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(54) Title: METHODS FOR THE TREATMENT OF CANCER USING CYTOKINES IN COMBINATION WITH LOW LEVEL DOSES OF CHEMOTHERAPY AND/OR RADIOThERAPY

(57) Abstract

Apoptosis, the main mechanism of programmed cell death, is a gene directed process responsible for the elimination of excessive cells during development and detrimental cell types in pathophysiological situations. The present invention provides a method for exploiting the molecular mechanisms which regulate the pathways leading to programmed cell death, and tumor regression without significant side-effects to the patient. Both low dose chemotherapy and radiotherapy induce DNA fragmentation, but not necessarily cell death, thereby positioning tumor cells to self-destruct by apoptosis. By infusing low doses of cytokines to patients undergoing chemotherapy and/or radiotherapy, tumor cells containing damaged DNA are induced into apoptosis resulting in tumor regression without significant side-effects to the patient.
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METHODS FOR THE TREATMENT OF CANCER
USING CYTOKINES IN COMBINATION WITH LOW LEVEL
DOSES OF CHEMOTHERAPY AND/OR RADIOTHERAPY

Description

Technical Field

The present invention relates to methods for the treatment of cancer, which reduce the side-effects or toxicity associated with conventional radiotherapy and chemotherapy. More particularly, the methods of treatment utilize chemotherapeutic agents and/or radiation in doses that produce cellular damage, but not cell death, in combination with the administration of cytokines which induce apoptosis in the damaged cells thereby resulting in tumor regression with minimal damage to normal tissues.

Background Art

Cancer is a group of diseases characterized by uncontrolled cellular growth with local invasion of normal tissue or systemic spread of the disease known as metastasis or both. Terms such as neoplasm, malignancy and tumor are used synonymously for cancer; however, tumors may be either benign or malignant. Normally, the division or growth of cells takes place in an orderly and controlled manner. Cancer cells lack this growth control mechanism, and as a result, cancer cells continue to divide and are capable of invading adjacent structures or breaking away from the original tumor mass and establishing new growth (metastasis) in other parts of the body. These cells do not function as normal cells and the malignant characteristics can ultimately cause death unless the process is halted.

The patient’s age, sex, underlying health conditions, history of previous treatment, as well as the type and extent of tumor involvement are all factors taken into consideration when determining the most appropriate therapy for the patient and the patient’s cancer. The major therapies are surgery, radiation and chemotherapy.

For many years, surgery was the only treatment capable of producing a cure for cancer. Surgery is used to diagnose the cancer, determine the extent of the disease, relieve symptoms, reconstruct anatomy damaged by the disease or treatment, as well as treat complications such as hemorrhage or perforation. Surgery can also be used to cure cancer when solid tumors are confined to one anatomic region or reduce the size by removing as much of the tumor as possible without damaging surrounding anatomy.

Radiotherapy, also referred to as radiation therapy, is the treatment of cancer and other diseases with ionizing radiation. Ionizing radiation deposits energy that injures or destroys cells in the area being treated, that is, the "target tissue" by damaging their genetic material, known as DNA,
RNA and some proteins, making it impossible for these cells to continue to grow. As radiation doses are increased, more cells are destroyed. Some cancers are very susceptible to radiation and can be cured by higher doses. However, since normal cells and tissue are damaged as well, the ability of the normal tissue surrounding the tumor to withstand the effects of radiation, determines the ability of cure with radiation. Radiotherapy may be used to treat localized solid tumors, such as cancers of the skin, tongue, larynx, brain, breast, or uterine cervix. It can also be used to treat leukemia and lymphoma.

One type of radiation therapy commonly used involves photons or "packets" of energy. X-rays were the first form of photon radiation to be used to treat cancer. Depending on the amount of energy they possess, the x-rays can be used to destroy cancer cells on the surface of or deeper in the body. The higher the energy of the x-ray beam, the deeper the x-rays will penetrate the target tissue. Betatrons and linear accelerators are machines that produce x-rays of increasingly greater energy. The use of machines to focus radiation on a cancer site is called external beam radiotherapy.

Gamma rays are another form of photons used in radiotherapy. Gamma rays are produced spontaneously as certain elements, such as radium, uranium, and cobalt 60 release radiation as they decompose, or decay. Each element decays at a specific rate and gives off energy in the form of gamma rays and other particles. X-rays and gamma rays have the same effect on cancer cells.

Another technique for delivering radiation to cancer cells is to place radioactive implants directly in a tumor or body cavity. This is called internal radiotherapy which includes brachytherapy, interstitial irradiation, and intracavitary irradiation. In this treatment, the radiation dose is concentrated in a small area, and the patient stays in the hospital for a few days. Internal radiotherapy is frequently used for cancers of the tongue, uterus, and cervix.

Several new approaches to radiation therapy are currently being evaluated to determine their effectiveness in treating cancer. One such technique is intraoperative irradiation, in which a large dose of external radiation is directed at the tumor and surrounding tissue during surgery. Another investigational approach is particle beam radiation therapy. This type of therapy differs from photon radiotherapy in that it involves the use of fast-moving subatomic particles to treat localized cancers. Some particles, such as, neutrons, pions, and heavy ions, deposit more energy along the path they take through tissue than do x-rays or gamma rays, thus causing more damage to the cells they hit.

This type of radiation is often referred to as high linear energy transfer (high LET) radiation.

Other recent radiotherapy research has focused on the use of radiolabeled antibodies to deliver doses of radiation directly to the cancer site (radioimmunotherapy). Antibodies are highly specific proteins that are made by the body in response to the presence of antigens. Some tumor cells contain specific antigens that trigger the production of tumor-specific antibodies. Large quantities of
these antibodies can be made in the laboratory and attached to radioactive substances using a process known as radiolabeling. Once injected into the body, the antibodies actively seek out and bind to the cancer cells, which are destroyed by the cytotoxic action of the radiation. This approach can minimize the risk of radiation damage to healthy cells. The success of this technique will depend upon both the identification of appropriate radioactive substances and determination of the safe and effective dose of radiation that can be delivered in this way.

Radiation therapy may be used alone or in combination with chemotherapy or surgery. Like all forms of cancer treatment, radiation therapy can have side-effects. Possible side-effects of treatment with radiation include temporary or permanent loss of hair in the area being treated, skin irritation, temporary change in skin color in the treated area, and tiredness. Other side-effects are largely dependent on the area of the body being treated.

Although radiation therapy and surgery can be very effective in treating localized tumors, their effectiveness in treating disseminated cancers is less than adequate. Many patients with cancer eventually develop disseminated disease that requires a more systemic (entire body) therapy, which may be accomplished through the use of chemotherapeutic agents or anticancer drugs. Today, there are more than 50 approved chemotherapeutic agents available in the United States, and many more in various stages of approval and development.

Chemotherapeutic agents are commonly administered to a cancer patient intravenously, orally, intramuscularly, intrathecally, or intraperitoneally and interfere with the way cells divide, or carry on metabolism. Chemotherapy attempts to cause tumor cells to ultimately fail or to reproduce and eventually die. In most cases, a combination of chemotherapeutic agents are used because each agent can damage the cell in different ways. Chemotherapeutic agents also damage normal cells, however, normal cells reproduce and grow much slower than cancer cells, and consequently, the chemotherapeutic agents are not as toxic to normal cells as they are to the rapidly dividing cancer cells. The slow growth of normal cells minimizes the damage to their reproductive mechanisms allowing them to quickly repair any damage caused by the therapy and return to normal function.

Those normal cells most effected are those which divide and grow rapidly, such as hair follicles, cells in the gastrointestinal (GI) tract, and bone marrow. Consequently, side-effects can occur, including hair loss, mouth sores, difficulty in swallowing, nausea, vomiting, constipation, diarrhea, infection, anemia, and increase risk of bleeding.

Immunotherapy includes the use of cytotoxic or cytostatic agents. Cytokines, hormones, genetically engineered vaccines, and immuno-modulating therapies called biological response modifiers. Biological response modifiers (BRMs) modify the relationship between the tumor and host by strengthening the host’s biological response to tumor cells. BRMs can be divided into three
major categories according to mechanisms of action: (1) agents that restore, augment, or modulate the host’s normal immunological mechanisms; (2) agents that have direct antitumor effects; and (3) agents that have other biologic effects, such as interference with a tumor cell’s ability to metastasize or survive after metastasis, promotion of cell differentiation, or interference with neoplastic transformation in cells.

Scientists began studying BRMs in cancer therapy in the 1960s, labeling this type of treatment immunotherapy. After promising results in animal studies, researchers initiated many large-scale clinical trials to stimulate cancer patients’ immune systems using the bacterial agents Bacillus Calmette-Guerin (BCG) and Corynebacterium parvum (C. parvum). The results of these trials were discouraging, so the research into immunotherapy as a possible modality for cancer treatment lost momentum. Recent technological advances, however, have prompted a renewed interest in BRMs, and today biological response modification, or biotherapy, is an important area in cancer research and treatment.

The immune response is an exceedingly complex and valuable homeostatic mechanism having the ability to create antibodies against foreign materials, such as, cancer cells, microbial pathogens, viruses or toxic products produced therefrom. These foreign materials, referred to as antigens, elicit the ultimate response of the host, the acquired immune system, which operates by means of antibodies. An antibody is said to be specific as it attacks and binds only the antigen that triggered its production, thereby inactivating the antigen. The antigen contains some molecular species, usually protein or glycoprotein, that is not normally present in the host organism. Therefore, cancer cell membranes or toxins produced by the cancer cells are considered antigenic in the host because they possess molecular species not normally present there. Following the initial exposure to an antigen, specific T and B cells of the immune system produce memory cells that allow a more vigorous response to subsequent exposures to the same antigen. These specific memory responses are generally divided into humoral and cell-mediated immunity.

Humoral immunity refers to the immunity conferred by the B-lymphocyte cells of the lymphoid system. These lymphocytes, also called B-cells, produce antibodies which seek out and bind complementary antigens. Cell-mediated immunity refers to the immunity conferred by the maturation of T-lymphocytes, which is believed to occur in the thymus gland. These lymphocytes, also called T-cells, directly or indirectly destroy viruses, malignant cells, cells infected with intracellular organisms, and cells of grafted organs. Both B- and T-cells produce chemicals that aid in regulating the immune response. These substances are referred to as mediators and are broadly referred to as cytokines.

The growth, differentiation, development and function of a variety of cell types are

The lymphokines are a group of soluble polypeptide cytokines produced by B- and T- cells which have a major role in mobilizing and activating haematopoietic cells in response to a variety of infective agents and inflammatory stimuli. Lymphokines also have a role, directly or indirectly, in the regeneration and repair of haematopoietic and nonhaematopoietic tissues.

Lymphokines have been recognized since 1964 (see studies of B.R. Bloom and B. Bennet, Science, 153:80, (1966) and J.R. David, J. Biol. Chem., 56:72, (1966)). The term "lymphokine" was coined by D.C. Dumonde et al., Nature, 224:38, (1969). Most lymphokines were originally detected and characterized through their effects on haematopoietic cells in vitro. The effects observed on target cells can include: (1) stimulation of mitogenesis and cell proliferation; (2) inhibition of proliferation; (3) induction of differentiation and acquisition of a mature cell phenotype; (4) modulation of the function of mature cells; and (5) promotion or inhibition of apoptosis depending on intrinsic cellular characteristics as well as on the extracellular milieu.


reactions has been described by A. Rios et al., Cancer, 44:1615, (1979). Recently, L.B. Schook et al. (Biochemical Characterization of Lymphokines, A.L. DeWeck (Ed.), Academic Press, (1980), p. 67) have enumerated a list of human lymphoblastoid cell lines active in lymphokine production.


Because lymphokines were originally characterized using in vitro assay systems that defined some biological effect, their nomenclature is not without problems. Examples of other names given to cytotoxic cell culture products are NK cell cytotoxic factor, hemorrhagic necrosis factor, macrophage cytotoxin or cytotoxic factor, interleukine-2, and tumor necrosis factor or lymphotoxin.

Lymphotoxin is the term applied to what has been described as a family of molecules. Lymphotoxin molecules have been identified as glycoproteins divided into five molecular weight classes, each of which in turn is heterogenous with respect to charge. The human alpha (MW 70-90,000 and beta (MW 25-50,000) classes appear to predominate in most lymphocyte supernatants. The alpha MW classes can be separated by charge into at least seven subclasses, while the beta subclass has been separated into two distinct subclasses. Collectively, the alpha and beta classes are commonly referred to as tumor necrosis factors or TNF-α and TNF-β, respectively, and comprise a ubiquitous family of cytokine proteins of pronounced activity in the human body. The family consists of nine known members, all but one of which are integral membrane proteins. Also identified have been complex (MW >200,000) and gamma (MW 10-20,000) lymphotoxin forms.

The various lymphotoxin forms and classes differ from one another in their stability and kinetics of appearance in culture.

Tumor necrosis factor beta (TNF-β), also known as lymphotoxin-alpha (LTα), and tumor necrosis factor alpha (TNF-α), are two structurally and functionally related proteins that bind to the P55 tumor necrosis factor receptor (TNF RI), Fas/Apo-1 and the P75 (TNF RII-P75) cell surface receptors of T-lymphocyte CD-4 cells, tumor cells as well as other cells in the body. The TNF's and many of the receptor molecules have characteristic "cysteine knot motifs" which engage the TNF proteins thereby inducing apoptosis, see Marsters, S. et al., J. Biol. Chem., 267:5747 (1992). Mature TNF-β and TNF-α share approximately 35% protein sequence homology and the biologically active secreted forms of both proteins are homotrimmers. Whereas TNF-α can exist as a type II membrane
protein, TNF-β possesses a typical signal peptide sequence and is a secreted protein. Recently, it has been shown that TNF-β is also present on the cell surface of activated T, B and LAK cells as a heteromeric complex with LT-β, a type II membrane protein that is another member of the TNF ligand family. The genes for TNF-α, TNF-β and LT-β are closely linked within the major histocompatibility complex. TNF-β is expressed in activated T- and B-lymphocytes.

In addition to its cytotoxic action on tumor cells, TNF-β has been shown to be a mediator of inflammation and immune function. Evidence is also accumulating that TNF-α is a mediator in the pathogenesis of certain autoimmune diseases. Recently, TNF-β has also been shown to have a role in lymphoid organ development, see Sacca, R., et al., *J. Immunol.* 159:4252-4260 (1997).

Apoptosis is a physiologic mechanism of cell death that is induced during development or reversible tissue expansion or after tissue damage to eliminate unwanted cells. The p55 tumor necrosis factor (TNF RI) receptor and Fas/Apo-1 receptor induce cell death via distinct regions in their intracellular domains when TNF-β and TNF-α are bound. The regions within these proteins that are involved in binding to the receptors share a common sequence motif, that of the "death domain." This study shows that the death domain motifs in MORT1, TRADD, and RIP bind to tumors by regulating cell breakdown and death. If these genes mutate, cancer cells may not be broken down, leading to their proliferation. Cytokines such as TNF-α, affect cell division and therefore can contribute to the process of apoptosis, or cell fragmentation. Laboratory experiments indicate that TNF-α has a direct effect on the rate of expression of the p53 gene. The product of the p53 gene is a tumor suppressor protein that contributes to apoptosis.

Partially purified lymphokine preparations from the lymphoid cell from line RPMI 1788 were investigated in Phase I studies in Missouri in 1985, using intravenous administration, to evaluate median toxic dosage and changes in clinical laboratory values. Large quantities of material (up to 3 X 105 units per 200 mls) were administered intravenously with ease, and it was evident that in the partially purified preparation, lymphotoxin activity augmented natural immune defense mechanisms.

Early studies between the years 1975-1988 on the RPMI-1788 cell line by Papermaster *et al.* using lymphocyte culture fluids in the treatment of cancer were confirmed in England by Dumonde *et al.* and at other institutions in the United States and Germany. At present, it is premature to ascribe the effects observed clinically to a specific cytokine. Extensive scientific knowledge of cytokines derived from cell line RPMI 1788 indicates that the cytokine TNF-β is the most prominent component. Pain palliation is one of the most dramatic effects in many patients but at present there are plausible mechanisms but no definite proof of how TNF-β blocks pain or why it stimulates tumor regression, when successful.
Since the eventual value of TNF-β therapy may be in treating patients with minimal tumor burden, the end point of the Phase I trials was to determine any toxicity found in doses of 200 mls or less by the intravenous route. No significant toxicity (over NCI Grade I) was found other than fever (10% of the patients), and only one patient had temperature elevation greater than 102°F (39°C). The reduction in the wasting symptoms seen in cancer patients is likely due to the presence of an inhibitor of tumor necrosis factor TNF-α. This cytokine is strongly implicated in the diarrhea and generalized loss of body mass that often accompanies cancer.

The conventional methods of cancer treatment (surgery, radiotherapy and chemotherapy) discussed above, produce response rates or prolongation of survival in only fifty percent of cancer patients. Furthermore, of these patients, radiation and/or chemotherapeutic agents are administered at doses that typically result in side-effects, such as, temporary or permanent loss of hair in the area being treated, skin irritation, temporary change in skin color in the treated area, mouth sores, difficulty in swallowing, nausea, vomiting, constipation, diarrhea, infection, anemia, and increase risk of bleeding.

There is still a need, therefore, for a method of treating cancer in a manner that greatly reduces the variety of adverse side-effects, discussed previously, associated with radiotherapy and chemotherapy while still achieving tumor regression.

**Disclosure of Invention**

Accordingly, it is an object of this invention to provide a method for the treatment of cancer which reduces the side-effects associated with radiotherapy.

Another object of this invention is to provide a method for the treatment of cancer which reduces the side-effects associated with chemotherapy.

It is a further object of this invention to provide a method for damaging cellular DNA in cancerous cells and then inducing apoptosis in the damaged cancerous cell.

It is yet another object of this invention to provide a method for causing tumor regression in cancer patients by exposing them to low doses of radiation and/or cytotoxic agents in combination with the administration of cytokines.

Additional objects, advantages, and novel features of the invention shall be set forth in part in the description that follows, and in part will become apparent to those skilled in the art upon examination of the following or may be learned by the practice of the invention. The objects and the advantages may be realized and attained by means of the instrumentalities and in combinations particularly pointed out in the appended claims.

To achieve the foregoing and other objects and in accordance with the purposes of the present invention, as embodied and broadly described herein, the method of this invention comprises
the creation of DNA damage in cancerous cells using low doses of radiation and/or a cytotoxic agents and then inducing apoptosis in the cancerous cells by exposing them to cytokines.

**Brief Description of the Drawings**

**Best Mode for Carrying out the Invention**

Apoptosis, the main mechanism of programmed cell death, is a gene directed process responsible for the elimination of excessive cells during development and detrimental cell types in pathophysiological situations. The present invention provides a method for exploiting the molecular mechanisms which regulate the pathways leading to programmed cell death, and tumor regression without significant side-effects to the patient. Both low dose chemotherapy and low dose radiotherapy induce DNA fragmentation, thereby positioning tumor cells to self-destruct by apoptosis. By infusing low doses of cytokines to patients prior to and during treatments of chemotherapy and/or radiotherapy, tumor cells containing damaged DNA are induced into apoptosis resulting in tumor regression without significant side-effects to the patient.

The surprising discovery that cytokines, and preferably TNF-β, may be used as an adjuvant to low dose radiotherapy and/or chemotherapy means that it is now possible to realize the benefits of tumor regression that may be accomplished using higher doses of radiation and/or cytotoxic agents while significantly reducing the typical side-effects that patients typically experience with these conventional treatments.

**EXPERIMENTAL PROTOCOL**

While experimental data is provided herein for the successful treatment of lung cancer, cancer of the large bowel (metastatic), and large clear cell lymphoma using the treatment protocol of the present invention, it is to be understood that the present treatment protocol can also be used for the treatment of other forms of cancer, such as, but not limited to leukemia lymphoma, non-small-cell lung cancer, colon cancer, central nervous system cancer, melanoma, ovarian cancer, renal cancer, prostate cancer, breast cancer and other carcinomas. The present invention provides an improvement in the treatment of all types of cancer which can be treated with radiation and/or chemotherapeutic agents, since by use of the administration protocol of the present invention, a decrease in side-effects and/or an increase in tumor regression are achieved than that associated with prior art protocols for treating cancer with radiation and/or chemotherapeutic agents.

**Patient Selection and Evaluation:**

The patients were treated with an effective dose of TNF-β in combination with low doses of radiation and/or chemotherapeutic agents. All patients had histologically proven malignancy, and clinical evidence of wide-spread metastatic disease. A measurable tumor mass as determined by
percutaneous measurement, radiologic, or other acceptable criteria of measurement was discernible; measurements consisted of the product of two transverse diameters of the tumor lesion(s) or other CT scan records in order for the patient to be declared evaluable for response. Patients could enter by self-election prior to conventional therapy or be unable to obtain therapy available that could be called either "curative" or standard salvage therapy (defined as having a 30% or greater chance of producing tumor response or partial response (PR). Signed informed consents were obtained from all participants. Patients had to be over 21 years in age with an Eastern Cooperative Oncology Group, ECOG, performance status of 0,1, or 2, or have a Karnovsky performance score of at least 60.

Furthermore, patients had to have a life expectancy of at least 60 days, and had to have recovered from all toxicities of previous treatment. No prior chemotherapy or radiotherapy were allowed within one month prior to entry into the study. There could be no evidence of additional primary malignancy of another tumor type other than non melanomatous skin cancer within past two years, and no evidence of active infection or open wounds or severe cardiac or other medical problems unrelated to the malignancy, and no evidence of psychosocial or psychiatric conditions, which may have interfered with the proposed therapeutic procedures or informed consent. Patients must have had central venous access line inserted, or agreed to such before beginning treatment and to necessary lab tests and studies during treatment and follow-up.

Adequate baseline values for the following were also required: white blood cell count of at least 2,000 mm$^3$, platelet count greater than 50,000 mm$^3$, serum bilirubin less than 2.5 mg/dl, serum glutamic oxalacetic transaminase (SGOT) less than three times the upper limit of normal, blood urea nitrogen (BUN) less than 30 mg/dl, serum creatinine less than 4.0 mg/dl and CD-4 absolute lymphocyte count greater than 250/ml. Pre-entry blood sample for CD-4/CD-8 lymphocyte levels, complete blood counts (CBC), and laboratory clinical chemical values were obtained.

During therapy, hematology data (hemoglobin, white blood cell count, granulocytes and platelets) were collected, and toxicity assessments were made. After each cycle, a physical history update was recorded, as well as tumor measurement (using photographs or x-rays, or CT scans with caliper recordings), performance status (Karnovsky), hematology, chemistry (serum creatinine, bilirubin, alkaline phosphatase, SGOT (AST), CA125) and a toxicity assessment. A quality of life assessment was also made after each cycle, and every two months until six months after treatment ceased.

Preparation of Cytokine for Clinical Use:

Lyophilized TNF-β was supplied by Baja Farmaceuticos, S.A. de C.V. and dissolved in a sterile saline solution (certified for intravenous use) containing 0.01% sterile human albumin (HSA), certified for human intravenous use by the Mexican government. A working stock solution of no
less than one and preferably two liters was prepared to contain 50 ng of TNF-β per ml in carrier. The stock solution is then added to sterile trace clean 20 ml vials in amounts of 5 or 10 ml. This prepares doses of 250ng (5ml) or 500ng (10ml). The sterile I.V. ready human albumin is used to avoid losses in activity due to non-specific binding to the inside surface of the vial. The final product is then aseptically dispensed into sterile 20 ml vaccine vials, frozen and/or lyophilized at -70°C, stoppered under vacuum and stored in a freezer or at 2° - 4° C in a refrigerator. The product is added to Mexican Government approved bags of sterile saline (50 or 100ml) pyrogen free, and consistent with the safety and sterility procedures established by the Mexican Authorities. While TNF-β in a sterile saline containing 0.01% sterile human albumin is utilized as a vehicle in the preferred embodiment, other pharmaceutically acceptable carriers for TNF-β, such as manitol, and other inert protein stabilizing agents, may also be used. Dose units are based on semi-quantitative immuno-assays and are standardized to 500 ng per dose. Details of methodology for standardization of the biological activity of this preparation are provided by Quantikine™: Human TNF-β Immunoassay: Catalogue No.: DTB 800. R&D Systems, 1997 Catalogue, P. 178, 614 McKinley Place N.E., Minneapolis, MN 55413.

Administration of Cytokine as an Adjuvant to Radiotherapy and/or Chemotherapy:

Prior to initiating infusion contents of the 10cc vial was taken up in a 10-12 ml syringe and injected into a 100 ml plastic bag of IV saline and TNF-β was then administered by intravenous infusion via a nylon mesh central line, having a .22 micron filter, as a continuous infusion in sterile bags, described previously, over a time period of 1 hour. The TNF-β IV solution is preferably administered at a rate of 1 ml/min.; however, it may be administered at a rate in the range of .6 ml/min. to 2 ml/min. A time period of 15 days constitutes one cycle and may be repeated at the discretion of the attending physician for up to 80 doses. The preferred assigned dose levels of 500 ng/day were maintained throughout the course of any additional cycle. While the preferred dosage level of TNF-β is 500 ng/day, 250 ng/day to 750 ng/day may also be administered, at the discretion of the attending physician.

A nurse remained with each patient during the infusion to continuously monitor vial signs, such as, temperature, pulse, blood pressure, and respiration. Precautionary procedures in place if a patient developed a temperature of over 99.5° F were that, Benadryl® 25 mg would be added intravenously to the infusion line, quarterly over four hours, as necessary; and Tylenol® 10 grains or Aspirin 650 mg (10 grains) quarterly over four hours for treatment of "flu-like" side-effects. Such reactions did not occur at the dosage used. Treatment was also to be stopped immediately at the first occurrence of a temperature exceeding 100° F (37.7° C), or at a sign of infection, or with any other adverse nonfebrile reaction.
Chemotherapy:

The administration of a chemotherapeutic agent or agents is begun preferably on the fourth day of treatment; however, it can proceed anywhere from the third to sixth day. The preferred chemotherapeutic agents utilized in the method of the present invention are agents such as 5-fluorouracil (500 mg/day for five days or 2500-7500 mg/cycle; Taxol® (up to 350 mg per dose or 50 mg/meter sq); Taxotere® (up to 50 mg/day); etoposide (up to 100 mg/day); chlorambucil (up to 2mg/day for three to four weeks); Dauxorubicin (Adriamycin) (up to 750 mg/cycle or 550 mg/meter² life time dose); methotrexate (10 mg to 25 mg/m²). Other chemotherapeutic agents with adjusted lower doses of which will be realized by one skilled in the art, include, but are not limited to, Actinomycin D, Asparaginase, Bleomycin, Busulphan, Carboplatin, Carmustine, Cisplatin, Cyclophosphamide, Cytarabine, Decarbazine, Epirubicin, Fludarabine, Fluorouracil (5-FU), Gemcitabine (Gemzar), Hydroxyurea, Idrubicin, Ifosfamide, Irinotecan (Campto), Lomustine, Melphalan, Mercaptopurine, Mitomycin, Mitozantrone, Procarbazine, Steroids, Streptozocin, Thioguanine, Thiotepa, Tomudex (Raltitrexed), Topotecan (Hycamtin), Treosulfan, Vinblastin, Vincristine, Vinodesine, and Vinorelbine (Navelbine). Hormonal Drugs include, but are not limited to, Anastrozole (Arimidex), Bicalutamide (Casodex), Buserelin (Suprefact), Cyproteron acetate (Cyprostat), Flutamide (Drogenil), Formestane (Lentaron), Goserelin (Zoladex), Letrozole (Femara), Leuprorelin (Prostap SR), Medroxyprogesterone acetate (Provera, Farlutal), Megestrol acetate (Megace), Tomoxifen (Nolvadex), Tormifene (Fareston), and Triptorelin (Decapeptyl).

Radiotherapy:

Beginning on the fifth day of treatment, radiotherapy is commenced usually for a period of five days. Effective radiation can be accomplished with any clinically acceptable radiation source. These are cobalt machines, electromagnetic photon generating sources, electron beam generating sources such as lineal accelerators, high linear energy transfer sources, radioimmunotherapy, brachterapy or implantation of radioactive seeds. Radiotherapy was discussed previously, in more detail, and it will be understood by one skilled in the art of the type of radiation that may be required, depending upon the specific cancer to be treated. Effective dosage ranges, which are acceptable for use in the present invention, will be those that result in damage to DNA without producing scarring or burns to the patient’s tissue (200 to 3,500 rads or cGy are acceptable). In the preferred embodiment, 2,000-3,000 cGy over a ten-day period were administered as calculated in a Target II simulator delivered in a General Electric Saturn 41 integrated radiation treatment to the calculated radiation target portals.
Response:

Pain was evaluated on a pain scale of 1-10 with 1 being no pain, 5 being intermediate pain, and 10 severe pain. Pain evaluation was made by interview daily from the attending physician and nurse on the pain scale from one to ten. A complete response consisted of reduction of measurable tumor to less than 50% of original size. A partial response will signify a reduction by at least 25% of the product of the transverse diameters of one or more measurable tumors as measurable by CT scan or other acceptable measure and no progression of the tumor or other appropriate indicators. It is to be understood that the following examples are for illustrative purposes only, and are not intended to limit the scope of the invention as herein described or as set forth in the subjoined claims.

EXAMPLES

Example I

LUNG Cancer

A 73 year old white male with a history of smoking one pack of cigarettes per day for 42 years was in generally good health prior to diagnosis. In 1996, following a cold in January, he noticed a persistent cough and some shortness of breath. He visited his family physician for treatment and a chest x-ray, CT scan, bronchoscopy and biopsy were taken. A thoracotomy confirmed squamous cell carcinoma in the right lung, Stage IIIb. The tumor appeared to be inoperable because of its location near the heart and great vessels. A bone scan was negative and the left lung appeared normal. Seven weeks of radiation along with a combination of three chemotherapy treatments were given with cisplatin and VP-16 (etoposide). These treatments did not affect metastasis of the patient’s tumor.

A follow-up CT scan revealed that the cancer had spread to the liver. A new round of chemotherapy using Taxol® was commenced. Following the first treatment, the patient developed pneumonia, which responded to antibiotics. Over the next few months, with three additional treatments using Taxol®, the patient’s condition declined, losing interest in food, losing weight, and developing a need for supplemental oxygen.

The patient elected to begin treatment with TNF-β in combination with Taxol® treatments. Though his activity level was low, he gained weight and responded well to these treatments. Following a conference with his oncologist, the patient came to our clinic for one week in March, approximately one year following his initial diagnosis, where he underwent installation of a port-a-cath and treatment via intravenous infusion of TNF-β once per day for five days. He received a supply for further home treatment, continuing on three administrations per week basis for six months. He remained active, began walking approximately one mile several times a week with no significant complaints, and with a renewed feeling of well-being. He continued on Taxol® at 330 mg once per month with control of toxicity as above, and received TNF-β at doses of 500 nanograms three times a
week for four months. A CT scan in August revealed stabilization of his lung tumor with no
evidence of metastatic disease in the liver. He then came to our clinic in Monterrey, Mexico, the
modern OCA hospital with an advanced immunotherapy program. This enabled thorough evaluation.
After a complete diagnostic work-up, including CT scans and ultrasound scans of the liver, his
tumor-free liver condition was confirmed, but his CD-4 lymphocytes were below normal (absolute
count normal = >500). The patient looked well and was in good spirits, and continued on with TNF-
\( \beta \). The patient remains ambulatory and active eleven months after beginning TNF-\( \beta \) and home
infusions for a total of 80 doses over the six-month period.

Example II

Lung Cancer (adenocarcinoma):

A 54 year old white male was referred for treatment of his lung cancer in January 1998. He
was previously having monthly follow-up treatment and management of his previously existing
congestive heart failure by his physician. His history included congenital hearing and speech
impairment, congestive heart failure, cardiomyopathy, and insulin-dependant diabetes mellitus.
The patient’s tumor was diagnosed beginning September 17, 1997 and corroborated on
December 22, 1997 by needle-aspiration biopsy as an adenocarcinoma of the right lung. Flat-plate
X-rays, CT scan, surgical biopsy, physical examination, and laboratory tests supported the tumor
diagnosis. The patient’s existing heart condition ruled out standard chemotherapy and radiation
treatment. He began a regimen of intravenous TNF-\( \beta \) at 500 nanograms/dose followed by 5-
fluorouracil at 500mg/dose for five days. This was followed by 10 sessions of radiotherapy for a
total dosage of 2500 cGy. He was discharged with additional cytokine doses of 500ng to be given
three times week .

Overall, the patient showed a 66% reduction in the size of his lung tumor at the time of
discharge, no evidence of toxicity from any of the treatment procedures, and was ambulatory and
cheerful throughout.

Example III

Lung Cancer – (non small cell carcinoma)

A 59 year old male, self-employed, in excellent physical condition and a non-smoker, with
no previous occupational hazard exposure, was diagnosed with non-small cell carcinoma of the lung.
CT scan showed a 6 cm diameter soft tissue density mass on the posterior segment of the left upper
lobe abutting the major fissure. In addition to this relatively large soft tissue density mass, a fairly
extensive pleural surface nodulation was noted that followed along the course of the major fissure.
Furthermore, there was irregular pleural fluid loculation of the left hemi thorax with other foci of
pleural surface nodulation and pleural surface thickening. The latter is most notable with respect to
the ipsilateral mediastinal pleura. No enlarged mediastinal masses and/or adenopathy were identified; however, a slightly prominent left pericardiophrenic node was also identified.

The patient began a 15-day course of TNF-β at 500ng/dose. Chemotherapy with 5-fluorouracil at 500mg/dose for 5 days (total of 2500 mg) began three days after beginning TNF-β.

Radiation treatment began and the patient received ten doses for a total of 2500 cGy. Examination in the Target II simulator revealed the patient had undergone a 37% reduction in tumor lesion size. The patient’s progress is being monitored by his oncologist. Further changes included up to 70% reduction in tumor size and fibrotic replacement of tumor tissue. The patient tolerated all treatment well, without signs of toxicity, and continues an active life, including exercise.

Example IV

Cancer of the Large Bowel-metastatic:

A seventy-eight year old gentleman with previous stroke and diabetes, was diagnosed with carcinoma of the bowel, for which he had a colon resection in 1996. In September 1997, a transverse colon polyp was found along with a cecal polyp. Upon surgery, a mass 8 cm in length was removed from the colon and positive lymph nodes were found. Subsequently, the patient had increased tumor growth in the thoracic and iliac lymph nodes which were detected by a CT scan and an increase in CEA. The patient had a CT scan on October 13, 1997, which showed enlarged lymph nodes in the thorax and abdomen. His CEA test gave a value of 34-35.

A PICC intravenous line was installed in the patient’s left arm which administered 500ng of TNF-β. Subsequently, he received further daily doses of TNF-β for eleven days. Chemotherapy was begun and he was given five infusions of 5-fluorouracil for five days at 500mg per treatment, for a total of 2500mg. He tolerated all treatments without signs of toxicity, although he complained of continuous fatigue at his initial examination. His laboratory values remain within normal limits, with the exception of RBC and Hb, showing slight decreases from chemotherapy.

Radiation treatment was begun and a regimen of radiation at 175 cGys per dose was carried out over the same time period for 20 sessions (cumulative dose = 3500 cGy). A CT scan following treatment indicated decreased size of the thoracic nodes. His CEA was reduced to 7.8. Other than an initial bout of diarrhea following his first radiation session, the patient experienced no toxicity during treatment and gained six pounds. He was released after six weeks of treatment with a CEA reduced to 7.8. Four months after treatment CT scan revealed periaortic abdominal nodes to be normal in size and a recent CEA was 2.8.
Example V

Large Clear cell Lymphoma:

An 80 year old white female presented with a history of mixed diffuse nodular large cell lymphoma, predominately cleaved cell stage 1A-E. Her disease was first diagnosed in November of 1990, when excised from the posterior neck. She had a recurrence in the left forearm, excised June 30, 1994, and finally, left groin recurrence in 1995. The reason for her visit was a CT scan in November 1997 showing lymphadenopathy in the pelvis in the left inguinal chain and left groin, which was considered a new finding.

The patient’s CT scans revealed enlarged inguinal nodes on the right, and a mass extending into the pelvis from the left inguinal node for approximately 3x5cm. She commenced on a four-day course of TNF-β at 500ng/dose, but developed bronchitis leading to pneumonitis. This was treated with oral Rotromicin (a macrolide erythromycin derivative) at 300mg/dose for three days, Amicacine at 1 gr./dose I.V. for three days, and Cefotoxime (a cephalosporin derivative) at 500mg I.V. for five days. Her cytokine was discontinued until her improvement three days later.

Chemotherapy of 5-fluorouracil at 500mg for five days was begun, along with the TNF-β, which was continued for a total of 15 doses. Radiation treatment was begun, and she received ten doses for at total of 2700 cGy. A CT scan on March 12, 1998 revealed that she had a complete remission of both inguinal masses and no evidence of tumor elsewhere. She had completely recovered from respiratory illness and was in excellent spirits.

The success of the use of low dose radiation and/or chemotherapeutic agent(s) in combination with the administration of TNF-β as disclosed in the present invention for the treatment of cancer, makes it readily apparent that tumor regression can be achieved with minimal side-effects. Thus, it is contemplated that the method of the present invention may be utilized to treat solid tumors, leukemias and lymphomas, such as, but not limited to, lung cancers, breast cancers, ovarian cancers, prostate cancers, colon cancer, central nervous system cancer, melanoma, and renal cancers.

It is to be understood that treatment of different forms of cancer may require the adjustment of the dosages of TNF-β, and of radiation and/or chemotherapeutic agents) to have optimal efficacy.

The foregoing description is considered as illustrative only of the principles of the invention. Furthermore, since a number modifications and changes will readily occur to those skilled in the art, it is not desired to limit the invention to the exact construction and process shown described above. Accordingly, all suitable modifications and equivalents may be resorted to falling within the scope of the invention as defined by the claims which follow.
Claims
The embodiments of the invention in which an exclusive property or privilege is claimed are defined as follows:

1. A method for inducing apoptosis in a population of normal and cancer cells comprising:
   fragmenting DNA in said population of cells, and
   inducing apoptosis in said cancer cells by administering a therapeutically effective dose of lymphotoxin.

2. The method of claim 1, wherein said population of normal cells and cancer cells is present in a mammal and said fragmenting step is performed by exposing said mammal to a therapeutically effective dose of energy which produces cellular damage but not cell death.

3. The method of claim 2, wherein apoptosis is induced in said damaged cancer cells by administering a therapeutically effective amount of a lymphotoxin.

4. The method of claim 2, wherein the mammal is a human.

5. The method of claim 2, wherein said energy is x-rays.

6. The method of claim 2, wherein said energy is gamma-rays.

7. The method of claim 2, wherein said energy is a subatomic particle.

8. The method of claim 2, wherein said energy is electron beam radiation.

9. The method of claim 2, wherein said radiation is delivered by way of cobalt machines, electromagnetic photon generating sources, high linear energy transfer sources, implantation of radioactive seeds, or radiolabeled antibodies.

10. The method of claim 2, wherein said therapeutically effective dose of energy is in the range of 200-3500 rads or cGys over a fifteen (15) to twenty-one (21) day cycle.

11. The method of claim 10, wherein said therapeutically effective dose of energy is 2,000 to 3,000 cGys over a 15 to 21 day period.

12. The method of claim 3, wherein said lymphotoxin is TNF-β.

13. The method of claim 12, wherein said therapeutically effective amount of TNF-β is 250 to 750 ng/day.

14. The method of claim 13, wherein said therapeutically effective amount of TNF-β is 500 ng/day.

15. The method of claim 1, wherein said population of normal cells and cancer cells is present in a mammal and said fragmenting step is performed by exposing said mammal to a therapeutically effective dose of a chemotherapeutic agent aimed at producing cellular damage but not cell death.
16. The method of claim 15, wherein the mammal is human.
17. The method of claim 15, wherein apoptosis is induced in said damaged cancer cells by administering a therapeutically effective amount of a lymphotoxin.
18. The method of claim 17, wherein said lymphotoxin is TNF-β.
19. The method of claim 18, wherein said TNF-β is delivered in a therapeutically effective dose in the range of 250 to 750 ng/day.
20. The method of claim 19, wherein said therapeutically effective dosage of TNF-β is 500 ng/day.
21. The method of claim 15, wherein said chemotherapeutic agent is selected from the group comprising: Actinomycin D, Asparaginase, Bleomycin, Busulphan, Carboplatin, Carmustine, Cisplatin, Cyclophosphamide, Cytarabine, Decarbazine, Epirubicin, Fludarabine, Fluorouracil (5-FU), Gemcitabine (Gemzar), Hydroxyurea, Idarubicin, Ifosfamide, Irinotecan (Campto), Lomustine, Melphalan, Mercaptopurine, Mitomycin, Mitozantrone, Procarbazine, Steroids, Streptozocin, Thioguanine, Thiopeta, Tomudex (Raltitrexed), Topotecan (Hycamtin), Treosulfan, Vinblastin, Vincristine, Vincedesine, and Vinorelbine (Navelbine). Hormonal Drugs include, but are not limited to, Anastrazole (Arimidex), Bicalutamide (Casodex), Buserelin (Suprefact), Cyproteron acetate (Cyprostat), Flutamide (Drogenil), Formestane (Lentaron), Goserelin (Zoladex), Letrozole (Femara), Leuprorelin (Prostap SR), Medroxyprogesterone acetate (Provera, Farlutal), Megestrol acetate (Megace), Tomoxifen (Nolvadex), Tormifene (Fareston), and Triptorelin (Decapeptyl).
22. The method of claim 21, wherein 5-fluorouracil is delivered in a therapeutically effective dosage in the range of 2500 to 3500 mg/15 days.
23. The method of claim 22 wherein 5-fluorouracil is delivered in a therapeutically effective dosage of 500 mg/day for 5 to 7 days.
24. The method of claim 21, wherein Taxol® is delivered in a therapeutically effective dosage of up to 350 mg/dose.
25. The method of claim 1, wherein said population of normal cells and cancer cells is present in a mammal and said fragmenting step is performed by exposing said mammal to therapeutically effective doses of energy and a chemotherapeutic agent, wherein said dosages are aimed at producing cellular damage but not cell death.
26. The method of claim 1, wherein said cancer cells are selected from the group of cancers comprising leukemia, non-small-cell lung cancer, colon cancer, central nervous system cancer, melanoma, ovarian cancer, renal cancer, prostate cancer, and breast cancer.
27. A method for minimizing common side-effects in patients suffering from cancer and undergoing conventional radiation therapy and/or chemotherapy comprising:
exposing a population of cells comprising normal cells and cancer cells to a dosage
of a DNA fragmenting agent in a therapeutically effective dosage that will produce cellular damage
but not cell death; and
administering a therapeutically effective dosage of a lymphotoxin.

28. The method of claim 27, wherein said DNA fragmenting agent is an ionizing
radiation.

29. The method of claim 27, wherein said DNA fragmenting agent is a chemotherapeutic
agent.

30. The method of claim 27, wherein said lymphotoxin is TNF-β

31. The method of claim 30, wherein said TNF-β is administered in said therapeutically
effective dosage in a range of 250 to 750 ng/day.

32. The method of claim 31, wherein said therapeutically effective dosage is 500 ng/day.

33. The method of claim 27, wherein said cancer cells are selected from the group of
cancers comprising leukemia, lymphoma, non-small-cell lung cancer, colon cancer, central nervous
system cancer, melanoma, ovarian cancer, renal cancer, prostate cancer, and breast cancer.

34. A method for the treatment of cancer comprising exposing a patient suffering from
cancer to a low dose of radiation and/or a chemotherapeutic agent in combination with a
therapeutically effective dosage of TNF-β.

35. The method of claim 34, wherein said low dose of radiation causes cellular damage
but not cell death, and said damaged cancer cells are induced into apoptosis by administering said
therapeutically effective dose of TNF-β.

36. The method of claim 35, wherein said low dose of radiation is 2000 to 3500 cGys.

37. The method of claim 36, wherein said low dose of radiation is 2700 cGys.

38. The method of claim 35, wherein said therapeutically effective dose of TNF-β is 250
to 750 ng/day.

39. The method of claim 38, wherein said therapeutically effective dose of TNF-β is 500
ng/day.

40. The method of claim 34, wherein said cancer is said cancer cells are selected from
the group of cancers comprising leukemia, non-small-cell lung cancer, colon cancer, central nervous
system cancer, melanoma, ovarian cancer, renal cancer, prostate cancer, and breast cancer.

41. The method of claim 34, wherein said low dose of chemotherapeutic agent causes
cellular damage but not cell death, and said damaged cancer cells are induced into apoptosis by
administering said therapeutically effective dose of TNF-β.

42. The method of claim 41, wherein said chemotherapeutic agent is selected from the
group comprising: Actinomycin D, Asparaginase, Bleomycin, Busulphan, Carboplatin, Carmustine, Cisplatin, Cyclophosphamide, Cytarabine, Decarbazine, Epirubicin, Fludarabine, Fluorouracil (5-FU), Gemcitabine (Gemzar), Hydroxyurea, Idarubicin, Ifosfamide, Irinotecan (Campto), Lomustine, Melphalan, Mercaptopurine, Mitomycin, Mitozantrone, Procarbazine, Steroids, Streptozocin, Thioguanine, Thiotepa, Tomudex (Raltitrexed), Toptecan (Hycamtin), Treosulfan, Vinblastin, Vincristine, Vindesine, and Vinorelbine (Navelbine). Hormonal Drugs include, but are not limited to, Anastrazole (Arimidex), Bicalutamide (Casodex), Buserelin (Suprefact), Cyproteron acetate (Cyprostat), Flutamide (Drogenil), Formestane (Lentaron), Goserelin (Zoladex), Letrozole (Femara), Leuprolelin (Prostap SR), Medroxyprogesterone acetate (Provera, Farlutal), Megestrol acetate (Megace), Tomoxifen (Nolvadex), Tormifene (Fareston), and Triptorelin (Decapeptyl).
INTERNATIONAL SEARCH REPORT

A. CLASSIFICATION OF SUBJECT MATTER

IPC(7) : A61K 38/19, 41/00
US CL : 514/2, 8, 12, 885; 424/49, 84, 85.1

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

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 514/2, 8, 12, 885; 424/49, 84, 85.1

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

NONE

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

Please See Extra Sheet.

C. DOCUMENTS CONSIDERED TO BE RELEVANT

<table>
<thead>
<tr>
<th>Category</th>
<th>Citation of document, with indication, where appropriate, of the relevant passages</th>
<th>Relevant to claim No.</th>
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<tbody>
<tr>
<td>A</td>
<td>US 4,920,196 A (AGGARWAL) 24 April 1990 (24/04/90), see entire document.</td>
<td>1-42</td>
</tr>
</tbody>
</table>

Further documents are listed in the continuation of Box C. See patent family annex.

Date of the actual completion of the international search 23 DECEMBER 1999

Date of mailing of the international search report

19 JAN 2000

Name and mailing address of the ISA/US Commissioner of Patents and Trademarks

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Form PCT/ISA/210 (second sheet)(July 1992)
B. FIELDS SEARCHED

Electronic data bases consulted (Name of data base and where practicable terms used):

WEST, CAS ONLINE, MEDLINE, EMBASE, BIOSIS
search terms: apoptosis, cell death, cancer cells, DNA fragmentation, lymphotoxin, tumor necrosis factor-beta, x-rays, gamma-rays, chemotherapeutic agent.