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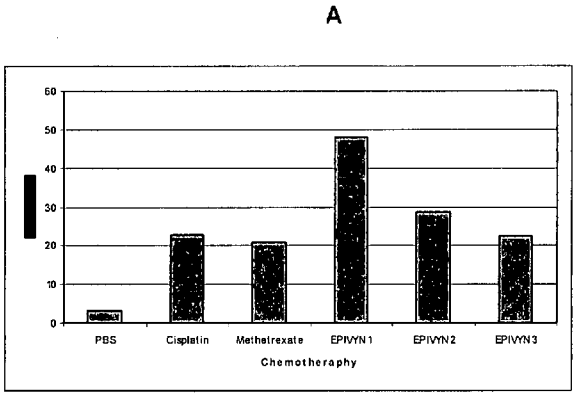
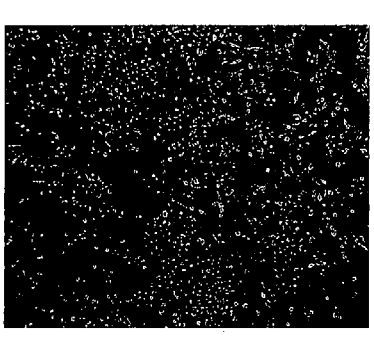
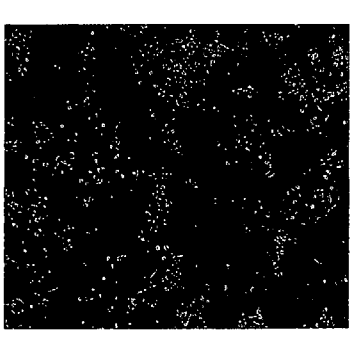



(57) Abstract: Methods and compositions for therapy of cancer are provided. Compositions comprising therapeutically effective amounts of gold(III) chloride are provided. Anti-proliferative, apoptotic and cytotoxic effects of specific gold(III) chlorides, such as hydrogen tetrachloroaurate and auric acid, are disclosed. Methods for preparation of gold(III) chlorides having anti-cancer effects are provided. Methods and compositions for therapy, treatment and prevention of cancer comprising gold(III) chlorides are provided. Methods for preparing copositions that induce apoptosis by the caspase pathway are provided.

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# GOLD(III) CHLORIDE COMPOSITIONS FOR CANCER

## TECHNICAL FIELD OF THE INVENTION

**[0001]** This invention relates generally to the field of using compositions comprising aurum muriaticum for ameliorating cancer. More specifically, the invention provides methods and compositions comprising auric chloride and/or hydrogen tetrachloroaurate for use in prevention and therapy of cancer, preventing the metastasis of cancer and arresting cell growth. In one aspect, the invention provides methods and compositions used to treat solid tumor cancers such as a carcinoma, melanoma, lymphoma, or sarcoma by administering an effective amount of compositions comprising auric chloride or hydrogen tetrachloroaurate.

## BACKGROUND OF THE INVENTION

**[0002]** It is estimated by the World Health Organization that about 10 million new cancer cases are occurring now annually around the world. That number is expected to reach 15 million by the year 2015, with two thirds of these new cases occurring in developing countries (World Health 48:22, 1995). For example, it is estimated that there is about 600,000 new cases of lung cancer per year worldwide; approaching 1 million new cases of breast cancer per year; and for head and neck cancer (the sixth most frequently occurring cancer worldwide) an incidence of 500,000 new cases annually. The National Cancer Institute of the United States estimates the overall annual costs for cancer at $107 billion. Treatment costs account for approximately $40 billion. Breast cancer is one of the most significant diseases that affects women. An estimated 212,920 new cases of invasive breast cancer are expected to occurs among women in the U.S. during 2006. Breast cancer is the most frequently diagnosed cancer in women. An estimated 234,460 new cases of prostate cancer in men, 92,700 (men) and 81,770 (women) new cases of lung cancer, 148,000 new cases of colorectal cancer in men and women are predicted for 2006. Cancer killed 6.7 million people around the world in 2002 and this figure is expected to rise to 10.3 million in 2010. (Cancer Facts and Figures 2006, American Cancer Society, Inc. ©2006).

**[0003]** Any tissue of an organism can end up proliferating uncontrollably. Tumors that form in epithelial cells are called carcinomas. Cells of the mucous membrane of intestines, stomach, lungs, uterus or bladder, cells of mammary glands, or skin and cells of certain inner organs like liver or kidneys are types of tissue in which growth can originate. These tumors constitute about 90 percent of all malignant growths. Thus, a majority of human cancers

originate from epithelial tissue. A common cancer of epithelial origin is nonmelanoma skin cancer (NMSC), including basal cell carcinoma (BCC) and squamous cell carcinoma (SCC), with more than 700,000 new cases diagnosed each year in the United States. Similar cancers are also seen in non-human animals such as domesticated animals and pets, including cats and dogs. BCC is rarely life-threatening because it is slow growing and is mostly localized. Unlike BCC, SCC metastasizes at a rate of 2% to 6% over several years after initial diagnosis. A highly malignant form invades and destroys tissue, and then metastasizes, initially to a regional lymph node before more distant organs such as the lung or brain are affected. SCC is commonly encountered in a number of epithelial tissues, including the oral cavity, esophagus, larynx, bronchi, intestines, colon, genital tract, and skin.

**[0004]** The three most prevalent forms of cancer therapy are surgery, chemotherapy and radiation, or a combination thereof. Following metastasis, localized therapies like surgery and radiation are no longer sufficient to stop the cancer from spreading. In this situation chemotherapy is the therapy of choice. The various kinds of cancer respond differently. In some cases the disease can be permanently cured, in others it is at least possible to reduce the size of the tumor, thus extending the patient's lifetime.

**[0005]** Several chemotherapeutic agents are in use in the treatment of cancer, including alkylating agents, antimetabolites antagonists, anticancer antibiotics, and plant-derived anticancer agents. Examples of "alkylating agents" include nitrogen mustard, nitrogen mustard-N-oxide hydrochloride, chlorambutyl, cyclophosphamide, ifosfamide, thiotepa, carboquone, improsulfan tosylate, busulfan, nimustine hydrochloride, mitobronitol, melphalan, dacarbazine, ranimustine, estramustine phosphate sodium, triethylenemelamine, carmustine, lomustine, streptozocin, pipobroman, etoglucid, carboplatin, cisplatin, miboplatin, nedaplatin, oxaliplatin, altretamine, ambamustine, dibrospidium hydrochloride, fotemustine, prednimustine, pumitepa, ribomustin, temozolomide, treosulphan, trophosphamide, zinostatin stimalamer, carboquone, adozelesin, cystemustine, and bizelesin. Examples of "antimetabolites" include mercaptopurine, 6-mercaptopurine riboside, thioinosine, methotrexate, enocitabine, cytarabine, cytarabine ocfosfate, ancitabine hydrochloride, 5-FU drugs (e.g., fluorouracil, tegafur, UFT, doxifluridine, carmofur, gallocitabine, emmitefur), aminopterine, leucovorin calcium, tabloid, butocine, folinate calcium, levofolinate calcium, cladribine, emitefur, fludarabine, gemcitabine, hydroxycarbamide, pentostatin, piritrexim, idoxuridine, mitoguazone, thiazophrine, and ambamustine, etc. Examples of "anticancer antibiotics" include actinomycin-D, actinomycin-

C, mitomycin-C, chromomycin-A3, bleomycin hydrochloride, bleomycin sulfate, peplomycin sulfate, daunorubicin hydrochloride, doxorubicin hydrochloride, aclarubicin hydrochloride, pirarubicin hydrochloride, epirubicin hydrochloride, neocarzinostatin, mithramycin, sarcomycin, carzinophilin, mitotane, zorubicin hydrochloride, mitoxantrone hydrochloride, and idarubicin hydrochloride, etc. Examples of "plant-derived anticancer agents" include etoposide phosphate, vinblastine sulfate, vincristine sulfate, vindesine sulfate, teniposide, paclitaxel, docetaxel, and vinorelbine, etc. For certain types of cancer, chemotherapy has been capable of rendering patients with responsive tumours free of disease. However, this responsive category does not include the most frequently encountered forms of malignant tumours.

**[0006]** The most common types of cancer in Western populations are colon, lung and breast cancer. Each of these can be treated to some extent with existing chemotherapy, with different drugs being used preferentially for each type of malignancy (for instance, doxorubicin, cyclophosphamide and methotrexate for breast cancer, 5-fluorouracil for colon cancer), but response rates are not good. In addition, melanoma is a disease which is increasing in incidence at an alarming rate among fair-skinned populations. In melanoma, only 25-30% of patients with disseminated disease respond to treatment, and only 5-10% sustain durable remission (Evans B. D., et al., Proc. Am. Soc. Clin. Oncol. 1990, 9, 276). Therefore there is a need for new types of cancer therapy, and a need for such treatments for the above cancers in particular.

**[0007]** There is increasing interest in the use of metal-containing compounds in medicine. (MJ Abrams and BA Murrer Science 6 August 1993 261: 725-730). Gold has been exploited for its putative medical properties throughout the history of civilisation (RV Parish, Interdisc. Sci. Rev., 1992, 17, 221). The earliest medical use of gold can be traced back to the Chinese in 2500 BC. A gold cordial could be found in the new pharmacopoeias of the 17th century and was advocated by Nicholas Culpepper for the treatment of ailments caused by a decrease in the vital spirits, such as melancholy, fainting, fevers, and falling sickness. Later in the 19th century a mixture of gold chloride and sodium chloride, "muriate of gold and soda ($Na[AuCl_4]$)," was used to treat syphilis. Aurum muriaticum natronatum (chloride of gold and sodium) also has been reported as remedy for sore throat symptoms. The use of gold compounds in modern, twentieth century medicine began with the discovery in 1890 by the German bacteriologist Robert Koch that gold cyanide $K[Au(CN)_2]$ was bacteriostatic towards the tubercle bacillus (Mycobacterium tuberculosis). Gold therapy for tuberculosis was

subsequently introduced in the 1920s (Benedek TG, Journal of the History of Medicine and Allied Sciences 2004 59(1):50-89). Development of water-soluble gold-containing compounds has been the subject of intensive research because of their potential in medical applications. (Kamei, H.et al. Cancer Biotherapy & Radiopharmaceuticals. 1998, 13:403). A gold-containing compound, auranofin, [(2,3,4,6-tetra-O-aceytl-1-thio-ß-D- glucopyranosato-S)-(triethylphosphine) gold(I)] has been shown to be effective for the treatment of rheumatoid arthritis (Berglof F. E. et al. J. Rheumatol. 1978, 5:68).

**[0008]** The discovery of the antitumour activity of cisplatin, cis-$[PtCl_2(NH_3)_2]$ in 1969 prompted the search for other metal-containing antitumour drugs (C.P. Shaw III Metal Compounds in Cancer Therapy, *ed.* S.P. Pricker, Chapman and Hall, London, 1994, p. 46). There has been intense efforts to identify a gold antitumor drug. Early indications noted that auranofin, used to treat rheumatoid arthritis, had limited antitumour activity in *in vitro* systems and in the P388 leukemia *in vivo*, however, it was nearly inactive against solid tumours. (GH Reem and NH Yeh, Science 27 July 1984 225: 429-430; Mirabelli CK et al. Cancer Res. 1985 Jan;45(1):32-39). Several other gold(I) phosphines with thiosugar ligands were investigated with similar results. (M. J. McKeage, et al., Cancer Chemother. Pharmacol., 2000, 46:343).

**[0009]** Indications were also noted with a series of digold phosphine complexes such as [dppe(AuCl)2]. The DPPE (N,N-diethyl-2-[4-(phenylmethyl)phenoxy] ethanamine) ligand itself is an intracellular histamine (HA) antagonist with chemopotentiating and cytoprotective properties, and has been the subject of clinical trials in breast and prostate cancer. (Brandes LJ, Cancer Chemother Pharmacol. 2000; 45(4):298-304). It is possible that the gold serves to protect the DPPE ligand from oxidation and aids in the delivery of the active species, however, there is substantial evidence to support a direct role for the gold in the anticancer activity of this complex. The proposed mechanism of action for $[Au(dppe)_2]Cl$ was the formation of DNA-protein crosslinks; the lack of affinity of Au(I) for oxygen and nitrogen-containing ligands resulting in poor reactivity with the bases of DNA (Berners, S. J. et al. J. Inorg. Chem. 1987, 26:3074). Although this compound had marked activity against peritoneal cancer cells only when administered intraperitonially, $[Au(dppe)_2]Cl$ showed no activity against 14 other tumour models. (Simon, T. M. Cancer Res. 1981, 41(1):94-97; Mirabelli CK et al. Cancer Res. 1985 Jan;45(1):32-9. ). Clinical development of $[Au(dppe)_2]Cl$ was precluded following the identification of severe cardiac, hepatic and vascular toxicities during pre-clinical toxicology studies (Hoke et al., 1989, Toxicol. Appl.

Pharmacol, 1989, 100:293). $[Au(dppe)_2]^+$ is a cation with eight hydrophobic phenyl substituents and these properties promote dissolution of the complex in the mitochondrial membrane where it disrupts mitochondrial function by uncoupling oxidative phosphorylation. (Hoke et al., 1988, Biol. Chem. 262 11:203).

**[0010]** One avenue of investigation has been to synthesize gold complexes containing ligands with known antitumour activity (C.P. Shaw III, in Metal Compounds in Cancer Therapy, *ed.* S.P. Pricker, Chapman and Hall, London, 1994, p. 46). Examples of this are a series of $Ph_3PAu(I)$-nucleotide complexes containing ligands such as 5-fluorouracil and 6-mercaptopurine; phosphinogold(I) ferrocene complexes such as [μ-1,1-bisbis(diphenyl phosphino)ferrocene]bis(chlorogold(I)], a series of novel nitrogen containing phosphinogold(I) ferrocenes (20), and a gold(III) complex of streptonigrin, a substituted 7-amino-quinoline-5,8,-dione (M. Viotte, et al. Metal Based Drugs, 1995, 2:311; C.P. Shaw III. *id.*). U.S. Patent No. 6,989,158 (Katti et al.) discloses hydroxyalkyl phosphine complexes of gold(I) as a chemotherapeutic agent. U.S. Patent No. 6,159,957 (Berners-Price et al.) discloses pyridyl-substituted gold(I)-diphosphine complexes as chemotherapeutic agents. A bifunctional antitumor agent, Au(III)-streptonigrin complex, with gold in the trivalent state and having the ablity to inhibit glutathione reductase activity, has been shown to exhibit antitumor activity against P-388 leukemia. (Moustatih A. et al. J Med Chem. 1989 Jul;32(7):1426-1431). Anti-angiogenic properties of gold nanoparticles formed from tetrachloroauric acid have been reported. (Mukherjee, P. *et al.* Clin. Cancer Res. 11(9):n 3530-3534 (2005).

**[0011]** Gold(III) complexes have not been as thoroughly investigated as gold(I) complexes, primarily because of their reactivity. Gold(III) is isoelectronic (d8) with platinum(ll) and likewise forms square planar complexes. It has been speculated that such complexes would have anti-tumor activity similar to cisplatin. A group of square planar gold(III) complexes, all containing at least two gold-chloride bonds in cis-position, isostructural and isoelectronic with platinum(II) complexes, showed significant cytotoxic effects when tested for their in vitro cytotoxicity on a panel of established human tumor cell lines. (Calamai P., et al. Anticancer Drug Des. 1998 Jan;13(1):67-80). While cisplatin is widely used as a chemotherapeutic agent in the treatment of human cancer its use is generally associated with severe toxic side effects that include decrease in blood cell numbers, kidney dysfunction, etc. Therefore, cisplatin cannot be used in treating cancer patients for longer periods of time.

**[0012]** Lack of identification of an effective gold-containing antitumor agent relates to problems with toxicity and level of activity toward specific tumors. Therefore, it is desirable to develop an anti-tumor gold compound which is not toxic to the patient.

## SUMMARY OF THE INVENTION

**[0013]** The present invention discloses Gold(III) chloride formulations capable of inducing profound apoptosis in cancer cells. The invention relates to a pharmacological anti-cancer activity of the formulation that displays at least equivalent and/or superior apoptotic effects in comparison to major oncological drugs including methotrexate, cisplatin and doxorubicin. This breakthrough invention enables a novel class of anti-cancer drugs that should clinically elicit low to very low toxicity in normal cells while actively inducing apoptosis in cancer cells.

**[0014]** The present invention relates to the novel finding that certain low toxicity chlorides of gold (Au[III]) induce apoptosis in cancer cells leading to a cytotoxic effect in tumors. Two typical gold(III) chloride compounds according to the invention are: (i) Gold Trichloride, Acid or Hydrogen tetrachloroaurate ($HAuCl_4$); and (ii) Gold Trichloride or Auric Chloride ($AuCl_3$).

**[0015]** These gold(III) chlorides, generally known as aurum muriaticum, and salts, esters, conjugates and derivatives thereof have been used by humans for many years without toxic side-effects. (Anshutz E.P., Pathogénésies de l'an 1900: New, Old And Forgotten Remedies. Philadelphia, January 2, 1900. Copyright © Robert Séror 1999; available at www.homeoint.org/seror/patho1900/aurummn.htm; downloaded September 25, 2006).

**[0016]** The invention provides novel compositions, extracts and compounds comprising gold(III) chlorides and their methods for manufacture and preparation. Use of such compounds in the treatment and therapy of cancer are also provided as are methods for preparation and formulation of compositions and medicaments for treatment using the compositions comprising gold(III) chlorides of this invention.

**[0017]** In one aspect, the invention relates to methods for using compositions comprising gold(III) chlorides for treatment of conditions characterized abnormal mammalian cell proliferation. In other aspects, abnormal mammalian cell proliferation includes sarcomas, melanomas and metastatic tumors.

**[0018]** In one aspect, the invention relates to methods for using compositions comprising gold(III) chlorides for treatment of conditions characterized abnormal epithelial cell proliferation. Such conditions include dermatological disorders, precancerous lesions, as well as tumors of epithelial origin.

**[0019]** In one aspect, the invention relates to methods for using compositions comprising gold(III) chlorides for use in adjuvant chemotherapy with surgery or radiation therapy.

**[0020]** In one aspect, the invention relates to methods for using compositions comprising gold(III) chlorides for use in combination chemotherapy with other chemotherapeutic agents. In one embodiment, the combinations comprise amounts of the gold(III) chlorides and the chemotherapeutic agents sufficient to exhibit synergy in efficacy or a measured activity.

**[0021]** In one aspect, the invention relates to methods and compositions comprising gold(III) chlorides for use in treatments having cytotoxic effects on tumor cells.

**[0022]** In one aspect, the invention relates to methods and compositions comprising gold(III) chlorides for use in treatments having apoptotic effects on cancer cells.

**[0023]** In one aspect, the invention relates to methods and compositions comprising gold(III) chlorides for use in treatments having anti-angiogenic effects on cells.

**[0024]** In one aspect, the invention relates to methods and compositions comprising gold(III) chlorides for use in prevention of cancer.

**[0025]** Formulations and compositions comprising gold(III) chlorides are provided. Formulations and methods suitable for administering the gold(III) chlorides by oral, parenteral, enteral, intraperitoneal, topical, transdermal (e.g., using any standard patch), ophthalmic, nasally, local, non-oral, such as aerosal, inhalation, subcutaneous, intramuscular, buccal, sublingual, rectal, vaginal, intra-arterial, and intrathecal routes are provided.

**[0026]** Methods for preparation of compositions comprising gold(III) chloride compounds, such as hydrogen tetrachloroaurate and auric chloride, and pharmaceutically acceptable compositions and formulations thereof, wherein the compositions are effective in the preparation of therapeutics and medicaments for the treatment of cancer, etc. are also provided. Also provided are unit dosage forms of compositions described herein, articles of manufacture comprising the inventive compositions or unit dosage forms in suitable packaging, and kits comprising the compositions. The invention also provides methods of making and using these compositions as described herein.

**[0027]** The present invention and other objects, features, and advantages of the present invention will become further apparent in the following Detailed Description of the Invention and the accompanying Figures and embodiments.

## BRIEF DESCRIPTION OF THE FIGURES

**[0028]** Figure 1A shows PBS-treated (control group) cells displaying a basal level of apoptosis with an apoptosis percentage of 3.1%.

**[0029]** Figure 1B shows Methotrexate (10μg/ml) treated cells displaying a positive apoptosis percentage of 21%.

**[0030]** Figure 1C shows Adriamycin (5μg/ml) treated cells displaying a positive apoptosis percentage of 64%.

**[0031]** Figure 1D shows Cisplatin (10μg/ml) treated cells displaying a positive apoptosis percentage of 23.1%.

**[0032]** Figure 1E shows Hydrogen tetrachloroaurate treated cells displaying a positive apoptosis percentage of 32.3%.

**[0033]** Figure 2A shows control group displaying a basal level of apoptosis with an apoptosis percentage of 2.2%.

**[0034]** Figure 2B shows a population of hydrogen tetrachloroaurate-treated cells displaying a positive apoptosis percentage of 12.7%

**[0035]** Figure 2C shows a population of hydrogen tetrachloroaurate-treated cells displaying a positive apoptosis percentage of 22.5%

**[0036]** Figure 2D shows a population of hydrogen tetrachloroaurate-treated cells displaying a positive apoptosis percentage of 31.7%

**[0037]** Figure 2E shows a population of hydrogen tetrachloroaurate-treated cells displaying a positive apoptosis percentage of 48.3%.

**[0038]** Figure 3 shows blood toxicology data for gold (III) compositions measuring WBC, RBC, HGB, HCT, MCHC and MCH. Group 1BS or 1B or1 was positive injection control had saline (NaCl 0. 9%) injection only; Group 2Y or 2 had high dosage Gold(III) solution (1 ng/ml); Group 3B or 3 had low dosage Gold(III) solution (1 pg/ml); and Group 4G or 4 had no injections (non-treatment control).

**[0039]** Figures 4A and 4B show blood (hepatic and plasma) toxicology data for gold (III) compositions measuring ALP, ALT, TP, AST, CPK, BUN, GLU, GLOB, and B/C. Group 1BS or 1B or1 was positive injection control had saline (NaCl 0. 9%) injection only; Group 2Y or 2 had high dosage Gold(III) solution (1 ng/ml); Group 3B or 3 had low dosage Gold(III) solution (1 pg/ml); and Group 4G or 4 had no injections (non-treatment control).

**[0040]** Figures 5A and 5B show blood toxicology data for gold (III) compositions measuring the electrolytes Ca, PHOS, $CO_2$, K, Na, Cl and Mg. Group 1BS or 1B or1 was positive injection control had saline (NaCl 0. 9%) injection only; Group 2Y or 2 had high dosage Gold(III) solution (1 ng/ml); Group 3B or 3 had low dosage Gold(III) solution (1 pg/ml); and Group 4G or 4 had no injections (non-treatment control).

**[0041]** Figure 6 shows blood lipid profile toxicology data for gold (III) compositions measuring LDH, HDL AND CHOL. Group 1BS or 1B or1 was positive injection control had saline (NaCl 0. 9%) injection only; Group 2Y or 2 had high dosage Gold(III) solution (1 ng/ml); Group 3B or 3 had low dosage Gold(III) solution (1 pg/ml); and Group 4G or 4 had no injections (non-treatment control).

**[0042]** Figure 7A shows relative apoptotic activities of methotrexate, cisplatin and gold (III) compositions, Epivyn 1, Epivyn 2 and Epivyn 3. Figures 7B-1 (PBS control) and 7B-2 (gold(III) chloride solution) show the cytotoxic effect on head and neck cancer cell lines in tissue culture of gold (III) compositions.

## DETAILED DESCRIPTION OF THE INVENTION

**[0043]** The invention relates to compositions comprising gold(III) chlorides, their salts, esters, derivatives and complexes. Chemical Names of two gold(III) chloride compounds are: (i) Gold Trichloride, Acid or hydrogen tetrachloroaurate ($HAuCl_4$); and (ii) Gold Trichloride or Auric Chloride ($AuCl_3$). Gold(III) chlorides have also been referred in the literature as Aurum Muriaticum, Gold Chloride, Auric Cloride, Chloride of Gold, Tetrachloroauric Acid, Gold trichloride, Chloroauric Acid, Auric Chloride, and Hydrogen tetrachloroaurate.

**[0044]** Hydrogen tetrachloroaurate is very soluble in water, alcohol; and soluble in ether. Gold trichloride has the molecular formula $AuCl_3$ (exists as $Au_2Cl_6$) and molar mass of 303.325 g/mol (anhydrous). Gold trichloride is soluble in water, alcohol and ether.

**[0045]** The invention further provides methods for treating a subject having a condition characterized by an abnormal mammalian cell proliferation. An abnormal mammalian cell

proliferation disorder or condition, as used herein, refers to a localized region of cells (e.g., a tumor) which exhibit an abnormal (e.g., increased) rate of division as compared to their normal tissue counterparts.

**[0046]** Conditions characterized by an abnormal mammalian cell proliferation, as used herein, include but are not limited to conditions involving solid tumor masses of benign, pre-malignant or malignant character. Although not wishing to be bound by a particular theory or mechanism, some of these solid tumor masses arise from at least one genetic mutation, some may display an increased rate of cellular proliferation as compared to the normal tissue counterpart, and still others may display factor independent cellular proliferation. Factor independent cellular proliferation is an example of a manifestation of loss of growth control signals which some, if not all, tumors or cancers undergo.

**[0047]** Hydrogen tetrachloroaurate and auric chloride have affinity for epithelial tissues. All carcinoma tumors (which make up most cancer tumors) are composed of malignant cells of epithelial origin. In one aspect, the invention provides a method for treating subjects having a condition characterized by an abnormal epithelial cell proliferation. Epithelial cells are cells occurring in one or more layers which cover the entire surface of the body and which line most of the hollow structures of the body, excluding the blood vessels, lymph vessels, and the heart interior which are lined with endothelium, and the chest and abdominal cavities which are lined with mesothelium. Examples of epithelium include anterius corneae, anterior epithelium of cornea, Barrett's epithelium, capsular epithelium, ciliated epithelium, columnar epithelium, epithelium corneae, corneal epithelium, cubical epithelium, cubical epithelium, cuboidal epithelium, epithelium eductus semicircularis, enamel epithelium, false epithelium, germinal epithelium, gingival epithelium, glandular epithelium, glomerular epithelium, laminated epithelium, epithelium of lens, epithelium lentis, mesenchymal epithelium, olfactory epithelium, pavement epithelium, pigmentary epithelium, pigmented epithelium, protective epithelium, pseudostratified epithelium, pyramidal epithelium, respiratory epithelium, rod epithelium, seminiferous epithelium, sense epithelium, sensory epithelium, simple epithelium, squamous epithelium, stratified epithelium, subcapsular epithelium, sulcular epithelium, tessellated epithelium, transitional epithelium.

**[0048]** One category of conditions characterized by abnormal epithelial cell proliferation is proliferative dermatologic disorders. These include conditions such as keloids, seborrheic keratosis, papilloma virus infection (e.g. producing verruca vulbaris, verruca plantaris, verruca plana, condylomata, etc.) and eczema.

**[0049]** An epithelial precancerous lesion is a skin lesion which has a propensity to develop into a cancerous condition. Epithelial precancerous skin lesions also arise from other proliferative skin disorders such as hemangiomas, keloids, eczema and papilloma virus infections producing verruca vulbaris, verruca plantaris and verruca planar. The symptoms of the epithelial precancerous lesions include skin-colored or red-brown macule or papule with dry adherent scales. Actinic keratosis is the most common epithelial precancerous lesion among fair skinned individuals. It is usually present as lesions on the skin which may or may not be visually detectable. The size and shape of the lesions varies. It is a photosensitive disorder and may be aggravated by exposure to sunlight. Bowenoid actinic keratosis is another form of an epithelial precancerous lesion. In some cases, the lesions may develop into an invasive form of squamous cell carcinoma and may pose a significant threat of metastasis. Other types of epithelial precancerous lesions include hypertrophic actinic keratosis, arsenical keratosis, hydrocarbon keratosis, thermal keratosis, radiation keratosis, viral keratosis, Bowen's disease, erythroplaquia of queyrat, oral erythroplaquia, leukoplakia, and intraepidermal epithelialoma.

**[0050]** Another category of conditions characterized by abnormal epithelial cell proliferation is tumors of epithelial origin. Thus, in one aspect, the invention provides a method for treating subjects having epithelial tumors. Epithelial tumors are known to those of ordinary skill in the art and include, but are not limited to, benign and premalignant epithelial tumors, such as breast fibroadenoma and colon adenoma, and malignant epithelial tumors. Malignant epithelial tumors include primary tumors, also referred to as carcinomas, and secondary tumors, also referred to as metastases of epithelial origin. Carcinomas intended for treatment with the methods of the invention include, but are not limited to, acinar carcinoma, acinous carcinoma, alveolar adenocarcinoma (also called adenocystic carcinoma, adenomyoepithelioma, cribriform carcinoma and cylindroma), carcinoma adenomatosum, adenocarcinoma, carcinoma of adrenal cortex, alveolar carcinoma, alveolar cell carcinoma (also called bronchiolar carcinoma, alveolar cell tumor and pulmonary adenomatosis), basal cell carcinoma, carcinoma basocellulare (also called basaloma, or basiloma, and hair matrix carcinoma), basaloid carcinoma, basosquamous cell carcinoma, breast carcinoma, bronchioalveolar carcinoma, bronchiolar carcinoma, bronchogenic carcinoma, cerebriform carcinoma, cholangiocellular carcinoma (also called cholangioma and cholangiocarcinoma), chorionic carcinoma, colloid carcinoma, comedo carcinoma, corpus carcinoma, cribriform carcinoma, carcinoma en cuirasse, carcinoma cutaneum, cylindrical carcinoma, cylindrical

cell carcinoma, duct carcinoma, carcinoma durum, embryonal carcinoma, encephaloid carcinoma, epibulbar carcinoma, epidermoid carcinoma, carcinoma epitheliale adenoides, carcinoma exulcere, carcinoma fibrosum, gelatiniform carcinoma, gelatinous carcinoma, giant cell carcinoma, gigantocellulare, glandular carcinoma, granulosa cell carcinoma, hair-matrix carcinoma, hematoid carcinoma, hepatocellular carcinoma (also called hepatoma, malignant hepatoma and hepatocarcinoma), Hurthle cell carcinoma, hyaline carcinoma, hypemephroid carcinoma, infantile embryonal carcinoma, carcinoma in situ, intraepidermal carcinoma, intraepithelial carcinoma, Krompecher's carcinoma, Kulchitzky-cell carcinoma, lenticular carcinoma, carcinoma lenticulare, lipomatous carcinoma, lymphoepithelial carcinoma, carcinoma mastitoides, carcinoma medullare, medullary carcinoma, carcinoma melanodes, melanotic carcinoma, mucinous carcinoma, carcinoma muciparum, carcinoma mucocellulare, mucoepidermoid carcinoma, carcinoma mucosum, mucous carcinoma, carcinoma myxomatodes, nasopharyngeal carcinoma, carcinoma nigrum, oat cell carcinoma, carcinoma ossificans, osteoid carcinoma, ovarian carcinoma, papillary carcinoma, periportal carcinoma, preinvasive carcinoma, prostate carcinoma, renal cell carcinoma of kidney (also called adenocarcinoma of kidney and hypemephoroid carcinoma), reserve cell carcinoma, carcinoma sarcomatodes, scheinderian carcinoma, scirrhous carcinoma, carcinoma scroti, signet-ring cell carcinoma, carcinoma simplex, small-cell carcinoma, solanoid carcinoma, spheroidal cell carcinoma, spindle cell carcinoma, carcinoma spongiosum, squarnous carcinoma, squamous cell carcinoma, string carcinoma, carcinoma telangiectaticum, carcinoma telangiectodes, transitional cell carcinoma, carcinoma tuberosum, tuberous carcinoma, verrucous carcinoma, carcinoma vilosum. In preferred embodiments, the methods of the invention are used to treat subjects having cancer of the breast, cervix, ovary, prostate, lung, colon and rectum, pancreas, stomach or kidney.

**[0051]** Other conditions characterized by an abnormal mammalian cell proliferation to be treated by the methods of the invention include sarcomas. Sarcomas are rare mesenchymal neoplasms that arise in bone and soft tissues. Different types of sarcomas are recognized and these include: liposarcomas (including myxoid liposarcomas and pleiomorphic liposarcomas), leiomyosarcomas, rhabdomyosarcomas, malignant peripheral nerve sheath tumors (also called malignant schwannomas, neurofibrosarcomas, or neurogenic sarcomas), Ewing's tumors (including Ewing's sarcoma of bone, extraskeletal [not bone] Ewing's sarcoma, and primitive neuroectodermal tumor [PNET]), synovial sarcoma, angiosarcomas, hemangiosarcomas, lymphangiosarcomas, Kaposi's sarcoma, hemangioendothelioma,

fibrosarcoma, desmoid tumor (also called aggressive fibromatosis), dermatofibrosarcoma protuberans (DFSP), malignant fibrous histiocytoma (MFH), hemangiopericytoma, malignant mesenchymoma, alveolar soft-part sarcoma, epithelioid sarcoma, clear cell sarcoma, desmoplastic small cell tumor, gastrointestinal stromal tumor (GIST) (also known as GI stromal sarcoma), osteosarcoma (also known as osteogenic sarcoma)-skeletal and extraskeletal, and chondrosarcoma.

**[0052]** The methods of the invention are also directed towards the treatment of subjects with melanoma. Melanomas are tumors arising from the melanocytic system of the skin and other organs. Examples of melanoma include lentigo maligna melanoma, superficial spreading melanoma, nodular melanoma, and acral lentiginous melanoma.

**[0053]** Other conditions characterized by an abnormal mammalian cell proliferation are cancers including, but not limited to, biliary tract cancer, endometrial cancer, esophageal cancer, gastric cancer, intraepithelial neoplasms, including Bowen's disease and Paget's disease, liver cancer, oral cancer, including squamous cell carcinoma, sarcomas, including fibrosarcoma and osteosarcoma, skin cancer, including melanoma, Kaposi's sarcoma, testicular cancer, including germinal tumors (seminoma, non-seminoma (teratomas, choriocarcinomas)), stromal tumors and germ cell tumors, thyroid cancer, including thyroid adenocarcinoma and medullar carcinoma, and renal cancer including adenocarcinoma and Wilms tumor.

**[0054]** According to other aspects of the invention, a method is provided for treating a subject having an abnormal proliferation originating in bone, muscle or connective tissue. Exemplary conditions intended for treatment by the method of the invention include primary tumors (i.e., sarcomas) of bone and connective tissue.

**[0055]** The methods of the invention are also directed towards the treatment of subjects with metastatic tumors. In some embodiments, the metastatic tumors are of epithelial origin. Carcinomas may metastasize to bone, as has been observed with breast cancer, and liver, as is sometimes the case with colon cancer. The methods of the invention are intended to treat metastatic tumors regardless of the site of the metastasis and/or the site of the primary tumor. In preferred embodiments, the metastases are of epithelial origin.

**[0056]** The present invention is advantageous in that it provides a compound that is suitable for use in the treatment of cancers such as breast cancer, ovarian cancer, endometrial cancer, sarcomas, melanomas, prostate cancer, pancreatic cancer etc. and other solid tumors.

The types of cancer that may be treated with gold(III) chlorides include but are not limited to: breast, colon, prostate, thyroid, testis, melanoma, corpus and uterus, Hodgkin's lymphoma, urinary, bladder, cervix, uteri, larynx, rectum, kidney and renal, pelvis, oral cancer, pharynx, non-Hodgkin lymphoma, leukemia, Kaposi's sarcoma, ovary, brain and ONS, myeloma, stomach, esophagus, lung and bronchus, mesothelioma, liver and pancreas. Many other types of cancers are also sensitive to hydrogen tetrachloroaurate and auric chloride.

**[0057]** Carcinomas, tumors that are made up of cells of epithelial origin, make up over 90% of all solid tumors. Hydrogen tetrachloroaurate and auric chloride have an affinity towards malignant epithelial cells and are able to induce apoptosis in carcinoma cells as early as within 24 hours of exposure and causes even greater cytotoxic effects at longer exposure time periods (48 and 72h).

**[0058]** In one aspect cell cycling is irreversibly prevented and/or inhibited and/or arrested by administration of the gold(III) chloride compounds. In one aspect cell cycling is inhibited and/or prevented and/or arrested in the $G_0/G_1$ to S phase transition.

**[0059]** According to another aspect of the invention, methods are provided for inhibiting angiogenesis in disorders having a pathology which requires angiogenesis by administration of gold(III) chlorides. Angiogenesis is defined as the formation of new blood vessels. One subset of these disorders is conditions characterized by abnormal mammalian cell proliferation. Another subset is non-cancer conditions including rheumatoid arthritis, diabetic retinopathy, neovascular glaucoma and psoriasis.

**[0060]** In some embodiments, the methods of the invention are aimed at inhibiting tumor angiogenesis. Tumor angiogenesis refers to the formation of new blood vessels in the vicinity or within a tumor mass. Solid tumor cancers require angiogenesis particularly for oxygen and nutrient supply. It has been previously shown that inhibition of angiogenesis in solid tumor can cause tumor regression in animal models. Thus in one aspect, the invention relates to a method for inhibiting angiogenesis by inhibiting the proliferation, migration or activation of endothelial cells, provided this angiogenesis is unrelated to wound healing in response to injury, infection or inflammation.

**[0061]** Thus in certain embodiments, the methods of the invention are intended for the treatment of diseases and processes that are mediated by angiogenesis including, but not limited to, hemangioma, solid tumors, tumor metastasis, benign tumors, for example hemangiomas, acoustic neuromas, neurofibromas and trachomas, Osler-Webber Syndrome,

telangiectasia, myocardial angiogenesis, angiofibroma, plaque neovascularization, coronary collaterals, ischemic limb angiogenesis, corneal diseases, rubiosis, neovascular glaucoma, diabetic retinopathy, retrolental fibroplasia, diabetic neovascularization, macular degeneration, keloids, ovulation, menstruation, and placentation.

**[0062]** In certain instances the gold(III) chloride compositions and methods of the invention may replace existing surgical procedures or drug therapies. In other instances the gold(III) chloride compositions are useful in improving the efficacy of existing therapies. Accordingly combination therapy may be used to treat the subjects. For example, the agent may be administered to a subject in combination with another anti-proliferative therapy. Suitable anti-cancer therapies include surgical procedures to remove the tumor mass, chemotherapy or localized radiation. The other anti-proliferative therapy may be administered before, concurrent with, or after treatment with the agent of the invention. There may also be a delay of several hours, days and in some instances weeks between the administration of the different treatments, such that the agent may be administered before or after the other treatment.

**[0063]** The agent may be administered in combination with surgery to remove an abnormal proliferative cell mass. As used herein, the phrase "in combination with surgery" means that the agent may be administered prior to, during or after the surgical procedure. Surgical methods for treating epithelial tumor conditions include intra-abdominal surgeries such as right or left hemicolectomy, sigmoid, subtotal or total colectomy and gastrectomy, radical or partial mastectomy, prostatectomy and hysterectomy. In these embodiments, the agent may be administered either by continuous infusion or in a single bolus. Administration during or immediately after surgery may include a lavage, soak or perfusion of the tumor excision site with a pharmaceutical preparation of the agent in a pharmaceutically acceptable carrier. In some embodiments, the agent is administered at the time of surgery as well as following surgery in order to inhibit the formation and development of metastatic lesions. The administration of the agent may continue for several hours, several days, several weeks, or in some instances, several months following a surgical procedure to remove a tumor mass.

**[0064]** The subjects can also be administered the agent in combination with non-surgical anti-proliferative (e.g., anti-cancer) drug therapy. In one embodiment, the agent may be administered in combination with an anti-cancer compound such as a cytostatic compound. A cytostatic compound is a compound (e.g., a nucleic acid, a protein) that suppresses cell growth and/or proliferation. In some embodiments, the cytostatic compound is directed

towards the malignant cells of a tumor. In yet other embodiments, the cytostatic compound is one which inhibits the growth and/or proliferation of vascular smooth muscle cells or fibroblasts.

**[0065]** Combination therapies involve different dosages of chemotherapeutic agents and drug cocktail configurations. In some embodimnts the gold(III) chloride compounds are combined with a chemotherapeutic agent in a synergistic combination. "Synergism" may be measured by combination index (CI). The combination index method was described by Chou and Talalay. (Chou, T.-C. The median-effect principle and the combination index for quantitation of synergism and antagonism, p. 61-102. *In* T.-C. Chou and D. C. Rideout (ed.), Synergism and antagonism in chemotherapy. Academic Press, San Diego, Calif. (1991); Chou, T.-C., and P. Talalay. Quantitative analysis of dose-effect relationships: the combined effects of multiple drugs on enzyme inhibitors. Adv. Enzyme Regul. 22:27-55 (1984)). A CI value of 0.90 or less is considered synergistic, with values of 0.85 being moderately synergistic and values below 0.70 being significantly synergistic. CI values of 0.90 to 1.10 are considered to be nearly additive and higher values are antagonistic.

**Table 1. Synergism/antagonism as a function of CI values**

| **CI Value** | **Interpretation** |
|---|---|
| >10 | Very strong antagonism |
| 3.3 – 10 | Strong antagonism |
| 1.45 – 3.3 | Antagonism |
| 1.2 – 1.45 | Moderate antagonism |
| 1.1 – 1.2 | Slight antagonism |
| 0.9 – 1.1 | Additive |
| 0.85 – 0.9 | Slight synergism |
| 0.7 – 0.85 | Moderate synergism |
| 0.3 – 0.7 | Synergism |
| 0.1 – 0.3 | Strong synergism |
| < 0.1 | Very strong synergism |

**[0066]** It is noted that determination of synergy may be affected by biological variability, dosage, experimental conditions (temperature, pH, oxygen tension, etc.), treatment schedule and combination ratio. Synergism is measured as combination index (CI) values where values of 0.7 or less is considered to be significant levels of synergism.

**[0067]** Suitable anti-proliferative drugs or cytostatic compounds to be used in combination with the agents of the invention include anti-cancer drugs. Anti-cancer drugs are well known and include: Acivicin®; Aclarubicin®; Acodazole Hydrochloride®; Acronine®; Adozelesin®; Aldesleukin®; Altretamine®; Ambomycin®; Ametantrone Acetate®; Aminoglutethimide®; Amsacrine®; Anastrozole®; Anthramycin®; Asparaginase®; Asperlin®; Azacitidine®; Azetepa®; Azotomycin®; Batimastat®; Benzodepa®; Bicalutamide®; Bisantrene Hydrochloride®; Bisnafide Dimesylate®; Bizelesin®; Bleomycin Sulfate®; Brequinar Sodium®; Bropirimine®; Busulfan®; Cactinomycin®; Calusterone®; Caracemide®; Carbetimer®; Carboplatin®; Carmustine®; Carubicin Hydrochloride®; Carzelesin®; Cedefingol®; Chlorambucil®; Cirolemycin®; Cisplatin®; Cladribine®; Crisnatol Mesylate®; Cyclophosphamide®; Cytarabine®; Dacarbazine®; Dactinomycin®; Daunorubicin Hydrochloride®; Decitabine®; Dexormaplatin®; Dezaguanine®; Dezaguanine Mesylate®; Diaziquone®; Docetaxel®; Doxorubicin®; Doxorubicin Hydrochloride®; Droloxifene®; Droloxifene Citrate®; Dromostanolone Propionate®; Duazomycin®; Edatrexate®; Eflornithine Hydrochloride®; Elsamitrucin®; Enloplatin®; Enpromate®; Epipropidine®; Epirubicin Hydrochloride®; Erbulozole®; Esorubicin Hydrochloride®; Estramustine®; Estramustine Phosphate Sodium®; Etanidazole®; Etoposide®; Etoposide Phosphate®; Etoprine®; Fadrozole Hydrochloride®; Fazarabine®; Fenretinide®; Floxuridine®; Fludarabine Phosphate®; Fluorouracil®; Flurocitabine®; Fosquidone®; Fostriecin Sodium®; Gemcitabine®; Gemcitabine Hydrochloride®; Hydroxyurea®; Idarubicin Hydrochloride®; Ifosfamide®; Ilmofosine®; Interferon Alfa-2a®; Interferon Alfa-2b®; Interferon Alfa-n1®; Interferon Alfa-n3®; Interferon Beta-I a®; Interferon Gamma-I b®; Iproplatin®; Irinotecan Hydrochloride®; Lanreotide Acetate®; Letrozole®; Leuprolide Acetate®; Liarozole Hydrochloride®; Lometrexol Sodium®; Lomustine®; Losoxantrone Hydrochloride®; Masoprocol®; Maytansine®; Mechlorethamine Hydrochloride®; Megestrol Acetate®; Melengestrol Acetate®; Melphalan®; Menogaril®; Mercaptopurine®; Methotrexate®; Methotrexate Sodium®; Metoprine®; Meturedepa®; Mitindomide®; Mitocarcin®; Mitocromin®; Mitogillin®; Mitomalcin®; Mitomycin®; Mitosper®; Mitotane®; Mitoxantrone Hydrochloride®; Mycophenolic Acid®; Nocodazole®; Nogalamycin®; Ormaplatin®; Oxisuran®; Paclitaxel®;

Pegaspargase®; Peliomycin®; Pentamustine®; Peplomycin Sulfate®; Perfosfamide®; Pipobroman®; Piposulfan®; Piroxantrone Hydrochloride®; Plicamycin®; Plomestane®; Porfimer Sodium®; Porfiromycin®; Prednimustine®; Procarbazine Hydrochloride®; Puromycin®; Puromycin Hydrochloride®; Pyrazofurin®; Riboprine®; Rogletimide®; Safingol®; Safingol Hydrochloride®; Semustine®; Simtrazene®; Sparfosate Sodium®; Sparsomycin®; Spirogermanium Hydrochloride®; Spiromustine®; Spiroplatin®; Streptonigrin®; Streptozocin®; Sulofenur®; Talisomycin®; Taxol®; Taxotere®; Tecogalan Sodium®; Tegafur®; Teloxantrone Hydrochloride®; Temoporfin®; Teniposide®; Teroxirone®; Testolactone®; Thiamiprine®; Thioguanine®; Thiotepa®; Tiazofurin®; Tirapazamine®; Topotecan Hydrochloride®; Toremifene Citrate®; Trestolone Acetate®; Triciribine Phosphate®; Trimetrexate®; Trimetrexate Glucuronate®; Triptorelin®; Tubulozole Hydrochloride®; Uracil Mustard®; Uredepa®; Vapreotide®; Verteporfin®; Vinblastine Sulfate®; Vincristine Sulfate®; Vindesine®; Vindesine Sulfate®; Vinepidine Sulfate®; Vinglycinate Sulfate®; Vinleurosine Sulfate®; Vinorelbine Tartrate®; Vinrosidine Sulfate®; Vinzolidine Sulfate®; Vorozole®; Zeniplatin®; Zinostatin®; Zorubicin Hydrochloride®.

**[0068]** Other anti-cancer drugs include: 20-epi-1,25 dihydroxyvitamin D3; 5-ethynyluracil; abiraterone; aclarubicin; acylfulvene; adecypenol; adozelesin; aldesleukin; ALL-TK antagonists; altretamine; ambamustine; amidox; amifostine; aminolevulinic acid; amrubicin; amsacrine; anagrelide; anastrozole; andrographolide; angiogenesis inhibitors; antagonist D; antagonist G; antarelix; anti-dorsalizing morphogenetic protein-1; antiandrogen, prostatic carcinoma; antiestrogen; antineoplaston; antisense oligonucleotides; aphidicolin glycinate; apoptosis gene modulators; apoptosis regulators; apurinic acid; ara-CDP-DL-PTBA; arginine deaminase; asulacrine; atamestane; atrimustine; axinastatin 1; axinastatin 2; axinastatin 3; azasetron; azatoxin; azatyrosine; baccatin III derivatives; balanol; batimastat; BCR/ABL antagonists; benzochlorins; benzoylstaurosporine; beta-lactam derivatives; beta-alethine; betaclamycin B; betulinic acid; bFGF inhibitor; bicalutamide; bisantrene; bisaziridinylspermine; bisnafide; bistratene A; bizelesin; breflate; bropirimine; budotitane; buthionine sulfoximine; calcipotriol; calphostin C; camptothecin derivatives; canarypox IL-2; capecitabine; carboxamide-amino-triazole; carboxyamidotriazole; CaRest M3; CARN 700; cartilage derived inhibitor; carzelesin; casein kinase inhibitors (ICOS); castanospermine; cecropin B; cetrorelix; chlorins; chloroquinoxaline sulfonamide; cicaprost; cis-porphyrin; cladribine; clomifene analogues; clotrimazole; collismycin A; collismycin B; combretastatin A4; combretastatin analogue; conagenin; crambescidin 816; crisnatol;

cryptophycin 8; cryptophycin A derivatives; curacin A; cyclopentanthraquinones; cycloplatam; cypemycin; cytarabine ocfosfate; cytolytic factor; cytostatin; dacliximab; decitabine; dehydrodidemnin B; deslorelin; dexifosfamide; dexrazoxane; dexverapamil; diaziquone; didemnin B; didox; diethylnorspermine; dihydro-5-azacytidine; dihydrotaxol, 9-; dioxamycin; diphenyl spiromustine; docosanol; dolasetron; doxifluridine; droloxifene; dronabinol; duocarmycin SA; ebselen; ecomustine; edelfosine; edrecolomab; eflomithine; elemene; emitefur; epirubicin; epristeride; estramustine analogue; estrogen agonists; estrogen antagonists; etanidazole; etoposide phosphate; exemestane; fadrozole; fazarabine; fenretinide; filgrastim; finasteride; flavopiridol; flezelastine; fluasterone; fludarabine; fluorodaunorunicin hydrochloride; forfenimex; formestane; fostriecin; fotemustine; gadolinium texaphyrin; gallium nitrate; galocitabine; ganirelix; gelatinase inhibitors; gemcitabine; glutathione inhibitors; hepsulfam; heregulin; hexamethylene bisacetamide; hypericin; ibandronic acid; idarubicin; idoxifene; idramantone; ilmofosine; ilomastat; imidazoacridones; imiquimod; immunostimulant peptides; insulin-like growth factor-I receptor inhibitor; interferon agonists; interferons; interleukins; iobenguane; iododoxorubicin; ipomeanol, 4-; irinotecan; iroplact; irsogladine; isobengazole; isohomohalicondrin B; itasetron; jasplakinolide; kahalalide F; lamellarin-N triacetate; lanreotide; leinamycin; lenograstim; lentinan sulfate; leptolstatin; letrozole; leukemia inhibiting factor; leukocyte alpha interferon; leuprolide+estrogen+progesterone; leuprorelin; levamisole; liarozole; linear polyamine analogue; lipophilic disaccharide peptide; lipophilic platinum compounds; lissoclinamide 7; lobaplatin; lombricine; lometrexol; lonidamine; losoxantrone; lovastatin; loxoribine; lurtotecan; lutetium texaphyrin; lysofylline; lytic peptides; maitansine; mannostatin A; marimastat; masoprocol; maspin; matrilysin inhibitors; matrix metalloproteinase inhibitors; menogaril; merbarone; meterelin; methioninase; metoclopramide; MIF inhibitor; mifepristone; miltefosine; mirimostim; mismatched double stranded RNA; mitoguazone; mitolactol; mitomycin analogues; mitonafide; mitotoxin fibroblast growth factor-saporin; mitoxantrone; mofarotene; molgramostim; monoclonal antibody, human chorionic gonadotrophin; monophosphoryl lipid A+myobacterium cell wall sk; mopidamol; multiple drug resistance gene inhibitor; multiple tumor suppressor 1-based therapy; mustard anti cancer compound; mycaperoxide B; mycobacterial cell wall extract; myriaporone; N-acetyldinaline; N-substituted benzamides; nafarelin; nagrestip; naloxone+pentazocine; napavin; naphterpin; nartograstim; nedaplatin; nemorubicin; neridronic acid; neutral endopeptidase; nilutamide; nisamycin; nitric oxide modulators; nitroxide antioxidant; nitrullyn; $O_6$-benzylguanine; octreotide; okicenone; oligonucleotides; onapristone;

ondansetron; ondansetron; oracin; oral cytokine inducer; ormaplatin; osaterone; oxaliplatin; oxaunomycin; paclitaxel analogues; paclitaxel derivatives; palauamine; palmitoylrhizoxin; pamidronic acid; panaxytriol; panomifene; parabactin; pazelliptine; pegaspargase; peldesine; pentosan polysulfate sodium; pentostatin; pentrozole; perflubron; perfosfamide; perillyl alcohol; phenazinomycin; phenylacetate; phosphatase inhibitors; picibanil; pilocarpine hydrochloride; pirarubicin; piritrexim; placetin A; placetin B; plasminogen activator inhibitor; platinum complex; platinum compounds; platinum-triamine complex; porfimer sodium; porfiromycin; propyl bis-acridone; prostaglandin J2; proteasome inhibitors; protein A-based immune modulator; protein kinase C inhibitor; protein kinase C inhibitors, microalgal; protein tyrosine phosphatase inhibitors; purine nucleoside phosphorylase inhibitors; purpurins; pyrazoloacridine; pyridoxylated hemoglobin polyoxyethylene conjugate; raf antagonists; raltitrexed; ramosetron; ras farnesyl protein transferase inhibitors; ras inhibitors; ras-GAP inhibitor; retelliptine demethylated; rhenium Re 186 etidronate; rhizoxin; ribozymes; RII retinamide; rogletimide; rohitukine; romurtide; roquinimex; rubiginone B1; ruboxyl; safingol; saintopin; SarCNU; sarcophytol A; sargramostim; Sdi 1 mimetics; semustine; senescence derived inhibitor 1; sense oligonucleotides; signal transduction inhibitors; signal transduction modulators; single chain antigen binding protein; sizofiran; sobuzoxane; sodium borocaptate; sodium phenylacetate; solverol; somatomedin binding protein; sonermin; sparfosic acid; spicamycin D; spiromustine; splenopentin; spongistatin 1; squalamine; stem cell inhibitor; stem-cell division inhibitors; stipiamide; stromelysin inhibitors; sulfinosine; superactive vasoactive intestinal peptide antagonist; suradista; suramin; swainsonine; synthetic glycosaminoglycans; tallimustine; tamoxifen methiodide; tauromustine; tazarotene; tecogalan sodium; tegafur; tellurapyrylium; telomerase inhibitors; temoporfin; temozolomide; teniposide; tetrachlorodecaoxide; tetrazomine; thaliblastine; thalidomide; thiocoraline; thrombopoietin; thrombopoietin mimetic; thymalfasin; thymopoietin receptor agonist; thymotrinan; thyroid stimulating hormone; tin ethyl etiopurpurin; tirapazamine; titanocene dichloride; topotecan; topsentin; toremifene; totipotent stem cell factor; translation inhibitors; tretinoin; triacetyluridine; triciribine; trimetrexate; triptorelin; tropisetron; turosteride; tyrosine kinase inhibitors; tyrphostins; UBC inhibitors; ubenimex; urogenital sinus-derived growth inhibitory factor; urokinase receptor antagonists; vapreotide; variolin B; vector system, erythrocyte gene therapy; velaresol; veramine; verdins; verteporfin; vinorelbine; vinxaltine; vitaxin; vorozole; zanoterone; zeniplatin; zilascorb; and zinostatin stimalamer.

**Anticancer therapies using gold(III) chlorides**

**[0069]** Members of the gold(III) chlorides, hydrogen tetrachloroaurate and auric chloride are contemplated as broad-spectrum drugs for cancer therapy of carcinoma solid tumors as a result of apoptosis-inducing and cytotoxic effects on carcinoma cells. Tumor formation is divided into two stages, the prevascular and vascular stages. Studies have shown that cells of prevascular tumors proliferate as rapidly as do cells from vascularized tumors. However, prevascular tumors rarely grow to more than 2-3 $mm^3$ because of the existence of an equilibrium between cell proliferation and cell death, the latter resulting from the hypoxic nature of the prevascular tumor (Folkman, 1995, Nat. Med. 1:27-31). The switch from the prevascular to vascular stage requires a shift in the balance of the regulatory factors of angiogenesis from a net balance favoring negative factors to one in which the positive factors, such as fibroblast growth factor (FGF) and vascular endothelial growth factor (VEGF), predominate (Cao, 1998, Prog. Mol. Subcell. Biol. 20:161-176). The shift in balance between regulatory factors is a result of the up-regulation of the angiogenic factors and the simultaneous down-regulation of anti-angiogenic factors (Folkman, 1995, N. Eng. J. Med. 333:1757-1763).

**[0070]** Cancers to be treated by gold(III) chlorides compositions described herein (such as a composition comprising auric chloride or tetrachloroauric acid) include, but are not limited to, carcinoma, lymphoma, blastoma, sarcoma, and leukemia. Examples of cancers that can be treated by compositions described herein include, but are not limited to, squamous cell cancer, lung cancer (including small cell lung cancer, non-small cell lung cancer, adenocarcinoma of the lung, and squamous carcinoma of the lung), cancer of the peritoneum, hepatocellular cancer, gastric or stomach cancer (including gastrointestinal cancer), pancreatic cancer, glioblastoma, cervical cancer, ovarian cancer, liver cancer, bladder cancer, hepatoma, breast cancer, colon cancer, melanoma, endometrical or uterine carcinoma, salivary gland carcinoma, kidney or renal cancer, liver cancer, prostate cancer, vulval cancer, thyroid cancer, hepatic carcinoma, head and neck cancer, colorectal cancer, rectal cancer, soft-tissue sarcoma, Kaposi's sarcoma, B-cell lymphoma (including low grade/follicular non-Hodgkin's lymphoma (NHL), small lymphocytic (SL) NHL, intermediate grade/follicular NHL, intermediate grade diffuse NHL, high grade immunoblastic NHL, high grade lymphoblastic NHL, high grade small non-cleaved cell NHL, bulky disease NHL, mantle cell lymphoma, AIDS-related lymphoma, and Waldenstrom's macroglobulinemia), chronic lymphocytic leukemia (CLL), acute lymphoblastic leukemia (ALL), myeloma, Hairy cell

leukemia, chronic myeloblastic leukemia, and post-transplant lymphoproliferative disorder (PTLD), as well as abnormal vascular proliferation associated with phakomatoses, edema (such as that associated with brain tumors), and Meigs' syndrome. In some embodiments, there is provided a method of treating metastatic cancer (that is, cancer that has metastasized from the primary tumor). In some embodiments, there is provided a method of reducing cell proliferation and/or cell migration. In some embodiments, there is provided a method of treating hyperplasia.

**[0071]** In one aspect, a method for treating breast, ovary, testicle, prostate, head, neck, eye, skin, mouth, throat, esophagus, chest, bone, lung, colon, sigmoid, rectum, stomach, kidney, liver, pancreas, brain, intestine, heart or adrenal cancer or neoplastic disease with gold(III) chloride compositions or pharmaceutically acceptable salts thereof as described herein (such as a composition comprising auric chloride or tetrachloroauric acid).

**[0072]** In some embodiments, methods of treating cancer at advanced stage(s) are provided. In some embodiments, there are provided methods of treating breast cancer (which may be HER2 positive or HER2 negative), including, for example, advanced breast cancer, stage IV breast cancer, locally advanced breast cancer, and metastatic breast cancer. In some embodiments, the cancer is lung cancer, including, for example, non-small cell lung cancer (NSCLC, such as advanced NSCLC), small cell lung cancer (SCLC, such as advanced SCLC), and advanced solid tumor malignancy in the lung. In some embodiments, the cancer is ovarian cancer, head and neck cancer, gastric malignancies, melanoma (including metastatic melanoma), colorectal cancer, pancreatic cancer, and solid tumors (such as advanced solid tumors). In some embodiments, the cancer is selected from the group consisting of breast cancer, colorectal cancer, rectal cancer, non-small cell lung cancer, non-Hodgkins lymphoma (NHL), renal cell cancer, prostate cancer, liver cancer, pancreatic cancer, soft-tissue sarcoma, Kaposi's sarcoma, carcinoid carcinoma, head and neck cancer, melanoma, ovarian cancer, mesothelioma, gliomas, glioblastomas, neuroblastomas, and multiple myeloma. In some embodiments, the cancer is a solid tumor. In some embodiments, the cancer is selected from the group consisting of prostate cancer, colon cancer, breast cancer, head and neck cancer, pancreatic cancer, lung cancer, and ovarian cancer.

**[0073]** In some embodiments, hydrogen tetrachloroaurate and auric chloride are effective treatments against sarcoma tumors and hematological cancers, as well as against non-cancer lesions including pre-cancer lesions, dysplasia and hyperplasia.

**[0074]** The present invention relates to methods of administering the compositions, e.g., to provide an effective amount of a chloride of gold(III), such as hydrogen tetrachloroaurate or auric chloride in an amount sufficient to cause a tumor to shrink and/or to decrease the growth rate of the tumor (such as to suppress tumor growth).

**[0075]** By the term "administering," it is meant that the compositions are delivered to the host in such a manner that it can achieve the desired purpose. As mentioned The compositions can be administered by an effective route, such as orally, topically, rectally, etc. The compositions can be administered to any host in need of treatment, e.g., vertebrates, such as mammals, including humans, male humans, female humans, primates, pets, such as cats and dogs, livestock, such as cows, horses, birds, chickens, etc.

**[0076]** An "effective amount" of the compositions are administered to such a host. Effective amounts are such amounts which are useful to achieve the desired effect, preferably a beneficial or therapeutic effect as described above. Such amount can be determined routinely, e.g., by performing a dose-response experiment in which varying doses are administered to cells, tissues, animal models to determine an effective amount for achieving a desired result. Amounts are selected based on various factors, including the milieu to which the composition is administered (e.g., a cancer patient, animal model, tissue culture cells, etc.), the site of the cells to be treated, the age, health, gender, and weight of a patient or animal to be treated, etc. Useful amounts include, 10 milligrams-100 grams, preferably, e.g., 100 milligrams-10 grams, 250 milligrams-2.5 grams, 1 gm, 2 gm, 3 gm, 500 milligrams-1.25 grams. etc., per dosage of different forms of the compositions depending upon the need of the recipients and the method of preparation.

**[0077]** The term "effective amount" used herein further refers to an amount of a compound or composition sufficient to treat a specified disorder, condition or disease such as ameliorate, palliate, lessen, and/or delay one or more of its symptoms. In reference to cancers or other unwanted cell proliferation, an effective amount comprises an amount sufficient to cause a tumor to shrink and/or to decrease the growth rate of the tumor (such as to suppress tumor growth). In some embodiments, an effective amount is an amount sufficient to delay development. In some embodiments, an effective amount is an amount sufficient to prevent occurrence and/or recurrence. An effective amount can be administered in one or more administrations. The compositions described herein can be administered alone or in combination with other pharmaceutical agents, including poorly water soluble pharmaceutical agents. For example, gold(III) chlorides can be co-administered with one or

more other chemotherapeutic agents including, but not limited to, carboplatin, navelbine® (vinorelbine), anthracycline (Doxil), lapatinib (GW57016), Herceptin, gemcitabine (Gemzar®), capecitabine (Xeloda®), alimta, cisplatin, 5-fluorouracil, epirubicin, cyclophosphamide, avastin, velcade®, etc. In some embodiments, the gold(III) chlorides are co-administered with a chemotherapeutic agent selected from the group consisting of antimetabolites (including nucleoside analogs), platinum-based agents, alkylating agents, tyrosine kinase inhibitors, anthracycline antibiotics, vinca alkloids, proteasome inhibitors, macrolides, and topoisomerase inhibitors. These other pharmaceutical agents can be present in the same composition as the gold(III) chlorides, or in a separate composition that is administered simultaneously or sequentially with the chloride of gold(III) composition.

**[0078]** In some embodiments the chloride of gold(III) composition is administered simultaneously or sequentially with one or more of the chemotherapeutic agents: Adriamycin®, Aldesleukin (Proleukin), Altretamine (Hexalen), Amifostine , Anastrazole (Arimidex), Arsenic trioxide (Trisenox), Asparaginase (Elspar), BCG (Bacillus Calmette-Gûerin), Bexarotene (Targretin), Bicalutamide (Casodex), Bleomycin (Blenoxane), Busulfan (Myleran), Capecitabine (Xelode), Carboplatin (Paraplatin), Carmustine, Chlorambucil (Leukoran), Cisplatin (Platinol), Cladribine (Leustatin), Cyclophosphamide (Cytoxan), Cytarabine (Cytosar), Dacarbazine, Dactinomycin, Daunorubicin (Cerubidine), Denileukin diftitox (Ontak), Dexrazoxane (Zinecard), Dexamethasone, (Taxotere), Doxorubicin , Epirubicin , Estramustine, Etoposide, Exemestane, Fludarabine, Fluorouracil, Flutamide, Gemcitabine, Gemtuzumab Ozogamicin (Mylotarg), Goserelin Acetate Implant (Zoladex), Hydroxyurea (Hydrea), Idarubicin, Ifosfamide, Interferon alfa-2a (Roferon), Irinotecan, Letrozole, Leuprolide (Lupron), Levimasole, Lomustine, Mechlorethamine, Megestrol, Melphalan, Mercaptopurine, Mesna, Methotrexate, Mitomycin, Mitomycin-C, Mitotane, Mitoxantrone, Nilutamide, Paclitaxel, Pegasparagase, Pentostatin (Nipent), Plicamycin, Procarbazine, Rituximab, Streptozotocin, Tomoxifen, Temozolomide, Teniposide, Thioguanine, Thiotepa, Topotecan, Toremifene, Trastuzumab (Herceptin), Tretinoin, Triptorelin, Valrubicin, Vinblastine, Vincristine, and Vinorelbine.

**[0079]** In some embodiments the gold(III) chlorides are administered in conjunction with other forms of cancer therapy such as, but not limited to, surgery, brachytherapy, and radiation therapy. The National Cancer Institute recommends that doctors should strongly consider giving chemotherapy at the same time as radiation therapy for women with invasive cervical cancer. Five major studies showed that chemotherapy that includes the drug

cisplatin, when given at the same time as radiation therapy, prolongs survival in women with this disease. Therefore, in one aspect of the invention compositions of gold(III) chlorides are administered at the same time as radiation therapy. For oral cancer, surgery and radiotherapy are equally effective in treating early stage disease. Surgery plus postoperative radiotherapy produces better local control rates and disease-free survival times than surgery or radiotherapy alone in late stage disease. Adjuvant chemotherapy with compositions of gold(III) chlorides according to the invention is likely to decrease local recurrence rates and extend disease-free survival times. Oral squamous cell carcinoma responds well to chemotherapy with combinations of Cisplatin and 5-fluorouracil (5-FU) giving the best results (93% response rate and 54% complete remission rate). (Rao RS, et al. American Journal of Surgery 1994;168:262-267). Treatment of oral carcinomas with hydrogen tetrachloroaurate or auric chloride in combination with an anti-metabolite, such as 5-fluorouracil (5-FU), is contemplated as one aspect of the invention.

**[0080]** The gold(III) chloride compounds of the present invention, including hydrogen tetrachloroaurate and auric chloride, include pharmaceutically acceptable salts of the compounds. The term "pharmaceutically acceptable salts" is meant to include salts of the active compounds which are prepared with relatively nontoxic acids or bases, depending on the particular substituents found on the compounds described herein. When compounds of the present invention contain relatively acidic functionalities, base addition salts can be obtained by contacting the neutral form of such compounds with a sufficient amount of the desired base, either neat or in a suitable inert solvent. Examples of pharmaceutically acceptable base addition salts include sodium, potassium, calcium, ammonium, organic amino, or magnesium salt, or a similar salt. When compounds of the present invention contain relatively basic functionalities, acid addition salts can be obtained by contacting the neutral form of such compounds with a sufficient amount of the desired acid, either neat or in a suitable inert solvent. Examples of pharmaceutically acceptable acid addition salts include those derived from inorganic acids like hydrochloric, hydrobromic, nitric, carbonic, monohydrogencarbonic, phosphoric, monohydrogenphosphoric, dihydrogenphosphoric, sulfuric, monohydrogensulfuric, hydriodic, or phosphorous acids and the like, as well as the salts derived from relatively nontoxic organic acids like acetic, propionic, isobutyric, oxalic, maleic, malonic, benzoic, succinic, suberic, fumaric, mandelic, phthalic, benzenesulfonic, p-tolylsulfonic, citric, tartaric, methanesulfonic, and the like. Also included are salts of amino acids such as arginate and the like, and salts of organic acids like glucuronic or galactunoric

acids and the like (see, for example, Berge et al. (1977) J. Pharm. Sci.66:1 19). Certain specific compounds of the present invention contain both basic and acidic functionalities that allow the compounds to be converted into either base or acid addition salts.

**[0081]** Neutral forms of the compounds may be regenerated by contacting the salt with a base or acid and isolating the parent compound in the conventional manner. The parent form of the compound differs from the various salt forms in certain physical properties, such as solubility in polar solvents, but otherwise the salts are equivalent to the parent form of the compound for the purposes of the present invention.

**[0082]** In addition to salt forms, the present invention provides compounds which are in a prodrug form. Prodrugs of the compounds described herein are those compounds that readily undergo chemical changes under physiological conditions to provide the compounds of the present invention. Additionally, prodrugs can be converted to the compounds of the present invention by chemical or biochemical methods in an ex vivo environment. For example, prodrugs can be slowly converted to the compounds of the present invention when placed in a transdermal patch reservoir with a suitable enzyme or chemical reagent. Prodrugs are often useful because, in some situations, they may be easier to administer than the parent drug. They may, for instance, be bioavailable by oral administration whereas the parent drug is not. The prodrug may also have improved solubility in pharmacological compositions over the parent drug. A wide variety of prodrug derivatives are known in the art, such as those that rely on hydrolytic cleavage or oxidative activation of the prodrug.

**[0083]** Certain compounds of the present invention can exist in unsolvated forms as well as solvated forms, including hydrated forms. In general, the solvated forms are equivalent to unsolvated forms and are intended to be encompassed within the scope of the present invention. Certain compounds of the present invention may exist in multiple crystalline or amorphous forms. In general, all physical forms are equivalent for the uses contemplated by the present invention and are intended to be within the scope of the present invention.

**[0084]** In accordance with the present invention, there are provided compositions and methods useful for in vivo delivery of the gold(III) chlorides in the form of nanoparticles that are suitable for parenteral administration in aqueous suspension. The average diameter of such nanoparticles in a composition according to the invention can range from 1-1000 nm, preferably 10-200 nm, more preferably 50-150 nm. In some embodiments the nanoparticles

are formed in combination with a stabilizer such as a polymer. The polymer is a biocompatible material, such as the protein albumin

**[0085]** It is known that the delivery of biologics in the form of a particulate suspension allows targeting to organs such as the liver, lungs, spleen, lymphatic circulation, and the like, due to the uptake in these organs, of the particles by the reticuloendothelial (RES) system of cells. Targeting to the RES containing organs may be controlled through the use of particles of varying size, and through administration by different routes. Suitable nontoxic pharmaceutically acceptable excipients for use in the compositions of the present invention will be apparent to those skilled in the art of pharmaceutical formulations and examples are described in REMINGTON: The Science and Practice of Pharmacy, 20th Edition, A. R. Gennaro, ed., (2000). The choice of suitable carriers will depend on the exact nature of the particular dosage form desired, e.g., whether the gold(III) chloride is to be formulated into microemulsions, suspensions, microparticles, or nanoparticles, as well as on the physicochemical properties of the gold(III) chloride.

**Cytotoxic effects of compositions comprising gold(III) chlorides**

**[0086]** Apoptosis, also known as programmed cell death, is the phenomenon by which a cell dies following a series of gene-mediated events, in response to a wide range of intracellular and extracellular agents. Apoptosis, a counterpart of mitosis, plays an important role in the development and homeostasis of many organisms and tissues. It serves to regulate cell numbers, to shape developing organisms and as a defense against potentially harmful agents. Apoptosis is not the only mode of cell death. Necrosis is a type of cell death which is nonspecific and frequently occurs when cells are exposed to high doses of toxic agents. Such exposure usually results in the loss of ionic homeostasis. Unlike apoptosis, necrosis does not seem to be genetically influenced.

**[0087]** Apoptotic and necrotic cells have different appearances which allow them to be distinguished microscopically. Necrotic cells and their mitochondria swell, the cell membrane eventually ruptures, and internal organelles become distended. As a result of the membrane rupture, inflammation occurs in the surrounding tissue. In contrast, the nuclei of apoptotic cells become fragmented into several smaller nuclear bodies, which are quickly recognized by phagocytes and engulfed, and no inflammatory response occurs. Therefore, it is useful to develop chemotherapeutics which induce apoptosis, rather than necrosis, in order to avoid inflammation and the toxic agents which are often released from necrotic tumor cells.

**[0088]** Apoptosis is a process of cell death characterized by cytoplasmic shrinkage, nuclear condensation, and DNA fragmentation (Kerr, J. F. R., et al., 1972, Br. J. Cancer 26, 239 257). Cell necrosis, on the other hand, is associated with both the translocation of PS to the external cell surface as well as the loss of membrane integrity (Vermes, I. et al., 1995, J. Immunol. Methods 184, 39 51). The cell membrane integrity of apoptotic cells can be established with a dye exclusion test using propidium iodide (PI).

**[0089]** Apoptosis is a key path of cellular death for most cells in the human body. This mechanism is often dysfunctional in cancer cells. However, hydrogen tetrachloroaurate and auric chloride are capable of inducing apoptosis in malignant cells that would otherwise remain dysfunctional (lack of apoptosis). Without being bound by theory, the fact that hydrogen tetrachloroaurate is capable of inducing apoptosis in carcinoma cells in many tissues types broadening the possible cancers types that may be sensitive to this drug. Most chemotherapy agents have broad target effects against more than one type of tissue. Cisplatin is used to treat testicular, ovarian, head and neck, bladder, cervical, lung and other types of tumors, and works by binding DNA and inducing genetic damage in all the cell types mentioned before. In other words Cisplatin is effective via the same mode of action against several cancer types which are sensitive to its effects.

**[0090]** At least one of the mechanisms by which hydrogen tetrachloroaurate induces cell death is by apoptosis. Cytotoxicity assays used to determine cell death were based on a commercially available cell death kit called M30 CYTODEATH$^{TM}$ (Roche). M30 is a fluorescent-linked monoclonal antibody that recognizes epitopes of the degraded epithelial protein cytokeratin 18 that is broken down by action of degradative enzymes known as caspases activated during apoptosis. The native non-degraded form of the cytokeratin 18 protein is not recognized by this antibody, and only the degraded form is detected with M30 by flow cytometry.

**[0091]** Apoptosis was assessed by measuring cell shrinkage and permeability to propidium iodide (PI) during their death. Propidium iodide (PI) is used with flow cytometry methods as a well established marker for cell death. PI only enters the cell and binds the cell nucleus when the cell's integrity has been compromised. Gold(III) chlorides, such as hydrogen tetrachloroaurate and auric chloride, produce cell death characterized by statistically significant high percentages of PI positive cells when compared to controls. Cytotoxicity assays of hydrogen tetrachloroaurate and auric chloride, based on flow cytometry following the incorporation of propidium iodide (PI) into cancer cells, show

reduction of cellular proliferation, cell apoptosis and cell death. Without being bound by theory, a logical pharmacological prediction would be that the mechanism of action of hydrogen tetrachloroaurate and auric chloride extends over carcinoma cells of several tissue types similar to other anti-cancer drugs like cisplatin.

**[0092]** Cisplatin (cis-diamminedichloroplatinum, cis-$Pt(NH_3)_2Cl_2$, molecular weight 300.05) has been used as a chemotherapeutic agent for many years since the discovery of its antitumor activity by B. Rosenberg, et al. (Nature 205:698-699 (1965)) Cisplatin is referred to commercially as Platinol® and is used to treat such cancers as testicular, ovarian, and bladder. (Physician's Desk Reference, PDR). Cisplatin is one of the most potent antitumor agents known, displaying clinical activity against a wide variety of solid tumors. Its cytotoxic mode of action is mediated by its interaction with DNA to form DNA adducts, primarily intrastrand crosslink adducts, which activate several signal transduction pathways, including those involving ATR, p53, p73, and MAPK, and culminate in the activation of apoptosis. (reviewed by Siddik Z. H., Oncogene. 2003 Oct 20;22(47):7265-7279.)

**[0093]** Without being bound by this theory, the inventors believe that whereas hydrogen tetrachloroaurate is a completely different molecule compared to Cisplatin (cis-diamminedichloroplatinum II) it may share some similarities regarding modes of action against tumor cells. Cisplatin is a molecule that is able to bind to DNA and inhibit or even impair cellular function by means of binding to the cell's genetic material and inducing cell damage or even death. Hydrogen tetrachloroaurate is likely to bind cellular DNA and induce damage to the cell in similar fashion to other chemotherapy agents that also bind DNA.

**Antiangiogenic effects of gold(III) compounds**

**[0094]** Angiogenesis, the process of growth of new capillaries from preexisting blood vessels, is a critical process for tumor growth and metastasis. (Carmeliet P. et al., Nature; 407:2249-257). The process of angiogenesis is very tightly regulated by an interplay between negative and positive factors, and in adults is normally restricted to the female reproductive cycle and wound repair (Malonne et al., 1999, Clin. Exp. Metastasis 17:1-14). Aberrant or abnormal regulation of angiogenesis has been implicated in many human disorders, including diabetic retinopathy, psoriasis, rheumatoid arthritis, cardiovascular disease, and tumorigenesis (Folkman, 1995, Nat. Med. 1:27-31).

**[0095]** Anti-angiogenic properties of gold nanoparticles prepared by the reduction of aqueous chloroaurate ions with sodium borohydride have been reported. (Mukherjee P. et al.

Clin. Cancer Res. 11(9):3530-3534 (2005)). Anti-angiogenic and anti-proliferative effects of gold chloride on human endothelial cells also have been reported. (Matsubara T., J. Clin. Investigation 79(5):1440-1446 (1987)).

**[0096]** Neoplastic growth is usually dependent on blood supply, and it is commonly accepted that this is provided by the formation of new vessels. However, tumors may be able to grow without neovascularization if they find a suitable vascular bed available. Alveolar or putative non-angiogenic growth is characterized by lack of parenchymal destruction and absence of both tumor associated stroma and new vessels. (Pezzella F. et al. Amer. J. Pathol. 151:1417-1423 (1997)).

**Formulations for Administration**

**[0097]** The compositions of the present invention can be administered in any form by any effective route, including, e.g., oral, parenteral, enteral, intraperitoneal, topical, transdermal (e.g., using any standard patch), ophthalmic, nasally, local, non-oral, such as aerosal, inhalation, subcutaneous, intramuscular, buccal, sublingual, rectal, vaginal, intra-arterial, and intrathecal, etc. It can be administered alone, or in combination with any ingredient(s), active or inactive, including in a medicinal form, or as a food or beverage additive.

**[0098]** In preferred embodiments of the invention, the compositions are administered orally in any suitable form, including, e.g., pills, capsules, granules, tablets, lozenges, aqueous or oily suspensions, granules, powders, emulsions, capsules, syrups, or elixirs. Orally administered compositions can contain one or more agents, for example, sweetening agents such as fructose, aspartame or saccharin; flavoring agents such as peppermint, oil of wintergreen, or cherry; coloring agents; and preserving agents, to provide a pharmaceutically palatable preparation. Moreover, where in tablet or pill form, the compositions can be coated to delay disintegration and absorption in the gastrointestinal tract thereby providing a sustained action over an extended period of time. Selectively permeable membranes surrounding an osmotically active driving compound are also suitable for orally administered compositions. In these latter platforms, fluid from the environment surrounding the capsule is imbibed by the driving compound, which swells to displace the agent or agent composition through an aperture. These delivery platforms can provide an essentially zero order delivery profile as opposed to the spiked profiles of immediate release formulations. A time-delay material such as glycerol monostearate or glycerol stearate can also be used. Oral compositions can include standard excipients such as mannitol, lactose, starch, magnesium

stearate, sodium saccharin, cellulose, and magnesium carbonate. In one embodiment, the excipients are of pharmaceutical grade.

**[0099]** In yet another embodiment, the compositions can be delivered in a controlled-release system or sustained-release system (see, e.g., Langer, Science 249:1527-1533 (1990)). In one embodiment, a pump can be used (Langer, Science 249:1527-1533 (1990); Sefton, CRC Crit. Ref. Biomed. Eng. 14:201 (1987); Buchwald et al., Surgery 88:507 (1980); and Saudek et al., N. Engl. J. Med. 321:574 (1989)). In another embodiment, polymeric materials can be used (see Medical Applications of Controlled Release (Langer and Wise eds., 1974); Controlled Drug Bioavailability, Drug Product Design and Performance (Smolen and Ball eds., 1984); Ranger and Peppas, J. Macromol. Sci. Rev. Macromol. Chem. 23:61 (1983); Levy et al., Science 228:190 (1985); During et al., Ann. Neurol. 25:351 (1989); and Howard et al., J. Neurosurg. 71:105 (1989)).

**[00100]** The present compositions can optionally comprise a suitable amount of a pharmaceutically acceptable excipient so as to provide the form for proper administration to the mammal. The present compositions can take the form of solutions, suspensions, emulsion, tablets, pills, pellets, capsules, capsules containing liquids, powders, sustained-release formulations, suppositories, emulsions, aerosols, sprays, suspensions, or any other form suitable for use. In one embodiment, the composition is in the form of a capsule (see e.g., U.S. Pat. No. 5,698,155). Other examples of suitable pharmaceutical excipients are described in Remington's Pharmaceutical Sciences 1447-1676 (Alfonso R. Gennaro ed., 19th ed. 1995), incorporated herein by reference. Examples of suitable carriers are well known in the art and can include, but are not limited to, any of the standard pharmaceutical carriers such as a phosphate buffered saline solutions, phosphate buffered saline containing Polysorb 80, water, emulsions such as oil/water emulsion and various type of wetting agents. Other carriers may also include sterile solutions, tablets, coated tablets pharmaceutical and capsules. Typically such carriers contain excipients such as such as starch, milk, sugar, certain types of clay, gelatin, stearic acid or salts thereof, magnesium or calcium stearate, talc, vegetable fats or oils, gums, glycols. Such carriers can also include flavor and color additives or other ingredients. Compositions comprising such carriers are formulated by well known conventional methods. Generally excipients formulated with the compositions are suitable for oral administration and do not deleteriously react with it, or other active components.

**[00101]** Suitable pharmaceutically acceptable carriers include but are not limited to water, salt solutions, alcohols, gum arabic, vegetable oils, benzyl alcohols, gelatin, carbohydrates

such as lactose, amylose or starch, magnesium stearate, talc, silicic acid, viscous paraffin, perfume oil, fatty acid monoglycerides and diglycerides, pentaerythritol fatty acid esters, hydroxy methylcellulose and the like. Other additives include, e.g., antioxidants and preservatives, coloring, flavoring and diluting agents, emulsifying and suspending agents, such as acacia, agar, alginic acid, sodium alginate, bentonite, carbomer, carrageenan, carboxymethylcellulose, cellulose, cholesterol, gelatin, hydroxyethyl cellulose, hydroxypropyl cellulose, hydroxypropyl methylcellulose, methylcellulose, octoxynol 9, oleyl alcohol, povidone, propylene glycol monostearate, sodium lauryl sulfate, sorbitan esters, stearyl alcohol, tragacanth, xanthan gum, and derivatives thereof, solvents, and miscellaneous ingredients such as microcrystalline cellulose, citric acid, dextrin, dextrose, liquid glucose, lactic acid, lactose, magnesium chloride, potassium metaphosphate, starch, and the like.

**[00102]** The compositions can also be formulated with other active ingredients, such as anti-oxidants, vitamins (A, C, ascorbic acid, B's, such as B1, thiamine, B6, pyridoxine, B complex, biotin, choline, nicotinic acid, pantothenic acid, B12, cyanocobalamin, and/or B2, D, D2, D3, calciferol, E, such as tocopherol, riboflavin, K, K1, K2). Preferred compounds, include, e.g., creatine monohydrate, pyruvate, L-Carnitine, α-lipoic acid, Phytin or Phytic acid, Coenzyme Q10, NADH, NAD, D-ribose, amino acids such as L-glutamine, Lysine, chrysin; pre-hormones such as 4-androstenedione, 5-androstenedione, 4(or 5-)androstenediol, 19-nor-4 (or 5-)-androstenedione, 19-nor-4 (or 5-)-androstenediol, ß-ecdysterone, and 5-Methyl-7-Methoxy Isoflavone.

**[00103]** Individuals suitable for receiving these compositions depend on the disease or condition or disorder to be treated and/or prevented. Accordingly, the term "individual" includes any vertebrates, mammals, and humans depending on intended suitable use. In some embodiments, the individual is a mammal. In some embodiments, the individual is any one or more of human, bovine, equine, feline, canine, rodent, or primate. In some embodiments, the individual is a human.

**[00104]** In another aspect, there is provided a method of treating a carcinoma in an individual, wherein the method comprises administering to the individual a composition comprising an effective amount of a chloride of gold(III) and a carrier protein (such as albumin). In some embodiments, the composition further comprises a stabilizing agent described herein, such as citrate. In some embodiments, the chloride of gold(III) used for preparation of the composition that is administered to the individual is in an anhydrous form. The gold(III) chlorides and the carrier protein may be present in the forms of nanoparticles.

**Dosages**

**[0100]** The dose of the inventive composition administered to an individual (such as human) will vary with the particular composition, the method of administration, and the particular disease being treated. The dose should be sufficient to effect a desirable response, such as a therapeutic or prophylactic response against a particular disease or condition. For example, the dosage of gold(III) chlorides administered can be about 1 to about 1000 $mg/m^2$, about 1 to about 500 $mg/m^2$, about 1 to about 300 $mg/m^2$, including for example about 10 to about 300 $mg/m^2$, about 30 to about 200 $mg/m^2$, and about 70 to about 150 $mg/m^2$. Typically, the dosage of gold(III) chlorides in the composition can be in the range of about 50 to about 200 $mg/m^2$ when given on a 3 week schedule, or about 10 to about 100 $mg/m^2$ when given on a weekly schedule. In addition, if given in a metronomic regimen (e.g., daily or a few times per week), the dosage may be in the range of about 1-50 $mg/m^2$.

**[0101]** Dosing frequency for the composition includes, but is not limited to, at least about any of once every three weeks, once every two weeks, once a week, twice a week, three times a week, four times a week, five times a week, six times a week, or daily. In some embodiments, the interval between each administration is less than about a week, such as less than about any of 6, 5, 4, 3, 2, or 1 day. In some embodiments, the interval between each administration is constant. For example, the administration can be carried out daily, every two days, every three days, every four days, every five days, or weekly. In some embodiments, the administration can be carried out twice daily, three times daily, or more frequent. Administration can also be continuous and adjusted to maintaining a level of the compound within any desired and specified range.

**[0102]** The administration of the composition can be extended over an extended period of time, such as from about a month up to about three years. For example, the dosing regime can be extended over a period of any of about 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 18, 24, 30, and 36 months. In some embodiments, there is no break in the dosing schedule. In some embodiments, the interval between each administration is no more than about a week.

**[0103]** The compositions described herein can be administered to an individual (such as human) via various routes, including, for example, intravenous, intra-arterial, intraperitoneal, intrapulmonary, oral, inhalation, intravesicular, intramuscular, intra-tracheal, subcutaneous, intraocular, intrathecal, transmucosal, and transdermal. For example, the inventive composition can be administered by inhalation to treat conditions of the respiratory tract.

The composition can be used to treat respiratory conditions such as pulmonary fibrosis, broncheolitis obliterans, lung cancer, bronchoalveolar carcinoma, and the like. In one embodiment of the invention, nanoparticles (such as albumin nanoparticles) of the inventive compounds can be administered by any acceptable route including, but not limited to, orally, intramuscularly, transdermally, intravenously, through an inhaler or other air borne delivery systems and the like.

**[0104]** When preparing the composition for injection, particularly for intravenous delivery, the continuous phase preferably comprises an aqueous solution of tonicity modifiers, buffered to a pH range of about 5 to about 8.5. The pH may also be below 7 or below 6. In some embodiments, the pH of the composition is no less than about 6, including for example no less than about any of 6.5, 7, or 8 (such as about 7.5 or 8).

**Arbitrary Units**

**[0105]** The percentage of apoptosis induced by a gold (III) chloride formulation can be quantified in arbitrary units. The units are based on the flow cytometry methods described *supra* under the section describing cytotoxic effects of compositions of gold(III) chlorides. The results can be obtained in forms of histograms and statistical percentages as shown in the examples and accompanying Figures 2A through 2E. One arbitrary unit is defined as the volume of gold (III) chloride formulation necessary to induce an increase of 1% of apoptosis above the statistical average of a non-treated control group of a cell culture dish containing $1x10^6$ cancer cells (for example CAL27 cell line). Most if not all untreated control groups render a normal positive average of fluorescent levels which is indicative of endogenous apoptosis due to physiologic conditions of the cell culture. It is normal and expected that a certain percentage of cells die in culture due to endogenous or physiological reasons. That physiological percentage of apoptosis is considered a baseline not caused by any external environment factors. Usually that percentage is less than 5% and varies from cell line to cell line. An arbitrary unit is the amount of volume of gold(III) chloride formulation that induces an increase of one percentage point above the previously established baseline. Statistical analysis is preformed using lineal regression in which the dependent variable is the average of fluorescent intensity (which renders a percentage in comparison to the control data) and the independent variable is the volume of gold(III) chloride formulation applied to the experiment.

**[0106]** 100 µl of gold(III) chloride formulation induces at least 5% to 8% apoptosis (above the control group baseline) in the CAL27 cell line under the conditions described in the patent application. Thus, 100µl of gold(III) chloride formulation is equivalent to 5 to 8 Arbitrary Units; activity refers to the increase in the average of fluorescence (due to a higher percentage of the M30 Cytodeath fluorescent antibody positive cells) compared to the average of fluorescence of the treatment group (baseline level) using the M30 Cytodeath® (Roche) kit examined by flow cytometry.

**Vehicles for Gold(III) Derived Solutions**

**[0107]** Lactose has been used as a vehicle in the development of gold(III) chloride formulations, including those of hydrogen tetrachloroaurate. The function of the sugar is to serve as vehicle for the active ingredient. However, other sugar derivatives can be used including but not limited to glucosamine, D-glucoronic acid, N-acetyl-D-glucosamine, and disaccharides like fructose, glucose, sucrose, mannose, galactose, ribose, and others trioses, tetroses, pentoses, hexoses, heptoses, octoses, and cellobiose, starch, amylose, oligosaccharides and polysaccharides and others can be used. In addition, lipids, proteins, nucleic acids can also be used as vehicles in gold(III) chloride formulations.

EXAMPLES

**[0108]** Without further elaboration, it is believed that one skilled in the art can, using the preceding description, utilize the present invention to its fullest extent. The following examples are illustrative only, and not limiting of the remainder of the disclosure in any way whatsoever.

**Example 1: Methods for preparation of active compositions of hydrogen tetrachloroaurate, $HAuCl_4$ and auric chloride**

**[0109]** The 10 methods described below produce gold(III) chloride-derived formulations that have anti-cancer effects. The degree of efficacy varies greatly from method to method. Other methods may produce gold(III) chloride-derived formulations that have none or insufficient anti-cancer effects. Each method must be assayed for producing gold(III) chloride-derived formulations that have desired properties. All procedures described below are performed under Good Manufacturing Practice (GMP) Regulations promulgated by the US Food and Drug Administration.

*Method 1*

**[0110]** Step 1: 0.5g to 175g of Gold(III) Chloride (Gold Trichloride, Acid or Hydrogen tetrachloroaurate ($HAuCl_4$) or Gold Trichloride or Auric Chloride ($AuCl_3$)) are placed in a beaker with 500mL of aqueous solution containing 4.3 mg of solid sodium borohydride. under vigorous stirring overnight. Nanogold thus formed were filtered through a 0.22-μm filter and used for experiments. This solution is called Nano-Gold Solution A.

**[0111]** Step 2: 0.5ml to 200ml of Nano-Gold Solution A are placed in a bottle and a range of 5ml to 900ml of double distilled water or sterile injectable grade solution of NaCl in a concentration that varies from .01 to 20% or double distilled water and alcohol (one to four volumens of water and nine to six volumens of alcohol) are mixed by agitation with Nano-Gold-Solution A. This mixture makes up Nano-Gold-Solution B.

**[0112]** Step 3: 0.5ml to 200ml of Nano-Gold-Solution B are placed in a second bottle and a range of 5ml to 900ml of double distilled water or sterile injectable grade solution of NaCl in a concentration that varies from .01 to 20% or alcohol are added and vigorously mixed. This solution is called Nano-Gold-Solution C.

**[0113]** Step 4: In this protocol, the name designation of the solutions is continuous, stemming from Nano Gold Solution A and follows a denominating method based on an alphabetical order for the gold-derived solutions. Step #3 is a reproducible step and key for the production methodology of downstream solutions. Step # 3 can be repeated under identical conditions with the logical exception that successional solutions are used as starter solutions that later are mixed with water or alcohol and produce a new downstream solution that in turn can be summitted to step # 3 but now as a starter solution to produce a new downstream solution. For example, if step #3 of this protocol is applied to Nano-Gold Solution B this process gives rise to Nano-Gold Solution C, if step # 3 is then applied to Nano-Gold Solution C (now considered the starter solution) it gives rise to Nano-Gold Solution D, if step #3 is applied to Nano-Gold Solution D this would give rise to Nano-Gold Solution E and so on and so on.

**[0114]** Step 5: The nano-gold(III) chloride derived products of this procedure are cytotoxic to cancer cells inducing apoptosis and cell death in percentages comparable to chemotherapy agents methotrexate and cisplatin. The number of subsequent steps (solution A to Z or further downstream AA,BB) that are necessary to induce apoptosis of different cells lines can vary and most likely will vary depending of the cell line examined and the

biological condition they are under. Some cells are sensitive (induction of apoptosis, DNA damage) at Nano-Gold Solutions F to H, while other cell types may need further downstream preparations. The volumen of each gold-derived solution used as therapy (dosage including concentration, volumens and timing) may be fixed or vary according to the different protocols and cell types being examined. The gold-derived solution is applied directly to the cell culture.

**[0115]** Step 6: Nano-Gold(III) chloride-derived solutions are tested on cell cultures by flow cytometry and other methods used for cytotoxicity and toxicology studies are then applied to observe the apoptotic effects of the gold-derived solutions as described elsewhere in the Specification.

**[0116]** Step 7: The results of Step 6 are analyzed and dosage and therapeutic indices, $LC_{50}$ can be established. Results are examined statistically and graphed. Several methods are used, one histogram method places all Nano-Gold derived solutions (for example A to Z) used in an experiment in the y-axis. The numbers of cells treated $1x10^6$, $2x10^6$, $3x10^6$ or a fixed number of cells are placed in the x-axis and the histogram examines one or several cellular or molecular events, for example apoptosis, necrosis, cell proliferation, DNA damage, cell cycle events, oncogene expression, cell adhesion, molecular markers (smads, cytokines, and others), toxicity, drug resistance and others. Histograms and statistical tables are then made. Definition of events can be established by percentages, number of cells, comparison to other drugs and molecules, protein concentration, DNA and RNA concentration, degradation, expression patterns and others. Arbitrary, international and/or relative units of activity, expression, damage, cell death, or others can be established. For *in vivo* studies, statistics are equally applied and the parameter mentioned above can be included in addition to the ones recommended for each particular study, serum levels, enzymes, general symptoms (fever, pain and others). Each clinical phase study includes all parameters required by the FDA.

*Method 2*

**[0117]** Step 1: 0.5g to 175g of Gold(III) Chloride (Gold Trichloride, Acid or Hydrogen tetrachloroaurate ($HAuCl_4$) or Gold Trichloride or Auric Chloride ($AuCl_3$)) are placed in a mortar grinder with 5g to 250g of lactose granules. These two ingredients are then crushed and grinded together in such a form that a uniform and a finely homogenized powder is accomplished. The grinding period may range from 10 to 35 minutes. Proceed to step 2.

**[0118]** Step 2: 1g to 250g of Gold powder obtained in step 1 are placed in a beaker and dissolved in 38.8 mM $Na_3C_6H_5O_7$ by dissolving the Stock powder in 500to 2,500mL of distilled water in order to obtain gold nanoparticles. The elements are then boiled for 5 to 35 minutes. This solution is called Gold Solution A.

**[0119]** Step 3: 0.5ml to 200ml of Gold Solution A are placed in a second bottle and a range of 5ml to 900ml of double distilled water or sterile injectable grade solution of NaCl in a concentration that varies from .01 to 20% or double distilled water and alcohol (one to four volumens of water and nine to six volumens of alcohol) are mixed by agitation with Gold-Solution A. This mixture makes up Gold-Solution B.

**[0120]** Step 4: 0.5ml to 200ml of Gold-Solution B are placed in a third bottle and a range of 5ml to 900ml of double distilled water or sterile injectable grade solution of NaCl in a concentration that varies from .01 to 20% or alcohol are added and vigorously mixed. This solution is called Gold-Solution C.

**[0121]** Step 5: The gold-derived Gold-Solution C is cytotoxic to cancer cells inducing apoptosis and cell death in percentages comparable to chemotherapy agents methotrexate and cisplatin. The volumen of gold-derived Solution C used as therapy (dosage including concentration, volumens and timing) may be fixed or vary according to the different protocols and cell types being examined. The gold-derived solution is applied directly to the cell culture.

**[0122]** Step 6: Nano-Gold(III) chloride-derived solutions are tested on cell cultures by flow cytometry and other methods used for cytotoxicity and toxicology studies are then applied to observe the apoptotic effects of the gold-derived solutions as described elsewhere in the Specification.

**[0123]** Step 7: The results of Step 6 are analyzed and dosage and therapeutic indices, $LC_{50}$ can be established. Results are examined statistically and graphed. Several methods are used, one histogram method places all Nano-Gold derived solutions (for example A to Z) used in an experiment in the y-axis. The numbers of cells treated $1x10^6$, $2x10^6$, $3x10^6$ or a fixed number of cells are placed in the x-axis and the histogram examines one or several cellular or molecular events, for example apoptosis, necrosis, cell proliferation, DNA damage, cell cycle events, oncogene expression, cell adhesion, molecular markers (smads, cytokines, and others), toxicity, drug resistance and others. Histograms and statistical tables are then made. Definition of events can be established by percentages, number of cells,

comparison to other drugs and molecules, protein concentration, DNA and RNA concentration, degradation, expression patterns and others. Arbitrary, international and/or relative units of activity, expression, damage, cell death, or others can be established. For *in vivo* studies, statistics are equally applied and the parameter mentioned above can be included in addition to the ones recommended for each particular study, serum levels, enzymes, general symptoms (fever, pain and others). Each clinical phase study includes all parameters required by the FDA.

*Method 3*

**[0124]** Step 1: 0.5g to 175g of Gold(III) Chloride (Gold Trichloride, Acid or Hydrogen tetrachloroaurate ($HAuCl_4$) or Gold Trichloride or Auric Chloride ($AuCl_3$)) are placed in a mortar grinder with 5g to 250g of lactose granules. These two ingredients are then crushed and grinded together in such a form that a uniform and a finely homogenized powder is accomplished. The first grinding period may range from 2 to 20 minutes. Proceed to step 2.

**[0125]** Step 2: After grinding, the mix is called Gold-Lactose Fine Powder Stock Mix and it is placed in a capped bottle (dark ambar bottles or other glass light protective bottles should be used) that protects the product from the light, this precaution should be included in the rest of the procedures since the product is light sensitive.

**[0126]** Step 3: Then, 1g to 50g of the Gold-Lactose Fine Powder Stock Mix is placed in a light protective glass bottle that contains a range of 0.5ml to 3,000ml of double distilled water or sterile injectable grade solution of NaCl in a concentration that varies from .01 to 20%, the elements are mixed and filtered. This Gold-Lactose solution is called Gold-Solution A.

**[0127]** Step 4: 0.5ml to 200ml of Gold-Solution A are placed in a third bottle and a range of 5ml to 900ml of double distilled water or sterile injectable grade solution of NaCl in a concentration that varies from .01 to 20% or alcohol are added and vigorously mixed. This solution is called Gold-Solution B.

**[0128]** Step 5: In this protocol, the name designation of the solutions is continuos, stemming from Gold-Lactose Fine Powder Stock Mix and follows a denominating method based on an alphabetical order for the gold-derived solutions. Step #4 is a reproducible step and key for the production methodology of downstream solutions. Step # 4 can be repeated under identical conditions with the logical exception that successional solutions are used as starter solutions that later are mixed with water or alcohol and produce a new downstream solution that in turn if needed can be summitted to step # 4 but now as a starter solution to

produce a new downstream solution. For example, if step #4 of this protocol is applied to Gold Solution B this process gives rise to Gold Solution C, if step # 4 is then applied to Gold Solution C (now considered the starter solution) it gives rise to Gold Solution D, if step #4 is applied to Gold Solution D this would give rise to Gold Solution E and so on and so on.

**[0129]** Step 6: The gold(III) chloride-derived products of this procedure are cytotoxic to cancer cells inducing apoptosis and cell death in percentages comparable to chemotherapy agents methotrexate and cisplatin. The number of subsequent steps (solution A to Z or further downstream AA,BB) that are necessary to induce apoptosis of different cells lines can vary and most likely will vary depending of the cell line examined and the biological condition they are under. Some cells are sensitive (induction of apoptosis, DNA damage) at Gold Solutions F to H, while other cell types may need further downstream preparations. The volumes of each gold(III) chloride-derived solution used as therapy (dosage including concentration, volumes and timing) may be fixed or vary according to the different protocols and cell types being examined. The gold(III) chloride-derived solution is applied directly to the cell culture.

**[0130]** Step 7: Nano-Gold(III) chloride-derived solutions are tested on cell cultures by flow cytometry and other methods used for cytotoxicity and toxicology studies are then applied to observe the apoptotic effects of the gold-derived solutions as described elsewhere in the Specification.

**[0131]** Step 8: The results of Step 7 are analyzed and dosage and therapeutic indices, $LC_{50}$ can be established. Results are examined statistically and graphed. Several methods are used, one histogram method places all Nano-Gold derived solutions (for example A to Z) used in an experiment in the y-axis. The numbers of cells treated $1x10^6$, $2x10^6$, $3x10^6$ or a fixed number of cells are placed in the x-axis and the histogram examines one or several cellular or molecular events, for example apoptosis, necrosis, cell proliferation, DNA damage, cell cycle events, oncogene expression, cell adhesion, molecular markers (smads, cytokines, and others), toxicity, drug resistance and others. Histograms and statistical tables are then made. Definition of events can be established by percentages, number of cells, comparison to other drugs and molecules, protein concentration, DNA and RNA concentration, degradation, expression patterns and others. Arbitrary, international and/or relative units of activity, expression, damage, cell death, or others can be established. For *in vivo* studies, statistics are equally applied and the parameter mentioned above can be included in addition to the ones recommended for each particular study, serum levels, enzymes,

general symptoms (fever, pain and others). Each clinical phase study includes all parameters required by the FDA.

*Method 4*

**[0132]** Step 1: 0.5g to 175g of Gold(III) Chloride (Gold Trichloride, Acid or Hydrogen tetrachloroaurate ($HAuCl_4$) or Gold Trichloride or Auric Chloride ($AuCl_3$)) are placed in a mortar grinder with 5g to 250g of lactose granules. These two ingredients are then crushed and grinded together in such a form that a uniform and a finely homogenized powder is accomplished. The first grinding period may range from 2 to 35 minutes. Proceed to step 2.

**[0133]** Step 2: Lactose (0g to 250g) is added to the mix and the addition of gold ranges from 0g to 40g and a second grinding period is applied to the mix (2 to 35 minutes). Proceed to step 3.

**[0134]** Step 3: Lactose (0g to 250g) is added to the mix and the addition of gold ranges from 0g to 40g and a third grinding period is applied to the mix (2 to 35 minutes). Proceed to step 4.

**[0135]** Step 4: After this third grinding period, the mix is called Gold-Lactose Fine Powder Stock Mix and it is placed in a capped bottle (dark ambar bottles or other glass light protective bottles should be used) that protects the product from the light, this precaution should be included in the rest of the procedures since the product is light sensitive.

**[0136]** Step 5: Then, 1g to 400g of the Gold-Lactose Fine Powder Stock Mix is placed in a light protective glass bottle that contains a range of 0.5ml to 7,500ml of double distilled water or sterile injectable grade solution of NaCl in a concentration that varies from .01 to 20%, the elements are mixed and filtered. This Gold-Lactose solution is called Gold-Solution A.

**[0137]** Step 6: 0.5ml to 200ml of Gold Solution A are placed in a second bottle and a range of 5ml to 900ml of double distilled water or sterile injectable grade solution of NaCl in a concentration that varies from .01 to 20% or double distilled water and alcohol (one to four volumens of water and nine to six volumens of alcohol) are mixed by agitation with Gold-Solution A. This mixture makes up Gold-Solution B.

**[0138]** Step 7: 0.1ml to 100ml of Gold-Solution B are placed in a third bottle and final bottle.The rest of the solution subtractions and adding of double distilled water or sterile injectable grade solution of NaCl in a concentration that varies from .01 to 20%, alcohol or

other solutions are done in this same final third bottle. A range of 5ml to 900ml of double distilled water or sterile injectable grade solution of NaCl in a concentration that varies from .01 to 20% or alcohol are added and vigorously mixed. This solution is called Gold-Solution C.

**[0139]** Step 8: 0.1ml to 300ml of Gold Solution C are suctioned out of the bottle and and replaced with 0.1ml to 300ml of water, alcohol or other solutions and are vigorously mixed. The final product is called Gold-Solution D.

**[0140]** Step 9: In this protocol, the name designation of the solutions is continuos, stemming from Gold-Lactose Fine Powder Stock Mix and follows a denominating method based on an alphabetical order for the gold-derived solutions. Step #8 is a reproducible step and key for the production methodology of downstream solutions. Step # 8 can be repeated under identical conditions with the logical exception that successional solutions are used as starter solutions that later are mixed with water or alcohol and produce a new downstream solution that in turn if needed can be summitted to step # 8 but now as a starter solution to produce a new downstream solution. For example, if step #8 of this protocol is applied to Gold Solution B this process gives rise to Gold Solution C, if step # 8 is then applied to Gold Solution C (now considered the starter solution) it gives rise to Gold Solution D, if step #8 is applied to Gold Solution D this would give rise to Gold Solution E and so on and so on.

**[0141]** Step 10: The gold(III) chloride-derived products of this procedure are cytotoxic to cancer cells inducing apoptosis and cell death in percentages comparable to chemotherapy agents methotrexate and cisplatin. The number of subsequent steps (solution A to Z or further downstream AA,BB) that are necessary to induce apoptosis of different cells lines can vary and most likely will vary depending of the cell line examined and the biological condition they are under. Some cells are sensitive (induction of apoptosis, DNA damage) at Gold Solutions F to H, while other cell types may need further downstream preparations. The volumes of each gold(III) chloride-derived solution used as therapy (dosage including concentration, volumes and timing) may be fixed or vary according to the different protocols and cell types being examined. The gold(III) chloride-derived solution is applied directly to the cell culture.

**[0142]** Step 11: Nano-Gold(III) chloride-derived solutions are tested on cell cultures by flow cytometry and other methods used for cytotoxicity and toxicology studies are then

applied to observe the apoptotic effects of the gold-derived solutions as described elsewhere in the Specification.

**[0143]** Step 12: The results of Step 11 are analyzed and dosage and therapeutic indices, $LC_{50}$ can be established. Results are examined statistically and graphed. Several methods are used, one histogram method places all Nano-Gold derived solutions (for example A to Z) used in an experiment in the y-axis. The numbers of cells treated $1x10^6$, $2x10^6$, $3x10^6$ or a fixed number of cells are placed in the x-axis and the histogram examines one or several cellular or molecular events, for example apoptosis, necrosis, cell proliferation, DNA damage, cell cycle events, oncogene expression, cell adhesion, molecular markers (smads, cytokines, and others), toxicity, drug resistance and others. Histograms and statistical tables are then made. Definition of events can be established by percentages, number of cells, comparison to other drugs and molecules, protein concentration, DNA and RNA concentration, degradation, expression patterns and others. Arbitrary, international and/or relative units of activity, expression, damage, cell death, or others can be established. For *in vivo* studies, statistics are equally applied and the parameter mentioned above can be included in addition to the ones recommended for each particular study, serum levels, enzymes, general symptoms (fever, pain and others). Each clinical phase study includes all parameters required by the FDA.

*Method 5*

**[0144]** Step 1: 0.5g to 175g of Gold(III) Chloride (Gold Trichloride, Acid or Hydrogen tetrachloroaurate ($HAuCl_4$) or Gold Trichloride or Auric Chloride ($AuCl_3$)) are placed in a mortar grinder with 5g to 250g of lactose granules. These two ingredients are then crushed and grinded together in such a form that a uniform and a finely homogenized powder is accomplished. The first grinding period may range from 2 to 35 minutes. Proceed to step 2.

**[0145]** Step 2: After grinding, the mix is called Gold-Lactose Fine Powder Stock Mix and it is placed in a capped bottle (dark ambar bottles or other glass light protective bottles should be used) that protects the product from the light, this precaution should be included in the rest of the procedures since the product is light sensitive.

**[0146]** Step 3: 1g to 250g of Gold-Lactose Fine Powder Stock Mix are placed in a beaker and mixed in 38.8 mM Na3C6H5O7 by dissolving the stock powder in 500-2,500 mL of distilled water in order to obtain gold nanoparticles. The elements are then boiled for 5 to 35 minutes. This solution is called Gold Solution A.

**[0147]** Step 4: 0.5ml to 200ml of Gold-Solution A are placed in a bottle and a range of 5ml to 900ml of double distilled water or sterile injectable grade solution of NaCl in a concentration that varies from .01 to 20% or alcohol are added and vigorously mixed. This solution is called Gold-Solution B.

**[0148]** Step 5: In this protocol, the name designation of the solutions is continuos, stemming from Gold-Lactose Fine Powder Stock Mix and follows a denominating method based on an alphabetical order for the gold-derived solutions. Step #4 is a reproducible step and key for the production methodology of downstream solutions. Step # 4 can be repeated under identical conditions with the logical exception that successional solutions are used as starter solutions that later are mixed with water or alcohol and produce a new downstream solution that in turn if needed can be summitted to step # 4 but now as a starter solution to produce a new downstream solution. For example, if step #4 of this protocol is applied to Gold Solution B this process gives rise to Gold Solution C, if step # 4 is then applied to Gold Solution C (now considered the starter solution) it gives rise to Gold Solution D, if step #4 is applied to Gold Solution D this would give rise to Gold Solution E and so on.

**[0149]** Step 6: The gold(III) chloride-derived products of this procedure are cytotoxic to cancer cells inducing apoptosis and cell death in percentages comparable to chemotherapy agents methotrexate and cisplatin. The number of subsequent steps (solution A to Z or further downstream AA,BB) that are necessary to induce apoptosis of different cells lines can vary and most likely will vary depending of the cell line examined and the biological condition they are under. Some cells are sensitive (induction of apoptosis, DNA damage) at Gold Solutions F to H, while other cell types may need further downstream preparations. The volumes of each gold(III) chloride-derived solution used as therapy (dosage including concentration, volumes and timing) may be fixed or vary according to the different protocols and cell types being examined. The gold(III) chloride-derived solution is applied directly to the cell culture.

**[0150]** Step 7: Nano-Gold(III) chloride-derived solutions are tested on cell cultures by flow cytometry and other methods used for cytotoxicity and toxicology studies are then applied to observe the apoptotic effects of the gold-derived solutions as described elsewhere in the Specification.

**[0151]** Step 8: The results of Step 7 are analyzed and dosage and therapeutic indices, $LC_{50}$ can be established. Results are examined statistically and graphed. Several methods are

used, one histogram method places all Nano-Gold derived solutions (for example A to Z) used in an experiment in the y-axis. The numbers of cells treated $1x10^6$, $2x10^6$, $3x10^6$ or a fixed number of cells are placed in the x-axis and the histogram examines one or several cellular or molecular events, for example apoptosis, necrosis, cell proliferation, DNA damage, cell cycle events, oncogene expression, cell adhesion, molecular markers (smads, cytokines, and others), toxicity, drug resistance and others. Histograms and statistical tables are then made. Definition of events can be established by percentages, number of cells, comparison to other drugs and molecules, protein concentration, DNA and RNA concentration, degradation, expression patterns and others. Arbitrary, international and/or relative units of activity, expression, damage, cell death, or others can be established. For *in vivo* studies, statistics are equally applied and the parameter mentioned above can be included in addition to the ones recommended for each particular study, serum levels, enzymes, general symptoms (fever, pain and others). Each clinical phase study includes all parameters required by the FDA.

*Method 6*

**[0152]** Step 1: 0.5g to 175g of Gold(III) Chloride (Gold Trichloride, Acid or Hydrogen tetrachloroaurate ($HAuCl_4$) or Gold Trichloride or Auric Chloride ($AuCl_3$)) is placed in a mortar crushed and grinded in such a form that a uniform and a finely homogenized powder is accomplished. After this grinding period, the mix is called Gold Fine Powder Mix.

**[0153]** Step 2: 10g to 350 of the Gold Fine Powder Mix is placed in a beaker and dissolved in 38.8 mM Na3C6H5O7 by dissolving the Stock powder in 100 to 1,000 mL of distilled water in order to obtain gold nanoparticles. The elements are boiled for 5 to 45 minutes and left to rest for at least 4 hours at 20°F. This solution is called Gold Solution A.

**[0154]** Step 3: 0.5ml to 200ml of Gold Solution A are placed in a bottle and a range of 5ml to 900ml of double distilled water or sterile injectable grade solution of NaCl in a concentration that varies from .01 to 20% or double distilled water and alcohol (one to four volumens of water and nine to six volumens of alcohol) are mixed by agitation with Gold-Solution A. This mixture makes up Gold-Solution B.

**[0155]** Step 4: 0.5ml to 200ml of Gold-Solution B are placed in a bottle and a range of 5ml to 900ml of double distilled water or sterile injectable grade solution of NaCl in a concentration that varies from .01 to 20% or alcohol are added and vigorously mixed. This solution is called Gold-Solution C.

**[0156]** Step 5: The Gold-Solution C derived products obtained in this procedure are tested for cytotoxicity to cancer cells and inducing apoptosis and cell death in percentages comparable to chemotherapy agents methotrexate and cisplatin.

**[0157]** Step 6: Nano-Gold(III) chloride-derived solutions are tested on cell cultures by flow cytometry and other methods used for cytotoxicity and toxicology studies are then applied to observe the apoptotic effects of the gold-derived solutions as described elsewhere in the Specification.

**[0158]** Step 7: The results of Step 6 are analyzed and dosage and therapeutic indices, $LC_{50}$ can be established. Results are examined statistically and graphed. Several methods are used, one histogram method places all Nano-Gold derived solutions (for example A to Z) used in an experiment in the y-axis. The numbers of cells treated $1x10^6$, $2x10^6$, $3x10^6$ or a fixed number of cells are placed in the x-axis and the histogram examines one or several cellular or molecular events, for example apoptosis, necrosis, cell proliferation, DNA damage, cell cycle events, oncogene expression, cell adhesion, molecular markers (smads, cytokines, and others), toxicity, drug resistance and others. Histograms and statistical tables are then made. Definition of events can be established by percentages, number of cells, comparison to other drugs and molecules, protein concentration, DNA and RNA concentration, degradation, expression patterns and others. Arbitrary, international and/or relative units of activity, expression, damage, cell death, or others can be established. For *in vivo* studies, statistics are equally applied and the parameter mentioned above can be included in addition to the ones recommended for each particular study, serum levels, enzymes, general symptoms (fever, pain and others). Each clinical phase study includes all parameters required by the FDA.

*Method 7*

**[0159]** Step 1: 0.5g to 175g of Gold(III) Chloride (Gold Trichloride, Acid or Hydrogen tetrachloroaurate ($HAuCl_4$) or Gold Trichloride or Auric Chloride ($AuCl_3$)) are placed in a beaker with 500mL of aqueous solution containing 4.3 mg of solid sodium borohydride and are placed under vigorous stirring overnight. This solution is called Nano-Gold Solution. Proceed to step 2.

**[0160]** Step 2: The Nano-Solution is then centrifuged for 12h at 45,000RPM. Once the run is done the liquid portion is decanted and the gold is resuspended in 500ml of double

distilled aqueous solution containing 4.3 mg of solid sodium borohydride and again placed under vigorous stirring overnight in order to obtain finer and uniform gold-nanoparticles.

**[0161]** Step 3: After the second stirring period is completed, the solution is called Gold Solution A.

**[0162]** Step 4: 0.5ml to 200ml of Gold-Solution A are placed in a bottle and a range of 5ml to 900ml of double distilled water or sterile injectable grade solution of NaCl in a concentration that varies from .01 to 20% or alcohol are added and vigorously mixed. This solution is called Gold-Solution B.

**[0163]** Step 5: The Gold-Solution C derived products obtained in this procedure are tested for cytotoxicity to cancer cells and inducing apoptosis and cell death in percentages comparable to chemotherapy agents methotrexate and cisplatin.

**[0164]** Step 6: Nano-Gold(III) chloride-derived solutions are tested on cell cultures by flow cytometry and other methods used for cytotoxicity and toxicology studies are then applied to observe the apoptotic effects of the gold-derived solutions as described elsewhere in the Specification.

**[0165]** Step 7: The results of Step 6 are analyzed and dosage and therapeutic indices, $LC_{50}$ can be established. Results are examined statistically and graphed. Several methods are used, one histogram method places all Nano-Gold derived solutions (for example A to Z) used in an experiment in the y-axis. The numbers of cells treated $1x10^6$, $2x10^6$, $3x10^6$ or a fixed number of cells are placed in the x-axis and the histogram examines one or several cellular or molecular events, for example apoptosis, necrosis, cell proliferation, DNA damage, cell cycle events, oncogene expression, cell adhesion, molecular markers (smads, cytokines, and others), toxicity, drug resistance and others. Histograms and statistical tables are then made. Definition of events can be established by percentages, number of cells, comparison to other drugs and molecules, protein concentration, DNA and RNA concentration, degradation, expression patterns and others. Arbitrary, international and/or relative units of activity, expression, damage, cell death, or others can be established. For *in vivo* studies, statistics are equally applied and the parameter mentioned above can be included in addition to the ones recommended for each particular study, serum levels, enzymes, general symptoms (fever, pain and others). Each clinical phase study includes all parameters required by the FDA.

*Method 8*

**[0166]** Step 1: 0.5g to 175g of Gold(III) Chloride (Gold Trichloride, Acid or Hydrogen tetrachloroaurate ($HAuCl_4$) or Gold Trichloride or Auric Chloride ($AuCl_3$)) are placed in a mortar grinder with 5g to 250g of lactose granules. These two ingredients are then crushed and grinded together in such a form that a uniform and a finely homogenized powder is accomplished. The first grinding period may range from 2 to 35 minutes. Proceed to step 2.

**[0167]** Step 2: Lactose (0g to 250g) is added to the mix and the addition of gold ranges from 0g to 40g and a second grinding period is applied to the mix (2 to 35 minutes). Proceed to step 3.

**[0168]** Step 3: Lactose (0g to 250g) is added to the mix and the addition of gold ranges from 0g to 40g and a third grinding period is applied to the mix (2 to 35 minutes). Proceed to step 4.

**[0169]** Step 4: After this third grinding period, the mix is called Gold-Lactose Fine Powder Stock Mix and it is placed in a capped bottle (dark ambar bottles or other glass light protective bottles should be used) that protects the product from the light, this precaution should be included in the rest of the procedures since the product is light sensitive.

**[0170]** Step 5: Then, 1g to 400g of the Gold-Lactose Fine Powder Stock Mix is placed in a light protective glass bottle that contains a range of 0.5ml to 7,500ml of double distilled water or sterile injectable grade solution of NaCl in a concentration that varies from .01 to 20%, the elements are mixed and filtered. This Gold-Lactose solution is called Gold-Solution A.

**[0171]** Step 6: 0.5ml to 200ml of Gold Solution A are placed in a second bottle and a range of 5ml to 900ml of double distilled water or sterile injectable grade solution of NaCl in a concentration that varies from .01 to 20% or double distilled water and alcohol (one to four volumens of water and nine to six volumens of alcohol) are mixed by agitation with Gold-Solution A. This mixture makes up Gold-Solution B.

**[0172]** Step 7: 0.5ml to 200ml of Gold-Solution B are placed in a third bottle and a range of 5ml to 900ml of double distilled water or sterile injectable grade solution of NaCl in a concentration that varies from .01 to 20% or alcohol are added and vigorously mixed. This solution is called Gold-Solution C.

**[0173]** Step 8: In this protocol, the name designation of the solutions is continuos, stemming from Gold-Lactose Fine Powder Stock Mix and follows a denominating method based on an alphabetical order for the gold-derived solutions. Step #7 is a reproducible step and key for the production methodology of downstream solutions. Step # 7 can be repeated under identical conditions with the exception that successional solutions are used as starter solutions that later are mixed with water or alcohol and produce a new downstream solution that in turn if needed can be summitted to step # 7 but now as a starter solution to produce a new downstream solution. For example, if step #7 of this protocol is applied to Gold Solution B this process gives rise to Gold Solution C, if step # 7 is then applied to Gold Solution C (now considered the starter solution) it gives rise to Gold Solution D, if step #7 is applied to Gold Solution D this would give rise to Gold Solution E and so on.

**[0174]** Step 9: The gold(III) chloride-derived products of this procedure are cytotoxic to cancer cells inducing apoptosis and cell death in percentages comparable to chemotherapy agents methotrexate and cisplatin. The number of subsequent steps (solution A to Z or further downstream AA,BB) that are necessary to induce apoptosis of different cells lines can vary and most likely will vary depending of the cell line examined and the biological condition they are under. Some cells are sensitive (induction of apoptosis, DNA damage) at Gold Solutions F to H, while other cell types may need further downstream preparations. The volumes of each gold(III) chloride-derived solution used as therapy (dosage including concentration, volumes and timing) may be fixed or vary according to the different protocols and cell types being examined. The gold(III) chloride-derived solution is applied directly to the cell culture.

**[0175]** Step 10: Nano-Gold(III) chloride-derived solutions are tested on cell cultures by flow cytometry and other methods used for cytotoxicity and toxicology studies are then applied to observe the apoptotic effects of the gold-derived solutions as described elsewhere in the Specification.

**[0176]** Step 11: The results of Step 10 are analyzed and dosage and therapeutic indices, $LC_{50}$ can be established. Results are examined statistically and graphed. Several methods are used, one histogram method places all Nano-Gold derived solutions (for example A to Z) used in an experiment in the y-axis. The numbers of cells treated $1x10^6$, $2x10^6$, $3x10^6$ or a fixed number of cells are placed in the x-axis and the histogram examines one or several cellular or molecular events, for example apoptosis, necrosis, cell proliferation, DNA damage, cell cycle events, oncogene expression, cell adhesion, molecular markers (smads,

cytokines, and others), toxicity, drug resistance and others. Histograms and statistical tables are then made. Definition of events can be established by percentages, number of cells, comparison to other drugs and molecules, protein concentration, DNA and RNA concentration, degradation, expression patterns and others. Arbitrary, international and/or relative units of activity, expression, damage, cell death, or others can be established. For *in vivo* studies, statistics are equally applied and the parameter mentioned above can be included in addition to the ones recommended for each particular study, serum levels, enzymes, general symptoms (fever, pain and others). Each clinical phase study includes all parameters required by the FDA.

*Method 9*

**[0177]** Step 1: 0.5g to 175g of Gold(III) Chloride (Gold Trichloride, Acid or Hydrogen tetrachloroaurate ($HAuCl_4$) or Gold Trichloride or Auric Chloride ($AuCl_3$)) are placed in a mortar grinder crushed and grinded from 2 to 10 minutes and form a finely homogenized powder called Gold Powder Stock Mix. Proceed to step 2.

**[0178]** Step 2: After grinding, the mix is called Gold Powder Stock Mix and it is placed in a capped bottle (dark ambar bottles or other glass light protective bottles should be used) that protects the product from the light, this precaution should be included in the rest of the procedures since the product is light sensitive.

**[0179]** Step 3: Then, 1g to 400g of the Gold Powder Stock Mix is placed in a light protective glass bottle that contains a range of 0.5ml to 7,500ml of double distilled water or sterile injectable grade solution of NaCl in a concentration that varies from .01 to 20%, the elements are mixed and filtered. Once filtered, the gold solution is place to boil for at least 45 minutes and no more than 180 minutes. This Gold solution is called Gold-Solution A.

**[0180]** Step 4: 0.5ml to 200ml of Gold Solution A are placed in a second bottle and a range of 5ml to 900ml of double distilled water or sterile injectable grade solution of NaCl in a concentration that varies from .01 to 20% or double distilled water and alcohol (one to four volumens of water and nine to six volumens of alcohol) are mixed by agitation with Gold-Solution A. This mixture makes up Gold-Solution B.

**[0181]** Step 5: The Gold-Solution B derived products of this procedure are cytotoxic to cancer cells inducing apoptosis and cell death in percentages comparable to chemotherapy agents methotrexate and cisplatin. The volumes of Gold-Solution B used as therapy (dosage including concentration, volumes and timing) may be fixed or vary according to the different

protocols and cell types being examined. The gold(III) chloride -derived solution is applied directly to the cell culture.

**[0182]** Step 6: Nano-Gold(III) chloride-derived solutions are tested on cell cultures by flow cytometry and other methods used for cytotoxicity and toxicology studies are then applied to observe the apoptotic effects of the gold-derived solutions as described elsewhere in the Specification.

**[0183]** Step 7: The results of Step 6 are analyzed and dosage and therapeutic indices, $LC_{50}$ can be established. Results are examined statistically and graphed. Several methods are used, one histogram method places all Nano-Gold derived solutions (for example A to Z) used in an experiment in the y-axis. The numbers of cells treated $1x10^6$, $2x10^6$, $3x10^6$ or a fixed number of cells are placed in the x-axis and the histogram examines one or several cellular or molecular events, for example apoptosis, necrosis, cell proliferation, DNA damage, cell cycle events, oncogene expression, cell adhesion, molecular markers (smads, cytokines, and others), toxicity, drug resistance and others. Histograms and statistical tables are then made. Definition of events can be established by percentages, number of cells, comparison to other drugs and molecules, protein concentration, DNA and RNA concentration, degradation, expression patterns and others. Arbitrary, international and/or relative units of activity, expression, damage, cell death, or others can be established. For *in vivo* studies, statistics are equally applied and the parameter mentioned above can be included in addition to the ones recommended for each particular study, serum levels, enzymes, general symptoms (fever, pain and others). Each clinical phase study includes all parameters required by the FDA.

*Method 10*

**[0184]** Step 1: 0.5g to 175g of Gold(III) Chloride (Gold Trichloride, Acid or Hydrogen tetrachloroaurate ($HAuCl_4$) or Gold Trichloride or Auric Chloride ($AuCl_3$)) are placed in a mortar grinder with 5g to 250g of lactose granules. These two ingredients are then crushed and grinded together in such a form that a uniform and a finely homogenized powder is accomplished. The grinding period may range from 2 to 180 minutes. Proceed to step 2.

**[0185]** Step 2: After the grinding period, the mix is called Gold-Lactose Fine Powder Stock Mix and it is placed in a capped bottle (dark ambar bottles or other glass light protective bottles should be used) that protects the product from the light, this precaution should be included in the rest of the procedures since the product is light sensitive.

**[0186]** Step 3: Then, 1g to 400g of the Gold-Lactose Fine Powder Stock Mix is placed in a light protective glass bottle that contains a range of 0.5ml to 7,500ml of double distilled water or sterile injectable grade solution of NaCl in a concentration that varies from .01 to 20%, the elements are mixed and filtered. This Gold-Lactose solution is called Gold-Solution A.

**[0187]** Step 4: 0.5ml to 200ml of Gold Solution A are placed in a second bottle and a range of 5ml to 900ml of double distilled water or sterile injectable grade solution of NaCl in a concentration that varies from .01 to 20% or double distilled water and alcohol (one to four volumens of water and nine to six volumens of alcohol) are mixed by agitation with Gold-Solution A. This mixture makes up Gold-Solution B.

**[0188]** Step 5: 0.5ml to 200ml of Gold-Solution B are placed in a third bottle and a range of 5ml to 900ml of double distilled water or sterile injectable grade solution of NaCl in a concentration that varies from .01 to 20% or alcohol are added and vigorously mixed. This solution is called Gold-Solution C.

**[0189]** Step 6: In this protocol, the name designation of the solutions is continuous, stemming from Gold-Lactose Fine Powder Stock Mix and follows a denominating method based on an alphabetical order for the gold-derived solutions. Step #5 is a reproducible step and key for the production methodology of downstream solutions. Step # 5 can be repeated under identical conditions with the logical exception that successional solutions are used as starter solutions that later are mixed with water or alcohol and produce a new downstream solution that in turn if needed can be summitted to step # 5 but now as a starter solution to produce a new downstream solution. For example, if step #5 of this protocol is applied to Gold Solution B this process gives rise to Gold Solution C, if step # 5 is then applied to Gold Solution C (now considered the starter solution) it gives rise to Gold Solution D, if step #5 is applied to Gold Solution D this would give rise to Gold Solution E and so on.

**[0190]** Step 7: The gold(III) chloride-derived products of this procedure are cytotoxic to cancer cells inducing apoptosis and cell death in percentages comparable to chemotherapy agents methotrexate and cisplatin. The number of subsequent steps (solution A to Z or further downstream AA,BB) that are necessary to induce apoptosis of different cells lines can vary and most likely will vary depending of the cell line examined and the biological condition they are under. Some cells are sensitive (induction of apoptosis, DNA damage) at Gold Solutions F to H, while other cell types may need further downstream preparations. The

volumes of each gold(III) chloride-derived solution used as therapy (dosage including concentration, volumes and timing) may be fixed or vary according to the different protocols and cell types being examined. The gold(III) chloride-derived solution is applied directly to the cell culture.

**[0191]** Step 8: Nano-Gold(III) chloride-derived solutions are tested on cell cultures by flow cytometry and other methods used for cytotoxicity and toxicology studies are then applied to observe the apoptotic effects of the gold-derived solutions as described elsewhere in the Specification.

**[0192]** Step 9: The results of Step 8 are analyzed and dosage and therapeutic indices, $LC_{50}$ can be established. Results are examined statistically and graphed. Several methods are used, one histogram method places all Nano-Gold derived solutions (for example A to Z) used in an experiment in the y-axis. The numbers of cells treated $1x10^6$, $2x10^6$, $3x10^6$ or a fixed number of cells are placed in the x-axis and the histogram examines one or several cellular or molecular events, for example apoptosis, necrosis, cell proliferation, DNA damage, cell cycle events, oncogene expression, cell adhesion, molecular markers (smads, cytokines, and others), toxicity, drug resistance and others. Histograms and statistical tables are then made. Definition of events can be established by percentages, number of cells, comparison to other drugs and molecules, protein concentration, DNA and RNA concentration, degradation, expression patterns and others. Arbitrary, international and/or relative units of activity, expression, damage, cell death, or others can be established. For *in vivo* studies, statistics are equally applied and the parameter mentioned above can be included in addition to the ones recommended for each particular study, serum levels, enzymes, general symptoms (fever, pain and others). Each clinical phase study includes all parameters required by the FDA.

**Example 2: Methods for preparation of compositions of hydrogen tetrachloroaurate, $HAuCl_4$ and auric chloride that display insufficient cytotoxicity**

**[0193]** The gold(III) derived compositions produced by methods listed below are known to display insufficient cytotoxicity to cells and are therefore not suitable for the anticancer effects described in the specification.

**[0194]** Method 1 is described in Sau T.K. et al., Size controlled synthesis of gold nanoparticles using photochemically prepared seed particles Journal of Nanoparticle Research 3: 257–261, 2001. According to this procedure, UV irradiation of chloroauric acid

was carried out with an ordinary germicidal lamp (Philips, Holland G15T8Uvc, 15 W). Appropriate amounts of chloroauric acid and TX-100 solutions were taken in 4 1 cm stoppered quartz cuvettes for photo-irradiation. The cuvettes were placed at a distance of 3 cm from the light source and were irradiated for 25 min. The photoactivation produced pink colored Au(0) particles. These particles were used as 'seeds' for further growth. Then various amounts of 'seed' particles and the Au(III) ions were taken in such a way that the total amount of gold remains constant whereas gradually [Au(III)]/[seed] ratio increases. The ratio was varied from 2 to 100. In the next step, ascorbic acid was added slowly to the unstirred mixture of 'seed' and Au(III) ions. The particles develop within a few minutes. The kinetics of particle development was followed at $\lambda$ = 532 nm at 28 ° UV–visible spectroscopy using 1 cm quartz cuvette with Shimadzu UV-160 (Kyoto, Japan) spectrophotometer. Transmission electron microscopy (TEM) were used to characterize the particles. Sample was prepared by placing a drop of solution containing nanoparticles on a carbon-coated Cu grid.

**[0195]** Method 2 is a method of preparation based on Sodium Citrate. Prepare 1.0 mM $HAuCl_4$ by dissolving 0.1 g of the solid in 500 mL of distilled water. Prepare a 38.8 mM $Na_3C_6H_5O_7$ (sodium citrate) by dissolving 0.5 g of the solid in 50 mL of distilled water. Hydrogen tetrachloroaurate trihydrate, $HAuCl_4\_3H_2O$, and sodium citrate dihydrate, $Na_3C_6H_5O_7\_2H_2O$. 1g $HAuCl_4\_3H_2O$ makes 5 L of stock solution. Unused Au nanoparticle solution made in Part A can be stored for several years in a brown bottle. 1.0 mM $HAuCl_4$ stock solution is unstable and lasts only a few days.

Part A. Preparation of 13 nm-Diameter Gold Nanoparticles

**[0196]** 1. Pour 20 mL of 1.0 mM HAuCl4 into a 50-mL beaker. Add a magnetic stir bar. Heat the solution to boiling on a stir/hot plate while stirring with the magnetic stir bar.

**[0197]** 2. After the solution begins to boil, add 2 mL of 38.8 mM Na3C6H5O7. Continue to boil and stir the solution until it is a deep red color (about 10 min). As the solution boils, add distilled water as needed to keep the total solution volume near 22 mL. Excess citrate anions in solution stick to the Au metal surface, giving an overall negative charge to each Au nanoparticle.

**[0198]** 3. When the solution is a deep red color, turn off the hot plate and stirrer. Cool the solution to room temperature before using it in Part B.

Part B.

**[0199]** 1. In a small container, dissolve 0.5 g of table salt (NaCl) in 10 mL of distilled water to make a 1 M solution.

**[0200]** 2. In a small container, dissolve 2 g of sucrose in 10 mL of distilled water to make a 1 M solution.

**[0201]** 3. Into each of four glass vials or clear, colorless plastic cups, place 3 mL of the gold nanoparticle solution you prepared in Part A. Add 3 mL distilled water to each vial.

**[0202]** 4. With a dropper, add 5–10 drops, one at a time, of the salt solution from part B, step 1 to one of the vials.

**[0203]** 5. With a dropper, add 5–10 drops, one at a time, of the sugar solution from part B, step 2 to one of the vials containing fresh nanoparticle solution.

**[0204]** Method 3 is described by Mukherjee et al., in "Antiangiogenic Properties of Gold Nanoparticles" Clin Cancer Res 2005;11(9)May 1, 2005. According to this reference, in a typical experiment, 50 mL of aqueous solution containing 4.3 mg of solid sodium borohydride was added to 100 mL of 100 μmol/L aqueous solution of tetracholoroauric acid under vigorous stirring that was continued overnight. Nanogold thus formed were filtered through a 0.22 μm filter and used for experiments.

**[0205]** Method 4 is described in "Networks of gold nanoparticles and bacteriophage as biological sensors and cell-targeting agents" by Glauco R. Souza et al. in Proc. Natl. Acad. Sci. USA (2006) vol. 103(5): 1215-1220. The 44±9-nm Au nanoparticle solution, verified by

TEM image analysis, was prepared following the common citrate-reduction (30) procedure [mass ratio of 0.8 sodium citrate: 1.0 Au(III) chloride]. Au(III) chloride (≥99.99%) was purchased from Sigma-Aldrich. Handley, D. A. (1989) in Colloidal Gold: Principles, Methods, and Applications, ed. Hayat, M. A. (Academic, San Diego), Vol. 1, pp. 23–27.

**[0206]** Method 5 is described in "Gold based clusters and nanomaterials induced by radiolysis" by H. Remita, et al. (*see* "Radiation-Induced and Chemical Formation of Gold Clusters", E.Gachard, H. Remita, J. Khatouri, J. Belloni, B.Keita, L.Nadjo, New.J.Chem., 1257-1265, (1998)). Remita describes radiation-induced reduction of metal ions as a powerful method to control the synthesis of metal nanoparticles and supported clusters. With electron beams, very monodispersed clusters and new alloys are obtained at room temperature while by gamma radiolysis as in chemical reduction a few systems are shown to yield intimately alloyed clusters. Efficient competition often occurs between the radiolytic reduction process of both types of ions and the electron transfer reaction from the less noble metal atoms to the other metal ions. This preferential reduction of more noble metal first results in a segregation between the metals and eventually in a core-shell structure of the cluster with the more noble in the core. A sudden and complete reduction of both types of metal ions by a train of irradiation pulses (electron beams) prevents this kind of redox processes through electron transfer. It was shown that with the same mixed ion system the metal clusters obtained change with increasing dose rate from bilayared structure to a bimetallic solid solution. Three bimetallic systems were disclosed: Au/Ag, Au/Pd and Au/Pt. (Radiation-induced synthesis of mono- and multimetallic clusters and nanocolloids, J. Belloni, et al New.J.Chem., 22, 1239-1255, (1998); "Silver-palladium alloyed clusters synthesized by radiolysis" H. Remita, Jet al. Z. Phys. D, 40, 127 (1997); "Dose-rate Effect on Radiolytic Synthesis of Gold-Silver Bimetallic Clusters in Solution" M. Tréger, et al., J. Phys. Chem.B, 102, 4310-4321, (1998); "Dose rate effect on gold-palladium bimetallic structure " , H. Remita, A. Etchebery, J. Belloni, J. Phys. Chem. B, 107, 31-36, (2003)).

**[0207]** Method 6 is disclosed by Fu W. et al, in "Biomedical Applications of Gold Nanoparticles Functionalized Using Hetero-Bifunctional Poly(ethylene glycol) Spacer" Mater. Res. Soc. Symp. Proc. Vol. 845 © 2005 Materials Research Society.

### Example 3: Preparation of CAL 27 cell line used to determine anticancer activity

**[0208]** The oral cancer cell line CAL 27 (ATCC Number CRL-2095) was used and propagated according to the American Type Culture Collection (ATCC) recommendations.

**[0209]** Cells of the CAL 27 oral cancer cell line were grown according to the recommendation of the American Type Culture Collection. ATCC complete growth medium comprises: Dulbecco's modified Eagle's medium with 4 mM L-glutamine adjusted to contain 1.5 g/L sodium bicarbonate and 4.5 g/L glucose, 90%; fetal bovine serum, 10%. Cells were grown at 37.0 °C. Subculturing protocol involved the following steps: remove spent medium, add fresh 0.25% trypsin, 0.53 mM EDTA solution, rinse and remove trypsin. Add fresh trypsin and let the culture sit at room temperature (or at 37°C) until the cells detach. Add fresh medium, aspirate and dispense into new flasks. A subcultivation ratio of 1:6 was recommended and medium renewed every 2 to 3 days. Doubling Time for the cell culture was about 35 hrs.

**[0210]** CAL 27 is tumorigenic in mice. Solid tumors developed within 6 weeks in nude mice inoculated with 2x $10^6$ cells subcutaneously.

**[0211]** The DNA Profile of the CAL 27 cell line is as follows: (STR): Amelogenin: X; CSF1PO: 10,12; D13S317: 10,11; D16S539: 11,12; D5S818: 11,12; D7S820: 10; THO1: 6,9.3; TPOX: 8; vWA: 14,17.

**[0212]** Cal 27 is aneuploid (modal number = 43) and was established in 1982 by J. Gioanni (Centre Antoine Lacassagne, Nice Cedex, France) from tissue taken prior to treatment from a 56 year old Caucasian male with a lesion of the middle of the tongue. (Gioanni J , et al. Eur. J. Cancer Clin. Oncol. 24: 1445-1455, 1988). CAL 27 cells are epithelial, shaped polygonal with a highly granular cytoplasm. Immunocytochemical studies show strong positive staining with anti keratin antibodies. The cells do not grow well in semi-solid medium.

**[0213]** Marked inhibition of thymidine incorporation was observed in the presence of VP16 (etoposide), CCNU (1-[2-chloroethyl]-3-cyclohexyl-1-nitrosourea), VM26 (teniposide), ADM (adriamycin), CPA (cyclophosphamide), and MTX (methotrexate).

**[0214]** CAL 27 cells were resistant to treatment with VDS (vindesine sulfate), CDP (cis-platinum) or ACTD (actinomycin D).

**Example 4: Anticancer activity of Hydrogen tetrachloroaurate, $HAuCl_4$.**

**[0215]** Compounds comprising hydrogen tetrachloroaurate have affinity for epithelial tissues. Hydrogen tetrachloroaurate was shown to display cytotoxic anti-cancer effects when applied to cells of the oral cancer cell line CAL 27 (ATCC Number CRL-2095) *in vitro*.

When applied to the CAL 27 epithelial cancer cells cytotoxicity was observed at 24, 48 and 72 hours. Cytotoxicity (measured in terms of $IC_{50}$) was compared with other leading chemotherapy drugs such as cisplatin, methotrexate and 5-fluorouracil. All drugs were tested *in vitro* under identical conditions. After 48 hours of exposure, hydrogen tetrachloroaurate achieved cytotoxicity that ranged from 30 to 42% of the total cells in culture that was comparable to 25 to 32% achieved by methotrexate, 34 to 43% for cisplatin and 42 to 52% of cells in apoptosis in culture that were treated with adriamycin.

**Example 5: Activation of apoptosis by hydrogen tetrachloroaurate**

**[0216]** The cytotoxic effect of hydrogen tetrachloroaurate has the characteristics of but is not limited to the apoptosis cell death pathway. Using commercial kits for cell death detection based on fluorescent monoclonal antibodies that recognize degraded cytokeratin 18 demonstrate caspase activity. Caspases are a family of enzymes that are activated during apoptosis and participate in internal degradation of cells on a path to cell death. In addition, caspases activity was determined.

**[0217]** Several caspases are thought to mediate very early stages of apoptosis. For instance, one of these, caspase 3 (CPP32) is required for the induction of apoptosis by certain effectors especially tumor necrosis factor and the cytotoxic T cell ligand effector, CD95 (also called Fas) (Enari et al. (1996) Nature 380:723–726).

**[0218]** The percentage of apoptotic cells was determined by M30 CytoDEATH™ antibody. The M30 CytoDEATH™ monoclonal antibody has been shown in numerous independent studies to be specific to the CK18-Asp396 caspase cleavage site (neo-epitope (M30)) and represents a well-established, validated apoptosis marker that does not react with viable or necrotic cells. Detection of apoptosis is accomplished by applying the M30-antibody to fixed samples, then using secondary detection systems based on immunohistochemistry, immunocytochemistry, and flow cytometry.

**[0219]** This is a monoclonal mouse immunoglobulin (Ig) G2b antibody (clone M30; Roche, Mannheim, Germany) that binds to a caspase-cleaved, formalin-resistant epitope of cytokeratin 18 cytoskeletal protein. The immunoreactivity of the M30 antibody is confined to the cytoplasm of apoptotic cells. Cells, treated with homocysteine (20 μmol/L) for 24 h, were fixed in 10% neutral-buffered formalin for 15 min, treated with 0.3% hydrogen peroxide in methanol for 10 min to block the endogenous peroxidase activity, washed in PBS, and then incubated with M30 antibody at room temperature for 1 h. In negative controls, preimmune

mouse serum instead of primary antibody was used. Immunoreactions were revealed by the avidin-biotin complex technique using diaminobenzidine (DAB) as substrate. The number of M30-positive cells in all fields was counted at 400x final magnification. For each slide, three randomly selected microscopic fields were observed and at least 100 cells/field were evaluated.

**Example 6: Effects of hydrogen tetrachloroaurate and auric chloride on different cancer types**

**[0220]** Cancer cell lines from different types of cancer such prostate, lung, breast, etc. are treated with formulations of hydrogen tetrachloroaurate and auric chloride to establish optimal dosage, therapeutic index, and conduct drug resistance assays. Cells derived from different human tissues that are used to examine the effects of hydrogen tetrachloroaurate and auric chloride include the following established cell lines: HSC3, HSC4, SCC25, CAL27, MCF-7, MDA-MB-361, MCF-10-2A, MDA-MB-134-VI, HBT30, HELA, HS-27, FF24, NK-92, HEP-G2, U-118 MG, DMS 114, RWPE 2, MDA-MB-134-VI, PC-3, T-47D, T24, Caki-1, Caki-2, 769-P, HTB-5, SK-MEL-24, CCD-25Sk, SK-MEL-3, HTB-3, HTB-1, HTB-182, HTB-33, HTB-43, HTB-59, HTB-58, HTB-107, HB-8307, HTB-178, CRL-2404, U937, K562. Cells are grown according to the specifications of the ATCC (American Type Culture Collection, Manassas, VA 20108 USA).

**[0221]** Hydrogen tetrachloroaurate has a cytotoxic effect and leads to apoptosis in these cell lines.

**Example 7: Mechanism of action: DNA binding assays of hydrogen tetrachloroaurate and auric chloride**

**[0222]** Without being bound by theory, a mechanism of action of the gold(III) chloride compositions of the invention is mediated by DNA binding. DNA binding of hydrogen tetrachloroaurate and auric chloride is carried out by the following method.

**[0223]** A double-stranded fragment of DNA is used as the initial tether to bind the hydrogen tetrachloroaurate and auric chloride nanoparticles to a solid surface. The gold particle spatial distribution and number are determined using dark-field optical microscopy and digital image capture. Site-specific cleavage of the DNA tether results in nanoparticle release. The methodology and validation of this approach for measuring enzyme-mediated, individual DNA cleavage events, rapidly, with high specificity, and in real-time are described. This approach is used to detect and discriminate between nonmethylated and

methylated DNA, and is a platform for high-throughput screening of modulators of enzyme activity (Glass, J. R., et al. (2006). "Enzyme-mediated individual nanoparticle release assay." Anal Biochem 353(2): 209-216.).

**[0224]** Hydrogen tetrachloroaurate and auric chloride nanoparticles bind DNA and cause DNA damage leading to apoptosis.

**Example 8: Comparison of induction *in vitro* of apoptosis in squamous cell carcinoma cell line CAL27, by hydrogen tetrachloroaurate, methotrexate, adriamycin and cisplatin.**

**[0225]** CAL27 (squamous cell carcinoma) cell line was used in this study. Cells were grown according to the recommendations of the American Tissue Type Collection (ATCC). $2x10^6$ cells were used in each experiment and incubated with the experimental drug for 48h. The cytotoxicty results are expressed as a percentage of apoptosis, each series of study was performed at least five times and these results reflect the average of those studies. All groups received a total of 100µl (control or test drug) of volumen. Group 1 was treated with 100µl of PBS as control. The dosage applied to each group was as follows: groups two (Methotrexate 10µg/ml), three (Adriamycin 5µg/ml) , four Cisplatin (10µg/ml) and five (hydrogen tetrachloroaurate 500µg to 1ng/ml).

**[0226]** Following incubation, cells were processed according to instructions by Roche for their M30 Cytodeath kit. M30 is a flourescent monoclonal antibody that recognizes the degraded form of Cytyokeratin 18 and yields a FITC emission detected by flow cytometry in FL1 channel. The percentage of cells in the M1 regions is considered to represent cells positive for M30 and indicative of apoptosis. The results were as follows:

**[0227]** Group 1 (Control group): showed a basal level of apoptosis, normal for any healthy cell culture with an apoptosis percentage of 3.1% as shown in Figure 1A.

**[0228]** Group 2 was treated with 10µg/ml Methotrexate (Trexall®, Rheumatrex®) and displayed positive apoptosis percentage of 21% as shown in Figure 1B.

**[0229]** Group 3 was treated with 5µg/ml Adriamycin® (doxorubicin) and displayed positive apoptosis percentage of 64% as shown in Figure 1C.

**[0230]** Group 4 was treated with Cisplatin® (10µg/ml) and displayed positive apoptosis percentage of 23.1% as shown in Figure 1D.

**[0231]** Group 5 was treated with hydrogen tetrachloroaurate (500μg to 1ng/ml) and displayed positive apoptosis percentage of 32.3% as shown in Figure 1E.

**[0232]** Hydrogen tetrachloroaurate displayed an anti-cancer effect comparable to at least cisplatin and methotrexate in doses that elicit less systemic toxicity than these two conventional chemotherapy agents. The conditions of all groups were identical and these results clearly show the potent apoptotic activity of hydrogen tetrachloroaurate .

**Example 9: Induction of apoptosis *in vitro* in squamous cell carcinoma cells by hydrogen tetrachloroaurate.**

**[0233]** CAL27 (squamous cell carcinoma) cells were used in all five groups examined. Cells were grown according to the recommendations of the American Tissue Type Collection (ATCC). $2x10^6$ cells were used in each experiment and incubated with the hydrogen tetrachloroaurate for 48h, each series was performed at least 5 times and the percentage of apoptosis is an average of those experiments. Group 1 (control) received 100μl of PBS as control. The dosage applied in groups two, three, four and five ranged from (500μg to 1ng of hydrogen tetrachloroaurate). After the incubation period, cells were processed according to instructions by Roche for M30 Cytodeath kit. M30 is a fluorescent monoclonal antibody that recognizes the degraded form of cytyokeratin 18 and yields a FITC emission detected by flow cytometry in FL1 channel. The percentage of cells in the M1 phase is considered to be representative of cells positive for M30 and indicative of apoptosis.

**[0234]** Group 1 (control): presented a basal level of apoptosis, normal of any healthy cell culture with an apoptosis percentage of 2.2% (Figure 2A).

**[0235]** Group 2: hydrogen tetrachloroaurate-treated cells presented a positive apoptosis percentage of 12.7% (Figure 2B).

**[0236]** Group 3: hydrogen tetrachloroaurate-treated cells presented a positive apoptosis percentage of 22.5% (Figure 2C).

**[0237]** Group 4: hydrogen hydrogen tetrachloroaurate-treated cells presented a positive apoptosis percentage of 31.7% (Figure 2D).

**[0238]** Group 5: hydrogen tetrachloroaurate-treated cells presented a positive apoptosis percentage of 48.3% (Figure 2E).

**[0239]** The results show that hydrogen tetrachloroaurate is a novel class of anti-cancer drug that induces apoptosis of cancer cells after 48h of incubation over a range of doses.

**Example 10: Measurement of apoptosis and necrosis in gold(III) chloride-treated cancer cells**

**[0240]** Propidium iodide (PI) was used as marker for the evaluation of apoptosis and necrosis. Differential fluorescent dye uptake and cellular morphology were used to distinguish viable and dead cells with apoptotic and/or necrotic morphologies. Propidium Iodide was used to stain DNA in dead cells. PI is a fluorescent dye that binds DNA, with an excitation wavelength of 485 nm and an emission wavelength of 620 nm. When a cell is damaged, the permeability of the cell membrane is increased and PI can enter the cell to bind DNA. The dead apoptotic and necrotic subpopulation, which has lost its membrane potential and organelle function, takes up Propidium Iodide. Under the fluorescence microscope with a DAPI filter, nuclei in these cells appear pinkish in color due to Propidium Iodide. Necrotic cells have intact nuclei while apoptotic cells have fragmented multi-nucleated bodies. In contrast, the viable apoptotic subpopulation has an intact membrane but inactive mitochondria. As a result, the fragmented multi-nucleated bodies (a hallmark of apoptotic cells) in these cells are unable to take up Propidium Iodide. The subpopulation of viable cells has both intact cell membranes and active mitochondria.

**[0241]** The permeability of the plasma membrane is also a central difference between necrosis and apoptosis. Large molecular weight DNA binding dyes, such as propidium iodide (PI), cannot enter intact cells because of their large size and, without permeabilization treatments, do not label apoptotic cells until the final lysis stage. On the other hand, smaller dyes, especially those that can attach to DNA, can label both apoptotic cells and normal cells. Using flow cytometry, one can distinguish apoptotic from necrotic cells as those that show internal DNA labeling with a small dye (such as DAPI, Hoechst 33342 or 33258, or calcein-AM), while not labeling with PI (Bussolati O, et al., (1995) Exp Cell Res 220:283-291; Gatti R, et al., (1998) J Histochem Cytochem 46:895-900). As cells generate apoptotic bodies that release some of their segmented nuclei, the strength of the internal DNA signal decreases, and this can be used as an assay of apoptotic progression. When the cell finally lyses and its membrane becomes permeable, then the larger-sized markers (such as PI) can label any DNA left within the cell.

**[0242]** To prepare cells for analysis of DNA content, cells were trypsinised (0.25% trypsin, 0.02% EDTA), washed with PBS and fixed with 70% ethanol. Cells were collected by centrifugation, re-suspended in PBS (1-2x $10^6$ cells/ml) and treated with RNase A (0.1 mg/ml) and stained with propidium iodide (0.05 mg/ml) for 30 min at room temperature.

Cells were analyzed with a flow cytometer (FACscan, Becton Dickinson Immunocytometry System, Bedford Mass.).

**Example 11: Assays for properties of of hydrogen tetrachloroaurate and auric chloride**

**[0243]** Cytotoxicity, drug resistance, dosage, mechanism(s) of action, apoptosis, necrosis, protein determination, comparative assays, therapeutic index and cell cycle effects of of hydrogen tetrachloroaurate and auric chloride are determined by flow cytometry, slide staining, microscopy, PCR, Q-PCR, Q-RT-PCR, Western blotting, and ELISA based assays using the following reagents according to the manufacturer`s instructions and including material and methods outlined in references included:

**[0244]** Cytotoxicity and apoptosis induction is measured by M30 Cytodeath Assay (Roche) (Vijayalakshmi, B., et al. (2006). "Chronic low vitamin intake potentiates cisplatin-induced intestinal epithelial cell apoptosis in WNIN rats." World J Gastroenterol 12(7): 1078-85); Propidium Iodide incorporation, Calcein AM (Invitrogen) (Santos, R. R., et al. (2006). "Vitrification of goat preantral follicles enclosed in ovarian tissue by using conventional and solid-surface vitrification methods." Cell Tissue Res. 2006 Aug 26; [Epub ahead of print]); and ApoSENSOR ADP/ATP (Biovision) (Cosen-Binker, L. I., et al. (2006). "Relaxin prevents the development of severe acute pancreatitis." World J Gastroenterol 12(10): 1558-68).

**[0245]** Vybrant DyeCycle (Invitrogen) (Endl, E., et al. (1997). "Analysis of cell cycle-related Ki-67 and p120 expression by flow cytometric BrdUrd-Hoechst/7AAD and immunolabeling technique." Cytometry 29(3): 233-41) is used for for cell proliferation an ELISA-based MTT assay (Sigma).

**[0246]** $^{3}$H-thymidine proliferation assay is carried out using ([methyl-$^{3}$H] Thymidine; Amersham- Pharmacia Biotech, Little Chalfont, England) (Momekov, G., et al. (2006). "Evaluation of the Cytotoxic and Pro-Apoptotic Activities of Eu(III) Complexes with Appended DNA Intercalators in a Panel of Human Malignant Cell Lines." Med Chem 2(5): 439-45).

**[0247]** Caspase determination using monoclonal antibodies are carried out using Vibrant FAM Poly Caspases FAM-VAD-FMK Hoechst 33342 and Propidium Iodide (Invitrogen) (Lovborg, H., et al. (2004). "Multiparametric evaluation of apoptosis: effects of standard cytotoxic agents and the cyanoguanidine CHS 828." Mol Cancer Ther 3(5): 521-6). Live-

Dead staining kit (Biovision), Bio-Rad DC Protein Assay Kit (Biorad) will also be included in the determinations.

**[0248]** PCR and Q-PCR primers are available from the University of California at San Francisco (UCSF) Comprehensive Cancer Center and Applied Biosciences (Ambion) (Cubedo, E., et al. (2006). "New symmetrical quinazoline derivatives selectively induce apoptosis in human cancer cells." Cancer Biol Ther 5(7): 850-9; Yong, A. S., et al. (2006). "Molecular profiling of CD34+ cells identifies low expression of CD7, along with high expression of proteinase 3 or elastase, as predictors of longer survival in patients with CML." Blood 107(1): 205-12).

**[0249]** Cytotoxicity and cell cycle disruptions are caused by administration of hydrogen tetrachloroaurate and auric chloride. Hydrogen tetrachloroaurate is a valid and potential therapeutic molecule for the treatment of different types of cancers, precancer lesions (including dysplasia) and others.

**Example 12: Cell cycle determination with propidium iodide**

**[0250]** An *in vitro* cell cycle assay is used to demonstrate that a compound of the invention induces cell-cycle arrest in CAL 27 human oral carcinoma cells. Tumor cells are synchronized by serum deprivation for 24 hours, then released by adding 10% serum to the cell culture with either hydrogen tetrachloroaurate or carrier vehicle (dimethylsulfoxide). The gold(III) chloride-treated CAL 27 human oral carcinoma cells are treated with trypsin/EDTA (Biochrom AG, L-2153), shaken off, collected in a 15 ml centrifugation tube, and centrifuged at 900 rpm for 3 minutes. The resulting supernatant is removed and 1 ml propidium iodide (PI) solution (50 μg/ml PI, 0.1% sodium citrate, and 0.1% Triton X-100) is added. After one hour of dark incubation at room temperature, the intensity of PI fluorescence is read by flow cytometry (FACSort). DNA content was analyzed by Flow Cytometry Coulter).

**[0251]** The gold(III) chloride-treated CAL 27 human oral carcinoma cells has $G_0/G_1$ to S phase transition blocked more efficiently than control vehicle. Thus, the gold(III) chloride compounds of the invention, significantly arrest cell cycle and, accordingly, are useful for treating or preventing human cancer.

**Example 13: Antitumor activity of auric chloride**

**[0252]** The antitumor activity of auric chloride on is measured *in vitro* on cell cultures issued from the human tumor line CAL27 (Gioanni J. et al., Eur. J. Cancer Clin. Oncol. 24:1445-1455 (1988)) which is sensitive to cis-platinum. The compounds are tested for exposure times with the cell medium from 2 to 120 hours, and the results are measured after 5 days. The cytotoxicity is measured as $IC_{50}$ (a concentration at which 50% of the cell growth is inhibited). Cisplatin is used as reference.

**[0253]** Auric chloride shows an antitumor activity on CAL 27. This activity increases with the exposure time. For example, after 5 hours exposure, auric chloride displays $IC_{50}$ range from 1 to 100 mM. These compounds have an antitumor activity comparable to that of cisplatin.

**Example 14: Ames test for measurement of mutagenicity of gold(III) chlorides**

**[0254]** The use of the Ames test is based on the assumption that in addition to causing tumors in animal cells, most carcinogens are mutagens. The bacterium used in the test is a strain of *Salmonella typhimurium* that caries a mutation in the *his* operon making it unable to synthesize the amino acid histidine (His) from the ingredients in its culture medium. with mutations in the *his* operon are histidine auxotrophs -- they are unable to grow without added histidine. Revertants that restore the $His^+$ phenotype will grow on minimal medium plates without histidine. This provides a simple, sensitive selection for revertants of *his* mutants as mutagens. (Ames, B., F. Lee, and W. Durston. 1973. An improved bacterial test system for the detection and classification of mutagens and carcinogens. Proc. Natl. Acad. Sci. USA 70: 782-786).

**[0255]** The Ames test shows whether the compositions tested are themselves mutagenic and thus potentially dangerous. The test could also show whether the extracts are beneficial by preventing mutations. Many compounds are altered in the liver to become mutagenic. The tests are thus performed in the presence and absence of liver enzymes (liver extract activated with NADPH and glucose-6-phosphate). 2-aminoantracene is used as positive control. The Ames test was performed on gold(III) chloride compounds at 20 μg per plate. None showed any significant mutagenicity.

**Example 15: Maximum tolerable dose of Gold(III) chloride compositions**

**[0256]** Solutions of hydrogen tetrachloroaurate or auric chloride representing 10x $IC_{50}$ is administered orally to SCID/nod mice. A solution of the extracted material is administered orally (1 ml/day/animal) to SCID/nod mice (25 gm; n=5) once a day for up to 14 days. The mice are monitored over a 28-day period for signs of stress following drug administration, including substantial loss of body weight, diarrhea, heavy panting, ruffling of hair, etc. On days 2 through 14, less than 15% body weight loss is observed and the animals are considered to be healthy. At the end of the period mice are terminated by $CO_2$ inhalation. Age-matched control mice (n=4) are treated with phosphate buffered saline at 1 ml/day for the 14 days. The data show that a daily dosage of the gold(III) chloride compositions is not toxic. This dosage is used in studies on the effect of the extract on tumor growth in a xenograft model system.

**Example 16: Efficacy study on xenografts of cancer cell lines**

**[0257]** Xenografts of established cell lines are used to produce tumors in immunodeficient mice and the animals are treated with hydrogen tetrachloroaurate (Curran, E. M., et al. (2006). "Estrogenic Regulation of Host Immunity against an Estrogen Receptor-Negative Human Breast Cancer." Clin Cancer Res 12(19): 5641-5647; Xu, J., et al. (2006). "Prostate cancer metastasis: Role of the host microenvironment in promoting epithelial to mesenchymal transition and increased bone and adrenal gland metastasis." Prostate 66(15): 1664-73).

**[0258]** A xenograft model is established for a variety of pre-cancerous and cancerous human tissues, including lung cancer tissue. Most importantly, the xenografts in the model retain the histological characteristics of the parental tissue. For selected types of cancer the xenografts respond to therapy in a manner similar to that observed in patients. For example, prostate cancer tissue grown in SCID mice show a dramatic response to androgen ablation therapy as regularly found in the clinic.

**[0259]** The xenografted tumors include breast, oral, prostate, lung, gastrointestinal, cervix and others. The examination of these animals with tumors are carried out by methods including histology, immunohistochemistry, in situ hybridization, serum tests, PCR, RT-PCR, toxicology testing and direct tumor measurements.

**[0260]** To determine the efficacy of hydrogen tetrachloroaurate or auric chloride on other cancer types and compare its performance to a standard chemotherapy regimen, tumor

xenografts from a prostate cell line DU145 are cut into 2 $mm^3$ pieces and grafted into SCOD/rod mice. Treatment is started at day 13 (mean volume = 15.6 $mm^3$). The mice are divided into equal groups for treatment with saline, gold(III) chloride compositions at $3xIC_{50}$, estramustine sodium phosphate (EMCYT®) and docetaxel (E+D), cisplatin and methotrexate. Gold(III) chloride compositions show a significant inhibitory effect comparable to the E+D , cisplatin and methotrexate regimen.

**[0261]** Histopathology shows differences in necrotic patterns. In untreated xenografts, necrosis is principally focal and central and reflects vaso-distal necrosis caused by rapid proliferation. The necrotic cells are predominantly located in the central portions of the tumor. Ki67 immunostaining shows usual increases in proliferation adjacent to areas of necrosis withput signs of repair. In gold(III) chloride treated xenografts, necrosis is increased and is principally confluent rather than focal. Necrosis is vasocentric and was present in juxtaposition to the advancing tumor margin.

**[0262]** Hydrogen tetrachloroaurate has direct and possibly indirect effects on xenografted tumors. Direct effects include but are not limited to apoptosis within the tumor cells, halting or reduction of cellular proliferation, deposition of sulfated glycosaminoglycans, and fibrous proteins including collagen and fibronectin.

**[0263]** Hydrogen tetrachloroaurate causes reduction in the expression and rate of epithelial to mesenchymal transition (Kim, K.K., et al. (2006). "Alveolar epithelial cell mesenchymal transition develops in vivo during pulmonary fibrosis and is regulated by the extracellular matrix." *Proc Natl Acad Sci USA* 103(35): 13180-5).

**[0264]** Animals treated with hydrogen tetrachloroaurate demonstrate longer survival periods when compared to the nontreated cohorts of animals. Kaplan-Meier statistical analysis is employed to establish mortality curves. A reduction of morbidity in the experimental group is observed in comparison to a non-treated group.

**Example 17: Preparation of hydrogen tetrachloroaurate and auric chloride nanoparticles**

**[0265]** Gold nanoparticles were synthesized by reduction of hydrogen tetrachloroaurate ($HAuCl_4$) with freshly prepared sodium citrate and allowed to boil under reflux conditions. The pale yellow-colored solution turned to deep red as the gold nanoparticles were formed and stabilized by adsorbed chloride ions. Particle size analysis was performed with a Coulter®

N-4 sub-micron particle size analyzer (Coulter Corporation, Miami, FL). The average sizes of the gold nanoparticles prepared by this method is in the range of 5 to 150nm.

**Example 18: Characterization of hydrogen tetrachloroaurate and auric chloride nanoparticles**

**[0266]** The size of particles comprising hydrogen tetrachloroaurate and auric chloride are determined and the particles examined for physical, chemical and biological properties. The examination of properties of hydrogen tetrachloroaurate and auric chloride preparations including gold particle size determination, electrical charge, ionic behaviors, arrangement patterns are carried out. In addition, the chemistry of the gold surfaces are tested and modified to enable agents bonded, tagged or linked to it. Such agents include DNA, RNA, RNAi, drugs, proteins, glycosaminoglycans, lipids, synthetic molecules, carbohydrates, dyes, molecular markers, antibodies and other diverse molecules (Dvorak, A. M. and E. S. Morgan (1999). "Ribonuclease-gold ultrastructural localization of heparin in isolated human lung mast cells stimulated to undergo anaphylactic degranulation and recovery in vitro." Clin Exp Allergy 29(8): 1118-28; McRae, R., et al. (2006). "Correlative microXRF and optical immunofluorescence microscopy of adherent cells labeled with ultrasmall gold particles." J Struct Biol 155(1): 22-9; Stadler, B. M., et al. (2006). "Light-induced in situ patterning of DNA-tagged biomolecules and nanoparticles." IEEE Trans Nanobioscience 5(3): 215-219).

**[0267]** The average sizes of the gold nanoparticles are in the range of 5 to 150nm. Each gold nanoparticle is surrounded by an ionic layer and the surface chemistry allows interactions sufficient to link other molecules and chemical entities.

**Example 19: Other applications and uses of hydrogen tetrachloroaurate and auric chloride nanoparticles**

**[0268]** These properties of the gold(III) chloride nanoparticles can also lead to non-biological applications.

**[0269]** Gold nanoparticles can be potent tools for nanotechnological applications. Based on the size of gold particles and their characteristics hydrogen tetrachloroaurate is a possible candidate for nanotechnology use. Hydrogen tetrachloroaurate nanoparticles have properties that are not shared by other gold preparations. The biological effects of hydrogen tetrachloroaurate are significantly different than those of other gold preparations. The different physical and chemical behavior of hydrogen tetrachloroaurate as compared to other forms of gold preparations make it suitable for engineering purposes that include but are not

limited to nanowires, nanodevices and microprocessors. The invention further contemplates use of hydrogen tetrachloroaurate and auric chloride as a tool and building block for chips and microprocessors.

**Example 20: Toxicology Studies in Animals**

**[0270]** Toxicology studies were carried out in Balb/C mice as follows: Animals Used: Balb/C Mice. Weight per animal: Approximately 20 g. Route of Injection: IV using the tail vein. Volume Injected: 150 µl. Time of evaluation: 48h.

**[0271]** Four groups were used in the experimental design.

**[0272]** Group 1BS or 1B also known as 1 had saline (NaCl 0. 9%) injection only, and used as positive injection control.

**[0273]** Group 2Y also known as 2 had high dosage, Gold(III) solution with 1 ng/ml.

**[0274]** Group 3B also known as 3 had low dosage, Gold(III) solution with 1 pg/ml.

**[0275]** Group 4G or also known as 4 had no injection, no treatment, and were used as non-treatment control.

**[0276]** The following data points were observed: general behaviour of the animals, body weigh, blood elements (described below) and blood chemistry (described below).

**[0277]** The blood elements that were observed (Figure 3) were: WBC, RBC, HGB, HCT, MCV, MCHC, RDW, PLT, MPV, %NEUT, % LYMPHS, %MONO, %EOS, %BASO, %BANDS, #NEUT, #LYMPHS, #MONO, #EOS, #BASO, #BANDS.

**[0278]** The blood chemistry elements ((hepatic/plasma (Figures 4A-4B); electrolytes (Figures 5A-5B); lipids (Figure 6)) that were observed were: ALB, TP, ALP, ALT, AST, CPK, TBIL, DBIL, BUN, CREAT, CA, CHOL, GLU, PHOS, CO2, NA, K, CL, LDH, HDL, LDL, MG, GLOB, A/G, B/C, IBIL and ANION.

**[0279]** Statistical Analysis: All data points were examined using ANOVA analysis. They were further examined using POST HOC analysis including Tukey HSD, Bonferroni, and Tamhane methods.

**[0280]** Results: No adverse visible reactions were observed in any of the animals in any of the groups examined (clinical evaluation). No significant weight changes were observed in any of the groups. No statistically significant changes could be observed in the blood elements or blood chemistry groups with the exception of MPV values (*see* Example 21)

among the experimental groups studied. The *in vivo* toxicity of the gold III solution described in this patent at the doses used display very low to no toxicity based on the toxicology studies performed. This suggests a high affinity and efficacy against malignant squamous cell carcinoma cells.

**Example 21: The *in vivo* effect of Gold III solution: Mean Platelet Volume (MPV) values.**

**[0281]** Mean Platelet Volume (MPV) is a measurement of the average size of platelets found in blood. Since the average platelet size is larger when the body is producing increased numbers of platelets, MPV test results can be used to make inferences about platelet production in bone marrow. Large platelets are young platelets and have better hemostatic function on average than older platelets.

**[0282]** Results: Statistical Differences were found between group 1BS when compared to group 3B and 4G. Statistical Differences were found between group 2Y when compared to group 3B and 4G. No Statistical Difference was found between group 1BS and group 2Y.

**[0283]** Interestingly, group 4G did not receive any injection and is the baseline size of platelets. The simple act of placing an injection in the body results in a change in MPV as observed in the 1BS (saline only group) and group 2Y. However, group 3B did not shift to a lower MPV value, it stayed within the non-injection size average. The gold(III) solutions of this invention has an effect on MPV values. The gold(III) formulations at the dosage examined and probably other dosages most likely have an effect on bone marrow cells of the platelet lineage. These results indicate that the gold(III) solutions of this invention could be used to treat disorders that are related, due to or caused by changes in MPV.

**Example 22: Comparison of *in vivo* apoptotic effect of Gold(III) solutions relative to methotrexate and cisplatin.**

**[0284]** Head and neck cancer cell lines were treated with phosphate-buffered saline (PBS), and equivalent $IC_{50}$ amounts of cisplatin, methotrexate and gold(III) compositions; Epivyn 1, Epivyn 2 and Epivyn 3. Figure 7A shows the relative efficacy of the gold(III) compositions is equal to or markedly greater than those of cisplatin and methotrexate. Figure 7B-1 (PBS control – healthy cells with high density) and Figure 7B-2 (gold(III)—large ares of cells lost due to apoptosis) show the cytotoxic effect of gold(III) compositions on tissue culture.

**[0285]** All publications and patent applications cited in this specification are herein incorporated by reference in their entirety as if each individual publication or patent application is specifically and individually indicated to be incorporated by reference.

**[0286]** Although the foregoing invention has been described in some detail by way of illustration and example for purposes of clarity of understanding, it will be readily apparent to those of ordinary skill in the art in light of the teachings of this invention that certain changes and modifications may be made thereto without departing from the spirit or scope of the appended claims.

# CLAIMS

WHAT IS CLAIMED IS:

1. A method for treating, preventing or inhibiting a cancer comprising administering to a subject a pharmaceutical composition comprising an effective amount of a gold(III) chloride or a pharmaceutically acceptable salt thereof.

2. A method for treating breast, ovary, testicle, prostate, head, neck, eye, skin, mouth, throat, esophagus, chest, bone, lung, colon, sigmoid, rectum, stomach, kidney, liver, pancreas, brain, intestine, heart or adrenal cancer or neoplastic disease comprising administering to a patient in need of such treatment an effective amount of a gold(III) chloride or a pharmaceutically acceptable salt thereof.

3. A method of treating a proliferative disease in an individual comprising administering to the individual:

a) an effective amount of a composition comprising a gold(III) chloride or a pharmaceutically acceptable salt thereof, and

b) an effective amount of at least one other chemotherapeutic agent, wherein said chemotherapeutic agent is selected from the group consisting of antimetabolites, platinum-based agents, alkylating agents, tyrosine kinase inhibitors, anthracycline antibiotics, vinca alkloids, proteasome inhibitors, macrolides, and topoisomerase inhibitors.

4. The method of claim 3, wherein the proliferative disease is cancer.

5. The method of claim 3, wherein the proliferative disease is a proliferative dermatologic disorder selected from the group consisting of: keloids, seborrheic keratosis, papilloma virus infection and eczema.

6. The method of claim 3, wherein the proliferative disease is a precancerous lesion.

7. The method of claim 3, wherein the proliferative disease is an epithelial tumor selected from the group consisting of: benign and premalignant epithelial tumor, breast fibroadenoma, colon adenoma, and malignant epithelial tumor.

8. The method of claim 3, wherein the composition comprising a gold(III) chloride or a pharmaceutically acceptable salt thereof, and the at least one other chemotherapeutic agent are in amounts sufficient to exhibit a synergistic effect.

9. A method of treating a tumor in an individual comprising:

a) a first therapy comprising administering to the individual an effective amount of a composition comprising a gold(III) chloride or a pharmaceutically acceptable salt thereof, and

b) a second therapy comprising radiation therapy, surgery, or combinations thereof..

10. The method according to any of claims 1-9, wherein the gold(III) chloride is hydrogen tetrachloroaurate or auric chloride.

11. The method according to any of claims 1-10, wherein the individual demonstrates a longer survival period.

12. The method according to any of claims 1-11, wherein the gold(III) chloride is in the form of nanoparticles.

13. The method of claim 12, wherein the nanoparticles have an average diameter of about 50 to about 150 nm.

14. The method according to any of claims 1-13, wherein the hydrogen tetrachloroaurate is prepared by any one of method 1 through method 10, wherein the hydrogen tetrachloroaurate has an apoptotic activity at least 5% greater than a basal level.

15. The method according to any of claims 1-13, wherein the hydrogen tetrachloroaurate is prepared by any one of method 1 through method 10, wherein the hydrogen tetrachloroaurate has a cytotoxic activity of at least 15% that of methotrexate or cisplatin.

16. A composition comprising a gold(III) chloride or a pharmaceutically acceptable salt thereof wherin the composition has an acivity selected from the group consisting or apoptosis, anti-angiogenesis, cytotoxic, anti-neoplastic and anti-hyperplastic.

17. A nanoparticle comprising a gold(III) chloride or a pharmaceutically acceptable salt thereof wherin the composition has an acivity selected from the group consisting or apoptosis, anti-angiogenesis, cytotoxic, anti-neoplastic and anti-hyperplastic.

18. A formulation comprising a gold(III) chloride or a pharmaceutically acceptable salt thereof wherin the composition has an acivity selected from the group consisting or apoptosis, anti-angiogenesis, cytotoxic, anti-neoplastic and anti-hyperplastic, wherin the formulation is suitable for administration by oral, parenteral, enteral, intraperitoneal, topical,

transdermal, ophthalmic, nasally, local, non-oral, such as aerosal, inhalation, subcutaneous, intramuscular, buccal, sublingual, rectal, vaginal, intra-arterial, and intrathecal routes.

19. A sealed vial containing an unit dosage form of a gold(III) chloride or a pharmaceutically acceptable salt thereof wherin the composition has an acivity selected from the group consisting or apoptosis, anti-angiogenesis, cytotoxic, anti-neoplastic and anti-hyperplastic.

20. The sealed vial of claim 19 containing a sub-therapeutic dose of the gold(III) chloride or a pharmaceutically acceptable salt thereof, for use in metronomic administration of the gold(III) chloride.

21. The composition of matter comprising gold(III) chloride or a pharmaceutically acceptable salt thereof according to any of claims 16-20 wherin the composition further comprises an effective amount of at least one other chemotherapeutic agent, wherein said chemotherapeutic agent is selected from the group consisting of antimetabolites, platinum-based agents, alkylating agents, tyrosine kinase inhibitors, anthracycline antibiotics, vinca alkloids, proteasome inhibitors, macrolides, and topoisomerase inhibitors.

22. The composition of claim 21, wherein the gold(III) chloride and the other chemotherapeutic agent are in amounts sufficient to exhibit a synergistic effect.

23. The composition according to any of claims 16-22, wherein the gold(III) chloride is hydrogen tetrachloroaurate or auric chloride.

24. The composition according to any of claims 16-23, comprising hydrogen tetrachloroaurate or auric chloride prepared according to a method described in the specification, wherein the composition exhibits an an acivity selected from the group consisting or apoptosis, anti-angiogenesis, cytotoxic, anti-neoplastic and anti-hyperplastic.

25. The composition according to any of claims 16-24, comprising nanoparticles comprising hydrogen tetrachloroaurate or auric chloride having an average diameter of about 50 to about 150 nm.

26. A formulation comprising a composition according to any of claims 16-25 suitable for treating a cancer selected from the group consisting of carcinoma, sarcoma, myeloma, leukemia, lymphoma, and a mixed type cancer.

27. The composition of matter comprising gold(III) chloride or a pharmaceutically acceptable salt thereof according to any of claims 16-20 wherein an amount of the composition having

equivalent level of efficacy displays reduced toxicity as compared to methotrexate and cisplatin.

28. The composition of matter comprising gold(III) chloride or a pharmaceutically acceptable salt thereof according to any of claims 16-20 comprising an amount of the composition effective to cause cell cycle arrest.

29. A gold (III) composition prepared by any one of method 1 through method 10, wherein the gold (III) composition has an apoptotic activity on cancer cells.

30. The gold (III) composition of claim 29, wherein the apoptosis is mediated by caspase pathway.

31. A method for producing a gold (III) composition, wherein the old (III) composition has an apoptotic activity on cancer cells.

32. The method of claim 31, wherein the apoptosis is mediated by caspase pathway.

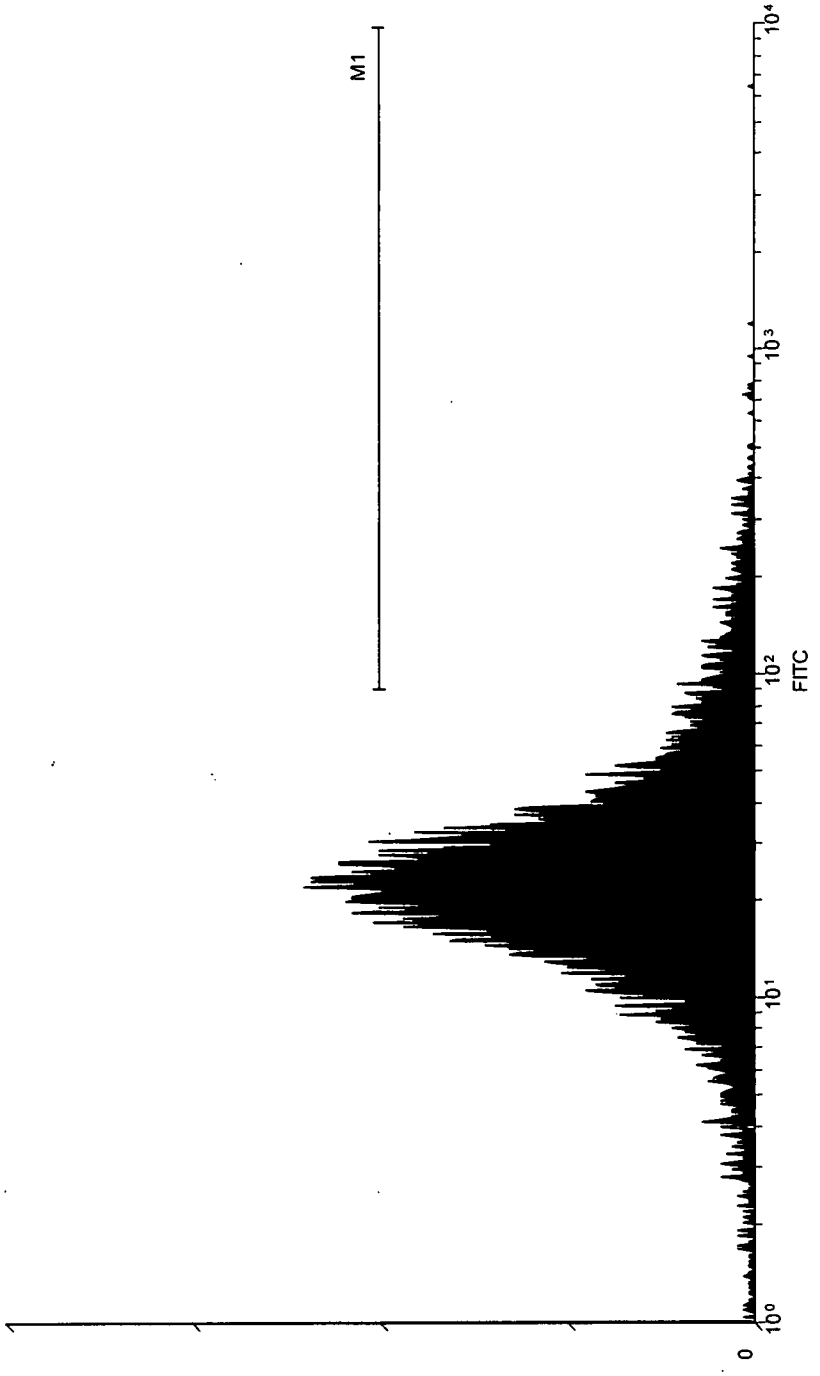



**Figure 1A. PBS-treated (control group) cells presented a basal level of apoptosis with an apoptosis percentage of 3.1%.**

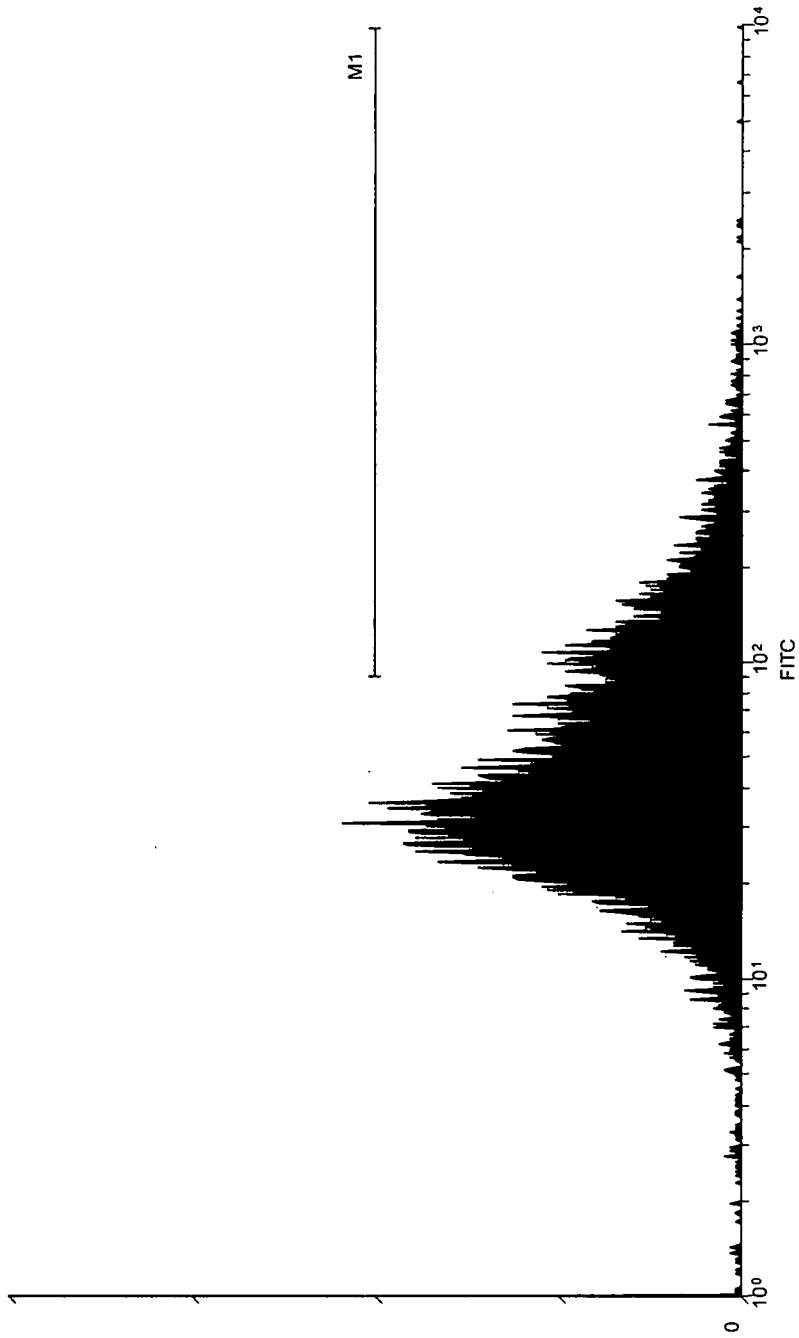



**Figure 1B. Methotrexate (10μg/ml) treated cells presented positive apoptosis percentage of 21%.**

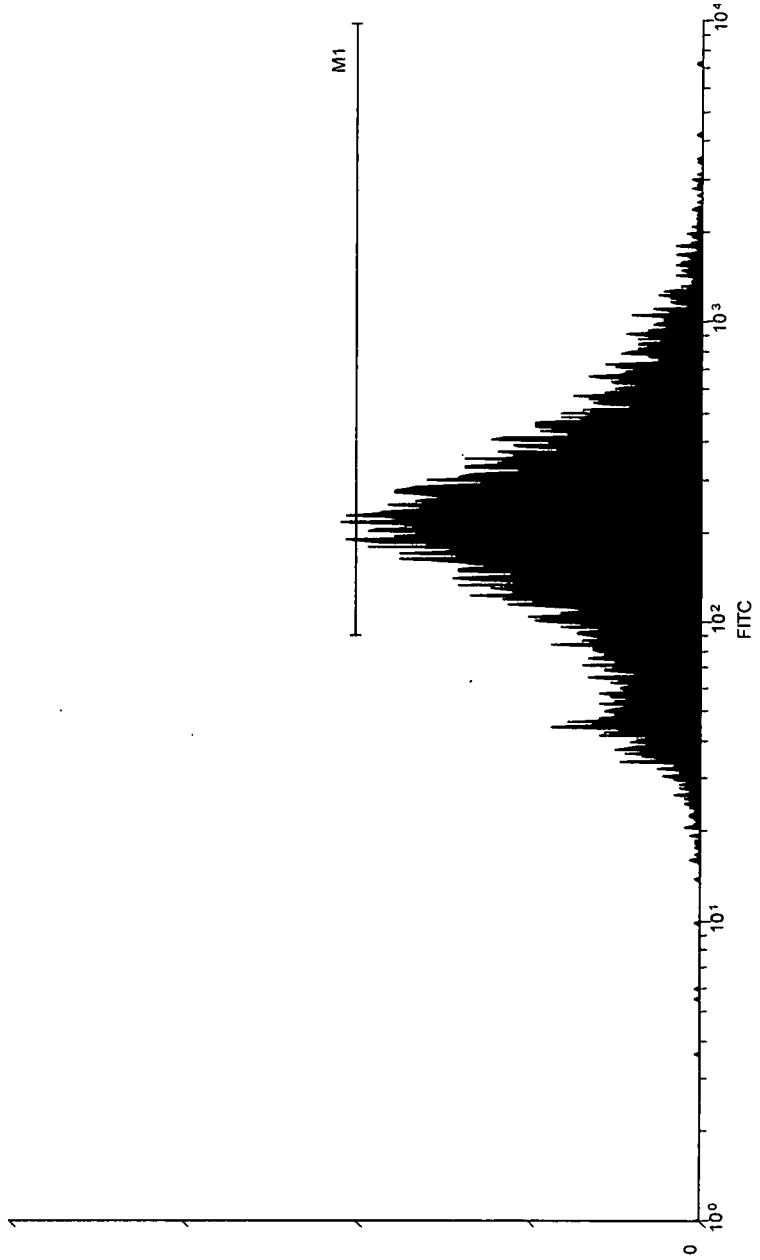



**Figure 1C. Adriamycin (5μg/ml) treated cells presented positive apoptosis percentage of 64%.**

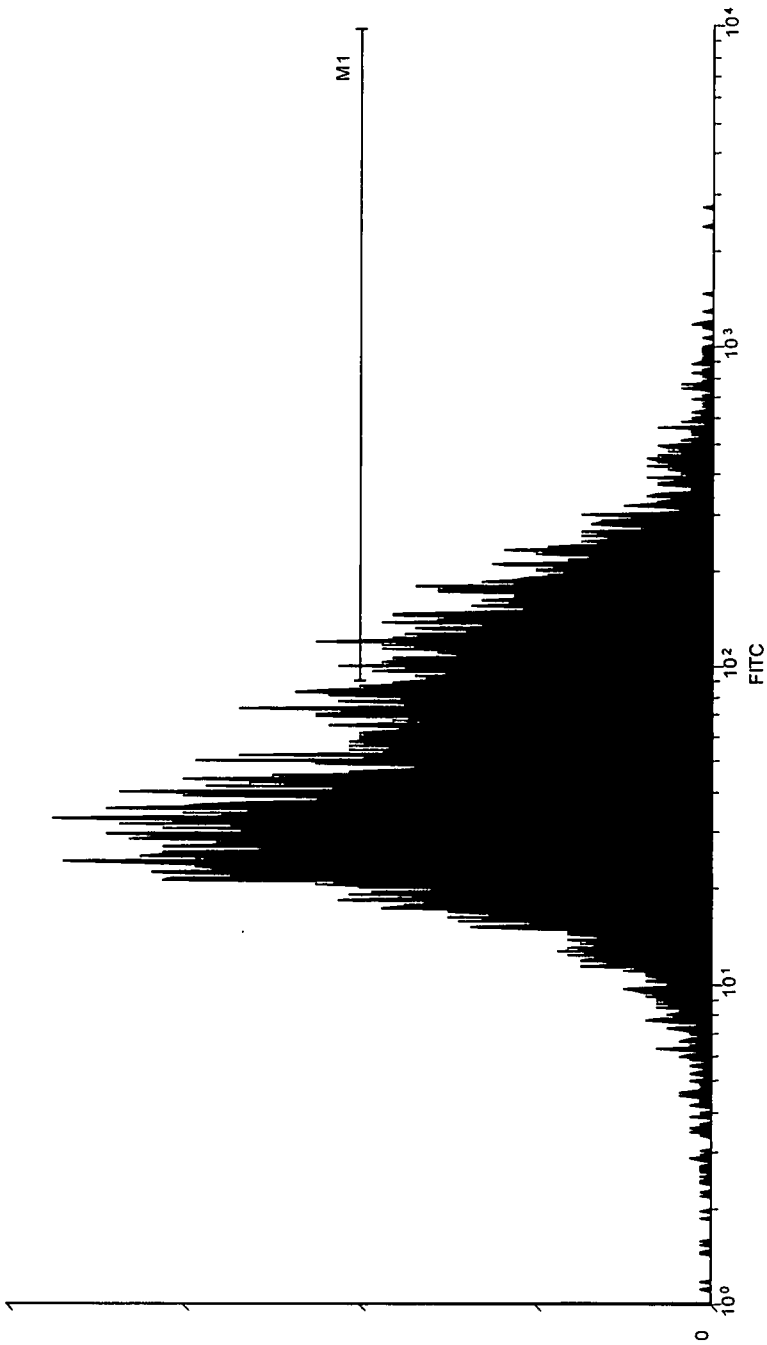



**Figure 1D. Cisplatin (10μg/ml) treated cells presented positive apoptosis percentage of 23.1%.**

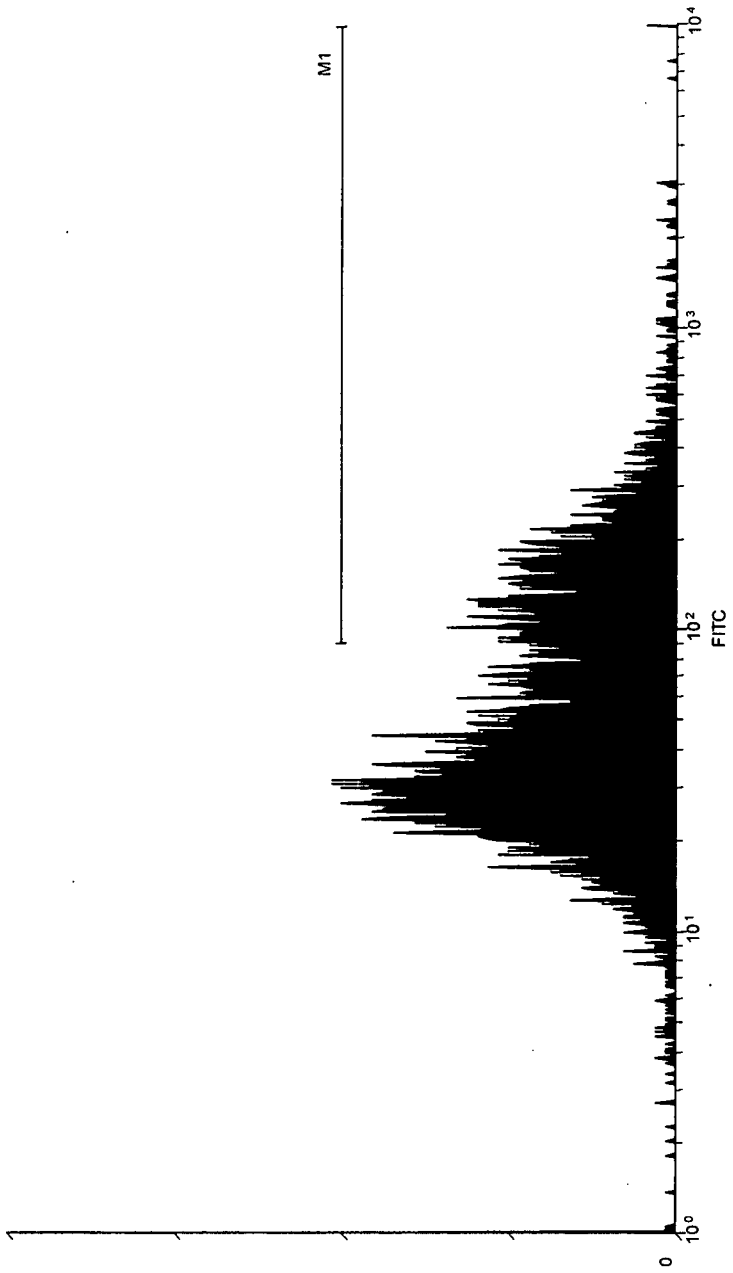



**Figure 1E. Hydrogen tetrachloroaurate treated cells presented positive apoptosis percentage of 32.3% .**

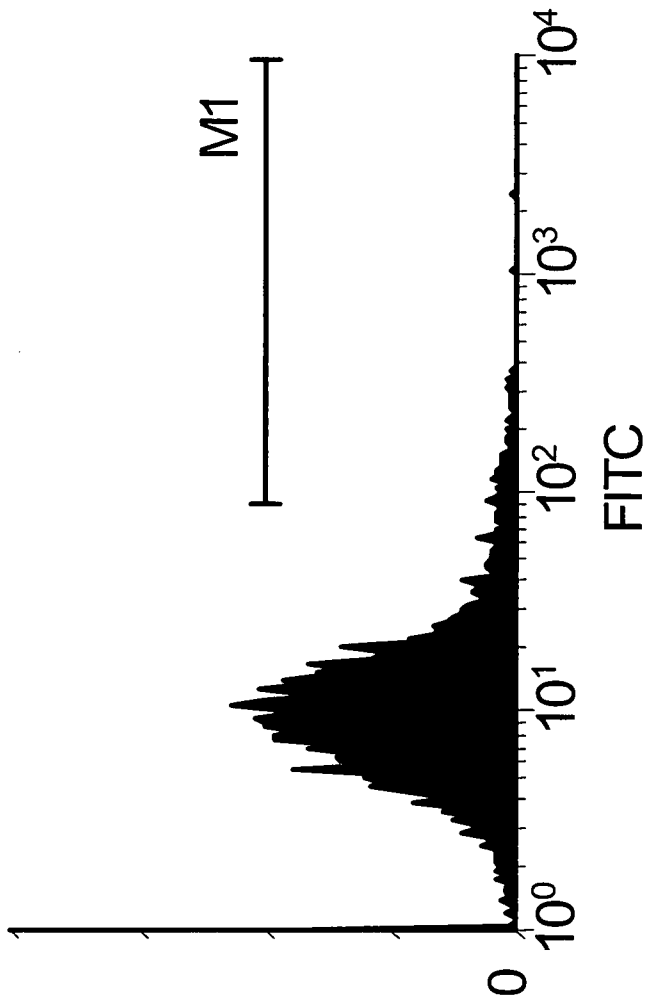



**Figure 2A. Control group presented a basal level of apoptosis with an apoptosis percentage of 2.2%.**

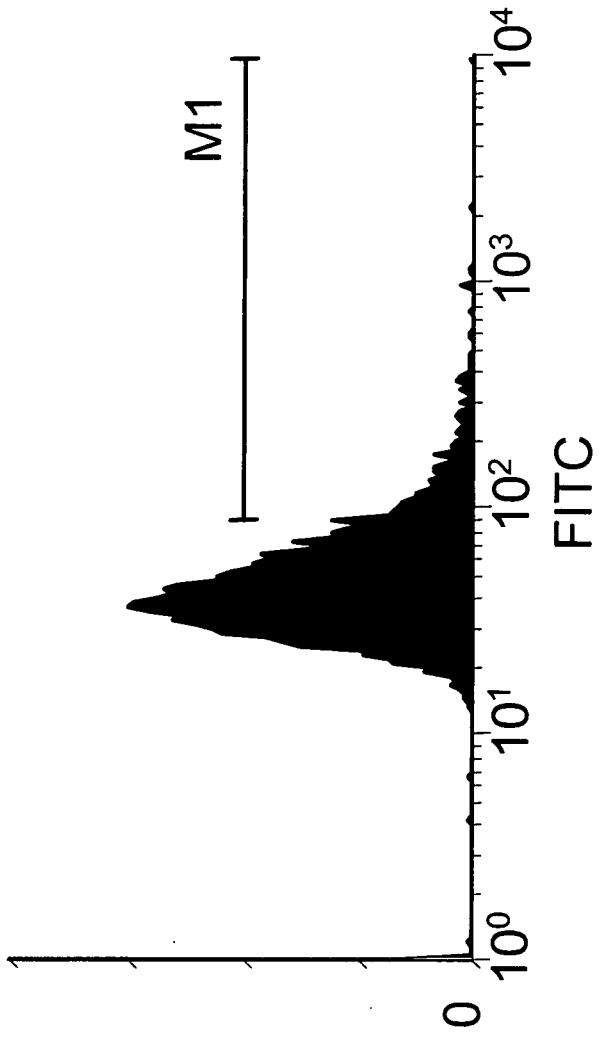



**Figure 2B. A population of hydrogen tetrachloroaurate-treated cells presented positive apoptosis percentage of 12.7%**

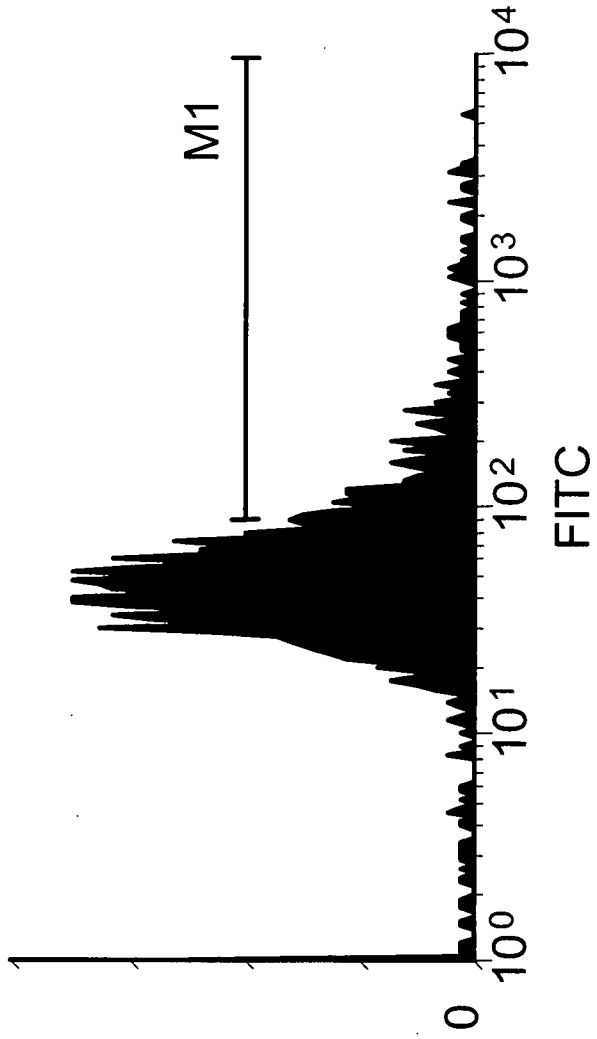



**Figure 2C. A population of hydrogen tetrachloroaurate-treated cells presented positive apoptosis percentage of 22.5%**

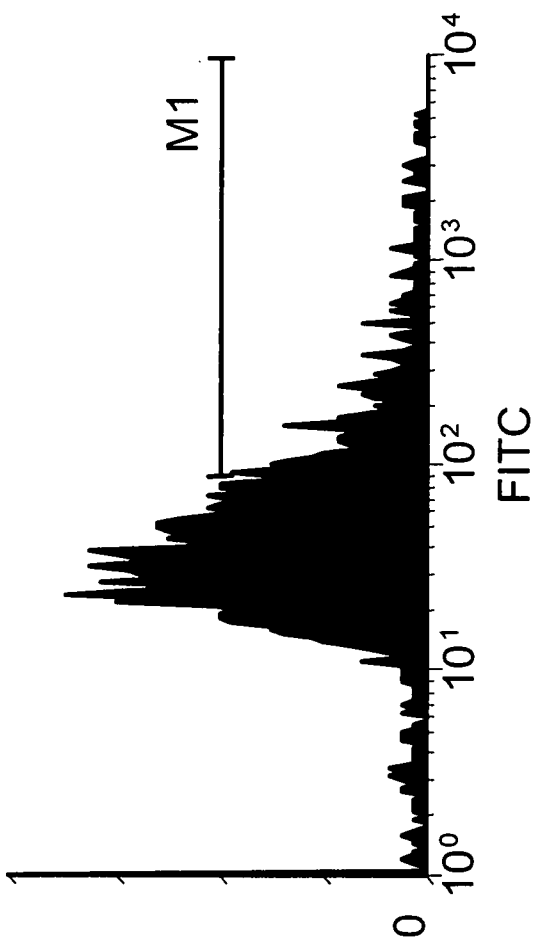



**Figure 2D. A population of hydrogen tetrachloroaurate-treated cells presented positive apoptosis percentage of 31.7%**

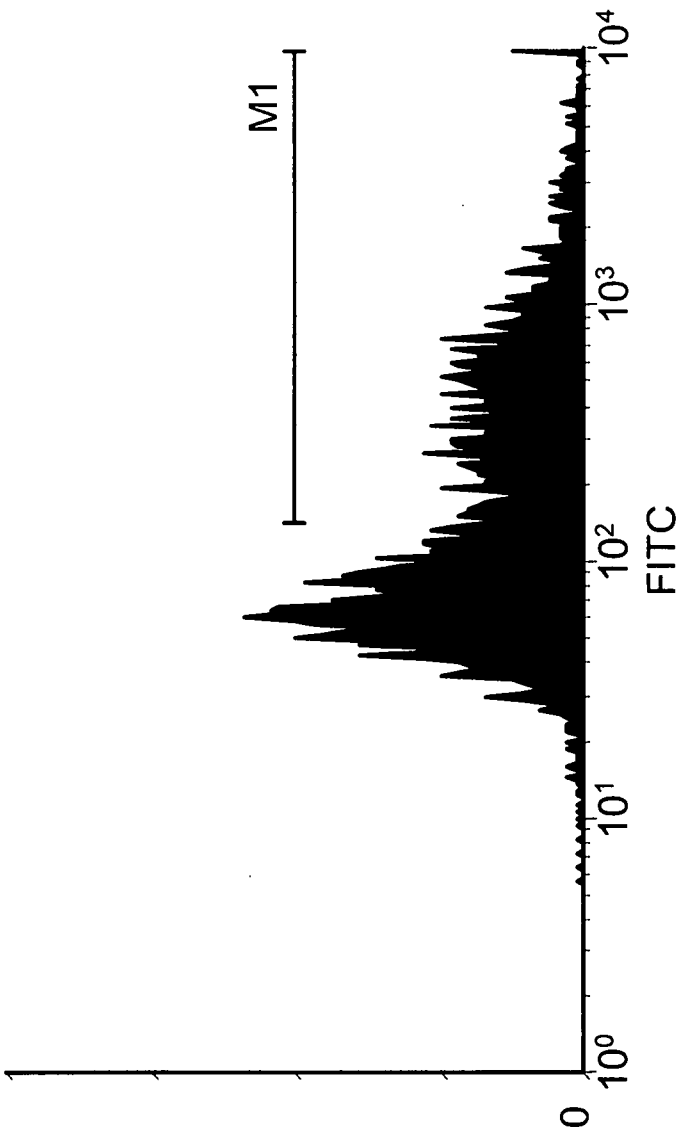



**Figure 2E. A population of hydrogen tetrachloroaurate-treated cells presented positive apoptosis percentage of 48.3%**

**Figure 3**

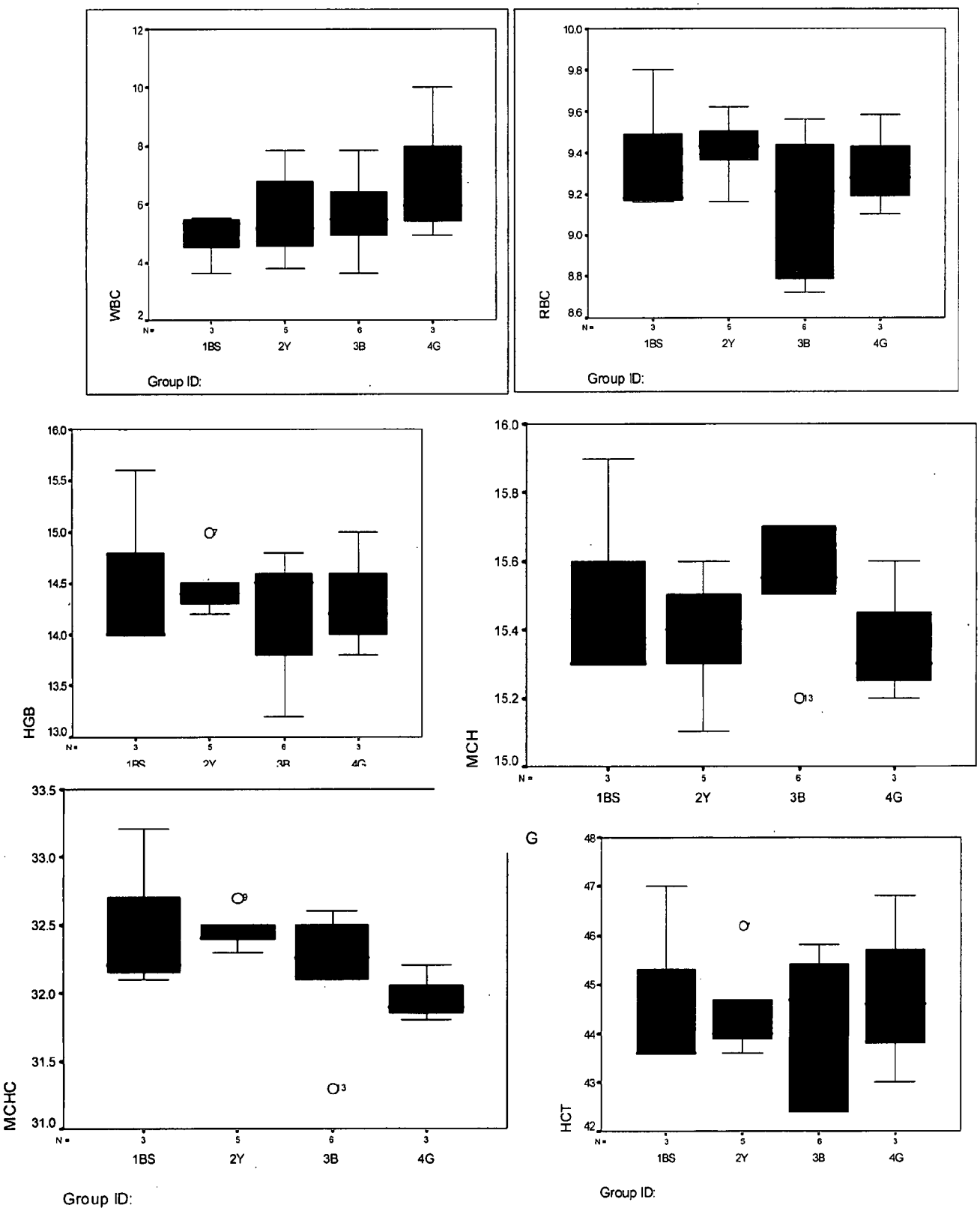
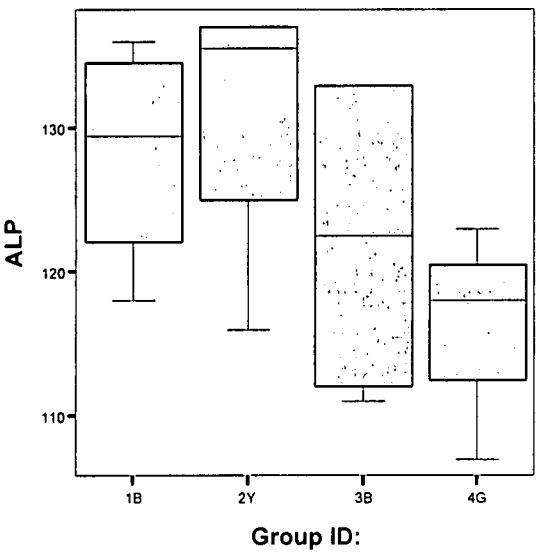
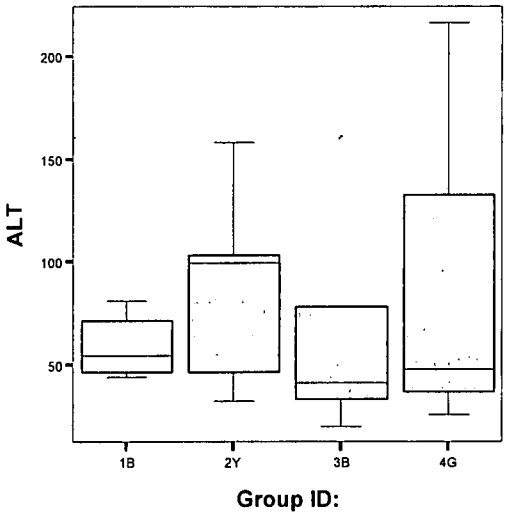
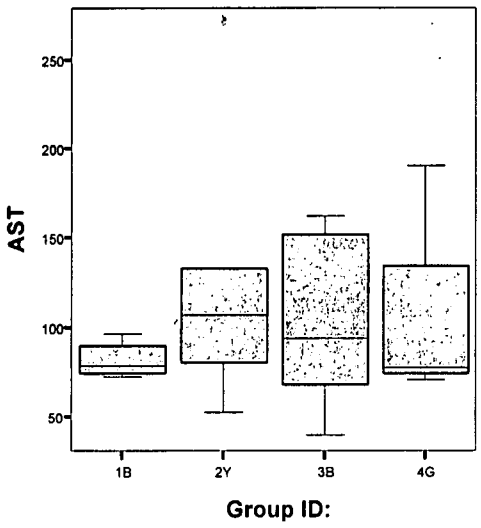
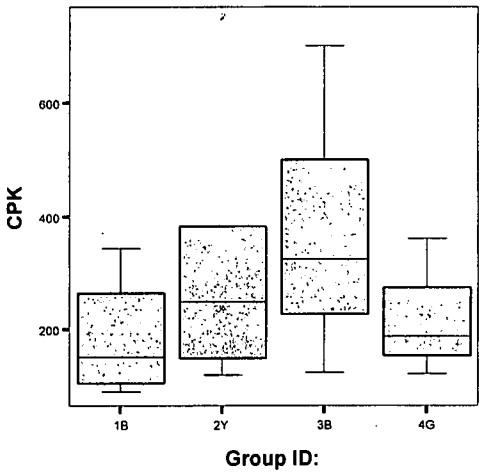


Figure 4A


ALP
130
120
110
1B
2Y
3B
4G
Group ID:


ALT
200
150
100
50
1B
2Y
3B
4G
Group ID:


AST
250
200
150
100
50
1B
2Y
3B
4G
Group ID:


CPK
600
400
200
1B
2Y
3B
4G
Group ID:

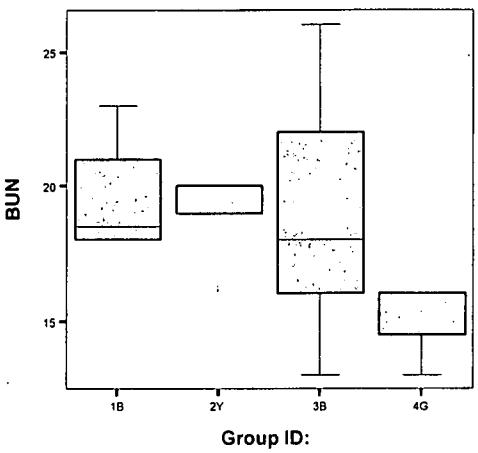
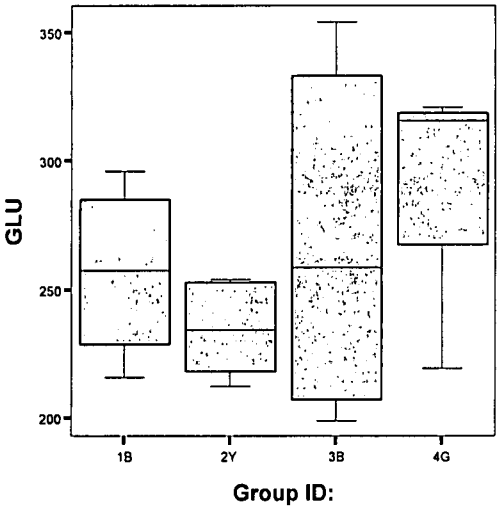
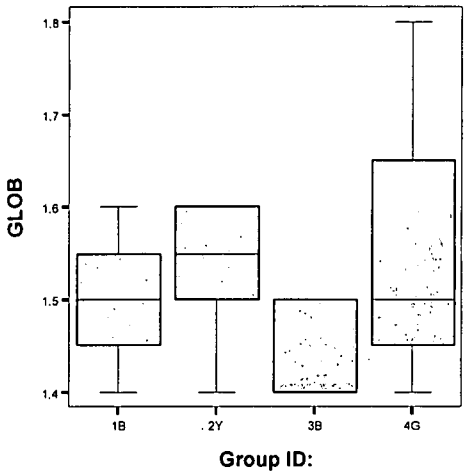
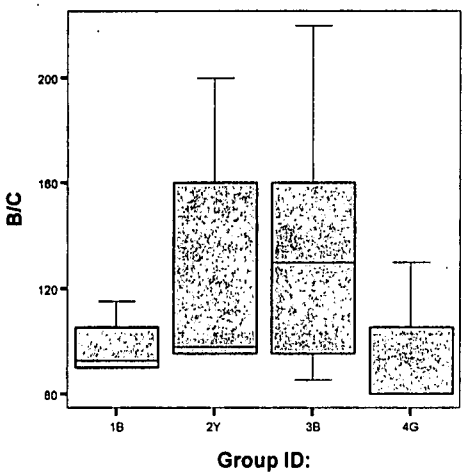

BUN
25
20
15
1B
2Y
3B
4G
Group ID:

**Figure 4B**


GLU
350
300
250
200
1B
2Y
3B
4G
Group ID:


GLOB
1.8
1.7
1.6
1.5
1.4
1B
2Y
3B
4G
Group ID:


B/C
200
160
120
80
1B
2Y
3B
4G
Group ID:

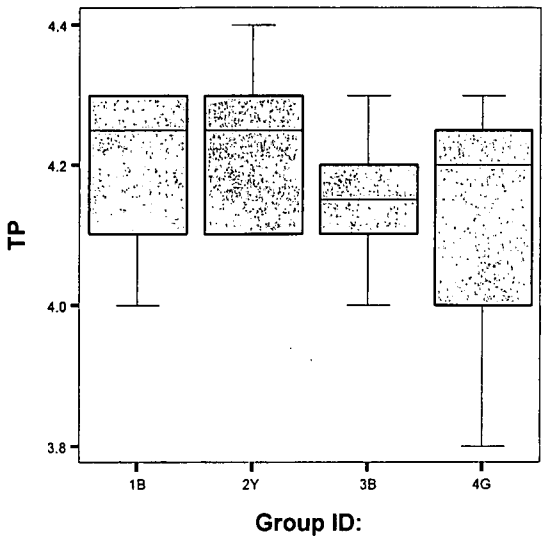
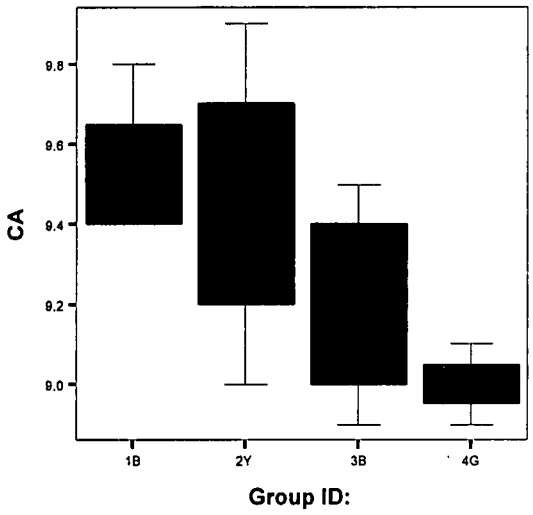
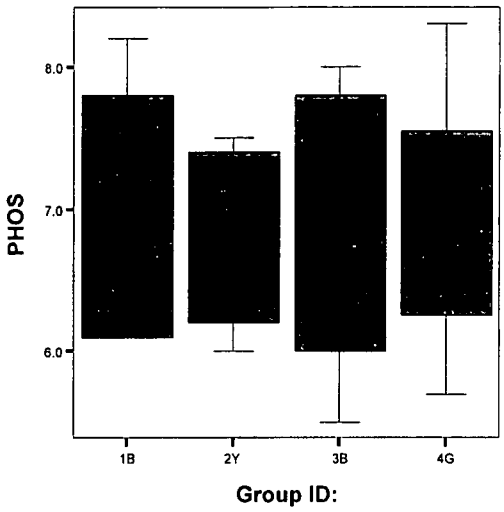
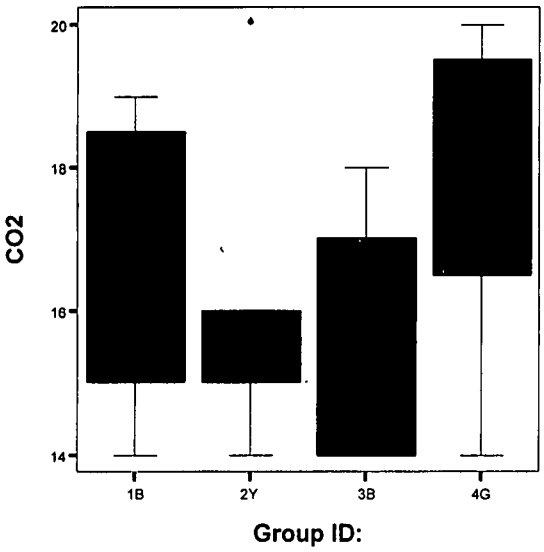

TP
4.4
4.2
4.0
3.8
1B
2Y
3B
4G
Group ID:

Figure 5A


CA
9.8
9.6
9.4
9.2
9.0
1B
2Y
3B
4G
Group ID:


PHOS
8.0
7.0
6.0
1B
2Y
3B
4G
Group ID:


CO2
20
18
16
14
1B
2Y
3B
4G
Group ID:

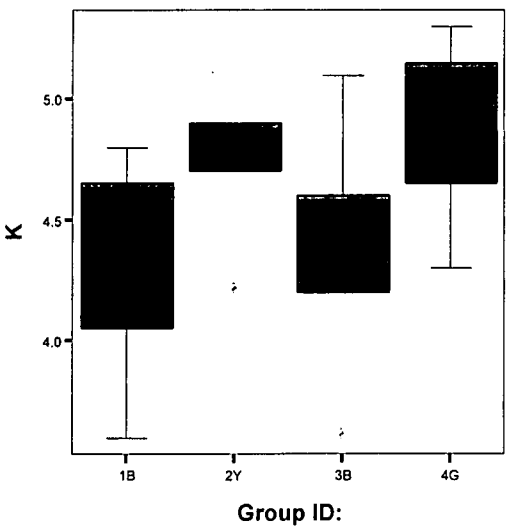
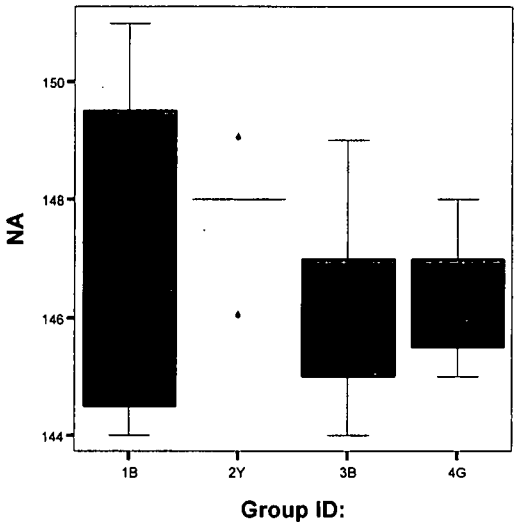
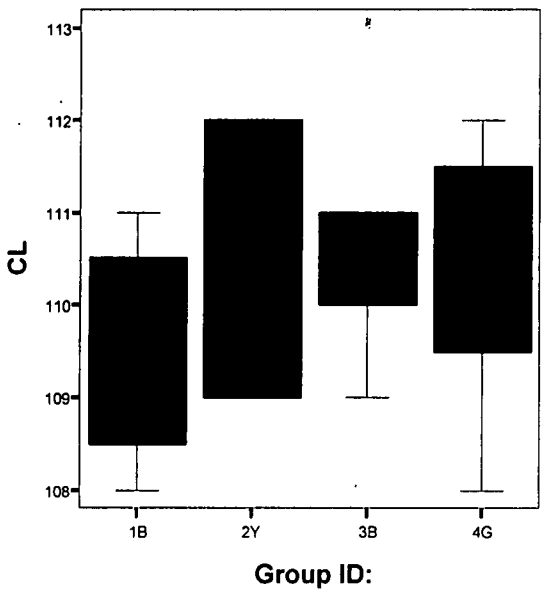

K
5.0
4.5
4.0
1B
2Y
3B
4G
Group ID:

Figure 5B


NA
150
148
146
144
1B
2Y
3B
4G
Group ID:


CL
113
112
111
110
109
108
1B
2Y
3B
4G
Group ID:

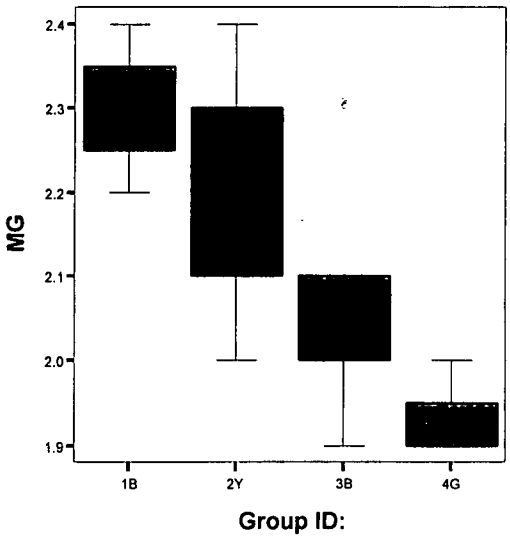

MG
2.4
2.3
2.2
2.1
2.0
1.9
1B
2Y
3B
4G
Group ID:

Figure 6

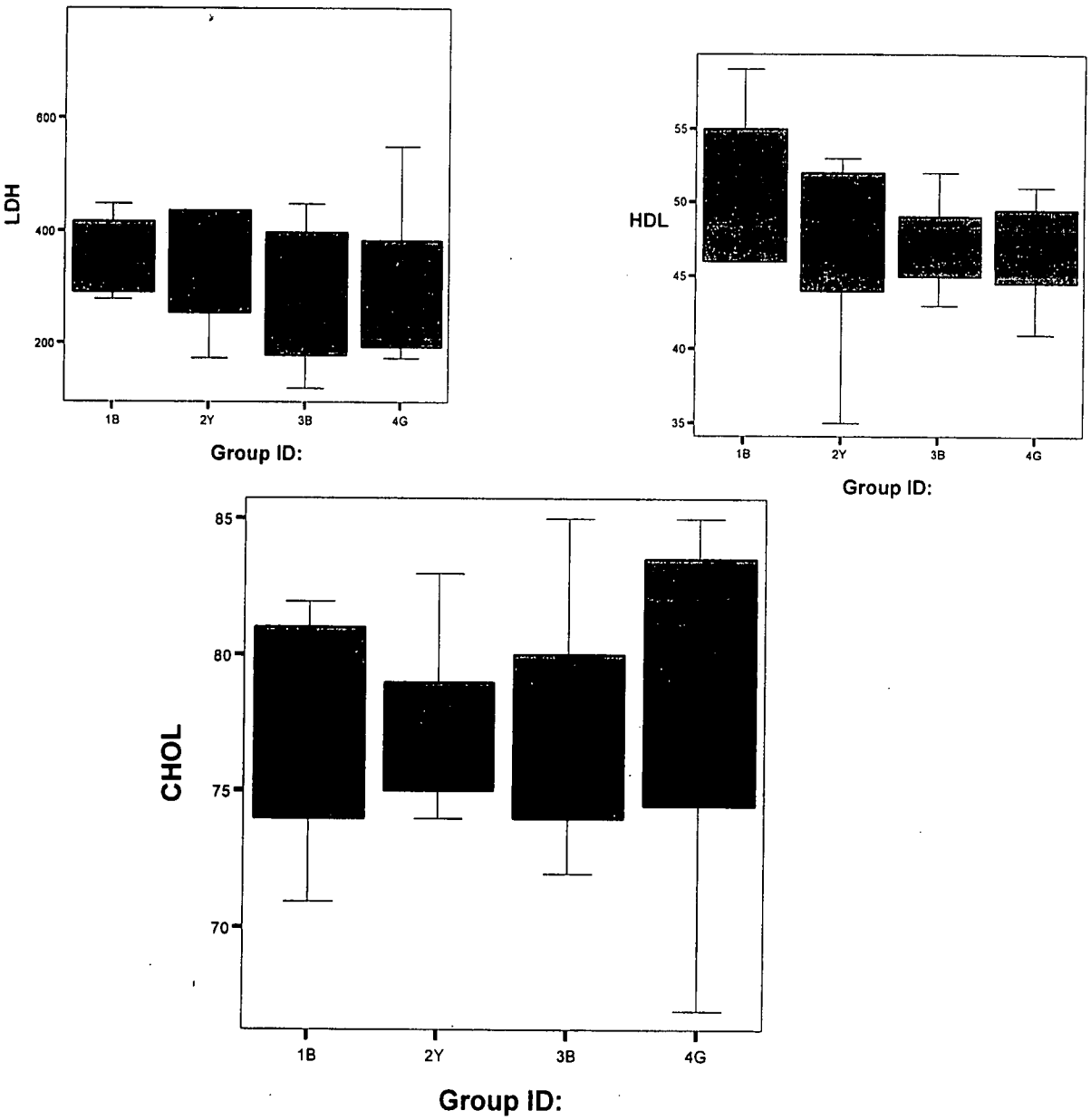

LDH
600
400
200
1B
2Y
3B
4G
Group ID:
HDL
55
50
45
40
35
1B
2Y
3B
4G
Group ID:
CHOL
85
80
75
70
1B
2Y
3B
4G
Group ID:

Figure 7A

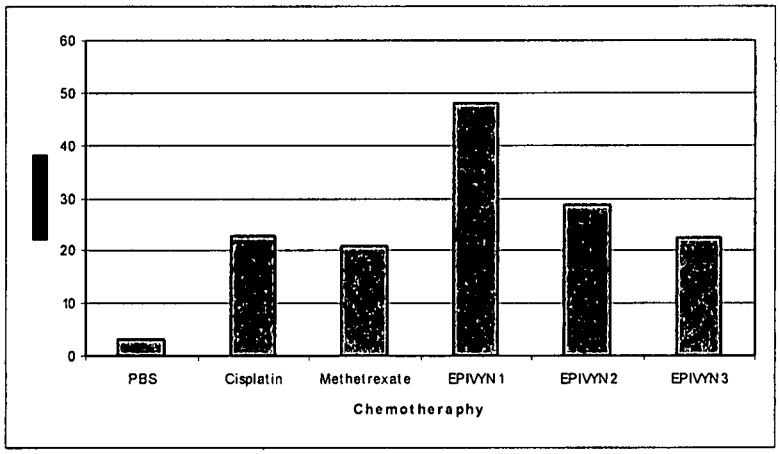



Figure 7B-1

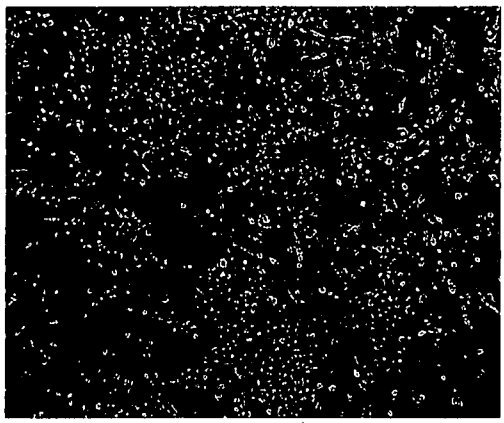


Figure 7B-2