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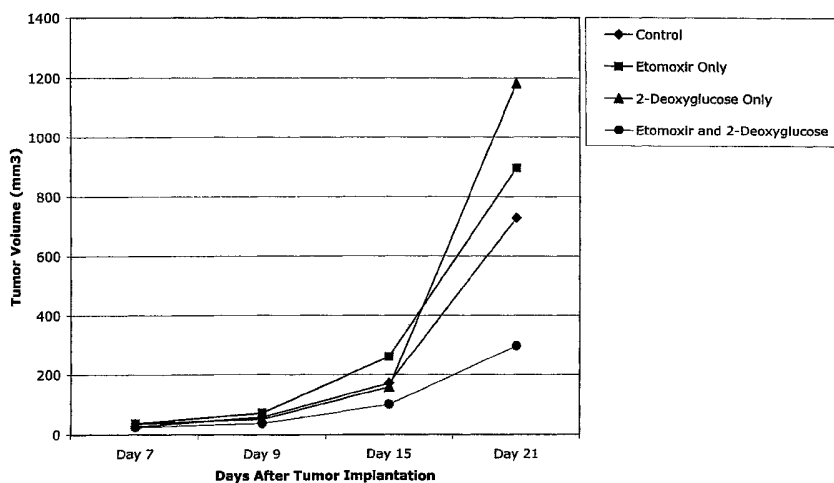
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(54) Title: SYSTEMS AND METHODS FOR TREATING HUMAN INFLAMMATORY AND PROLIFERATIVE DISEASES AND WOUNDS, WITH FATTY ACID METABOLISM INHIBITORS AND/OR GLYCOLYTIC INHIBITORS

HL60 MDR Tumor in Nude Mice Treated with Etomoxir, 2-Deoxyglucose, or Both



(57) Abstract: Systems and methods for treating inflammatory and proliferative diseases, and wounds, using as a pharmacon a fatty acid metabolism inhibitor, a glycolytic inhibitor, and/or an agent able to alter cellular production of reactive oxygen, or combination thereof, optionally in combination with one or more chemotherapeutic agents. In preferred embodiments, the invention combines an oxirane carboxylic acid, represented by etomoxir, with a 2-deoxyglucose compound, represented by 2-deoxy-D-glucose, and/or an antibody against UCP and/or Fas antigen. The systems and methods of the invention can be used to treat drug-resistant or multi-drug resistant cancers.



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**SYSTEMS AND METHODS FOR TREATING HUMAN INFLAMMATORY AND
PROLIFERATIVE DISEASES AND WOUNDS, WITH FATTY ACID METABOLISM
INHIBITORS AND/OR GLYCOLYTIC INHIBITORS**

RELATED APPLICATIONS

[0001] This application claims the benefit of: U.S. Provisional Patent Application Serial No. 60/477,873 filed June 12, 2003, entitled "Systems and Methods for Treating Cancers and Wounds," by M. Karen Newell, *et al.*; U.S. Provisional Patent Application Serial No. 60/478,646 filed June 12, 2003, entitled "Systems and Methods for Treating Cancers and Wounds," by Martha Karen Newell Rogers, *et al.*; and U.S. Provisional Patent Application Serial No. 60/490,587, filed July 28, 2003, entitled "Systems and Methods for Treating Cancers and Wounds With Oxirane Carboxylic Acids," by M. Karen Newell, *et al.*

FEDERALLY SPONSORED RESEARCH

[0002] This invention was sponsored by the NIH, Grant No. RO1 GM62562. The Government may have certain rights to this invention.

FIELD OF INVENTION

[0003] The field of the invention generally relates to systems and methods for treating inflammatory and proliferative diseases, and wounds, using a fatty acid metabolism inhibitor, a glycolytic inhibitor, and/or an agent able to alter cellular production of reactive oxygen, or combination thereof, optionally in combination with one or more chemotherapeutic agents.

BACKGROUND

[0004] Normal tissue develops, and is maintained by, processes of cell division and cell death. In many diseases, such as cancer, diabetes mellitus Type I, and autoimmune disease, the normal balance between cell division and cell death is disrupted, causing either a rapid growth of unwanted and potentially dangerous cells, and/or a loss of cells essential to maintaining the functions of tissue. Inappropriate cell division or cell death can result in serious life-threatening diseases. Diseases associated with increased cell division include cancer and

atherosclerosis. Diseases resulting from increased cell death include AIDS, neurodegenerative diseases (e.g., Alzheimer's disease, Parkinson's disease, amyotrophic lateral sclerosis, retinitis pigmentosa), aplastic anemia, atherosclerosis (e.g., myocardial infarction, stroke, reperfusion injury), and toxin-induced liver disease.

[0005] The immune system, a complex organization of cells, tissues and organs, serves to protect us from potential harm. Extraordinary advances in our understanding of the immune system have been made in the last hundred years, especially since the discovery of the T cell and B cell [3-5]. Native T-cells require two signals for activation. These are recognition of antigens in Major Histocompatibility Complex-encoded (MHC) molecules [3], and a co-stimulation signal [6-10] provided by the B7/CD28 family members or other co-stimulatory molecules such as Fas (CD95) [11]. Previously activated T cells can be reactivated by co-stimulation alone [12, 13]. In the absence of activation, T-cells disregard the tissue. If a T cell is activated the consequences can be: 1) destruction of the damaged cells or 2) repair of damaged cells by promoting regeneration either directly or indirectly.

[0006] A natural question is: why doesn't the immune system destroy tumor cells as they arise? In fact, there is substantial evidence that the immune system does play an extensive role in suppressing cancer. For example, it is known that people on immune-suppressive therapy have a much higher cancer rate as do people with AIDS [14] and the very young and very old. However, it is also clear that the ability of the immune system to control cancer is not perfect.

[0007] Researchers have for many years tried to stimulate the immune system as a therapeutic strategy against cancer [15, 16]. These attempts have generally been ineffective, although there have been some recent successes [17]. There are many reasons for this variability. These include: 1) the inability to activate T cells that can destroy the tumor due to the absence of signal one (lack of recognition of the appropriate tumor antigen); 2) the presence of a signal two that results in the production of the wrong cytokines by T cells which may lead to the growth of tumors, or 3) the failure of activated T cells to kill cancerous cells [18].

[0008] Anti-cancer agents may work to promote the death of tumor cells in multiple ways. First, these agents may work by direct cytolysis requiring active participation of the tumor cell in the death process [25]. Second, chemotherapeutics may promote the ability of the tumor cell to be recognized by cells of the immune system and to be killed by immune-directed cell death [26]. Third, these agents may work to "rewire" the death inducing receptor/ligand pairs

which include Fas and FasL [23, 25, 26]. The second and third possibilities are not mutually exclusive and may work in concert to result in tumor cell death.

[0009] It has been discovered that uncoupling protein ("UCP") is normally present in the plasma membrane of rapidly dividing cells, but it is not typically found on the plasma membrane of growth-arrested or chemotherapy-resistant tumor cells. UCP, which is also present in the mitochondria, can regulate cell division by manipulating the manner in which the cell processes and stores energy. It was found that the UCP in the plasma membrane plays an important role in the signal processes that determine whether a cell will undergo cellular division, cellular differentiation, or cellular death. These findings have important implications on the ability to regulate cell division as well as sensitivity and resistance to chemotherapeutic agents and, therefore, for treating diseases associated with excessive cellular division, aberrant differentiation, and premature cellular death, e.g., for the treatment of cancers, autoimmune disease, degenerative diseases, regeneration, etc.

[0010] Mitochondrial uncoupling proteins (UCP 1-5) are a family of molecules, first described in brown adipose tissue, that dissipate of the proton gradient, lower mitochondrial membrane potential, induce of a metabolic shift to fatty acids as a source of fuel in mitochondria, promote of high rates of glucose utilization in the cytosol and increased oxygen consumption in the mitochondria; and reduce production of reactive oxygen species [29,30]. UCP-mediated uncoupling requires transport of the anionic fatty acids head groups by UCP and proton transport via the bilayer [31]. UCP-2 has been shown to be expressed at higher levels in the mitochondria of cells that are protected from apoptosis-inducing stimuli. [32]. Data is consistent with the report that the production of reactive oxygen species and subsequent cellular damage induced by hyperglycemia in endothelial cells is prevented by transfection with UCP-1. UCP are inducible proteins. For example, it has been shown that lipopolysaccharides can induce synthesis of UCP-2 [33] and superoxide can activate UCP-2 within the mitochondria [34]. UCP-2 mRNA and protein are induced in the cerebral cortex after trauma {Bechmann, 2002 #81} [35] ischemic preconditioning in vivo or intraperitoneal injection of kainic acid [36,37], and UCP-4 is inducible in neurons in vitro by glycolytic inhibitors [38]. Significant neuroprotective actions of UCP-induced uncoupling have been shown in vitro models of oxygen-glucose deprivation [39] and in vivo [39] models of trauma ischemia, seizures [39] and excitotoxicity [37] in mice overexpressing UCP.

[0011] Several cell surface proteins have previously been identified as cell death proteins. These proteins are believed to be involved in initiating a signal which instructs the cell to die. Cell death proteins include, for example, Fas/CD95 (Trauth, *et al.*, *Science*, 245:301, 1989), tumor necrosis factor receptors, immune cell receptors such as CD40, OX40, CD27 and 4-1BB (Smith, *et al.*, *Cell*, 76:959, 1994), and RIP (U.S. Patent No. 5,674,734). These proteins are believed to be important mediators of cell death. These mediators, however, do not always instruct a cell to die. In some cases, these mediators actually instruct a cell to undergo cell division. The intracellular environment, and particularly the status of the proton motor force and the source of fuel for mitochondrial metabolism, determines whether stimulation of the cell death protein will lead to a signal for death or cell division (see, e.g., U.S. Patent Application Serial No. 09/277,575, incorporated herein by reference).

[0012] These findings have important implication in the present invention in dealing with drug resistant tumors. Every year at least 6.2 million people die worldwide from cancer [1]. Many cancer patients will be treated by chemotherapy. For some people this treatment will be effective, but in many cases chemotherapy is not successful, in part because of the development of drug resistance. It is commonly observed in treating cancers, that initial treatments, such as with chemotherapy and/or radiation therapy, are effective to destroy significant numbers of tumor cells, only to leave behind a small number of tumor cells that are resistant to the treatment, which then multiply to form newly detected tumors that are increasingly resistant to treatment as new rounds of therapy are tried. The growing popularity of “cocktails” of chemotherapy drugs has given rise to multidrug resistant (“MDR”) tumor cells, which are ever more difficult to destroy. Drug sensitive tumor cells, under the selective pressure of treatment with drugs, develop into drug resistant versions of the same tumor cell type. It is the drug resistant cells that take over, and with each round of chemotherapy the proportion of drug resistant cells to drug sensitive cells increases, to the point where recovery becomes more and more difficult, and eventually the cancer becomes untreatable. Indeed, drug resistance, either acquired or inherent, is the leading cause of death in cancer [27]. Mechanisms which have been suggested to account for drug resistance include over-expression of a multi-drug resistance transporter (pgp -1) [27], failure to express death inducing receptors [27, 28], and a metabolic strategy that may provide protection from a variety of stresses. Because drug resistance is such an important problem, one of the goals of the present invention is to provide methods to overcome this problem. Methods

for dealing with MDR tumor cells have been proposed, but without practical, clear clinical success at entirely eliminating such cells and providing a cure for patients with MDR tumors. For example, in Lampdis and Priebe U.S. Patent No. 6,670,330, entitled: "Cancer Chemotherapy with 2-Deoxy-D-Glucose", incorporated herein in its entirety by reference, a class of glycolytic inhibitors are described for use in combination with standard chemotherapy protocols in treating solid tumors by attacking anaerobic cells at the center of the tumor.

SUMMARY OF INVENTION

[0013] The invention generally relates to systems and methods for treating inflammatory and proliferative diseases, and wounds, using as a pharmacological agent a fatty acid metabolism inhibitor, a glycolytic inhibitor, and/or an agent able to alter cellular production of reactive oxygen, or combination thereof, optionally in combination with one or more chemotherapeutic agents. In preferred embodiments, the invention combines an oxirane carboxylic acid, represented by etomoxir, with a 2-deoxyglucose compound, represented by 2-deoxy-D-glucose.

[0014] The subject matter of this invention involves, in some cases, interrelated products, alternative solutions to a particular problem, and/or a plurality of different uses of one or more systems and/or articles. In contrast to therapies that concentrated primarily on activating the immune system, the present invention can use chemotherapeutics in concert with the immune system and derives some of its effectiveness from the immune response. Tuning the immune response enables an increase in effectiveness of cancer therapy. In the present invention, cellular metabolism plays a dominating role in controlling the intercellular communication between a cancer cell and a T cell and thereby plays a dominant role in T cell activation. In particular there is a significant correlation between cellular metabolism and the expression of cell-surface Fas, one of the key co-stimulatory molecules. Fas is expressed on most rapidly dividing and self-renewing cells, including a wide variety of tumor cells [19]. While Fas is widely known as a "death receptor" and can induce programmed cell death when engaged by Fas Ligand (FasL, CD95L) or anti-Fas antibodies [20, 21], paradoxically, Fas engagement can also result in accelerated proliferation of T lymphocytes and some tumor cells [22, 23].

[0015] As an example of the connection between metabolism and Fas expression, it has been shown that cell surface Fas expression is upregulated in response to increasing glucose

concentrations *in vitro*, in transformed cell lines and in freshly isolated cells from a variety of tissue origins [19]. Obviously glucose is a key source of fuel and the availability of glucose drives metabolic activity. A further example of the connection between cellular metabolism and Fas is found in the work of Bhushan et al [2] who showed that drug resistant tumor cells fail to express cell surface Fas and the work of Harper et al [24] who showed that drug resistant tumor cells also demonstrated a unique metabolic strategy, quite different from drug-sensitive tumor cells.

[0016] In one set of embodiments of the invention, a cell is exposed to a pharmakon of the invention. In another set of embodiments, an immunity profile of a tumor cell is altered by exposing the tumor cell to a pharmakon of the invention. In another set of embodiments, the method includes an act of administering a pharmakon of the invention to a subject susceptible to or exhibiting symptoms of drug-resistant cancer, in some cases where the subject is not otherwise indicated for treatment with the pharmakon. In another set of embodiments, the method includes an act of administering a pharmakon of the invention to a subject susceptible to or exhibiting symptoms of cancer, in some cases, where the subject is not otherwise indicated for treatment with the compound.

[0017] The method, in yet another set of embodiments, includes an act of administering, to a subject susceptible to or exhibiting symptoms of cancer, a therapeutically acceptable amount of a composition comprising an inhibitor of a fatty acid reductase or fatty acid isomerase. The subject, in some cases, may not otherwise be indicated for treatment with the inhibitor of the fatty acid reductase or fatty acid isomerase.

[0018] According to another set of embodiments, the method includes an act of administering, to a wound in a subject, a therapeutically acceptable amount of a pharmakon of the invention that includes an agent able to alter production of reactive oxygen in cells located within or proximate the wound.

[0019] The method, in yet another set of embodiments, includes acts of surgically removing a tumor from a subject; and inserting a pharmakon of the invention into the subject. In another set of embodiments, the invention provides an act of inserting a pharmakon of the invention into or proximate a tumor. The invention, in still another set of embodiments, includes acts of removing cells from a tumor, exposing the cells to a pharmakon of the invention, and inserting the cells into a subject. In one set of embodiments, the method includes an act of

altering an immunity profile of a tumor cell by exposing the tumor cell to a pharmacon of the invention. In another set of embodiments, the method includes an act of administering a pharmacon of the invention to a subject having or at risk of developing an autoimmune disease.

[0020] In another aspect, the invention provides a composition that includes a cell exposed to a pharmacon of the invention. In yet another set of embodiments, the composition includes a pharmacon of the invention and a pharmaceutically acceptable carrier.

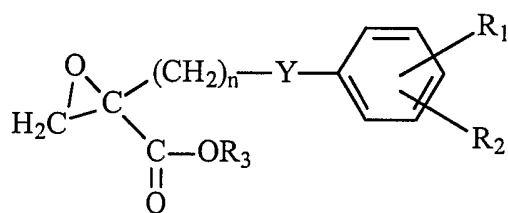
[0021] In yet another aspect, the invention provides an article that includes a substrate comprising cells exposed to an agent able to alter production of reactive oxygen in a cell. In another set of embodiments, the article includes isolated tumor cells exposed to a pharmacon of the invention.

[0022] The invention, in still another aspect, provides a kit. The kit, according to one set of embodiments, includes a pharmacon of the invention.

[0023] In another aspect, the present invention is directed to a method of making one or more of the embodiments described herein. In yet another aspect, the present invention is directed to a method of using one or more of the embodiments described herein. In still another aspect, the present invention is directed to a method of promoting one or more of the embodiments described herein.

[0024] In particular embodiments, the pharmacon of the invention comprises a therapeutically acceptable amount of an oxirane carboxylic acid compound capable of inhibiting fatty acid metabolism, or a pharmacologically acceptable salt thereof. The method is useful for treating cancer that has become drug resistant including cancer that is multi-drug resistant. It has been found that drug resistant cancer cells derive a majority of their metabolic energy through fatty acid metabolism, which is inhibited by the fatty acid metabolism inhibitors used in the methods of this invention, and which are able to bind to a mitochondrial fatty acid transporter to inhibit beta-oxidation. Preferred fatty acid metabolism inhibitors are exemplified by oxirane carboxylic acid compounds..

[0025] The oxirane carboxylic acid compound preferably has the formula:

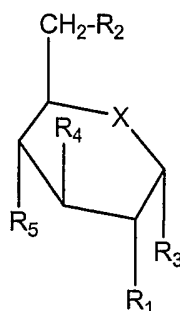


wherein: R_1 represents a hydrogen atom, a halogen atom, a 1 – 4C alkyl group, a 1 – 4C alkoxy group, a nitro group or a trifluoromethyl group; R_2 has one of the meanings of R_1 ; R_3 represents a hydrogen atom or a 1 – 4C alkyl group; Y represents the grouping $—O—(CH_2)_m—$; m is 0 or a whole number from 1 to 4; and n is a whole number from 2 to 8 wherein the sum of m and n is a whole number from 2 to 8.

[0026] The oxirane carboxylic acid compound is preferably etomoxir, which is 2-(6-(4-chlorophenoxy)-hexyl)-oxirane-2-carboxylic acid ethyl ester, and a preferred method of treatment for drug resistant cancers comprises administering to a subject susceptible to or exhibiting symptoms of drug-resistant cancer, a therapeutically acceptable amount of etomoxir or a pharmacologically acceptable salt of etomoxir.

[0027] Other examples of fatty acid metabolism inhibitors that can be used in the invention include oxfenicine, methyl palmoxirate, metoprolol, amiodarone, perhexiline, aminocarnitine, hydrazonopropionic acid, 4-bromocrotonic acid, trimetazidine, ranolazine, hypoglycin, dichloroacetate, methylene cyclopropyl acetic acid, or beta-hydroxy butyrate.

[0028] In other particular embodiments, the pharmacon of the invention comprises a therapeutically acceptable amount of a glycolytic inhibitor. Preferred glycolytic inhibitors are 2-deoxyglucose compounds, defined herein as homologs, analogs, and/or derivatives of 2-deoxy-D-glucose. Glycolytic inhibitors particularly useful herein can have the formula:



wherein: X represents an O or S atom; R_1 represents a hydrogen atom or a halogen atom; R_2 represents a hydroxyl group, a halogen atom, a thiol group, or $CO-R_6$; and R_3 , R_4 , and R_5 each represent a hydroxyl group, a halogen atom, or $CO-R_6$ wherein R_6 represents an alkyl group of from 1 to 20 carbon atoms, and wherein at least two of R_3 , R_4 , and R_5 are hydroxyl groups. The halogen atom is as described above with respect to the oxirane carboxylic acid compounds, and in R_2 , R_3 , R_4 , and R_5 . The halogen atom is preferably F, and R_6 is preferably a $C_3 - C_{15}$ alkyl group. A preferred glycolytic inhibitor is 2-deoxy-D-glucose

[0029] In other particular embodiments, an anti-cancer agent, such as methotrexate, trimetrexate, adriamycin, or taxotere, can also administered to the subject, preferably as part of the same treatment regimen. Similarly, immunotherapies, such as tumor cell vaccines, and biotherapies such as cytokine therapies, are useful for stimulating a specific immune response against a cancer antigen. The procedure can be applied along with or after radiation treatment, and after surgically removing a tumor from the subject.

[0030] Other advantages and novel features of the present invention will become apparent from the following detailed description of various non-limiting embodiments of the invention when considered in conjunction with the accompanying figures. In cases where the present specification and a document incorporated by reference include conflicting and/or inconsistent disclosure, the present specification shall control.

BRIEF DESCRIPTION OF DRAWINGS

[0031] Figures. 1 illustrates certain compounds for use with the invention;

[0032] Figures 2A-2D illustrates flow cytometry data, illustrating the treatment of cancer cells according to one embodiment of the invention;

[0033] Figures 3A-3D illustrates flow cytometry data, illustrating the treatment of cancer cells according to another embodiment of the invention;

[0034] Figures 4A-4F illustrates, *in vitro*, the treatment of cancer cells according to one embodiment of the invention;

[0035] Figures 5A-5D illustrates, *in vivo*, the treatment of cancer cells according to another embodiment of the invention;

[0036] Figure 6A is a plot showing levels of cell-surface Fas expression on drug sensitive L1210 cells as measured by flow cytometry;

[0037] Figure 6B is a plot showing levels of cell-surface Fas expression on drug resistant L12100/DDP cells as measured by flow cytometry;

[0038] Figure 7 is a chart showing the levels of cell surface Fas expression on L1210 and L1210 DDP cells after 23 hours of incubation with etomoxir;

[0039] Figure 8 is a chart showing the levels of DCFDA staining on L1210 DDP cells treated (or not) with methotrexate, etomoxir, or both, after 24 hours of incubation;

[0040] Figure 9 shows scatter diagrams for drug sensitive and drug resistant tumor cells in which change in pH across the inner mitochondrial membrane is plotted against mitochondrial membrane potential;

[0041] Figure 10 is a plot showing the rates of oleate oxidation with glucose competition;

[0042] Figure 11 is a chart showing the levels of mitochondrial membrane potential (by MitoTracker) of L1210 DDP cells treated (or not) with methotrexate, etomoxir, or both, after 24 hours of incubation;

[0043] Figure 12A is a chart showing the levels of DCFDA staining on L1210 cells treated (or not) with methotrexate, etomoxir, or both, after 24 hours of incubation;

[0044] Figure 12B is a chart showing the levels of mitochondrial membrane potential (by MitoTracker) of L1210 cells treated (or not) with methotrexate, etomoxir, or both, after 24 hours of incubation;

[0045] Figure 13 is a chart showing the levels of mitochondrial membrane potential (by MitoTracker) of RU937 human leukemia cells treated (or not) with methotrexate, etomoxir, or both, after 24 hours of incubation;

[0046] Figure 14 is a chart showing the levels of DCFDA staining on HL60 human leukemia cells treated (or not) with methotrexate or etomoxir after 24 hours of incubation.

[0047] Figure 15 is a dot plot as a function of live versus dead B16F1 melanoma cells untreated or treated with etomoxir at the indicated concentrations for 48 hours;

[0048] Figure 16 shows the percent death of B16F1 melanoma cells after 48 hours of treatment with indicated concentrations of etomoxir;

[0049] Figure 17 shows cell surface Fas (CD95) expression on live B16F1 melanoma cells after 48 hours in etomoxir;

[0050] Figure 18 shows cell surface Fas (CD95) expression on dead B16F1 melanoma cells after 48 hours in etomoxir;

[0051] Figure 19 shows intracellular reactive intermediates in B16F1 melanoma cells untreated or treated with etomoxir at the indicated concentrations for 24 hours;

[0052] Figure 20 shows mitochondrial membrane potential in B16F1 melanoma cells untreated or treated with etomoxir at the indicated concentrations for 24 hours;

[0053] Figure 21 shows the effects of etomoxir and/or taxol on tumor size in *in vivo* tumor transplantation and treatment with etomoxir and taxol;

[0054] Figure 22. shows percent death after 72 hours in etomoxir with or without 24 hours of adriamycin treatment on the human leukemic cell HL60;

[0055] Figure 23 shows percent death after 72 hours in etomoxir with or without 24 hours of taxotere treatment on the multidrug resistant human leukemic cell HL60MDR;

[0056] Figure 24 is a dot plot as a function of live versus dead A204 human rhabdomyosarcoma cells untreated or treated with etomoxir at the indicated concentrations for 24, 48, & 72 hours;

[0057] Figure 25 shows the “fold” increases in death of A204 human rhabdomyosarcoma cells after 24, 48, & 72 hours of treatment with indicated concentrations of etomoxir;

[0058] Figure 26 shows the “fold” increases in cell surface UCP2 expression on A-204 human rhabdomyosarcoma cells after 24, 48, & 72 hours in etomoxir.

[0059] Figure 27 is a dot plot as a function of live versus dead multidrug resistant human leukemia cell HL60 MDR untreated or treated with etomoxir, 2-deoxy-D-glucose, or both, at the indicated concentrations for 24 hours;

[0060] Figure 28 shows the percent death of HL60 MDR cells untreated or treated for 24 hours with etomoxir, 2-deoxy-D-glucose, or both, at the indicated concentrations;

[0061] Figure 29 is a dot plot as a function of live versus dead RD cells untreated or treated with etomoxir, 2-deoxy-D-glucose, or both, at the indicated concentrations for 24 hours;

[0062] Figure 30 shows the percent death of RD cells untreated or treated for 24 hours with etomoxir, 2-deoxy-D-glucose, or both, at the indicated concentrations;

[0063] Figure 31 is a dot plot as a function of live versus dead HL60 MDR cells untreated or treated with etomoxir, 2-deoxy-D-glucose, or both, at the indicated concentrations for 48 hours;

[0064] Figure 32 shows the percent death of HL60 MDR cells untreated or treated for 48 hours with etomoxir, 2-deoxy-D-glucose, or both, at the indicated concentrations;

[0065] Figure 33 is a dot plot as a function of live versus dead RD cells untreated or treated with etomoxir, 2-deoxy-D-glucose, or both, at the indicated concentrations for 48 hours;

[0066] Figure 34 shows the percent death of RD cells untreated or treated for 48 hours with etomoxir, 2-deoxy-D-glucose, or both, at the indicated concentrations; and

[0067] Figure 35 shows tumor volume of an HL60 MDR tumor implanted in nude mice, treated etomoxir, 2-deoxy-D-glucose, or both, at the indicated days after tumor implantation.

DETAILED DESCRIPTION

[0068] The present invention proceeds by recognizing that cells have available to them a number of different metabolic pathways that are brought into play depending on the nature and degree of stress applied to the cells, that cell apoptosis is brought about to a significant extent because the target cells are recognized by the immune system, and that MDR cells are to a significant extent invisible to the immune system. The invention targets cellular metabolic pathways of defective cells, tissues or organs, and the immune system to treat human inflammatory and proliferative diseases, such as cancer, autoimmunity, heart disease, and chronic infectious disease. The methods herein are also useful in tissue regeneration, including neural regeneration, transplantation, and wound healing.

[0069] Every cell in the body uses carbohydrates, protein, and fat in different proportions for energy. The cell's choice of fuel, its metabolic strategy, will change depending on its state of activation or differentiation. For example, a cell that is rapidly dividing has different energy demands than one that is not dividing. The same is true for cells that are under stress or are infected. The present invention proceeds from the discovery of a unique metabolic strategy, widely used by drug resistant cells, that is characterized by the ability to burn fat under conditions of stress, including the stress of chemotherapy or radiation. When cells are rapidly dividing, they use glucose at very high rates, but under conditions of stress, cells, if capable, use fat in a greater proportion as a protective strategy. Respiration, oxygen use, and external stresses can generate a variety of toxic by-products (including free radicals) that can cause damage to cells. Tumor cells upregulate proteins that allow them to burn fat as a protective strategy against such by-products. The immune system can monitor the metabolic state of individual cells and destroy those in an inappropriate state. However, tumor cells can survive this surveillance by changing their metabolic strategy to one that protects the tumor cell by causing the cell to be invisible to the immune system.

[0070] UCPs are often expressed in the plasma membrane of rapidly dividing cells. According to certain embodiments of this invention, by manipulating UCP expression, inhibition of cellular, metabolic, and/or immunological responses may occur, for example, which may be useful in the treatment of cancers, wounds, or the like. Additionally, cells in cancers or wounds often derive a majority of their metabolic energy through fatty acid metabolism. Thus, the cells may be manipulated to increase the amount of UCP in the plasma membrane (and/or decrease mitochondrial UCP), for example, by exposure of the cells to a fatty acid metabolism inhibitor and/or an agent able to alter cellular production of reactive oxygen, singularly or in combination. Additionally, altered production of reactive oxygen may be associated with changes in cellular immunogenic profiles and thus, in some embodiments, reactive oxygen production may be altered to stimulate or inhibit cellular and/or immunological responses, for example, to cancer cells or wounds. In some cases, the cells may be manipulated to increase the amount of Fas expressed on the cell surface. The ability to manipulate the plasma membrane potential of a cell thus may provide the ability to control or at least influence the fate of the cell. When the plasma membrane potential of a cell is increased in a cell, by causing an increase in the expression of UCP in the plasma membrane, the cell may be able to respond by rapid cell division or cell death, depending on the signal. In certain instances, the systems and methods of the invention can be used in conjunction with conventional therapies.

[0071] An increase in UCP levels in the plasma membrane may be related to an increased dependence on UCP for energy production or regulation by the cell. Thus, various systems and methods of the invention provide for the ability to control UCP levels within the cell. As an example, the amount of UCP in the plasma membrane of the cells may be increased by exposing the cells to a composition including a fatty acid metabolism inhibitor, and/or by delivering a nucleic acid to a cell such that the expression of UCP in the plasma membrane is increased, as further described herein.

[0072] The present invention generally relates to systems and methods for treating inflammatory or proliferative diseases such as cancers, immunological conditions, autoimmune diseases, and treating wounds. Various aspects of the invention involve the treatment of cells with fatty acid metabolism inhibitors, glycolytic inhibitors, and/or agents able to alter cellular production of reactive oxygen. The manipulation of the cells may be performed simultaneously or sequentially, in any order. The cells may be manipulated under any suitable condition, i.e., *in*

vivo, *ex vivo*, *in vitro*, etc. By inhibiting fatty acid metabolism, the cell is forced to resume glucose metabolism, thus exhibiting UCP and/or Fas on its cell surface to become visible to the immune system. Thus the invention uses a pharmakon of this invention that targets one or both of the predominant metabolic pathways: a fatty acid metabolism inhibitor and/or a glycolytic inhibitor, and/or an agent able to alter cellular production of reactive oxygen. As a result of inhibition of fatty acid metabolism, UCP and/or Fas is exposed on the cell surface so that the cell becomes susceptible to attack by an antibody. In a preferred embodiment, the fatty acid metabolism inhibitor is an oxirane carboxylic acid, exemplified by etomoxir, and the glycolytic inhibitor is a 2-deoxyglucose compound, exemplified by 2-deoxy-D-glucose.

[0073] The following applications are incorporated by reference. U.S. Provisional Patent Application Serial No. 60/477,873 filed June 12, 2003, entitled "Systems and Methods for Treating Cancers and Wounds," by M. Karen Newell, *et al.*; U.S. Provisional Patent Application Serial No. 60/478,646 filed June 12, 2003, entitled "Systems and Methods for Treating Cancers and Wounds," by Martha Karen Newell Rogers, *et al.*; U.S. Provisional Patent Application Serial No. 60/490,587, filed July 28, 2003, entitled "Systems and Methods for Treating Cancers and Wounds With Oxirane Carboxylic Acids," by M. Karen Newell, *et al.*; U.S. Patent Application Serial No. 10/272,432, filed October 15, 2002, entitled "Methods for Regulating Co-Stimulatory Molecule Expression with Reactive Oxygen," by M. K. Newell, *et al.*, published as 2004/0005291 on January 8, 2004; and International Patent Application No. PCT/US00/17245, filed June 22, 2000, entitled "Methods and Products for Manipulating Uncoupling Protein Expression," by M. K. Newell, *et al.*, published as WO 00/78941 on December 28, 2000.

Fatty Acid Metabolism Inhibitors

[0074] According to one set of embodiments, the cells are exposed to a fatty acid metabolism inhibitor. A "fatty acid metabolism inhibitor," as used herein, is a compound able to inhibit (e.g., prevent, or at least decrease or inhibit the activity by an order of magnitude or more) a reaction within the fatty acid metabolism pathway, such as an enzyme-catalyzed reaction within the pathway. The inhibitor may inhibit the enzyme, e.g., by binding to the enzyme or otherwise interfering with operation of the enzyme (for example, by blocking an active site or a docking site, altering the configuration of the enzyme, competing with an enzyme substrate for the active site of an enzyme, etc.), and/or by reacting with a coenzyme, cofactor, etc. necessary

for the enzyme to react with a substrate. The fatty acid metabolism pathway is the pathway by which fatty acids are metabolized within a cell for energy (e.g., through the synthesis of ATP and the breakdown of fatty acids into simpler structures, such as CO₂, acyl groups, etc.).

[0075] The fatty acid metabolism pathway includes several enzymatic reactions, which uses various enzymes such as reductases or isomerases. Specific examples of enzymes within the fatty acid metabolism pathway include 2,4-dienoyl-CoA reductase, 2,4-dienoyl-CoA isomerase, butyryl dehydrogenase, etc, as further discussed below. In one embodiment, the fatty acid metabolism inhibitor is an inhibitor able to inhibit a beta-oxidation reaction in the fatty acid metabolism pathway. In another embodiment, the inhibitor is an inhibitor for a fatty acid transporter (e.g., a transporter that transports fatty acids into the cell, or from the cytoplasm into the mitochondria for metabolism). In yet another embodiment, the inhibitor may react or otherwise inhibit key steps within the fatty acid metabolism pathway. In still another embodiment, the inhibitor may be an inhibitor of fatty acids as a source of energy in the mitochondria. For example, the inhibitor may inhibit the breakdown of intermediates such as butyryl CoA, glutaryl CoA, or isovaleryl CoA.

[0076] 2,4-dienoyl-CoA reductase is an enzyme within the fatty acid metabolism pathway that catalyzes reduction reactions involved in the metabolism of polyunsaturated fatty acids. Certain fatty acids are substrates for 2,4-dienoyl-CoA reductases located within the mitochondria. In some cases, fatty acids may be transported into the mitochondria through uncoupling proteins. The uncoupling protein may, in certain instances, increase the mitochondrial metabolism to increase the availability of fatty acids within the mitochondria and/or increase the throughput of beta-oxidation within the mitochondria.

[0077] The enzyme 2,4-dienoyl-CoA isomerase is an enzyme within the fatty acid metabolism pathway that catalyzes isomerization of certain fatty acids. One step in the metabolism of certain polyunsaturated fatty acids may be protective against reactive oxygen intermediates ("ROI"). Thus, by generating substrates and antagonists for the activity of 2,4-dienoyl-CoA isomerase, the metabolic production of reactive oxygen intermediates may be enhanced and/or reduced. This, in turn, may affect certain disease states, such as cancer.

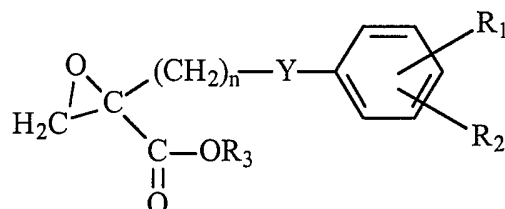
[0078] Thus, it is to be understood that, as used herein, compounds useful for inhibiting fatty acid metabolism (i.e., "fatty acid metabolism inhibitors") are also useful for altering cellular production of reactive oxygen; compounds described in reference to fatty acid

metabolism inhibition should also be understood herein to be able to alter reactive oxygen production within a cell. For example, by altering the ability of a cell to metabolize a fatty acid, the ability of the cell to produce reactive oxygen may also be affected, since one pathway for a cell to produce reactive oxygen intermediates is through the metabolism of fatty acids.

Alteration of the production of reactive oxygen in a cell may be associated with changes in the immune profile of cells, i.e., how immune cells respond to the cell. Thus, in some cases, the production of reactive oxygen can be affected by exposing a cell to, or removing a cell from, a fatty acid metabolism inhibitor. The alteration of the production of reactive oxygen may be useful in treating conditions such as cancers or wounds (as further discussed below), as the alteration of the immune profile of cells within the cancer site or the wound may stimulate the immune system and/or other wound-healing processes.

[0079] In a preferred embodiment of the invention, the fatty acid inhibitor is an oxirane carboxylic acid compound. In accordance with a discovery of this invention, such compounds, exemplified by etomoxir, are able to alter cellular production of reactive oxygen. Preferred oxirane carboxylic acid compounds have the formula:

:



wherein: R_1 represents a hydrogen atom, a halogen atom, a 1 – 4C alkyl group, a 1 – 4C alkoxy group, a nitro group or a trifluoromethyl group; R_2 has one of the meanings of R_1 ; R_3 represents a hydrogen atom or a 1 – 4C alkyl group; Y represents the grouping $—O—(CH_2)_m—$; m is 0 or a whole number from 1 to 4; and n is a whole number from 2 to 8 wherein the sum of m and n is a whole number from 2 to 8. More preferred are oxirane carboxylic acid compounds wherein R_1 is a halogen atom, R_2 is a hydrogen atom, m is 0, and n is 6, and more particularly where R_3 is an ethyl group.

[0080] It is most particularly preferred to use etomoxir, i.e., 2-(6-(4-chlorophenoxy)-hexyl)-oxirane-2-carboxylic acid ethyl ester. Examples of other oxirane carboxylic acid compounds useful in the invention are 2-(4-(3-chlorophenoxy)-butyl)-oxirane-2-carboxylic acid ethyl ester, 2-(4-(3-trifluoromethylphenoxy)-butyl)-oxirane-2-carboxylic acid ethyl ester, 2-(5-

(4-chlorophenoxy)-pentyl)-oxirane-2-carboxylic acid ethyl ester, 2-(6-(3,4-dichlorophenoxy)-hexyl)-oxirane-2-carboxylic acid ethyl ester, 2-(6-(4-fluorophenoxy)-hexyl)-oxirane-2-carboxylic acid ethyl ester, and 2-(6-phenoxyhexyl)-oxirane-2-carboxylic acid ethyl ester, the corresponding oxirane carboxylic acids, and their pharmacologically acceptable salts.

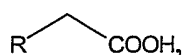
[0081] The foregoing class of oxirane carboxylic acid compounds, including etomoxir, has been described by Horst Wolf and Klaus Eistetter in United States Patent 4,946,866 for the prevention and treatment of illnesses associated with increased cholesterol and/or triglyceride concentration, and by Horst Wolf in United States Patent 5,739,159 for treating heart insufficiency. The preparation of oxirane carboxylic acid compounds, and their use for blood glucose lowering effects as an antidiabetic agent, is described in Jew et al United States Patent 6,013,666. Etomoxir has been described as an inhibitor of mitochondrial carnitine palmitoyl transferase-I by Mannaerts, G. P., L. J. Debeer, J. Thomas, and P. J. De Schepper. "Mitochondrial and peroxisomal fatty acid oxidation in liver homogenates and isolated hepatocytes from control and clofibrate-treated rats," J. Biol. Chem. 254:4585-4595, 1979. United States Patent Application 20030036199 by Bamdad et al, entitled: "Diagnostic tumor markers, drug screening for tumorigenesis inhibition, and compositions and methods for treatment of cancer", published February 20, 2003, describes treating a subject having a cancer characterized by the aberrant expression of MUC1, comprising administering to the subject etomoxir in an amount effective to reduce tumor growth. In a preferred aspect of this embodiment, subjects for whom the methods of the invention involving treatment with etomoxir are not intended are those diagnosed with diseases which already call for treatment with etomoxir, particularly those subjects who have MUC1-dependant tumors, nor those diagnosed with diabetes, or diseases associated with increased cholesterol and/or triglyceride concentration, or chronic heart failure (e.g., failing cardiac hypertrophy associated with an inadequate sarcoplasmic reticulum function) calling for treatment with etomoxir.

[0082] The foregoing United States Patents 4,946,866, 5,739,159, and 6,013,666, United States Patent Application 20030036199, and the foregoing publication by Mannaerts, G. P., L. J. Debeer, J. Thomas, and P. J. De Schepper, are incorporated herein by reference. In addition, U.S. Patent Application Serial No. 10/272,432, filed October 15, 2002, entitled "Methods for Regulating Co-Stimulatory Molecule Expression with Reactive Oxygen," by M. K. Newell, *et al.* is incorporated herein by reference in its entirety.

[0083] In a specific embodiment, the method includes the step of administering, to a subject susceptible to or exhibiting symptoms of cancer, preferably a subject susceptible to or exhibiting symptoms of drug-resistant cancer, a therapeutically acceptable amount of the oxirane carboxylic acid compound, exemplified by etomoxir. A "subject," as used herein, means a human or non-human mammal, the latter including, but not limited to, a dog, cat, horse, cow, pig, sheep, goat, chicken, primate, rat, and mouse.

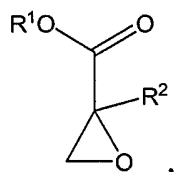
[0084] Other, non-limiting examples of fatty acid metabolism inhibitors include fatty acid transporter inhibitors, beta-oxidation process inhibitors, reductase inhibitors, and/or isomerase inhibitors within the fatty acid metabolism pathway. Specific examples of other fatty acid metabolism inhibitors include, but are not limited to, cerulenin, 5-(tetradecyloxy)-2-furoic acid, oxfenicine, methyl palmoxirate, metoprolol, amiodarone, perhexiline, aminocarnitine, hydrazonopropionic acid, 4-bromocrotonic acid, trimetazidine, ranolazine, hypoglycin, dichloroacetate, methylene cyclopropyl acetic acid, and beta-hydroxy butyrate. Structural formulas for these inhibitors are shown in Figs. 1A-1C. As a another example, the inhibitor may be a non-hydrolyzable analog of carnitine.

[0085] In one embodiment, the fatty acid metabolism inhibitor is a carboxylic acid. In some cases, the carboxylic acid may have the structure:

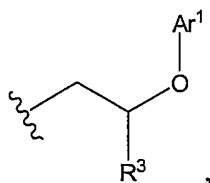


where R comprises an organic moiety, as further described below. In some cases, R may include at least two nitrogen atoms, or R may include an aromatic moiety (as further described below), such as a benzene ring, a furan, etc.

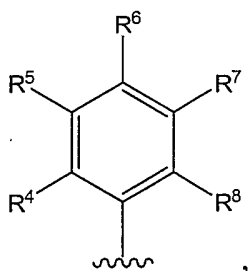
[0086] In another embodiment, the fatty acid metabolism inhibitor has the structure:



where each of R^1 and R^2 independently comprises organic moiety. In some instances, either or both of R^1 and R^2 may independently be an alkyl, such as a straight-chain alkyl, for instance, methyl, ethyl, propyl, etc. In certain cases, R^2 may have at least 5 carbon atoms, at least 10 carbon atoms, or at least 15 or more carbon atoms. For example, in one embodiment, R^2 may be a tetradecyl moiety. In other cases, R^2 may include an aromatic moiety, for example, a benzene ring. In still other cases, R^2 may have the structure:

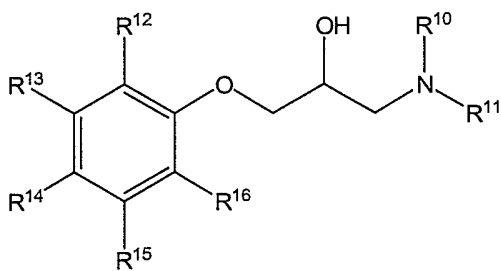


where R^3 comprises an organic moiety and Ar^1 comprises an aromatic moiety. R^3 may be an alkyl, such as a straight-chain alkyl. In some instances, Ar^1 may be a benzene ring or a derivative thereof, i.e., having the structure:

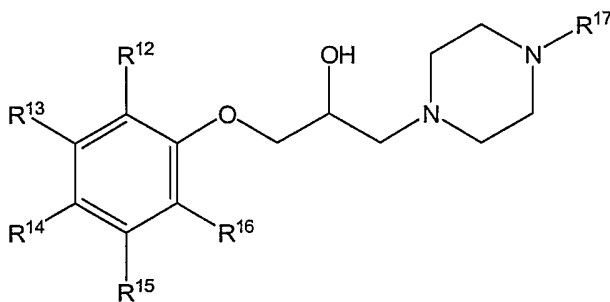


wherein each of R^4 , R^5 , R^6 , R^7 , and R^8 is hydrogen, a halogen, an alkyl, an alkoxy, etc.

[0087] In yet another embodiment, the fatty acid metabolism inhibitor has the structure:

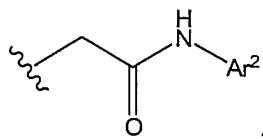


where each of R^{10} , R^{11} , R^{12} , R^{13} , R^{14} , R^{15} and R^{16} independently comprises hydrogen, a halogen, or an organic moiety, such as an alkyl, an alkoxy, etc. In some cases, R^{10} and R^{11} together may define an organic moiety, such as a cyclic group. For example, the fatty acid metabolism inhibitor may have the structure:



wherein R^{17} comprises an organic moiety, such as an alkyl, an alkoxy, an aromatic moiety, an

amide, etc. An example, of R¹⁷ is:



wherein Ar² comprises an aromatic moiety, such as a benzene ring or a benzene derivative, as previously described.

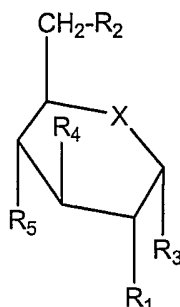
[0088] In still another embodiment, the fatty acid metabolism inhibitor includes a dominant negative plasma membrane polypeptide. The end result of the use (e.g., expression) of a dominant negative polypeptide in a cell may be a reduction in functional enzymes present within the fatty acid metabolism pathway. One of ordinary skill in the art can assess the potential for a dominant negative variant of a protein or enzyme, and use standard mutagenesis techniques to create one or more dominant negative variant polypeptides. For example, one of ordinary skill in the art can modify the sequence of an enzyme coding region by site-specific mutagenesis, scanning mutagenesis, partial gene deletion or truncation, and the like. See, e.g., U.S. Patent No. 5,580,723 and Sambrook, *et al.*, *Molecular Cloning: A Laboratory Manual*, Second Edition, Cold Spring Harbor Laboratory Press, 1989. One of ordinary skill in the art then can test the population of mutagenized polypeptides for diminution in a selected and/or for retention of such activity of the protein or enzyme. Other similar methods for creating and testing dominant negative variants of a protein will be apparent to one of ordinary skill in the art.

[0089] In another set of embodiments, the cells may be exposed to an agent that inhibits the synthesis or production of one or enzymes within the fatty acid metabolism pathway. Exposure of the cells to the agent thus inhibits fatty acid metabolism within the cell. For example, in one embodiment, an antisense oligonucleotide may be used that selectively binds to regions encoding enzymes present within the fatty acid metabolism pathway, such as 2,4-dienoyl-CoA reductase or 2,4-dienoyl-CoA isomerase. Antisense oligonucleotides are discussed in more detail below.

Glycolytic inhibitor

[0090] Preferred glycolytic inhibitors are 2-deoxyglucose compounds, defined herein as 2-deoxy-D-glucos, and homologs, analogs, and/or derivatives of 2-deoxy-D-glucose. While the levo form is not prevalent, and 2-deoxy-D-glucose is preferred, the term "2-deoxyglucose" is

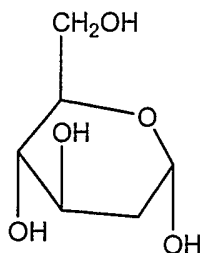
intended to cover *inter alia* either 2-deoxy-D-glucose and 2-deoxy-L-glucose, or a mixture thereof. In general glycolytic inhibitors can have the formula:



wherein: X represents an O or S atom; R_1 represents a hydrogen atom or a halogen atom; R_2 represents a hydroxyl group, a halogen atom, a thiol group, or CO-R_6 ; and R_3 , R_4 , and R_5 each represent a hydroxyl group, a halogen atom, or CO-R_6 wherein R_6 represents an alkyl group of from 1 to 20 carbon atoms, and wherein at least two of R_3 , R_4 , and R_5 are hydroxyl groups. The halogen atom is as described above with respect to the oxirane carboxylic acid compounds, and in R_2 , R_3 , R_4 , and R_5 . The halogen atom is preferably F, and R_6 is preferably a $\text{C}_3\text{--C}_{15}$ alkyl group.

[0091] Examples of 2-deoxyglucose compounds useful in the invention are: 2-deoxy-D-glucose, 2-deoxy-L-glucose; 2-bromo-D-glucose, 2-fluoro-D-glucose, 2-iodo-D-glucose, 6-fluoro-D-glucose, 6-thio-D-glucose, 7-glucosyl fluoride, 3-fluoro-D-glucose, 4-fluoro-D-glucose, 1-O-propyl ester of 2-deoxy-D-glucose, 1-O-tridecyl ester of 2-deoxy-D-glucose, 1-O-pentadecyl ester of 2-deoxy-D-glucose, 3-O-propyl ester of 2-deoxy-D-glucose, 3-O-tridecyl ester of 2-deoxy-D-glucose, 3-O-pentadecyl ester of 2-deoxy-D-glucose, 4-O-propyl ester of 2-deoxy-D-glucose, 4-O-tridecyl ester of 2-deoxy-D-glucose, 4-O-pentadecyl ester of 2-deoxy-D-glucose, 6-O-propyl ester of 2-deoxy-D-glucose, 6-O-tridecyl ester of 2-deoxy-D-glucose, 6-O-pentadecyl ester of 2-deoxy-D-glucose, and 5-thio-D-glucose, and mixtures thereof.

[0092] A preferred glycolytic inhibitor is 2-deoxy-D-glucose, which has the structure:



General Considerations

[0093] One or more of the fatty acid metabolism inhibitors described herein may be an isolated molecule in certain cases. An "isolated molecule," as used herein, is a molecule that is substantially pure and is free of other substances with which it is ordinarily found in nature or *in vivo* systems to an extent practical and appropriate for its intended use. In particular, the molecular species may be sufficiently pure and may be sufficiently free from other biological constituents of host cells so as to be useful in, for example, producing pharmaceutical preparations, or for sequencing, e.g., if the molecular species is a nucleic acid, peptide, or polysaccharide. Because an isolated molecular species of the invention may be admixed with a pharmaceutically-acceptable carrier in a pharmaceutical preparation, and/or other physiologically-active agents, the molecular species may comprise only a small percentage by weight of the preparation. The molecular species is nonetheless substantially pure in that it has been substantially separated from the substances with which it may be associated in living systems. As an example, a fatty acid metabolism inhibitor may be associated with other molecules, such as a glycolytic inhibitor and/or a pharmaceutically acceptable carrier.

[0094] In some aspects of the invention, the systems and methods described herein have broad utility in regulating mammalian cell growth and death *in vitro*, *in vivo*, and/or *ex vivo*. For example, as further discussed below, the systems and methods of the invention may be used in the treatment of cancers, tumors, and other conditions involving rapidly dividing cell populations that may be uncontrolled.

[0095] The *in vitro* methods of the invention are useful for a variety of purposes. For instance, the systems and methods of the invention may be useful for identifying drugs which have an effect, such as a preventative effect, on cellular division, cancers, or cell death, by

contacting cells manipulated by the invention to undergo cellular division or death upon exposure to putative compounds.

[0096] In addition to *in vitro* methods, certain methods of the invention may be performed *in vivo* or *ex vivo* in a subject to manipulate one or more cell types within a subject. A “subject” as used herein, means a human or non-human mammal, including but not limited to, a dog, cat, horse, cow, pig, sheep, goat, chicken, primate, rat, and mouse. *In vivo* methods are well known in the art. Thus, the invention is useful for therapeutic purposes as well as research purposes, such as testing in animal or *in vitro* models of certain medical, physiological or metabolic pathways or conditions. An “*ex vivo*” method, as used herein, is a method which involves isolation of a cell from a subject, manipulation of the cell outside of the body, and reimplantation of the manipulated cell into the subject. The *ex vivo* procedure may be used on autologous or heterologous cells, and is typically used on autologous cells. In some embodiments, the *ex vivo* method is performed on cells that are isolated from bodily fluids, such as peripheral blood or bone marrow; however, the cells may be isolated from any source of cells. When returned to the subject, the manipulated cell can be programmed for cell death or division, depending on the treatment to which it was exposed. *Ex vivo* manipulation of cells has been described in several references in the art, including Engleman, *Cytotechnology*, 25:1, 1997; Van Schooten, *et al.*, *Molecular Medicine Today*, June, 255, 1997; Steinman, *Experimental Hematology*, 24:849, 1996; and Gluckman, *Cytokines, Cellular and Molecular Therapy*, 3:187, 1997. The *ex vivo* activation of cells of the invention may be performed by routine *ex vivo* manipulation steps known in the art.

[0097] In addition to the systems and methods of manipulating cells described herein, the invention is also useful, in some embodiments, for screening cells such as cancer cells, tumor cells or other cells that rapidly divide uncontrolled, to determine if those cells are susceptible to cellular division or cellular death, alone or in conjunction with treatment with a chemotherapeutic agent or other cell signal and kits for performing these screening assays. The screening method can be accomplished by isolating a tumor cell from a subject and detecting the ability of the tumor cell to use fat for fuel. The use of fat for fuel indicates the tumor cell is susceptible to treatment with a compound of the invention.

[0098] According to one aspect of the invention, the systems and methods described herein are useful in treating cancers, tumors, and other conditions involving rapidly dividing cell

populations that are typically uncontrolled. A “rapidly dividing cell,” as used herein, is a cell which is undergoing mitotic growth. Such cells are well known in the art and include, but are not limited to, tumor cells, cancer cells, lymphocytes (T cells or B cells), bacteria, and pancreatic beta (β) cells. Rapidly dividing cells, for example, cancer cells such as drug and multi-drug resistant cancer cells, are often able to derive a majority of their metabolic energy through fatty acid metabolism, i.e., the main source of energy (ATP) for the cancer cells comes from the oxidation of fatty acids instead of sugars such as glucose. Thus, in certain embodiments, the invention provides an inhibitor for a reaction of the fatty acid metabolism pathway, which, in some cases, may kill the rapidly dividing cells (“cytotoxic”) or at least prevent the cells from further division and/or growth.

[0099] Thus, the systems and methods of the invention, in some embodiments, are useful for inducing cell death in many types of mammalian cells, for example, tumor cells. As used herein, the term “cell death” is used to refer to either of the processes of apoptosis or cell lysis. In both apoptosis and cell lysis, the cell dies, but the processes occur through different mechanisms and/or different metabolic states of the cell. Apoptosis is a process of cell death in which the cell undergoes shrinkage and fragmentation, followed by phagocytosis of the cell fragments. Apoptosis is well known in the art and can be assessed by any art-recognized method. For example, apoptosis can easily be determined using flow cytometry, which is able to distinguish between live and dead cells.

[00100] In one set of embodiments, the invention includes a method of treating a subject susceptible to or exhibiting symptoms of cancer. In some cases, the cancer is drug-resistant or multi-drug resistant. As used herein, a “drug-resistant cancer” is a cancer that is resistant to conventional commonly-known cancer therapies. Examples of conventional cancer therapies include treatment of the cancer with agents such as methotrexate, trimetrexate, adriamycin, taxotere, doxorubicin, 5-fluorouracil, vincristine, vinblastine, pamidronate disodium, anastrozole, exemestane, cyclophosphamide, epirubicin, toremifene, letrozole, trastuzumab, megestrol, tamoxifen, paclitaxel, docetaxel, capecitabine, goserelin acetate, etc. A “multi-drug resistant cancer” is a cancer that resists more than one type or class of cancer agents, i.e., the cancer is able to resist a first drug having a first mechanism of action, and a second drug having a second mechanism of action. In some cases, the subject is not otherwise indicated for treatment with the inhibitor, for example, the subject is not indicated for obesity treatment.

[00101] In one embodiment, the systems and methods of the invention can be used in conjunction with one or more other forms of cancer treatment. For example, a fatty acid metabolism inhibitor, a glycolytic inhibitor, and/or an agent able to alter cellular production of reactive oxygen may be used in conjunction with an anti-cancer agent, chemotherapy, radiotherapy, etc. (e.g., simultaneously, or as part of an overall treatment procedure). As another non-limiting example, a cell may be manipulated to increase the amount of UCP or Fas in the plasma membrane, and also exposed to another form of cancer treatment. The term “cancer treatment” as used herein, may include, but is not limited to, chemotherapy, radiotherapy, adjuvant therapy, vaccination, or any combination of these methods. Parameters of cancer treatment that may vary include, but are not limited to, dosages, timing of administration or duration of therapy; and the cancer treatment can vary in dosage, timing, or duration. Another treatment for cancer is surgery, which can be utilized either alone or in combination with any of the previously treatment methods. One of ordinary skill in the medical arts can determine an appropriate treatment for a subject.

[00102] A “tumor cell,” as used herein, is a cell which is undergoing unwanted mitotic proliferation. A tumor cell, when used in the *in vitro* embodiments of the invention, can be isolated from a tumor within a subject, or may be part of an established cell line. A tumor cell in a subject may be part of any type of cancer. Cancers include, but are not limited to, biliary tract cancer; bladder cancer; brain cancer including glioblastomas and medulloblastomas; breast cancer; cervical cancer; choriocarcinoma; colon cancer; endometrial cancer; esophageal cancer; gastric cancer; hematological neoplasms including acute lymphocytic and myelogenous leukemia; multiple myeloma; AIDS-associated leukemias and adult T-cell leukemia lymphoma; intraepithelial neoplasms including Bowen’s disease and Paget’s disease; liver cancer; lung cancer; lymphomas including Hodgkin’s disease and lymphocytic lymphomas; neuroblastomas; oral cancer including squamous cell carcinoma; ovarian cancer including those arising from epithelial cells, stromal cells, germ cells and mesenchymal cells; pancreatic cancer; prostate cancer; rectal cancer; sarcomas including leiomyosarcoma, rhabdomyosarcoma, liposarcoma, fibrosarcoma, and osteosarcoma; skin cancer including melanoma, Kaposi’s sarcoma, basocellular cancer, and squamous cell cancer; testicular cancer including germinal tumors such as 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. Commonly encountered cancers include breast, prostate, lung, ovarian, colorectal, and brain cancer. In general, an effective amount of a composition for treating a cancer will be that amount necessary to inhibit mammalian cancer cell proliferation in situ. Those of ordinary skill in the art are well-schooled in the art of evaluating effective amounts of anti-cancer agents.

[00103] In some cases, the cancer treatment may include treatment with an anti-cancer agent or drug, for example, a conventionally-known anti-cancer agent or drug. Examples of suitable anti-cancer agents and drugs include, but are not limited to, methotrexate, trimetrexate, adriamycin, taxotere, 5-fluorouracil, vincristine, vinblastine, pamidronate disodium, anastrozole, exemestane, cyclophosphamide, epirubicin, toremifene, letrozole, trastuzumab, megestrol, tamoxifen, paclitaxel, docetaxel, capecitabine, and goserelin acetate. Additional examples of suitable anti-cancer agents and drugs include, but are not limited to, 20-epi-1,25 dihydroxyvitamin D3, 4-ipomeanol, 5-ethynyluracil, 9-dihydrotaxol, abiraterone, acivicin, aclarubicin, acodazole hydrochloride, acronine, acylfulvene, adecypenol, adozelesin, aldesleukin, all-tk antagonists, altretamine, ambamustine, ambomycin, ametantrone acetate, amidox, amifostine, aminoglutethimide, aminolevulinic acid, amrubicin, amsacrine, anagrelide, andrographolide, angiogenesis inhibitors, antagonist D, antagonist G, antarelix, anthramycin, anti-dorsalizing morphogenetic protein-1, antiestrogen, antineoplaston, antisense oligonucleotides, aphidicolin glycinate, apoptosis gene modulators, apoptosis regulators, apurinic acid, ARA-CDP-DL-PTBA, arginine deaminase, asparaginase, asperlin, asulacrine, atamestane, atrimustine, axinastatin 1, axinastatin 2, axinastatin 3, azacitidine, azasetron, azatoxin, azatyrosine, azetepa, azotomycin, baccatin III derivatives, balanol, batimastat, benzochlorins, benzodepa, benzoylstaurosporine, beta lactam derivatives, beta-alethine, betaclamycin B, betulinic acid, BFGF inhibitor, bicalutamide, bisantrene, bisantrene hydrochloride, bisaziridinylspermine, bisnafide, bisnafide dimesylate, bistratene A, bizelesin, bleomycin, bleomycin sulfate, BCR/ABL antagonists, breflate, brequinar sodium, bropirimine, budotitane, busulfan, buthionine sulfoximine, cactinomycin, calcipotriol, calphostin C, calusterone, camptothecin derivatives, canarypox IL-2, caracemide, carbetimer, carboplatin, carboxamide-amino-triazole, carboxyamidotriazole, carest M3, carmustine, carn 700, cartilage derived inhibitor, carubicin hydrochloride, carzelesin, casein kinase inhibitors, castanospermine, cecropin B, cedefingol, cetorelix, chlorambucil, chlorins, chloroquinoxaline sulfonamide,

cicaprost, cirolemycin, cisplatin, cis-porphyrin, cladribine, clomifene analogs, clotrimazole, collismycin A, collismycin B, combretastatin A4, combretastatin analog, conagenin, crambescidin 816, crisnatol, crisnatol mesylate, cryptophycin 8, cryptophycin A derivatives, curacin A, cyclopentantraquinones, cycloplatam, cypemycin, cytarabine, cytarabine ocfosfate, cytolytic factor, cytostatin, dacarbazine, dacliximab, dactinomycin, daunorubicin hydrochloride, decitabine, dehydrididemnin B, deslorelin, dexifosfamide, dexormaplatin, dexrazoxane, dexverapamil, dezaguanine, dezaguanine mesylate, diaziquone, didemnin B, didox, diethylnorspermine, dihydro-5-azacytidine, dioxamycin, diphenyl spiromustine, docosanol, dolasetron, doxifluridine, doxorubicin hydrochloride, droloxifene, droloxifene citrate, dromostanolone propionate, dronabinol, duazomycin, duocarmycin SA, ebselen, ecomustine, edatrexate, edelfosine, edrecolomab, eflornithine, eflornithine hydrochloride, elemene, elsamitrucin, emitefur, enloplatin, enpromate, epipropidine, epirubicin hydrochloride, epristeride, erbulozole, erythrocyte gene therapy vector system, esorubicin hydrochloride, estramustine, estramustine analog, estramustine phosphate sodium, estrogen agonists, estrogen antagonists, etanidazole, etoposide, etoposide phosphate, etoprine, fadrozole, fadrozole hydrochloride, fazarabine, fenretinide, filgrastim, finasteride, flavopiridol, flezelastine, floxuridine, fluasterone, fludarabine, fludarabine phosphate, fluorodaunorubicin hydrochloride, flurocitabine, forfenimex, formestane, fosquidone, fostriecin, fostriecin sodium, fotemustine, gadolinium texaphyrin, gallium nitrate, galocitabine, ganirelix, gelatinase inhibitors, gemcitabine, gemcitabine hydrochloride, glutathione inhibitors, hepsulfam, heregulin, hexamethylene bisacetamide, hydroxyurea, hypericin, ibandronic acid, idarubicin, idarubicin hydrochloride, idoxifene, idramantone, ifosfamide, ilmofofosine, ilomastat, imidazoacridones, imiquimod, immunostimulant peptides, insulin-like growth factor-1 receptor inhibitor, interferon agonists, interferon alpha-2A, interferon alpha-2B, interferon alpha-N1, interferon alpha-N3, interferon beta-1A, interferon gamma-1B, interferons, interleukins, iobenguane, iododoxorubicin, iroplatin, irinotecan, irinotecan hydrochloride, iroplact, irsogladine, isobengazole, isohomohalicondrin B, itasetron, jasplakinolide, kahalalide F, lamellarin-N triacetate, lanreotide, lanreotide acetate, leinamycin, lenograstim, lentinan sulfate, leptolstatin, leukemia inhibiting factor, leukocyte alpha interferon, leuprolide acetate, leuprolide/estrogen/progesterone, leuprorelin, levamisole, liarozole, liarozole hydrochloride, linear polyamine analog, lipophilic disaccharide peptide, lipophilic platinum compounds, lissoclinamide 7, lobaplatin, lombricine, lometrexol, lometrexol sodium, lomustine,

lonidamine, losoxantrone, losoxantrone hydrochloride, lovastatin, loxoribine, lurtotecan, lutetium texaphyrin, lysofylline, lytic peptides, maitansine, mannostatin A, marimastat, masoprocol, maspin, matrilysin inhibitors, matrix metalloproteinase inhibitors, maytansine, mechlorethamine hydrochloride, megestrol acetate, melengestrol acetate, melphalan, menogaril, merbarone, mercaptopurine, meterelin, methioninase, methotrexate sodium, metoclopramide, metoprine, meturedopa, microalgal protein kinase C inhibitors, MIF inhibitor, mifepristone, miltefosine, mirimostim, mismatched double stranded RNA, mitindomide, mitocarcin, mitocromin, mitogillin, mitoguazone, mitolactol, mitomalcin, mitomycin, mitomycin analogs, mitonafide, mitosper, mitotane, mitotoxin fibroblast growth factor-saporin, mitoxantrone, mitoxantrone hydrochloride, 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 anticancer agent, mycaperoxide B, mycobacterial cell wall extract, mycophenolic acid, myriaporone, n-acetyldinaline, nafarelin, nagrestip, naloxone/pentazocine, napavin, naphterpin, nartograstim, nedaplatin, nemorubicin, neridronic acid, neutral endopeptidase, nilutamide, nisamycin, nitric oxide modulators, nitroxide antioxidant, nitrullyn, nocodazole, nogalamycin, n-substituted benzamides, O6-benzylguanine, octreotide, okicenone, oligonucleotides, onapristone, ondansetron, oracin, oral cytokine inducer, ormaplatin, osaterone, oxaliplatin, oxaunomycin, oxisuran, paclitaxel analogs, paclitaxel derivatives, palauamine, palmitoylrhizoxin, pamidronic acid, panaxytriol, panomifene, parabactin, pazelliptine, pegaspargase, peldesine, peliomycin, pentamustine, pentosan polysulfate sodium, pentostatin, pentozole, peplomycin sulfate, perflubron, perfosfamide, perillyl alcohol, phenazinomycin, phenylacetate, phosphatase inhibitors, picibanil, pilocarpine hydrochloride, pipobroman, piposulfan, pirarubicin, piritrexim, piroxantrone hydrochloride, placetin A, placetin B, plasminogen activator inhibitor, platinum complex, platinum compounds, platinum-triamine complex, plicamycin, plomestane, porfimer sodium, porfiromycin, prednimustine, procarbazine hydrochloride, propyl bis-acridone, prostaglandin J2, prostatic carcinoma antiandrogen, proteasome inhibitors, protein A-based immune modulator, protein kinase C inhibitor, protein tyrosine phosphatase inhibitors, purine nucleoside phosphorylase inhibitors, puromycin, puromycin hydrochloride, purpurins, pyrazofurin, 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, riboprime, ribozymes, RII retinamide, RNAi, rogletimide, rohitukine, romurtide, roquinimex, rubiginone B1, ruboxyl, safinol, safinol hydrochloride, saintopin, sarcnu, sarcophytol A, sargramostim, SDI 1 mimetics, semustine, senescence derived inhibitor 1, sense oligonucleotides, signal transduction inhibitors, signal transduction modulators, simtrazene, single chain antigen binding protein, sizofiran, sobuzoxane, sodium borocaptate, sodium phenylacetate, solverol, somatomedin binding protein, sonermin, sparfosate sodium, sparfosic acid, sparsomycin, spicamycin D, spirogermanium hydrochloride, spiromustine, spiroplatin, splenopentin, spongistatin 1, squalamine, stem cell inhibitor, stem-cell division inhibitors, stipiamide, streptonigrin, streptozocin, stromelysin inhibitors, sulfinosine, sulofenur, superactive vasoactive intestinal peptide antagonist, suradista, suramin, swainsonine, synthetic glycosaminoglycans, talisomycin, tallimustine, tamoxifen methiodide, tauromustine, tazarotene, tecogalan sodium, tegafur, tellurapyrylium, telomerase inhibitors, teloxantrone hydrochloride, temoporfin, temozolomide, teniposide, teroxirone, testolactone, tetrachlorodecaoxide, tetrazomine, thaliblastine, thalidomide, thiamiprine, thiocoraline, thioguanine, thiotepa, thrombopoietin, thrombopoietin mimetic, thymalfasin, thymopoietin receptor agonist, thymotrinan, thyroid stimulating hormone, tiazofurin, tin ethyl etiopurpurin, tirapazamine, titanocene dichloride, topotecan hydrochloride, topsentin, toremifene citrate, totipotent stem cell factor, translation inhibitors, trestolone acetate, tretinoin, triacetyluridine, triciribine, triciribine phosphate, trimetrexate, trimetrexate glucuronate, triptorelin, tropisetron, tubulazole hydrochloride, turosteride, tyrosine kinase inhibitors, tyrphostins, UBC inhibitors, ubenimex, uracil mustard, uredepa, urogenital sinus-derived growth inhibitory factor, urokinase receptor antagonists, vapreotide, variolin B, velaesol, veramine, verdins, verteporfin, vinblastine sulfate, vincristine sulfate, vindesine, vindesine sulfate, vinepidine sulfate, vinglycinate sulfate, vinleurosine sulfate, vinorelbine, vinorelbine tartrate, vinrosidine sulfate, vinxaltine, vinzolidine sulfate, vitaxin, vorozole, zanoterone, zeniplatin, zilascorb, zinostatin, zinostatin stimalamer, and zorubicin hydrochloride, as well as salts, homologs, analogs, derivatives, enantiomers and/or functionally equivalent compositions thereof.

[00104] In another embodiment, cells may be removed from a tumor or other rapidly dividing cell mass (e.g., a tumor from a subject, a tumor growing *in vitro*, etc.) and exposed in some fashion to the systems and methods described herein. For example, the cells may be

manipulated to increase the amount of UCP in the plasma membrane, e.g., by exposure to a fatty acid metabolic inhibitor. After suitable exposure, the exposed cells may be introduced into a subject. Exposure of the cells may alter the immunological profile of the tumor cells in some fashion, for example, such that a subject's immune system is able to recognize the tumor cells. The subject's immune system, after interacting with the exposed cells, may then be able to recognize tumors present within the subject, thus causing the cancer (or other rapidly dividing cell mass) to decrease. If the subject has a tumor, the cells may be injected into the tumor, proximate the tumor, and/or systemically or locally delivered in a region of the body away from the tumor. In some cases, a tumor may be removed from a subject, then the exposed cells may be inserted, e.g., into the cavity created upon removal of the tumor, or to another site within the body. Optionally, other cancer treatment methods, such as radiation or exposure to conventional anti-cancer agents, may also be used in conjunction with these methods. In some cases, the subject may not have a cancer or tumor, but the cells may be injected to stimulate the immune system to produce antibodies against future cancers and/or other uncontrolled cellular growths, i.e., "immunizing" the subject from cancer and/or other uncontrolled cellular growths. In some the cancer cells are antigenic and can be targeted by the immune system. Thus, the combined administration of the systems and methods of the invention and cancer medicaments, particularly those which are classified as cancer immunotherapies, can be very useful for stimulating a specific immune response against a cancer antigen. A "cancer antigen" as used herein is a compound, such as a peptide, associated with a tumor or cancer cell surface, and which is capable of provoking an immune response when expressed on the surface of an antigen-presenting cell in the context of an MHC molecule. Cancer antigens, such as those present in cancer vaccines or those used to prepare cancer immunotherapies, can be prepared from crude cancer cell extracts, e.g., as described in Cohen, *et al.*, *Cancer Research*, 54:1055, 1994, or by partially purifying the antigens, using recombinant technology, or *de novo* synthesis of known antigens. Cancer antigens can be used in the form of immunogenic portions of a particular antigen, or in some instances, a whole cell or a tumor mass can be used as the antigen. Such antigens can be isolated or prepared recombinantly or by any other means known in the art.

[00105] The systems and methods of the invention can be used in combination with immunotherapeutics, according to another embodiment. The goal of immunotherapy is to augment a subject's immune response to an established tumor. One method of immunotherapy

includes the use of adjuvants. Adjuvant substances derived from microorganisms, such as *bacillus Calmette-Guerin*, can heighten the immune response and enhance resistance to tumors in animals. Immunotherapeutic agents are often medicaments which derive from antibodies or antibody fragments that specifically bind to or otherwise recognize a cancer antigen. Binding of such agents can promote an immune response, such as an antigen-specific immune response. Antibody-based immunotherapy may function by binding to the cell surface of a cancer cell, which can stimulate the endogenous immune system to attack the cancer cell.

[00106] As used herein, a “cancer antigen” is broadly defined as an antigen expressed by a cancer cell. The antigen can be expressed at the cell surface of the cancer cell. In many cases, the antigen is one which is not expressed by normal cells, or at least not expressed at the same level or concentration as in cancer cells. As examples, some cancer antigens are normally silent (i.e., not expressed) in normal cells, some are expressed only at certain stages of differentiation, and others are only temporally expressed (such as embryonic and fetal antigens). Other cancer antigens are encoded by mutant cellular genes, such as oncogenes (e.g., activated ras oncogene), suppressor genes (e.g., mutant p53), fusion proteins resulting from internal deletions or chromosomal translocations, or the like. Still other cancer antigens can be encoded by viral genes, such as those carried on RNA and DNA tumor viruses. The differential expression of cancer antigens in normal and cancer cells can be exploited in order to target cancer cells in some cases. As used herein, the terms “cancer antigen” and “tumor antigen” are used interchangeably.

[00107] The theory of immune surveillance is that a prime function of the immune system is to detect and eliminate neoplastic cells before a tumor forms. A basic principle of this theory is that cancer cells are antigenically different from normal cells and thus can elicit immune reactions similar to those that cause rejection of immunologically incompatible allografts. Studies have confirmed that tumor cells differ, qualitatively or quantitatively, in their expression of antigens. For example, “tumor-specific antigens” are antigens that are specifically associated with tumor cells but not normal cells. Examples of tumor specific antigens are viral antigens in tumors induced by DNA or RNA viruses. “Tumor-associated” antigens are present in both tumor cells and normal cells but are present in a different quantity or a different form in tumor cells. Examples of such antigens are oncofetal antigens (e.g., carcinoembryonic antigen), differentiation antigens (e.g., T and Tn antigens), and oncogene products (e.g., HER/neu).

[00108] Different types of cells that can kill tumor targets *in vitro* and *in vivo* have been identified: natural killer cells (NK cells), cytolytic T lymphocytes (CTLs), lymphokine-activated killer cells (LAKs), and activated macrophages. NK cells can kill tumor cells without having been previously sensitized to specific antigens, and the activity does not require the presence of class I antigens encoded by the major histocompatibility complex (MHC) on target cells. NK cells are thought to participate in the control of nascent tumors and in the control of metastatic growth. In contrast to NK cells, CTLs can kill tumor cells only after they have been sensitized to tumor antigens and when the target antigen is expressed on the tumor cells that also express MHC class I. CTLs are thought to be effector cells in the rejection of transplanted tumors and of tumors caused by DNA viruses. LAK cells are a subset of null lymphocytes distinct from the NK and CTL populations. Activated macrophages can kill tumor cells in a manner that is not antigen-dependent, nor MHC-restricted, once activated. Activated macrophages are thought to decrease the growth rate of the tumors they infiltrate. *In vitro* assays have identified other immune mechanisms such as antibody-dependent, cell-mediated cytotoxic reactions, and lysis by antibody plus complement. However, these immune effector mechanisms are thought to be less important *in vivo* than the function of NK, CTLs, LAK, and macrophages *in vivo* (for a review, see Piessens, "Tumor Immunology," in *Scientific American Medicine*, Vol. 2, Scientific American Books, p. 1-13, 1996).

[00109] In some cases, the immunotherapeutic agent may function as a delivery system for the specific targeting of toxic substances to cancer cells. For example, the agent may be conjugated to toxins such as ricin (e.g., from castor beans), calicheamicin, maytansinoids, radioactive isotopes such as iodine-131 and yttrium-90, chemotherapeutic agents, and/or to biological response modifiers. In this way, the toxic substances can be concentrated in the region of the cancer and non-specific toxicity to normal cells can be minimized.

[00110] In certain instances, the immunotherapeutic agent may be directed towards the binding of vasculature, such as those which bind to endothelial cells. This is because solid tumors are generally dependent upon newly formed blood vessels to survive, and thus most tumors are capable of recruiting and stimulating the growth of new blood vessels. As a result, one strategy of many cancer medicaments is to attack the blood vessels feeding a tumor and/or the connective tissues (or stroma) supporting such blood vessels.

[00111] In another set of embodiments, the combined administration of the systems and methods of the invention and an apoptotic chemotherapeutic agent may be used. An “apoptotic chemotherapeutic agent,” as used herein, includes molecules which function by a variety of mechanisms to induce apoptosis in rapidly dividing cells. Apoptotic chemotherapeutic agents are a class of chemotherapeutic agents which are well known to those of ordinary skill in the art. Chemotherapeutic agents include those agents disclosed in Chapter 52, “Antineoplastic Agents” (Paul Calabresi and Bruce A. Chabner), and the introduction thereto, p. 1202-1263, of Goodman and Gilman’s *The Pharmacological Basis of Therapeutics*, Eighth Edition, McGraw-Hill, Inc. Health Professions Division, 1990, incorporated herein by reference. Suitable chemotherapeutic agents may have various mechanisms of action. Classes of suitable chemotherapeutic agents include, but are not limited to: (a) alkylating agents, such as nitrogen mustard (e.g. mechlorethamine, cyclophosphamide, ifosfamide, melphalan, chlorambucil), ethylenimines and methylmelamines (e.g. hexamethylmelamine, thiotepa), alkyl sulfonates (e.g. busulfan), nitrosoureas (e.g. carmustine, which is also known as BCNU, lomustine which is also known as CCNU, semustine, which is also known as methyl-CCNU, chlorozotacin, streptozocin), and triazines (e.g. dicarbazine, which is also known as DTIC); (b) antimetabolites, such as folic acid analogs (e.g. methotrexate), pyrimidine analogs (e.g. 5-fluorouracil, floxuridine, cytarabine, and azauridine and its prodrug form azaribine), and purine analogs and related materials (e.g. 6-mercaptopurine, 6-thioguanine, pentostatin); (c) natural products, such as the vinca alkaloids (e.g. vinblastine, vincristine), epipodophylotoxins (e.g. etoposide, teniposide), antibiotics (e.g. dactinomycin, which is also known as actinomycin-D, daunorubicin, doxorubicin, bleomycin, plicamycin, mitomycin, epirubicin, which is 4-epidoxorubicin, idarubicin which is 4-dimethoxydaunorubicin, and mitoxanthrone), enzymes (e.g. L-asparaginase), and biological response modifiers (e.g. interferon alfa); (d) miscellaneous agents, such as the platinum coordination complexes (e.g. cisplatin, carboplatin), substituted ureas (e.g. hydroxyurea), methylhydiazine derivatives (e.g. procarbazine), adreocortical suppressants (e.g. mitotane, aminogluthetimide) taxol; (e) hormones and antagonists, such as adrenocorticosteroids (e.g. prednisone or the like), progestins (e.g. hydroxyprogesterone caproate, medroxyprogesterone acetate, megestrol acetate), estrogens (e.g. diethyestilbestrol, ethinyl estradiol, or the like), antiestrogens (e.g. tamoxifen), androgens (e.g. testosterone propionate, fluoxymesterone, or the like), antiandrogens (e.g. flutamide), and gonadotropin-releasing hormone analogs (e.g.

leuprolide), and (f) DNA damaging compounds, such as adriamycin. The combined administration of the systems and methods of the invention and an apoptotic chemotherapeutic agent effective to inhibit growth of the tumor cell is that amount effective to induce apoptosis of the tumor cell in some cases.

[00112] In yet another set of embodiments, the systems and methods of the invention may be used in conjunction with a vaccine, such as a cancer vaccine. Cancer vaccines are medicaments which are intended to stimulate an endogenous immune response against cancer cells. Currently-produced vaccines predominantly activate the humoral immune system (i.e., the antibody dependent immune response). Other vaccines currently in development are focused on activating the cell-mediated immune system including cytotoxic T lymphocytes which are capable of killing tumor cells. Cancer vaccines generally enhance the presentation of cancer antigens to both antigen presenting cells (e.g., macrophages and dendritic cells) and/or to other immune cells such as T cells, B cells, and NK cells.

[00113] Although cancer vaccines may take one of several forms, their purpose is to deliver cancer antigens and/or cancer associated antigens to antigen presenting cells (APC) in order to facilitate the endogenous processing of such antigens by APC and the ultimate presentation of antigen presentation on the cell surface in the context of MHC class I molecules. One form of cancer vaccine is a whole cell vaccine which is a preparation of cancer cells which have been removed from a subject, treated *ex vivo* and then reintroduced as whole cells in the subject. Lysates of tumor cells can also be used as cancer vaccines to elicit an immune response in certain cases. Another form of cancer vaccine is a peptide vaccine which uses cancer-specific or cancer-associated small proteins to activate T cells. Cancer-associated proteins are proteins which are not exclusively expressed by cancer cells (i.e., other normal cells may still express these antigens). However, the expression of cancer-associated antigens is generally consistently upregulated with cancers of a particular type. Yet another form of cancer vaccine is a dendritic cell vaccine which includes whole dendritic cells that have been exposed to a cancer antigen or a cancer-associated antigen *in vitro*. Lysates or membrane fractions of dendritic cells may also be used as cancer vaccines in some instances. Dendritic cell vaccines are able to activate antigen-presenting cells directly. Other non-limiting examples of cancer vaccines include ganglioside vaccines, heat-shock protein vaccines, viral and bacterial vaccines, and nucleic acid vaccines.

[00114] Other cancer vaccines can take the form of dendritic cells which have been exposed to cancer antigens *in vitro*, have processed the antigens and are able to express the cancer antigens at their cell surface in the context of MHC molecules for effective antigen presentation to other immune system cells.

[00115] In some embodiments, cancer vaccines may be used along with adjuvants. Adjuvants are substances which activate the subject's immune system, and can be used as an adjunct therapy in any of the systems or methods of the invention. Adjuvants include, for example, alum, QS-Stimulon (Aquila), MF-59 (Chiron), Detox (Ribi), Optivax (Vaxcels) and LeIF (Corixa).

[00116] The invention, in still another set of embodiments, is useful for treating other diseases associated with rapidly dividing cells, such as rheumatoid arthritis and scleroderma. Rheumatoid arthritis is associated in its early stages with the rapid division of synoviocytes. This process is referred to a pannus formation. The rapidly dividing cells produce a substance that kills osteocytes leading to the hardening of the tissue.

[00117] In another aspect, the systems and methods of the invention are useful for treating or preventing disorders associated with a specific antigenic immune response. Thus, in some embodiments of the invention, the methods are used to treat mammals at risk of, or afflicted with, autoimmune disease. Autoimmune disease is a disorder in which the host's immune response is defective and results in the production of a specific immune response against the individual's own antigens or components. In an autoimmune disease, an individual's own antibodies react with host tissue or in which immune effector T cells are autoreactive to endogenous self peptides and cause destruction of tissue. It is well established that MHC class II alleles act as major genetic elements in susceptibility to a variety of autoimmune diseases. The structures recognized by T cells, the cells that cause autoimmunity, are complexes comprised of class II MHC molecules and antigenic peptides. When the T cells react with the host's class II MHC molecules-peptide complexes derived from a host's own gene products, autoimmune disease can result. If these class II MHC/peptide complexes are inhibited from being formed, the autoimmune response is reduced or suppressed, and thus is inhibited according to the invention. The peptide-antigen of autoimmune disorders are self-antigens. Any autoimmune disease in which class II MHC/peptide complexes play a role may be treated according to the methods of the present invention. Such autoimmune diseases include, but are not limited to, juvenile-onset

diabetes (insulin-dependent), multiple sclerosis, pemphigus vulgaris, Graves disease, myasthenia gravis, systemic lupus erythematosus (SLE), celiac disease rheumatoid arthritis, and Hashimoto's thyroiditis. The invention includes a method for determining an individuals susceptibility to developing autoimmune disease. As used herein, "susceptibility to autoimmune disease" indicates a likelihood of at least greater than the average of developing autoimmune disease, and in some embodiments at least about 10% greater. Thus the invention also includes systems and methods for treating a subject having autoimmune disease to reduce associated cell death.

[00118] The methods of the invention also include methods for treating a subject having autoimmune disease to reduce associated cell death, according to one set of embodiments. One method is based on the ability to selectively remove gamma delta ($\gamma\delta$) T cells which specifically recognize MHC class II HLA-DR on the surface of a self cell. When the gamma delta T cells recognize a tissue having significant amounts of MHC class II HLA-DR these T cells become activated and proliferate in order to kill more of the recognized cells. The methods of treatment are based on the concept of eliminating the activated gamma delta T cells from the body. These cells can be removed by contacting a gamma delta T cell with an amount of a plasma membrane targeted UCP inhibitor in an amount effective to induce gamma delta T cell death. This selective killing of the gamma delta cells inhibits cell death associated with autoimmune disease.

[00119] When used with mammalian cells *in vitro*, certain systems and methods may have utility for loading of specific antigens within the MHC molecules. Cells with specific antigen loading in class II molecules have utility in a variety of analytical and diagnostic assays. These cells are also useful as therapeutic agents. For instance, the cells can be used in culture to study immune responses or to screen the effect of putative drugs on inhibiting or promoting antigen-specific immune responses. Additionally, the cells could be administered to a mammalian subject to promote an antigen-specific T cell response. When administered to a subject, the class II MHC/antigen complexes on the surface of the cell can interact with endogenous T cells, inducing an immune cascade, and thus can produce an antigen-specific immune response. In some embodiments, the cells manipulated *in vitro* have been isolated from the same subject *ex vivo*.

[00120] The systems and methods of the invention can also be used for treating a mammalian subject *in vivo* to induce an antigen-specific immune response, according to another set of embodiments. It is useful to produce antigen-specific immune responses against any foreign antigen, whether it is capable of causing a pathological state and/or any damage to its mammalian host. The terms “foreign antigen” or “antigen” are used synonymously to refer to a molecule capable of provoking an immune response in a host wherein the antigen is not a self-antigen, as defined above. Thus, these terms specifically excludes self-antigens. Self-antigens are used herein to refer to the peptide-antigens of autoimmune disorders. An immune response against the self-antigen results in an autoimmune disorder. The term “self-antigen” does not include, however, antigens such as cancer antigens, which are recognized by the host as foreign and which are not associated with autoimmune disease. Thus, the term “antigen” specifically excludes self-antigens and broadly includes any type of molecule (e.g. associated with a host or foreign cell) which is recognized by a host immune system as being foreign. Antigens include, but are not limited to, cancer antigens and microbial antigens and may be composed of cells, cell extracts, polysaccharides, polysaccharide conjugates, lipids, glycolipids, carbohydrates, peptides, proteins, viruses, viral extracts, etc. A “cancer antigen,” as used herein, is a compound which is associated with a tumor or cancer cell surface and which is capable of provoking an immune response when expressed on the surface of an antigen-presenting cell in the context of a class II MHC molecule. Cancers or tumors include those described above.

[00121] Cancer antigens include but are not limited to Melan-A/MART-1, Dipeptidyl peptidase IV (DPP-IV), adenosine deaminase-binding protein (ADAbp), cyclophilin b, Colorectal associated antigen (CRC)--C017-1A/GA733, Carcinoembryonic Antigen (CEA) and its immunogenic epitopes CAP-1 and CAP-2, etv6, aml1, Prostate Specific Antigen (PSA) and its immunogenic epitopes PSA-1, PSA-2, and PSA-3, prostate-specific membrane antigen (PSMA), T-cell receptor/CD3-zeta chain, MAGE-family of tumor antigens (e.g., MAGE-A1, MAGE-A2, MAGE-A3, MAGE-A4, MAGE-A5, MAGE-A6, MAGE-A7, MAGE-A8, MAGE-A9, MAGE-A10, MAGE-A11, MAGE-A12, MAGE-Xp2 (MAGE-B2), MAGE-Xp3 (MAGE-B3), MAGE-Xp4 (MAGE-B4), MAGE-C1, MAGE-C2, MAGE-C3, MAGE-C4, MAGE-C5), GAGE-family of tumor antigens (e.g., GAGE-1, GAGE-2, GAGE-3, GAGE-4, GAGE-5, GAGE-6, GAGE-7, GAGE-8, GAGE-9), BAGE, RAGE, LAGE-1, NAG, GnT-V, MUM-1, CDK4, tyrosinase, p53, MUC family, HER2/neu, p21ras, RCAS1, alpha-fetoprotein, E-cadherin, alpha-catenin, beta-

catenin and gamma-catenin, p120ctn, gp100^{Pmel117}, PRAME, NY-ESO-1, brain glycogen phosphorylase, SSX-1, SSX-2 (HOM-MEL-40), SSX-1, SSX-4, SSX-5, SCP-1 and CT-7, cdc27, adenomatous polyposis coli protein (APC), fodrin, P1A, Connexin 37, Ig-idiotypic, p15, gp75, GM2 and GD2 gangliosides, viral products such as human papilloma virus proteins, Smad family of tumor antigens, Imp-1, EBV-encoded nuclear antigen (EBNA)-1, or c-erbB-2.

[00122] In some embodiments, cancers or tumors escaping immune recognition and tumor-antigens associated with such tumors (but not exclusively), include acute lymphoblastic leukemia (etv6; aml1; cyclophilin b), B cell lymphoma (Ig-idiotypic), glioma (E-cadherin; alpha-catenin; beta-catenin; gamma-catenin; p120ctn), bladder cancer (p21ras), biliary cancer (p21ras), breast cancer (MUC family; HER2/neu; c-erbB-2), cervical carcinoma (p53; p21ras), colon carcinoma (p21ras; HER2/neu; c-erbB-2; MUC family), colorectal cancer (Colorectal associated antigen (CRC)--C017-1A/GA733; APC), choriocarcinoma (CEA), epithelial cell-cancer (cyclophilin b), gastric cancer (HER2/neu; c-erbB-2; ga733 glycoprotein), hepatocellular cancer (alpha-fetoprotein), hodgkins lymphoma (Imp-1; EBNA-1), lung cancer (CEA; MAGE-3; NY-ESO-1), lymphoid cell-derived leukemia (cyclophilin b), melanoma (p15 protein, gp75, oncofetal antigen, GM2 and GD2 gangliosides), myeloma (MUC family; p21ras), non-small cell lung carcinoma (HER2/neu; c-erbB-2), nasopharyngeal cancer (Imp-1; EBNA-1), ovarian cancer (MUC family; HER2/neu; c-erbB-2), prostate cancer (Prostate Specific Antigen (PSA) and its immunogenic epitopes PSA-1, PSA-2, and PSA-3; PSMA; HER2/neu; c-erbB-2), pancreatic cancer (p21ras; MUC family; HER2/neu; c-erbB-2; ga733 glycoprotein), renal (HER2/neu; c-erbB-2), squamous cell cancers of cervix and esophagus (viral products such as human papilloma virus proteins), testicular cancer (NY-ESO-1), T cell leukemia (HTLV-1 epitopes), and melanoma (Melan-A/MART-1; cdc27; MAGE-3; p21ras; gp100^{Pmel117}).

[00123] For examples of tumor antigens which bind to either or both MHC class I and MHC class II molecules, see the following references: Coulie, *Stem Cells*, 13:393-403, 1995; Traversari, *et al.*, *J. Exp. Med.*, 176:1453-1457, 1992; Chaux, *et al.*, *J. Immunol.*, 163:2928-2936, 1999; Fujie, *et al.*, *Int. J. Cancer*, 80:169-172, 1999; Tanzarella, *et al.*, *Cancer Res.*, 59:2668-2674, 1999; van der Bruggen, *et al.*, *Eur. J. Immunol.*, 24:2134-2140, 1994; Chaux, *et al.*, *J. Exp. Med.*, 189:767-778, 1999; Kawashima *et al.*, *Hum. Immunol.*, 59:1-14, 1998; Tahara, *et al.*, *Clin. Cancer Res.*, 5:2236-2241, 1999; Gaugler, *et al.*, *J. Exp. Med.*, 179:921-930, 1994; van der Bruggen, *et al.*, *Eur. J. Immunol.*, 24:3038-3043, 1994; Tanaka, *et al.*, *Cancer Res.*, 57:4465-

4468, 1997; Oiso, *et al.*, *Int. J. Cancer*, 81:387-394, 1999; Herman, *et al.*, *Immunogenetics*, 43:377-383, 1996; Manici, *et al.*, *J. Exp. Med.*, 189:871-876, 1999; Duffour, *et al.*, *Eur. J. Immunol.*, 29:3329-3337, 1999; Zorn, *et al.*, *Eur. J. Immunol.*, 29:602-607, 1999; Huang, *et al.*, *J. Immunol.*, 162:6849-6854, 1999; Boël, *et al.*, *Immunity*, 2:167-175, 1995; Van den Eynde, *et al.*, *J. Exp. Med.*, 182:689-698, 1995; De Backer, *et al.*, *Cancer Res.*, 59:3157-3165, 1999; Jäger, *et al.*, *J. Exp. Med.*, 187:265-270, 1998; Wang, *et al.*, *J. Immunol.*, 161:3596-3606, 1998; Aarnoudse, *et al.*, *Int. J. Cancer*, 82:442-448, 1999; Guilloux, *et al.*, *J. Exp. Med.*, 183:1173-1183, 1996; Lupetti, *et al.*, *J. Exp. Med.*, 188:1005-1016, 1998; Wölfel, *et al.*, *Eur. J. Immunol.*, 24:759-764, 1994; Skipper, *et al.*, *J. Exp. Med.*, 183:527-534, 1996; Kang, *et al.*, *J. Immunol.*, 155:1343-1348, 1995; Morel, *et al.*, *Int. J. Cancer*, 83:755-759, 1999; Brichard, *et al.*, *Eur. J. Immunol.*, 26:224-230, 1996; Kittlesen, *et al.*, *J. Immunol.*, 160:2099-2106, 1998; Kawakami, *et al.*, *J. Immunol.*, 161:6985-6992, 1998; Topalian, *et al.*, *J. Exp. Med.*, 183:1965-1971, 1996; Kobayashi, *et al.*, *Cancer Res.*, 58:296-301, 1998; Kawakami, *et al.*, *J. Immunol.*, 154:3961-3968, 1995; Tsai, *et al.*, *J. Immunol.*, 158:1796-1802, 1997; Cox, *et al.*, *Science*, 264:716-719, 1994; Kawakami, *et al.*, *Proc. Natl. Acad. Sci. U.S.A.*, 91:6458-6462, 1994; Skipper, *et al.*, *J. Immunol.*, 157:5027-5033, 1996; Robbins, *et al.*, *J. Immunol.*, 159:303-308, 1997; Castelli *et al.*, *J. Immunol.*, 162:1739-1748, 1999; Kawakami, *et al.*, *J. Exp. Med.*, 180:347-352, 1994; Castelli, *et al.*, *J. Exp. Med.*, 181:363-368, 1995; Schneider, *et al.*, *Int. J. Cancer*, 75:451-458, 1998; Wang, *et al.*, *J. Exp. Med.*, 183:1131-1140, 1996; Wang, *et al.*, *J. Exp. Med.*, 184:2207-2216, 1996; Parkhurst, *et al.*, *Cancer Res.*, 58:4895-4901, 1998; Tsang, *et al.*, *J. Natl. Cancer Inst.*, 87:982-990, 1995; Correale, *et al.*, *J. Natl. Cancer Inst.*, 89:293-300, 1997; Coulie, *et al.*, *Proc. Natl. Acad. Sci. U.S.A.*, 92:7976-7980, 1995; Wölfel, *et al.*, *Science*, 269:1281-1284, 1995; Robbins, *et al.*, *J. Exp. Med.*, 183:1185-1192, 1996; Brändle, *et al.*, *J. Exp. Med.*, 183:2501-2508, 1996; ten Bosch, *et al.*, *Blood*, 88:3522-3527, 1996; Mandruzzato, *et al.*, *J. Exp. Med.*, 186:785-793, 1997; Guéguen, *et al.*, *J. Immunol.*, 160:6188-6194, 1998; Gjertsen, *et al.*, *Int. J. Cancer*, 72:784-790, 1997; Gaudin, *et al.*, *J. Immunol.*, 162:1730-1738, 1999; Chiari, *et al.*, *Cancer Res.*, 59:5785-5792, 1999; Hogan, *et al.*, *Cancer Res.*, 58:5144-5150, 1998; Pieper, *et al.*, *J. Exp. Med.*, 189:757-765, 1999; Wang, *et al.*, *Science*, 284:1351-1354, 1999; Fisk, *et al.*, *J. Exp. Med.*, 181:2109-2117, 1995; Brossart, *et al.*, *Cancer Res.*, 58:732-736, 1998; Röpke, *et al.*, *Proc. Natl. Acad. Sci. U.S.A.*, 93:14704-14707, 1996; Ikeda, *et al.*, *Immunity* 6:199-208, 1997;

Ronsin, *et al.*, *J. Immunol.*, 163:483-490, 1999; or Vonderheide, *et al.*, *Immunity*, 10:673-679, 1999. These antigens as well as others are disclosed in PCT Application PCT/US98/18601.

[00124] The systems and methods of the invention are also useful for treating mammals at risk of, or afflicted with, allergic responses, in yet another set of embodiments. An “allergic response” as used herein is a disorder in which the host’s immune response to a particular antigen is unnecessary or disproportionate, resulting in pathology. An allergic response may occur, in part, because a T cell recognizes a particular class II MHC/peptide complex and triggers a cascade of immune response. If the class II MHC/peptide complex is inhibited from being formed, the allergic response is reduced or suppressed. Any allergic response in which class II MHC/peptide complexes play a role may be treated according to the methods of the present invention. Allergies arising from an allergic response include, but are not limited to, allergies to pollen, ragweed, shellfish, domestic animals, (e.g., cats and dogs), B-venom, and the like. A subset of allergic responses produce asthma. Allergic asthmatic responses are also included within the definition of the term “allergic response.” It is particularly desirable to treat severe or life-threatening allergic responses, such as those arising during asthmatic attacks or anaphylactic shock, according to the systems and methods of the invention.

[00125] In another aspect, the systems and methods of the invention are useful in treating wounds in subjects. As used herein, the term “wound” is used to describe skin wounds as well as tissue wounds. A “skin wound” is defined herein as a break in the continuity of skin tissue which is caused by direct injury to the skin. Skin wounds are generally characterized by several classes including punctures, incisions, including those produced by surgical procedures, excisions, lacerations, abrasions, atrophic skin, or necrotic wounds and burns. The systems and methods of the invention are useful for enhancing the healing of all wounds of the skin.

[00126] A “tissue wound,” as used herein, is a wound to an internal organ, such as a blood vessel, intestine, colon, etc. The systems and methods of the invention are useful for enhancing the wound healing process in tissue wounds, whether they arise naturally, or as the result of surgery. For instance, during the repair of arteries an artery may need to be sealed and wound healing promoted as quickly as possible. The systems and methods of the invention can speed up that process in many cases. The invention may also be particularly useful for the treatment of damaged tissue in the colon. In addition to promoting wound healing of the

damaged colon, in some cases, the systems and methods of the invention can provide an antimicrobial effect.

[00127] The cells treated according to the present invention may be used to treat a wound, for example cells exposed to a fatty acid metabolism inhibitor, a glycolytic inhibitor, and/or an agent able to alter cellular production of reactive oxygen in cells. As an example, *ex vivo* cells may be attached to a bandage or other substrate, and the substrate positioned over a wound, at least partially covering the wound. In some cases, the bandage or other substrate may be adhered to the subject, for example, through the use of adhesives. Suitable adhesives can be selected by those of ordinary skill in the art; some suitable adhesives are further described below.

[00128] The systems and methods of the invention may also include additional therapeutic and/or pharmacologically acceptable agents. For instance, the compositions or methods may involve other agents for the treatment of wounds such as, for instance, dexpanthenol, growth factors, enzymes or hormones, povidon-iodide, fatty acids, such as cetylphridinium chloride, antibiotics, and analgesics. In some embodiments, the compositions may also include growth factors. Growth factors include, but are not limited to, fibroblast growth factor (FGF), FGF-1, FGF-2, FGF-4, platelet-derived growth factor (PDGF), insulin-binding growth factor (IGF), IGF-1, IGF-2, epidermal growth factor (EGF), transforming growth factor (TGF), TGF- α , TGF- β , cartilage inducing factors –A and –B, osteoid-inducing factors, osteogenin and other bone growth factors, collagen growth factors, heparin-binding growth factor –1 or –2, and/or their biologically active derivatives. The compositions may also include antiseptics in some embodiments.

[00129] In another aspect, the systems and methods of the invention are useful for treating mammals which have undergone or about to undergo, an organ transplant or tissue graft. In tissue transplantation (e.g., kidney, lung, liver, heart) or skin grafting, when there is a mismatch between the class II MHC genotypes (HLA types) of the donor and recipient, there may be a severe “allogeneic immune response” against the donor tissues which results from the presence of non-self or allogeneic class II MHC molecules presenting antigenic peptides on the surface of donor cells.

[00130] The systems and methods of the invention, in yet another aspect, are useful for treating mammals having an inflammatory disease or condition. An “inflammatory disease or

condition,” as used herein, refers to any condition characterized by local inflammation at a site of injury or infection and includes autoimmune diseases, certain forms of infectious inflammatory states, undesirable neutrophil activity characteristic of organ transplants or other implants and virtually any other condition characterized by unwanted neutrophil activation. These conditions include, but are not limited to, meningitis, cerebral edema, arthritis, nephritis, adult respiratory distress syndrome, pancreatitis, myositis, neuritis, connective tissue diseases, phlebitis, arteritis, vasculitis, allergy, anaphylaxis, ehrlichiosis, gout, organ transplants and/or ulcerative colitis.

[00131] In one aspect, the systems and methods of the invention can also be used in combination with other therapies, such as radiation therapy. When a combination of therapies are used the effective amount to achieve the desired result, inhibition of cell proliferation may be less. This may reduce or eliminate any side effects associated with high concentrations of the individual therapies. One example is a combination of one or more compositions of the invention and radiation therapy. In some cases, the radiation therapy may also contribute to the inhibition of UCP in the plasma membrane. Radiation-sensitive cells are those cells that express UCP in the plasma membrane, and radioresistant cells do not express plasma membrane UCP. The invention also includes, in some instances, systems and methods of treating radioresistant cells by inducing UCP expression in the plasma membrane and treating them with radiation.

[00132] Optionally, in some embodiments, a targeting mechanism can be used to target one or more compositions of the invention to a specific cell, tumor, wound, or the like. It is desirable in many instances to specifically target a cell type to increase the efficiency and specificity of administration of the composition, thus avoiding the effects that can damage or destroy unrelated cells. Thus, a delivery system which enables the delivery of such drugs specifically to target cells is provided. The delivery system may increase the efficacy of treatment and reduce the associated “side effects” of such treatment.

[00133] Methods of targeting drugs and other compositions to target cells (such as cancer cells or cells within a wound) are well known in the art. One method of targeting involves antibody or receptor targeting. Receptor or antibody targeting involves linking the compound of the invention to a ligand or an antibody which has an affinity for a receptor or cell surface molecule expressed on the desired target cell surface. Using this approach, a composition of the invention is intended to adhere to the target cell following formation of a ligand-receptor or antibody-cell surface antigen complex on the cell surface. The type of

receptor or antibody used to target the cell will depend on the specific cell type being targeted. A target molecule may be attached by a peptide or other type of bond such as a sulfhydryl or disulfide bond. Targeting molecules are described, for instance in US Patent No. 5,849,718, as well as many other references.

[00134] In general, the targeting moiety can be coupled to a composition of the invention. The molecules may be directly coupled to one another, such as by conjugation, or may be indirectly coupled to one another where, for example, the targeting moiety is on the surface of a liposome and one or more compositions of the invention are contained within the liposome. If the molecules are linked to one another, then the targeting moiety can be covalently or noncovalently bound to the compound of the invention in a manner that preserves the targeting specificity of the targeting moiety. As used herein, "linked" or "linkage" means two entities are bound to one another by any physiochemical means. It is important that the linkage be of such a nature that it does not impair substantially the effectiveness of the compositions of the invention or the binding specificity of the targeting moiety. Keeping these parameters in mind, any linkage known to those of ordinary skill in the art may be employed, covalent or noncovalent. Such means and methods of linkage are well known to those of ordinary skill in the art.

[00135] Linkages according to the invention need not be direct linkage. The compositions of the invention may be provided with functionalized groups to facilitate their linkage and/or linker groups may be interposed therebetween to facilitate their linkage. In some instances, the components of the present invention may be synthesized in a single process, whereby the composition is regarded as a single entity. For example, a targeting moiety specific for a tumor cell could be synthesized together with a VCP inhibitor and a fatty acid metabolism inhibitor of the invention. These and other modifications are intended to be embraced by the present invention.

[00136] Specific examples of covalent bonds include those where bifunctional cross-linker molecules can be used. The cross-linker molecules may be homobifunctional or heterobifunctional, depending upon the nature of the molecules to be conjugated. Homobifunctional cross-linkers have two identical reactive groups. Heterobifunctional cross-linkers have two different reactive groups that allow sequential conjugation reaction. Various types of commercially available cross-linkers are reactive with one or more of the following

groups, such as primary amines, secondary amines, sulfhydryles, carboxyls, carbonyls and carbohydrates.

[00137] Non-covalent methods of conjugation also may be used to join the targeting moiety and the composition in some cases. Non-covalent conjugation may be accomplished by direct or indirect means, including hydrophobic interaction, ionic interaction, intercalation, binding to major or minor grooves of a nucleic acid, and other affinity interactions.

[00138] Covalent linkages may be noncleavable in physiological environments, or cleavable in physiological environments, such as linkers containing disulfide bonds. Such molecules may resist degradation and/or may be subject to different intracellular transport mechanisms. One of ordinary skill in the art will be able to ascertain, without undue experimentation, the preferred bond for linking the targeting moiety and the compositions of the invention, based on the chemical properties of the molecules being linked and the preferred characteristics of the bond, for a given application.

[00139] For indirect linkage, the targeting moiety may be part of a particle, such as a liposome, which is targeted to a specific cell type. The liposome, in turn, may contain the compositions of the invention. The manufacture of liposomes containing compositions of the invention is fully described in the literature. Many for example, are based upon cholesteric molecules as starting ingredients and/or phospholipids. They may be synthetically derived or isolated from natural membrane components. Virtually any hydrophobic substance can be used, including cholesteric molecules, phospholipids and fatty acids preferably of medium chain length (i.e., 12 to 20 carbons), for example, naturally occurring fatty acids of between 14 and 18 carbons in length. These molecules can be attached to one or more compositions of the invention, for example, with the lipophilic anchor inserting into the membrane of a liposome and the compositions tethered on the surface of the liposome for targeting the liposome to the cell. In other cases, one or more compositions of the invention may be present in the interior of the liposome.

[00140] Each of the compositions described herein (or portions thereof) may optionally be associated with a delivery system or vector, according to one aspect of the invention. In its broadest sense, a "vector" is any vehicle capable of facilitating: (1) delivery of a composition to a target cell or (2) uptake of a composition by a target cell, if uptake is important. Optionally, a "targeting ligand" (in addition to, or the same as, the plasma membrane targeting

molecule) can be attached to the vector to selectively deliver the vector to a cell which expresses on its surface the cognate receptor for the targeting ligand. In this manner, the vector (containing one or more compositions of the invention) can be selectively delivered to a cell in, e.g., a tumor, a wound, etc. In general, the vectors useful in the invention are divided into two classes: colloidal dispersion systems and biological vectors. Other example compositions that can be used to facilitate uptake by a target cell of compositions of the invention include calcium phosphate and other chemical mediators of intracellular transport, microinjection compositions, and electroporation.

[00141] Suitable vectors include plasmid vectors. Plasmid vectors have been extensively described in the art and are well-known to those of skill in the art. See e.g., Sambrook, *et al.*, *Molecular Cloning: A Laboratory Manual*, Second Edition, Cold Spring Harbor Laboratory Press, 1989. Plasmid vectors have been found to be particularly advantageous for delivering genes to cells *in vivo* because of their inability to replicate within and integrate into a host genome. These plasmids, however, having a promoter compatible with the host cell, can express a peptide from a gene operatively encoded within the plasmid. Some commonly used plasmids include pBR322, pUC18, pUC19, pRC/CMV, SV40, and pBlueScript. Other plasmids are well-known to those of ordinary skill in the art. Additionally, plasmids may be custom designed using restriction enzymes and/or ligation reactions to remove and add specific fragments of DNA.

[00142] It has also been discovered that gene carrying plasmids can be delivered to the cells *in vivo* using bacteria. Modified forms of bacteria such as *Salmonella* can be transfected with the plasmid and can thus be used as delivery vehicles in some cases. The bacterial delivery vehicles can be administered to a host subject orally or by other administration means. The bacteria in some instances can pass through the gut barrier. High levels of expression have been established using this methodology.

[00143] Compaction agents also can be used alone, or in combination with, a vector of the invention. A "compaction agent," as used herein, refers to an agent, such as a histone, that neutralizes the negative charges on the nucleic acid and thereby permits compaction of the nucleic acid into a fine granule. Compaction of the nucleic acid facilitates the uptake of the nucleic acid by the target cell. The compaction agents can be used alone, i.e., to deliver the

compositions in a form that is more efficiently taken up by the cell or, more preferably, in combination with one or more of the above-described vectors.

[00144] In one aspect, the invention provides a method of administering any of the compositions described herein to a subject. When administered, the compositions are applied in a therapeutically effective, pharmaceutically acceptable amount as a pharmaceutically acceptable formulation. As used herein, the term “pharmaceutically acceptable” is given its ordinary meaning. Pharmaceutically acceptable compositions are generally compatible with other materials of the formulation and are not generally deleterious to the subject. Any of the compositions of the present invention may be administered to the subject in a therapeutically effective dose. The dose to the subject may be such that a therapeutically effective amount of one or more active compounds reaches the active site(s) within the subject. A “therapeutically effective” or an “effective” dose, as used herein, means that amount necessary to delay the onset of, inhibit the progression of, halt altogether the onset or progression of, diagnose a particular condition being treated, or otherwise achieve a medically desirable result, i.e., that amount which is capable of at least partially preventing, reversing, reducing, decreasing, ameliorating, or otherwise suppressing the particular condition being treated. A therapeutically effective amount can be determined on an individual basis and will be based, at least in part, on consideration of the species of mammal, the mammal’s age, sex, size, and health; the composition used, the type of delivery system used; the time of administration relative to the severity of the disease; and whether a single, multiple, or controlled-release dose regiment is employed. A therapeutically effective amount can be determined by one of ordinary skill in the art employing such factors and using no more than routine experimentation.

[00145] The terms “treat,” “treated,” “treating,” and the like, when used herein, refer to administration of the systems and methods of the invention to a subject, which may, for example, increase the resistance of the subject to development or further development of cancers, to eliminate or at least control a cancer or a wound, and/or to reduce the severity of the cancer or wound. The pharmaceutical preparations of the invention are administered to subjects in effective amounts. When administered to a subject, effective amounts will depend on the particular condition being treated and the desired outcome. A therapeutically effective dose may be determined by those of ordinary skill in the art, for instance, employing factors such as those further described below and using no more than routine experimentation.

[00146] In administering the systems and methods of the invention to a subject, dosing amounts, dosing schedules, routes of administration, and the like may be selected so as to affect known activities of these systems and methods. Dosage may be adjusted appropriately to achieve desired drug levels, local or systemic, depending upon the mode of administration. The doses may be given in one or several administrations per day. As one example, if daily doses are required, daily doses may be from about 0.01 mg/kg/day to about 1000 mg/kg/day, and in some embodiments, from about 0.1 to about 100 mg/kg/day or from about 1 mg/kg/day to about 10 mg/kg/day. Parental administration, in some cases, may be from one to several orders of magnitude lower dose per day, as compared to oral doses. For example, the dosage of an active compound, when parentally administered, may be between about 0.1 micrograms/kg/day to about 10 mg/kg/day, and in some embodiments, from about 1 microgram/kg/day to about 1 mg/kg/day or from about 0.01 mg/kg/day to about 0.1 mg/kg/day.

[00147] In some embodiments, the concentration of the active compound(s) of the composition, if administered systemically, is at a dose of about 1.0 mg to about 2000 mg for an adult of 70 kg body weight, per day. In other embodiments, the dose is about 10 mg to about 1000 mg/70 kg/day. In yet other embodiments, the dose is about 100 mg to about 500 mg/70 kg/day. If applied topically, the concentration may be about 0.1 mg to about 500 mg/g of ointment or other base, about 1.0 mg to about 100 mg/g of base, or about 30 mg to about 70 mg/g of base. The specific concentration partially depends upon the particular composition used, as some are more effective than others. The dosage concentration of the composition actually administered is dependent, at least in part, upon the particular disorder being treated, the final concentration of composition that is desired at the site of action, the method of administration, the efficacy of the particular composition, the longevity of the particular composition, and the timing of administration relative to the severity of the disease. Preferably, the dosage form is such that it does not substantially deleteriously effect the mammal.

[00148] The dosage may be given in some cases at the maximum amount while avoiding or minimizing any potentially detrimental side effects within the subject. The dosage actually administered can be dependent upon factors such as the final concentration desired at the active site, the method of administration to the subject, the efficacy of the composition, the longevity of the composition within the subject, the mode and/or timing of administration, the effect of concurrent treatments (e.g., as in a cocktail), etc. The dose delivered may also depend

on conditions associated with the subject, and can vary from subject to subject in some cases. For example, the age, sex, weight, size, environment, physical conditions, active site of the cancer or wound, or current state of health of the subject may also influence the dose required and/or the concentration of the composition at the active site. Variations in dosing may occur between different individuals or even within the same individual on different days. These factors are well known to those of ordinary skill in the art and can be addressed with no more than routine experimentation. It is preferred that a maximum dose be used, that is, the highest safe dose according to sound medical judgment.

[00149] In the event that the response of a particular subject is insufficient at such doses, even higher doses (or effectively higher doses by a different, more localized delivery route) may be employed to the extent that subject tolerance permits. Multiple doses per day are also contemplated, in some cases, to achieve appropriate systemic levels within the subject or within the active site of the subject. In certain instances, dosing amounts, dosing schedules, routes of administration, and the like may be selected as described herein, whereby therapeutically effective levels of the composition are provided.

[00150] In certain embodiments where cancers are being treated, a composition of the invention is administered to a subject who has a family history of cancer, or to a subject who has a genetic predisposition for cancer. In other embodiments, the composition is administered to a subject who has reached a particular age, or to a subject more likely to get cancer. In yet other embodiments, the compositions is administered to subjects who exhibit symptoms of cancer (e.g., early or advanced). In still other embodiments, the composition may be administered to a subject as a preventive measure. In some embodiments, the inventive composition may be administered to a subject based on demographics or epidemiological studies, or to a subject in a particular field or career.

[00151] Administration of a composition of the invention to a subject may be accomplished by any medically acceptable method which allows the composition to reach its target. The particular mode selected will depend of course, upon factors such as those previously described, for example, the particular composition, the severity of the state of the subject being treated, the dosage required for therapeutic efficacy, etc. As used herein, a "medically acceptable" mode of treatment is a mode able to produce effective levels of the active compound(s) of the composition within the subject without causing clinically unacceptable

adverse effects. The administration may be localized (i.e., to a particular region, physiological system, tissue, organ, or cell type) or systemic, depending on the condition being treated. For example, the composition may be administered orally, vaginally, rectally, buccally, pulmonary, topically, nasally, transdermally, through parenteral injection or implantation, via surgical administration, or any other method of administration where suitable access to a target is achieved. Examples of parenteral modalities that can be used with the invention include intravenous, intradermal, subcutaneous, intracavity, intramuscular, intraperitoneal, epidural, or intrathecal. Examples of implantation modalities include any implantable or injectable drug delivery system. Oral administration may be preferred in some embodiments because of the convenience to the subject as well as the dosing schedule. Compositions suitable for oral administration may be presented as discrete units such as hard or soft capsules, pills, cachettes, tablets, troches, or lozenges, each containing a predetermined amount of the composition. Other oral compositions suitable for use with the invention include solutions or suspensions in aqueous or non-aqueous liquids such as a syrup, an elixir, or an emulsion. In one set of embodiments, the composition may be used to fortify a food or a beverage.

[00152] Injections can be e.g., intravenous, intradermal, subcutaneous, intramuscular, or interperitoneal. For example, the inhibitor can be injected intravenously or intramuscularly for the treatment of multiple sclerosis, or can be injected directly into the joints for treatment of arthritic disease, or can be injected directly into the lesions for treatment of pemphigus vulgaris. The composition can be injected interdermally for treatment or prevention of infectious disease, for example. In some embodiments, the injections can be given at multiple locations. Implantation includes inserting implantable drug delivery systems, e.g., microspheres, hydrogels, polymeric reservoirs, cholesterol matrixes, polymeric systems, e.g., matrix erosion and/or diffusion systems and non-polymeric systems, e.g., compressed, fused, or partially-fused pellets. For systemic administration, it may be useful to encapsulate the composition in liposomes.

[00153] Inhalation includes administering the composition with an aerosol in an inhaler, either alone or attached to a carrier that can be absorbed.

[00154] In general, the compositions of the invention may be delivered using a bioerodible implant by way of diffusion, or more preferably, by degradation of the polymeric matrix. Exemplary synthetic polymers which can be used to form the biodegradable delivery system include: polyamides, polycarbonates, polyalkylenes, polyalkylene glycols, polyalkylene

oxides, polyalkylene terephthalates, polyvinyl alcohols, polyvinyl ethers, polyvinyl esters, polyvinyl halides, polyvinylpyrrolidone, polyglycolides, polysiloxanes, polyurethanes and copolymers thereof, alkyl cellulose, hydroxyalkyl celluloses, cellulose ethers, cellulose esters, nitro celluloses, polymers of acrylic and methacrylic esters, methyl cellulose, ethyl cellulose, hydroxypropyl cellulose, hydroxypropyl methyl cellulose, hydroxybutyl methyl cellulose, cellulose acetate, cellulose propionate, cellulose acetate butyrate, cellulose acetate phthalate, carboxylethyl cellulose, cellulose triacetate, cellulose sulphate sodium salt, poly(methyl methacrylate), poly(ethyl methacrylate), poly(butylmethacrylate), poly(isobutyl methacrylate), poly(hexylmethacrylate), poly(isodecyl methacrylate), poly(lauryl methacrylate), poly(phenyl methacrylate), poly(methyl acrylate), poly(isopropyl acrylate), poly(isobutyl acrylate), poly(octadecyl acrylate), polyethylene, polypropylene, poly(ethylene glycol), poly(ethylene oxide), poly(ethylene terephthalate), poly(vinyl alcohols), polyvinyl acetate, poly vinyl chloride, polystyrene, polyvinylpyrrolidone, and polymers of lactic acid and glycolic acid, polyanhydrides, poly(ortho)esters, poly(butic acid), poly(valeric acid), and poly(lactide-co-caprolactone), and natural polymers such as alginate and other polysaccharides including dextran and cellulose, collagen, chemical derivatives thereof (substitutions, additions of chemical groups, for example, alkyl, alkylene, hydroxylations, oxidations, and other modifications routinely made by those of ordinary skill in the art), albumin and other hydrophilic proteins, zein and other prolamines and hydrophobic proteins, copolymers and mixtures thereof. In general, these materials degrade either by enzymatic hydrolysis or exposure to water *in vivo*, by surface or bulk erosion. Examples of non-biodegradable polymers include ethylene vinyl acetate, poly(meth)acrylic acid, polyamides, copolymers and mixtures thereof.

[00155] Bioadhesive polymers of particular interest in some cases include, but are not limited to, the bioerodible hydrogels described by Sawhney, *et al.*, *Macromolecules*, 26:581-587, 1993, the teachings of which are incorporated herein, as well as polyhyaluronic acids, casein, gelatin, gluten, polyanhydrides, polyacrylic acid, alginate, chitosan, poly(methyl methacrylates), poly(ethyl methacrylates), poly(butylmethacrylate), poly(isobutyl methacrylate), poly(hexylmethacrylate), poly(isodecyl methacrylate), poly(lauryl methacrylate), poly(phenyl methacrylate), poly(methyl acrylate), poly(isopropyl acrylate), poly(isobutyl acrylate), and poly(octadecyl acrylate).

[00156] The systems and methods of the invention can be administered by any method which allows the composition of the invention to reach the target cells, e.g., tumor cells. These methods include, e.g., injection, infusion, deposition, implantation, anal or vaginal supposition, oral ingestion, inhalation, topical administration, or any other method of administration where access to the target cells by the inhibitor is obtained. In some embodiments, topical administration is preferred, due to the high concentration of APCs in the skin. One method for accomplishing topical administration includes transdermal administration, such as iontophoresis. Iontophoretic transmission can be accomplished by using commercially-available patches which deliver a compound continuously through unbroken skin for periods of hours to days to weeks, depending on the particular patch. This method allows for the controlled delivery of the composition through the skin in relatively high concentrations. One example of an iontophoretic patch is the LECTRO PATCHTM sold by General Medical Company of Los Angeles, CA. The patch provides dosages of different concentrations which can be continuously or periodically administered across the skin using electronic stimulation of reservoirs containing the composition. Topical administration also includes epidermal administration which involves the mechanical or chemical irritation of the outermost layer of the epidermis sufficiently to provoke an immune response to the irritant. The irritant attracts APCs to the site of irritation where they can then take up the composition. One example of a mechanical irritant is a tyne-containing device. Such a device contains tynees which irritate the skin and deliver the drug at the same time, for instance, the MONO VACCTM manufactured by Pasteur Merieux of Lyon, France. The device contains a syringe plunger at one end and a tyne disk at the other. The tyne disk supports several narrow diameter tynees which are capable of scratching the outermost layer of epidermal cells. Chemical irritants include, for instance, keratinolytic agents, such as salicylic acid, and can be used alone or in conjunction with other irritants such as mechanical irritants.

[00157] In certain embodiments of the invention, the administration of the composition of the invention may be designed so as to result in sequential exposures to the composition over a certain time period, for example, hours, days, weeks, months, or years. This may be accomplished, for example, by repeated administration of a composition of the invention by one of the methods described above, and/or by a sustained or controlled release delivery system in which the composition is delivered over a prolonged period, usually without repeated administrations. Administration of the composition using such a delivery system may be, for

example, by oral dosage forms, bolus injections, transdermal patches or subcutaneous implants. Maintaining a substantially constant concentration of the composition may be desirable in some cases.

[00158] Other delivery systems suitable for use with the present invention include time-release, delayed release, sustained release, or controlled release delivery systems. Such systems may avoid repeated administrations in many cases, increasing convenience to the subject and the physician. Many types of release delivery systems are available and known to those of ordinary skill in the art. They include, for example, polymer-based systems such as polylactic and/or polyglycolic acids, polyanhydrides, polycaprolactones, copolyoxalates, polyesteramides, polyorthoesters, polyhydroxybutyric acid, and/or combinations of these. Microcapsules of the foregoing polymers containing drugs are described in, for example, U.S. Patent 5,075,109. Other examples include nonpolymer systems that are lipid-based including sterols such as cholesterol, cholesterol esters, and fatty acids or neutral fats such as mono-, di- and triglycerides, hydrogel release systems, liposome-based systems, phospholipid based-systems, silastic systems, peptide based systems, wax coatings, compressed tablets using conventional binders and excipients, or partially fused implants. Specific examples include, but are not limited to, erosional systems in which the composition is contained in a form within a matrix (for example, as described in U.S. Patent Nos. 4,452,775, 4,675,189, 5,736,152, 4,667,014, 4,748,034 and 5,239,660), or diffusional systems in which an active component controls the release rate (for example, as described in U.S. Patent Nos. 3,832,253, 3,854,480, 5,133,974 and 5,407,686). The formulation may be present as, for example, microspheres, hydrogels, polymeric reservoirs, cholesterol matrices, or polymeric systems. In some embodiments, the system may allow sustained or controlled release of the composition to occur, for example, through control of the diffusion or erosion/degradation rate of the formulation containing the composition. In addition, a pump-based hardware delivery system may be used to deliver one or more embodiments of the invention in some cases.

[00159] Examples of systems in which release occurs in bursts includes, e.g., systems in which the composition is entrapped in liposomes which are encapsulated in a polymer matrix, the liposomes being sensitive to specific stimuli, e.g., temperature, pH, light or a degrading enzyme and systems in which the composition is encapsulated by an ionically-coated microcapsule with a microcapsule core-degrading enzyme. Examples of systems in which

release of the inhibitor is gradual and continuous include, e.g., erosional systems in which the composition is contained in a form within a matrix and effusional systems in which the composition permeates at a controlled rate, e.g., through a polymer. Such sustained release systems can be e.g., in the form of pellets, or capsules.

[00160] Use of a long-term release implant may be particularly suitable in some embodiments of the invention. "Long-term release," as used herein, means that the implant containing the composition is constructed and arranged to deliver therapeutically effective levels of the composition for at least 30 or 45 days, and preferably at least 60 or 90 days, or even longer in some cases. Long-term release implants are well known to those of ordinary skill in the art, and include some of the release systems described above.

[00161] In certain embodiments of the invention, a composition may include a suitable pharmaceutically acceptable carrier, for example, as incorporated into a liposome, incorporated into a polymer release system, or suspended in a liquid, e.g., in a dissolved form or a colloidal form, such as in a colloidal dispersion system. In general, pharmaceutically acceptable carriers suitable for use in the invention are wellknown to those of ordinary skill in the art. As used herein, a "pharmaceutically acceptable carrier" refers to a non-toxic material that does not significantly interfere with the effectiveness of the biological activity of the active compound(s) to be administered, but is used as a formulation ingredient, for example, to stabilize or protect the active compound(s) within the composition before use. The term "carrier" denotes an organic or inorganic ingredient, which may be natural or synthetic, with which one or more active compounds of the invention are combined to facilitate the application of the composition. The carrier may be co-mingled or otherwise mixed with one or more active compounds of the present invention, and with each other, in a manner such that there is no interaction which would substantially impair the desired pharmaceutical efficacy. The carrier may be either soluble or insoluble, depending on the application. Examples of well-known carriers include glass, polystyrene, polypropylene, polyethylene, dextran, nylon, amylase, natural and modified cellulose, polyacrylamide, agarose and magnetite. The nature of the carrier can be either soluble or insoluble. Those skilled in the art will know of other suitable carriers, or will be able to ascertain such, using only routine experimentation.

[00162] As used herein, a "colloidal dispersion system" refers to a natural or synthetic molecule, other than those derived from bacteriological or viral sources, capable of delivering to

and releasing the composition in a subject. Colloidal dispersion systems include macromolecular complexes, nanocapsules, microspheres, beads, and lipid-based systems including oil-in-water emulsions, micelles, mixed micelles, and liposomes. A preferred colloidal system of the invention is a liposome. Liposomes are artificial membrane vessels which are useful as a delivery vector *in vivo* or *in vitro*. It has been shown that large unilamellar vessels (LUV), which range in size from 0.2 micrometers to 4.0 micrometers can encapsulate large macromolecules within the aqueous interior and these macromolecules can be delivered to cells in a biologically active form (Fraley, *et al.*, *Trends Biochem. Sci.*, 6:77, 1981).

[00163] Lipid formulations for transfection are commercially available, e.g., from QIAGEN, for example as EFFECTENETM (a non-liposomal lipid with a special DNA condensing enhancer) and SUPER-FECTTM (a novel acting dendrimeric technology) as well as Gibco BRL, for example, as LIPOFECTINTM and LIPOFECTACETM, which are formed of cationic lipids such as N-[1-(2,3-dioleoyloxy)-propyl]-N,N,N-trimethylammonium chloride (DOTMA) and dimethyl dioctadecylammonium bromide (DDAB). Methods for making liposomes are well known in the art and have been described in many publications. Some liposomes were described in a review article by Gregoriadis, *Trends in Biotechnol.*, 3:235-241, 1985, which is hereby incorporated by reference.

[00164] In one embodiment, the vehicle is a biocompatible microparticle or implant that is suitable for implantation into the mammalian recipient. Exemplary bioerodible implants that are useful in accordance with this method are described in PCT International Application No. PCT/US/03307 (Publication No. WO 95/24929, entitled "Polymeric Gene Delivery System." PCT/US/0307 describes a biocompatible, preferably biodegradable polymeric matrix for containing an exogenous gene under the control of an appropriate promoter. The polymeric matrix is used to achieve sustained release of the exogenous gene in the subject. In accordance with the present invention, the compositions of the invention described herein can be encapsulated or dispersed within the biocompatible, optionally biodegradable polymeric matrix disclosed in PCT/US/03307.

[00165] The polymeric matrix can be in the form of a microparticle such as a microsphere (where the composition is dispersed throughout a solid polymeric matrix) or a microcapsule (where the composition is stored in the core of a polymeric shell). Other forms of the polymeric matrix for containing the composition include films, coatings, gels, implants, and

stents. The size and composition of the polymeric matrix device can be selected to result in favorable release kinetics in the tissue into which the matrix is introduced. The size of the polymeric matrix can also be selected according to the method of delivery which is to be used, typically injection into a tissue or administration of a suspension by aerosol into the nasal and/or pulmonary areas. When an aerosol route is used the polymeric matrix and composition can be encompassed in a surfactant vehicle. The polymeric matrix composition can be selected to have both favorable degradation rates and/or to be formed of a material which is bioadhesive, e.g., to further increase the effectiveness of transfer when the matrix is administered to a nasal and/or pulmonary surface that has sustained an injury. The matrix composition can also be selected not to degrade, but rather, to release by diffusion over an extended period of time. In another embodiment, the matrix is a biocompatible microsphere that is suitable for oral delivery. Such microspheres are disclosed in Chickering, *et al.*, *Biotech. and Bioeng.*, 52:96-101, 1996, and Mathiowitz, *et al.*, *Nature*, 386:410-414, 1997.

[00166] Both non-biodegradable and biodegradable polymeric matrices can be used to deliver the compositions of the invention to the subject. Such polymers may be natural or synthetic polymers. The polymer may be selected based on the period of time over which release is desired, generally in the order of a few hours, to a year or longer. Typically, release over a period ranging from between a few hours and three to twelve months is desirable. The polymer optionally is in the form of a hydrogel that can absorb up to about 90% of its weight in water and further, optionally is cross-linked with multivalent ions or other polymers.

[00167] In some embodiments, the compositions of the invention may include pharmaceutically acceptable carriers with formulation ingredients such as salts, carriers, buffering agents, emulsifiers, diluents, excipients, chelating agents, fillers, drying agents, antioxidants, antimicrobials, preservatives, binding agents, bulking agents, silicas, solubilizers, or stabilizers. For example, if the formulation is a liquid, the carrier may be a solvent, partial solvent, or non-solvent, and may be aqueous or organically based. Examples of suitable formulation ingredients include diluents such as calcium carbonate, sodium carbonate, lactose, kaolin, calcium phosphate, or sodium phosphate; granulating and disintegrating agents such as corn starch or algenic acid; binding agents such as starch, gelatin or acacia; lubricating agents such as magnesium stearate, stearic acid, or talc; time-delay materials such as glycerol monostearate or glycerol distearate; suspending agents such as sodium carboxymethylcellulose,

methylcellulose, hydroxypropylmethylcellulose, sodium alginate, polyvinylpyrrolidone; dispersing or wetting agents such as lecithin or other naturally-occurring phosphatides; thickening agents such as cetyl alcohol or beeswax; buffering agents such as acetic acid and salts thereof, citric acid and salts thereof, boric acid and salts thereof, or phosphoric acid and salts thereof; or preservatives such as benzalkonium chloride, chlorobutanol, parabens, or thimerosal. Suitable carrier concentrations can be determined by those of ordinary skill in the art, using no more than routine experimentation. The compositions of the invention may be formulated into preparations in solid, semi-solid, liquid or gaseous forms such as tablets, capsules, elixirs, powders, granules, ointments, solutions, depositories, inhalants or injectables. Those of ordinary skill in the art will know of other suitable formulation ingredients, or will be able to ascertain such, using only routine experimentation.

[00168] Preparations include sterile aqueous or nonaqueous solutions, suspensions and emulsions, which can be isotonic with the blood of the subject in certain embodiments. Examples of nonaqueous solvents are polypropylene glycol, polyethylene glycol, vegetable oil such as olive oil, sesame oil, coconut oil, arachis oil, peanut oil, mineral oil, injectable organic esters such as ethyl oleate, or fixed oils including synthetic mono or di-glycerides. Aqueous carriers include water, alcoholic/aqueous solutions, emulsions or suspensions, including saline and buffered media. Parenteral vehicles include sodium chloride solution, 1,3-butandiol, Ringer's dextrose, dextrose and sodium chloride, lactated Ringer's or fixed oils. Intravenous vehicles include fluid and nutrient replenishers, electrolyte replenishers (such as those based on Ringer's dextrose), and the like. Preservatives and other additives may also be present such as, for example, antimicrobials, antioxidants, chelating agents and inert gases and the like. In addition, sterile, fixed oils are conventionally employed as a solvent or suspending medium. For this purpose, any bland fixed oil may be employed including synthetic mono- or di-glycerides. In addition, fatty acids such as oleic acid may be used in the preparation of injectables. Carrier formulation suitable for oral, subcutaneous, intravenous, intramuscular, etc. administrations can be found in, for example, *Remington's Pharmaceutical Sciences*, Mack Publishing Co. Those of ordinary skill in the art can readily determine the various parameters for preparing and formulating the compositions of the invention without resort to undue experimentation.

[00169] In some embodiments, the present invention includes the step of forming a composition of the invention by bringing an active compound into association or contact with a

suitable carrier, which may constitute one or more accessory ingredients. The final composition may be prepared by any suitable technique, for example, by uniformly and intimately bringing the composition into association with a liquid carrier, a finely divided solid carrier or both, optionally with one or more formulation ingredients as previously described, and then, if necessary, shaping the product.

[00170] In some embodiments, the compositions of the present invention may be present as pharmaceutically acceptable salts. The term “pharmaceutically acceptable salts” includes salts of the composition, prepared in combination with, for example, acids or bases, depending on the particular compounds found within the composition and the treatment modality desired. Pharmaceutically acceptable salts can be prepared as alkaline metal salts, such as lithium, sodium, or potassium salts; or as alkaline earth salts, such as beryllium, magnesium or calcium salts. Examples of suitable bases that may be used to form salts include ammonium, or mineral bases such as sodium hydroxide, lithium hydroxide, potassium hydroxide, calcium hydroxide, magnesium hydroxide, and the like. Examples of suitable acids that may be used to form salts include inorganic or mineral acids such as hydrochloric, hydrobromic, hydroiodic, hydrofluoric, nitric, carbonic, monohydrogencarbonic, phosphoric, monohydrogenphosphoric, dihydrogenphosphoric, sulfuric, monohydrogensulfuric, phosphorous acids and the like. Other suitable acids include organic acids, for example, acetic, propionic, isobutyric, maleic, malonic, benzoic, succinic, suberic, fumaric, mandelic, phthalic, benzenesulfonic, *p*-tolylsulfonic, citric, tartaric, methanesulfonic, glucuronic, galacturonic, salicylic, formic, naphthalene-2-sulfonic, and the like. Still other suitable acids include amino acids such as arginate, aspartate, glutamate, and the like.

[00171] In one aspect, the present invention provides any of the above-mentioned compositions in kits, optionally including instructions for use of the composition e.g., for the treatment of cancers or wounds. The “kit” typically defines a package including one or more compositions of the invention and the instructions, or homologs, analogs, derivatives, enantiomers and functionally equivalent compositions thereof. That is, the kit can include a description of use of the composition for participation in any biological or chemical mechanism disclosed herein associated with cancers or wounds. The kits can further include a description of activity of the cancers or wounds in treating the pathology, as opposed to the symptoms. The kit can include a description of use of the compositions as discussed herein. The kit also can include

instructions for use of a combination of two or more compositions of the invention, or instruction for use of a combination of a composition of the invention and one or more other compounds indicated for treatment of a cancer, a wound, etc. Instructions also may be provided for administering the composition by any suitable technique as previously described, for example, orally, intravenously, pump or implantable delivery device, or via another known route of drug delivery. The instructions may be of any form provided in connection with the composition in a manner such that a clinical professional will clearly recognize that the instructions are to be associated with the specific composition.

[00172] The kits described herein may also contain one or more containers, which may contain the inventive composition and other ingredients as previously described. The kits also may contain instructions for mixing, diluting, and/or administering the compositions in some cases. The kits also can include other containers with one or more solvents, surfactants, preservative and/or diluents (e.g., normal saline (0.9% NaCl), or 5% dextrose) as well as containers for mixing, diluting or administering the components to a subject in need of such treatment.

[00173] The compositions of the kit may be provided as any suitable form, for example, as liquid solutions or as dried powders. When the composition provided is a dry powder, the composition may be reconstituted by the addition of a suitable solvent, which may also be provided. In embodiments where liquid forms of the composition are used, the liquid form may be concentrated or ready to use. The solvent will depend on the active compound(s) within the composition and the mode of use or administration. Suitable solvents are well known, for example as previously described, and are available in the literature. The solvent will depend on the compound and the mode of use or administration.

[00174] The invention also involves, in another aspect, promotion of the treatment of cancers, wounds, etc. according to any of the systems or methods described herein. In some embodiments, one or more compositions of the invention may be promoted for treatment of cancers or wounds, or include instructions for treatment of cancers or wounds. In some cases, the invention provides a method involving promoting the prevention or treatment of cancers, wounds, etc. via administration of any one of the compositions of the present invention, and homologs, analogs, derivatives, enantiomers and functionally equivalent compositions thereof in which the invention is able to treat cancer, wounds, etc. As used herein, "promoted" includes all

methods of doing business including methods of education, hospital and other clinical instruction, pharmaceutical industry activity including pharmaceutical sales, and any advertising or other promotional activity including written, oral and electronic communication of any form, associated with compositions of the invention in connection with treatment of cancers or wounds. "Instructions" can define a component of promotion, and typically involve written instructions on or associated with packaging of compositions of the invention. Instructions also can include any oral or electronic instructions provided in any manner.

Examples

[00175] The following examples illustrate the invention with the use of a fatty acid metabolism inhibitor, a glycolytic inhibitor, and combinations thereof, alone or in combination with a chemotherapeutic, to produce changes in immunologically important co-stimulatory levels, and induce tumor cell death by the action of the immune system. The examples demonstrate that treatment of some cancer cells by etomoxir and chemotherapeutics produces substantially enhanced co-stimulatory signals, and that the treatment of cancer cells by chemotherapeutics and immune system involvement results in substantially higher membrane potential and levels of intracellular reactive intermediates—thereby resulting in tumor cell death. The examples also demonstrate efficacious, synergistic combinations of the foregoing inhibitors,

Materials and Methods.

[00176] Except to the extent stated otherwise in the examples, materials and methods used in the examples are as follows:

[00177] **Cell Culture.** All cell lines were cultured in RPMI 1640 culture medium. The medium is supplemented with 5% fetal bovine serum (FBS), 2mM L-Glutamine, 500 units/mL penicillin/500 µg/mL of streptomycin, 10 mM HEPES Buffer, 10^{-5} M 2-mercaptoethanol (2-ME), 1 mM MEM Sodium Pyruvate, and .04 µg/mL of Gentamicin (All reagents from Gibco BRL). Cells were maintained at 37°C in a humidified atmosphere under 5% CO₂ in air.

[00178] **Flow Cytometry.** Cells were harvested, counted, and resuspended at 10^6 cells/ 100 µl of PBS containing 2.5% fetal calf serum in preparation for flow cytometric analysis. Cells were stained for intracellular H₂O₂ using 6-carboxy-2',7'-

dichlorodihydrofluorescein diacetate (DCF-DA, Molecular Probes, Eugene, Oregon). Briefly, cells were incubated at 37° with 1 mM DCF-DA for 20 minutes, washed twice in PBS containing 2.5 % fetal calf serum and analyzed flow cytometrically. Mitochondrial membrane potential was assessed using Mitotracker Red (CM-H₂XROS, Molecular Probes, Eugene, Oregon). The cells were resuspended in PBS containing 2.5% fetal calf serum containing a final concentration of 0.5 micromolar Mitotracker dye. The cells were incubated at 37° for 20 minutes, washed twice in PBS containing 2.5 % fetal calf serum and analyzed flow cytometrically. Data were acquired on a Coulter Elite Epics or Excel flow cytometer (Coulter, Hialeah, Florida) and analyzed with CellQuest software, (Becton Dickinson, San Jose, California). The Coulter Epics Elite flow cytometer has a single excitation wavelength (488 nm) and band filters for PE (575 nm), FITC (525 nm) and Red613 (613 nm) that was used to analyze the stained cells. Each sample population was classified for cell size (forward scatter) and complexity (side scatter), gated on a population of interest and evaluated using 5,000 cells. Each figure describing flow cytometric data represents one of at least four replicate experiments.

[00179] **Cell Counting.** Cells were harvested and resuspended in 1mL of RPMI medium. A 1:20 dilution of the cell suspension was made by using 50 µL of trypan blue (Sigma chemicals), 45µL of Phosphate Buffered Saline (PBS) supplemented with 2.5% FBS, and 5µL of the cell suspension. Live cells were counted using a hemacytometer and the following calculation was used to determine cell number: Average # of Cells x Dilution x 10⁴.

[00180] **Preparation of Cell for Staining.** For staining protocols, between 0.5 X 10⁶ and 1.0 X 10⁶ cells were used; all staining was done in a 96-well U-bottom staining plate. Cells were harvested by centrifugation for 5 minutes at 300 x g, washed with PBS/2.5% FBS, and resuspended into PBS/2% FBS for staining. Cells were plated into wells of a labeled 96-well plate in 100 µL of PBS/2.5% FBS.

[00181] **Cell Surface Staining.** Non-permeabilized cells were stained with antibodies to the cell surface receptors Fas (CD95) (Pharmingen) or with antibodies to uncoupling proteins (anti-UCP2 antibody) (Alpha Diagnostic International). Antibodies for both the isotype control and actual stain were added to the cell suspension, mixed, and then placed on ice for and incubation of 25 minutes in the dark. Subsequently the cells were centrifuged at 300 xg for 5 minutes and the supernatant removed. The cells were washed one time with 100 µL of

PBS/2.5% FBS and then transferred into flow cytometric tubes containing 500 μ L of PBS/2.5% FBS for analysis.

[00182] **Metabolic Activity Assay.** Cells were prepared as previously described. The specific metabolic dye was added and mixed into the cell suspension. This plate was then placed into the 37° incubator for a 20-minute incubation. After incubation, the cells were centrifuged at 300 xg for 5 minutes, and the supernatant was removed. The cells were then washed once with PBS/2.5% FBS and transferred into flow cytometric tubes containing 500 μ L of PBS/2.5% FBS for analysis

[00183] **MitoTracker Red CM-H₂XROS (Molecular Probes).** One vial of MitoTracker Red provides 10 tests and contains 50 μ g. This is unstable and can't be stored. Therefore, when each vial was opened, 43 μ L of Dimethylsulfoxide (DMSO) were added. Once this was mixed well, 4.0 μ L of this was added to each well containing the cells to be tested for their mitochondrial membrane potential. MitoTracker was used at a final concentration of 46 ng for each test.

[00184] **5-(and-6)-Chloromethyl-2', 7'-dichlorodihydrofluorescein diacetate (CMH₂DCFda) (Molecular Probes).** One vial of DCFda provides 10 tests and contains 50 μ g. This is unstable and can't be stored. Therefore, each vial was opened and 43 μ L of DMSO was added. Once this was mixed well, 4.0 μ L of this was added to each well containing the cells to be tested. DCFda was used at a final concentration of 46 ng for each test.

[00185] **Dot plots as a function of live versus dead cells.** The cells were cultured at a concentration of .5 to 1 million cells per ml. Cells were untreated or treated with etomoxir or 2-deoxy-D-glucose at a concentration of 2.5 mM at the indicated concentrations for either 24 or 48 hours. In additional experiments, under each of these culture conditions, the cells were cultured for 24 hours at which time point, polyclonal anti-UCP-2 was added at a 1:1000 dilution of stock provided by Alpha Diagnostics, San Antonio, Texas. Cells were harvested and analyzed by flow cytometry. Each dot on the dot plots of Figures 1, 3, 5, 7, 9, and 11 represents one cell. Five thousand cells were assessed for forward scatter (FS), as a function of cell size, versus side scatter (SS), as a function of cellular granularity. The upper ellipse represents live cells by these criterion and the lower ellipse in each dot plot represents the dead cells.

[00186] **Statistical Analysis, Percents, and Geometric Mean Values Percents.** Gating is a tool provided by Cell Quest software and allows for the analysis of a certain

population of cells. Gating around both the live and dead cell populations gave a percent of the cell numbers that was in each population. After the gates were drawn, a percent value of dead cells was calculated by taking the number of dead cells divided by the number of total cells and multiplying by one hundred.

[00187] **Standard Error.** When experiments were done in triplicate, a standard error of the mean value was determined using the Excel program (Microsoft). This identified the value given for the error bars seen on some figures.

[00188] **Geometric Mean Fluorescence.** When analyzing data on Cell Quest software, a geometric mean value will be given for each histogram plotted. Once the stained sample was plotted against the control (isotype or unstained), geometric mean fluorescence values were obtained for both histogram peaks. The stained control sample value was subtracted from sample to identify the actual fluorescence of the stained sample over that of the control.

[00189] **Tumor implantation.** Mice were purchased from the Animal Production Program of National Cancer Institute, Frederick, Maryland. The animals used were Athymic Ncr- nu/nu (strain code 01B74). HL60 MDR cells were harvested and reconstituted at 1 million cells per 100 microliters of phosphate buffered saline. One million tumor cells per injection site were implanted subcutaneously on two sites of the animal's back. Treatments began at exactly seven days post tumor implantation. Tumor size/volume was monitored twice weekly using measurement calipers, with the formula $\text{Tumor Volume} = \pi \times (\text{short diameter})^2 \times (\text{long diameter})/6$.

[00190] **Mice experiments with fatty acid metabolism and glycolytic inhibitors.** Fatty acid metabolism inhibitors were exemplified by etomoxir at 200 micrograms/day. Glycolytic inhibitors were exemplified by 2-deoxy-D-glucose at 2.5mM in 100 microliters daily. The combination was administered daily, etomoxir at 200 micrograms and 2-deoxy-D-glucose at 2.5mM in 100 microliters. All drugs were administered intra-peritoneally. 5-Fluoro-uracil, at 20 mg per kilogram mouse, was injected intra-peritoneally once a week

Example 1

[00191] This example illustrates an embodiment of the invention, where multi-drug resistant cancer cells were exposed to compositions of the invention. Analysis of the cells was performed using flow cytometry. The flow cytometry experiments were performed as follows.

The cells were harvested, counted, and resuspended as described above. For DNA staining, the cells were washed and resuspended in ice-cold PBS and fixed by the dropwise addition of 95% ethanol. The cells were then incubated for 30 minutes on ice, washed, and resuspended in PBS containing 1% formaldehyde and 0.01% Tween. The cells were incubated with 50 units of DNase and washed with PBS containing 5% DNase. The cells were then incubated with anti DNA Fab (166, kind gift of Dr. Susan Wallace, University of Vermont), then washed and stained with a fluorescein conjugated second step anti-mouse immunoglobulin. The cells were then washed and resuspended in PBS/3% BSA/1% formaldehyde. The cells were stained for intracellular H₂O₂ using 6-carboxy-2',7'-dichlorodihydrofluorescein diacetate (DCF-DA, Molecular Probes, Eugene, Oregon). The cells were incubated with 1 mM DCF-DA for 20 minutes, washed twice in PBS containing 5% fetal calf serum and analyzed using flow cytometry. The mitochondrial membrane potential was assessed using MitoTracker Red (CM-H₂XROS, Molecular Probes, Eugene, Oregon). The cells were resuspended in warm (37 °C) PBS containing a final concentration of 0.5 micromolar of dye. The cells were then incubated for 30 minutes, pelleted, and resuspended in prewarmed medium for analysis. The data was acquired on a Coulter Elite Epics or Excel flow cytometer (Coulter, Hialeah, Florida) and analyzed with CellQuest software, (Becton Dickinson, San Jose, California). The Coulter Epics Elite flow cytometer used in this example had a single excitation wavelength (488 nm) and band filters for PE (575 nm), FITC (525 nm) and Red 613 (613 nm) that was used to analyze the stained cells. Each sample population was classified for cell size (forward scatter) and complexity (side scatter), gated on a population of interest and evaluated using 40,000 cells. Each figure describing flow cytometric data represents one of at least four replicate experiments.

[00192] All cell lines in this example were cultured in RPMI 1640 culture medium. The medium was supplemented with 5% fetal bovine serum ("FBS"), 2 mM L-Glutamine, 500 units/mL penicillin/500 micrograms/mL of streptomycin, 10 mM HEPES Buffer, 10⁻⁵ M 2-mercaptoethanol ("2-ME"), 1 mM MEM Sodium Pyruvate, and 0.04 micrograms/mL of Gentamicin (All reagents from Gibco BRL). The cells were maintained at 37 °C in a humidified atmosphere under 5% CO₂ in air using standard cell culture techniques known to those of ordinary skill in the art.

[00193] The cells were counted, prepared for staining, and the cell surfaces were stained, as described above. Techniques used to prepare the cells for intracellular staining are as

follows. The cells were prepared as described above with respect to cell surface staining. The cell membranes of the cells then was permeabilized using a Cytofix/Cytoperm kit (Pharmagin). 100 microliters of Cytofix solution was added to all the cell suspensions and mixed. The solutions were then placed on ice for an incubation time of 30 minutes in darkness. The cells were then washed twice with 100 microliters of 1X PermWash buffer, then resuspended into 100 microliters of 1X PermWash buffer for staining. The cells will be stained according to the cell surface staining protocol, described above. After staining and washing, the cells were then transferred into flow cytometric tubes containing 500 microliters of PBS/2% FBS for further analysis.

[00194] A metabolic activity assay was conducted and MitoTracker Red and 5-(and-6)-Chloromethyl-2',7'-dichlorodihydrofluorescein diacetate molecular probes were prepared, as described above. LysoSensor Green DND-189 molecular probes were provided by the manufacturer at a concentration of 1 mM in 50 microliters of DMSO. The LysoSensor Solution was thawed to room temperature immediately before use and 0.5 microliters was added to each well containing the cells to be tested. The LysoSensor Solution was used at a final concentration of 5nM for each test.

[00195] For all death assays between 0.5×10^6 and 1.0×10^6 cells were used. Cells were harvested and placed into a 48-well plate in 1 mL of RPMI medium. To a first well, no treatment was added. To a second well 20 microliters of 37% hydrochloric acid was added. To a third well 1 microliter of purified mouse IgG1 kappa isotype control NA/LE was added (to a final concentration of 1 microgram/mL). To a fourth well, 1 microliter of purified anti-human CD95 (Fas) antibody NA/LE was added (to a final concentration of 1 microgram/mL). These treatment groups were incubated at 37 °C for 24 hours. After the incubation, the cells were examined for viability using flow cytometry.

[00196] For all death assays between 0.5×10^6 and 1.0×10^6 cells were used. Cells were harvested and placed into a 48-well plate in 1 mL of RPMI medium. The plate was prepared for this assay by adding 10 micrograms/mL of purified mouse IgG1 kappa isotype control NA/LE to a control well and 10 micrograms/mL of purified CD95 (Fas) antibody NA/LE to an experimental well. This antibody dilution solution was plated in the control and experimental wells and incubated for 1 hour at 37 °C. The wells were then washed twice with 1 mL of PBS/2% FBS. The cell suspension was then added to the treated wells in complete RPMI

medium and placed into the 37 °C incubator for a 24-hour period. After the incubation, the cells were examined for viability using flow cytometry.

[00197] The flow cytometry experiments were performed as follows. Once the samples were prepared and transferred into flow cytometric tubes, the samples were analyzed on a Becton/Dickinson Flow Cytometer. For antibodies that were PE conjugated and for the MitoTracker Red experiments, a program for red-colored fluorochromes was utilized. For antibodies that were FITC-conjugated and for the DCFDA, LysoSensor, and LysoTracker experiments, a program for green-colored fluorochromes was used. Statistical analysis, percents, and geometric mean values percents were calculated as described above.

[00198] As the experiments were performed in triplicate, a standard error of the mean value was determined using the Excel program (Microsoft). This is shown as error bars in some of the figures.

[00199] The geometric mean fluorescence was determined as follows. When analyzing data on Cell Quest software, a geometric mean value was calculated for each histogram plotted. The stained sample was plotted against the control (isotype or unstained), and the geometric mean fluorescence values was obtained for both histogram peaks. The stained sample value was divided by the control sample to identify the actual fluorescence of the stained sample over that of the control.

[00200] Flow cytometry profiles from these experiments are shown in Figs. 2A-2D and 3A-3D. The experimental protocols and the % of cell death for these experiments are shown in Tables 1 and 2. In these experiments, multidrug resistant cancer cells (HL60 MDR and L1210 DDP) showed greater amounts of cell death after treatment with cerulenin, compared to control and non-multidrug resistant cancer cells.

Table 1

B16F1 % Death Data		
Treated or Not with 25ug/mL Cerulenin		
Time Frame	No Treatment	Treated
5.0 Hours	44	61
24 Hours	50	90
HL60 % Death Data		
Treated or Not with 25ug/mL Cerulenin		
Time Frame	No Treatment	Treated
2.0 Hours	10	11
24 Hours	7	44
HL60 MDR % Death Data		
Treated or Not with 25ug/mL Cerulenin		
Time Frame	No Treatment	Treated
2.0 Hours	6.3	13
24 Hours	6.1	99.6

Table 2

B16F1 % Death Data		
Treated or Not with 25ug/mL Cerulenin		
Time Frame	No Treatment	Treated
5.0 Hours	43	67
24 Hours	39	72
L1210 % Death Data		
Treated or Not with 25ug/mL Cerulenin		
Time Frame	No Treatment	Treated
7.0 Hours	1.7	27

B16F1 % Death Data		
24 Hours	2.3	89
L1210 DDP % Death Data		
Treated or Not with 25 ug/mL Cerulenin		
Time Frame	No Treatment	Treated
7.0 Hours	1.4	14
24 Hours	2	92

[00201] In Fig. 4, microscopy images of HL60 and HL60 MDR (“multi-drug resistant”) cells treated with cerulenin (“Treated”) and not treated with cerulenin (“No Strn”) are shown. Fig. 4A-4B show non-treated HL60 cells, while Fig. 4C shows HL60 cells treated with cerulenin. For the HL60 cells, large numbers of the cells survived treatment with cerulenin. In contrast, Fig. 4D shows non-treated HL60 MDR, and Figs. 4E and 4F show HL60 MDR cells treated with cerulenin. In these figures, exposure of drug-resistant HL60 MDR cells to cerulenin resulted in large numbers of cell death.

Example 2

[00202] In this example, *in vivo* experiments were performed on mice in accordance with one embodiment of the invention.

[00203] Highly carcinogenic B16F1 melanoma cells were either exposed (“treated”) or not exposed to cerulenin, then injected into male mice. Experimental protocols, data, and observations from these experiments are shown in Table 3. It was found that mice exposed to untreated melanoma cells developed lethal cancers within a few days. In contrast, mice exposed to melanoma cells treated with cerulenin did not develop lethal cancers during the experiment.

Table 3

Control Group

Mouse #1

Day 0 @ 2:45 pm injected with PBS only

Day 9 No apparent tumors active

Day 12 Picture – see Fig. 5A

Day 13 Still no apparent tumors still active

Day 14 Still no apparent tumors still active

Mouse #2

Day 0 @ 2:45 pm injected with PBS only

Day 9 No apparent tumors – active

Day 13 Still no apparent tumors-active

Day 14 Still no apparent tumors still active

Mouse #3

Day 0 @ 2:45 pm injected with PBS only

Day 9 no apparent tumors – active

Day 13 still no apparent tumors-active (Fig. 5B)

Day 14 Still no apparent tumors still active

No treatment group

Mouse #4

Day 0 @ 2:45 pm injected with B16F1 10^6 cells in PBS, No Treatment

Day 9 possible start of tumor behind R shoulder and upper R leg - relatively active

Day 10 tumor development lower belly near left leg - still active

Day 12 tumor progressing inactive possibly in process of dying

Day 13 Very inactive, labored breathing, close to death

Mouse #5

Day 0 @ 2:45 pm injected with B16F1 10^6 cells with PBS, No Treatment

Day 9 no apparent tumors – active

Day 10 Tumor developing

Day 12 tumor still progressing still active

Day 13 tumor same, still active

Mouse #6

Day 0 @ 2:45 pm injected B16F1 10^6 cells with PBS, No Treatment

Day 9 tumor developing lower belly, near genitals left side – active

Day 12 tumor still progressing also second tumor appeared on right side—still active (Fig. 5C)

Day 13 Tumors larger, beginning to be inactive

Treatment group

Mouse #7

Day 0 @ 2:45 pm injected with B16F1 10^6 cells in PBS, Treatment w/ 25 ug/ml cerulenin

5.0 hrs

Day 9 no visible tumors - very active

Day 12 no visible tumors - very active

Day 13 no visible tumors - very active

Mouse #8

Day 0 injected w/ B16F1 10^6 cells in PBS, Treatment w/ 25 ug/ml cerulenin 5.0 h

Day 9 no visible tumors - very active

Day 12 no visible tumors - very active (Fig. 5D)

Day 13 no visible tumors - very active

Mouse #9

Day 0 @ 2:45 pm injected w/ B16F1 (10^6 cells in PBS) Treatment w/ 25ug/ml cerulenin

5 h

Day 9 no visible sign of tumors - very active

Day 12 no visible tumors - very active

Day 13 no visible tumors - very active

[00204] It was found that mice exposed to untreated melanoma cells developed lethal cancers within a few days. In contrast, mice exposed to melanoma cells treated with cerulenin did not develop lethal cancers during the experiment.

Example 3

[00205] Drug sensitive L1210 cells and drug resistant L12100/DDP cells were cultured in the presence of chemotherapeutic agents overnight. Figures 6A and 6B show the levels of cell-surface Fas expression on the drug sensitive L1210 cells (Figure 6A) and on the drug resistant L12100/DDP cells (Figure 6B), as measured by flow cytometry. The drug sensitive cells show increases in Fas as a result of the drug. In contrast, the drug resistant cells

are unchanged with treatment. The horizontal axis in each panel shows intensity of the fluorescence and the vertical axis shows the number of cells at each intensity. Peaks which appear farther to the right have higher levels of Fas per cell. Note that the drug sensitive cells have significantly higher levels of cell surface Fas.

Example 4

[00206] Drug sensitive L1210 cells and drug resistant L1210/DDP cells were cultured with etomoxir for 24 hours (Tx indicates treatment with etomoxir). The level of cell-surface Fas was determined using fluorochrome conjugated anti-Fas antibodies and flow cytometry. The Fas levels are measured relative to a control for staining artifacts. Percent values indicate % per cent dead cells. Figure 7 shows the expression of cell-surface Fas as a function of treatment with etomoxir. The etomoxir induced significant increases in cell surface Fas in both cell lines.

Example 5

[00207] In a third series of experiments, we used etomoxir with and without methotrexates to determine the effects of combination therapy on Fas expression, Figure 8.

[00208] In Figure 8, we see that etomoxir increases the methotrexate-induced increases in Fas on L1210 and promotes increases in cell surface Fas on methotrexate-treated L1210 DDP, drug resistant, tumor cells.

Example 6

[00209] The foregoing examples demonstrate that treatment of some cancer cells by etomoxir and chemotherapeutics produces substantially enhanced co-stimulatory signals, and that the treatment of cancer cells by chemotherapeutics and immune system involvement results in substantially higher membrane potential and levels of intracellular reactive intermediates—thereby resulting in tumor cell death. Thus, drug sensitive cancer cells and drug resistant tumor cells have been shown to have very different metabolic properties. As is well known, cell metabolism is largely governed by the action of the mitochondria. This, in turn, depends on some key parameters including the potential difference across the inner mitochondrial membrane and

the change in acidity across the same membrane. Figure 9 shows scatter diagrams for drug sensitive and drug resistant tumor cells in which the horizontal axis is a measure of the change in pH across the inner mitochondrial membrane and the vertical axis measures mitochondrial membrane potential, and indicates the range of both of these parameters. Figure 9 shows that the drug resistant cells, in general, have a lower membrane potential and the pH gradient is also significantly reduced for these cells. These data have been substantiated and extended to other cell lines (including HL60 and HL60/MDR) by other measurements, providing strong evidence that drug resistance is correlated with altered metabolic behavior.

Example 7

[00210] A second example of how drug resistant cells exhibit a different metabolic behavior compared to drug sensitive cells is provided by the rate of mitochondrial oleate consumption in the two different types of cells, L1210 and L1210 DDP. Figure 10 is a plot showing the rates of oleate oxidation with glucose competition and shows that the oxidation rates for drug resistant cells are generally higher than those for drug sensitive cells and this becomes more pronounced at low glucose levels. The drug resistant cells typically have higher levels of oleate consumption, and as glucose becomes limited, the rate of consumption increases dramatically in the drug resistant cells only. This demonstrates that the ability to "burn fat" is substantially different between the two types of cells. These data provided the rationale for the use of etomoxir as etomoxir has been reported to inhibit an enzyme, carnitine palmitoyl transferase (CPT) that is used when cells burn fat for fuel.

Example 8

[00211] We measured the effects of etomoxir on metabolism by measuring mitochondrial membrane potential with a fluorescent dye (Mitotracker Red, Molecular Probes, Eugene, Oregon) which fluoresces as a function of mitochondrial potential. We also measured levels of reactive intermediates using dichloro-difluoro-diacetate ester (DCF-da, Molecular Probes, Eugene, Oregon) which fluoresces as a function of intracellular reactive intermediates. We expected and, referring to Figure 11, found that etomoxir would increase the mitochondrial membrane potential of the drug resistant cells to a point that correlates with the potential of drug sensitive cells. Figure 11 is a chart showing the levels of mitochondrial membrane potential (by

MitoTracker) of L1210 DDP cells treated (or not) with methotrexate, etomoxir, or both, after 24 hours of incubation.

Example 9

[00212] Figure 12A is a chart showing the levels of DCFDA staining on L1210 cells and Figure 12B is a chart showing the levels of mitochondrial membrane potential (by MitoTracker) of L1210 cells, in each case treated (or not) with methotrexate, etomoxir, or both, after 24 hours of incubation. As expected etomoxir caused an increase in free radicals (reactive intermediates), an important aspect in susceptibility of the cells to immune mediated cell death.

Example 10

[00213] The effects of etomoxir on human leukemia RU937 cells was determined. Figure 13 is a chart showing the levels of mitochondrial membrane potential (by MitoTracker) of RU937 cells treated (or not) with methotrexate, etomoxir, or both, after 24 hours of incubation. Etomoxir increased the levels of mitochondrial membrane potential in human leukemia RU937.

Example 11

[00214] The effects of etomoxir on human leukemia HL60 cells was determined. Figure 14 is a chart showing the levels of DCFDA staining on HL60 cells treated (or not) with methotrexate or etomoxir after 24 hours of incubation. Etomoxir induces increases in reactive oxygen intermediates in human leukemia HL60.

Example 12

[00215] B16F1 melanoma cells were cultured at a concentration of 0.5 to 1 million cells per ml. Cells were untreated or treated with etomoxir at concentrations indicated in Figure 15 for 48 hours. Cells were harvested and analyzed by flow cytometry. Dot plots are shown in Figure 15 as functions of live versus dead cells. Each dot on the dot plot represents one cell. Five thousand cells were assessed for forward scatter (FS), as a function of cell size, versus side scatter (SS), as a function of cellular granularity. The upper ellipse represents live cells by these criterion and the lower ellipse in each dot plot represents the dead cells. Figure 16 shows the

percent death of the B16F1 melanoma cells, calculated by taking the number of dead cells divided by the total number of cells and multiplying by 100.

Example 13

[00216] Referring to Figures 17 and 18, B16F1 melanoma cells of Example 12 were untreated or treated with etomoxir at the indicated concentrations for 48 hours. Cells were harvested, stained with fluorochrome conjugated anti-Fas antibody (Pharmingen) and analyzed by flow cytometry. Results indicate etomoxir induced a dose dependent increase in cell surface Fas expression in the live cell (1b) or dead cell (1c) populations after 48 hours.

Example 14

[00217] B16F1 melanoma cells of Example 12 were untreated or treated with etomoxir at the indicated concentrations for 48 hours. Cells were harvested, stained with DCFDA as described in Materials and Methods, and analyzed by flow cytometry. The value of DCFDA was then calculated as described in Materials and Methods. Referring to Figure 19, etomoxir induced a dose dependent increase the reactive oxygen species as measured by DCFDA.

Example 15

[00218] B16F1 melanoma cells of Example 12 were untreated or treated with etomoxir at the indicated concentrations for 48 hours. Cells were harvested, stained with Mitotracker Red (Molecular Probes) as described in Materials and Methods, and analyzed by flow cytometry. The value of Mitotracker Red was then calculated as described in Materials and Methods. Referring to Figure 20, etomoxir induced an increase in mitochondrial membrane potential as measured by Mitotracker Red..

Example 16

[00219] C57black 6 mice were injected with 750,000 tumor cells intradermally in two locations on the back. One week later the animals were treated with etomoxir and taxol as indicated in Figure 21 and tumor size was monitored for three to four weeks. The results, as shown in Figure 21, indicate the effects of etomoxir at 50 μ g/dose and/or taxol at 480 μ g/dose on tumor size.

Example 17

[00220] HL60 cells were cultured at a concentration of 0.5 to 1 million cells per ml. Referring to Figure 22, cells were untreated or treated with etomoxir with or without adriamycin at the indicated concentrations for 24 to 72 hours. Cells were harvested and analyzed by flow cytometry. Results shown in Figure 22 represent percent death after 72 hours in etomoxir with or without 24 hours of adriamycin treatment.

Example 18

[00221] HL60MDR cells were cultured at a concentration of 0.5 to 1 million cells per ml. Referring to Figure 23, cells were untreated or treated with etomoxir with or without taxotere at the indicated concentrations for 24 to 72 hours. Cells were harvested and analyzed by flow cytometry. Results shown in Figure 23 represent per cent death after 72 hours in etomoxir with or without 24 hours of taxotere treatment.

Example 19

[00222] A204 human rhabdomyosarcoma cells were cultured at a concentration of 0.5 to 1 million cells per ml. Cells were untreated or treated with etomoxir at at concentrations indicated in Figure 24 for 24, 48, & 72 hours. Cells were harvested and analyzed by flow cytometry. Dot plots are shown in Figure 24 as functions of live versus dead cells. Each dot on the dot plot represents one cell. Five thousand cells were assessed for forward scatter (FS), as a function of cell size, versus side scatter (SS), as a function of cellular granularity. The upper ellipse represents live cells by these criterion and the lower ellipse in each dot plot represents the dead cells. Figure 25 shows the “fold” increases in death of the B16F1 melanoma cells.

Example 20

[00223] Cells were untreated or treated with etomoxir at the concentration indicated in Figure 26 at the indicated concentration for 24, 48, & 72 hours. Cells were harvested, stained with fluorochrome conjugated with anti-UCP2 antibody and analyzed by flow cytometry. Results shown in Figure 26 indicate etomoxir induced a time dependent “fold” increases in in cell surface UCP2 expresssion in the live cells.

Example 21

[00224] HL60 MDR human leukemia cells were cultured as described above under “Materials and Methods”. Cells were untreated or treated with etomoxir, with 2-deoxy-D-glucose, or with a combination of etomoxir and 2-deoxy-D-glucose, at concentrations indicated in Figure 27 for 24 hours. Cells were harvested and analyzed by flow cytometry. Dot plots are shown in Figure 27 as functions of live versus dead cells, as described above under “Materials and Methods” – “Dot plots as a function of live versus dead cells”. Figure 28 shows the percent death of the HL60 MDR cells, calculated as described above under “Materials and Methods” – “Statistical Analysis, Percents.” The percent of cell deaths for HL60 MDR cells after 24 hours was: (a) 2% when untreated, (b) 11% when treated with etomoxir alone, (c) 16% when treated with 2-deoxy-D-glucose alone, and (d) 98% when treated with the combination of etomoxir and 2-deoxy-D-glucose. Thus the combination of glycolytic inhibitor and fatty acid metabolism inhibitor had a dramatically synergistic effect in killing the multi drug resistant HL60 MDR human leukemia cells.

Example 22

[00225] Referring to Figures 29 and 30, RD cells (rhabdomyosarcoma cells – not drug resistant), cultured as described above under “Materials and Methods” were untreated or treated with etomoxir, with 2-deoxy-D-glucose, or with a combination of etomoxir and 2-deoxy-D-glucose, at concentrations indicated in Figure 29 for 24 hours. Cells were harvested and analyzed by flow cytometry. Dot plots are shown in Figure 29. Figure 30 shows the percent death of the RD cells. The percent of cell deaths for RD cells after 24 hours was: (a) 31% when untreated, (b) 57% when treated with etomoxir alone, (c) 46% when treated with 2-deoxy-D-glucose alone, and (d) 66% when treated with the combination of etomoxir and 2-deoxy-D-glucose. The combination of glycolytic inhibitor and fatty acid metabolism inhibitor still had a synergistic effect in killing the RD cells but not nearly as much as with the multi drug resistant cells in Example 21.

Example 23

[00226] Referring to Figures 31 and 32, HL60 MDR cells, cultured as described in Example 21, were untreated or treated with etomoxir, with 2-deoxy-D-glucose, or with a combination of etomoxir and 2-deoxy-D-glucose, at concentrations indicated in Figure 31 for 48 hours. Cells were harvested and analyzed by flow cytometry. Dot plots are shown in Figure 31. Figure 32 shows the percent death of the HL60 cells. The percent of cell deaths for HL60 MDR cells after 48 hours was: (a) 9% when untreated, (b) 14% when treated with etomoxir alone, (c) 38% when treated with 2-deoxy-D-glucose alone, and (d) 100% when treated with the combination of etomoxir and 2-deoxy-D-glucose. Thus, increasing exposure time increased cell deaths generally, but most dramatically with the combination of glycolytic inhibitor and fatty acid metabolism inhibitor, where 100% of the HL60 MDR cells were killed.

Example 24

[00227] Referring to Figures 33 and 34, RD cells, cultured as described in Example 22, were untreated or treated with etomoxir, with 2-deoxy-D-glucose, or with a combination of etomoxir and 2-deoxy-D-glucose, at concentrations indicated in Figure 33 for 48 hours. Cells were harvested and analyzed by flow cytometry. Dot plots are shown in Figure 33. Figure 34 shows the percent death of the RD cells. The percent of cell deaths for RD cells after 48 hours was: (a) 36% when untreated, (b) 64% when treated with etomoxir alone, (c) 63% when treated with 2-deoxy-D-glucose alone, and (d) 68% when treated with the combination of etomoxir and 2-deoxy-D-glucose. Increasing the treatment time for RD cells only modestly increased the number of cells killed.

Example 25

[00228] HL60 MDR human leukemia cells were cultured and implanted in nude mice as described above under "Materials and Methods". The mice were given 200ug of Etomoxir, 2.5mM 2-Deoxy-D-glucose, or both 200ug of Etomoxir and 2.5mM 2-Deoxy-D-glucose given daily by IP injection. Treatment began exactly one week after tumor implantation. Tumor growth was observed for 21 days with tumor volume measured on days 7, 9, 15 and 21. The following shows the tumor volume in mm³ for the four classes of mice over the 21 day period:

	<u>Day 7</u>	<u>Day 9</u>	<u>Day 15</u>	<u>Day 21</u>
Control	26	58.12	171.27	728.87
Etomoxir only	36	71.73	261.35	895.09
2-Deoxy-D-glucose only	35	51.56	158.55	1,181.61
Etomoxir and 2-deoxy-D-glucose	24	37.72	99.76	296.83

[00229] Figure 39 shows plots of the observed tumor volume over the 21 day period. The etomoxir alone and the 2-deoxy-D-glucose alone each caused an increase in tumor growth, likely because by each restricting only one of the fatty acid and glycolytic pathways, the other pathway became dominant. However, the combination of fatty acid metabolism inhibitor and glycolytic inhibitor had a dramatically synergistic effect, limiting tumor growth to 40% of the growth of the control.

Definitions

[00230] Following are several definitions which will aid in understanding of the scope of the compounds described above and in understanding the invention. All definitions, as defined and used herein, should be understood to control over dictionary definitions, definitions in documents incorporated by reference, and/or ordinary meanings of the defined terms.

[00231] As used herein, the term “halogen,” or equivalently, “halogen atom,” is given its ordinary meaning as used in the field of chemistry. The halogens include fluorine, chlorine, bromine, iodine, and astatine. Preferably, the halogen atoms used in the present invention include one or more of fluorine, chlorine, bromine, or iodine. In certain embodiments of the invention, the halogen atoms found within the structure are fluorine, chlorine, and bromine; fluorine and chlorine; chlorine and bromine, or a single type of halogen atom.

[00232] As used herein, “alkyl” is given its ordinary meaning as used in the field of organic chemistry. Alkyl (i.e., aliphatic) moieties useful for practicing the invention can contain any of a wide number of carbon atoms, for example, between and 1 and 25 carbon atoms, between 1 and 20 carbon atoms, between 1 and 15 carbon atoms, between 1 and 10 carbon atoms, or between 1 and 5 carbon atoms. In some embodiments, the alkyl moiety will contain at least 1 carbon atom, at least 3 carbon atoms, at least 5 carbon atoms, or at least 10 carbon atoms;

in other embodiments, the alkyl moiety will have at most 10 carbon atoms, at most 5 carbon atoms, or at most 3 carbon atoms. Typically, an alkyl moiety is a non-cyclic moiety.

[00233] The carbon atoms within the alkyl moiety may be arranged in any configuration within the alkyl moiety, for example, as a straight chain (i.e., a *n*-alkyl such as methyl, ethyl, propyl, butyl, pentyl, hexyl, heptyl, octyl, etc.) or a branched chain, i.e., a chain where there is at least one carbon atom that is covalently bonded to at least three carbon atoms (e.g., a *t*-butyl moiety, an isoalkyl moiety such as an isopropyl moiety or an isobutyl moiety, etc.). The alkyl moiety may contain only single bonds, or may contain one or more double and/or triple bonds within its structure, for example, as in an alkene, an alkyne, an alkadiene, an alkadiyne, an alkenyne, etc. In some cases, the alkyl moiety contains only carbon and hydrogen atoms; however, in other embodiments, the alkyl moiety may also contain one or more substituents, i.e., a non-carbon and non-hydrogen moiety may be present within the alkyl moiety. For example, in certain embodiments, the alkyl moiety can include a halogen, an alkoxy moiety (e.g., methoxy or ethoxy), an amine moiety (e.g., a primary, secondary, or tertiary amine), a carbonyl (e.g., an aldehyde and/or a ketone) or a hydroxide as a substituent. If more than one substituent is present within the alkyl moiety, then the substituents may each be the same or different.

[00234] Similarly, a “cyclic” moiety, as used herein, is given its ordinary definition as used in the field of organic chemistry, i.e., a moiety structure that contains at least one ring of atoms, and may contain more than one ring of atoms. That is, a cyclic structure has at least one chain of atoms that does not have a terminal end. The chain may have, for example, three, four, five, six, or more atoms arranged to form a ring. In some embodiments, the cyclic moiety has a maximum size of at most ten atoms, at most eight atoms, or at most seven atoms. In some cases, the cyclic moiety may only include carbon and hydrogen atoms; however, in other cases, the atoms within the ring may also include, besides carbon atoms, nitrogen atoms, oxygen atoms, sulfur atoms, silicon atoms, or any other atom able to covalently bond to at least two different atoms (i.e., a “heterocyclic” moiety). If the cyclic moiety contains more than one ring, the rings may be arranged in any orientation with respect to each other, e.g., the rings may be fused (i.e., at least two rings have more than one atom in common, for example, as in bicyclic moieties, tricyclic moieties, etc.), spiro (i.e., two rings have only one atom in common), a ring may be a substituent on another ring, two or more rings may be connected through an alkyl moiety, etc. In

some embodiments of the invention, one or more substituents may be present on the cyclic moiety. The substituents may be any substituent, as previously described in connection with alkyl moieties, for example, a halogen, an alkoxy, an amine, a hydroxide, or the like. In some embodiments, the substituents may also be alkyl moieties, as previously described, for example, methyl, ethyl, propyl, etc.

[00235] The cyclic moiety may be a saturated cyclic moiety (i.e., a moiety not containing any double or triple bonds, such as a cyclopentyl moiety, a cyclohexyl moiety, a cycloheptyl moiety, a cyclooctyl moiety, etc.) or an unsaturated cyclic moiety (i.e., a moiety containing at least one double or triple bond, such as a cycloalkenyl moiety, a cycloalkynyl moiety, an aromatic moiety, etc.). An "aromatic" moiety is given its ordinary meaning as used in the art, i.e., a moiety having at least one ring in which some electrons are delocalized in the ring. For instance, the aromatic moiety may include a benzene moiety, a naphthalenyl moiety, an anthracenyl moiety, a pyridinyl moiety, a furanyl moiety, etc. Similarly, a "non-aromatic" structure is a structure in which aromaticity of the cyclic moiety is not present. For example, a non-aromatic cyclic structure may be a saturated cyclic structure, a cycloalkenyl moiety such as a cyclopentenyl moiety or a cyclohexenyl moiety, a cycloalkynyl moiety such as a cyclooctynyl moiety or a cyclodecynyl moiety, etc.

[00236] Some of the compounds described herein are commercially available compounds, are derived from commercially available compounds, or are synthesized *de novo* using routine chemical synthetic procedures known to those of ordinary skill in the art and/or described herein.

[00237] In some embodiments, the systems and methods of the invention described herein may include homologs, analogs, derivatives, enantiomers and/or functionally equivalent compositions thereof of the compositions described herein, for example, as shown in Fig. 1. Such homologs, analogs, derivatives, enantiomers and functionally equivalent compositions thereof may be used in any of the systems and methods described herein. "Functionally equivalent" also refers to compositions capable of treatment of a subject that is wounded or exhibits symptoms of cancer (or other conditions described herein), a subject susceptible to or otherwise at increased risk for cancer, or a subject not exhibiting symptoms of cancer, but for whom it is desired to decrease the risk of cancer (e.g., a vaccination or a prophylactic treatment), etc. It will be understood that one of ordinary skill in the art will be able to manipulate the

conditions in a manner to prepare such homologs, analogs, derivatives, enantiomers and functionally equivalent compositions as necessary. Homologs, analogs, derivatives, enantiomers and/or functionally equivalent compositions that are about as effective or more effective than the parent compound are also intended for use in the systems and methods of the invention. The synthesis of such compositions may be accomplished through typical chemical modification methods such as those routinely practiced by those of ordinary skill in the art.

[00238] The indefinite articles “a” and “an,” as used herein in the specification and in the claims, unless clearly indicated to the contrary, should be understood to mean “one or more.”

[00239] The phrase “and/or,” as used herein in the specification and in the claims, should be understood to mean “either or both” of the elements so conjoined, i.e., elements that are conjunctively present in some cases and disjunctively present in other cases. Other elements may optionally be present other than the elements specifically identified by the “and/or” clause, whether related or unrelated to those elements specifically identified. Thus, as a non-limiting example, a reference to “A and/or B” can refer, in one embodiment, to A only (optionally including elements other than B); in another embodiment, to B only (optionally including elements other than A); in yet another embodiment, to both A and B (optionally including other elements); etc.

[00240] As used herein in the specification and in the claims, unless clearly indicated to the contrary, “or” should be understood to have the same meaning as “and/or” as defined above. For example, when separating items in a list, “or” and “and/or” each shall be interpreted as being inclusive, i.e., the inclusion of at least one, but also including more than one, of a number or list of elements, and, optionally, additional unlisted items. In general, the term “or” as used herein shall only be interpreted as indicating exclusive alternatives (i.e. “one or the other but not both”) when preceded by terms of exclusivity, such as “only one of” or “exactly one of.”

[00241] As used herein in the specification and in the claims, the phrase “at least one,” in reference to a list of one or more elements, should be understood to mean at least one element selected from any one or more of the elements in the list of elements, but not necessarily including at least one of each and every element specifically listed within the list of elements and not excluding any combinations of elements in the list of elements. This definition also allows that elements may optionally be present other than the elements specifically identified within the list of elements that the phrase “at least one” refers to, whether related or unrelated to those

elements specifically identified. Thus, as a non-limiting example, “at least one of A and B” (or, equivalently, “at least one of A or B,” or, equivalently “at least one of A and/or B”) can refer, in one embodiment, to at least one, optionally including more than one, A, with no B present (and optionally including elements other than B); in another embodiment, to at least one, optionally including more than one, B, with no A present (and optionally including elements other than A); in yet another embodiment, to at least one, optionally including more than one, A, and at least one, optionally including more than one, B (and optionally including other elements); etc.

[00242] It should also be understood that, unless clearly indicated to the contrary, in any methods claimed herein that include more than one act, the order of the acts of the method is not necessarily limited to the order in which the acts of the method are recited.

[00243] In the claims, as well as in the specification above, all transitional phrases such as “comprising,” “including,” “carrying,” “having,” “containing,” “involving,” “holding,” and the like are to be understood to be open-ended, i.e., to mean including but not limited to. Only the transitional phrases “consisting of” and “consisting essentially of” shall be closed or semi-closed transitional phrases, respectively, as set forth in the United States Patent Office Manual of Patent Examining Procedures, Section 2111.03.

[00244] While several embodiments of the present invention have been described and illustrated herein, those of ordinary skill in the art will readily envision a variety of other means and/or structures for performing the functions and/or obtaining the results and/or one or more of the advantages described herein, and each of such variations and/or modifications is deemed to be within the scope of the present invention. More generally, those skilled in the art will readily appreciate that all parameters, dimensions, materials, and configurations described herein are meant to be exemplary and that the actual parameters, dimensions, materials, and/or configurations will depend upon the specific application or applications for which the teachings of the present invention is/are used. Those skilled in the art will recognize, or be able to ascertain using no more than routine experimentation, many equivalents to the specific embodiments of the invention described herein. It is, therefore, to be understood that the foregoing embodiments are presented by way of example only and that, within the scope of the appended claims and equivalents thereto, the invention may be practiced otherwise than as specifically described and claimed. The present invention is directed to each individual feature, system, article, material, kit, and/or method described herein. In addition, any combination of

two or more such features, systems, articles, materials, kits, and/or methods, if such features, systems, articles, materials, kits, and/or methods are not mutually inconsistent, is included within the scope of the present invention.

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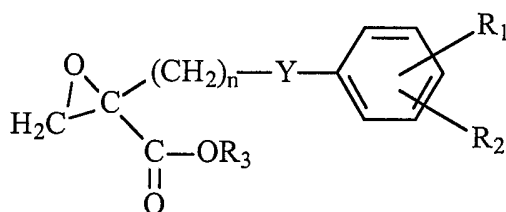
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CLAIMS

What is claimed is:

1. A method for treating an inflammatory or proliferative disease, or a wound, comprising administering to a subject susceptible to or exhibiting symptoms of such a disease, or suffering from such a wound, a pharmacon comprising a therapeutically acceptable amount of a fatty acid metabolism inhibitor, a glycolytic inhibitor, and/or an agent able to alter cellular production of reactive oxygen, or combination thereof..
2. The method of claim 1 wherein the pharmacon is a fatty acid metabolism inhibitor.
3. The method of claim 2 wherein the fatty acid metabolism inhibitor is able to inhibit beta-oxidation in the cell.
4. The method of claim 2 wherein the fatty acid metabolism inhibitor is able to bind to a reductase.
5. The method of claim 4 wherein the reductase is 2,4-dienoyl-CoA reductase.
6. The method of claim 2 wherein the fatty acid metabolism inhibitor is able to bind to a fatty acid isomerase.
7. The method of claim 6 wherein the isomerase is 2,4-dienoyl-CoA isomerase.
8. The method of claim 2 wherein the fatty acid metabolism inhibitor is able to bind to a fatty acid transporter.
9. The method of claim 8 wherein the fatty acid transporter is a mitochondrial fatty acid transporter.

10. The method of claim 2 wherein the fatty acid metabolism inhibitor inhibits breakdown of butyryl CoA.
11. The method of claim 2 wherein the fatty acid metabolism inhibitor inhibits breakdown of glytryl CoA.
12. The method of claim 2 wherein the fatty acid metabolism inhibitor inhibits breakdown of isovaleryl CoA.
13. The method of claim 2 wherein the fatty acid metabolism inhibitor inhibits butyryl dehydrogenase.
14. The method of claim 2 in which the fatty acid metabolism inhibitor is an oxirane carboxylic acid compound capable of inhibiting fatty acid metabolism, or a pharmacologically acceptable salt thereof, wherein the subject is not otherwise indicated for treatment with the compound.
15. The method of claim 14 wherein the oxirane carboxylic acid compound has the formula:



wherein

R₁ represents a hydrogen atom, a halogen atom, a 1 – 4C alkyl group, a 1 – 4C alkoxy group, a nitro group or a trifluoromethyl group,

R_2 has one of the meanings of R_1 .

R₃ represents a hydrogen atom or a 1 – 4C alkyl group,

Y represents the grouping $\text{—O—(CH}_2\text{)}_m\text{—}$,

m is 0 or a whole number from 1 to 4, and

n is a whole number from 2 to 8 wherein the sum of m and n is a whole number

from 2 to 8.

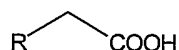
16. The method of claim 15 wherein R_1 is a halogen atom, R_2 is a hydrogen atom, m is 0, and n is 6.

17. The method of claim 16 wherein R_3 is an ethyl group.

18. The method of claim 15 wherein the oxirane carboxylic acid compound is etomoxir.

19. The method of claim 2 wherein the fatty acid metabolism inhibitor comprises a carboxylic acid.

20. The method of claim 19 wherein the fatty acid metabolism inhibitor has the structure:



wherein R comprises an organic moiety.

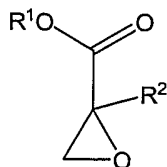
21. The method of claim 19 wherein R comprises at least two nitrogen atoms.

22. The method of claim 19 wherein R comprises an aromatic moiety.

23. The method of claim 19 wherein R comprises a benzene ring.

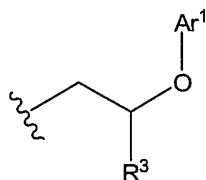
24. The method of claim 19 wherein R comprises a furan.

25. The method of claim 2 wherein the fatty acid metabolism inhibitor has a structure:



wherein each of R^1 and R^2 independently comprises an organic moiety.

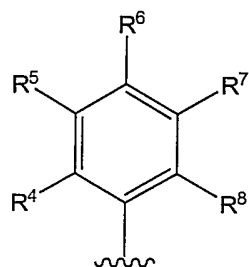
26. The method of claim 25 wherein R^1 is an alkyl.
27. The method of claim 25 wherein R^1 is a straight-chain alkyl.
28. The method of claim 27 wherein R^1 is methyl.
29. The method of claim 25 wherein R^2 is an alkyl.
30. The method of claim 29 wherein R^2 is a straight-chain alkyl.
31. The method of claim 30 wherein R^2 is tetradecyl.
32. The method of claim 25 wherein R^2 comprises an aromatic moiety.
33. The method of claim 32 wherein R^2 comprises a benzene ring.
34. The method of claim 32 wherein R^2 comprises a structure:



wherein R^3 comprises an organic moiety, and Ar^1 comprises an aromatic moiety.

35. The method of claim 34 wherein R^3 is an alkyl.
36. The method of claim 35 wherein R^3 is a straight-chain alkyl.
37. The method of claim 36 wherein R^3 is butyl.

38. The method of claim 34 wherein Ar¹ comprises a structure:



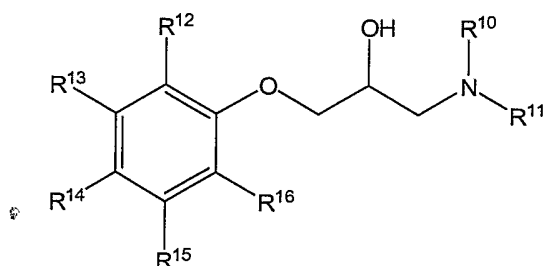
wherein each of R⁴, R⁵, R⁶, R⁷, and R⁸ is independently hydrogen or a halogen.

39. The method of claim 38 wherein one or more of R⁴, R⁵, R⁶, R⁷, and R⁸ is a halogen.

40. The method of claim 39 wherein R⁶ is a halogen.

41. The method of claim 40 wherein R⁶ is chlorine.

42. The method of claim 2 wherein the fatty acid metabolism inhibitor has a structure:



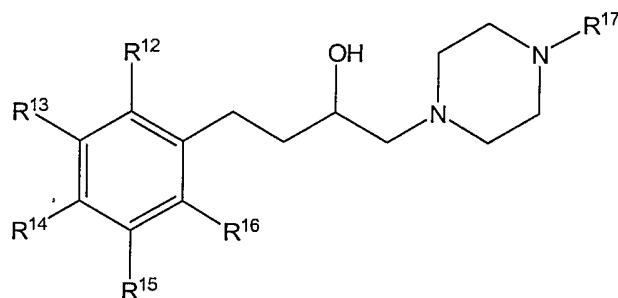
wherein each of R¹⁰, R¹¹, R¹², R¹³, R¹⁴, R¹⁵ and R¹⁶ independently comprises hydrogen or an organic moiety.

43. The method of claim 42 wherein one or more of R¹², R¹³, R¹⁴, R¹⁵ and R¹⁶ comprises alkoxy.

44. The method of claim 42 wherein R¹⁴ comprises an alkoxy.

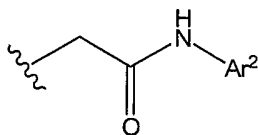
45. The method of claim 42 wherein R¹⁴ is 3-methoxypropyl.

46. The method of claim 42 wherein R^{16} comprises an alkoxy.
47. The method of claim 42 wherein R^{16} is methoxy.
48. The method of claim 42 wherein R^{10} comprises an alkyl.
49. The method of claim 48 wherein R^{10} comprises isopropyl.
50. The method of claim 42 wherein R^{10} and R^{11} together comprise a cyclic structure.
51. The method of claim 42 wherein the fatty acid metabolism inhibitor has a structure:



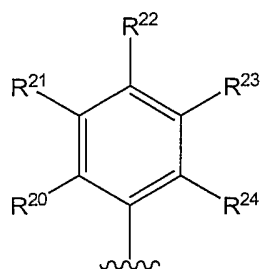
wherein R^{17} comprises an organic moiety.

52. The method of claim 51 wherein R^{17} comprises an aromatic moiety.
53. The method of claim 51 wherein R^{17} comprises an amide moiety.
54. The method of claim 42 wherein R^{17} comprises a structure:



wherein Ar^2 comprises an aromatic moiety.

55. The method of claim 54 wherein Ar² comprises a structure:



wherein each of R²⁰, R²¹, R²², R²³, and R²⁴ independently is hydrogen or an alkyl.

56. The method of claim 55 wherein R²⁰ is an alkyl.

57. The method of claim 55 wherein R²⁴ is an alkyl.

58. The method of claim 55 wherein R²⁰ and R²⁴ are each methyl.

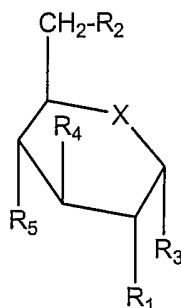
59. The method of claim 2 wherein the fatty acid metabolism inhibitor comprises a non-hydrolyzable analog of carnitine.

60. The method of claim 2 wherein the fatty acid metabolism inhibitor includes at least one of etomoxir, cerulenin, 5-(tetradecyloxy)-2-furoic acid, oxfenicine, methyl palmoxirate, metoprolol, amiodarone, perhexiline, aminocarnitine, hydrazonopropionic acid, 4-bromocrotonic acid, trimetazidine, ranolazine, hypoglycin, dichloroacetate, methylene cyclopropyl acetic acid, or beta-hydroxy butyrate.

61. The method of claim 2 wherein the pharmacon is a glycolytic inhibitor.

62. The method of claim 61 in which the glycolytic inhibitor is a 2-deoxyglucose compound.

63. The method of claim 62 in which the 2-deoxyglucose compound has the formula:



wherein

X represents an O or S atom;

R₁ represents a hydrogen atom or a halogen atom;

R₂ represents a hydroxyl group, a halogen atom, a thiol group, or CO-R₆;

R₃, R₄, and R₅ each represent a hydroxyl group, a halogen atom, or CO-R₆,

R₆ represents an alkyl group of from 1 to 20 carbon atoms, and

at least two of R₃, R₄, and R₅ are hydroxyl groups.

64. The method of claim 63 in which the 2-deoxyglucose compound is 2-deoxy-D-glucose.

65. The method of claim 1 in which the pharmacon comprises a combination of a fatty acid metabolism inhibitor and a glycolytic inhibitor.

66. The method of claim 65 in which the fatty acid metabolism inhibitor is an oxirane carboxylic acid compound capable of inhibiting fatty acid metabolism, or a pharmacologically acceptable salt thereof and the glycolytic inhibitor is a 2-deoxyglucose compound.

67. The method of claim 65 in which the fatty acid metabolism inhibitor is etomoxir and the glycolytic inhibitor is 2-deoxy-D-glucose.

68. The method of claim 1 for treating cancer.

69. The method of claim 68 wherein the cancer is multi-drug resistant.
70. The method of claim 68 wherein the therapeutically acceptable amount is in an amount cytotoxic to cells comprising the cancer.
71. The method of claim 68 wherein the subject is not indicated for treatment of cardiac insufficiency.
72. The method of claim 68 wherein the cells comprising the cancer derive a majority of their metabolic energy through fatty acid metabolism.
73. The method of claim 1 wherein the composition further includes a pharmaceutically acceptable carrier.
74. The method of claim 68 further comprising administering to the subject one or more chemotherapeutic agents.
75. The method of claim 74 wherein the one or more chemotherapeutic agents and the pharmacon are applied as part of the same treatment regimen.
76. The method of claim 75 wherein one or more of the chemotherapeutic agents is selected from the group consisting of methotrexate, trimetrexate, adriamycin, taxotere, doxorubicin, 5-fluorouracil, vincristine, vinblastine, pamidronate disodium, anastrozole, exemestane, cyclophosphamide, epirubicin, toremifene, letrozole, trastuzumab, megestrol, tamoxifen, paclitaxel, docetaxel, capecitabine, and goserelin acetate.
77. The method of claim 1 in which the pharmacon is administered to the subject by exposing cells of the subject to said pharmacon.
78. The method of claim 77 in which the cells are cancer cells.

79. The method of claim 78 wherein the pharmacon is administered *in vivo*.
80. The method of claim 78 wherein the pharmacon is administered *ex vivo*.
81. The method of claim 77 wherein the cells are lymphocyte cells.
82. The method of claim 77 wherein the cells are pancreatic beta cells.
83. The method of claim 77 wherein the cells are B cells.
84. The method of claim 78 in which the pharmacon comprises a glycolytic inhibitor, and/or an agent able to alter cellular production of reactive oxygen and/ or a fatty acid metabolism inhibitor.
85. The method of claim 84 in which the fatty acid metabolism inhibitor is an oxirane carboxylic acid compound capable of inhibiting fatty acid metabolism or a pharmacologically acceptable salt thereof, and the glycolytic inhibitor is a 2-deoxyglucose compound capable of inhibiting glycolysis.
86. The method of claim 84 in which the fatty acid metabolism inhibitor is etomoxir and the glycolytic inhibitor is 2-deoxy-D-glucose or the pharmaceutically acceptable salt of either or both..
87. The method of claim 86 further comprising administering to the subject a form of cancer treatment.
88. The method of claim 87 wherein the cancer treatment includes radiation.
89. The method of claim 87 wherein the cancer treatment includes one or more chemotherapeutic agents.

90. A composition, comprising:
a therapeutically acceptable combination of at least two of a fatty acid metabolism inhibitor, a glycolytic inhibitor, and/or an agent able to alter cellular production of reactive oxygen.
91. The composition of claim 90 comprising a fatty acid metabolism inhibitor.
92. The composition of claim 91 wherein the fatty acid metabolism inhibitor is able to inhibit beta-oxidation in the cell.
93. The composition of claim 91 wherein the fatty acid metabolism inhibitor is able to bind to a reductase.
94. The composition of claim 93 wherein the reductase is 2,4-dienoyl-CoA reductase.
95. The composition of claim 91 wherein the fatty acid metabolism inhibitor is able to bind to a fatty acid isomerase.
96. The composition of claim 95 wherein the isomerase is 2,4-dienoyl-CoA isomerase.
97. The composition of claim 91 wherein the fatty acid metabolism inhibitor is able to bind to a fatty acid transporter.
98. The composition of claim 97 wherein the fatty acid transporter is a mitochondrial fatty acid transporter.
99. The composition of claim 91 wherein the fatty acid metabolism inhibitor inhibits breakdown of butyryl CoA.

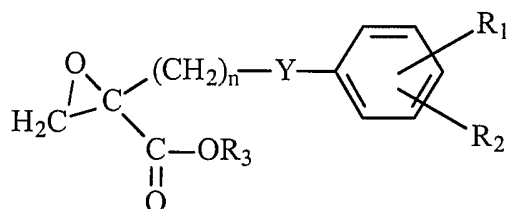
100. The composition of claim 91 wherein the fatty acid metabolism inhibitor inhibits breakdown of glytrayl CoA.

101. The composition of claim 91 wherein the fatty acid metabolism inhibitor inhibits breakdown of isovaleryl CoA.

102. The composition of claim 91 wherein the fatty acid metabolism inhibitor inhibits butyryl dehydrogenase.

103. The composition of claim 91 in which the fatty acid metabolism inhibitor is an oxirane carboxylic acid compound capable of inhibiting fatty acid metabolism, or a pharmacologically acceptable salt thereof, wherein the subject is not otherwise indicated for treatment with the compound.

104. The composition of claim 103 wherein the oxirane carboxylic acid compound has the formula:



wherein

R₁ represents a hydrogen atom, a halogen atom, a 1 – 4C alkyl group, a 1 – 4C alkoxy group, a nitro group or a trifluoromethyl group,

R_2 has one of the meanings of R_1 ,

R₃ represents a hydrogen atom or a 1 – 4C alkyl group,

Y represents the grouping $\text{—O—(CH}_2\text{)}_m\text{—}$,

m is 0 or a whole number from 1 to 4, and

n is a whole number from 2 to 8 wherein the sum of m and n is a whole number from 2 to 8.

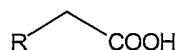
105. The composition of claim 104 wherein R_1 is a halogen atom, R_2 is a hydrogen atom, m is 0, and n is 6.

106. The composition of claim 105 wherein R_3 is an ethyl group.

107. The composition of claim 104 wherein the oxirane carboxylic acid compound is etomoxir.

108. The composition of claim 91 wherein the fatty acid metabolism inhibitor comprises a carboxylic acid.

109. The composition of claim 108 wherein the fatty acid metabolism inhibitor has the structure:



wherein R comprises an organic moiety.

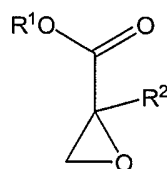
110. The composition of claim 108 wherein R comprises at least two nitrogen atoms.

111. The composition of claim 108 wherein R comprises an aromatic moiety.

112. The composition of claim 108 wherein R comprises a benzene ring.

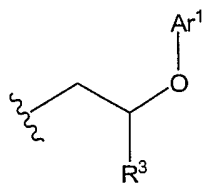
113. The composition of claim 108 wherein R comprises a furan.

114. The composition of claim 91 wherein the fatty acid metabolism inhibitor has the structure:



wherein each of R^1 and R^2 independently comprises an organic moiety.

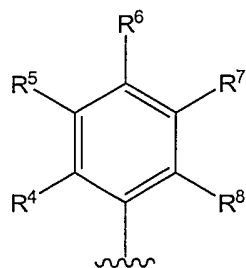
115. The composition of claim 114 wherein R^1 is an alkyl.
116. The composition of claim 114 wherein R^1 is a straight-chain alkyl.
117. The composition of claim 116 wherein R^1 is methyl.
118. The composition of claim 114 wherein R^2 is an alkyl.
119. The composition of claim 118 wherein R^2 is a straight-chain alkyl.
120. The composition of claim 119 wherein R^2 is tetradecyl.
121. The composition of claim 114 wherein R^2 comprises an aromatic moiety.
122. The composition of claim 121 wherein R^2 comprises a benzene ring.
123. The composition of claim 121 wherein R^2 comprises the structure:



wherein R^3 comprises an organic moiety, and Ar^1 comprises an aromatic moiety.

124. The composition of claim 123 wherein R^3 is an alkyl.
125. The composition of claim 124 wherein R^3 is a straight-chain alkyl.
126. The composition of claim 125 wherein R^3 is butyl.

127. The composition of claim 123 wherein Ar¹ comprises the structure:



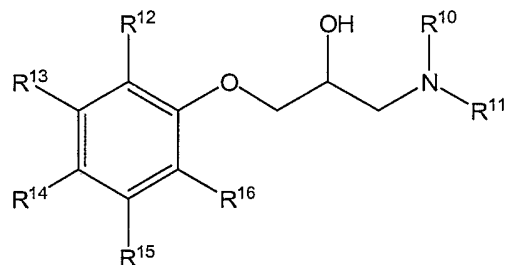
wherein each of R⁴, R⁵, R⁶, R⁷, and R⁸ is independently hydrogen or a halogen.

128. The composition of claim 127 wherein one or more of R⁴, R⁵, R⁶, R⁷, and R⁸ is a halogen.

129. The composition of claim 128 wherein R⁶ is a halogen.

130. The composition of claim 129 wherein R⁶ is chlorine.

131. The composition of claim 91 wherein the fatty acid metabolism inhibitor has the structure:



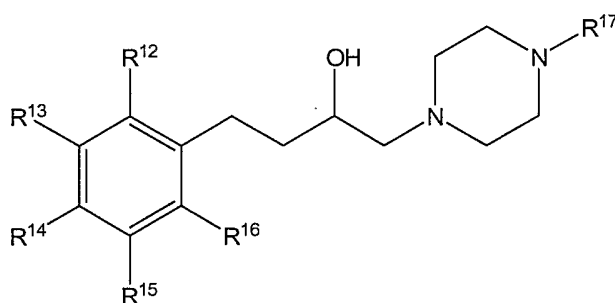
wherein each of R¹⁰, R¹¹, R¹², R¹³, R¹⁴, R¹⁵ and R¹⁶ independently comprises hydrogen or an organic moiety.

132. The composition of claim 131 wherein one or more of R¹², R¹³, R¹⁴, R¹⁵ and R¹⁶ comprises alkoxy.

133. The composition of claim 131 wherein R¹⁴ comprises an alkoxy.

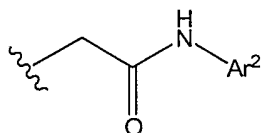
134. The composition of claim 131 wherein R¹⁴ is 3-methoxypropyl.

135. The composition of claim 131 wherein R^{16} comprises an alkoxy.
136. The composition of claim 131 wherein R^{16} is methoxy.
137. The composition of claim 131 wherein R^{10} comprises an alkyl.
138. The composition of claim 137 wherein R^{10} comprises isopropyl.
139. The composition of claim 131 wherein R^{10} and R^{11} together comprise a cyclic structure.
140. The composition of claim 131 wherein the fatty acid metabolism inhibitor has the structure:



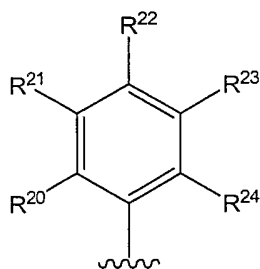
wherein R^{17} comprises an organic moiety.

141. The composition of claim 140 wherein R^{17} comprises an aromatic moiety.
142. The composition of claim 140 wherein R^{17} comprises an amide moiety.
143. The composition of claim 131 wherein R^{17} comprises the structure:



wherein Ar^2 comprises an aromatic moiety.

144. The composition of claim 143 wherein Ar^2 comprises the structure:



wherein each of R^{20} , R^{21} , R^{22} , R^{23} , and R^{24} independently is hydrogen or an alkyl.

145. The composition of claim 144 wherein R^{20} is an alkyl.

146. The composition of claim 144 wherein R^{24} is an alkyl.

147. The composition of claim 144 wherein R^{20} and R^{24} are each methyl.

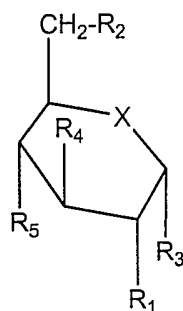
148. The composition of claim 91 wherein the fatty acid metabolism inhibitor comprises a non-hydrolyzable analog of carnitine.

149. The composition of claim 91 wherein the fatty acid metabolism inhibitor includes at least one of etomoxir, cerulenin, 5-(tetradecyloxy)-2-furoic acid, oxfenicine, methyl palmoxirate, metoprolol, amiodarone, perhexiline, aminocarnitine, hydrazonopropionic acid, 4-bromocrotonic acid, trimetazidine, ranolazine, hypoglycin, dichloroacetate, methylene cyclopropyl acetic acid, or beta-hydroxy butyrate.

150. The composition of claim 90 comprising a glycolytic inhibitor.

151. The composition of claim 150 in which the glycolytic inhibitor is a 2-deoxyglucose compound.

152. The composition of claim 151 in which the 2-deoxyglucose compound has the formula:



wherein

X represents an O or S atom;

R₁ represents a hydrogen atom or a halogen atom;

R₂ represents a hydroxyl group, a halogen atom, a thiol group, or CO-R₆;

R₃, R₄, and R₅ each represent a hydroxyl group, a halogen atom, or CO-R₆,

R₆ represents an alkyl group of from 1 to 20 carbon atoms, and

at least two of R₃, R₄, and R₅ are hydroxyl groups.

153. The composition of claim 152 in which the 2-deoxyglucose compound is 2-deoxy-D-glucose.

154. The method of claim 90 in which the pharmacon comprises a combination of a fatty acid metabolism inhibitor and a glycolytic inhibitor.

155. The method of claim 154 in which the fatty acid metabolism inhibitor is an oxirane carboxylic acid compound capable of inhibiting fatty acid metabolism, or a pharmacologically acceptable salt thereof and the glycolytic inhibitor is a 2-deoxyglucose compound.

156. The method of claim 154 in which the fatty acid metabolism inhibitor is etomoxir and the glycolytic inhibitor is 2-deoxy-D-glucose.

157. The composition of claim 90 further comprising a pharmaceutically acceptable carrier.

158. The composition of claim 90 further comprising one or more chemotherapeutic agents.

159. The composition of claim 158 wherein one or more of the chemotherapeutic agents is selected from the group consisting of methotrexate, trimetrexate, adriamycin, taxotere, doxorubicin, 5-fluorouracil, vincristine, vinblastine, pamidronate disodium, anastrozole, exemestane, cyclophosphamide, epirubicin, toremifene, letrozole, trastuzumab, megestrol, tamoxifen, paclitaxel, docetaxel, capecitabine, and goserelin acetate.

160. A composition, comprising:
a cell exposed to a composition of claim 90.

161. The composition of claim 160 wherein the cell is a cancer cell.

162. The composition of claim 161 wherein the cell is in a subject and the cell is exposed *in vivo*.

163. A method, comprising:
administering, to a subject susceptible to or having cancer, a therapeutically acceptable amount of the composition of claim 90.

164. The method of claim 163 wherein the cancer is drug-resistant.

165. The method of claim 163 wherein the cancer is multi-drug resistant.

166. The method of claim 163 wherein the therapeutically acceptable amount is in an amount cytotoxic to cells comprising the cancer.

167. The method of claim 163 wherein the subject is not indicated for treatment for obesity.

168. The method of claim 163 wherein a majority of cells comprising the cancer derive a majority of their metabolic energy through fatty acid metabolism.

169. A method, comprising:
surgically removing a tumor from a subject; and
inserting, into a site within the subject where the tumor was removed therefrom,
the composition of claim 90.

170. A method, comprising:
surgically removing a tumor from a subject; and
inserting, into the tumor, the composition of claim 90.

171. The composition of claim 170 wherein the composition includes a substrate positionable on a subject.

172. The composition of claim 171 further comprising an adhesive material.

173. The composition of claim 171 further comprising a backing.

174. A kit, comprising:
a container housing a therapeutically acceptable amount of a first pharmakon selected from a fatty acid metabolism inhibitor, a glycolytic inhibitor, and/or an agent able to alter cellular production of reactive oxygen, and a container housing a second therapeutically acceptable amount of a pharmakon selected from a fatty acid metabolism inhibitor, a glycolytic inhibitor, and/or an agent able to alter cellular production of reactive oxygen, said second pharmakon being different from said first pharmakon.

175. The kit of claim 174 further comprising a container housing a UCP molecule detection reagent.

176. The kit of claim 175 wherein the UCP molecule detection reagent is attached to a solid surface.

177. The kit of claim 175 further comprising instructions for using the UCP molecule detection reagent for detecting the presence of a UCP molecule on the plasma membrane of the tumor cell.

178. The kit of claim 175 further comprising a container housing a chemotherapeutic agent.

179. The kit of claim 175 further comprising a panel of chemotherapeutic agents, housed in separate compartments.

180. A method, comprising:
administering, to a subject susceptible to or exhibiting symptoms of drug-resistant cancer, a therapeutically acceptable amount of a composition of Claim 90 wherein the subject is not otherwise indicated for treatment with the compound.

181. The method of claim 1 wherein the composition comprises at least one of etomoxir, oxfenicine, methyl palmoxirate, metoprolol, amiodarone, perhexiline, aminocarnitine, hydrazonopropionic acid, 4-bromocrotonic acid, trimetazidine, ranolazine, hypoglycin, dichloroacetate, methylene cyclopropyl acetic acid, or beta-hydroxy butyrate.

182. A method, comprising:
administering, to a wound in a subject, a therapeutically acceptable amount of a composition comprising an agent able to alter production of reactive oxygen in cells located within or proximate the wound.

183. The method of claim 182 in which the agent is a composition of claim 90.

184. An article, comprising:
a substrate comprising cells exposed to an agent able to alter production of reactive oxygen in a cell.
185. The article of claim 184 further comprising an adhesive material.
186. The article of claim 184 further comprising a backing.
187. A method, comprising:
removing cells from a tumor;
exposing the cells to a composition of claim 90; and
inserting the cells into a subject.
188. The method of claim 187 wherein the tumor is derived from the subject.
189. The method of claim 187 wherein the tumor is located within the subject.
190. The method of claim 187 wherein the composition is a fatty acid metabolism inhibitor.
191. The method of claim 190 wherein the fatty acid metabolism inhibitor comprises at least one of etomoxir, cerulenin, 5-(tetradecyloxy)-2-furoic acid, oxfenicine, methyl palmoxirate, metoprolol, amiodarone, perhexiline, aminocarnitine, hydrazonopropionic acid, 4-bromocrotonic acid, trimetazidine, ranolazine, hypoglycin, dichloroacetate, methylene cyclopropyl acetic acid, or beta-hydroxy butyrate.
192. The method of claim 190 wherein the fatty acid metabolism inhibitor comprises etomoxir.
193. The method of claim 190 wherein the fatty acid metabolism inhibitor comprises cerulenin

194. An article, comprising:
isolated tumor cells exposed to a a composition of claim 90.
195. The article of claim 194 wherein the fatty acid metabolism inhibitor comprises at least one of etomoxir, cerulenin, 5-(tetradecyloxy)-2-furoic acid, oxfenicine, methyl palmoxirate, metoprolol, amiodarone, perhexiline, aminocarnitine, hydrazonopropionic acid, 4-bromocrotonic acid, trimetazidine, ranolazine, hypoglycin, dichloroacetate, methylene cyclopropyl acetic acid, or beta-hydroxy butyrate.
196. The article of claim 194 wherein the fatty acid metabolism inhibitor comprises etomoxir.
197. The article of claim 194 further comprising a pharmaceutically acceptable carrier.
198. A method, comprising:
altering an immunity profile of a tumor cell by exposing the tumor cell to a a composition of claim 90.
199. A method, comprising:
administering to a subject having or at risk of developing an autoimmune disease a a composition of claim 90.
200. A composition, comprising:
a a composition of claim 90; and
a tumor cell vaccine.
201. A composition, comprising:
a composition of claim 90; and
a cytokine.
202. A method of treating a subject who has a tumor, comprising:
surgically removing the tumor from the subject; and

inserting, into the subject, a composition of claim 90.

203. The method of claim 202 wherein the steps are performed in the order recited.

204. An article, comprising:

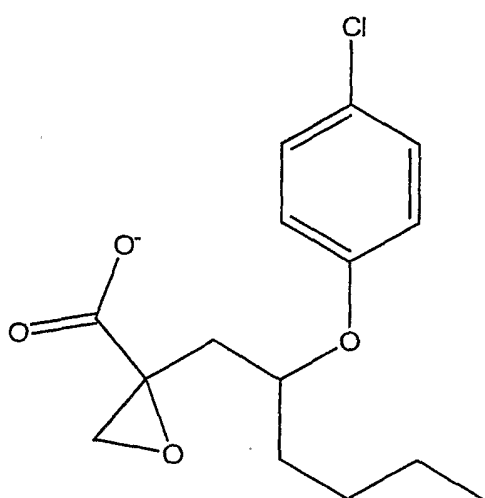
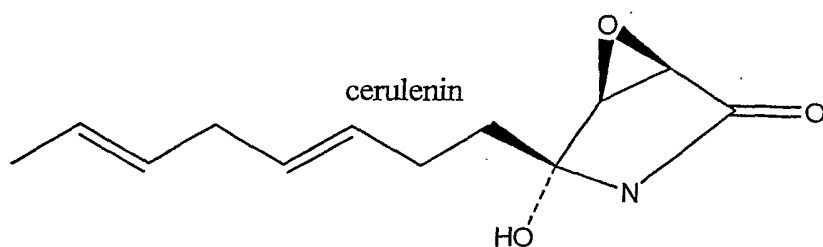
isolated tumor cells exposed to a composition of claim 90.

205. A method of treating a tumor cell, comprising:

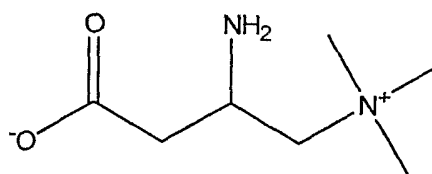
altering an immunity profile of the tumor cell by exposing it to a composition of claim 90.

206. A method, comprising:

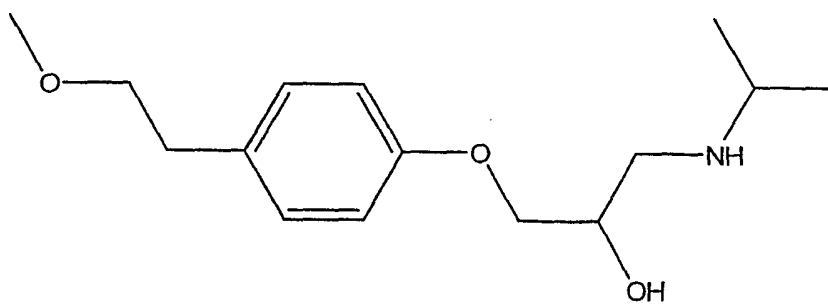
administering to a subject having or at risk of developing an autoimmune disease a composition of claim 90.



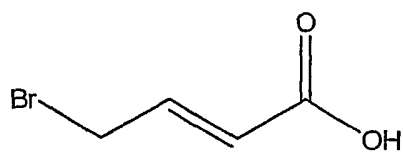
2-[b-(4-chlorophenoxy)-hexyl]oxirane-2-carboxylate



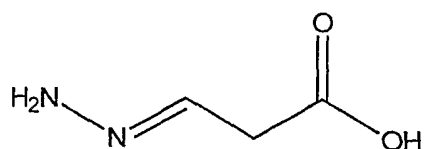
aminocarnitine



metoprolol



4-bromocrotonic acid



hydrazonopropionic acid

FIG. 1A

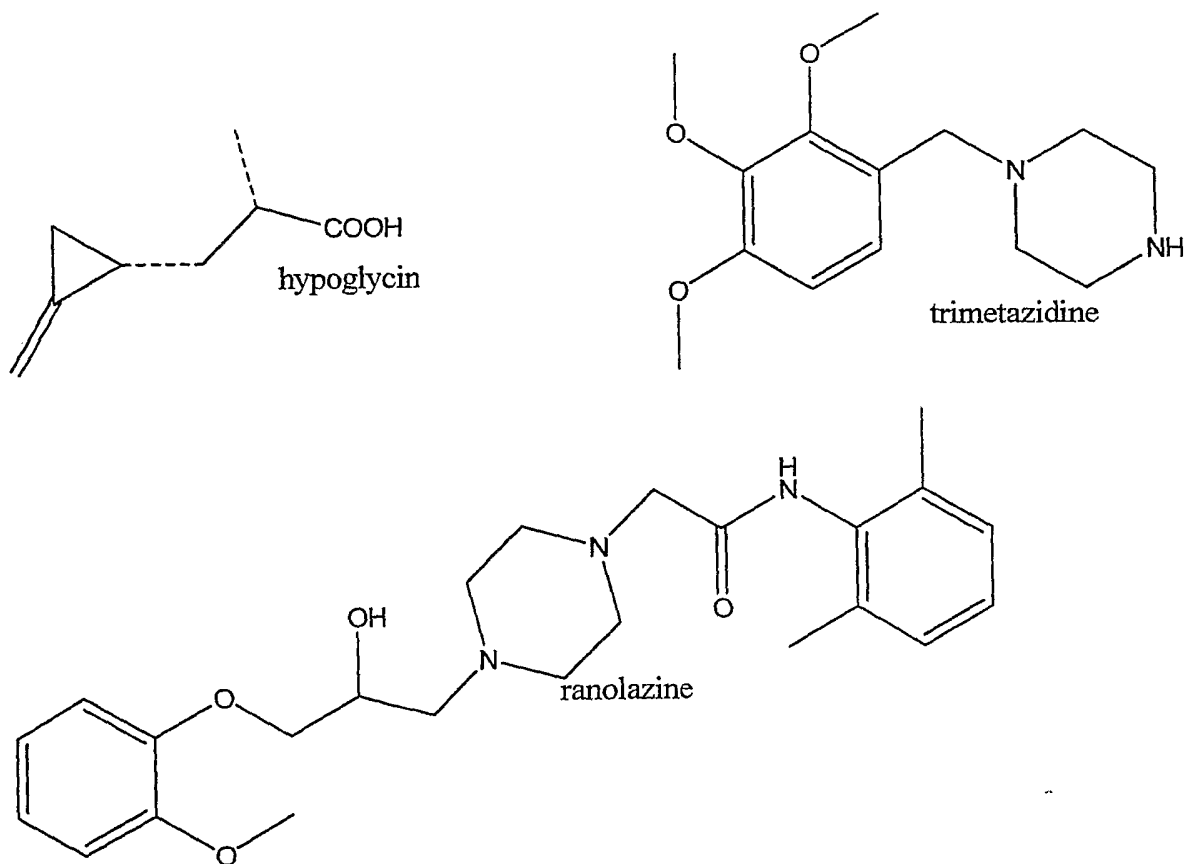
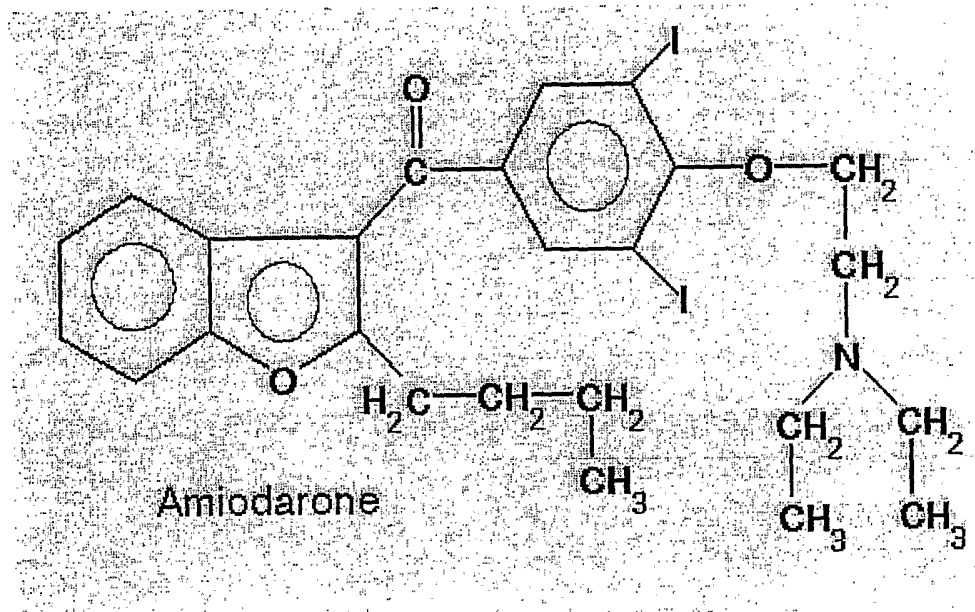
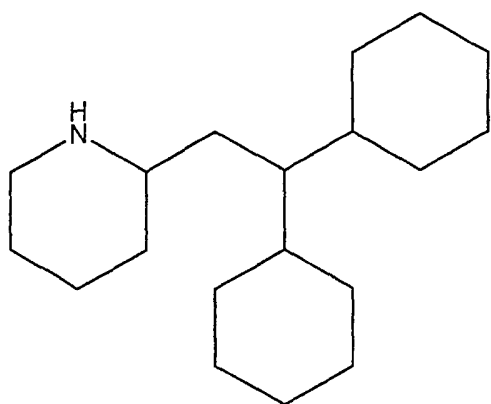
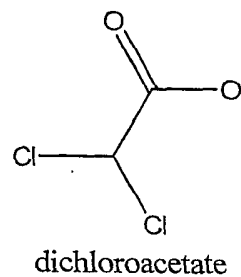


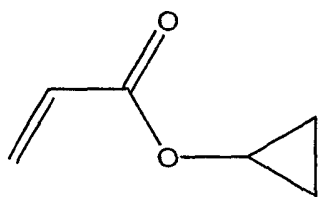
FIG. 1B



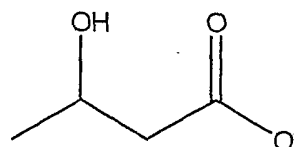
perhexiline



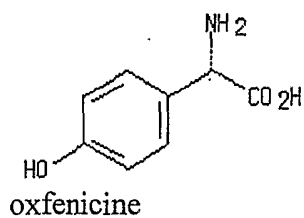
dichloroacetate



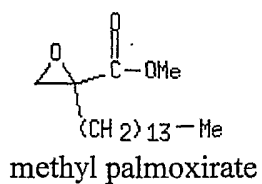
methylene cyclopropyl acetic acid



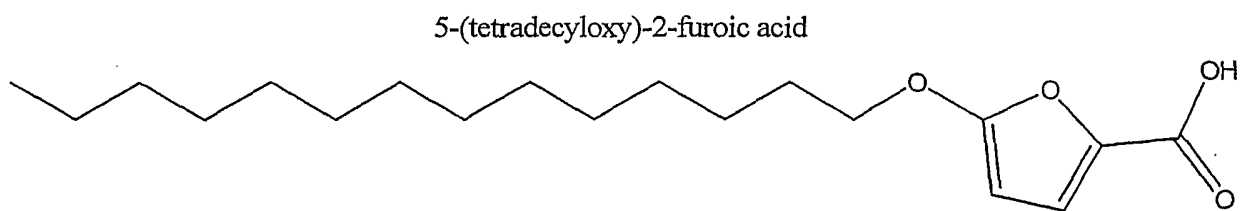
3-hydroxybutyrate
beta-hydroxybutyrate



oxfenicine



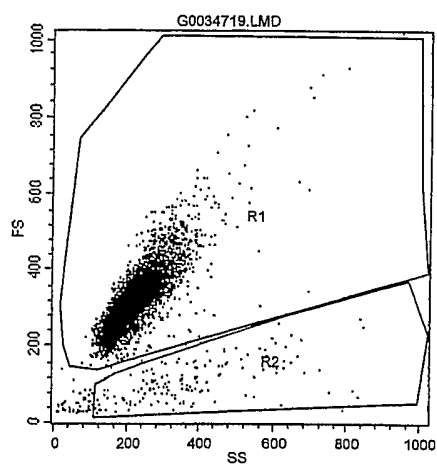
methyl palmoxirate



5-(tetradecyloxy)-2-furoic acid

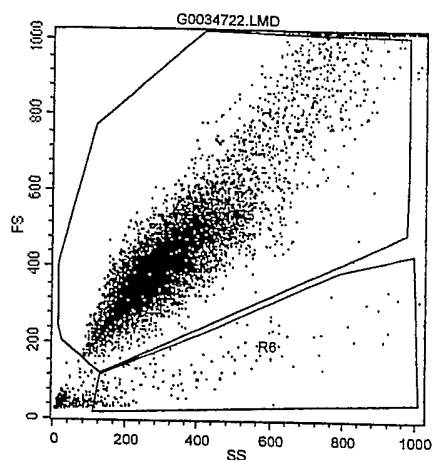
FIG.1C

FIG. 2A

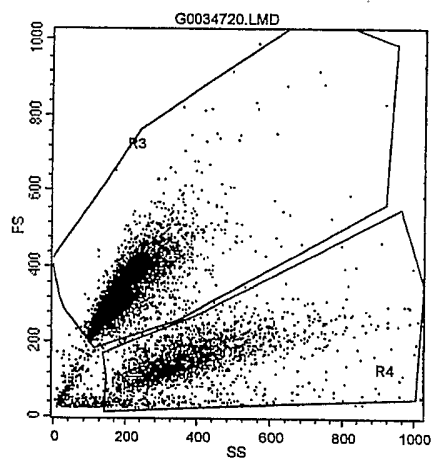


L1210 No Treatment
7.0 Hours
1.7 % Death

FIG. 2B

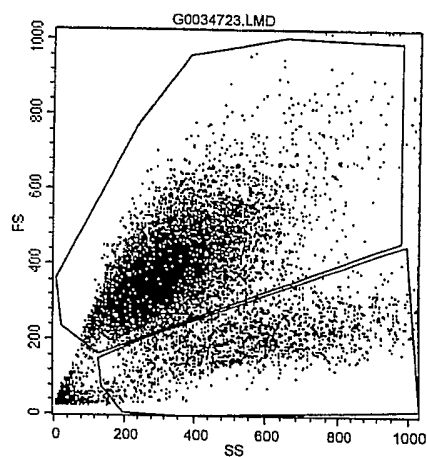


L1210 DDP No Treatment
7.0 Hours
1.4 % Death



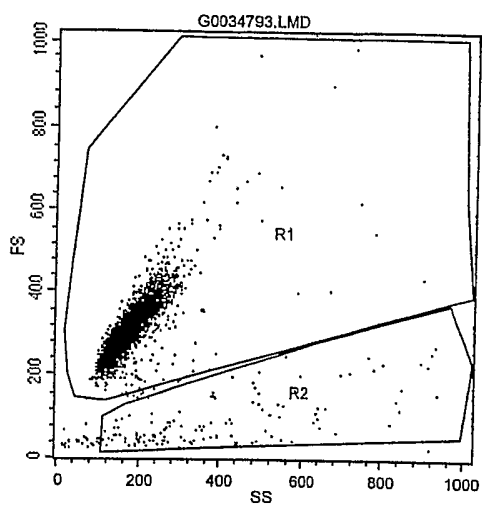
L1210 @ 25ug/mL Cerulenin
7.0 Hours
27 % Death

FIG. 2C



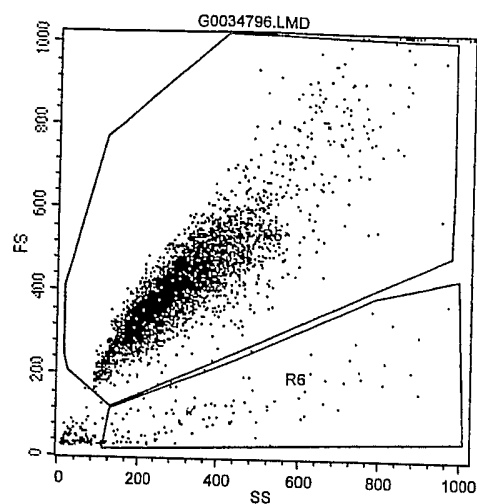
L1210 DDP @ 25ug/mL Cerulenin
7.0 Hours
14 % Death

FIG. 2D



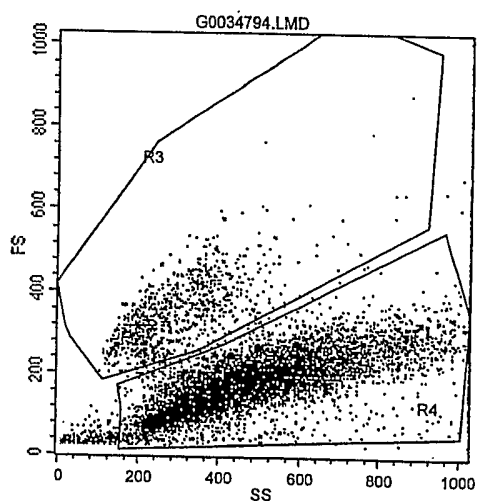
L1210 No Treatment
24 Hours
2.3 % Death

FIG. 3A



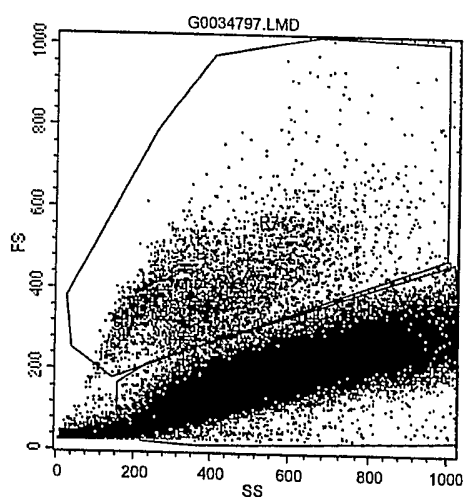
L1210 DDP No Treatment
24 Hours
2.0 % Death

FIG. 3B



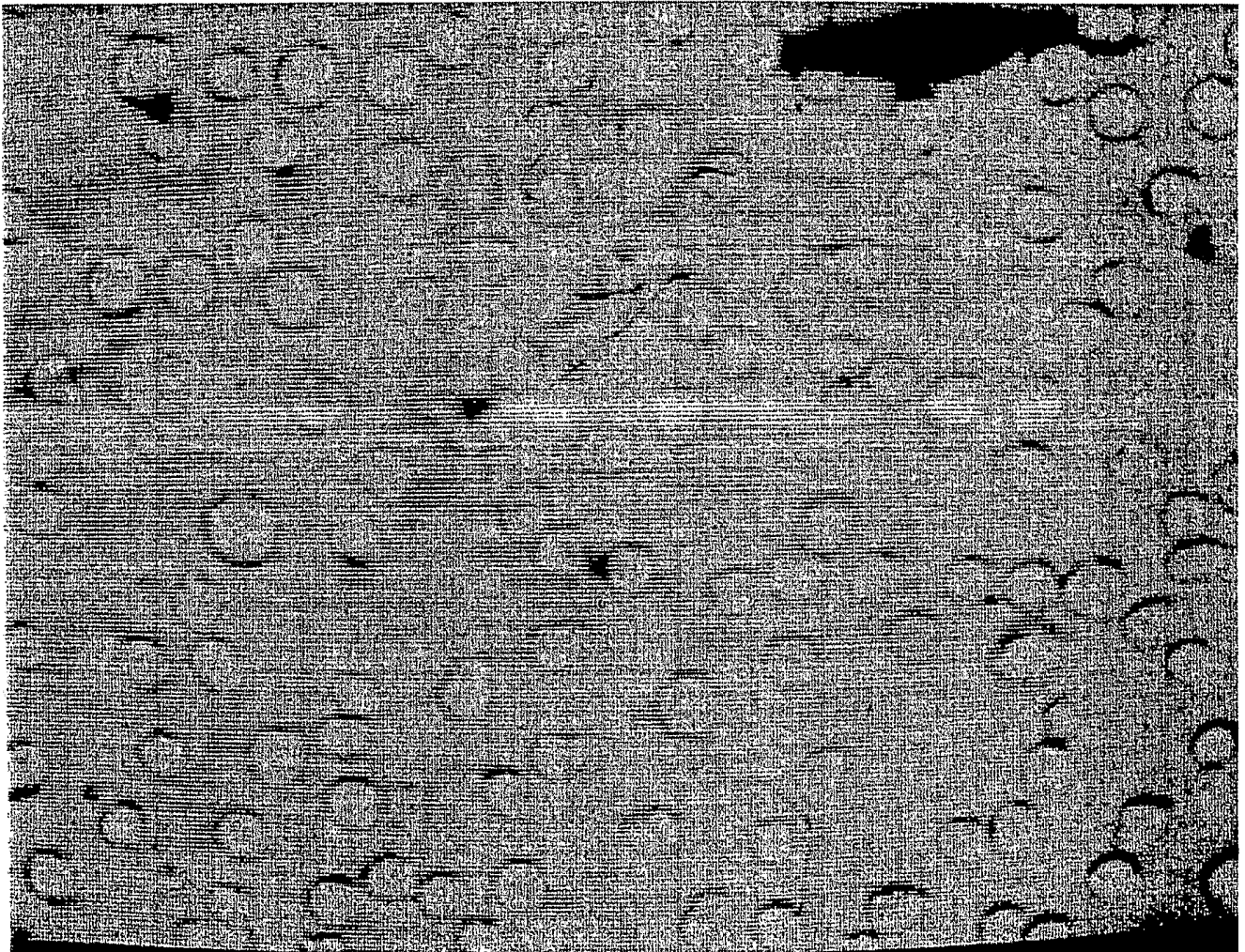
L1210 @ 25ug/mL Cerulenin
24 Hours
89 % Death

FIG. 3C



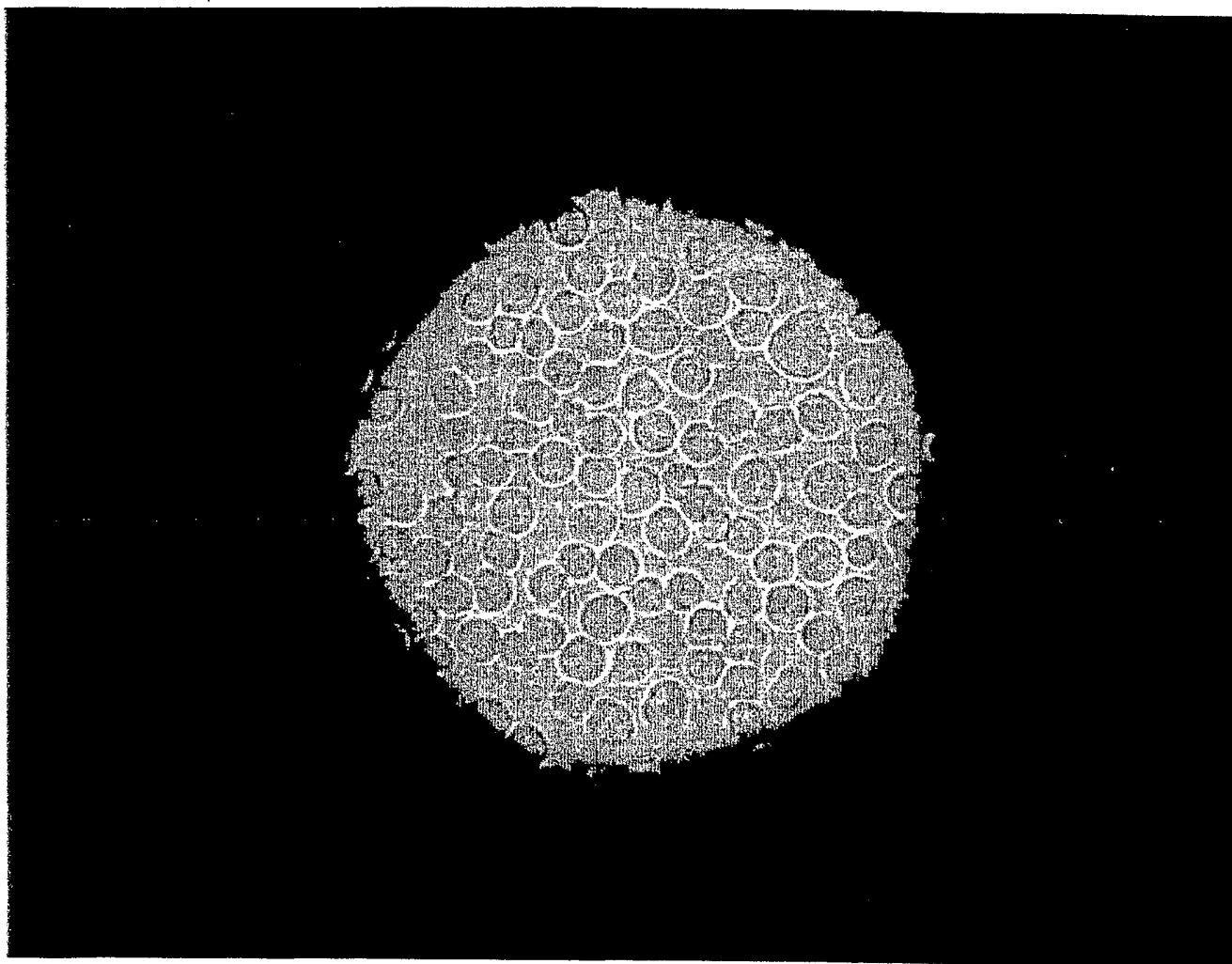
L1210 DDP @ 25ug/mL Cerulenin
24 Hours
92 % Death

FIG. 3D



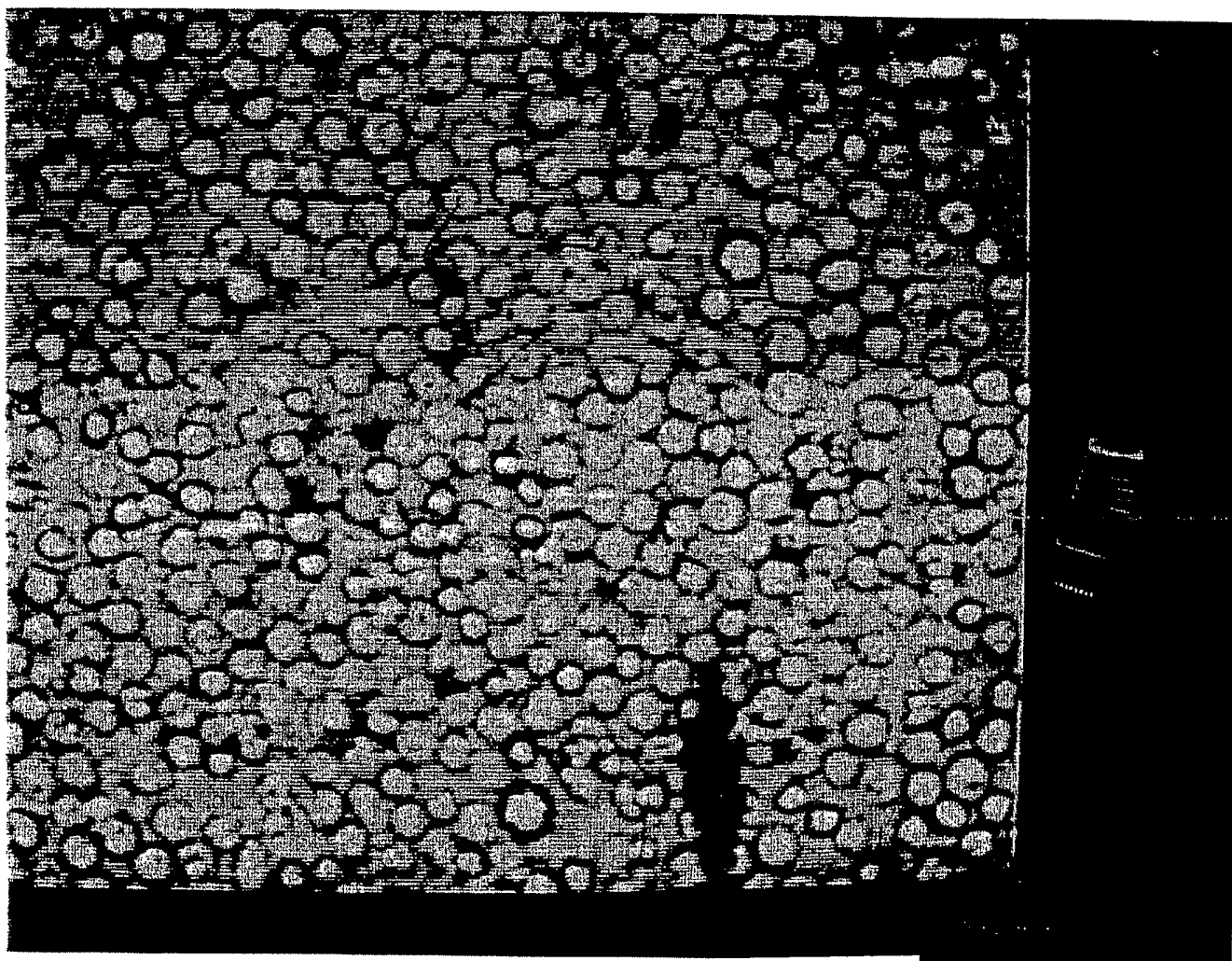
HL60 No Stn

FIG. 4A



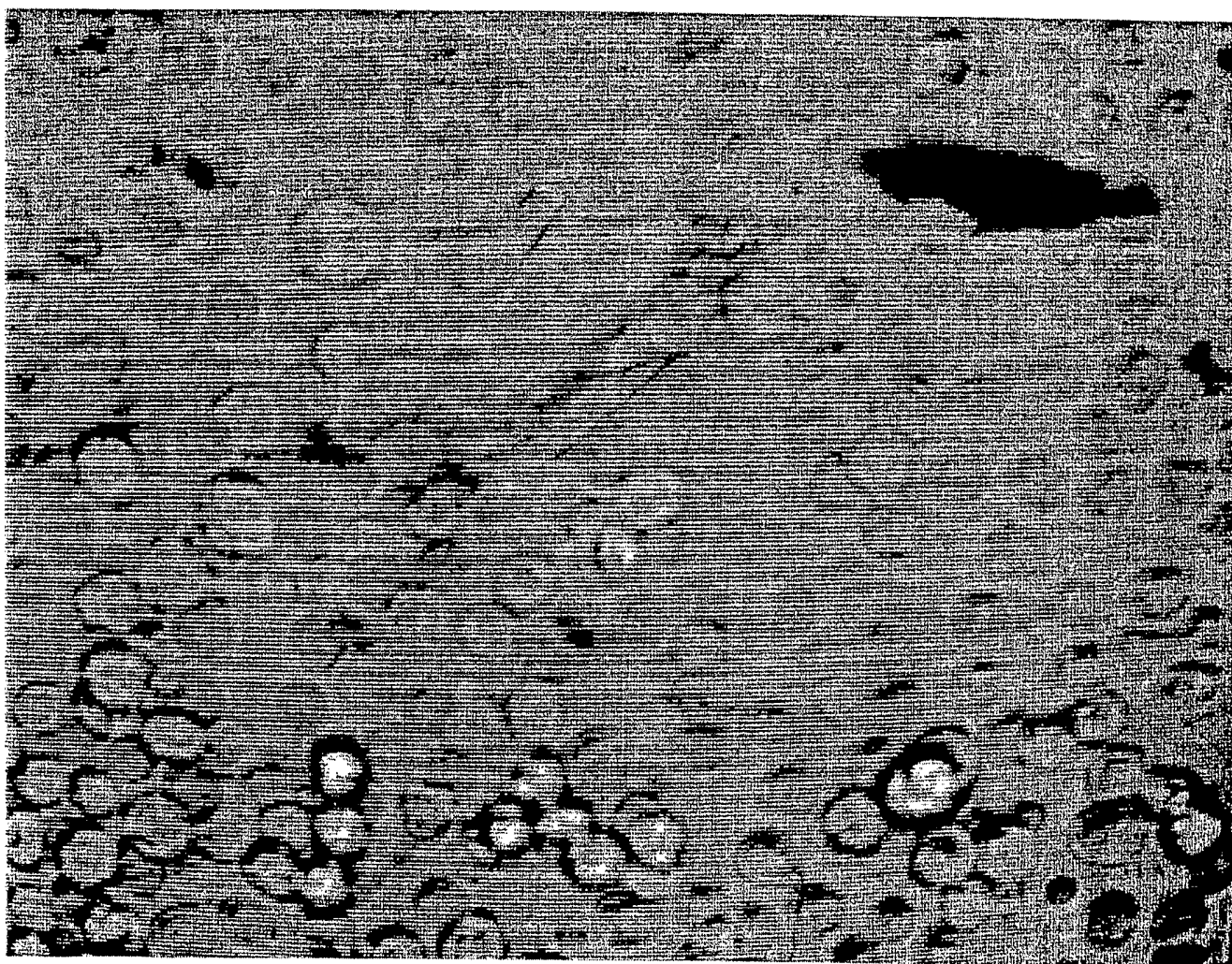
HL60 No Stn

FIG. 4B



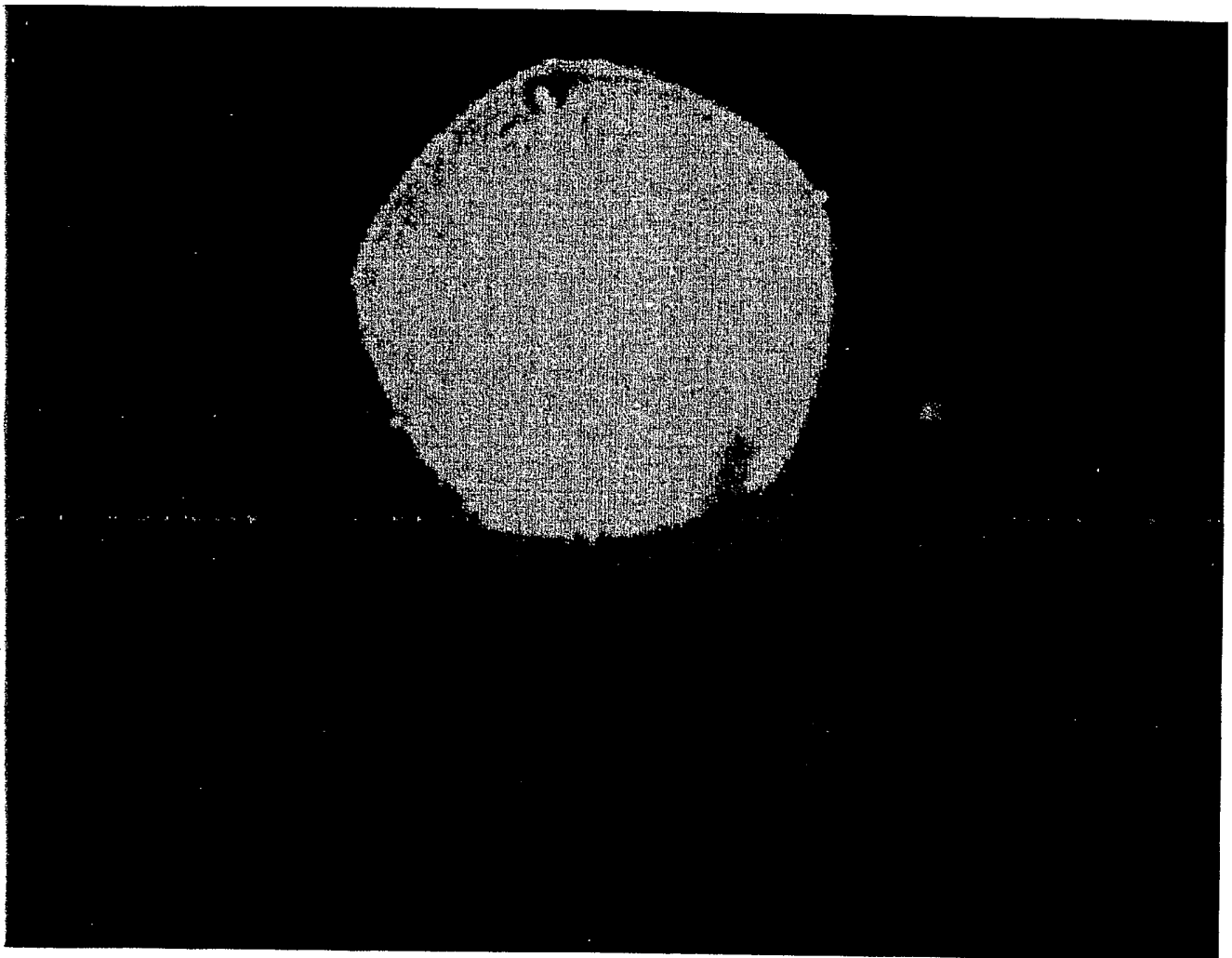
HL60 Treated Cells

FIG. 4C



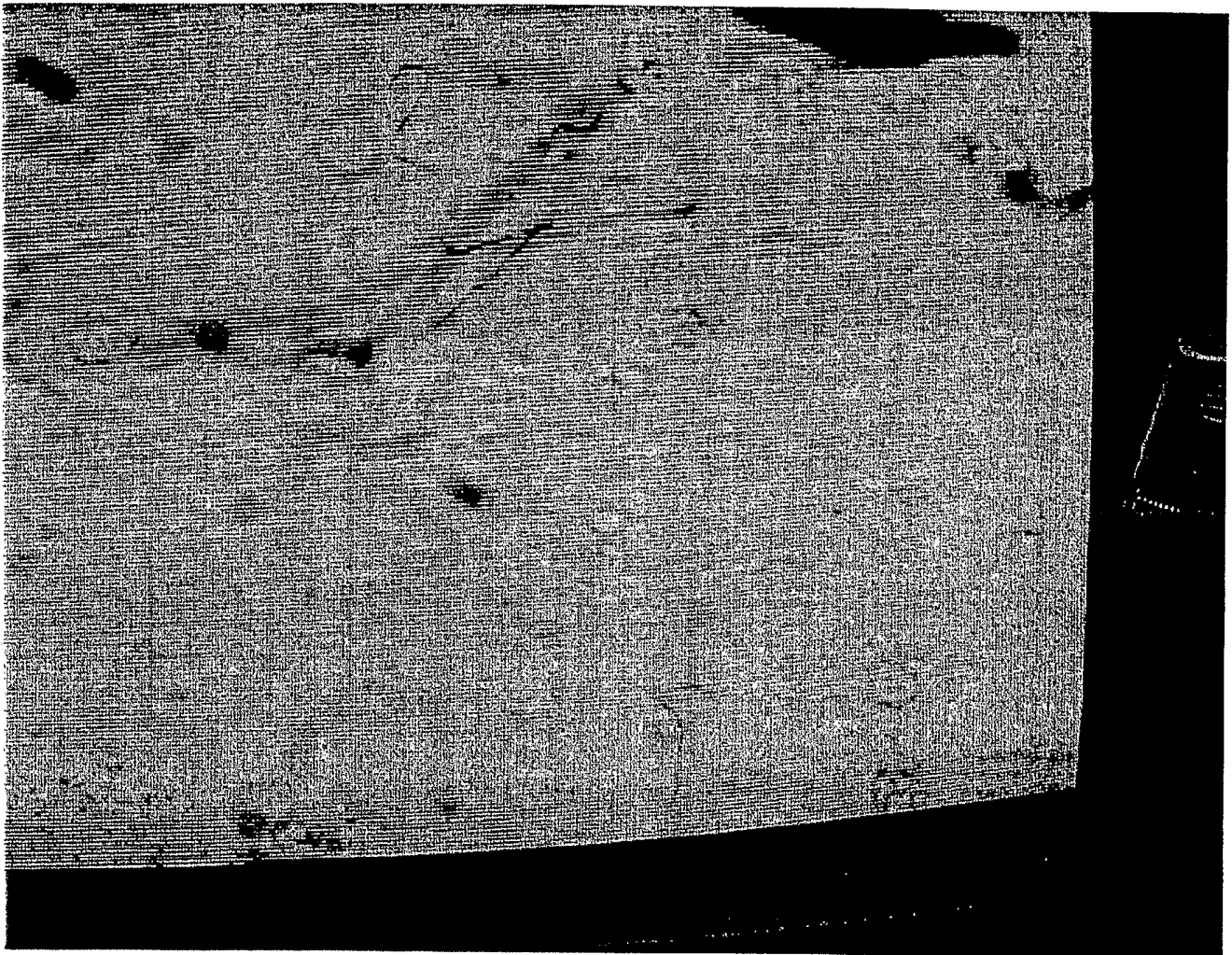
HL60 MDR Cells No Stn

FIG. D



HL60 MDR Cells Treated Cells

FIG. 4E



HL60

MDR Cells

Treated Cells

FIG. 4F



FIG. 5A

Mouse #1 Day 12
PBS Injection

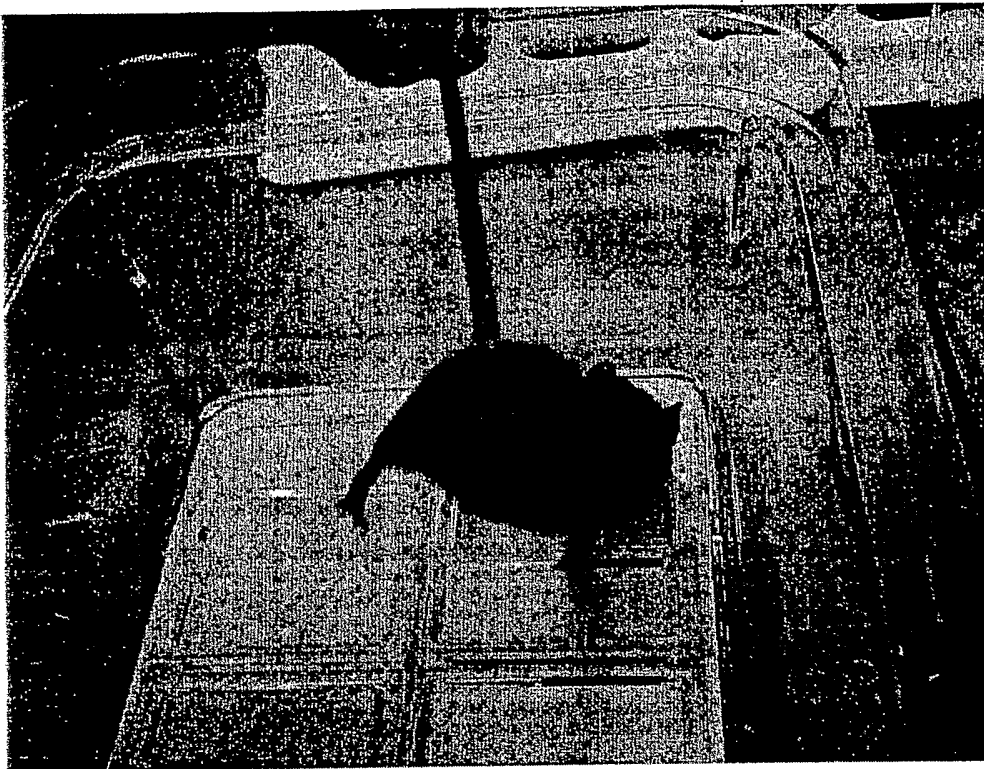


FIG. 5B

Mouse #3 Day 13
PBS Injection

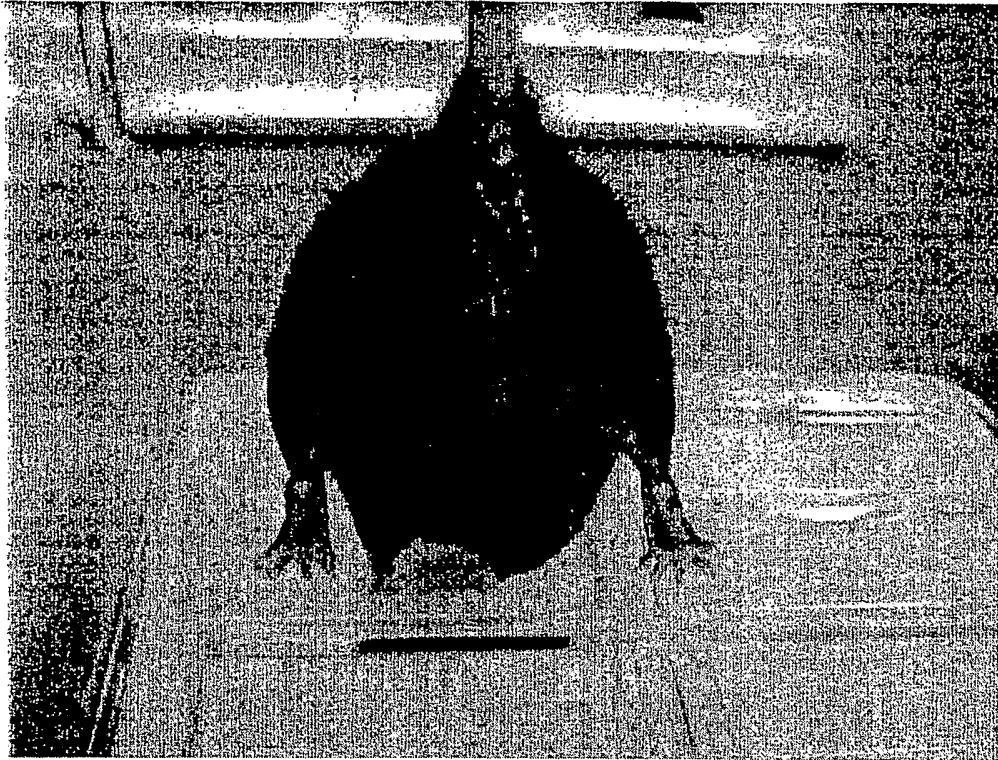


FIG. 5C

Mouse #6 Day 12
B16 fl Melanoma
No treatment

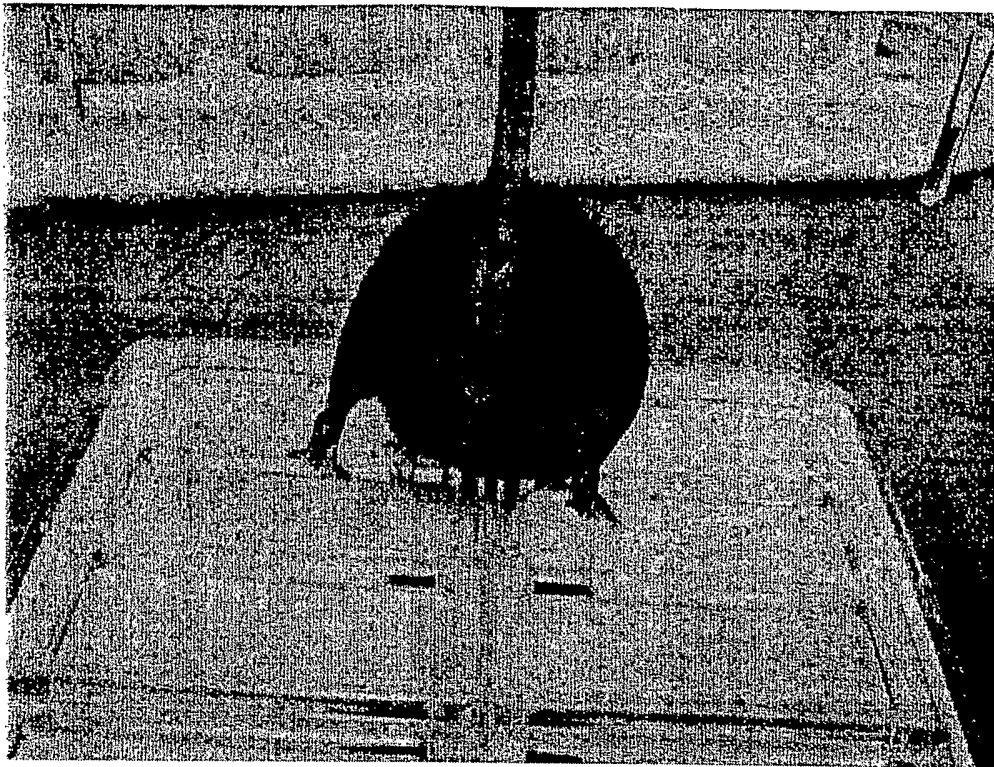


FIG. 5D

Mouse #8 Day 12
B16 fl Melanoma
Treated with Cerlonin

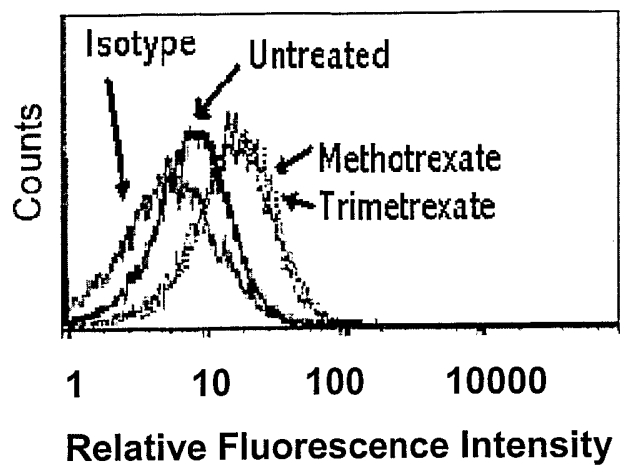


FIG. 6A

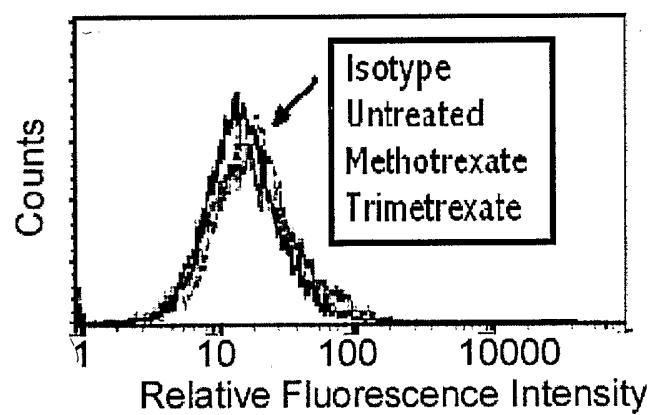


FIG. 6B

L1210 & L1210 DDP Cells
Post 23 Hour Incubation with Etomoxir @ 25ug/mL
Fas Staining

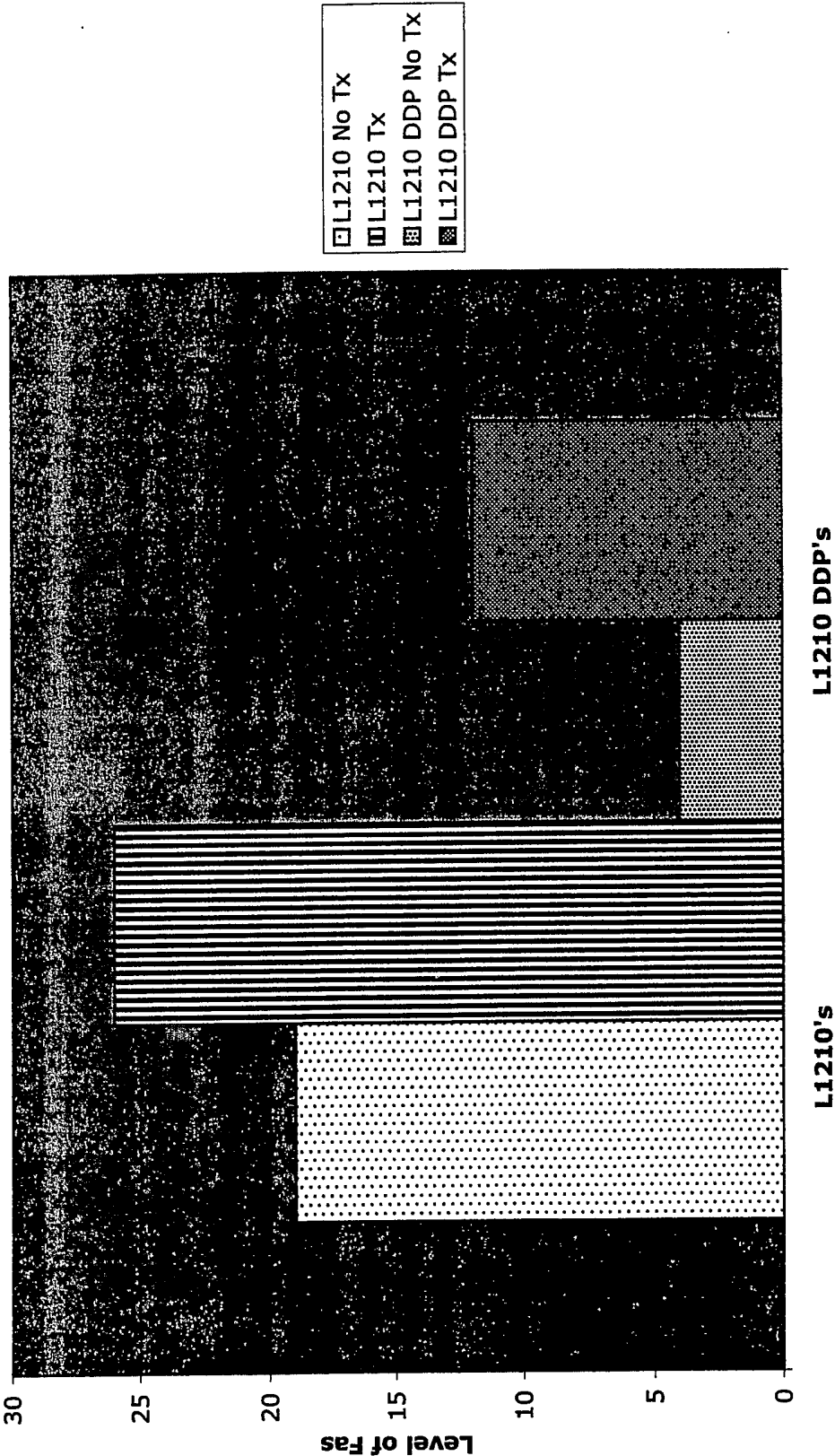


FIG. 7

**L1210 DDP Cells Treated (or not) with:
Methotrexate @ 10⁻⁸M, Eotmoxir @ 25ug/mL, or Both-24Hrs
DCFDA Staining**

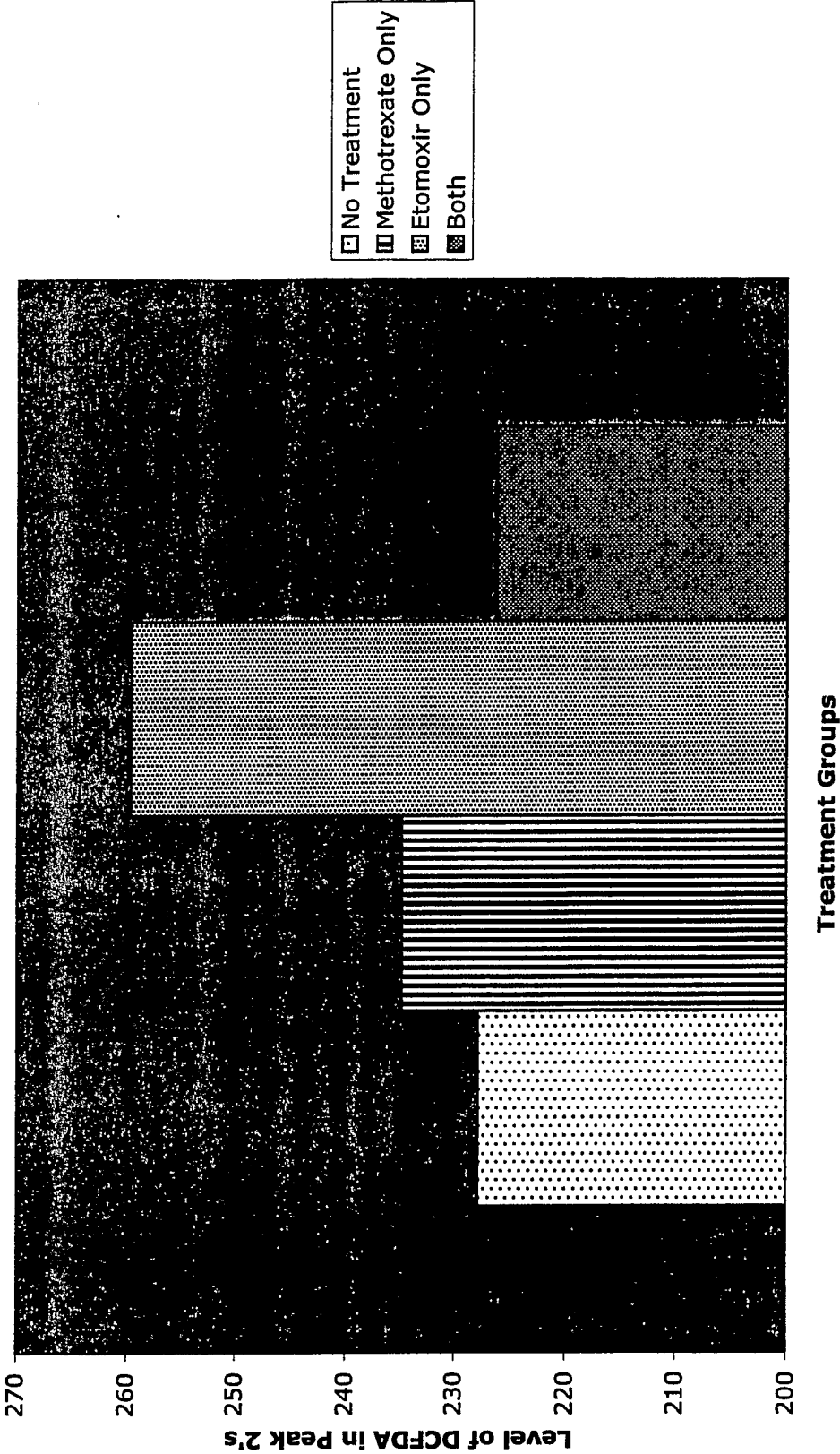


FIG. 8

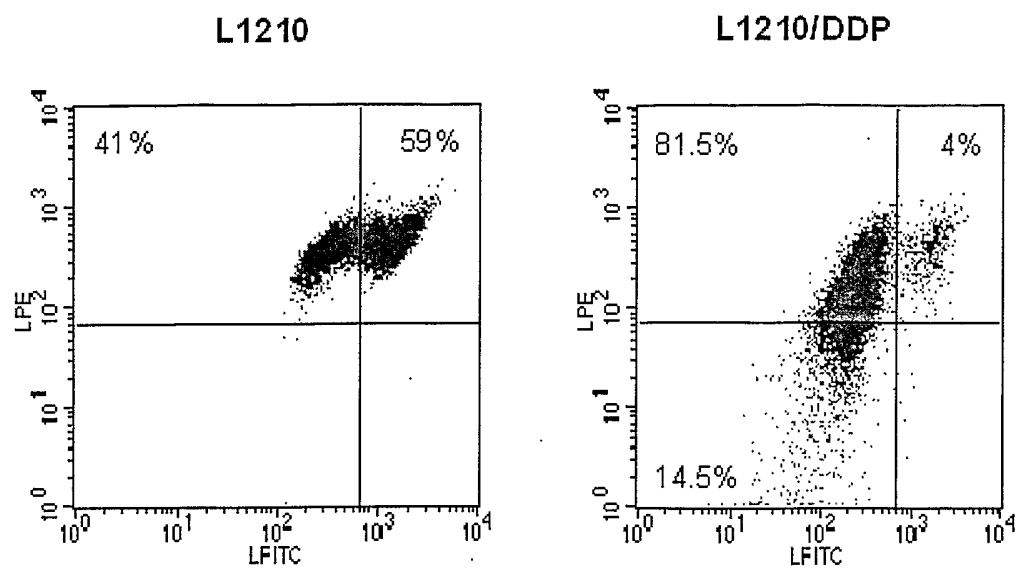


FIG. 9

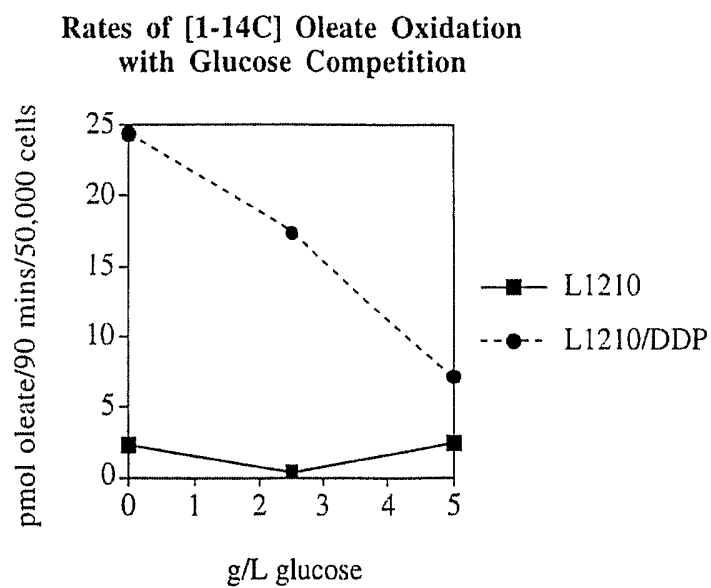


FIG. 10

**L1210 DDP Cells Treated (or not) with:
Methotrexate @ 10⁻⁸M, Etomoxir @ 25ug/mL, or Both-24Hrs
MitoTracker Staining**

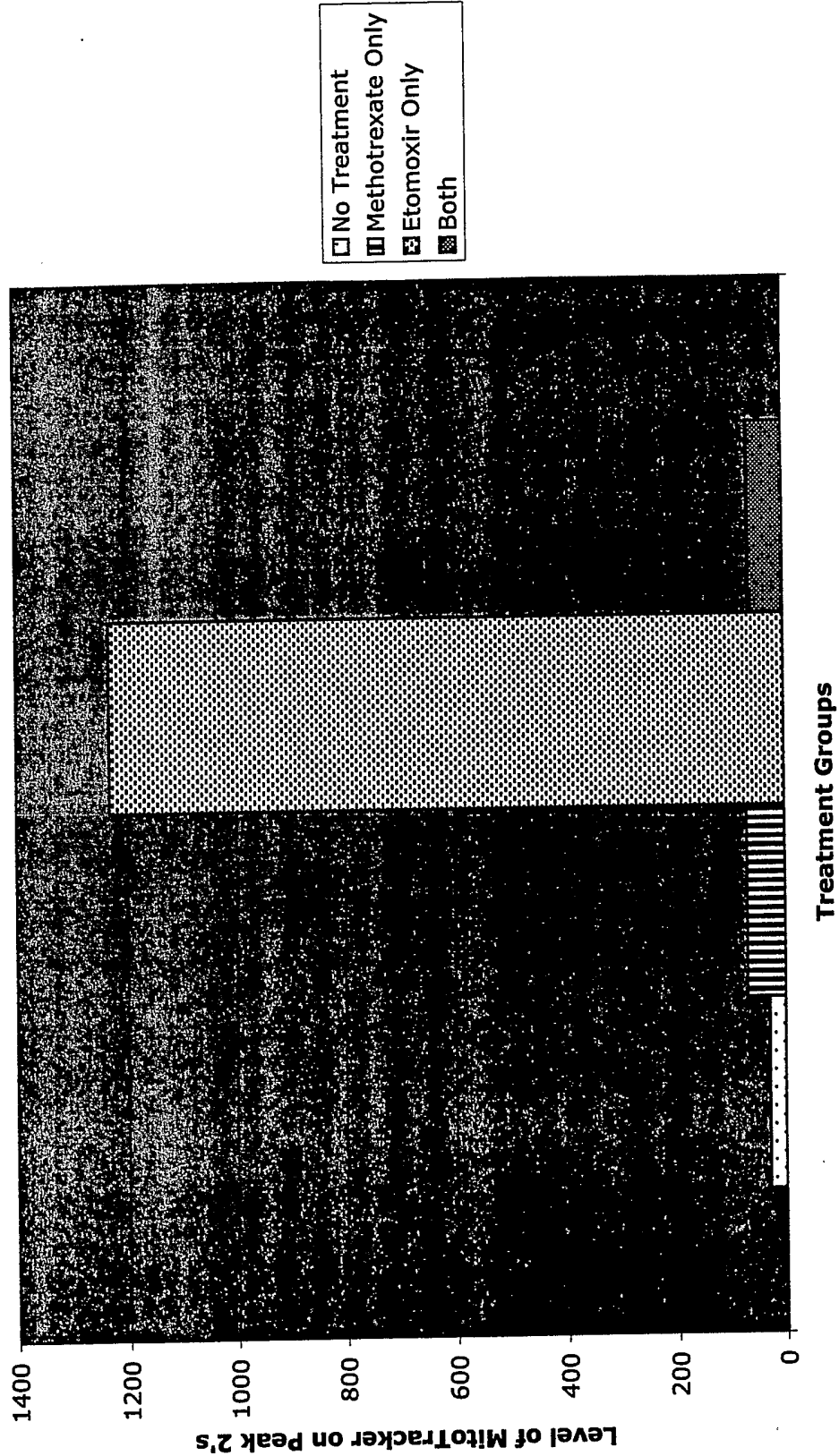


FIG. 11

**L1210 Cells Treated (or Not) with:
Methotrexate @ 10⁻⁸M, Etomoxir @ 25ug/ml, or Both-24Hrs
DCFDA Staining**

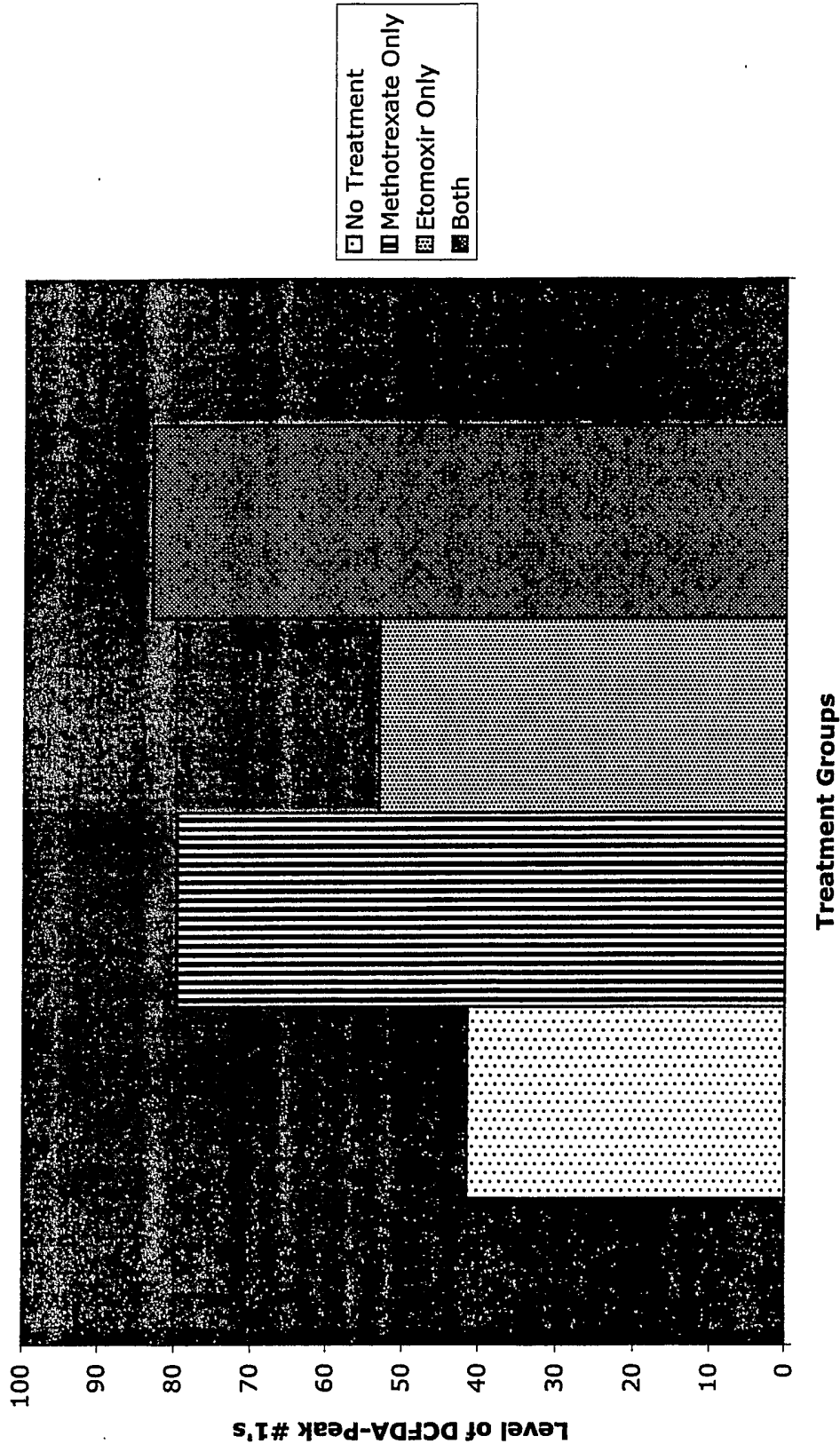


FIG. 12A

**L1210 Cells Treated (or not) with:
Methotrexate @ 10⁻⁸M, Etomoxir @ 25ug/mL, or Both-24Hrs
MitoTracker Staining**

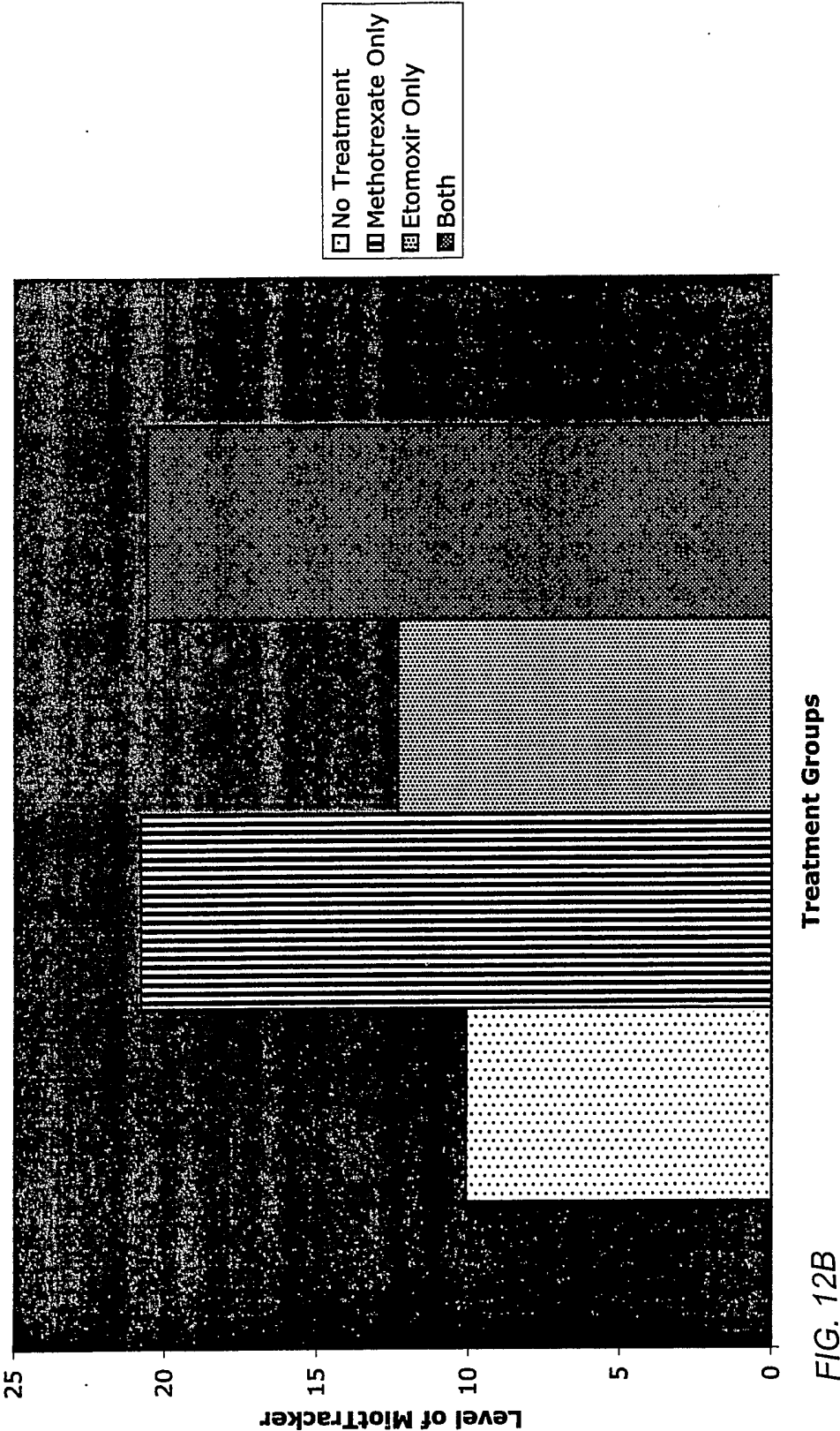


FIG. 12B

**RU937 Cells treated (or not) with:
Methotrexate @ 10⁻⁸M, Etomoxir @ 25ug/mL, or both-24Hrs
MitoTracker Staining**

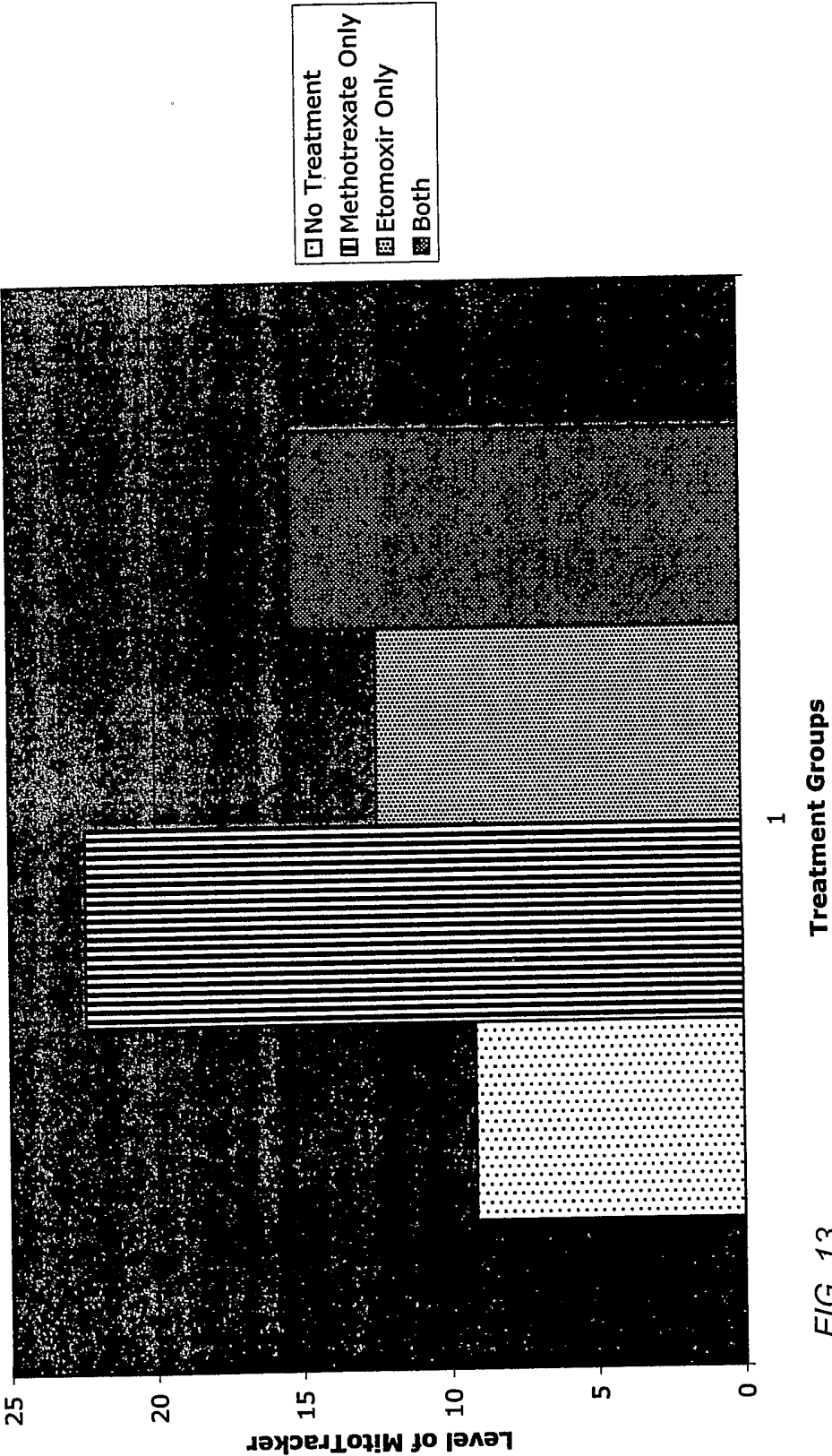


FIG. 13

**HL60 Cells Treated (or not) with:
Methotrexate @ 10⁻⁸M, Etomoxir @ 25ug/mL, or both-24Hrs
DCFDA Staining**

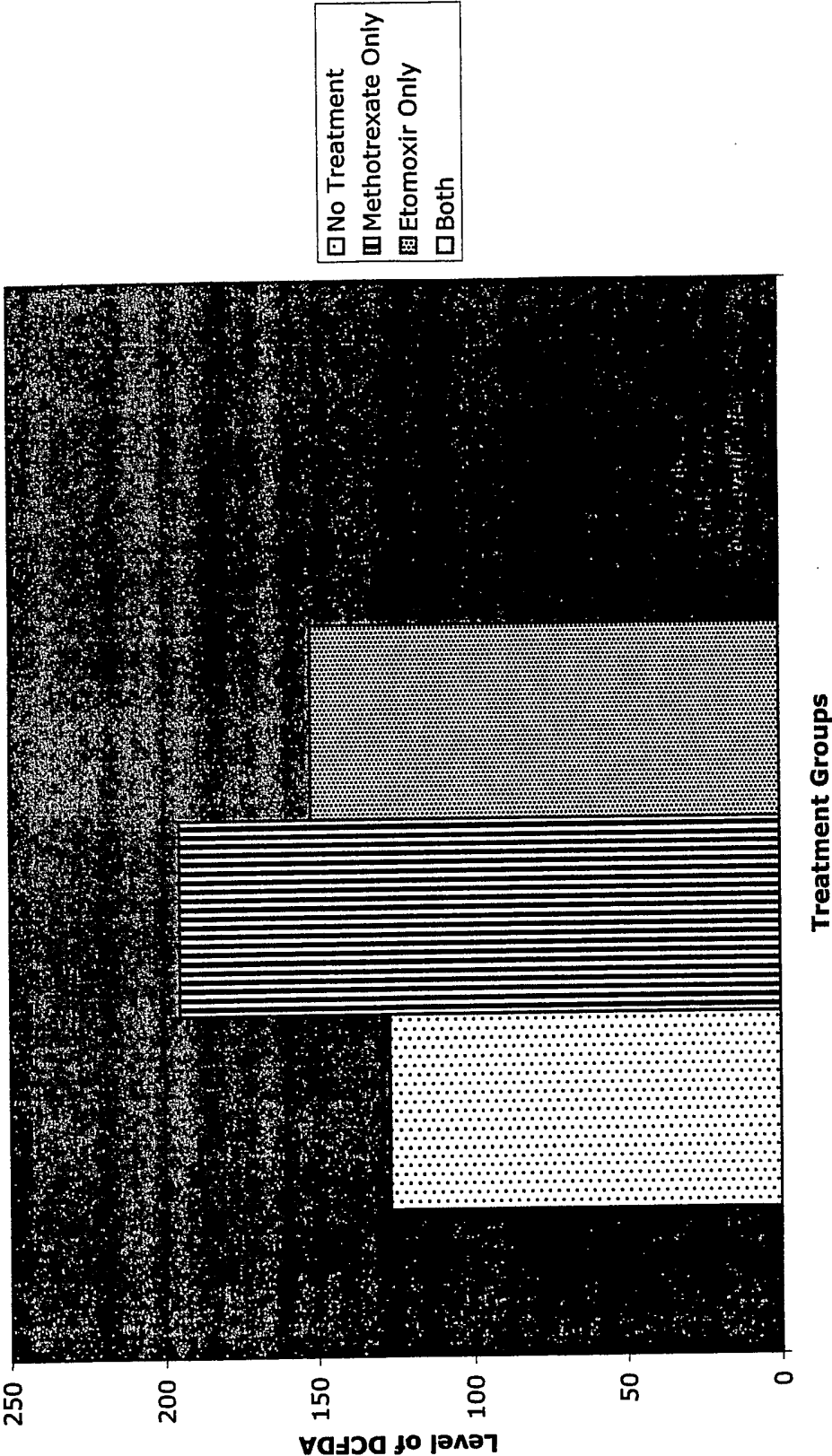
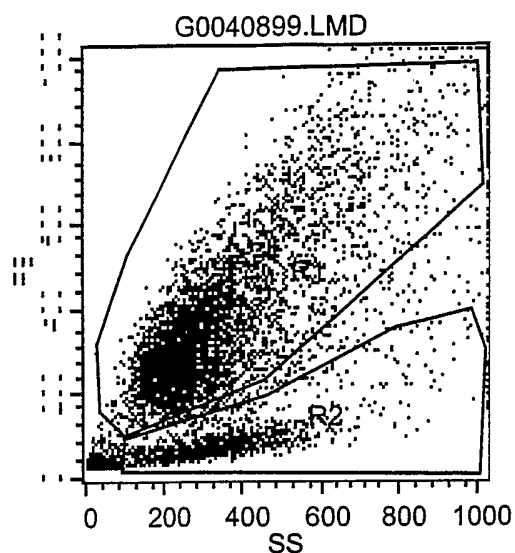
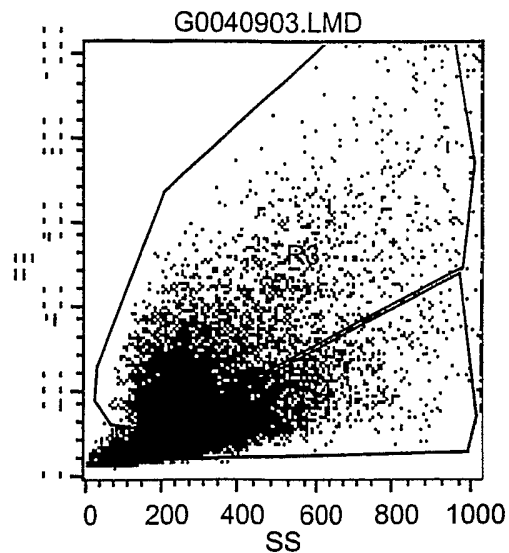


FIG. 14

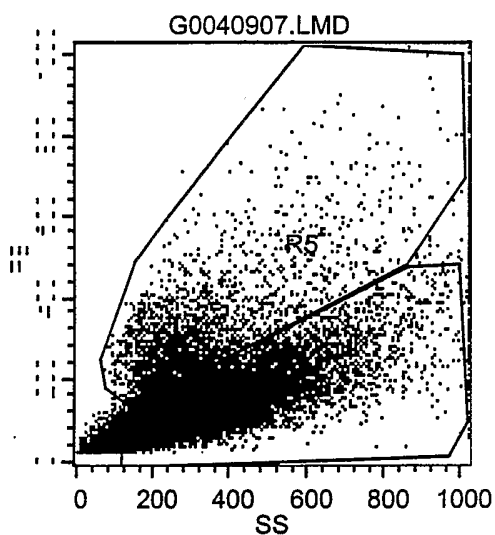
B16F1 Melanoma Data
Treated (or not) with Etomoxir for 48 Hours



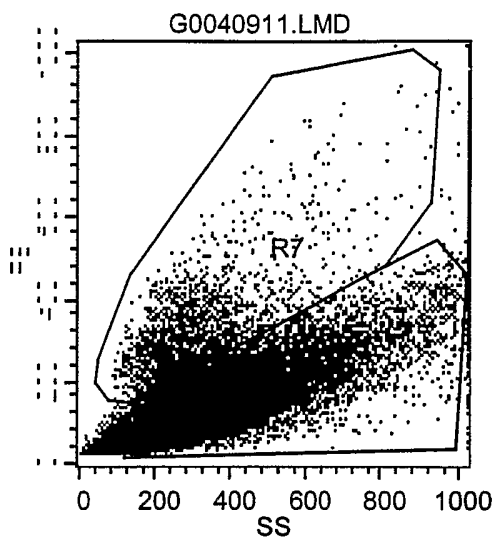
B16F1 Melanoma Cells
Untreated
Percent Death = 19%



B16F1 Melanoma Cells
100ug/mL Etomoxir
Percent Death = 65%



B16F1 Melanoma Cells
25ug/mL Etomoxir
Percent Death = 89.5%



B16F1 Melanoma Cells
500ug/mL Etomoxir
Percent Death = 91.3%

FIG. 15

B16F1 Melanoma Cells
Treated (or not) with Etomoxir for 48 Hours

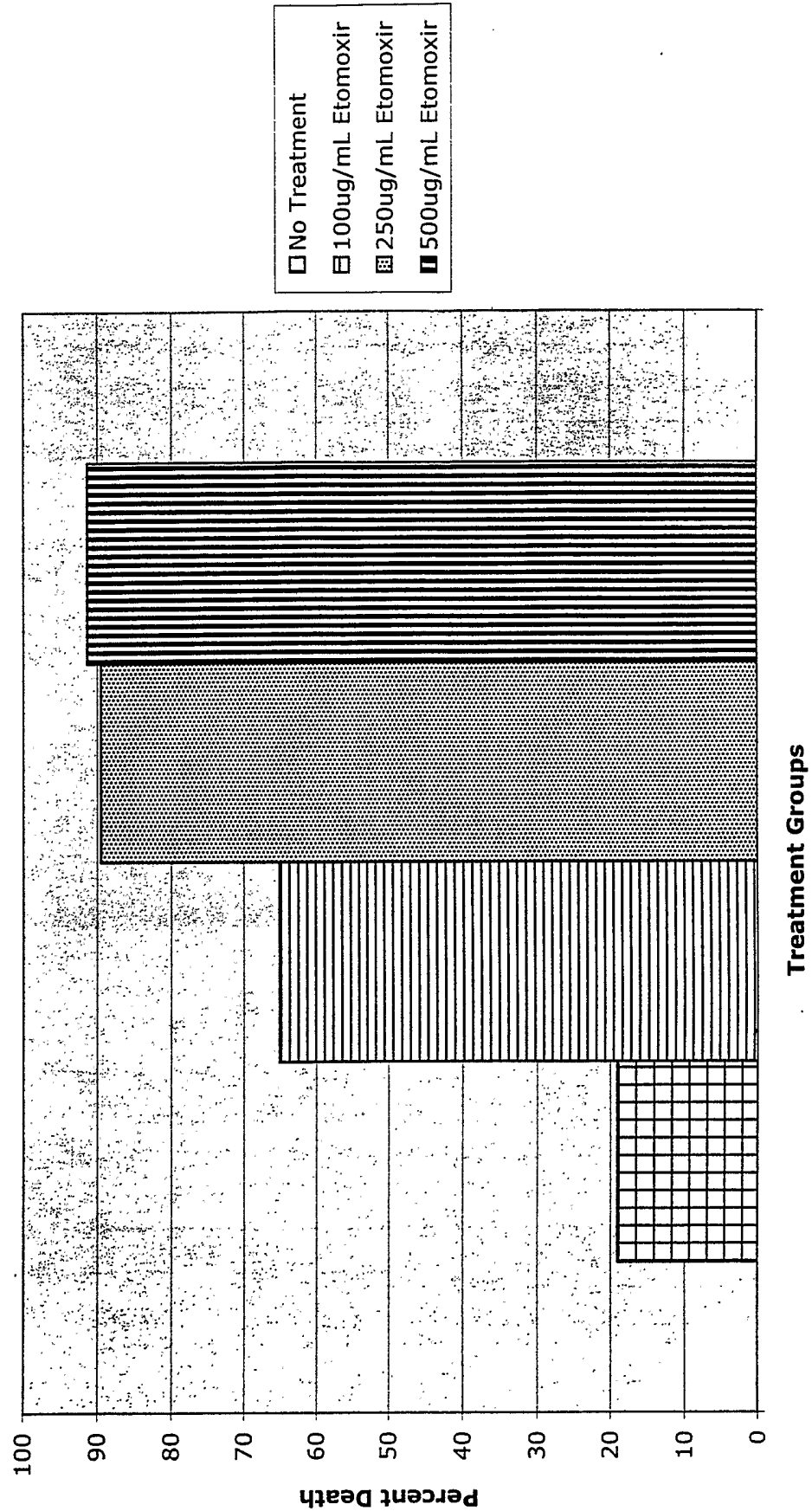


FIG. 16

B16F1 Melanoma Cells
Treated (or not) with Etomoxir for 48 Hours

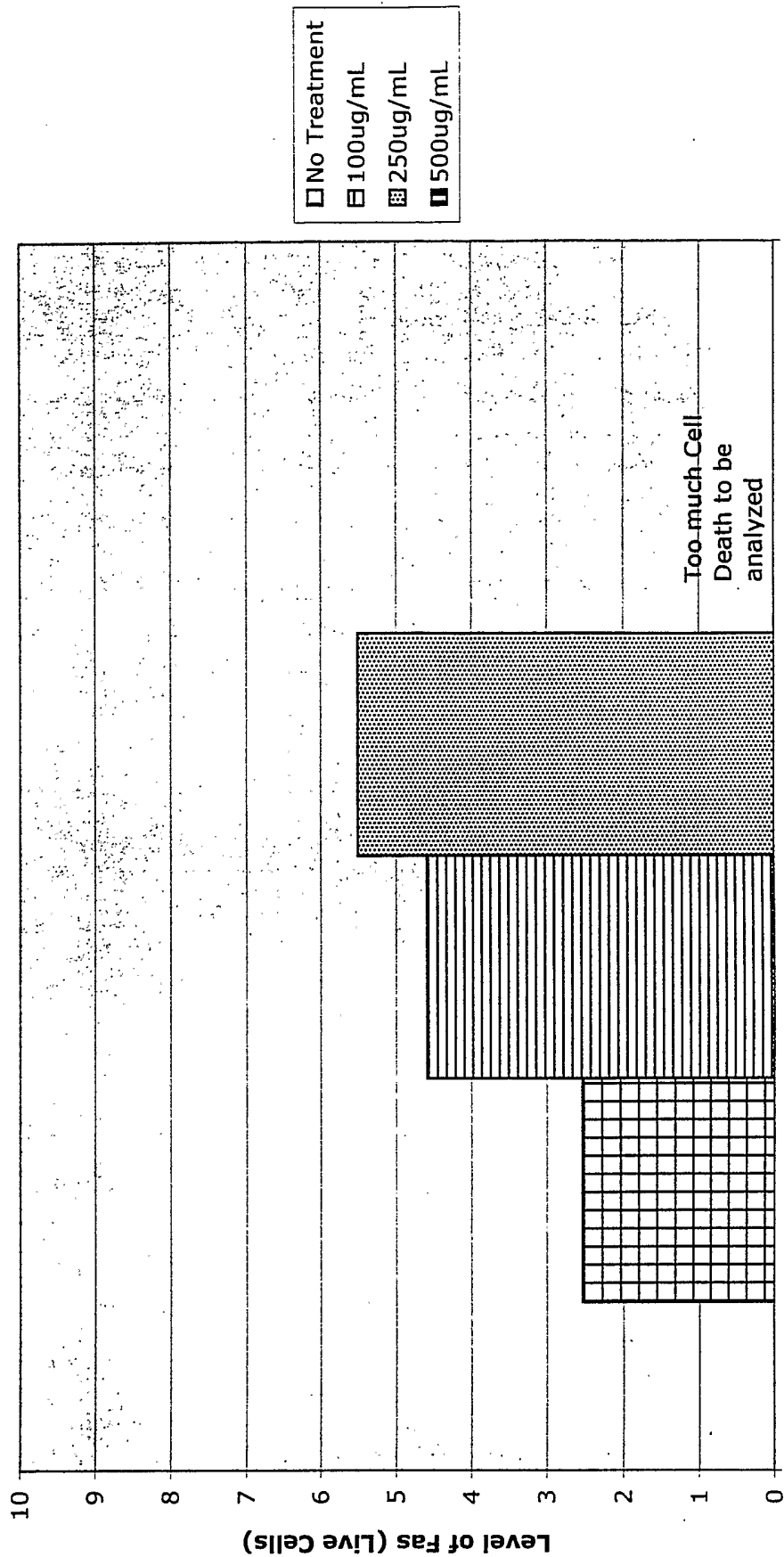
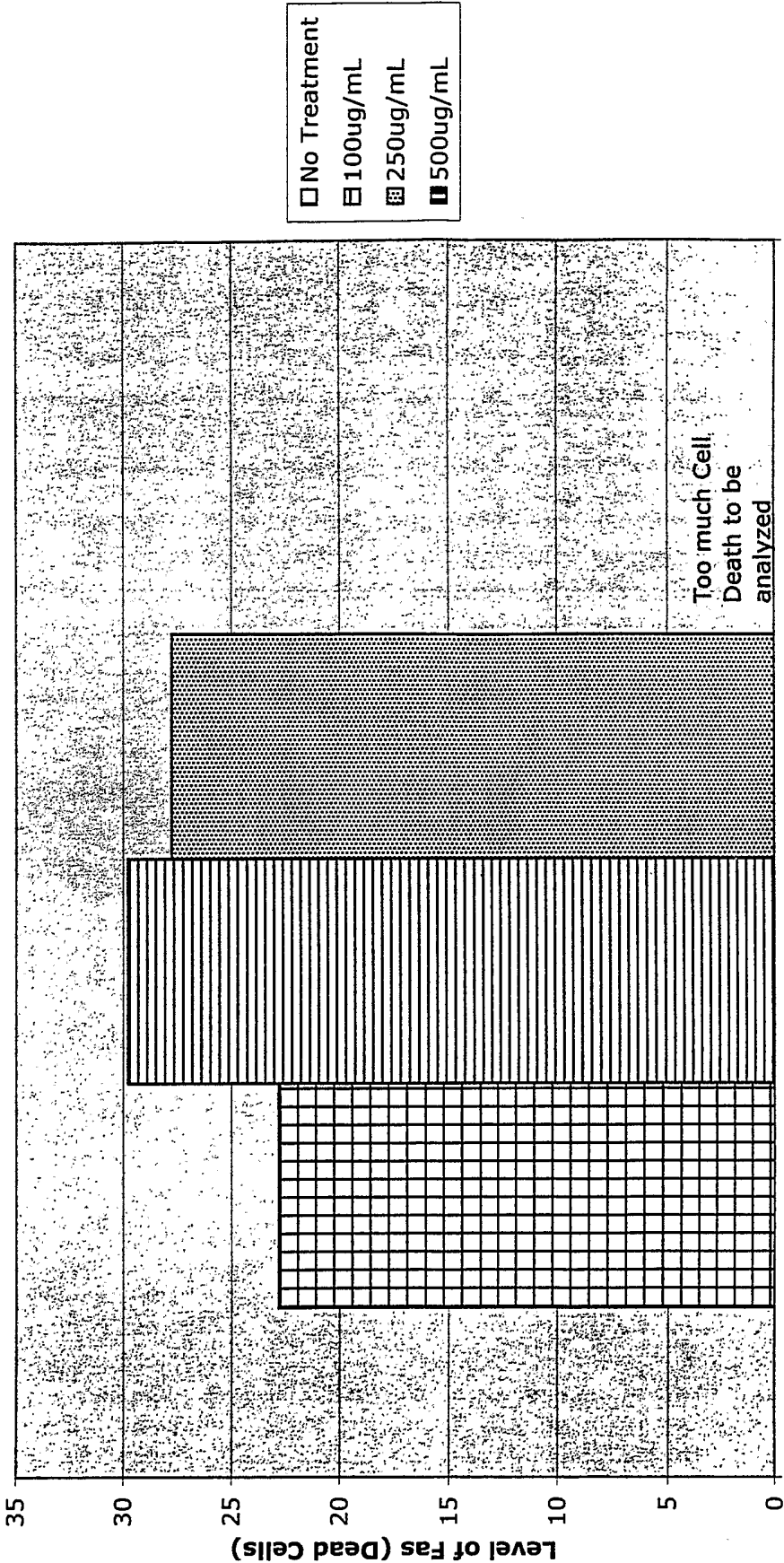


FIG. 17

B16F1 Melanoma Cells
Treated (or not) with Etomoxir for 48 Hours



Treatment Groups

FIG. 18

B16F1 Melanoma Cells
Treated (or not) with Etomoxir for 24 Hours

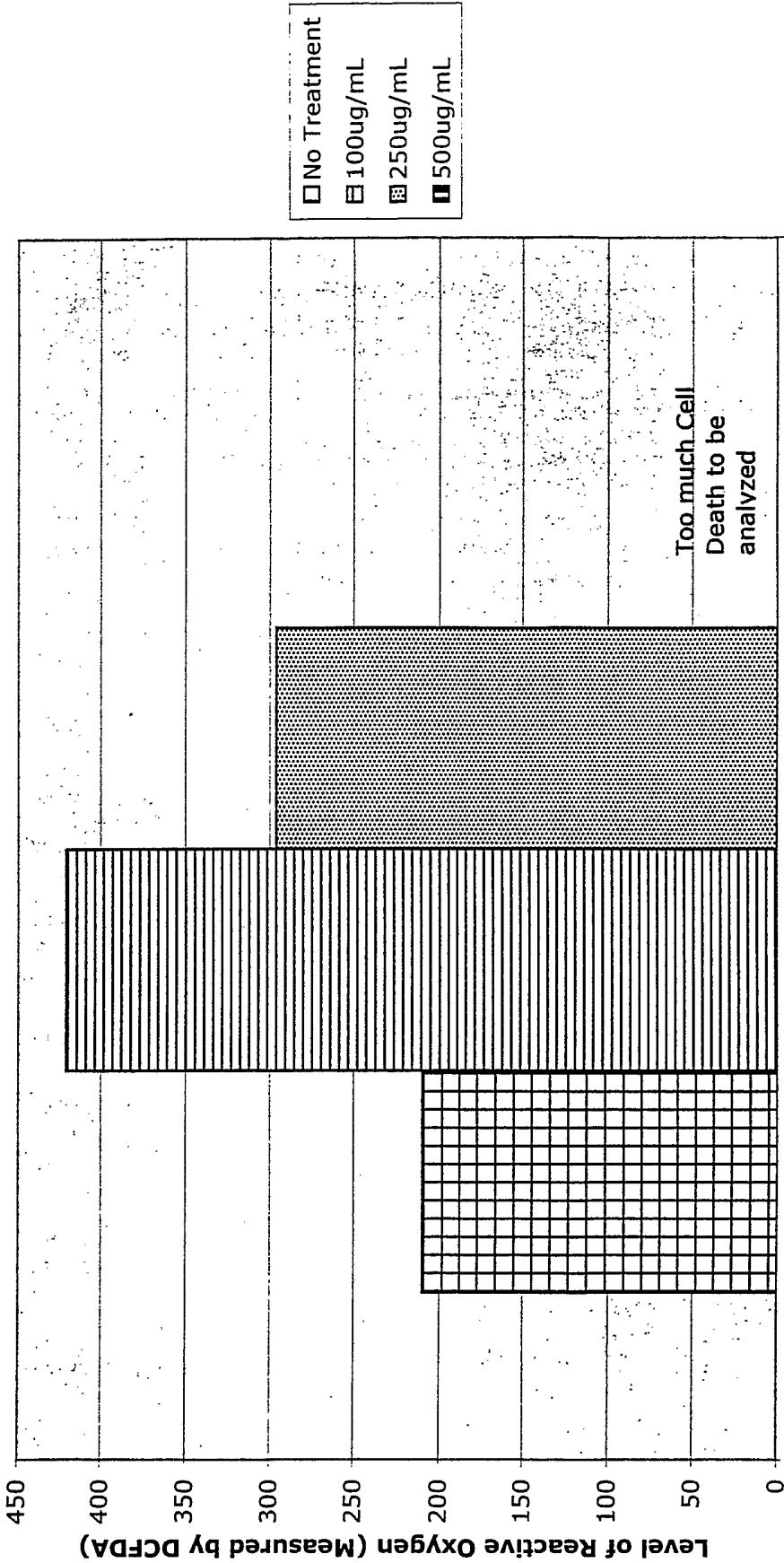


FIG. 19

Treatment Groups

B16F1 Melanoma Cells
Treated (or not) with Etomoxir for 24 Hours

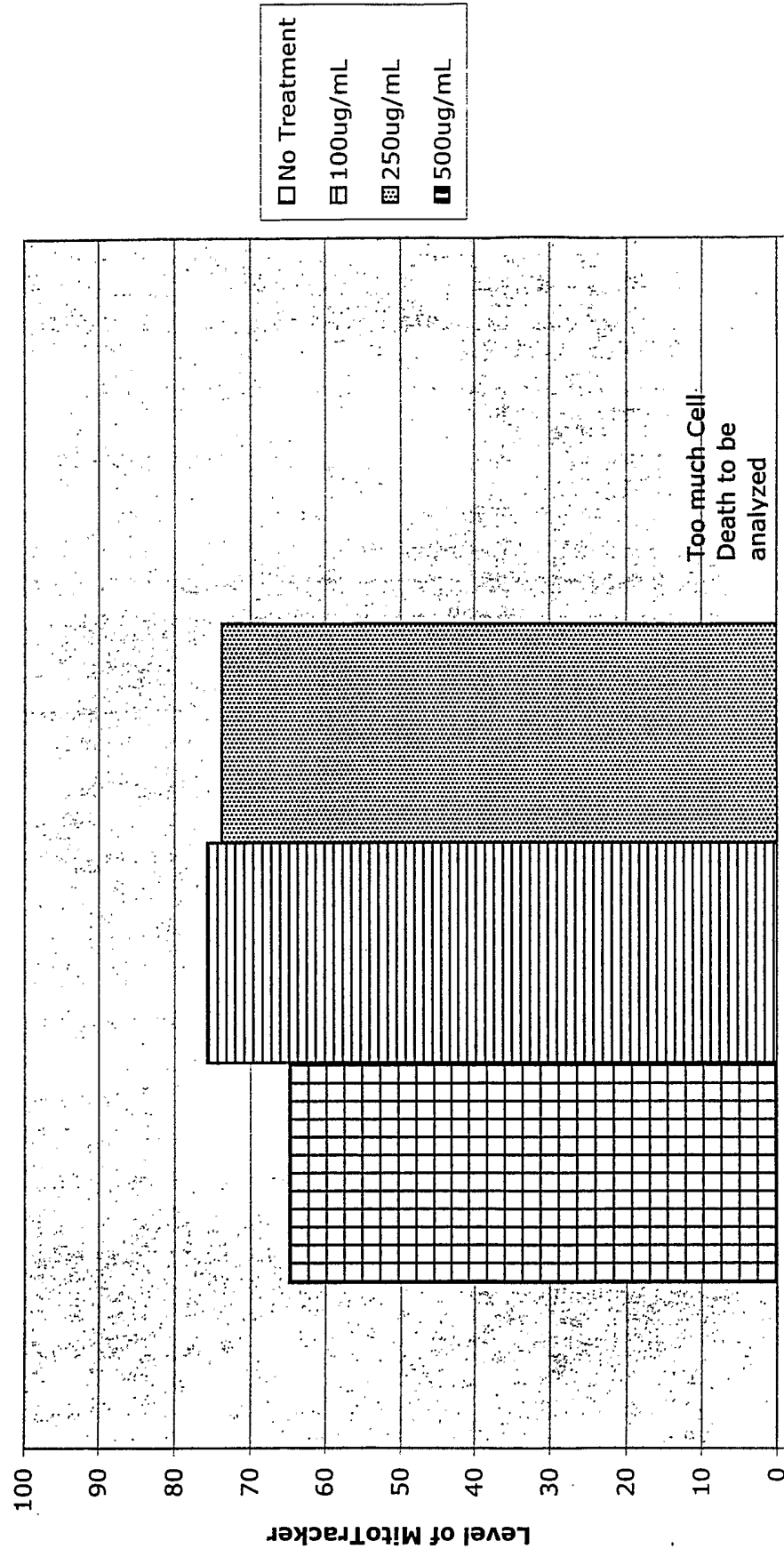


FIG. 20

Treatment Groups

B16F1 Melanoma Data
Preliminary "Live Model" work
Effects of Etomoxir on Tumor size

Effects of ETO on B16F1 / C57B6 Mice

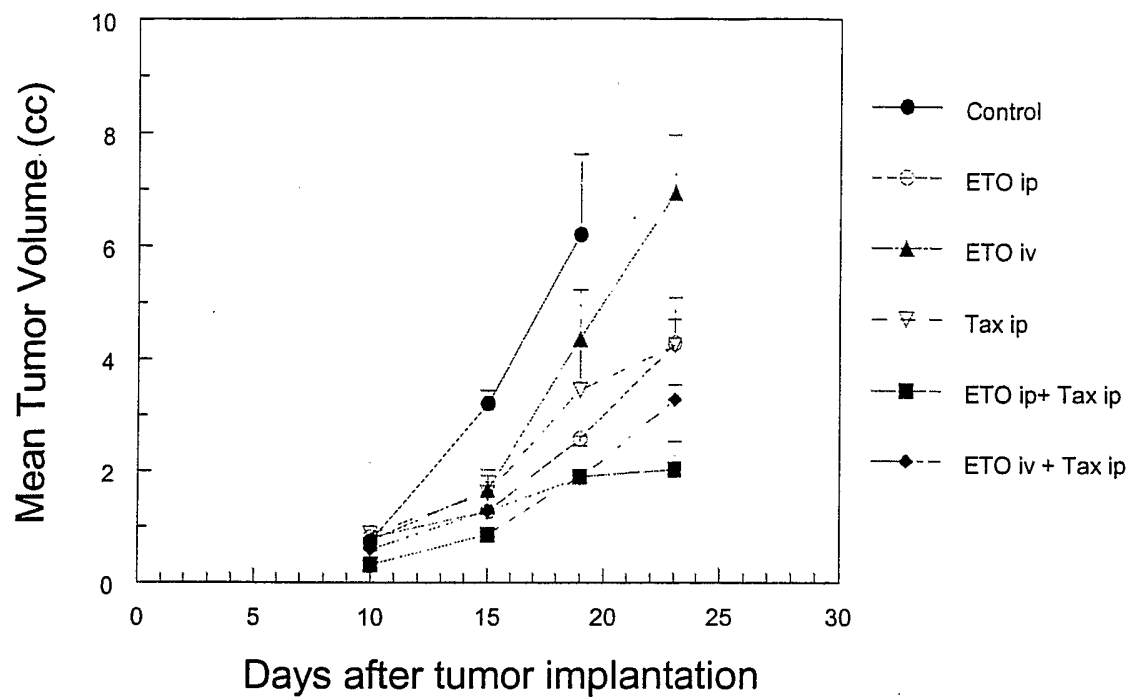


FIG. 21

HL60 Cells
Treated (or not) with Etomoxir for 72 Hours and Adriamycin for 24 Hours

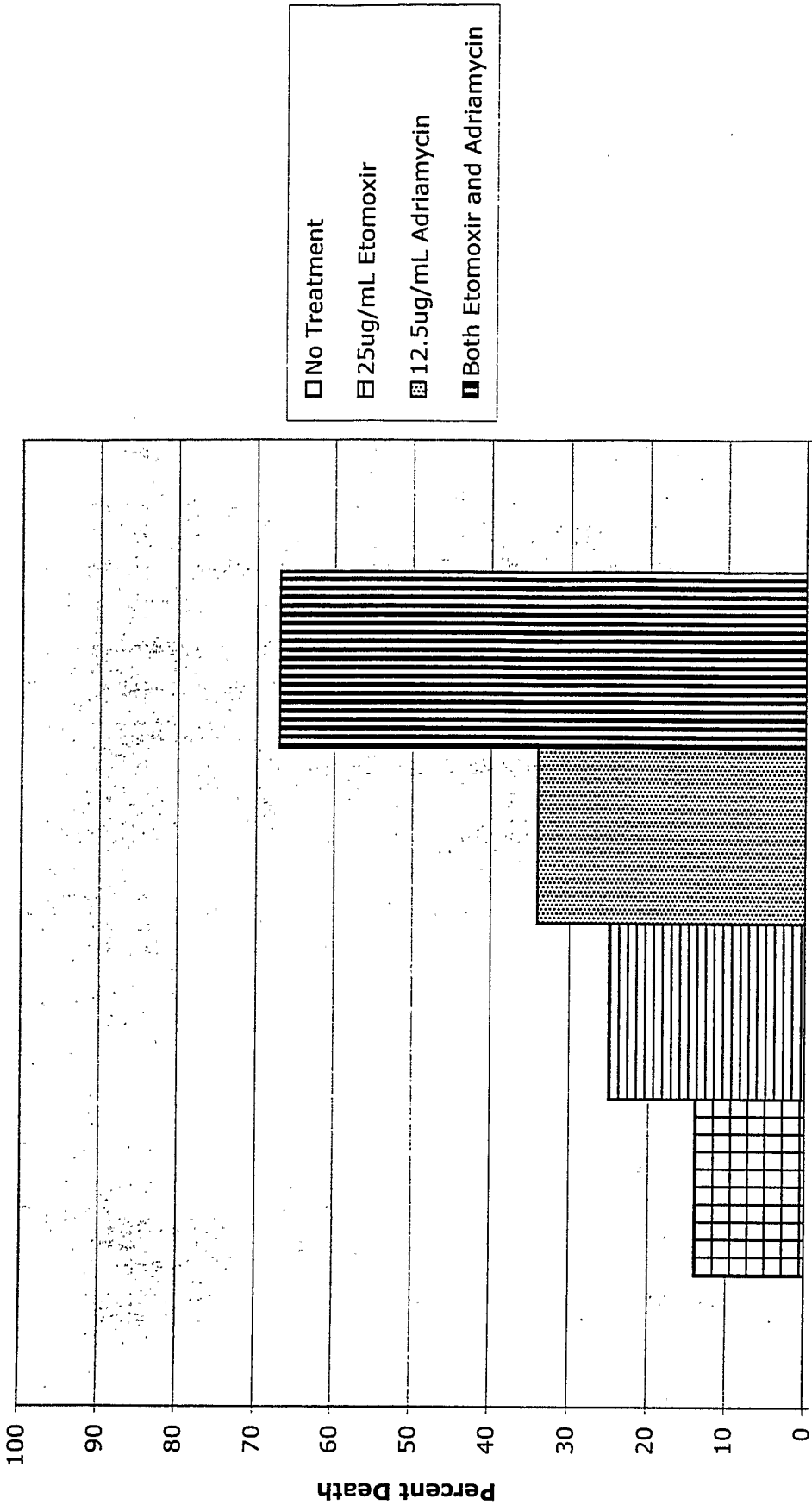
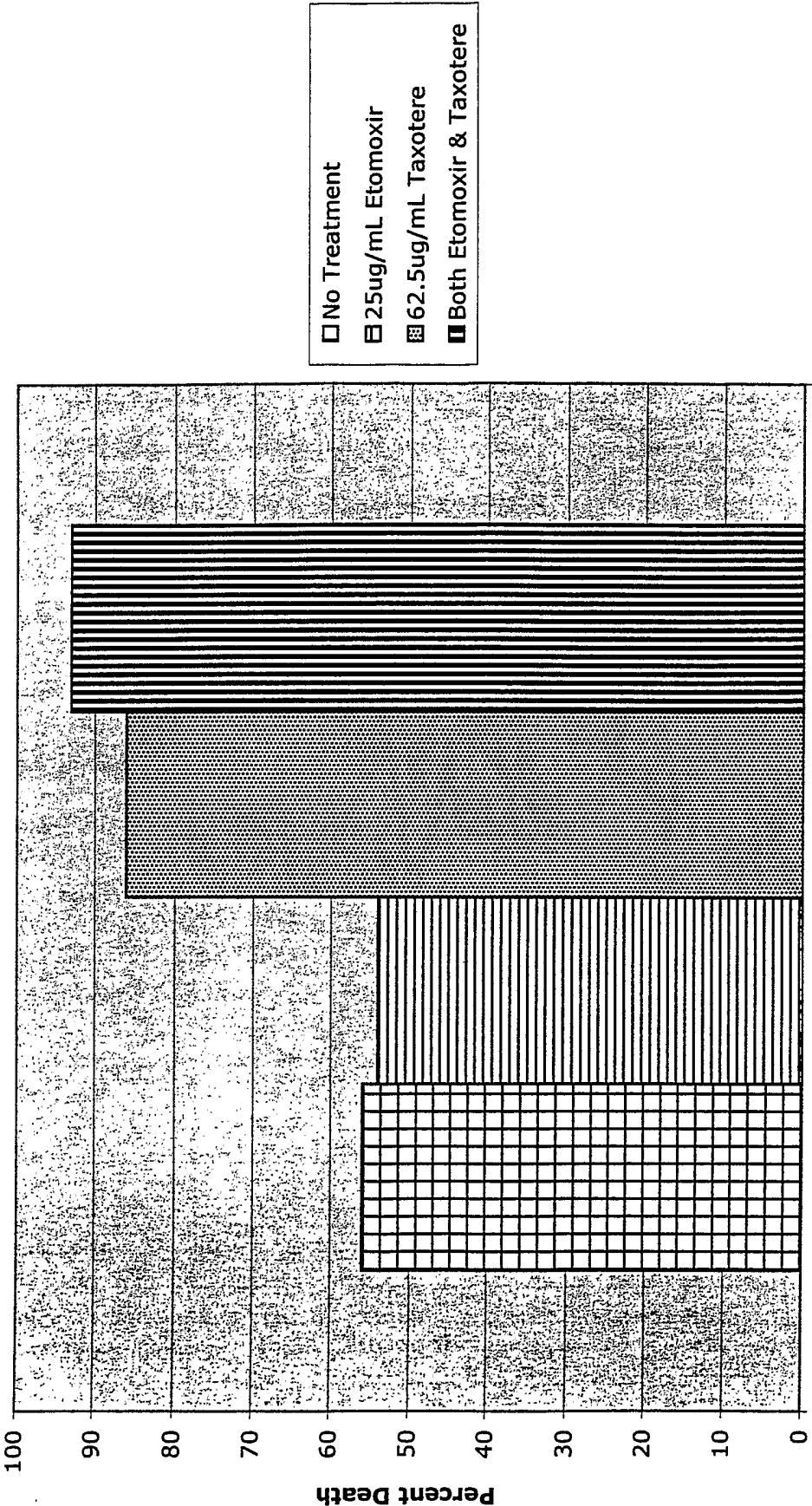


FIG. 22

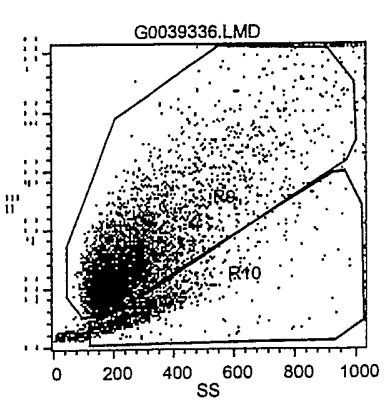
HL60 MDR Cells
Treated (or not) with Etomoxir for 72 Hours and Taxotere for 24 Hours



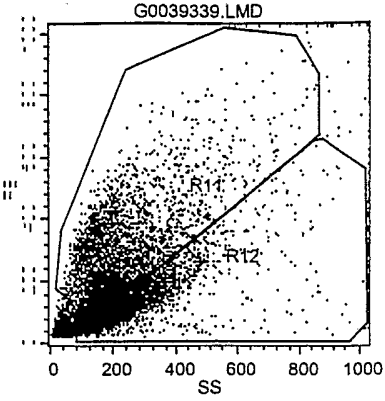
Treatment Groups

FIG. 23

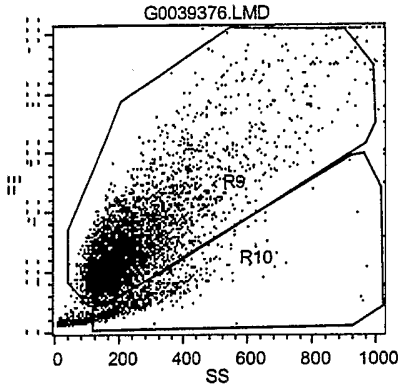
A-204 Rhabdomyosarcoma Data
Treated (or not) with Etomoxir for 24, 48, & 72 Hours



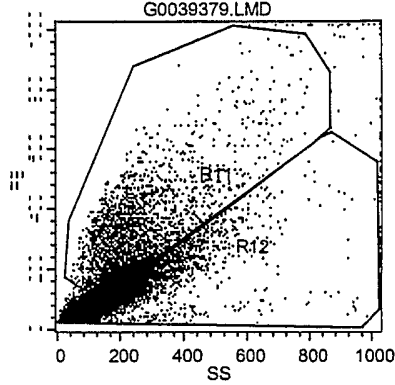
A-204 Rhabdomyosarcoma Cells
Untreated
Percent Death = 17%



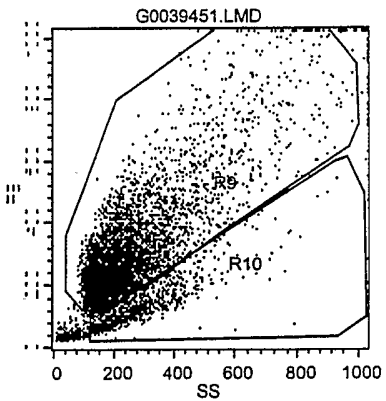
A-204 Rhabdomyosarcoma Cells
250ug/mL Etomoxir 24 Hrs
Percent Death = 66%



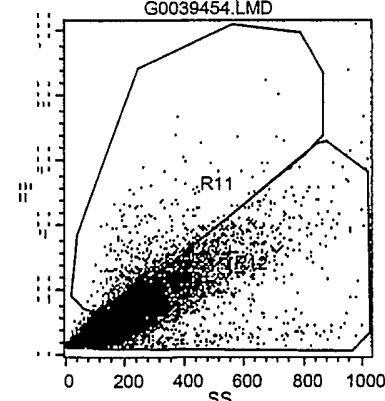
A-204 Rhabdomyosarcoma Cells
Untreated
Percent Death = 10%



A-204 Rhabdomyosarcoma Cells
250ug/mL Etomoxir 48 Hrs
Percent Death = 69%



A-204 Rhabdomyosarcoma Cells
Untreated
Percent Death = 13%



A-204 Rhabdomyosarcoma Cells
250ug/mL Etomoxir 72 Hours
Percent Death = 94%

FIG. 24

A-204 Rhabdomyosarcoma Cells
Treated (or not) with 250ug/mL of Etomoxir for 24, 48, & 72 Hours
Representing the "fold" increase in death over time

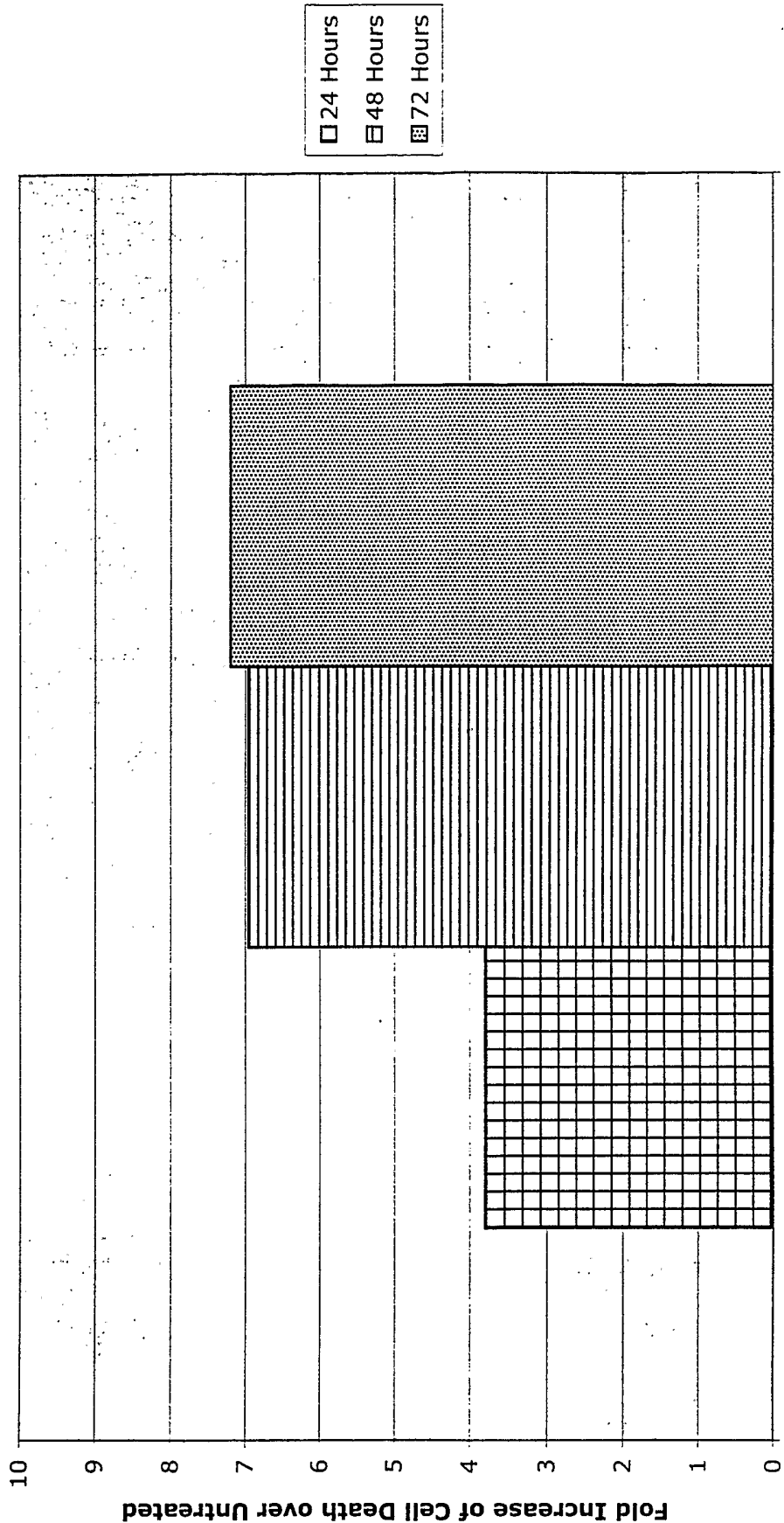


FIG. 25

Time of Treatment

A-204 Rhabdomyosarcoma Cells
Treated (or not) with 25ug/mL of Etomoxir for 24, 48, & 72 Hours
Representing a Fold increase over background

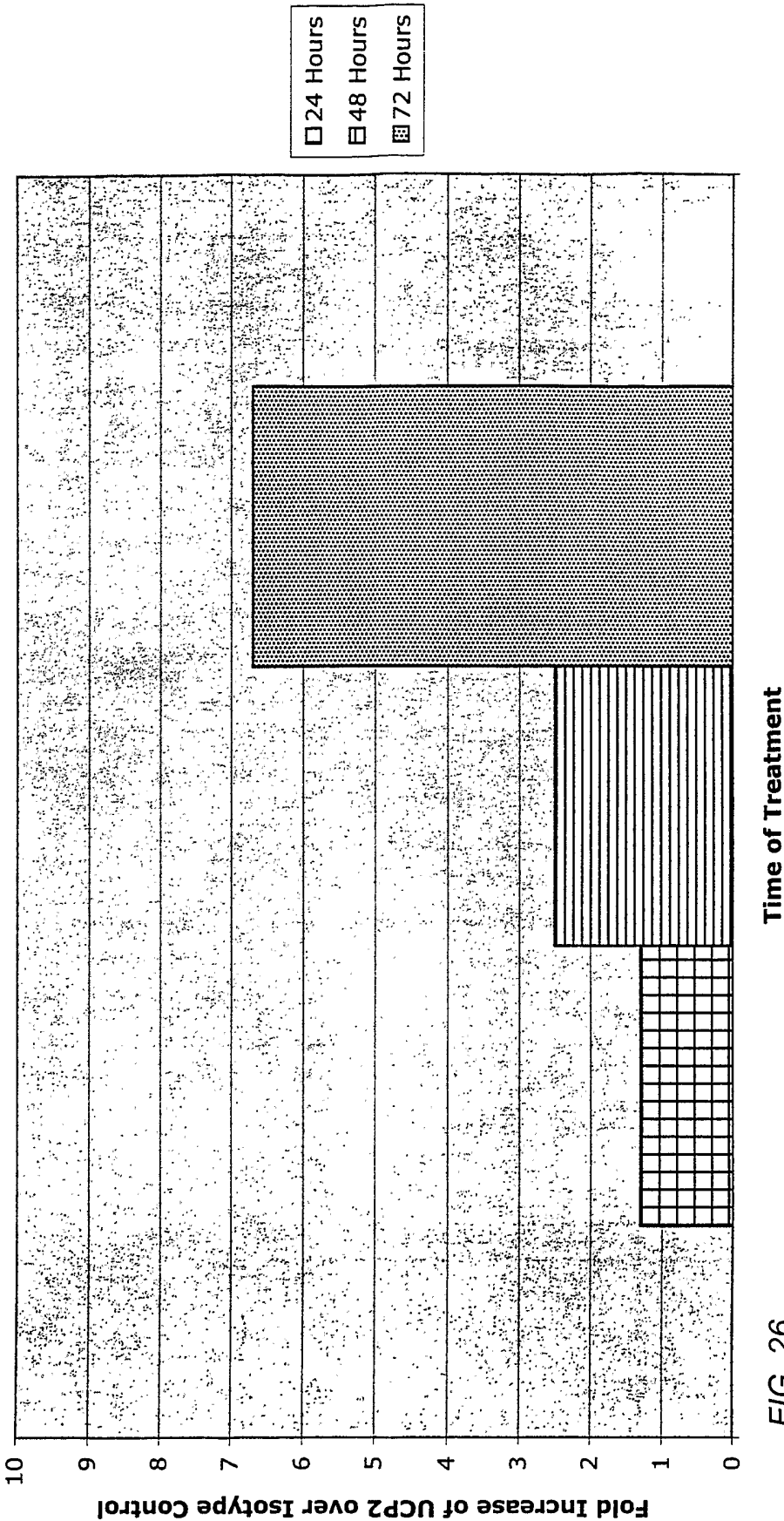


FIG. 26

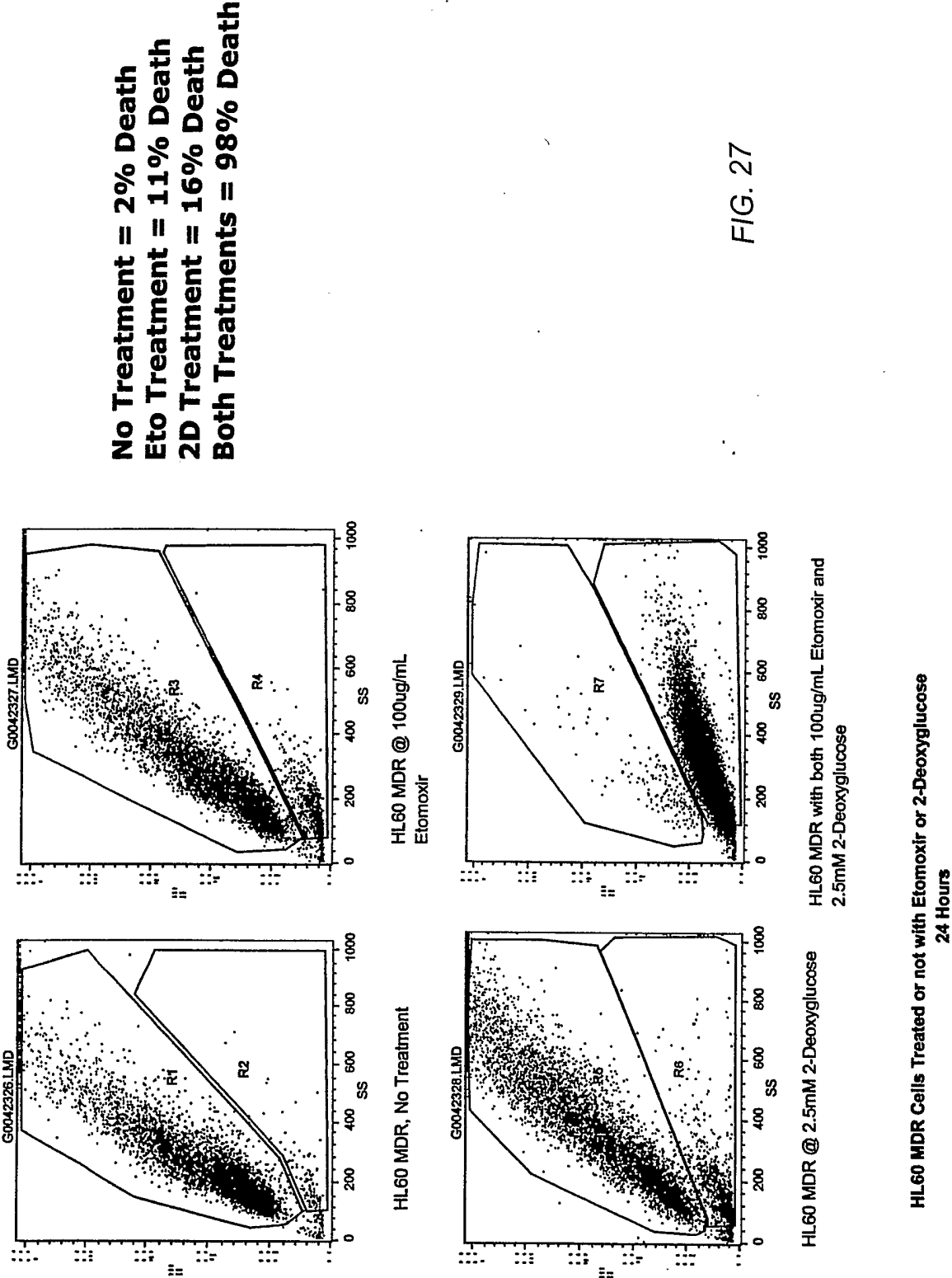


FIG. 27

**HL60 MDR Cells Treated (or Not) with Etomoxir,
2-Deoxyglucose, or Both
24 Hour Treatment**

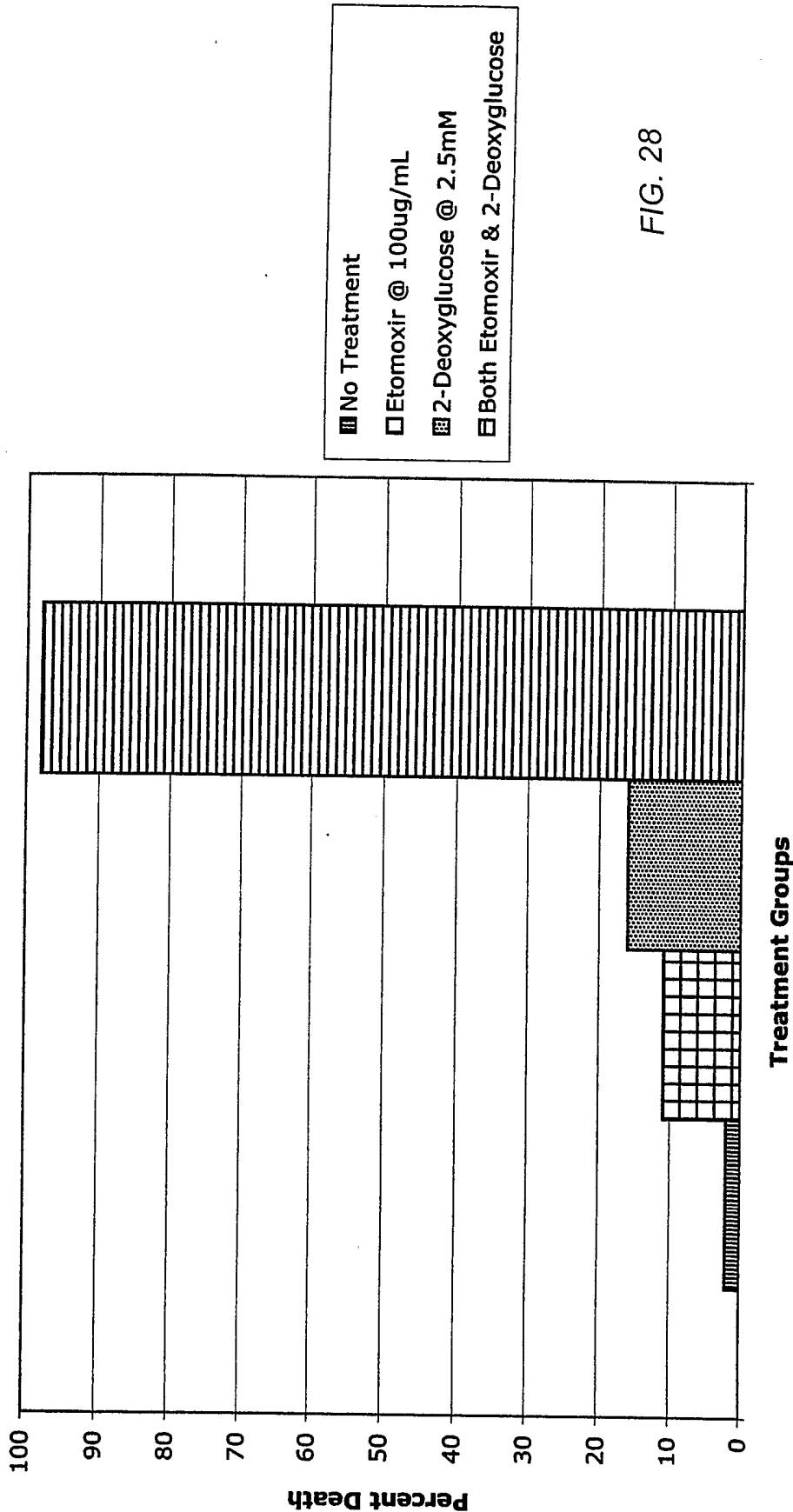
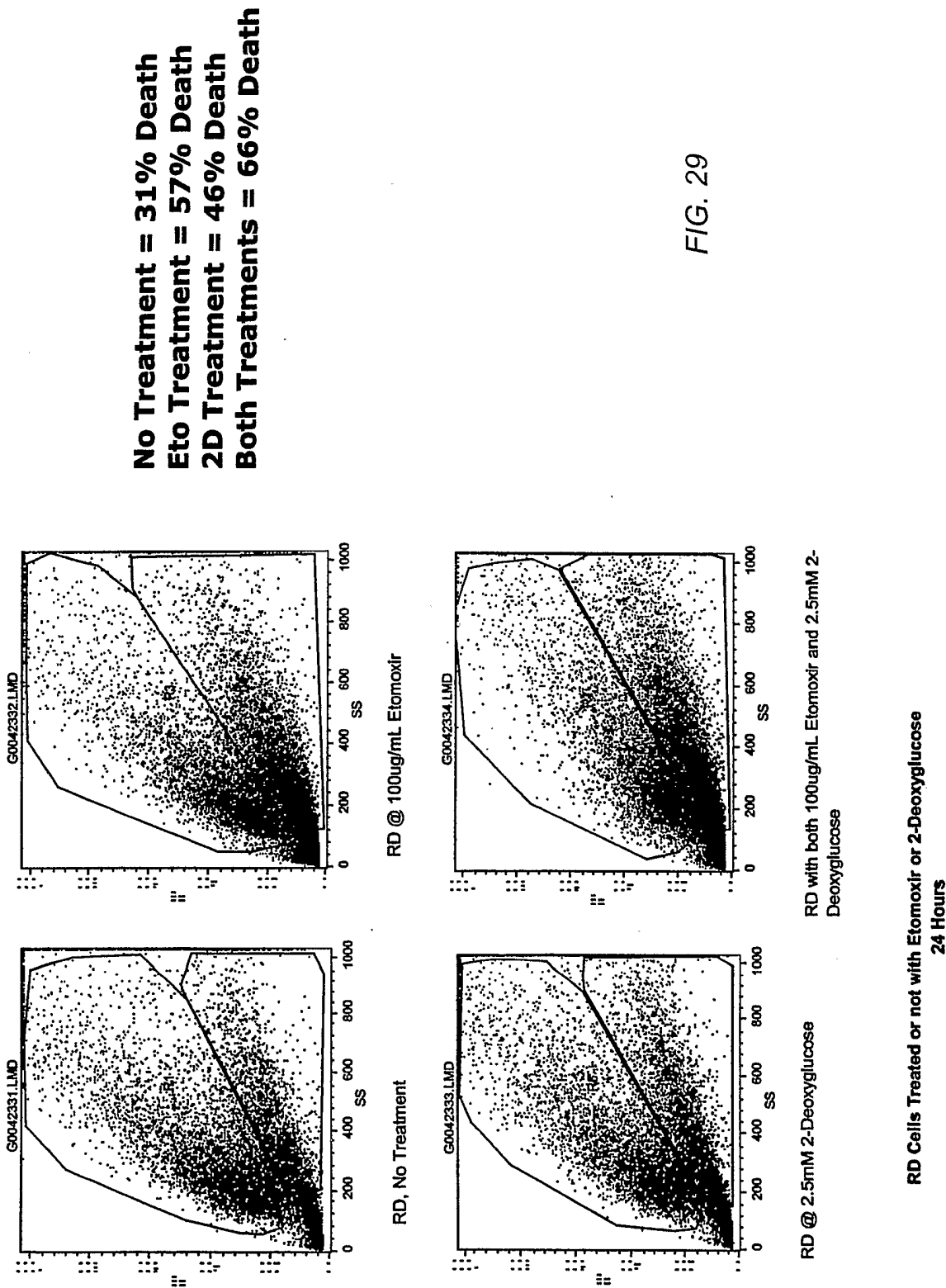


FIG. 28



**RD Cells Treated (or not) with Etomoxir,
2-Deoxyglucose, or Both
24 Hour Treatment**

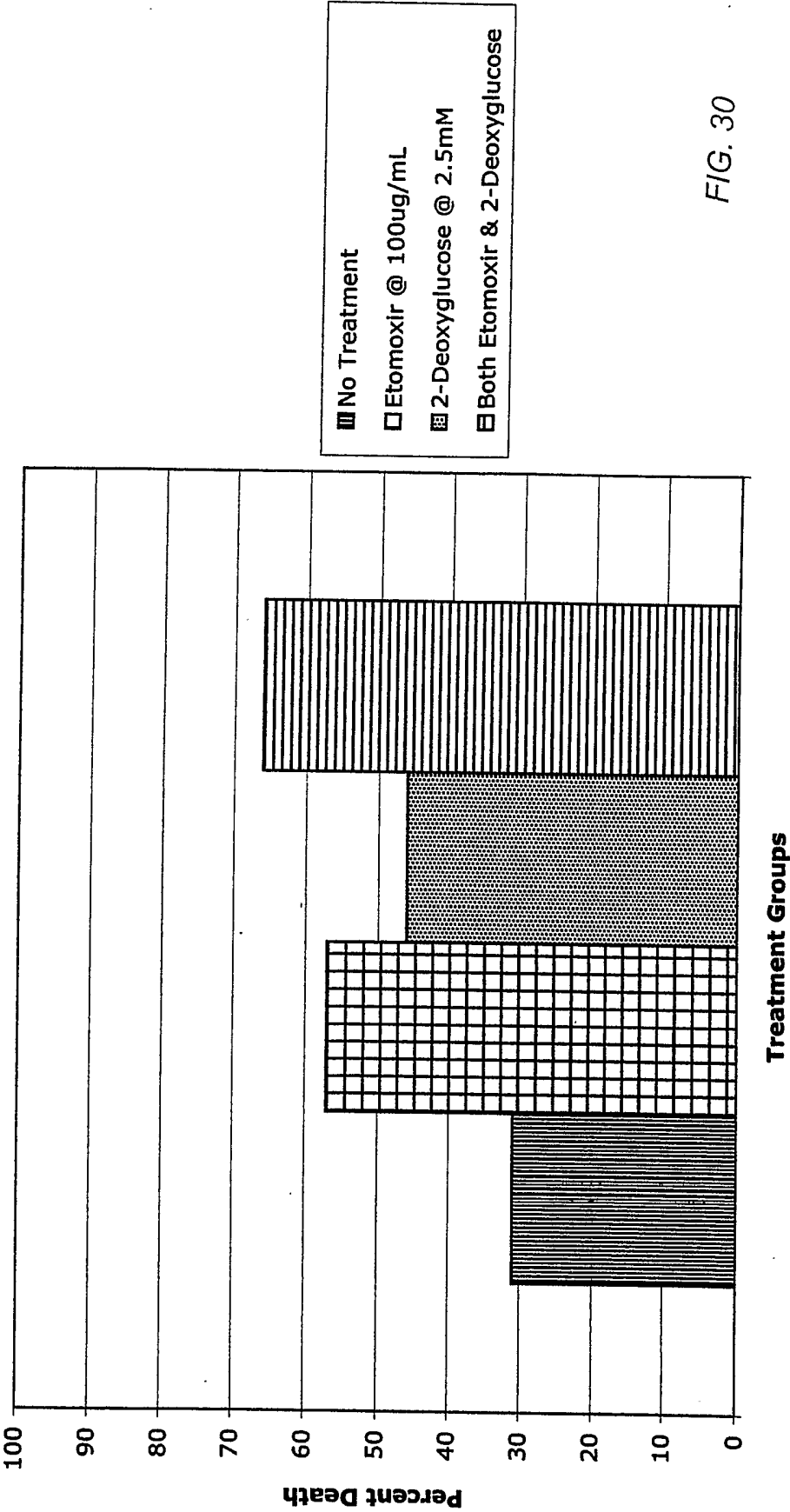


FIG. 30

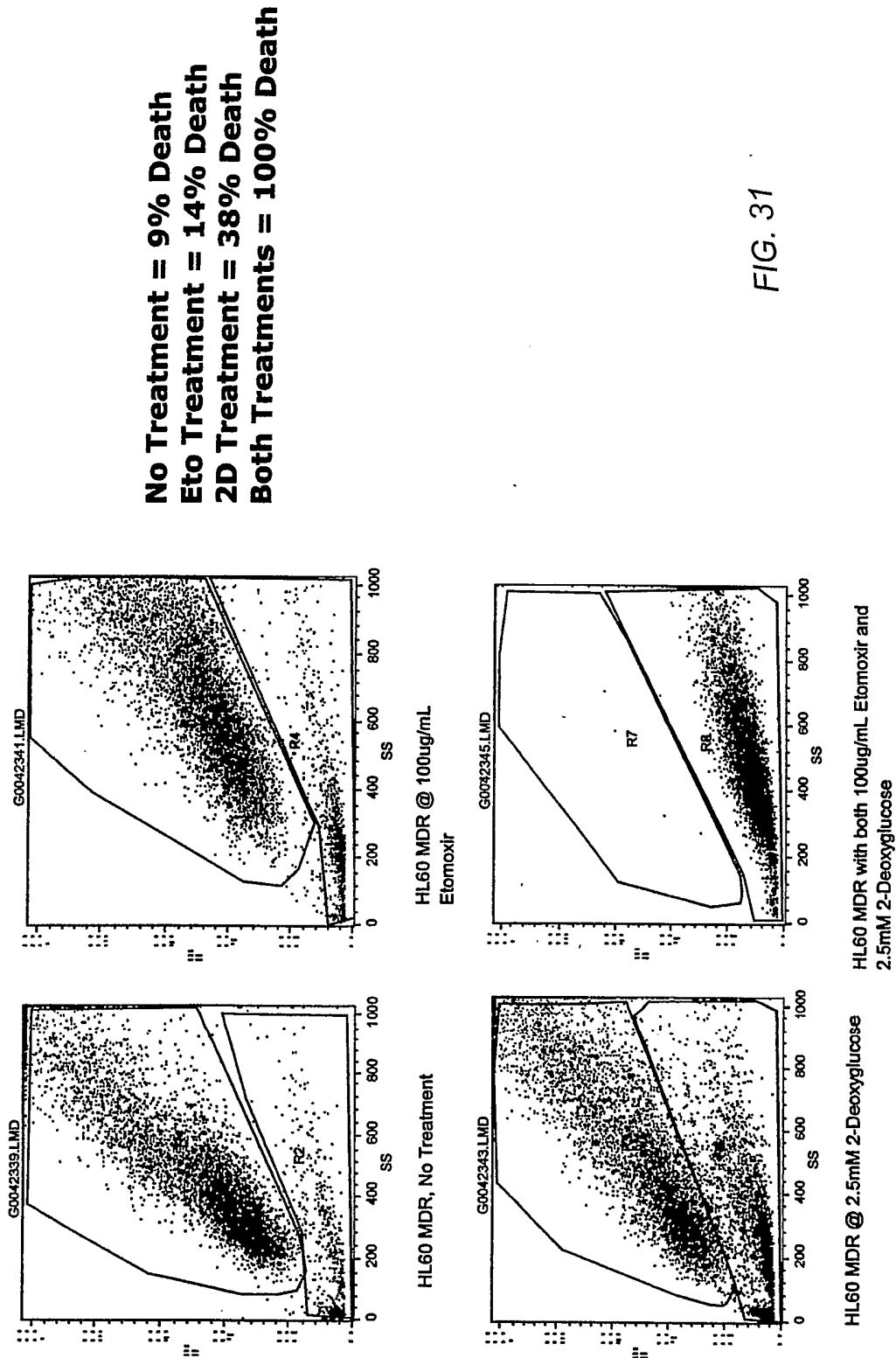


FIG. 31

HL60 MDR Cells Treated or not with Etomoxir,
2-Deoxyglucose, or Both
48 Hours

**HL60 MDR Cells Treated (or Not) with Etomoxir,
2-Deoxyglucose, or Both
48 Hours Treatment**

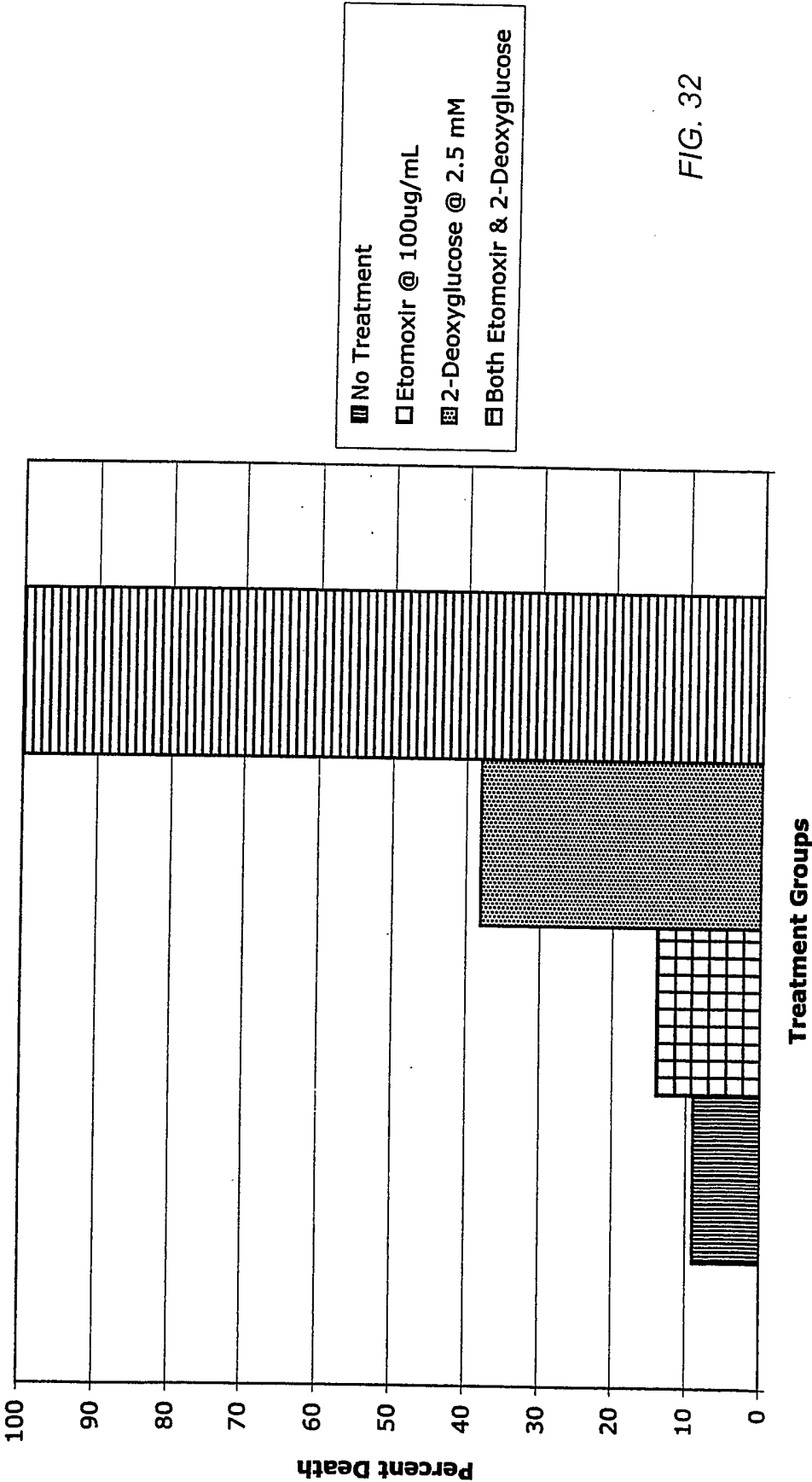
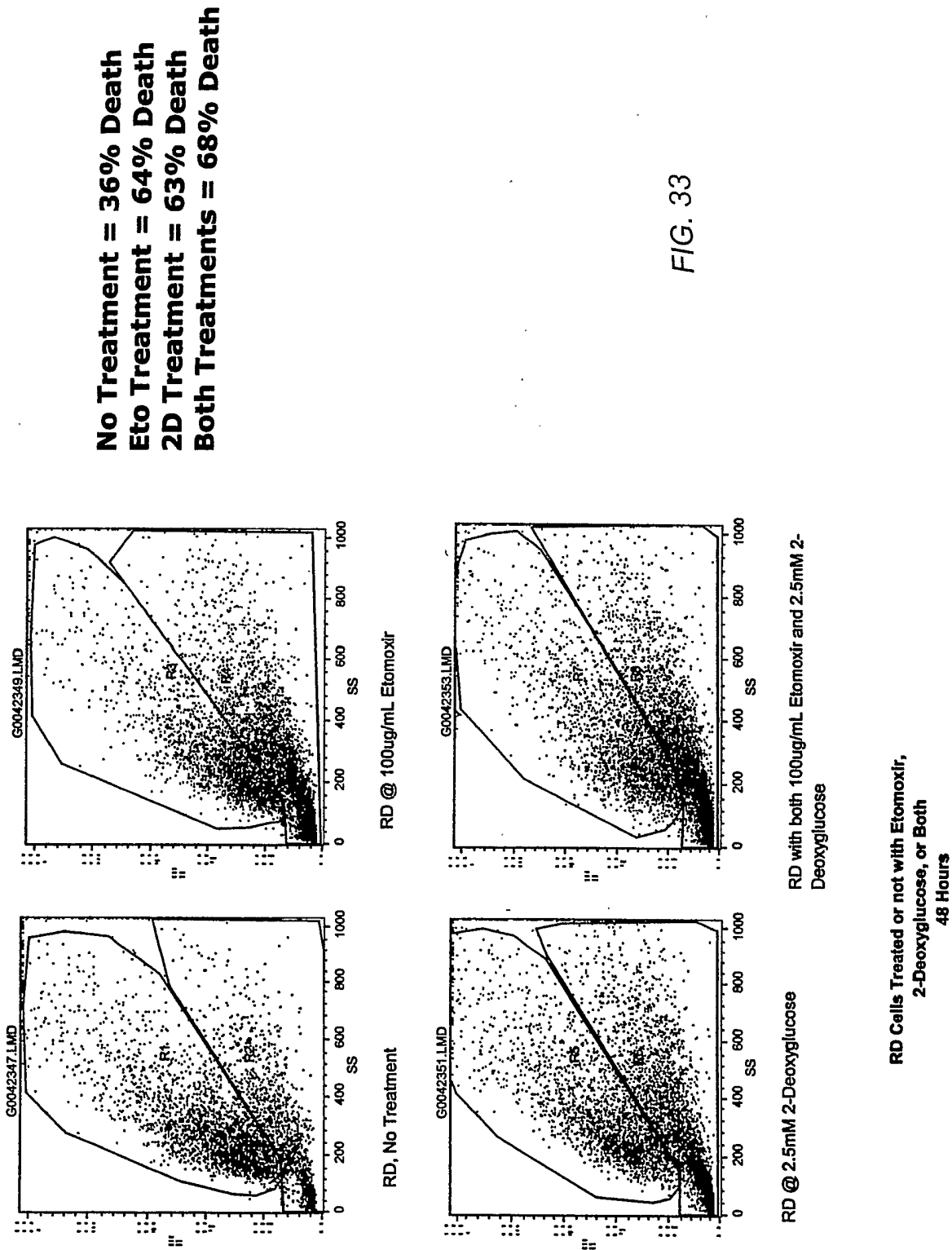


FIG. 32



**RD Cells Treated (or Not) with Etomoxir, 2-Deoxyglucose, or Both
48 Hours**

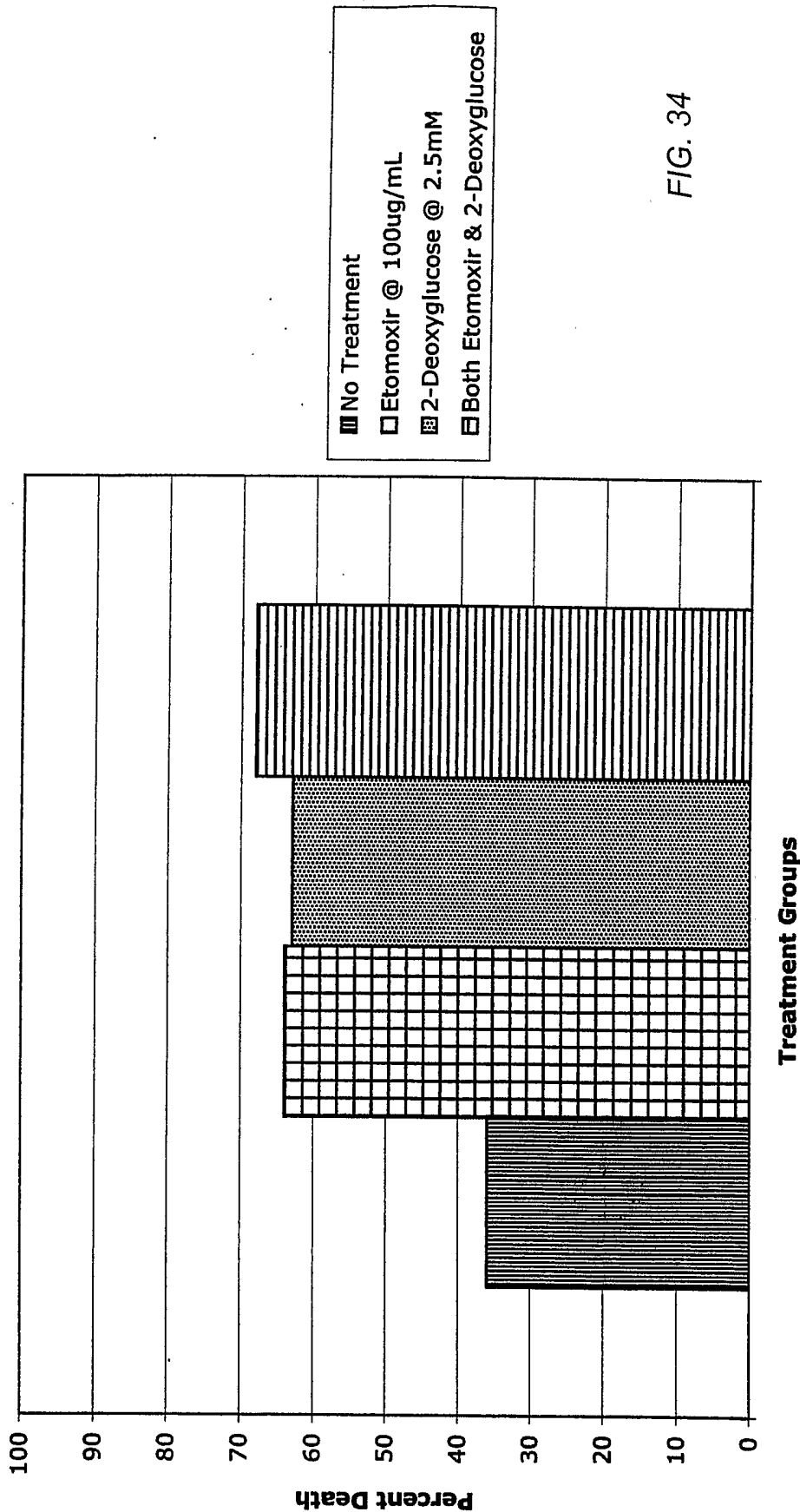


FIG. 34

HL60 MDR Tumor in Nude Mice Treated with Etomoxir, 2-Deoxyglucose, or Both

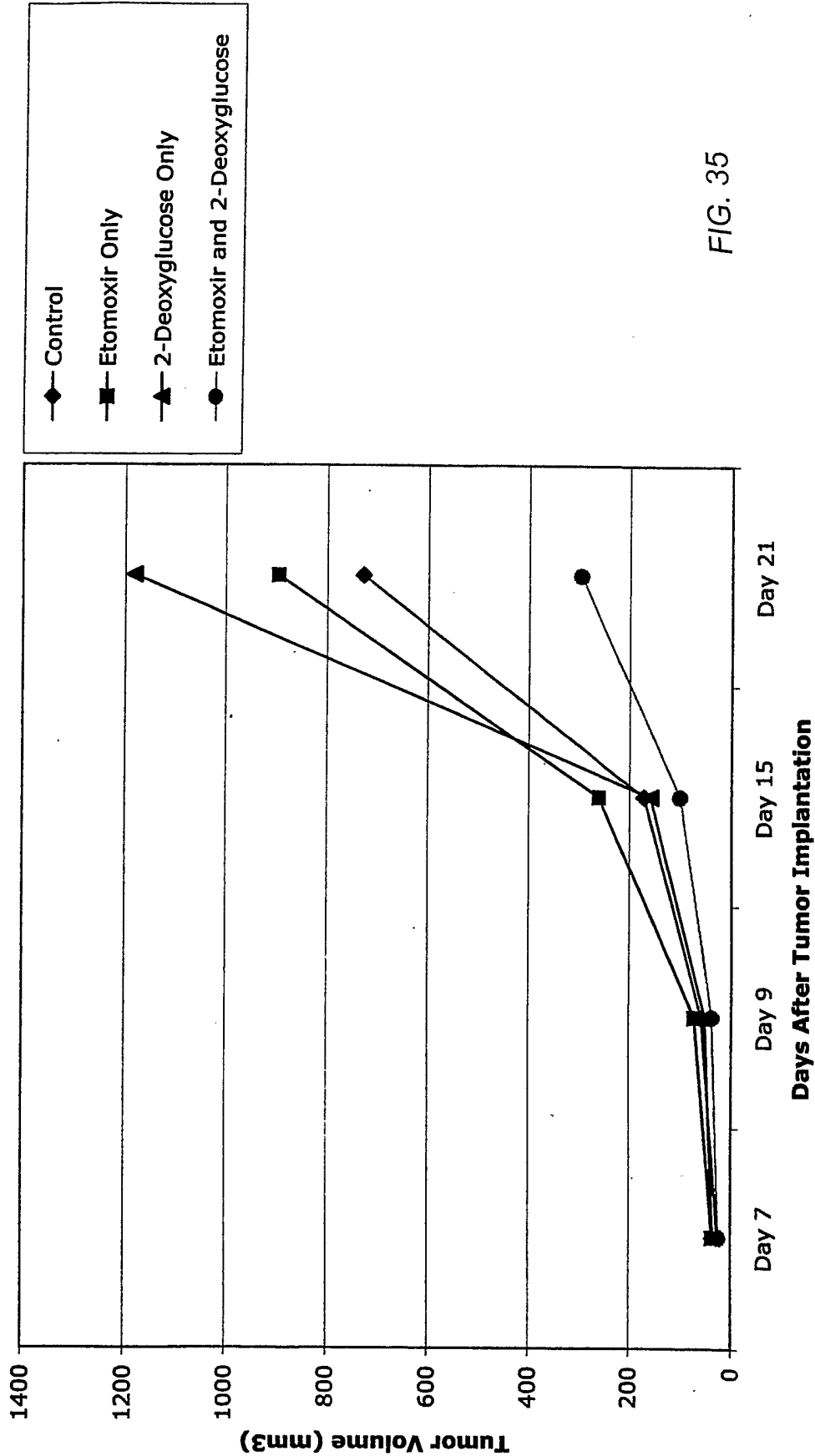


FIG. 35