicted in A) represent the mean of 4-5 mice per group.

Example 5B. Illustrating the influence of a heat inactivated *Klebsiella pneumoniae* antigenic composition and a heat inactivated *E. coli* antigenic composition on monocyte/macrophage, dendritic cell, and effector cell populations in mice

[00433] The following methods and materials were utilized in this Example:

[00434] *Mice*. C57BL/6 female mice 7-8 weeks of age were ordered from Harlan Labs (Livermore, CA) for these studies.

[00435] *Antibodies and reagents*. The following antibodies were used: anti-I-A/I-E FITC (MHC Class II; M5/114.15.2); anti-Gr-1 PE (RB6-8C5); anti-CD11b PerCP-Cy5 (M1/70); anti-CD11c APC (N418); anti-CD4 FITC (GK1.5); anti-NK1.1 PE (PK136); anti-CD8a eFluor780 (53-6.7); anti-CD44 APC (IM7). All antibodies were acquired from eBioscience (San Diego, CA). Liberase TM and DNAse I was acquired from Roche. All media was from HyClone (Fisher).

[00436] *Treatment with antigenic compositions*. Heat-killed *K. pneumoniae* with phenol (*K. pneumoniae*; lot KO12; 5.0 OD600 units) was diluted 1/10 in PBS containing 0.4% phenol and 100ul was injected subcutaneously on day 0, 2, 4, and 6 into 5 mice. Heat-killed *E. coli* (lot ; 5.0 OD600 units) was diluted 1/10 in containing 0.4% phenol and 100µl was injected subcutaneously on day 0, 2, 4, and 6 into 5 mice. Control mice (n=5) were injected on day 0, 2, 4, and 6 with PBS.

[00437] *Brochoalveolar lavage.* On day 7 mice were sacrificed and a bronchoalveolar lavage (BAL) was performed by exposing the trachea followed by insertion of a 22G catheter attached to a 1ml syringe. 1ml of PBS was injected into the lungs and removed and placed into a 1.5ml microcentrifuge tube. The lungs were subsequently washed 3 more times with 1ml of PBS and the fluid was pooled. The first wash from each mouse was centrifuged at 400xg and the supernatant was frozen for cytokine analysis. The final 3ml of lavage fluid was centrifuged and the cells were pooled with the cell pellet from the first lavage. The cells were counted and stained with antibodies specific for MHC class II, Ly6G/C, CD11b, and CD11c. After staining the cells were washed and analyzed on a FACS Calibur flow cytometer.

[00438] *Lung digestion.* After BAL was performed the lungs were placed in 5ml of RPMI containing 417.5µg/ml Liberase TL (Roche) and 200µg/ml DNAse I (Roche). The lungs were then digested at 37°C for 30mins. After digestion the lungs were forced through a 70µm cell strainer to create a single cell suspension. The cells were then centrifuged, washed, resuspended in FACS Buffer (PBS with 2%FCS and 5mM EDTA) and counted. After counting the cells were stained and analyzed by FACS using the same antibodies as for the BAL cells.

[00439] *Peritoneal lavage.* 1ml of PBS was injected into the peritoneum of mice using a 1ml syringe attached to a 25G needle after BAL. The abdomen was massaged for 1 minute and 0.5ml of PBS was recovered from the peritoneum using a 1ml pipet. The lavage fluid was put in a 1.5ml centrifuge tube, centrifuged at 400xg for 5mins, and resuspended in FACS buffer prior to staining and FACS analysis.

[00440] *Spleen and lymph node analysis.* The spleen and draining lymph node were removed after BAL and peritoneal lavage and placed in PBS. The spleen was disrupted by mashing through a 70µm cell strainer (Fisher) and the lymph node was disrupted using the rubber end of the plunger from a 1ml syringe. After disruption, the single cell suspension from the spleen and lymph nodes was centrifuged, washed once

with FACS Buffer, and resuspended in FACS Buffer prior to counting, staining, and FACS analysis.

[00441] *FACS Analysis.* Cells were stained on ice for 20mins in 96 well plates using 50µl of antibodies diluted in FACS buffer. After 20mins, 100µl of FACs buffer was added to the wells and the plates were centrifuged at 400xg for 5mins. Subsequently the media was removed and the cells were washed 1 more time with FACS buffer. After the final wash the cells were resuspended in 200µl of FACS buffer and the data was acquired using a FACS Calibur flow cytometer (BD). A minimum of 20,000 live events were collected for all samples except the BAL where a minimum of 5,000 events was collected.

[00442] The following results were obtained in this Example:

[00443] As illustrated in Figure 18, mice were treated on day 0, 2, 4, and 6 with either a *K. pneumoniae* antigenic composition, an *E. coli* antigenic composition or PBS. On day 7 the mice were sacrificed and the total number of inflammatory monocytes (CD11b+ Gr-1+ cells) and dendritic cells (CD11c+ MHC class II+ cells) were determined by flow cytometry in the peritoneal lavage fluid, lungs, lymph node and spleen. Error bars in Figure 18 represent the standard deviation from 5 mice. *p-value < .05 using a Student's t-test.

[00444] Figure 18 illustrates that treatment with a *K. pneumoniae* antigenic composition, but not an *E. coli* antigenic composition treatment, markedly increased the number of monocytes and DCs in the lungs of mice. In contrast to the lungs, *K. pneumoniae* did not lead to an increase in monocytes in the peritoneum of the mice whereas *E. coli* did. Importantly, there was only a slight increase in the number of inflammatory monocytes and no increase in DCs in the spleens of mice treated with either *K. pneumoniae* or *E. coli* suggesting that the effects of the therapie are not general and are, in fact, specific for a particular organ site. In addition to looking at the

effects of treatment on inflammatory monocytes and DCs in the lungs of mice, we also looked at changes in other leukocytes such as cytotoxic CD8 T cells, CD4 T helper cells, and natural killer (NK) cells all of which can potentially play a role in anti-tumour immunity.

[00445] Figure 19 illustrates that a *K. pneumoniae* antigenic composition, but not PBS or an *E. coli* antigenic composition, resulted in a marked increase in the frequency and total numbers of NK cells, CD4 and CD8 T cells in the lungs of treated mice. This Example is the first demonstration to our knowledge that subcutaneous injection of a killed bacterial species which normally causes lung infection can promote the accumulation of leukocytes in the lungs without the presence of any inflammation in that site. In addition, we have demonstrated that this effect is specific to the targeted site and that it is also specific to the bacterial constituents of the treatment used.

[00446] As illustrated in Figure 19, mice were treated on day 0, 2, 4, and 6 with either a *K. pneumoniae* antigenic composition, an *E. coli* antigenic composition, or PBS. On day 7, the mice were sacrificed and the total number of CD4 T cells, CD8 T cells, and natural killer (NK) cells were determined by flow cytometry. Error bars represent the sd of values obtained from 5 mice per group. *p-value <.05 using a Student's t-test.

Example 5C. Illustrating the effects of heat and phenol inactivated *Klebsiella pneumoniae* (*K. pneumoniae*) antigenic compositions on anti-tumor response in mice, and the status of inflammatory monocytes and dendritic cells following treatment in tumor-bearing mice.

[00447] The following methods and materials were utilized in this Example:

[00448] *Tumour cell inoculations*. The Lewis lung carcinoma cell line derived from the C57BL/6 background were acquired from ATCC (Manassas, VA). The cells were

maintained in Dulbecco's Modified Eagles Media (ATCC, Manassas, VA) containing 10% FCS. The cells were grown in a humidified 37°C incubator with 5% CO_2. Prior to tumour inoculation, cells were detached from culture plates using 0.25% trypsin and 0.53mM EDTA. The cells were washed in PBS and resuspended at $8x10^6$ cells/ml and 200ul ($4x10^5$ cells) was injected intravenously into mice.

[00449] *Treatment with antigenic composition*. The following antigenic compositions were used in this study: a heat-inactivated *K. pneumoniae* antigenic composition with phenol (lot KO12) and a phenol-inactivated *K .pneumoniae* antigenic composition (lot KO25). Both heat-inactivated *K. pneumoniae* and phenol-inactivated *K .pneumoniae* are concentrated to 5.0 OD600 units. 0.1ml of *K .pneumoniae* diluted 1/10 in PBS with 0.4% phenol was injected subcutaneously every 2 days starting on day 2 after tumour injection.

[00450] *Analysis of inflammatory monocytes, DCs, T cells, and NK cells*. All analysis was done according to the methods used in Example 5B above.

Table 18. Experimental groupings and dose schedule for Example 5C

Group	Number of animals/sex	LL/2 Tumor cells and dose	Site of LL/2 Tumor Injection	Treatment	Concentration of *K. Pneumoniae* (OD units) Injections	Schedule of Treatment	Injection Volume (ml)	Date of Sacrific
1A	5F	$4x10^5$ LL/2	Intravenous	DPBSWP[1]	None	Once daily on day 2, 4, 6, and 8	0.1	Day 9
2A	5F			HKWP[2]	0.05			
3A	5F			PHWP[3]	0.05			
1B	5F			DPBSWP[1]	None	Once daily on day 2, 4, 6, 8, 10, 12, 14,		Day 16
2B	5F			HKWP[2]	0.05			
3B	5F			PHWP[3]	0.05			
1C	5F			DPBSNP[1]	None	Once daily on day 2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22		Day 23
2C	5F			HKWP[2]	0.05			
3C	5F			PHWP[3]	0.05			

[1] Dulbecco's Phosphate Buffered Saline with phenol [2] Heat killed with phenol [3] Phenol killed with phenol

[00451] The following results were obtained in this Example:

[00452] This example was designed to determine if the presence of tumour impacted the recruitment of cells to the lungs and whether the treatment effect increases over time resulting in further increases in cell recruitment with ongoing treatment and thus, potentially, more optimal therapeutic effect with prolonged

treatment. In addition, we wanted to determine whether a *K. pneumoniae* antigenic composition inactivated by phenol was more effective than a *K. pneumoniae* antigenic composition inactivated by heat.

[00453] Figure 20 clearly demonstrates that by day 9 (*i.e.*, 4 treatments with a *K. pneumoniae* antigenic composition) there is already a marked increase in the number of acute inflammatory monocytes, DCs, T cells, and NK cells in the lungs of mice treated with a *K. pneumoniae* antigenic composition inactivated by heat or phenol, many times greater than that of the lungs of mice treated with placebo (normal saline = PBS), again, clearly demonstrating the marked targeted cellular immune response in the lungs triggered by *K. pneumoniae* antigenic composition therapy. On day 9, there was a suggestion that phenol inactivation was more effective than heat inactivation, but this trend reversed by day 16. Importantly, however, with reference to the cell numbers in the lungs on day 9 and day 16, it is evident that there is a cumulative effect of bacterial treatment on the recruitment of cells to the lungs. For example in the group treated with a phenol inactivated *K. pneumoniae* antigenic composition there are about 100,000 acute inflammatory monocytes in the lungs of the mice at day 9 and this number increases substantially to 400,000 by day 16, demonstrating a substantially increasing response with ongoing treatment. The same increased treatment response with ongoing treatment occurred in mice treated with heat inactivated bacteria. Importantly, this cumulative treatment effect is observed for all other cell types analyzed as well. In this study, there was no demonstrable statistically significant difference in immune cell recruitment with heat or phenol inactivated *K. pneumoniae* antigenic compositoins.

[00454] As illustrated in Figure 20, mice were injected with 4×10^5 lewis lung carcinoma cells intravenously on day 0. The mice were subsequently treated every other day starting on day 2 with a *K. pneumoniae* antigenic composition generated by heat inactivation, or phenol inactivation, or with PBS. On day 9 and day 16 the mice were sacrificed and the total numbers of (A) inflammatory monocytes (CD11b+ Gr-1+)

and DCs (CD11c+ lab+) or (B) CD4 T cells, CD8 T cells, and natural killer (NK) cells were determined by flow cytometry. Bars represent respectively: the PBS treated group, the mice treated with a heat-inactivated *K. pneumoniae* antigenic composition and mice treated with a phenol-inactivated *K. pneumoniae* antigenic composition. Error bars represent the sd of values obtained from 5 mice per group. *p-value < .05 using a Student's t-test.

[00455] The results of this study demonstrate an increasing immune response within the targeted tissue with ongoing treatment.

Example 5D. Illustrating the effects of heat, irradiation, and phenol inactivation on *K. pneumoniae* antigenic compositions, including leukocyte recruitment into the lungs of mice, and the effects of phenol as a preservative has any effects.

[00456] The following methods and materials were utilized in this Example:

[00457] *Mice.* C57BL/6 female mice 7-8 weeks of age were ordered from Harlan Labs (Livermore, CA) for these studies.

[00458] *Antigenic compositions.* Heat killed *K. pneumoniae* antigenic composition with phenol (KO12), heat killed *K. pneumoniae* antigenic composition without phenol (KO25), irradiated *K. pneumoniae* antigenic composition without phenol (KO24), and phenol killed *K. pneumoniae* antigenic composition without phenol (KO25) were used in this study. All bacterial formulations were at a concentration of 5.0 OD units in saline. For 1/10 dilution, 1ml of bacterial formulation was added to 9ml of DPBS and mixed immediately and then again prior to injection. For 1/100 dilution, 0.1ml of bacterial formulation will be added to 9.9ml of DPBS and mixed immediately and then again prior to injection. For dilutions of heat-killed *Klebsiella pneumoniae* antigenic composition with phenol, the dilutions were carried out as above using a DPBS solution containing 0.4% phenol (w/v). To prepare the 0.4% phenol in DPBS, first a 5% phenol solution

was prepared by adding 0.5g of solid phenol (Sigma Aldrich, St. Louis, MO) to 10ml of DPBS (Hyclone, Logan, UT) This solution was filtered through a 0.22um filter (Millipore, Billerica, MA) and stored at 4°C. Immediately prior to use the 5% phenol solution was diluted 1ml in 12.5ml DPBS and used to prepare the bacterial formulations.

[00459] *Treatment with antigenic compositions.* 5 mice per group were treated subcutaneously on day 0, 2, 4, and 6 with 0.1ml of a heat-killed *K. pneumoniae* antigenic composition diluted 1/10 in PBS or PBS with 0.4% phenol, 0.1ml of an irradiated *K. pneumoniae* antigenic composition diluted 1/10 in PBS, or a phenol inactivated *K. pneumoniae* antigenic composition diluted 1/10 with PBS or PBS with 0.4% phenol. On day 7 the mice were sacrificed and leukocyte recruitment to the lungs was analyzed as in Example 5B.

[00460] The following results were obtained in this Example:

[00461] In this example, we used leukocyte recruitment to the lungs as a surrogate of efficacy to compare the efficacy of *K. pneumoniae* antigenic compositions inactivated by various methods. Figure 21 illustrates that, for both heat killed and phenol killed *K. pneumoniae* antigenic compositions, the addition of phenol (0.4%) as a preservative, increased efficacy, as measured by cellular recruitment. In some embodiements, a small amount of phenol (i.e., 0.4% as a preservative) may stabilize a component of the bacterial cell wall, for example a component that is important in antigen pattern recognition and activating an optimal targeted response. In comparing the 3 formulations containing phenol as a preservative (i.e., heat killed, phenol killed and radiation killed), irradiated *K. pneumoniae* antigenic composition led to the greatest recruitment of acute inflammatory monocytes, DCs, NK cells, and T cells to the lungs, followed by phenol killed *K. pneumoniae* antigenic composition, with heat killed *K. pneumoniae* antigenic composition resulting in the least cellular recruitment.

[00462] As illustrated in Figure 21, mice were treated on day 0, 2, 4, and 6 with *K. pneumoniae* antigenic composition inactivated by heat (HKWP) or without (HKnp) phenol preservative, inactivated with phenol with (PKWP) or without (PKnp) phenol preservative, or *K. pneumoniae* antigenic composition inactivated by irradiation with phenol preservative (IRWP). On day 7 the mice were sacrificed and the total numbers of (A) inflammatory monocytes (CD11b+ Gr-1+) and DCs (CD11c+ Iab+) or (B) CD4 T cells, CD8 T cells, and natural killer (NK) cells were determined by flow cytometry. Error bars represent the sd of values from 5 mice per group. *p-value <.05 compared to mice treated with IRWP using a Student's t-test.

EXAMPLE 6: Clinical Studies involving advanced epithelial cancers

Overview of treatment

[00463] The following patients with different advanced cancers have undergone treatment with heat inactivated targeted bacterial antigenic compositions. Fully informed written consent was received for each patient and in every case. The treatment consisted of repeated (3x/week) subcutaneous injections of exact amounts of the vaccine in the abdominal region. The dosage was gradually increased in each patient to achieve a sufficient skin response (3-5cm redness lasting for ca. 24h). For each patient a case report form (CRF) documented skin reaction and possible clinical effects and/or side effects related to the treatment. Characteristic and concomitant treatments as well as the response to therapy of the patients are briefly described.

Patient #1:

[00464] 53y old male patient with advanced melanoma, ICD10: C43, 1st diagnosed 11/2005 lesion under right big toe nail, histology one year later 12/2006: advanced malignant melanoma; patient refused amputation of the big toe; 5/2008 lymphatic metastasis to the right leg, at the time of first presentation for targeted bacterial antigenic composition treatment (9/2008) leg swollen with 100% increase in circumference: Karnofsky 80%, no pretreatment.

[00465] Treatments over 6 month 09/2008-04/2009:

- 12 x intraperitoneal Ozone (O3) insufflation;
- 42 x locoregional radiofrequency hyperthermia (13.56 Mhz);
- 18 x moderate whole body hyperthermia 38.5°C;
- 6 month treatment with *Staph. aureus* antigenic composition s.c.;
- Orthomolecular medicine: high dose vitamin C infusions (0.5g/kg/BW), Vitamin D3; 2.000iu/day, Artesunate 200mg/day, Celebrex 100mg/day, low dose naltrexone, medical; mushroom (cordyceps, reishi, shitake), selenium 200uc/day, curcumin 3.000mg/day, proteolytic enzymes (Wobemugos)

[00466] Epicrisis details:

- PET 7/2008: SUV in right big toe 4.81, knee: 5.01
- PET 12/2008: SUV in right big toe 3.80, knee: 4.02
- We started treatment end of 9/2008.
- At that time (9/2008) there were clinically already lymph nodes in the groin palpable which were not seen on the PET from 7/2008.
- 05/2010: good clinical condition, PET confirms complete remission, Karnofsky 100%.

Patient #2:

[00467] 48y old female patient with advanced bilateral breast cancer, ICD10: C50.9 1st diagnosed 4/2008; histology ductal infiltrating adenocarcinoma of the breast, ER/PR pos., Her2 not known, T1/T2, N1 (sentinel node axilla), M0 G3; multiple treatments with sodium bicarbonate injections and repeated surgery to both breasts; patient refused the proposed bilateral mastectomy and was never resected "in sano" (*e.g.*, margins of the lumpectomies were never free of tumor cells). Patient also refused Chemotherapy and/or hormone therapy. Karnofsky 90%, no pretreatment.

[00468] Treatments over 8 month 03/2009-11/2009:

- 3 x autologous Dendritic cell therapy in combination with:

- 3 x long-duration moderate whole body hyperthermia 40° over 8h
- 57 x locoregional radiofrequency hyperthermia to both breasts (13.56 Mhz)
- 6 month treatment with *Staph. aureus* antigenic composition s.c.
- Orthomolecular medicine: high dose vitamin C infusions (0.5g/kg/BW), low dose naltrexone, medical mushroom (cordyceps, reishi, shitake), curcuma 3.000mg/day, zinc

[00469] Epicrisis details:

- Treatment started end of 3/2009.
- 05/2010: bilateral breast MRI shows no abnormality detected, good clinical condition, Karnofsky 100%

Patient #3:

[00470] 73y old female patient with advanced NSCLC cancer FIGO IIc, ICD10: C34, 1st diagnosed 6/2009; histology clear cell adenocarcinoma of the lungs, T4, N1, M0 G3; she underwent neoadjuvant CHT; the restaging after 3 cycles neoadjuvant CHT demonstrated T2 tumor; she then underwent left pneumectomy R0 resection and mediastinal lymphadenectomy; Karnofsky 90%.

[00471] Treatments over 7 month 08/2009-03/2010:

- 08/2009 Began chemotherapy with Taxan/Cisplatin until 10/2009 in combination with:
- 4 x long-duration moderate whole body hyperthermia 40° over 8h (1-2/2009)
- 20 x locoregional radiofrequency hyperthermia to the thorax (13.56 Mhz) (8-10/2009)
- 10/2009 le Pneumectomy R0 resection (Oncology decided against further adjuvant CHT)
- 6 month treatment with *Klebsiella pneumoniae* antigenic composition s.c. (08/2009-02/2010)

• Orthomolecular medicine: thymus peptides i.m., indometacin, cimetidine, high dose vitamin C infusions (0.5g/kg/BW), ALA/N protocol (low dose naltrexone and alpha lipoic acid), medical mushroom (reishi), curcuma 3.000mg/day, zinc, melatonin, inhalation of ionized oxygen

[00472] Epicrisis details:

• Started treatment end of 08/2009.

• 05/2010: CT Thorax and Tumor marker CEA, NSE and CYFRA show complete remission, good clinical condition, Karnofsky 100%.

Patient #4:

[00473] 50y old female patient with advanced Breast cancer, ICD10: C50, with disseminated hepatic and pulmonary metastasis, 1st diagnosed 8/1990; histology undifferentiated cirrhoses type adenocarcinoma of the breast, pT1c, N1, M0 G3; she underwent multiple chemotherapy courses over 20 years; 11/2004 first scar reoccurrence, 12/2004 ablation left breast, 6x CHT Epitax and radiation of thoracic wall; 9/2005 1st diagnosis of disseminated liver and pulmonary metastases: again 8x CHT with Epitax until 3/2006. Restaging showed progressive disease at which time she began targeted bacterial antigenic composition treatment. Karnofsky 90%.

[00474] Treatments over 4 years 03/2006-03/2010:

- 03/2006 Began treatment with Polyvaccinum forte vaccine in combination with:
- 3 x autologous Dendritic cell therapy in combination with (6-8/2006):
- 3 x long-duration moderate whole body hyperthermia (LD-WBH) 40° over 8h (1-2/2009)
- 25 x locoregional radiofrequency hyperthermia to the thorax & liver (13.56 Mhz) (3-6/2006)
- 8 month treatment with *Klebsiella pneumoniae* antigenic composition (11/2008-7/2009)
- 10/2009 TM CEA and CA15/3 started rising again
- 02-03/2009 2x autologous Dendritic cell therapy without LD-WBH
- 04/2010 thermal ablation of liver mets (No change of lung metastasis)
- Orthomolecular medicine: thymus peptides i.m., indometacin, cimetidine, high dose vitamin C infusions (0.5g/kg/BW), curcuma 3.000mg/day, zinc, proteolytic enzymes

[00475] Epicrisis details:

- We started treatment end of 03/2006.
- 05/2010: CT Thorax show stable disease over 4 years, progressive disease of liver mets, good clinical condition, Karnofsky 100%.

Patient #5:

[00476] 66y old male patient with advanced prostate cancer, ICD10: C61, with disseminated bone and lymphatic metastasis, 1st diagnosed 01/1997; histology undifferentiated adenocarcinoma of the prostate, pT3, N1, M1 G3; he underwent multiple Hormone- and CHT over 13 years; patient is in good clinical condition surviving metastatic prostate cancer for 13 years since diagnosis; Karnofsky 90%.

[00477] Treatments over 11 years 11/1999-05/2010:

- 13 years anti-androgen with Suprefact, Zoladex, Casodex, Trenantone, Estracyt, ß-Sitosterol
- 06/2006-12/2007 Began Mixed bacterial vaccine treatment with Polyvaccinum forte vaccine
- Since 03/2008 regular chemotherapy with Taxotere 140mg every 3-4 weeks
- 50 x moderate whole body hyperthermia 39° over 3h (1999-2009)
- 18 month treatment with *Staph. aureus* antigenic composition (11/2008-05/2010, ongoing)
- 05-06/2009 2x autologous Dendritic cell therapy without moderate WBH 39°, 3h
- Orthomolecular medicine: wheat grass, cimetidine, Zometa, high dose vitamin C infusions (0.5g/kg/BW), curcuma 3.000mg/day, boswellia serrata (Indian) 400mg 4x4/day, zinc, proteolytic enzymes

[00478] Epicrisis details:

- We started treatment end of 1999.
- 05/2010: Bone scan shows stable disease; PSA currently 89 ng/ml, good clinical condition, Karnofsky 90%.

Patient #6:

[00479] 52y old female patient with advanced primary cancer of the peritoneum, ICD10: C48.2, with disseminated peritoneal carcinosis, 1st diagnosed 06/2003; histology undifferentiated adenocarcinoma of the peritoneum, pT3, N1, M1 G3; she underwent Debulking OP with ovarectomy bilaterly and hysterectomy and adjuvant chemotherapy with Taxol/Paraplatin: progressive disease and change to Taxol/Paraplatin with SD until 8/2008; progressive disease with disseminated peritoneal LK metastasis and 3rd line CHT with Carboplatin and beginning of treatment with targeted bacterial antigenic composition; patient was in good clinical condition; Karnofsky 100%.

[00480] Treatments over 4 month 05-09/2009:

- 5x Carboplatin chemotherapy (3rd line) followed by:
- 5 x long-duration moderate whole body hyperthermia (LD-WBH) 40° over 8h (05-09/2009)
- 20 x locoregional radiofrequency hyperthermia to the abdomen (13.56 Mhz) (05-09/2009)
- 2 month treatment with *E.coli* (colon) antigenic composition (05-07/2009)
- Orthomolecular medicine: high dose vitamin C infusions (0.5g/kg/BW), high dose artichoke and sylimarin extracts (liver)

[00481] Epicrisis details:

- We started treatment end of 5/2009.
- 04/2010: CT abdomen and tumour marker demonstrate complete remission; good clinical condition, Karnofsky 100%

Patient #7:

[00482] 50y old female patient with inoperable pancreatic cancer, ICD10: C25.9, with the cancer infiltrating the large vessels; Sonographic and CT evidence suggested invasion into superior mesenteric vein. first diagnosed 02/2009; histology

undifferentiated adenocarcinoma of the pancreas, pT3, N1, M1 G3 with peritoneal carcinosis; she underwent local radiation and low dose Xeloda as radiation sensitizer; when she began treatment at our clinic the cancer was still unresectable; Karnofsky 100%.

[00483] Treatments over 2 month 06-07/2009: (E. coli SSI for 11 months)

- 1 x autologous NDV-primed Dendritic cell therapy in combination with:
- 1 x long-duration moderate whole body hyperthermia 40° over 8h (7-10/2009)
- 4 x moderate whole body hyperthermia 38.5°
- 15 x locoregional radiofrequency hyperthermia to the abdomen (13.56 Mhz) (06-07/2009)
- 11 months treatment with *E.coli* (colon) antigenic composition (06/2009 until today, ongoing)
- Orthomolecular medicine: thymus peptides i.m; medical mushroom (reishi, cordyceps, shitake;)high dose vitamin C infusions (0.5g/kg/BW), high dose proteolytic enzyme therapy (wobenzym phlogenzym), cimetidine.

[00484] Epicrisis details:

- We started treatment end of 6/2009.
- 05/2010: complete remission, NED; PET 02/2010 demonstrated no glucose uptake; tumour marker normal CA19/9: 4; good clinical condition, Karnofsky 100%

EXAMPLE 7: Dosage studies (QB28 study)

[00485] In an effort to examine the role of dosing, mice were treated with either different doses of *K. pneumoniae* antigenic composition or a PBS control. All mice (C57BL/6) began treatment with *K. pneumoniae* antigenic composition or PBS on day -10, -8, -6, -4 and -2 using the following injection sites (1st injection: ventral inguinal right; 2nd injection: ventral axillary right, 3rd injection: ventral axillary left; 4th injection: ventral inguinal left *etc.*). All mice then received a tumor inoculation dose of $3x10^5$ Lewis Lung carcinoma cells via intravenous injection into the lateral tail vein of the

mice. The doses of antigenic compositions or PBS were as follows: i) PBS 0.1ml; ii) *K. pneumoniae* 0.1mL of OD600= 1.67; iii) *K. pneumoniae* 0.1mL of OD600=0.5. Mice received *K.pneumoniae* antigenic compositions or PBS, treatment on days 2, 4, 6, and 8. The experiment was termination on day 10; all mice were sacrificed, their lungs were surgically removed, rinsed in water, weighed, and then placed into Bouins fluid for fixation and counting 24 hours later. As shown in Figure 22, each data point represents the number of tumour nodules from one mouse. Photographs of representative lungs from these experiments are shown in Figure 23. Figure 23 shows that the lungs from mice treated with the PBS control show significant numbers of nodules. By way of comparison, Figure 23 demonstrates that the lungs from mice treated with *K.pneumoniae* antigenic composition shows fewer numbers of nodules at a rate equivalent to those shown in Figure 22.

[00486] **QB30 study**. In further studies on the effect of dosage of *K.pneumoniae* antigenic compositions, it was demonstrated that if a dosage of a *K.pneumoniae* antigenic composition is too low, it is not effective for the treatment of lung cancer. In these studies, the results of which are shown in Figure 24, all mice (C57BL/6) began treatment with *K. pneumoniae* antigenic composition or PBS on day -10, -8, -6, -4 and -2 using the following injection sites (1st injection: ventral inguinal right; 2nd injection: ventral axillary right, 3rd injection: ventral axillary left; 4th injection: ventral inguinal left *etc.*). All mice then received a tumor inoculation dose of $3x10^5$ Lewis Lung carcinoma cells via intravenous injection into the lateral tail vein of the mice. The doses of antigenic compositions or PBS was as follows: i) PBS 0.1ml; ii) *K. pneumoniae* 0.1mL of OD600= 0.5; iii) *K. pneumoniae* 0.1mL of OD600=0.05. Mice continued to receive *K. pneumoniae* antigenic compositions, or PBS, treatment on day 2, 4, 6, 8, 10, 12, 14, and 16. The experiment was termination on day 18; all mice were sacrificed, their lungs were surgically removed, rinsed in water, weighed, and then placed into Bouins fluid for fixation and counting 24 hours later. As shown in Figure 24, each data point represents the number of tumour nodules from one mouse.

[00487] The results from the QB28 and QB30 studies suggest that extremely high doses of *K. pneumoniae* antigenic compositions are ineffective, perhaps due to over-stimulation of the host immune system. The results from these studies also suggest that low doses are ineffective, perhapse due to insufficient stimulation of the host immune system.

EXAMPLE 8: Cisplatin efficacy studies

[00488] In an effort to examine the role between Cisplatin and chemotherapy and doses of *K. pneumoniae* antigenic compositions, the QB38 study was completed. Briefly, all mice received a tumor inoculation dose of $3x10^5$ Lewis Lung carcinoma cells via intravenous injection into the lateral tail vein on day 0 of the study. Following this inoculation, all mice were pooled into a single cage and subsequently distributed into their respective cages at random to control against biased data. On the morning of day +2 of this study, mice within the Cisplatin group were injected with 10mg/kg of this drug intraperitoneally. Control mice were injected with control (PBS). Thereafter, on the afternoon of day +2 of this study, mice received *K. pneumoniae* antigenic compositions, or PBS; these injections continued on days 4, 6, 8, 10, and 12. The study was terminated on day 14; thereafter, all mice were sacrificed, their lungs were surgically removed, rinsed in water, weighed, and then placed into Bouins fluid for fixation and counting 24 hours later. As shown in Figure 25, each data point represents the number of tumour nodules from one mouse based on whether the mice were treated with PBS, *K. pneumoniae* antigenic compositions, *K. pneumoniae* antigenic compositions combined with Cisplatin, or Cisplatin alone. The results summarized in Figure 25 indicate that there were fewer nodules in mice treated with *K. pneumoniae* antigenic compositions and Cisplatin compared with those treated with Cisplatin alone, *K. pneumoniae* antigenic compositions alone, or the PBS control.

[00489] A further study was designed to determine whether *K. pneumoniae* antigenic composition therapy could work in synergy with platinum-based chemotherapy to extend the survival of mice bearing pulmonary Lewis Lung tumors.

Four groups of five C57BL/6 female mice all received 10^5 Lewis Lung carcinoma cells *i.v.* on day 0. On day 2, groups 3 and 4 received a single i.p. dose of 10mg/kg Cisplatin. Beginning on day 4 the mice received either *K. pneumoniae* or placebo (PBS) every 2 days until death. Figure 26 illustrates the survival of all of the mice and the table shows the p-values of the various survival curves calculated using the Log-rank Test. Treatment of mice with Cisplatin alone increased the survival of mice with lung tumors with median survival increasing from 16 days in the PBS group to 23 days in the PBS + Cisplatin group. Treatment with *K. pneumoniae* alone also increased survival of the mice compared to PBS (median survival 19 days for *K. pneumoniae* vs. 16 days for PBS). Most importantly Cisplatin chemotherapy together with *K. pneumoniae* therapy had the greatest therapeutic benefit (median survival of 32 days) demonstrating the synergy between platinum-based chemotherapy and antigenic composition therapy in a mouse model of lung cancer. The p-values for the data generated in this QB45 study is shown in Table 19 below.

Table 19. Survival Date (QB45)

	PBS	*K. pneumoniae*	PBS + cisplatin	*K. pneumoniae* + cisplatin
Median survival (days)	16	19	23	32
P value (to PBS)		0.037	0.002	0.002
P value (to PBS + cisplatin)				0.008

[00490] As shown in this Example, antigenic compositions can be used on the same day as Cisplatin or at a period after Cisplatin administration. As used herein, the

term "antigenic composition" refers to a composition that includes antigens of one or more microbial species. As used herein, the term "microbial species" may refer to a viral pathogen or a bacterial pathogen or a fungal pathogen, as detailed herein.

[00491] A further study was designed to explore whether there is any synergy between platinum based chemotherapy and *K. pneumoniae* antigenic composition therapy for the treatment of lung cancer in mice. All of the mice received 10^5 Lewis Lung carcinoma cells iv on day 0. The mice received antigenic compositions or PBS subcutaneously every 2 days starting on day 0. On day 12, some of the mice were treated with cisplatin (10mg/kg) intraperitoneally. The following day (day 13), 3 mice from each group were bled and the blood cells were stained with anti-CD11b antibody and analyzed by FACS. The plots in Figure 27 depict the frequency of CD11b+ myeloid cells in the blood on day 13. Each point represents a single mouse. The results of this study demonstrate that antigenic composition therapy can enhance the frequency of myeloid cells (monocytes, macrophages, granulocytes, dendritic cells) in the blood when given with cisplatin. This may enhance immunity in the animals by providing a greater number of these innate myeloid cells which are capable of responding to the tumors or pathogens. In addition, since bone marrow suppression is a clinically important side-effect of chemotherapy treatment, with resultant reduction in myeloid cells in the blood, which can result in postponement/discontinuation of chemotherapy or susceptibility to infection, the enhancement of myeloid cells with antigenic composition therapy during chemotherapy, may be helpful to reduce the risk of this important and clinically relevant side effect of chemotherapy.

EXAMPLE 9: Macrophage studies (MOA13 study)

[00492] To examine the role of macrophages with respect to embodiments of the invention, the MOA13 study was completed. Briefly, mice were either inoculated with $3x10^5$ Lewis Lung carcinoma cells or were injected with HBSS as a vehicle control. For mice inoculated with Lewis Lung carcinoma cells, they were pooled into a single cage where they were subsequently transferred at random to their respective cages.

Thereafter, mice were treated with either *K. pneumoniae* antigenic compositions, or PBS, on days 2, 4, 6 and 8. Thereafter, all mice were sacrificed on day 9. To obtain information with respect to myeloid and NK cell frequencies, 20 mice (5 from each group) had their lungs surgically removed, and the lungs were digested using Liberase TL and DNAse. Following the digesting and cell preparation, a single cell suspension was generated. Thereafter, a portion of the cells were transferred into 96 round bottom plates for staining using myeloid specific antibodies (CD11b+, NK1.1+) and NK specific antibodies (NK1.1+, CD11b+). For the former cell type, gates were used to select only CD11b+ and NK1.1- cell populations. Thereafter, cell data acquisition was performed using BD FACSCalibur and analysis was performed using FlowJo. Statistical analysis and graphical representation was performed using Excel and GraphPad Prism. The results are shown in Figure 28. Briefly, treatment with *K. pneumoniae* antigenic compositions leads to an increase in both myeloid cells (likely monocytes and macrophages) as well as CD11b+ NK cells in the lungs of mice so treated.

[00493] With a focus of obtaining information with regard to cytokines being produced within lung tissue, the following experiments were conducted. Mice were either inoculated with $3x10^5$ Lewis Lung carcinoma cells or were injected with HBSS as a vehicle control. For mice inoculated with Lewis Lung carcinoma cells, they were pooled into a single cage where they were subsequently transferred at random to their respective cages. Thereafter, mice were treated with either *K. pneumoniae* antigenic compositions, or PBS, on days 2, 4, 6 and 8. Thereafter, all mice were sacrificed on day 9. To obtain information re: cytokine within lung tissue, a bronchoalveolar lavage was performed on the lungs. Thereafter, the lungs were surgically removed immediately after the lavage and placed into pre-weighed vials containing PBS and protease inhibitors. Thereafter, the lung tissue was homogenized, centrifuged, and the supernatant was applied to ELISA kits (eBioscience); the ELISA assay was carried out according to manufacturer's guidelines. Statistical analysis and graphical representation was performed using Excel and GraphPad Prism. Data is represented as pg of cytokine per mg of original lung tissue. Each data point in Figure 29 is the

value from a single mouse. As shown in Figure 29, treatment with K. pneumoniae antigenic compositions leads to an increase in anti-tumour (IL-12, MCP-1, GMCSF, IL-6) cytokine production in the lung tissue.

[00494] With a focus of obtaining information with regard to cytokine production in the bronchoalveolar lavage (BAL) fluid, the following experiments were conducted. Mice were either inoculated with $3x10^5$ Lewis Lung carcinoma cells or were injected with HBSS as a vehicle control. For mice inoculated with Lewis Lung carcinoma cells, they were pooled into a single cage where they were subsequently transferred at random to their respective cages. Thereafter, mice were treated with either *K. pneumoniae* antigenic compositions, or PBS, on days 2, 4, 6 and 8 of the experiment. Thereafter, all mice were sacrificed on day 9. To obtain information with regard to cytokine production in the lungs, bronchoalveolar lavage was performed on the lungs of the mice. The fluid from the lavage was placed into vials, and stored at -80^0C until a time when ELISA assay was performed. Thereafter, the ELISA assay was carried out according to manufacturer's guidelines. Statistical analysis and graphical representation was performed using Excel and GraphPad Prism. Data is represented as pg/ml of lavage fluid as shown in Figure 30. Each data point represents the value obtained from one mouse. As shown in Figure 30, treatment with *K. pneumoniae* antigenic compositions had no effect on cytokine production in the BAL fluid.

[00495] With a focus on examining the M1/M2 macrophage phenotypes in the model described herein, NOS2 and Arg1 levels were monitored in the lung. The experiments conducted were as follows: briefly, mice were either inoculated with $3x10^5$ Lewis Lung carcinoma cells or were injected with HBSS as a vehicle control. For mice inoculated with Lewis Lung carcinoma cells, they were pooled into a single cage where they were subsequently transferred at random to their respective cages. Thereafter, mice were treated with either *K. pneumoniae* antigenic compositions, or PBS, on days 2, 4, 6 and 8 of the experiment. Thereafter, all mice were sacrificed on day 9. To obtain information with respect to NOS2 and Arg1 gene expression, 20 mice (5 from each

group) had their lungs surgically removed. Thereafter, a small portion of these lungs were cut using sterile techniques and placed into RNAlater to stabilize the RNA material for future gene analysis. Total RNA was extracted from the lung tissue using a kit from Qiagen and used according to manufacturer's protocol. A cDNA kit was used to convert an amount of RNA into cDNA (Qiagen), again by following instructions supplied by manufacturer. qPCR was conducted using primers designed to specifically amplify Nos2 and Arg1(Nos2: forward –CGCTTTGCCACGGACGAGA; reverse –AGGAAGGCAGCGGGCACAT; Arg1:forward - GGTCCACCCTGACCTATGTG; reverse –GCAAGCCAATGTACACGATG). Statistical analysis was conducted using Excel and GraphPad Prism. As shown in Figure 31, treatment with *K. pneumoniae* antigenic compositions leads to an increase in the Nos2/Arg1 ratio in the lungs, which corrlates with an enhanced anti-tumour response.

[00496] With a focus on investigating the M1/M2 response in the *in vivo* model described herein, the expression of CD206 (mannose receptor) was monitored. Briefly, mice were either inoculated with $3x10^5$ Lewis Lung carcinoma cells or were injected with HBSS as a vehicle control. For mice inoculated with Lewis Lung carcinoma cells, they were pooled into a single cage where they were subsequently transferred at random to their respective cages. Thereafter, mice were treated with either *K. pneumoniae* antigenic compositions, or PBS, on days 2, 4, 6 and 8 of the experiment. Thereafter, all mice were sacrificed on day 9. To obtain information with regard to CD206 expression, 20 mice (5 from each group) had their lungs surgically removed, and digested using Liberase TL and DNAse. Following the digesting and cell preparation, a single cell suspension was generated. A portion of the cells were transferred into 96 round bottom plates for staining using CD206 specific antibodies (clone MR5D3), acquired from Cedarlane Labs (Burlington, ON). As CD206 is located both intra- and extracllularly, the cells were first fixed using paraformaldehyde, and staining with the antibody in permeabilization solution. Analysis was performed using FlowJo. Statistical analysis and graphical representation was performed using Excel and GraphPad Prism. The results are shown in Figure 32. As shown in Figure 32,

treatment with *K. pneumoniae* antigenic compositions leads to a reduction in CD206 expression on lung macrophages both in the presence (PBS + LL2 and *K. pneumoniae* 1/10X + LL2) or absence of lung tumours (PBS and *K. pneumoniae* 1/10X). In the presence of lung cancer, *K. pneumoniae* antigenic compositions reduce the frequence of CD206 expression from about 20% (PBS + LL2 group) to about 10% (*K. pneumoniae* 1/10X + LL2 gorup). A similar reduction in CD206 expression was seen in *K. pneumoniae* treated animals without any lung cancer (PBS and *K. pneumoniae* 1/10X).

[00497] With a focus on investigating the M1/M2 phenotypes in the *in vivo* model described herein, the expression of F4/80+ macrophages was monitored. Briefly, mice were either inoculated with $3x10^5$ Lewis Lung carcinoma cells or were injected with HBSS as a vehicle control. For mice inoculated with Lewis Lung carcinoma cells, they were pooled into a single cage where they were subsequently transferred at random to their respective cages. Thereafter, mice were treated with either *K. pneumoniae* antigenic compositions, or PBS, on days 2, 4, 6 and 8 of the experiment. Thereafter, all mice were sacrificed on day 9. To obtain information with respect to F4/80+ expression in macrophages, 20 mice (5 from each group) had their lungs surgically removed, and digested using Liberase TL and DNAse. Following digesting and cell preparation, a single cell suspension was generated. A portion of the cells were transferred into 96 round bottom plates for staining using F4/80 monoclonal antibodies. Cell data acquisition was performed using BD FACSCalibur. Analysis was performed using FlowJo. Statistical analysis and graphical representation was performed using Excel and GraphPad Prism. As shown in Figure 33, treatment with *K. pneumoniae* antigenic compositions leads to a reduction in F4/80+ macrophages in the lungs. This reduction is thought to correlate with a reduction in M2-like macrophages.

EXAMPLE 10: Site specificity studies (MOA14 study)

[00498] With a focus on investigating the M1/M2 phenotypes in the in vivo model described herein which is used in conjunction with the antigenic compositions

described herein, the following experiments were performed. Briefly, 5 mice per group were treated on day 0, 2, 4, and 6 with either PBS, *E. coli* colon antigenic compositions, or K. pneumoniae antigenic compositions. On day 7 of the experiment, the mice were sacrificed and a bronchoalveolar lavage was performed. Subsequently the lungs and proximal colon were removed and enzymatically digested. After digestion, the recovered cells were washed and stained with antibodies specific for I-A/I-E FITC (MHC class II; M5/114.15.2); anti-Gr-1 PE (RB6-8C5_; anti-CD11b PerCP-Cy5 (M1/70); anti-CD11c APC (N418). All antibodies were acquired from eBioscience (San Diego, CA). The lung cells were counted to determine the total number of cells (the colon was not counted because we did not remove equal amounts of colon between samples). After staining for 20mins the cells were washed and analyzed by FACS. Each data point shown in corresponding Figure 34 represents the frequency of CD11b+Gr-1+ cells in the live gate for one mouse. As shown in Figure 34, treatment with *E. coli* antigenic compositions leads to an increased frequency of inflammatory monocytes in the colon of treated mice.

[00499] Further, and as shown in Figure 35, when monocytes in the lungs were examined based on the experimental methods detailed herein, it was found that while both *E. coli* and *K. pneumoniae* antigenic compositions increase the frequency of monocytes in the lungs of mice, *K. pneumoniae* antigenic compositions were more effective when counting for total numbers. Referring to Figure 35, the left-most panel shows the frequency of CD11b+Gr-1+ (inflammatory monocyte) cells in the lungs; the right-most panel shows the total number of CD11b+Gr-1+ cells in the lung.

[00500] In an effort to examine the phenotype of macrophages present in tumours using the *in vivo* model described herein which is used in conjunction with the antigenic compositions described herein, M1-like and M2-like macrophages were examines. As shown in Figure 36, this Figure represents the frequency of M1-like (see left panel of Figure 36) TAMs (tissue associated macrophages) or M2-like (see right panel of Figure 36) in subQ 4T1 tumours on day 8 post tumour implantation. As

detailed herein, M1-like macrophages were defined as being CD11b+/Gr-1-/MHC Class II high; M2-like macrophages were defined as being CD11b+/Gr-1-/MHC Class II low.

EXAMPLE 11: Indomethacin efficacy and anti-inflammatory studies

[00501] In an effort to examine the efficacy of combining antigenic composition treatment with indomethacin. Briefly, experiments were designed wherein there were 10 or 11 mice in each of 4 groups with all treatments beginning on day 4 after tumour inoculation. All mice received 50,000 4T1 mammary carcinoma cells subQ on day 0. Thereafter, the 4 groups were treated as follows: 1) Indomethacin daily (in drinking water) + PBS subQ every 2 days; 2) Indomethacin daily (in drinking water) + S. aureus antigenic compposition subQ every 2 days; 3) Control vehicle daily (in drinking water) + PBS subQ every 2 days; and 4) Control vehicle daily (in drinking water) + S. aureus antigen composition subQ every 2 days. With respect to Figure 37, the left-most panel of the Figure demonstrates the tumour volume for the various groups on day 15 of the experiment. The right-most panel demonstrates the frequency and makeup of the CD11b+ cells in the tumors on day 11 of the experiment. The frequency of CD11b+ cells is significantly increased in both of the Indomethacin treated groups relative to the control. These results demonstrate the efficacy of combining *Staph. aureus* antigenic compositions with anti-inflammatories, such as indomethacin. Further, and as shown in Figure 38 herein, at the day 22 time point, indomethacin treatment resulted in an increase in CD11b+ cells.

[00502] To further examine the efficacy of combining antigenic composition treatment with indomethacin, a study was designed. Briefly, four (4) groups of 10-11 Balb/c mice per group received 10,000 4T1 carcinoma calls SQ on day 0. Thereafter, treatments were as follows: the first group received indomethacin (14 µg/ml in water) starting on day 4 + PBS on days 4, 6, 8 etc.; the second group recevived indomethacin (14 µg/ml in water) and *S. aureus* antigenic compositions [0.1 ml of 0.5 OD 600nm stock] starting on day 4 + PBS on days 4, 6, 8 etc.; the third group received PBS starting on days 4, 6, 8 etc.; and the fourth group received *S. aureus* antigenic

compositions [0.1 ml of 0.5 OD 600nm stock] on days 4, 6, 8 etc. Tumor volume measurements were made on days 15, 19, and 22 and this data appears in Figure 39 for the four (4) groups of mice. The data decpicted in Figure 39 sows that there is synergy between antigenic composition treatment and an anti-inflammatory (*e.g.*, Indomethacin) in a subcutaneous cancel model.

[00503] On day 11 of the Experiment detailed above, tumors were excised and digested. Thereafter. The digested tumors were stained with anti-CD11b and analyzed by FACS (n=3 per group of mice). The results are depicted in Figure 40 herein and demonstrate that there is an increased frequency of CD11b+ cells in the tumors of indomethacin treated mice at day 11 post-inoculation.

[00504] On day 22 of the Experiment detailed above, tumors were excised and digested. Thereafter. The digested tumors were stained with anti-CD11b and analyzed by FACS (n=7-8 per group of mice). The results are depicted in Figure 41 herein and demonstrate that there is an increased frequency of CD11b+ cells in the tumors of indomethacin treated mice at day 22 post-inoculation. Further, Figure 42 demonstrates that indomethacin treatment causes an increase in CD11b+ CD94+ myeloid cells in the tumors as found at day 22 post-inoculation.

[00505] To further examine whether the antigenic composition treatment was associated with an anti-inflammatory response, at day 22 of the Experiment described above, whole tumor samples were subjected to quantitative PCR by known methods. The gene products Fizz1 and Ym1 were targeted. Both Fizz1 and Ym1 are reportedly associated with M2 macrophages (see, for *e.g.*, Wong *et al.* (2010) *Eur. J. Immunol.* 40(8): 2296-307). The results are depicted in Figure 43 herein. As shown in Figure 43, the relative expression of both Fizz1 and Ym1 was increased in tumors where there was treatment of both indomethacin and antigenic compositions (compared, for example, to indomethacin alone).

[00506] The relative expression levels of Arg1 and Fizz1 was examined in both the tumors and spleens of mice at day 22 of the Experiment. As shown in Figure 44, both Arg1 and Fizz1 expression increased in the spleens of mice treated with indomethacin and antigenic compositions. The relative expression levels of Nos2 and Ym1 was examined in both the tumors and spleens of mice at day 22 of the Experiment. The relative expression levels are depicted in Figure 45 herein for the four (4) groups of mice.

[00507] To further examine the anti-inflammatory response associated with the antigenic composition treatment, an experiment was designed as follows. Briefly, mice were given Lewis Lung tumors on day 0 [PBS and *K. pneumoniae* antigenic composition] or no tumors [PBS (no tumor)] or *K. pneumoniae* antigenic composition (no tumor)]. The mice received *K. pneumoniae* antigenic composition [0.1ml of 0.5 OD500nm stock] or PBS on day 2, 4, 6, 8. The mice were sacrificed on day 9 and their lungs were removed and homogenized. IFNγ was measured in the lung homogenate using ELISA. The results are shown in Figure 46 herein. Treatment with *K. pneumoniae* antigenic composition reduced IFNγ production in the lungs of mice with and without lung tumors.

[00508] To further examine the anti-inflammatory response associated with antigenic composition treatment, the following experiment was designed. Briefly, bone marrow macrophages were cultured overnight either in media, or in medial supplanted with LPS, or in media supplanted with *K. pneumoniae* antigenic compositions. The media was tested for IL-12 and IL-10. The results are shown in Figure 47 herein and demonstrate that IL-12 was not identified. As shown in Figure 47, bone marrow macrophages cultured overnight with *K. pneumoniae* antigenic compositions produced IL-10. IL-10 is known to be associated with an anti-inflammatory response (see, for *e.g.*, Bazzoni *et al.* (2010) *Eur. J. Immunol.* 40(9): 2360-8).

[00509] To further examine the anti-inflammatory response associated with antigenic composition treatment, the following experiment was designed. Briefly, the 4T1 tumor model was utilized to determine the effects of *Staph aureus* (SA) antigenic composition therapy to work synergistically with the anti-inflammatory drug indomethacin. In this study, there were four (4) groups of 10 Balb/c female mice which all received 50,000 4T1 mammary carcinoma cells subcutaneously on day 0. Group 1 received PBS subcutaneously every 2 days starting on day 4. Group 2 received SA [0.1ml of 0.5 OD 600nm] subcutaneously every 2 days starting at day 4. Group 3 and 4 received 14ug/ml of indomethacin (indo) in their drinking water and subcutaneously received PBS and SA respectively every 2 days beginning on day 4. On day 22 the mice were sacrificed and tumors from 7-8 mice per group were harvested and placed in an RNA preserving agent. Subsequently we analyzed the expression of IL-10 in the tumors by real-time PCR. The results in Figure 48 show that mice treated with indomethacin and PBS have a significant increase in IL-10 expression in the tumors. Treatment with indomethacin and SA leads to an even greater amount of IL-10 suggesting that SA enhances the anti-inflammatory effects of indomethacin in this tumor model. Since IL-10 is widely known to be an anti-inflammatory cytokine these results demonstrates that SA works synergistically with indomethacin in an anti-inflammatory manner. It is important to note that the tumors in the PBS group were the largest followed by the SA group and the Indo-PBS group. The tumors in the Indo-SA group were the smallest on day 22. The anti-inflammatory properties detailed herein can be employed for use against a variety of inflammatory diseases (see Table 20 herein).

Table 20. List of Chronic Inflammatory Diseases

Acne vulgaris
Acute disseminated encephalomyelitis
Acute hemorrhagicleukoencephalitis
Addison's Disease
Agammaglobulinemia

Allergies
Alopecia areata
Alzheimer's
Amyotrophic Lateral Sclerosis
Anaemia, autoimmune hemolytic
Anaemia, pernicious
Ankylosing spondylitis
Anti-GBM/TBM Nephritis
Antiphospholipid syndrome
Antisynthetase syndrome
Arteritis, temporal (also known as "giant cell arteritis")
Arthritis, juvenile
Arthritis, psoriatic
Arthritis, reactive (Reiter's syndrome, rea)
Arthritis, rheumatoid
Asthma
Atherosclerosis
Atopic allergy
Atopic dermatitis
Autoimmune enteropathy
Autoimmune aplastic anemia
Balo disease/Balo concentric sclerosis
Bartter syndrome
Bechets Syndrome
Berger's disease
Bickerstaff's encephalitis
Blau syndrome
Bronchitis, chronic
Bullous pemphigoid

Bursitis
Cardiomyopathy, autoimmune
Castleman's disease
Celiac disease
Celiac disease
Chronic fatigue syndrome
Chronic inflammatory demyelinating polyneuropathy
Chronic recurrent multifocal osteomyelitis
Churg-Strauss syndrome
Cicatricialpemphigoid
Cirrhosis, primary biliary
Cogan syndrome
Cold agglutinin disease
Colitis
Complement component 2 deficiency
Connective tissue disease, mixed
Connective tissue disease, undifferentiated
COPD (chronic obstructive lung disease)
Cranial arteritis
CREST syndrome
Cryoglobulinemia
Cushing's Syndrome
Cutaneous leukocytoclasticangiitis
Cystitis, interstitial
Dacryadenitis
Dego's disease
Dercum's disease
Dermatitis
Dermatitis herpetiformis

Dermatitis, autoimmune progesterone
Dermatomyositis
Diabetes
Diabetes insipidus, nephrogenic
Diabetes mellitus type 1
Diffuse cutaneous systemic sclerosis
Discoid lupus erythematosus
Diverticulitis
Dressler's syndrome
Dysmenorrhea (menstrual cramps/pain)
Eczema
Eczema
Endometriosis
Enthesitis-related arthritis
Eosinophilic fasciitis
Eosinophilic gastroenteritis
Epidermolysisbullosaacquisita
Erythema nodosum
Essential mixed cryoglobulinemia
Evan's syndrome
Fibrodysplasiaossificansprogressiva
Fibromyalgia
Fibrosingaveolitis
Gastritis, atrophic
Gastritis, atrophic
Gastrointestinal pemphigoid
Giant cell arteritis
Glomerulonephritis
Glomerulonephritis

Goodpasture's syndrome
Gout, acute
Gout, arthritic
Graves' disease
Guillain-Barré syndrome (GBS)
Haemolytic anaemia
Hashimoto's encephalitis
Hashimoto's thyroiditis
Hemolyticanemia, autoimmune
Henoch-Schonleinpurpura
Hepatitis, autoimmune
Hepatitis, viral
Herpes gestationis
Hypogammaglobulinemia
Idiopathic Inflammatory Demyelinating Diseases
Idiopathic pulmonary fibrosis
Iga nephropathy
Ileus (bowel obstruction)
Inclusion body myositis
Inflammatory bowel disease, Crohn's disease
Inflammatory bowel disease, ulcerative colitis
Inflammatory demyelinating polyneuopathy
Inner ear disease, autoimmune
Interstitial cystitis
Irritable bowel syndrome (IBS)
Juvenile idiopathic arthritis
Juvenile rheumatoid arthritis
Kawasaki's Disease
Kidney stones

Lambert-Eaton myasthenic syndrome
Leukocytoclasticvasculitis
Lichen planus
Lichen sclerosus
Linear iga disease (LAD)
Lou Gehrig's disease (Also Amyotrophic lateral sclerosis)
Lupoid hepatitis
Lupus
Lupus erythematous
Lymphoproliferative syndrome, autoimmune
Majeed syndrome
Ménière's disease
Meningitis
Microscopic polyangiitis
Miller-Fisher syndrome
Morphea
Mucha-Habermann disease
Multiple sclerosis
Multiple sclerosis
Myasthenia gravis
Myositis
Myositis, inclusion body
Nephritis
Nephrotic syndrome
Neuromyelitisoptica (Also Devic's Disease)
Neuromyotonia
Occularcicatricialpemphigoid
Ocular inflammation (acute and chronic non-bacterial inflammation of the anterior part of the eyes)

Opsoclonus myoclonus syndrome
Ord thyroiditis
Osteoarthritis
Paget's disease of bone
Palindromic rheumatism
Pancreatitis, autoimmune
PANDAS (pediatric autoimmune neuropsychiatric disorders associated with streptococcus)
Paraneoplastic cerebellar degeneration
Parkinson's
Paroxysmal nocturnal hemoglobinuria (PNH)
Parry Romberg syndrome
Pars planitis
Parsonnage-Turner syndrome
Pelvic inflammatory disease
Pemphigus
Pemphigus vulgaris
Pericarditis, non-rheumatic
Peripheral neuropathy, autoimmune
Perivenous encephalomyelitis
POEMS syndrome
Polyarteritisnodosa
Polychondritis, relapsing
Polyendocrine syndrome, autoimmune
Polymyalgia rheumatica
Polymyalgia rheumatica
Polymyositis
Primary sclerosing cholangitis
Progressive inflammatory neuropathy

Prostatitis, chronic
Pseudogout
Psoriasis
Psoriasis
Pure red cell aplasia
Pyodermagangrenosum
Rasmussen's encephalitis
Raynaud phenomenon
Reiter's syndrome
Restless leg syndrome
Retinopathy of prematurity
Retroperitoneal fibrosis
Rheumatoid fever
Rhinitis, allergic
Sarcoidosis
Sarcoidosis
Schmidt syndrome
Schnitzler syndrome
Scleritis
Scleroderma
Sclerosis, systemic
Sjögren's syndrome
Spondyloarthropathy
Still's disease
Subacute bacterial endocarditis (SBE)
Susac's syndrome
Sweet's syndrome
Sydenham chorea
Sympathetic ophthalmia

Takayasu's arteritis
Temporomandibular joint disorder (TMJD or TMD), or TMJ syndrome
Thrombocytopenic purpura, autoimmune
Thrombocytopenic purpura, idiopathic
Tolosa-Hunt syndrome
Transplant rejection
Transverse myelitis
Undifferentiated spondyloarthropathy
Urticaria
Uveitis, autoimmune
Valvular disease, non -rheumatic
Vasculitis
Vitiligo
Wegener's granulomatosis

EXAMPLE 12: Inflammatory bowl disease studies

[00510] This example provides an illustration of the clinically effective use of an antigenic formulation comprising killed *E. coli* in the treatment of a patient with Crohn's disease, over a three-month course of treatment. During the course of treatment, the patient became symptom free and ceased using anti-inflammatory medications.

[00511] The patient initially presented reporting pain in the area of the large intestine, while under treatment with prednisone and Imuran™.

[00512] Treatment was initiated with a subcutaneous inoculum of a killed formulation of whole E. coli, derived from an E. coli strain collected from a patient with an E. coli colonic infection. The dosing schedule involved a subcutaneous dose every

second day, beginning with a 0.05 ml dose, gradually increasing in volume until a 2 inch diameter light pink/red skin response was achieved within 24 hours after injection at the injection site. The dose eventually required to achieve this skin response was 0.09-0.11 ml in this patient, and this dose has been continued as a maintenance dose every second day.

[00513] One week following the initiation of treatment with the antigenic preparation of whole killed E. coli, the patient reported that the pain was gone. Within about two months, the patient discontinued treatment with prednisone while continuing on a daily does of 150mg of Imuran™. Subsequently, the patient also discontinued use of Imuran™.

[00514] By two-months following the initiation of treatment with the *E. coli* composition, the patient was self-administering 0.09-0.11ml of the E.coli preparation every other day. The patient self-adjusted this dosage so as to provoke a local inflammatory reaction evidenced by a pink spot of approximately 2 inches in diameter that persisted at the site of administration for about two days.

EXAMPLE 13: Fungal studies

[00515] Experiments utilizing the lung mouse model system described in detail herein were conducted which determined that mice that had received water contaminated with a particular fungal species (*Penicillium marneffei*), which is known to cause respiratory infections in both mice and humans, demonstrated decreased tumor burden as has been demonstrated and discussed with respect to bacterial lung pathogens.

[00516] Numerous fungal species are known to be associated with various organs or tissues as detailed in Table 21 below that water contaminated with fungal species also resulted. Accordingly, and based on the experimental principles detailed herein,

the specificity demonstrated herein with respect to bacterial and viral pathogens should be extendable to fungal pathogens as well.

Table 21. Fungal species and associations with organs/tissues

Fungus	Skin	Respiratory	Genital	GU system	Mouth	Stomach	Duo-denum Jejunum	Ileum	Colon	Other
Actinomadura species	X									
Alternaria species	X									sinuses
Aspergillus fumigatus		X								sinuses, brain
Bipolaris species	X									sinuses
Blastomyces species	X	X		X						bones
Candida alibcans Candida krusei	X		X	X	X					œsophagus
Cladophialophora carrionii	X									sinuses
Coccidiodes immitis Coccidiodes posadasii		X								
Cryptococcus neoformans Cryptococcus gattii		X								
Curvularia species	X									sinuses
Epidermophyton species	X									
Exophiala species	X									sinuses
Fonsecæa pedrosoi	X									
Fusarium species	X	X								sinuses
Histoplasma capsulatum		X								
Microsporum species	X									
Nocardia species	X									
Paecilomyces species	X	X								sinuses
Paracoccidioides brasiliensis	X	X			X					

Pityrosporum species	X									
Penicillium marneffei	X	X								systemic infection
Pneumocystis jiroveci		X								
Pseudallescheria boydii (Scedosporium apiospermum)	X	X								sinuses
Rhizopus species (Rhizo)Mucor species Absidia species Cunninghamella species	X	X				X		X	X	sinuses, orbits, brain,
Scedosporium prolificans	X	X								sinuses
Sporothrix schenckii	X									
Stachybotrys chartarum		X								
Tinea capitis Tinea cruris Tinea pedis	X									
Trichoderma longibrachiatium	X	X								sinuses
Trichophyton species	X									
Trichosporon species	X	X								sinuses

OTHER EMBODIMENTS

[00515] Although various embodiments of the invention are disclosed herein, many adaptations and modifications may be made within the scope of the invention in accordance with the common general knowledge of those skilled in this art. Such modifications include the substitution of known equivalents for any aspect of the invention in order to achieve the same result in substantially the same way. Numeric ranges are inclusive of the numbers defining the range. In the specification, the word "comprising" is used as an open-ended term, substantially equivalent to the phrase "including, but not limited to", and the word "comprises" has a corresponding meaning.

Citation of references herein shall not be construed as an admission that such references are prior art to the present invention. All publications are incorporated herein by reference as if each individual publication were specifically and individually indicated to be incorporated by reference herein and as though fully set forth herein. The invention includes all embodiments and variations substantially as hereinbefore described and with reference to the examples and drawings.

WHAT IS CLAIMED IS:

1. Use of an anti-inflammatory composition to formulate a medicament for treating an individual for an inflammatory bowel disease (IBD) characterized by inflammation in a region of the gastrointestinal tract (GIT), wherein the anti-inflammatory composition comprises antigenic determinants selected or formulated so that together they are specific for at least one microbial pathogen that is pathogenic in the region of the GIT, and wherein:

the region is a small bowel region, and the pathogen is selected from the group consisting of *Escherichia coli, Clostridium difficile, Bacteroides fragilis, Bacteroides vulgatus, Bacteroides thetaiotaomicron, Clostridium perfringens, Salmonella enteriditis, Yersinia enterocolitica, Shigella flexneri,* adenoviruses, astroviruses, caliciviruses, noroviruses, rotaviruses, and cytomegalovirus; or,

the region is a colon or rectum region, and the pathogen is selected from the group consisting of *Escherichia coli, Clostridium difficile, Bacteroides fragilis, Bacteroides vulgatus, Bacteroides thetaiotaomicron, Clostridium perfringens, Salmonella enteriditis, Yersinia enterocolitica, Shigella flexneri,* adenoviruses, astroviruses, caliciviruses, noroviruses, rotaviruses, and cytomegalovirus; or,

the region is an anus region, and the pathogen is selected from the group consisting of E. *coli, Streptococcus pyogenes, Bacteroides* spp., *Fusobacterium* spp., anaerobic streptococci, *Clostridium* spp., *Enterobacter* spp., *Pseudomonas aeruginosa, Treponema pallidum,* and herpes simplex virus; or,

the region is an oral region, and the pathogen is selected from the group consisting of *Prevotella melaninogenicus, anaerobic streptococci, viridans streptococci, Actinomyces* spp., *Peptostreptococcus* spp., *Bacteroides* spp., herpes simplex virus, coxsackieviruses, and Epstein-Barr virus; or,

the region is a stomach region, and the pathogen is selected from the group consisting of *Streptococcus pyogenes, Helicobacter pylori,* cytomegalovirus, herpes simplex virus, Epstein-Barr virus, rotaviruses, noroviruses, and adenoviruses.

2. The use according to claim 1, wherein the IBD is selected from the group consisting of Crohn's disease, ulcerative colitis, collagenous colitis, lymphocytic colitis, ischaemic colitis, diversion colitis, Becet's syndrome or indeterminate colitis.

3. The use according to claim 1 or 2, wherein the anti-inflammatory composition is formulated for use at an administration site in successive doses given at a dosage interval of between one hour and one month, over a dosage duration of at least 2 weeks, 2 months or 6 months.

4. The use according to any preceding claim, wherein the anti-inflammatory composition is formulated for use in a dose so that each dose is effective to cause a visible localized inflammatory immune response at the administration site.

5. The use according to any preceding claim, wherein the anti-inflammatory composition is formulated for use intradermally or subcutaneously.

6. The use according to anyone of claims 1 to 4, wherein the anti-inflammatory composition is formulated for use by a route that is not enteric.

7. The use according to any preceding claim, wherein the anti-inflammatory composition is formulated for use so that visible localized inflammation at the administration site occurs within 1 to 48 hours.

8. The use according to claim 7, wherein the visible localized inflammation is an area of inflammation from 2mm to 100mm in diameter, optionally appearing 2 to 48 hours after administration, and optionally lasting from 2 to 72 hours.

9. The use according to any preceding claim, wherein the human patient is a patient receiving an effective amount of an anti-inflammatory or an immune suppressant medicament, wherein the anti-inflammatory or an immune suppressant medicament is sulfasalazine, mesalamine, a corticosteroid, azathioprine, mercaptopurine, infliximab, adalimumab, certolizumab pegol, methotrexate, cyclosporine or natalizumab.

10. The use according to any preceding claim, wherein the anti-inflammatory composition consists essentially of antigenic determinants of one or more pathogens that are each pathogenic in the region of the GIT.

11. The use according to any preceding claim, wherein the patient is diagnosed as having suffered from a prior pathogenic exposure to the pathogen.

12. The use according to any preceding claim, wherein the pathogen is a bacteria, and the anti-inflammatory composition is a whole killed bacterial cell composition.

13. The use according to any preceding claim, wherein the IBD is Crohn's disease, and the pathogen is *E. coli.*