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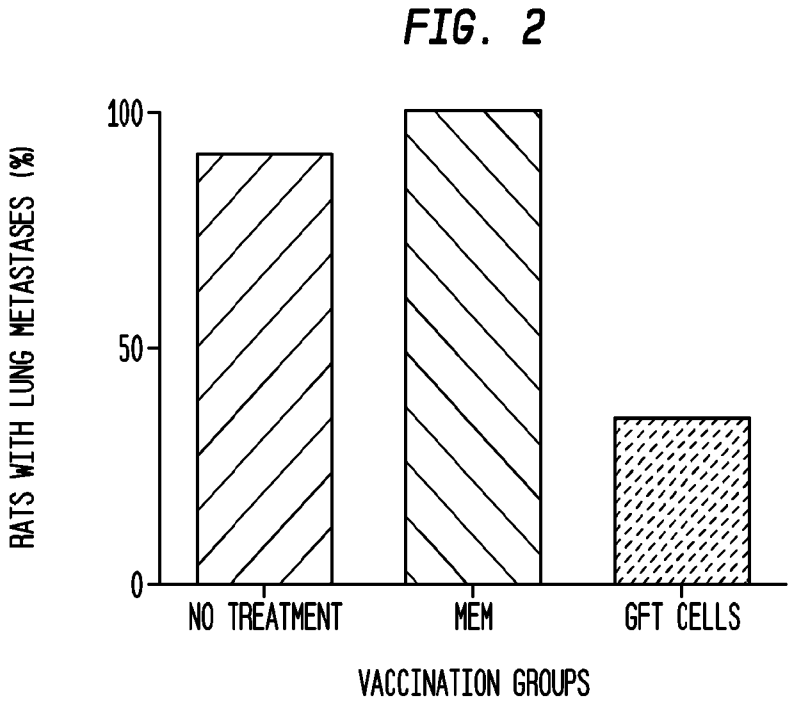
(54) Title: METASTASIS INHIBITION PREPARATIONS AND METHODS



(57) Abstract: Disclosed are compositions and pharmaceutical preparations suitable for inhibiting metastasis of a malignant cancer in an animal. Methods for inhibiting and/or eliminating metastasis in an animal are also provided. In some embodiments, the preparations and compositions comprise a whole cell tumor preparation comprising tumor tissue cells and tumor connective tissue stroma. The disclosure also provides methods for preparing the preparations and pharmaceutical preparations thereof. Inhibition of metastasis of malignant prostate cancer to the lung is shown *in vivo*.

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METASTASIS INHIBITION PREPARATIONS AND METHODS

BACKGROUND OF THE INVENTION

[0001] Metastasis is a complex series of steps in which cancer cells leave the original tumor site and migrate to other parts of the body via the bloodstream or the lymphatic system. To do so, malignant cells break away from the primary tumor and attach to and degrade proteins that make up the surrounding extracellular matrix (ECM), which separates the tumor from adjoining tissue. By degrading these proteins, cancer cells are able to breach the ECM and escape. When oral cancers metastasize, they commonly travel through the lymph system to the lymph nodes in the neck. The body resists metastasis by a variety of mechanisms through the actions of a class of proteins known as metastasis suppressors of which about a dozen are known.

[0002] Cancer researchers studying the conditions necessary for cancer metastasis have discovered that one of the critical events required is the growth of a new network of blood vessels, called tumor angiogenesis. Angiogenesis inhibitors have therefore been proposed in preventing the growth of metastases.

[0003] Whether or not a cancer is local or has spread to other locations affects treatment and survival. If the cancer spreads to other tissues and organs, it may decrease a patient's likelihood of survival. When cancer has metastasized, it may be treated with radiosurgery, chemotheraphy, chemotheraphy, radiation therapy, biological therapy, hormone therapy, surgery, laser immunotheraphy, or a combination of these. The choice of treatment generally depends on the type of primary cancer, the size and location of the metastasis, the patient's age and general health, and the types of treatments used previously. Unfortunately, current treatment options are rarely able to cure metastatic cancer.

[0004] Cancer of the prostate may metastasize to the bones and/or to the lungs. In a similar manner, colon cancer has the tendency to metastasize to the liver. Stomach cancer often metastasizes to the ovary in women, where it is then called a Krukenberg tumor. It is difficult for cancer cells to survive outside their region of origin, so in order to metastasize they must find a location with similar characteristics.

[0005] Prostate cancer is a significant cause of morbidity and mortality among men in the Western world. In advanced cases, the disease becomes refractory to conventional

treatments and death of the patient typically results from sequelae related to metastasis to sites including the bone and lungs.

[0006] Adenocarcinoma of the prostate is one of the most common malignancies. It is estimated that there are 220,000 new cases of prostate cancer will be diagnosed in the United States in 2007, and that it will cause more than 30,000 deaths during the year. In fact, prostate adenocarcinoma is the second leading cause of cancer-related mortality among men in the United States.

[0007] With prostate cancer, as with all solid tumors, it is the metastatic encroachment of the tumor on other vital function that causes the demise of the patient. Approximately 10% of patients are diagnosed initially with metastatic disease. Ultimately, 30-40% of patients with this cancer will develop metastatic disease. Once metastasis occurs, the cancer follows a relentless progression.

[0008] Invasion is a prerequisite for migration of tumor cells in connective tissue stroma and basement membranes form the major physical barriers to the migration process. Invasion of the local extracellular matrix (ECM) by tumor cells thus can be marked as the first step in metastasis. The sequential biochemical mechanism first involves cell attachment to specific components of ECM followed by a progressive cascade of proteolytic dissolution. Prostate cancers which grow to a critical size exhibit extracapsular invasion and metastasize to specific anatomical sites apparently in response to stromal cell secretory proteins which are necessary for their growth and proliferation. Invasive migration of tumor cells within the prostate gland may occur as a function of chemokinesis along anatomical paths of least resistance which include the perineural duct. Further establishment of metastasis relies upon successful penetration of the circulatory or lymphatic system, and vessel extravasation at the secondary organ which for prostate cancer is frequently bone and/or lung tissue. Nearly all of these steps, including attachment, matrix degradation and migration, can be modeled experimentally *in vitro* by measuring invasion of a reconstituted basement membrane (RBM) barrier in response to fibroblast-conditioned medium (FCM) used as a chemo-attractant.

[0009] Individual molecules associated with prostate cancer have been studied for their utility as vaccine antigens. For example, prostate-specific antigen (PSA), prostate-specific membrane antigen (PSMA), and prostatic acid phosphatase (PAP) have all been identified as immunogenic. PAP, as a vaccine antigen, has been shown to induce Th1 immunity in patients and conferred moderate clinical improvement. Clinical trials showed that patients vaccinated with dendritic cells loaded with recombinant PAP/granulocyte-

macrophage-colony-stimulating factor (GM-CSF) protein had moderation of PSA levels and prolonged survival. Some patients having hormone-refractory prostate cancer showed moderation of PSA levels following vaccination with dendritic cells pulsed with various antigens, including PSA and PSMA. While some clinical success has been achieved with these antigens, none have resulted in long-term survival of patients

[0010] Use of autologous whole cell vaccines have been examined for a variety of cancers, including melanoma, lung cancer, colon cancer, and renal tumors. Varying degrees of efficacy were reported.

[0011] A phase I clinical trial of irradiated GM-CSF-secreting autologous prostate tumor cell vaccine therapy reported that the vaccine was well tolerated by patients and induced both B-cell and T-cell immune responses against antigens associated with prostate cancer cells. However, those investigators concluded that, while promising, autologous vaccines for prostate cancer were limited by the low yield of cells recovered from tumor harvest, even after expansion in cell culture. Instead, investigators have focused on the use of preparations composed of irradiated allogeneic prostate cancer cells, these cultured cells having been engineered to secrete GM-CSF, or allogenic cells with a *Bacillus* adjuvant as a means for treatment of prostate cancer. One study reported that vaccination slowed the rise of PSA in 40% of vaccinated patients, and an increased average time to disease progression of 58 weeks, compared to historical experience of 29-30 weeks.

[0001] Despite these and other reports, a need continues to exist in the medical and clinical arts for more effective methods and compositions for inhibiting metastasis and the spread of cancer.

SUMMARY

[0012] The present invention, in a general and overall sense, provides preparations and methods of using these preparations for inhibiting and/or halting metastasis, as well as the cancer disease progression associated with metastasis. These methods and preparations may be used in both human and non-human animals. For example, the present methods and preparations may be employed in the treatment of non-human animals including companion animals, such as cats, dogs and horses. Other types of non-human animals envisioned for treatment according to the present methods include commercially important animals, including sheep, swine, cattle and others.

[0013] In some aspects, the types of metastasis that may be inhibited and/or eliminated include metastasis to the lung and/or bone (such as to the spine). It is envisioned

that the present methods and preparations will also find utility in reducing and/or preventing the metastasis of tumor/cancer cells to other organs, such as, by way of example and not limitation, metastasis to ovary, liver, brain, kidney, spleen, intestines, adrenal glands, or any other tissue and/or organ or combination of tissues and/or organs. It therefore is an object of the present invention to provide methods for inhibiting or preventing metastasis.

[0014] It is another aspect of the present invention to provide preparations for inhibiting or preventing metastasis.

[0015] In accomplishing the foregoing objects, there has been provided, in accordance with one aspect of the present invention, a method for preventing or inhibiting metastasis of a cancer, for example, a cancer of epithelial cell origin, comprising the step of administering a composition comprising a preparation as described herein. In some embodiments, the preparation may be described as comprising a tumor tissue preparation.

[0016] In some embodiments, the preparation and/or composition comprises a tumor tissue preparation that has been treated so as to inactivate any proliferating malignant cells. By way of example, this may be accomplished by treating a cell suspension of a tumor tissue preparation with an inactivating process, such as a chemical or other than chemical process. By way of example, and not limitation, a chemical inactivating process may comprise treatment and/or exposure of a tissue preparation to an inactivating amount of glutaraldehdye, formalin, or any other like inactivating chemical substance. Alternatively, the tumor tissue preparation may be treated and/or exposed to a non-chemical inactivating treatment, such as to radiation. For example, a tumor tissue preparation may be exposed to a radiation dose sufficient to eliminate malignant cell activity and/or malignant cell characteristics in the tumor tissue preparation. In some embodiments, the chemical and/or non-chemical inactivating treatment may be described as rendering the tumor tissue preparation essentially free of malignant cell activity and/or malignant cell characteristics.

[0017] In some embodiments, the tissue vaccine preparation may be described as comprising a glutaraldyhyde-fixed tumor (GFT) cell vaccine. In other embodiments, the tumor tissue preparation comprises a prostate tumor tissue preparation of glutaraldyhyde-fixed prostate tumor (GFPT) cells.

[0018] In another regard, some embodiments of the present method provide a method for inhibiting and/or eliminating metastasis attendant a hormone resistant prostate cancer and/or tumor in an animal. The animal may be a human or non-human animal.

[0019] In further embodiments, the method is used in conjunction with additional treatments. In this regard, some treatments may include surgical intervention, radiation therapy, hormonal therapy, immunotherapy, chemotherapy or cryotherapy.

[0020] In accordance with another aspect of the present invention, there is provided a pharmaceutical composition for inhibiting or preventing metastasis of a cancer and/or tumor, particularly that metastasis attendant the spread of a cancer of epithelial cell origin, comprising: (i) a composition comprising a tumor tissue preparation and (ii) a carrier. The carrier may be further described as a carrier that is effective for the therapeutic administration of said composition to the animal.

[0021] In some embodiments, the metastasis inhibition preparation comprises a tumor tissue preparation.

[0022] Other objects, features and advantages of the invention will be apparent from the following detailed description. It should be understood, however, that the detailed description and the specific examples, while providing general and specific descriptions and indicating preferred embodiments of the invention, are given by way of illustration only. Various changes and modifications within the spirit and scope of the invention will become apparent to those skilled in the art from the detailed description and other aspects of the present disclosure.

BRIEF DESCRIPTION OF THE FIGURES

[0023] Figure 1. Section from a prostate tumor of a non-vaccinated Lobund-Wistar rat. The tissue mass shown is a typical adenocarcinoma, with scattered acinar structures in an abundant connective tissue stroma. Section is stained with H & E, magnified x200.

[0024] Figure 2. Percentage of rats having pulmonary metastases from primary prostate tumor. Tumor bearing rats underwent weekly vaccination with either media (MEM) or a tissue vaccine, the tissue vaccine comprising a suspension of glutaraldehyde-fixed tumor (GFT) cells. In addition, an untreated group was included. Most untreated and (sham) MEM-vaccinated rats had evidence of pulmonary metastases. In contrast, 70% of GFT cell-vaccinated rats were completely free of metastasis in the lungs.

[0025] Figure 3 A-3B. IFN-γ (3A) and TNF-α (3B) levels in supernatants of cultured splenocytes from vaccinated rats. Treatment groups are vaccinated with MEM and pulsed with MEM (MEM/M); vaccinated with MEM and pulsed with GFT cells (MEM/G); vaccinated with GFT cells and pulsed with MEM (GFT/M); and vaccinated with GFT cells

and pulsed with GFT cells (GFT/G). Supernatants were harvested after 72 h and cytokines measured using a general multiplex assay protocol in a sandwich immunoassay system employing microspheres and using the Luminex-100 (Luminex Corp.), a dual-laser flow analyzer. For both IFN-γ and TNF-α, the mean value for GFT/G supernatants was significantly ($P \leq 0.001$) greater than for all other groups. No other significant differences between groups were found. Values are in pg/ml.

DETAILED DESCRIPTION

[0026] The present invention provides both preparations and/or compositions and methods of using these preparations and/or compositions for the inhibition and/or treatment of metastasis in human and non-human animals. In particular aspects, a preparation and/or composition and a method of using the preparation and/or composition as part of a method of inhibiting and/or treating metastasis attendant prostate cancer by vaccination is provided.

[0027] Among other benefits, the present preparations and/or compositions and methods are characterized by an absence of adverse side-effects relative to the experience of treatments that include chemotherapy or radiation treatment, and additional benefits as an alternative to undergoing radical prostatectomy. Moreover, with the presently disclosed methods, metastatic cancers can be targeted more specifically by use of a multivalent mixture of antigens associated with the targeted type of cancer being treated, while sparing normal adjacent tissue.

[0028] Because metastatic forms of cancer are a complex mixture of neoplastic cells, connective tissue cells, and matrix, the present multivalent vaccine captures the greatest range of relevant antigens, and therefore is of significant clinical utility. In this regard, the tissue vaccines of the present invention are made of harvested tumor material, and as such, are composed of a rich antigenic menu. In addition, the tissue vaccines provided herein are shown to be well tolerated by the animal/patient *in vivo*.

[0029] The whole cell vaccines described herein comprise harvested tumor material, and thereby provide a large number of different and relevant antigenic targets to the immune system, providing a highly effective multivalent vaccine preparation. These multivalent whole cell vaccines, comprising inactivated tumor tissue, are demonstrated to inhibit and/or eliminate metastasis of cancer *in vivo*. In particular, the present compositions prove a 70% reduction in any evidence of metastasis (such as pulmonary metastasis) in an animal having a tumor growth. This is particularly significant in that the reduced metastatic burden represents

animals essentially free of metastasis. The present methods and compositions/preparations also evidence a significant reduction in the size or number of metastatic foci.

[0030] The present preparations and/or compositions and methods are demonstrated to be effective in the treatment of hormone-refractory cancers, as well as the metastasis of these cancers. For example, the present compositions and methods are effective in the treatment and/or inhibition of hormone refractory prostate cancer and metastasis of these cancers. This is significant because, among other reasons, cancers that have become hormone refractory have been historically recognized as more difficult to contain and treat. Thus, the activity of the present methods and preparations for effectively inhibiting these types of cancers reduces and/or eliminates technical challenges in available treatment options for these patients. The availability of adequate amounts of autologous tumor material does not constitute a limiting factor when considering this as a treatment option among this particular group of patients.

[0031] Tumor material harvested from other sources may also be used for preparation of the presently described whole cell vaccines. For example, a xenogeneic tissue vaccine composed of harvested subcutaneous PAIII prostate tumors (in for example, LW rats) stimulates sufficient immunity in immunocompetent animals. Specifically, Ncr-Foxn1<nu> mice splenocytes were incubated with human PC346C prostate cancer cells and administered to syngeneic nu/nu mice. Nearly 70% of the immunodeficient mice were completely free of tumor growth compared to none of the controls [35]. While not intending to be limited to any particular mechanism of action and/or theory, this protective response may be mediated by Th1 immunity. In the present methods and compositions, immunization with the GFT cell vaccine stimulated increases in the Th-1-associated cytokines, IFN-γ and TNF-α, suggesting concordance with the earlier results in the xenogeneic system. Xenogeneic tumor tissue represents a source from which material can be harvested for construction of tissue vaccines. This approach, among others, overcomes the limitations identified by earlier investigators [31].

[0032] Vaccination of rats with glutaraldehdye-fixed material harvested directly from tumors completely eliminates metastasis in 70% of rats bearing autochthonous prostate tumors. Thus, the present preparations and/or compositions and methods provide a tissue vaccine having utility in the prevention and/or inhibition of metastasis of prostate cancer.

Definitions:

[0033] The abbreviations and terms in the present disclosure are employed in contemplation of their fullest meaning consistent with the disclosed and claimed invention. The following brief explanations are entirely illustrative and neither exhaustively define nor limit the invention disclosed and claimed herein. The full meaning of the terms will be clear from an understanding of the invention based on contemplation of the disclosure as a whole in light of a full understanding of the pertinent arts.

[0034] METASTASIS: As set out in Hill, R. P., Chapter 11, Metastasis, pp178-195 in The Basic Science of Oncology, Tannock et al., Eds., McGraw-Hill, New York (1992), which is incorporated by reference herein in its entirety, metastasis is "The ability of cells of a cancer to disseminate and form new foci of growth at noncontiguous sites (i.e., to form metastases)".

[0035] Similarly, metastasis is described in Aznavoorian et al., Cancer 71: 1368-1383 (1993) (incorporated by reference in its entirety) as "The transition from in situ tumor growth to metastatic disease as defined by the ability of tumor cells of the primary site to invade local tissues and to cross tissue barriers. To initiate the metastatic process, carcinoma cells must first penetrate the epithelial basement membrane and then invade the interstitial stroma. For distant metastases, intravasation requires tumor cell invasion of the subendothelial basement membrane that must also be negotiated during tumor cell extravasation. The development of malignancy is also associated with tumor-induced angiogenesis that not only allows for expansion of the primary tumor, but also permits easy access to the vascular compartment due to defects in the basement membranes of newly formed vessels."

[0036] MALIGNANT: from the Latin roots *mal-* = "bad" and *-genus* = "born") is a medical term used to describe a severe and progressively worsening disease. The term is most familiar as a description of cancer. A *malignant tumor* may be contrasted with a non-cancerous benign tumor in that a *malignancy* is not self-limited in its growth, is capable of invading into adjacent tissues, and may be capable of spreading to distant tissues (metastasizing), while a *benign tumor* has none of those properties.

[0037] EPITHELIAL CELL ORIGIN: derived from an epithelial cell, of a tissue.

[0038] INHIBITION: inhibition of metastasis may be measured by many parameters in accordance with the present invention and, for instance, may be assessed by delayed appearance of secondary tumors, slowed development of primary or secondary tumors, decreased occurrence of secondary tumors, slowed or decreased severity of secondary effects

of disease, arrested tumor growth and regression of tumors, among others. In the extreme, complete inhibition, is referred to herein as prevention. In addition, the inhibition of metastasis may be identified by a reduction in metastatic foci present in the animal.

[0039] PREVENTION: in relation to metastasis, virtually complete inhibition, no metastasis if it had not occurred, no further metastasis if there had already been metastasis of a cancer. See INHIBITION.

[0040] COMPOSITIONS: Any non-toxic, inert and effective carrier may be used to formulate compositions of the present invention. Well known carriers used to formulate other therapeutic compounds for administration to humans particularly will be useful in the compositions of the present invention. Pharmaceutically acceptable carriers, excipients and diluents in this regard are well known to those of skill, such as those described in the MERCK INDEX, 11th Ed., Budavari et al., Eds., Merck & Co., Inc., Rahway, N.J. (1989), which is incorporated by reference herein in its entirety. Examples of such useful pharmaceutically acceptable excipients, carriers and diluents include distilled water, physiological saline, Ringer's solution, dextrose solution, Hank's solution and DMSO, which are among those preferred for use in the present invention.

[0041] In particular, for instance Mantile et al., J. Biol Chem. 268.: 20343-20351 (1993), incorporated herein by reference above, report on sterile formulations, that may also be useful in preparing the present compositions.

[0042] CANCERS: Methods and compositions of the present invention may be applied to the treatment of a variety of metastasis attendant a cancer, such as a cancer of epithelial cell origin. Among these are metastatic cancers of breast, lung, colon, bladder, prostate, gastrointestinal tract, endometrium, tracheal-bronchial tract, pancreas, liver, uterus, nasopharynges and the skin. In some aspects, the target cancer is prostate cancer of epithelial cell origin.

[0043] The following detailed discussion of prostate cancers is provided in illustration of the compositions and methods of the invention not only as to prostate cancers, but also other cancers that may be treated in analogous or identical fashion, in accordance with the present invention.

[0044] Metastatic potential of prostate cancers of epithelial cells origin can be inhibited by compositions and methods of the invention. In particular, metastasis of these cancers can be inhibited and/or eliminated by a preparation and/or composition comprising a whole tissue preparation of prostate tumor cells.

[0045] DOSE: The quantity of the whole cell vaccine for effective therapy will depend upon a variety of factors, including the type of cancer, means of administration, physiological state of the patient, other medications administered, and other factors.

[0046] Treatment dosages generally may be titrated to optimize safety and efficacy. Typically, dosage-effect relationships from *in vitro* initially can provide useful guidance on the proper doses for patient administration. Studies in animal models also generally may be used for guidance regarding effective dosages for treatment of metastatic cancers in accordance with the present invention.

[0047] These considerations, as well as effective formulations and administration procedures are well known in the art and are described in standard textbooks, such as GOODMAN AND GILMAN'S: THE PHARMACOLOGICAL BASES OF THERAPEUTICS, 8th Ed., Gilman et al. Eds. Pergamon Press (1990) and REMINGTON'S PHARMACEUTICAL SCIENCES, 17th Ed., Mack Publishing Co., Easton, Pa. (1990), both of which are incorporated by reference herein in their entirety.

[0048] It should be understood that various changes and modifications to the presently preferred embodiments described herein will be apparent to those skilled in the art. Such changes and modifications can be made without departing from the spirit and scope of the present subject matter and without diminishing its intended advantages. It is therefore intended that such changes and modifications be covered by the appended claims.

Example 1 – Materials and Methods

[0049] The present example provides a description of the materials and some of the particular methods employed in the present invention.

Materials and Methods

Animals

[0050] Male LW rats were obtained from the LW rat breeding colony at the University of Notre Dame. This line arose spontaneously from a breeding colony of germfree inbred Wistar rats [8]. Large, autochthonous tumors arise in the prostate/seminal vesicle complex; these tumors metastasize to the lungs via the lymphatics. The rats were housed in polycarbonate cages provided with hardwood shavings. A natural ingredient diet, Teklad L-485 (Harlan Teklad, Inc., Madison, WI) and fresh water were provided *ad libitum*. All animal studies were conducted in a facility accredited by the Association for Assessment and

Accreditation of Laboratory Animal Care, International; studies were approved by the University of Notre Dame Institutional Animal Care and Use Committee.

Vaccine Preparation

[0051] A glutaraldehyde-fixed tumor (GFT) cell vaccine was prepared as previously described [7]. Briefly, 3 g of a LW rat subcutaneous tumor, produced by administration of prostate adenocarcinoma (PAIII) cells which were originally isolated from an autochthonous, metastatic adenocarcinoma in a LW rat were harvested. The tissue was finely minced, and the cells separated using an 80-mesh screen to create a cell suspension in modified Eagle's medium (MEM). The cell suspension was then incubated in 2.5% glutaraldehyde (v/v) at 37°C for 60 min and then washed thoroughly with medium. This preparation was washed three (3) times in a modified Eagles media. The washed preparation was then suspended in a weight/volume ratio of 1:1 in a Freunds complete adjuvant. The "booster" tissue vaccine preparations were prepared in a Freund's incomplete adjuvant. The overall concentration of the preparations was about 1 mg (tissue)/ml, expressed as minced cell mass per ml adjuvant.

Example 2 -Tumor Metastasis Inhibition *In Vivo*

[0052] The present example is provided to demonstrate, among other things, the utility of the present invention for reducing and/or inhibiting tumor metastasis in an animal. The present example further demonstrates the utility of the present methods and preparations in animals and humans. The present example also demonstrates the utility of the invention as a method for inhibiting the metastasis of prostate cancer to the lung.

[0053] A group of sixty 3-4 month old, male LW rats were administered a single intravenous dose (30 mg/kg) of methylnitrosourea (MNU). At subsequent 2-month intervals, rats were anesthetized with an intramuscular dose of ketamine (90 mg/kg) and xylazine (10 mg/kg) and a silastic capsule containing 20 mg of testosterone propionate aseptically implanted into the subcutaneous space of the dorsal thorax. This method results in 70-80% of treated rats developing autochthonous, metastasizing hormone-refractory prostate tumors. These tumors developed within 8 months of MNU inoculation [8].

[0054] Beginning at 4 months after MNU inoculation, rats were palpated weekly for caudal intraabdominal masses indicative of prostate tumors. Based upon experience with this system, tumors may be readily detected when they reach approximately 0.5 cm in diameter. Tumor-bearing rats were randomly assigned to one of three groups: no treatment (11 rats); vaccination with MEM (10 rats); or vaccination with the GFT cell vaccine (19 rats). Animals

were vaccinated initially when tumors were first palpated and weekly until the time of euthanasia; the minimum number of vaccinations was two and the maximum number was nine, with an average of 3.6 overall (3.6 for GFT cell vaccinated rats and 3.7 for MEM-vaccinated rats).

[0055] Rats were euthanized by exsanguinations under halothane anesthesia when they became clinically debilitated. Debilitation was typically the result of hydroureter and hydronephrosis resulting from the tumor mass in the caudal abdomen. Four rats in the GFT cell vaccination group were observed to have complete regression of tumors; one was euthanized and found to have a renal abscess, the others were euthanized after four, six, or nine weekly vaccinations to confirm that the tumor had regressed.

Necropsy and histopathology

[0056] At the time of euthanasia, animals underwent necropsy. Prostate-seminal vesicle (PSV) complexes, including tumors, were weighed and fixed in 10% neutral buffered formalin for 24 hours and then placed in 70% isopropyl alcohol. Lungs were examined for the presence of any metastatic foci on pleural surface, typical of metastasis in this model. After fixation, PSV complexes were serially sectioned at 4-5 μm and stained with hematoxylin and eosin.

Splenocyte culture and supernatant cytokine production

[0057] Ten rats from groups vaccinated with either MEM or GFT cells were euthanized and single cell suspensions prepared by puncturing the splenic capsule with a thin syringe and squeezing the cells out. The single cell suspensions were washed in sterile PBS and then incubated on ice for 10 min in TRIS buffer with ammonium chloride. After two washes in RPMI 1640 medium, the cells were resuspended in culture medium and counted. Culture medium consisted of RPMI 1640 with 10% fetal calf serum, 100 U/ml penicillin, 100 μg/ml streptomycin, 50 μM 2-mercaptoethanol and 2 mM L-glutamine. To each well of sterile 24-well microplates, 3×10^6 splenocytes were added. Splenocytes were pulsed with either GFT cells (5×10^4 GFT cells per well) or sterile RPMI medium (as a control). Cultures were incubated for 72 h in 5% CO_2. Supernatants from cultured splenocytes were collected and frozen at -80°C until evaluated for cytokine production.

[0058] Concentrations of TNF-α and IFN-γ were measured by a general multiplex assay protocol in a sandwich immunoassay system employing microspheres and using the Luminex-100 (Luminex Corp., Austin, TX), a dual-laser flow analyzer. All cytokines were simultaneously measured from a single specimen.

Statistical analysis

[0059] Group differences in weights of PSV complexes were evaluated for significance using the Bonferroni multiple comparisons test with significance reached when $p \leq 0.05$. Results for presence of pulmonary metastases were compared between groups using the χ^2 test with two degrees of freedom. Differences were considered to be significant when $p \leq 0.05$.

Example 3 - Vaccination Inhibits Pulmonary Metastasis

[0060] The present example demonstrates the utility of the present invention for preventing and/or reducing metastasis in an animal. The present example also demonstrates the utility of the method for inhibiting and/or preventing metastasis, and in particular, metastasis of a tumor of prostate origin, to another organ. For example, metastasis may be prevented from progressing to the lung.

[0061] Vaccination with the GFT cell vaccine induces protective immunity against metastasis from autochthonous prostate cancer. To demonstrate this, non-vaccinated and MEM- and GFT cell-vaccinated rats were evaluated for the presence of metastasis in the lungs, the typical site of metastasis in the Lobund-Wistar rat model. Nearly all rats in the non-vaccinated (10/11) and the MEM-vaccinated (10/10) groups had metastatic foci in the lungs (Figure 2). In contrast, a significant ($p \leq 0.001$) reduction in the incidence of rats having pulmonary metastasis was noted in tumor-bearing GFT cell-vaccinated rats (5/15).

[0062] Serial sections of lung lobes from rats noted to be free of grossly observable metastatic foci showed that they were also free of histologic evidence of neoplasia.

Example 4 - Cell-Mediated Immune Response

[0063] The production of IFN-γ and TNF-α in supernatants of cultured splenocytes was examined in response to vaccination to demonstrate that vaccination with the whole cell tissue preparations as described herein (GFT treated) induced a Th1 response (T-cell, cell-mediated immune response). There exist several advantages to the demonstration of a cell-mediated response, rather than a humoral, or B-cell mediated response, with the whole cell preparations disclosed. In addition, the observation of this characteristic may be used to distinguish the present preparations and methods from other cancer and/or metastasis inhibiting preparations and methods.

[0064] Vaccine preparations and compositions provided here, that are specifically shown to enhance cell-mediated immunity verses humoral immunity, can be anticipated to further enhance an animal's protective immune response. Further, because cell-mediated immunity provides a variety of clinical benefits in patients, vaccination and/or treatment with the whole cell vaccine preparations provided in the present disclosure and variants thereof, yield substantial clinical advantage in the overall care and management of disease in the patient.

[0065] Figure 3 summarizes the cytokine content of supernatants from cultured splenocytes of rats from all treatment groups. Compared to media-treated controls, rats immunized with the GFT cell vaccine had significantly increased ($P \leq 0.001$) levels of IFN-γ and TNF-α, cytokines associated with a Th1 immune response. Further, splenocytes from rats vaccinated with the GFT cells had significantly increased ($P \leq 0.001$) levels of both cytokines when pulsed with GFT cells versus media.

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CLAIMS

The invention is claimed as follows:

1. A method for inhibiting metastasis in an animal comprising:

administering to an animal having a metastatic tumor a composition comprising a whole cell preparation; and

inhibiting metastasis of said tumor to an organ or tissue, wherein said whole cell preparation is inactivated and non-malignant and comprises tumor tissue cells and tumor connective tissue stroma.

2. The method of claim 1 wherein the metastasis is lung metastasis.

3. The method of claim 1 wherein the tumor whole cell preparation is an inactivated tumor whole cell preparation.

4. The method of claim 1 wherein the tumor whole cell preparation is a prostate tumor whole cell preparation.

5. The method of claim 1 wherein the animal is a non-human animal.

6. The method of claim 1 wherein the animal is a human.

7. The method of claim 4 wherein the whole cell preparation is prepared by a method comprising the steps of:

mincing a volume of tumor tissue and tumor stromal tissue harvested from an animal to provide a minced whole cell preparation;

processing said minced whole cell preparation so as to provide a suspension of whole tumor cells; and

inactivating said suspension of whole tumor cells so as to provide a non-malignant whole tumor cell preparation.

8. The method of claim 7 wherein the tumor tissue is prostate tumor tissue.

9. The method of claim 7 wherein the suspension of whole tumor cells is inactivated by a chemical treatment.

10. The method of claim 9 wherein the chemical treatment is a treatment with glutaraldehdye.

11. The method of claim 7 wherein a suspension of the minced cell preparation is provided by screening the minced cell preparation through an 80-mesh screen.

12. The method of claim 4 wherein the metastatic tumor is a autochthonous, metastasizing hormone-refractory prostate tumor.

13. The method of claim 2 wherein the metastasis that is inhibited is lung metastasis.

14. The method of claim 14 wherein the inhibition of lung metastasis is evidenced by the absence of metastatic foci on a pleural lung surface of an animal.

15. The method of claim 1 wherein the metastasis that is inhibited is bone metastasis.

16. A whole cell preparation capable of inhibiting metastasis comprising tumor tissue and tumor stromal tissue, said preparation being essentially free of malignant cell activity.

17. The whole cell preparation of claim 16 wherein said tumor tissue is prostate tumor tissue.

18. The whole cell preparation of claim 16 wherein the metastasis is lung metastasis.

19. The whole cell preparation of claim 16 prepared by a process comprising the steps of:

mincing a volume of tumor tissue and tumor stromal tissue to provide a minced whole cell preparation of tumor tissue and tumor stromal tissue; and

processing said minced whole cell preparation so as to provide a suspension of whole cells essentially free of malignant cell activity.

20. The whole cell preparation of claim 19 wherein said tumor tissue is prostate tumor tissue.

FIG. 1

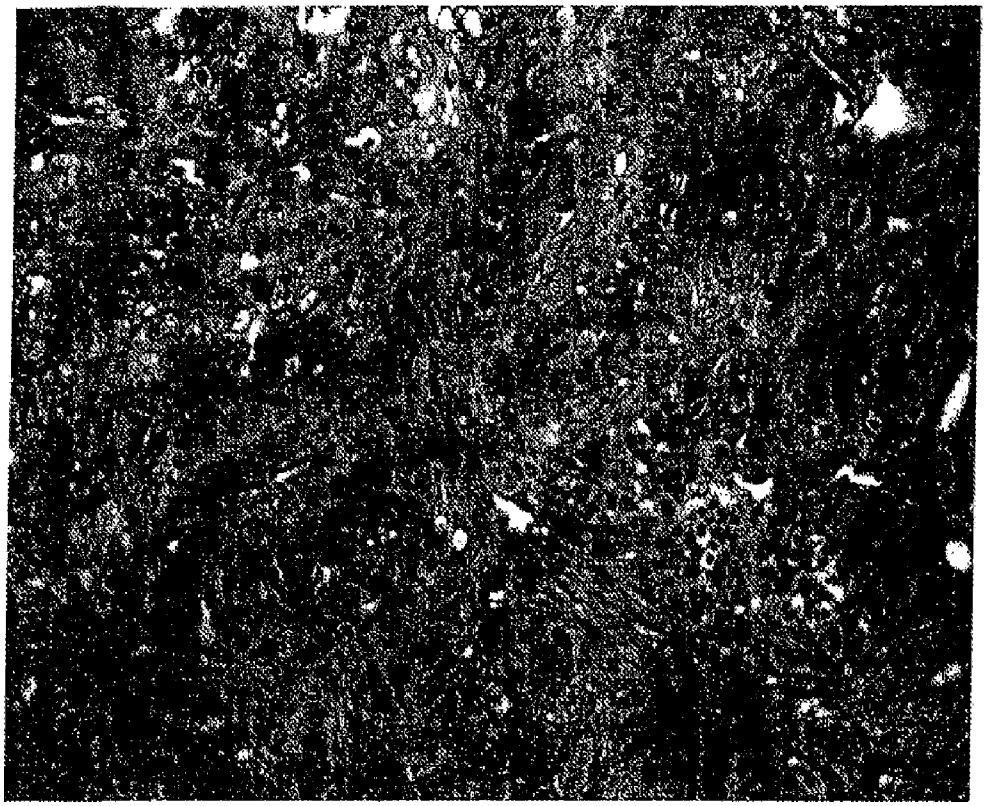


FIG. 2

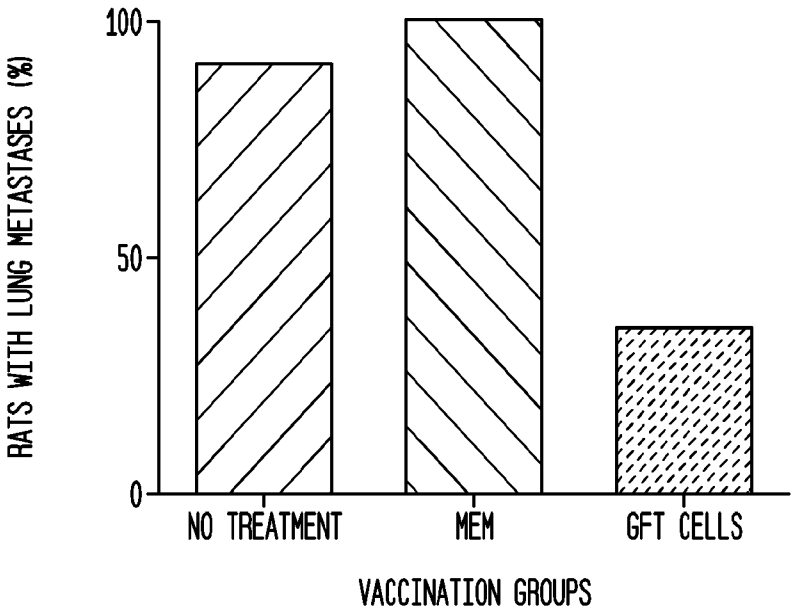
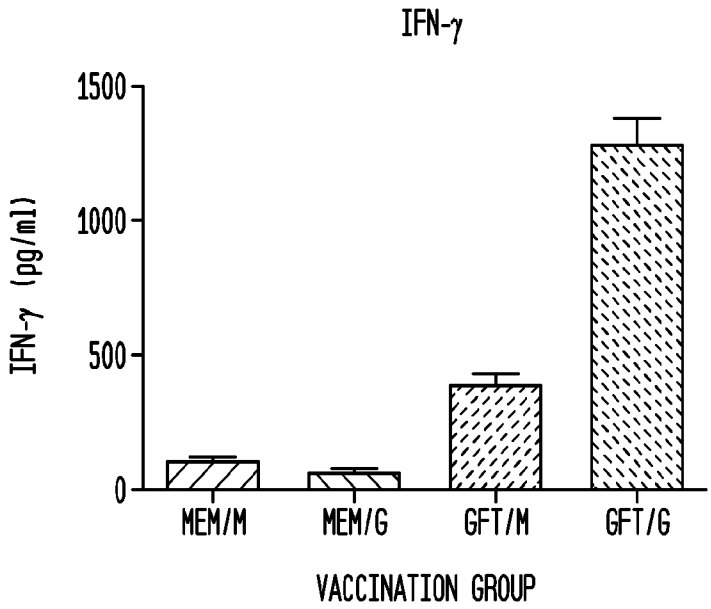

100
50
0
RATS WITH LUNG METASTASES (%)
NO TREATMENT
MEM
GFT CELLS
VACCINATION GROUPS

FIG. 3A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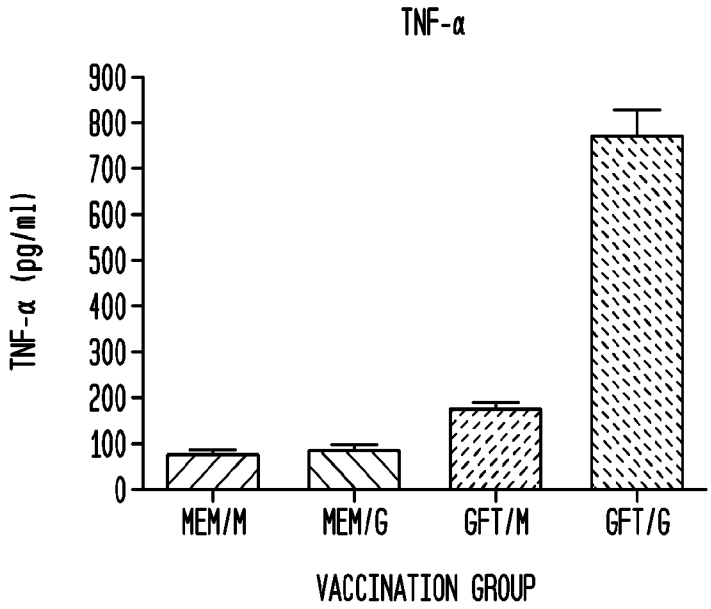
1000
500
0
MEM/M
MEM/G
GFT/M
GFT/G
VACCINATION GROUP

FIG. 3B


TNF-α
TNF-α (pg/ml)
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700
600
500
400
300
200
100
0
MEM/M
MEM/G
GFT/M
GFT/G
VACCINATION GROUP

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 09/35062

A. CLASSIFICATION OF SUBJECT MATTER
IPC(8) - A61K 31/41 (2009.01)
USPC - 514/382
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
USPC-514/382

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched
USPC-514/382; 435/375;424/93.7 and NPL

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
PubWest (US Patent, PgPub, OCR: classification), Google, GoogleScholar; metastasis tumor cell prostate glutaraldehyde bone cancer malignant mesh prostate autochthonous inactivate hormone whole cell vaccine GM-CSF glutaraldehyde-fixed tumor cell vaccine

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X -- Y	US 6,227,368 B1 (Hiserodt et al.) 21 Aug 2001 (21.08.2001) abstract, col. 11, ln 42-50; col. 22, ln 58-61; col 22, ln 58 - col. 23, ln 8; col. 33, ln 35-45; col. 14, ln 30-39; col. 26, ln 53-55; col. 15, ln 14-16;col. 12, ln 29-38	1-10,12-20 ---------- 11
Y	US 2001/0006631 A1 (Hiserodt et al.) 05 July 2001 (05.07.2001) para [0287]	11

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

* Special categories of cited documents:
"A" document defining the general state of the art which is not considered to be of particular relevance
"E" earlier application or patent but published on or after the international filing date
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"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
12 July 2009 (12.07.2009)	22 JUL 2009
Name and mailing address of the ISA/US Mail Stop PCT, Attn: ISA/US, Commissioner for Patents P.O. Box 1450, Alexandria, Virginia 22313-1450 Facsimile No. 571-273-3201	Authorized officer: Lee W. Young PCT Helpdesk: 571-272-4300 PCT OSP: 571-272-7774

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