METHODS FOR TREATMENT OF HUMAN CANCERS
USING MUSHROOM COMPOSITIONS

CROSS-REFERENCE TO RELATED APPLICATIONS

[1]   This claims the benefit of United States Provisional Patent Application No. 63/143,959 filed February 1, 2021, the entirety of which is incorporated herein by reference.

BACKGROUND

Field of the Invention

[2]   This relates generally to methods useful in the treatment of human cancers and for treating patients with cancer diagnoses using compositions comprising mushrooms and/ or mushroom extracts. More particularly this relates to nutraceutical and/ or pharmaceutical compositions comprising mushrooms and/ or mushroom extracts, with or without added cannabis and/ or cannabis extracts or combinations thereof useful for treating cancer, with or without additional therapeutics.

Description of Related Art

[3]   Cancer remains one of the leading causes of death in the modern world. Many cancers remain recalcitrant to treatment, although some types have become more treatable over time. Treatment can be difficult, expensive, physically and emotionally painful, and even deadly. In order to quickly and effectively kill the cancer cells, highly toxic pharmaceuticals compounds are used which have a vast many well-known side effects.

[4]   Depending on the nature of the cancer and how advanced it is/how far it has progressed, treatment options may be limited. Some people with cancer have only one option presented for medical treatment, while multiple options or a combination of available treatments may be presented to other patients depending on their own health status and genetics, the type and stage of the cancer at issue (including its detailed genotype and phenotype), and their ability to withstand the various options physically, emotionally, and/ or financially.

[5]   Generally, possible cancer treatments include surgery, chemotherapy, radiation therapy, immunotherapy, targeted therapy, hormone therapy, and combinations of any of the foregoing. Common types of cancer vary depending on the geography (i.e. the country or part of the world), dietary habits, environmental considerations, exposure to carcinogens, genetics, phenotype, and more, however, some common types of human cancers include breast

cancer, including HER2+, ER+ /PR+, and HER2- ER- PR- ("Triple Negative"), bladder cancer, colon cancer, lung cancer including epithelial, non-small cell, and small cell lung cancers, prostate cancer, including hormone-sensitive and hormone-resistant, and skin cancer (epithelial).

[6]   Cancers are generally characterized by genetic changes that result in uncontrolled cell growth, lack of normal differentiation, failure to respond to apoptosis and other normal cell signals, promoting aberrant angiogenesis, and abnormal immune response to the cancer cells. The genetic changes are most frequently in connection with proto-oncogenes, tumor suppressor genes, and DNA repair genes.

[7]   Treatments such as immunotherapy, targeted therapy, and hormone therapy can be great but are limited in their application. Immunotherapy requires the presence of one or more specific proteins or biomarkers that allow the cancer to be targeted. If the cancer cell lack those biomarkers, the immunotherapy is unlikely to be an appropriate treatment. If a particular cancer is responsive to an immunotherapeutic, e.g. a monoclonal antibody, a person may have a very positive outcome, and often with fewer side effects. Yet another similarly- situated patient my not respond to the immunotherapeutic for a variety of reasons, only some of which are understood. Likewise, hormone therapy is only applicable to certain types of cancers that are sensitive to the hormonal treatment, and not everyone responds in the same way. Targeted therapies using e.g. electromagnetic radiation of particular wavelengths may also be very successful, but a cancer or tumor must be situated such that it can be successfully targeted using the available equipment. These newer therapies, which often receive a lot of very positive press, can create high expectations and hopes (e.g. of avoiding nausea and hair loss, not being sick, maintaining their schedule, etc.) in cancer patients considered for them. Unfortunately, they can result in a highly negative emotional impact on a patient who does not qualify for the treatment (e.g. genetically), or for whom they do not work. This can cause a loss of time in starting more aggressive but less desirable treatment options, as well as have a negative impact on the outcome for patients who lose that hope.

[8]   Regardless of the nature of any therapy for cancer, there are only a few common routes of attack that are frequently used to treat cancer. On one hand, treatment includes the use of direct cytotoxic agents that can directly inhibit or kill tumor cells (or indirectly do so, e.g., by eliminating the required blood supply or other cellular factors required for the tumor cells to thrive). On the other hand, a number of treatments involve the use of compounds that work via the immune system.

[9]   The former treatments include use of classic chemotherapeutic agents grouped as alkylating agents, antimetabolites, topoisomerase inhibitors, antibiotics, mitotic inhibitors, and protein kinase inhibitors. Examples include commonly used compounds such as doxorubicin (e.g. Adriamycin®), paclitaxel (e.g. Taxol®), methotrexate (e.g. Trexall®), 5-fluorouracil (e.g. Fluroplex®), and gemcitabine (e.g. Gemzar®).

[10]   Treatment that works via the immune system includes treatments/ compounds that can trigger antibody-dependent cellular phagocytosis ("ADCP", i.e. immune-stimulated macrophage killing of cancer cells, and treatments/ compounds that can trigger antibody-dependent cellular cytotoxicity ("ADCC", i.e. immune-stimulated T-cell killing of cancer cells). Examples of ADCC promoting treatments include pembrolizumab (e.g. Keytruda®) and atezolizumab (e.g. Tecentriq®). Examples of ADCP promoting treatments include trastuzumab (e.g. Herceptin®, or several biosimilars such as Herzuma®, Kanjinti™, Ogivri®, Ontruzant®, and Trazimera®. By some estimates, the market size for Herceptin® alone is around $7 billion.

[11]   There are ongoing concerns about available treatments, side effects, toxicity, and effectiveness of therapeutics for cancer. And the economic and emotional impact of these conditions is enormous on the personal level for those directly impacted by cancer, as well as for their families, and on the societal level. Accordingly, people have searched for new treatments for cancer and other diseases from sources such as the natural world.

[12]   Mushrooms have been used for medicinal and therapeutic purposes for centuries throughout Asia (e.g. in traditional Chinese and Japanese medicines) and around the world. Among the multitude of potentially therapeutic compounds present in mushrooms are complex sugars and polysaccharides (substituted or not) (e.g. glucans, glycosides, glycopeptides, and glycoproteins), terpenes and/ or terpenoids, sterols, peptides, amino acids, and other small and large molecules.

[13]   Various mushrooms are considered to have anti-cancer, antioxidant, antitumor, antiviral, antibacterial, anti-diabetic, anti-hypercholesterolemic, anti- arthritic, anti-asthmatic, anti-obesity, anti-allergenic, anti-thrombotic, anti- inflammatory, anti-bacterial, anti-mutagenic, anti-osteoporotic, and anti-aging therapeutic properties. For example, reishi mushrooms have been reported to calm the central nervous system and/ or have neuroprotective effect, stimulate the immune system, and act as a prebiotic to support gut health. Reishi has been reported to have a beneficial effect on the adrenals, and to be anxiolytic, reducing anxiety and promoting sleep. Reishi has also been associated with improved memory, and sharpened

concentration and focus. Lion's Mane reportedly calms mental activity and modulates certain neurotransmitters. Cordyceps has been reported to have adaptagenic properties and stimulate the adrenals glands and modulates the nervous system.

[14]   *Cannabis* spp. have also been used medicinally for centuries. Their therapeutic value is the subject of many current studies. The endogenous endocannabinoid system and related endocannabinoid biology was originally believed to be primarily directed to neurological and psychiatric effects of naturally occurring and exogenous cannabinoids. However, cannabinoids are increasingly recognized as having role(s) in both inflammation and cancer.

[15]   The ongoing discovery of less abundant cannabinoids continues to broaden our knowledge about the range of cannabinoids in plants, and their ability to function in regulation of the endocannabinoid system in humans. Our understanding of the role and potential therapeutic value of such compounds is ongoing. Thus, the use of exogenous cannabinoids and their ability to regulate (particularly upregulate) the endocannabinoid system as a therapeutic approach is being studied.

[16]   Other compounds such as terpenes, flavonoids and various botanicals are known to provide beneficial and healthful functions when consumed or administered, and are also found in cannabis.

[17]   There is an ongoing need for new treatment compositions and protocols that are useful for cancers and which provide significant new features and benefits.

SUMMARY

[18]   In a first of the several aspects of this disclosure, the inventor has discovered that certain pharmaceutical and/ or nutraceutical compositions generally comprising combinations of one or more edible or medicinal mushrooms or an extract, fraction, or isolate thereof, appear to have powerful anti-cancer effect bot directly, in terms of cytotoxicity, and indirectly via the immune system, through immune-mediated cytotoxicity and/ or immune mediated phagacytosis.

[19]   The mushroom or mushroom extract present in the compositions is from any edible or medicinal mushroom species. The edible or medicinal mushroom in various embodiments comprises one or more of *Agaricus, Auricularia, Clitocybe, Ganoderma, Grifola, Hericium, Lentinus, Leucopaxillus, Phellinus, Pleurotus, Sarcodona, and Trametes.* In other embodiments, the edible or medicinal mushroom comprises one or more of *Albatrellus, Antrodia, Calvatia, Cordyceps,*

Flammulina, Fames, Funlia, Inocybe, Inonotus, Lactarius, Russula, Schizophyllum, Suillus, or Xerocomus.

[20] In presently preferred embodiments, the mushrooms commonly known as Lions Mane, Cordyceps, Shitake, Turkey Tail, Maitake, Chaga, Reishi, and Cubensis may be useful herein.

[21] Mushrooms may be used in their entirety, or an extract or fraction may be derived therefrom with the relevant activity.

[22] In a series of experiments with human cancers in culture, the direct cytotoxicity, and indirect ability to eliminate cancer cells via the immune system was tested for a series of novel mushroom extracts, microemulsions, and/ or nanoemulsions. Surprisingly, some of the extracts were extremely potent with respect to direct and/ or indirect cytotoxicity against common humans cancer cell lines including: Hormone sensitive and insensitive prostate cancers (22Rvl and PC-3), skin cancer (A-431), large and epithelial lung cancers (A-549 and NCI- H460), HER2+ breast cancer, ER+ /PR+ breast cancer, and 'triple negative' breast cancer BT-474, MDA-MB-231, and T-47D cell lines), colon cancer (HT-29), and bladder cancer (T24).

[23] The results have provided new information that provides improved compositions that can be delivered as food or pharmaceuticals with a great deal of safety (i.e. little to no risks of side effects for the vast majority of people who can consume mushrooms without problems). These compositions are highly effective at directly killing cancer cells in one or more ways and/ or stimulating a subject's immune system to destroy the cancer cells via immune-mediated systems for cytotoxicity and/ or phagocytosis.

[24] The compositions can comprise any extracts of the mushrooms, however the inventor has previously disclosed methods of preparing microemulsions and nanoemulsions of mushrooms and cannabis that appear to be particular effective at extracting the biologically active components from the mushrooms, and thus these preparations are presently preferred for use herein.

[25] All nine of the mushrooms tested showed some level of direct cytotoxicity against one or more cancer cell lines. The tested extracts of reishi and chaga were particularly effective with significant direct cytotoxicity against breast cancer, lung cancer, and colon cancer cells, and other cancer types tested, as can be clearly seen in the results. In the various results the mushrooms are coded as fl through f9, as detailed in the Code/Key Table below.

[26] In addition to the nine mushroom extracts, six additional mushroom compositions were prepared and tested for direct cytotoxic killing of cancer cells. These additional compositions include one or cannabis compounds or extracts (e.g. extracts, oils, cannabinoids, or other

cannabis compounds) combined with the mushroom components. These samples are identified throughout the results as ml through m6, as further detailed in the Code/Key Table..

[27]   The cannabinoids can be derived from any *Cannabis* spp., including plants that may be classified as *Cannabis sativa, Cannabis indica,* or *Cannabis ruderalis.*

[28]   In one of its several aspects, provided are methods of treating a subject suffering from cancer. The methods generally comprise administering a therapeutically effective dose of a mushroom composition to the subject. The compositions generally comprise one or more edible or medicinal mushrooms or an extract, fraction, or isolate thereof. The compositions are preferably administered through a route that allows them to have maximal cytotoxicity on the cancer cells, directly or as mediated through the immune system. Additional components may be included to enhance the anticancer properties of the compositions, or to support the patient's nutritional status, general health, immune status, or the like.

[29]   Generally, the cancer comprises a common type of cancer such as bladder cancer, breast cancer, lung cancer, skin cancer, colorectal cancer, prostate cancer, cervical cancer, endometrial cancer, esophageal cancer, gastric cancer, head and neck cancers, brain tumors, Kaposi sarcoma, kidney (renal cell) cancer, leukemia, liver cancer, lymphoma, melanoma, non-Hodgkin lymphoma, neuroblastoma, ovarian cancer, osteosarcoma and other bone cancers, pancreatic cancer, pituitary tumors, retinoblastoma, testicular cancer, thyroid cancer, or uterine cancer. Of particular interest in some embodiments are breast (including HER2+ cancers), lung cancers (including large and epithelial types), and colon cancers.

[30]   In another aspect hereof, a beneficial mushroom composition can be administered to a subject on its own, or together with another treatment such as a chemotherapeutic agent, or an immunotherapeutic agent, particularly where such agents are the standard of care for a particular type, form, or stage of the cancer involved. In various embodiments, the mushroom composition can be administered together in a single formulation, or together in separate forms and/ or by separate routes of delivery. F or example, a chemotherapeutic or immunotherapeutic agent may be administered intravenously or by injection, and the mushroom composition according to this disclosure may be administered orally, parenterally, or by other useful means, which may be informed by the nature of the cancer. For example, rectal administration (e.g. via a suppository, microenema, or the like) may be well-considered for a subject suffering from colorectal cancer. Inhalation may be possible with sufficiently purified compositions, and suitable for deliver to a subject with lung cancer. Liquid delivery may be useful for bladder cancer, particularly for compounds that can pass through the kidney

to the bladder. The mushroom composition and the other treatment or agent may be administered at the same time, or at different times, including on different days or even different weeks, depending on the best interests of the patient and the nature of the cancer, and the response to either the mushroom composition or the other treatment.

[31]   Preferably, the co-administration is performed such that the use of the mushroom composition enhances the effectiveness of the other treatment. Such enhancement in various embodiments includes a more rapid or more complete reduction in the cancer cell population in the subject. In one embodiment, the use of the mushroom composition allows reduction of the amount of a chemotherapeutic agent used, thereby decreasing the incidence and severity of side effects and adverse events, and/or increasing the overall health of the patient, and/or increasing the likelihood of survival at 1 year, 2 years, 5 years and the like. In yet another embodiment, the use of the mushroom composition can at least partially reduce the cost of the treatment with another agent, or decrease the length of time for which the subject requires such treatment, or the frequency with such treatment needs to be administered. If the amount of the chemotherapeutic or especially the immunotherapeutic agent required to achieve a positive outcome can be reduced, and the cost concomitantly reduced, it may increase the ability to treat more patients since insurance coverage may be broadened and thus allow more people to reap the benefits of a successful therapeutic.

[32]   In one embodiment, the cancer is a HER2+ breast cancer and the mushroom composition can be administered with or without trastuzumab. Preferably the use of the mushroom composition (with or without cannabis components) increases the effectiveness of the trastuzumab treatment.

[33]   In another aspect hereof, a mushroom composition for the treatment of subject having cancer is created by identifying and selecting two or more compounds; each of said compounds having at least of the following properties: direct cytotoxicity on the cancer cells, the ability to stimulate antibody-dependent cellular cytotoxicity ("ADCC) against the cancer cells, or the ability to stimulate antibody-dependent cellular phagocytosis of the cancer cells; wherein the composition has at least two have the foregoing on the whole; and wherein at least one of the compounds is a mushroom compound.

[34]   The disclosure also provides a plurality of dosing regimens that utilize the compositions, and variations thereof, on various schedules as dictated by the physiological or psychological health of the subject, and the status of the cancer.

[35]   In yet another aspect, methods are provided for optimizing a composition for use in treatment of a subject suffering from cancer. The methods generally employ the use of artificial intelligence algorithms, such as classification algorithms, regression algorithms, clustering algorithms, or a combination thereof.

[36]   The methods generally comprise:

[37]   a)   providing data on the therapeutic effect on the cancer of each of:

[38]   i)   a plurality of edible or medicinal mushrooms, mushroom extracts, or components thereof; and

[39]   ii)   a plurality of cannabis extracts, cannabinoids or combinations thereof; and optionally,

[40]   iii)   a plurality of optional components comprising terpenes, triterpenes, flavonoids or combinations thereof;

[41]   b)   using an artificial intelligence algorithm to analyze the data for the mushrooms or extracts, cannabis extracts or cannabinoids, and any optional components; and

[42]   c)   generating one or more profiles of compositions optimized for therapeutic treatment of the cancer;

[43]   wherein the therapeutic effects include one or more of direct cytotoxicity on the cancer cells, the ability to stimulate antibody-dependent cellular cytotoxicity ("ADCC) against the cancer cells, or the ability to stimulate antibody-dependent cellular phagocytosis of the cancer cells.

[44]   The data for use in the artificial intelligence algorithm can be obtained from original experiments (including cell culture experiments) or literature review.

[45]   In a final aspect, this disclosure provides methods for treating cancer in a patient in need thereof. The methods generally comprise the step of administering a composition comprising at least one edible or medicinal mushroom or extract thereof, in combination with at least one cannabinoid, at least one terpene, and at least one flavonoid. The at least one cannabinoid, at least one terpene, and at least one flavonoid are conveniently administered separately from, sequentially to, or simultaneously with the edible or medicinal mushroom. The at least one edible or medicinal mushroom or extract thereof is also administered separately from, sequentially to, or simultaneously with the cannabinoid, terpene, and flavonoid.

[46]   These and / or further aspects, features, and advantages of the present invention will become apparent to those skilled in the art in view of this disclosure.

BRIEF DESCRIPTION OF THE DRAWINGS

[46] Fig. 1: Several graphs (with corresponding cytotoxicity data) for mushroom compositions with human cancer cell lines showing cancer cell survival as a percentage of untreated control for mushroom compositions (described below).

[47] Fig. 2: Schematic diagram showing a proposed three-prong approach to attacking cancer cells. This is useful in designing mushroom compositions that will target particular cancer cell types or cancer cells with particular biomarkers.

[48] Fig. 3: Several bar charts (with corresponding assay data) for mushroom compositions with human cancer cell lines in ADCC assays using human HER2+ breast cancer cells. Each composition was tested at 6 different concentrations (see the Experimental Methods below).

[47] Fig. 4: Several bar charts (with corresponding assay data) for mushroom compositions with human cancer cell lines in ADCP assays using human HER2+ breast cancer cells. Each composition was tested at 6 different concentrations (see the Experimental Methods below).

DETAILED DESCRIPTION

[48] Provided herein are mushroom compositions and methods for treating cancers including prevalent cancers, but the methods are also applicable to any metastatic or neoplastic disease in a subject. Surprisingly, the compositions allow a modern practitioner to combine the benefits of certain compounds found in edible and medicinal mushrooms (such as used for centuries in Chinese and other traditional medicine practices, and by e.g. herbalists throughout the world), with the positive benefits of another natural substance, cannabis, also used for centuries. Used properly, these compositions have little risk, few side effects, and are effective for producing measurable and lasting results in patients suffering from cancers or neoplastic diseases.

Definitions & Abbreviations

[49] Unless expressly defined otherwise, all technical and scientific terms, terms of art, and acronyms used herein have the meanings commonly understood by one of ordinary skill in the

art in the field(s) of the invention, or in the field(s) where the term is used. In accordance with this description, the following abbreviations and definitions apply.

[50]   The term "cancer" as used herein includes any type of disease characterized by uncontrolled cell growth. Cancer broadly means any type of neoplastic or malignant disease, including metastatic and non-metastatic diseases. Examples of common cancers include breast cancer, colorectal cancer, lung cancer, gastric cancer, bladder cancer, kidney (renal cell) cancer, leukemia, liver cancer, lymphoma, pancreatic cancer, prostate cancer, skin cancer, thyroid cancer, uterine cancer, non-Hodgkin lymphoma, melanoma, endometrial cancer, testicular cancer, ovarian cancer, osteosarcoma and other bone cancers, brain tumors, cervical cancer, esophageal cancer, retinoblastoma, Kaposi sarcoma, head and neck cancers, neuroblastoma, and pituitary tumors.

[51]   As the skilled artisan will appreciate, as used herein the term "edible" does not mean merely capable of being eaten. In that overly broad sense, even poisonous or toxic mushrooms are 'edible' however lethal or sickening or the like. In contrast "edible mushrooms" is used herein in the sense of mushrooms that are used traditionally or in modern times as sources of food, nutrients, nutraceuticals, flavors, and the like. Edible mushrooms are neither toxic or poisonous as consumed.

[52]   "Medicinal mushrooms" as used herein means any mushroom species that has been used traditionally or in modern times as a source of medicinal or therapeutic benefits, healing properties, and / or healthful compounds.

[53]   Generally, mushrooms that are edible or medicinal may be grouped together, as there may be many crossovers and it may be difficult to clearly distinguish between the two groups. Examples of edible and / or medicinal mushrooms useful herein include mushrooms of the genera *Agaricus, Auricularia, Clitocybe, Ganoderma, Grifola, Hericium, Lentinus, Leucopaxillus, Phellinus, Pleurotus, Sarcodona,* and *Trametes* (aka *Coriolus, Polyporus,* or *Polystictus)* are all suitable for use herein. Also useful herein are mushrooms of the genera *Albatrellus, Antrodia, Calvatia, Cordyceps, Flammulina, Fames, Funlia, Inocybe, Inonotus, Lactarius, Russula, Schizophyllum, Suillus,* and *Xerocomus.* Species of particular interest include *Agaricus blazei, Albatrellus confluens, Antrodia camphorate, Boletus badius, Clitocybe maxima, Cordyceps militaris, Flanulina velutipes, Fames fomentarius, Funalia trogii, Ganoderma lucidum, Grifoloa fondosa, Hericium erinaceus, Inocybe umbrinella, Ionatus olbiquus, Lactarius flavidulus, Lentinula edodes, Phellinus linteus, Pleurotus ostreatus, Schizophyllum commune, Suillus placidus, Trametes versicolor,* as well as *Cordyceps sinensis,* C. *liangshanensis,* C. *gunnii, or* C. *cicadicola.*

[54]   More generally edible and/ or medicinal mushrooms can be useful in connection with the current disclosure for treating cancer, directly or indirectly. In presently preferred embodiments, the composition has one or more of the following properties: direct cytotoxicity on the cancer cells, the ability to stimulate antibody-dependent cellular cytotoxicity ("ADCC) against the cancer cells, or the ability to stimulate antibody-dependent cellular phagocytosis of the cancer cells.

[55]   More generally the therapeutic mushroom compositions may also include function such as preventing and/ or treating cancer, helping recover from chemotherapy and/ or other treatments with significant toxicity, stimulating and/ or supporting the immune system, treating primary or secondary infections, or providing antibacterial or antifungal properties, reducing the side effects of radiation therapy, supporting non-chemotherapeutic approaches to treatment, reducing or mitigating the psychological affects (e.g. stress, anxiety, or the like) of a cancer diagnosis, generally promoting of health, providing antioxidant functionality, reducing nausea or stimulating appetite, stimulating or promoting cellular health, or the like.

[56]   Specific anticancer or antitumor therapeutic properties include functioning as a reactive oxygen species inducer, a mitotic kinase inhibitor, an anti-mitotic, an angiogenesis inhibitor, a topoisomerase inhibitor, a stimulator of apoptosis, a stimulator of DNA editing and/ or repair functions, or as a general immunomodulatory or immunostimulatory compound.

[57]   Immune system functions can include stimulating cellular aspect of immunity such as monocytes, natural killer (NK) cells, and dendritic cells. Other potential functions include stimulating T-cell activity, or preventing T-cell apoptosis.

[58]   "Mushroom compositions" means any compound, extract, or composition comprising a medical or edible mushroom. Preferably, the mushroom provides a meaningful or useful proportion or function of the overall composition. As used herein, mushroom composition may also comprise cannabis extracts or components. These are sometimes referred to herein as mixtures.

[59]   *"Cannabis"* or *"Cannabis spp."* as used herein refers to any plant of the genus *Cannabis,* including plants that may be classified as *Cannabis sativa, Cannabis indica,* or *Cannabis ruderalis.* It is well-known that despite the foregoing list, some experts believe that there are only 2 species, and still others consider that there is only a single species (generally, C. *sativa).* Whatever nomenclature is used, for purposes of this disclosure, *"Cannabis"* includes all

possible members of the genus, without regard to the species to which they are assigned. "*Cannabis*" or "*Cannabis spp.*" as used herein refers to any plant of the genus *Cannabis*, including plants that may be classified as *Cannabis sativa*, *Cannabis indica*, or *Cannabis ruderalis*. It is well known that despite the foregoing list, some experts believe that there are only 2 species, and still others consider that there is only a single species (generally, *C. sativa*). Whatever nomenclature is used, for purposes of this disclosure, "*Cannabis*" includes all possible members of the genus, without regard to the species to which they are assigned.

[60]   As used herein 'cannabinoids' means any of a class of compounds that generally can interact with one or more cannabinoid receptors, including the receptors of the endocannabinoid system, in particular, CB1 and CB2. Cannabinoids include e.g., phytocannabinoids and synthetic cannabinoids. Phytocannabinoids are found in several plant species, especially *Cannabis* spp. Among the most prevalent and most studied cannabinoids are tetrahydrocannabinol (THC), and cannabidiol (CBD). However, there are at least ~120 known cannabinoids that have been identified in *Cannabis* within certain classes including the tetrahydrocannabinols, cannabidiols (including e.g. cannabidol (CBD) and cannabidivarin (CBDV)), cannabigerols, cannabinols, cannabichromenes, and cannabinodiol. Other cannabinoids, such as cannabicyclol, cannabieslsoin, and cannabitriol are currently classed as 'miscellaneous' by some researchers. THC is not only a major cannabinoid in *Cannabis* spp., it is generally the compound responsible for the psychoactive effects of consuming *Cannabis*. However, other cannabinoids, such as cannabinol may also be at least mildly psychoactive. Certain other cannabinoids such as CBD may help regulate or attenuate the psychoactive effects of other cannabinoids. For purposes herein, compositions may be created with various ratios of cannabinoids, such as the ratio of CBD to THC or other ratios depending the specific person or the specific condition being treated.

[61]   "Herbal extracts" as used herein comprise extracts from one or more of *Rehmanniae* spp., *Achyranthis* spp., *Corni* spp., *Moutan* spp., *Alismatis* spp., *Dioscorea* spp., *Plantaginis* spp., *Hoelen* spp., *Aconiti* spp., *Cinnamomi* spp., *Barosma betulina*, *Galium aparine*, cornsilk from *Zea mays*, horsetail (*Equisetum* spp.), resiniferatoxin (or extract from *Euphorbia resinifera*), capsaicin, saw palmetto, bearberry, cranberry, St. John's Wort, stinging nettle, and/or combinations thereof.

[62]   Additional nutraceutical compounds that may be useful herein include but are not limited S-adenosylmethionine, methylfolate, polyphenols, D-mannose, antioxidants, omega-3 fatty

acids, or a B vitamin, vitamin A, vitamin C, vitamin D, vitamin E, or a compound providing a biologically-available form thereof, or combinations thereof.

[63]   As used herein, "curcuminoids" means any of the compounds associated with turmeric or curcumin, as derived rhizome of the plant *Curcuma longa*, or synthetic versions or derivative thereof. Curcuminoids include but are not limited to curcumin (aka diferuloylmethane), analogs of curcumin such as demethoxycurcumin (DMC), bisdemethoxycurcumin (BDMC), turmerones, and turmeric oil. Also include are metabolites of curcumin such as tetrahydrocurcumin (THCU), hexahydrocurcumin, and octahydrocurcumin.

[64]   Conjugates, such as curcumin glucuronide and curcumin sulfate, are also included herein. Conjugation may also provide opportunities for improved delivery of curcumins herein, for example, conjugation to peptide carriers, or polylactic-co-glycolic acid [PLGA]; as well as complexation with essential oils; coadministration with piperine; and encapsulation into nanoparticles, liposomes, phytosomes, polymeric micelles, and cyclodextrins may also be useful herein.

[65]   As used herein, "terpenes" means any of the organic compounds commonly known as terpenes or terpenoids. Terpenes are generally aromatic compounds classified as isoprene derivatives. Terpenes suitable for use herein include hemiterpenes, monoterpenes, sesquiterpenes, diterpenes, sesterterpenes, triterpenes, sesquaterpenes, tetraterpenes, polyterpenes, and norisoprenoids. Exemplary terpenes that are particularly useful herein include alpha bisabolol, alpha pinene, beta caryophyllene, beta pinene, borneol, camphor, camphene, caryophyllene, cineole, delta-3 carene, eucalyptol, farnesenes, farnesol, fenchol, fenchone, geraniol, guaiol, humulene, isopulegol, limonene, linalool, menthol, myrcene, nerol, nerolidol, ocimene, pinene, phytol, pulegone, terpinene, terpineol, terpinolene, and valencene. In other embodiments phytol, limonene, humulene, myrcene, cineol, phellandrene, caryophyllene, terpinolene, linalool, ocimene, pinene, or a combination thereof are presently preferred.

[66]   As used herein, "flavonoids" includes any of the class of polyphenolic molecules containing 15 carbon atoms that are naturally produced in plants and are soluble in water. Also included herein as "flavonoids" are natural or synthetic derivative or analogs thereof that have biological activity. Flavonoids of use herein can generally be divided in to 6 groups of structurally related compounds: chalcones, flavones, isoflavonoids, flavanones, anthoxanthins, and anthocyanins. Also useful are flavanols and catechins, as well as glucosides or other derivatives or analogs of any of the foregoing. The flavonoids are found in most fruits and

vegetables, particular colorful ones. They are also prevalent in legumes (including soybeans), grains, green and black teas, as well as red wine.

[67]   Flavonoids have numerous functions in plants, and act as important cell messengers. Various flavonoids are believed to provide healthful benefits and functions to humans such as anti-cancer, anti-inflammatory, anti-allergic, and anti-oxidant properties. They may also be cardio-protective, cholesterol-lowering, and anti-atherosclerotic. Natural or synthetic flavonoids from any source may be used herein. Generally natural flavonoids are preferred. Flavonoids isolated from *Cannabis*, such as cannaflavins A, B, and or C, are of interest in certain applications, as are vitexin, isovitexin, apigenin, kaempferol, quercetin, orientin, and luteolin, as well as the catechins found in *Cannabis*.

[68]   "Traditional Jamaican medicinal plants" means any plant that has been used in traditional or indigenous medicine or herbalism practices in Jamaica or other Caribbean states. The book, "*Common Medicinal Plants of Portland, Jamaica*" by Thomas and Austin, provides a useful list of a number of such plants. The book was published in its second edition in 2010 by CIEER and is incorporated herein by reference. For purposes herein, the definition of such traditional Jamaican medicinal plants" expressly excludes *Cannabis* spp.

[69]   As used herein, the singular form of a word includes the plural, and vice versa, unless the context clearly dictates otherwise. Thus, the references "a", "an", and "the" are generally inclusive of the plurals of the respective terms. For example, reference to "a composition" or "a cannabis extract" includes a plurality of such "compositions" or "cannabis extracts."

[70]   The words "comprise", "comprises", and "comprising" are to be interpreted inclusively rather than exclusively. Likewise, the terms "include", "including" and "or" should all be construed to be inclusive, unless such a construction is clearly prohibited from the context. Further, forms of the terms "comprising" or "including" are intended to include embodiments encompassed by the phrases "consisting essentially of" and "consisting of". Similarly, the phrase "consisting essentially of" is intended to include embodiments encompassed by the phrase "consisting of".

[71]   Where used herein, ranges are provided in shorthand, so as to avoid having to list and describe each and every value within the range. Any appropriate value within the range can be selected, where appropriate, as the upper value, lower value, or the terminus of the range.

[72]   The methods and devices and/or other advances disclosed here are not limited to particular methodology, protocols, and/or structures described herein because, as the skilled artisan will appreciate, they may vary. Further, the terminology used herein is for the purpose

of describing particular embodiments only, and is not intended to, and does not, limit the scope of that which is disclosed or claimed.

[73]   Although any devices, methods, articles of manufacture, or other means or materials similar or equivalent to those described herein can be used in the practice of the present invention, the preferred compositions, methods, articles of manufacture, or other means or materials are described herein.

[74]   All patents, patent applications, publications, technical and/or scholarly articles, and other references cited or referred to herein are in their entirety incorporated herein by reference to the extent permitted under applicable law. Any discussion of those references is intended merely to summarize the assertions made therein. No admission is made that any such patents, patent applications, publications or references are prior art, or that any portion thereof is either relevant or material to the patentability of what is claimed herein. Applicant specifically reserves the right to challenge the accuracy and pertinence of any assertion that such patents, patent applications, publications, and other references are prior art, or are relevant, and/or material.

Abbreviations

[75]   The following abbreviations apply unless indicated otherwise:

ADCC:	antibody-dependent cellular cytotoxicity;
ADCP:	antibody-dependent cellular phagocytosis;
APM:	"Apollon Medical" strain of *C. sativa*
CBD:	cannabidiol;
CBG:	cannabigerol;
CBN:	cannabinol;
NK:	natural killer cells;
SVM:	Support Vector Machines;
THC:	tetrahydrocannabinol; and
THCA:	tetrahydrocannabolic acid.

Detailed Description of Illustrative Embodiments

[76]   In a first of its several aspects, compositions are provided for treatment of cancers, the compositions generally comprising one or more edible or medicinal mushrooms or extracts,

fractions, isolates, or components thereof. The compositions can optionally comprise one or more terpenes, triterpenes, and one or more flavonoids.

[77]   The compositions in various embodiments also include cannabinoids, comprising one or more of cannabidiol (CBD), cannabinol (CBN), cannabigerol (CBG), tetrahydrocannabinol (THC), or tetrahydrocannabinolic acid (THCA).

[78]   The example compositions used for the experiments disclosed herein represent only a few possible compositions hereunder.

[79]   Code/Key Table for the Exemplary Mushroom Compositions and Mixtures with *Cannabis* components

Code	Mushroom ID	Cannabis *YIN*	*Cannabis* ID/ Compounds
Fl	Lions Mane	N	n/a
F2	Cordyceps	N	n/a
F3	Shiitake	N	n/a
F4	Turkey Tail	N	n/a
F5	Reishi	N	n/a
F6	Maitake	N	n/a
F7	Chaga	N	n/a
F8	Reishi Triterpenes	N	n/a
F9	Cubensis	N	n/a
Ml	Reishi Triterpenes	y	THC, CBD, CBG, THCA
M2	Mushroom Compounds Reishi, Shitaki, Maitaki	y	Ringo's Gift+ Cannabinoid Blend*
M3	Mushroom Compounds Reishi, Shitaki, Maitaki	y	THC, CBD, CBG, THCA+ Cannabinoid Blend*
M4	Mushroom Compounds TurkeyTail, Chaga, Lion's Mane, Cordyceps	y	Ringo's Gift+ Cannabinoid Blend*
MS	Mushroom Compounds TurkeyTail, Chaga, Lion's Mane, Cordyceps	y	Ringo's Gift+ Cannabinoid Blend*

M6	Cubensis	y	Ringo's Gift+ Cannabinoid Blend*

* Blend: equal parts of THC, CBD, CBG, THCA, and terpenes

[80]   In various embodiments, the edible or medicinal mushroom(s) comprise one or more of *Agaricus, Auricularia, Clitocybe, Ganoderma, Grifola, Hericium, Lentinus, Leucopaxillus, Phellinus, Pleurotus, Sarcodona,* and *Trametes.* In other embodiments, the edible or medicinal mushroom(s) comprises one or more of *Albatrellus, Antrodia, Calvatia, Cordyceps, Flammulina, Fames, Funlia, Inocybe, Inonotus, Lactarius, Russula, Schizophyllum, Suillus,* or *Xerocomus*

[81]   These mushrooms are all well-known edible and/ or medicinal mushrooms with a long history of use. Without limiting the invention to any particular theory of operation, mushrooms, such as Turkey Tail, Reishi, shitake, maitake, and many others have a plethora of beneficial and potentially therapeutic compounds present in them. For example, the polysaccharide content in Reishi mushroom, particularly the beta-1,3 D-glucan, has been shown to up-regulate the production of certain lymphocytes, T-helper cells, T-killer cells, and macrophages. The same glycan has also been shown to be involved with suppressing tumor necrosis factor (TNF-a). Despite the name, the cytokine TNF has shown in certain cancers to actual interfere with processes that would otherwise fight cancer or eliminate cancer cells.

[82]   Regardless of the mechanism(s) responsible, the compositions preferably comprise the benefits of such edible or medicinal mushroom(s) as described above.

[83]   The mushroom(s) in certain presently preferred embodiments   comprises one or more species such as *Agaricus blazei, Albatrellus confluens, Antrodia camphorate, Boletus badius, Clitocybe maxima, Cordyceps militaris, Cordyceps sinensis, Cordyceps liangshanensis, Cordyceps gunnii, Cordyceps cicadicola, Flanulina velutipes, Fames fomentarius, Funalia trogii, Ganoderma lucidum, Grifoloa fondosa, Hericium erinaceus, Inocybe umbrinella, Ionatus olbiquus, Lactarius flavidulus, Lentinula edodes, Phellinus linteus, Pleurotus ostreatus, Schizophyllum commune, Suillus placidus,* or *Trametes versicolor.*

[84]   In various presently preferred embodiments, the cannabinoids are derived from *Cannabis* spp. or an extract thereof. *Cannabis* can generally be concentrated or extracted (e.g. via mechanical or chemical means) to obtain cannabinoids. Mechanical means of extracting oils from plants, such as pressing, have been used for centuries, and may be suitable for use herein. Extraction via chemical means includes extraction with various volatile solvents that range from hydrocarbon solvents such as butane, hexanes or propane, to supercritical fluids,

alcohol (e.g. isopropanol, butanol, or ethanol), steam, or even water. Two very common methods are extraction with supercritical carbon dioxide, or ethanol, both of which are particularly useful herein. Extracts can be also be distilled e.g. to remove additional compounds of interest, or to concentrate them. Certain components can be removed, e.g. by treatment with steam to strip certain volatiles, which can be captured as an additional component from the *Cannabis.*

[85]   In various embodiments, cannabinoids present in an ethanolic extract or supercritical CO, extract of *Cannabis sativa* are preferred for use herein. The extract comprises one or more of Ringo's Gift, Harle Tsu, ACDC, Charlotte's Web, The Gift, or Pineberry strains of *Cannabis sativa.* "Apollon Medical" ("APM"), a proprietary strain commercially available from Apollon Formularies, is also useful herein.

[86]   The composition in various embodiments include terpenes comprising one or more monoterpenes, one or more sesquiterpenes, or a combination thereof. The terpenes comprise one or more of alpha bisabolol, alpha pinene, beta caryophyllene, beta pinene, borneol, camphor, camphene, caryophyllene oxide, cineole, delta-3 carene, eucalyptol, farnesenes, farnesol, fenchol, fenchone, geraniol, guaiol, humulene, isopulegol, limonene, linalool, menthol, myrcene, nerol, nerolidol, ocimene, pinene, phytol, pulegone, terpinene, terpineol, terpinolene, or valencene.

[87]   In one embodiment, the terpenes comprise phytol, limonene, humulene, myrcene, phellandrene, caryophyllene, linalool, pinene, or a combination thereof. In other embodiments, the terpenes preferably comprise one or more of limonene, myrcene, beta-caryophyllene, linalool, alpha pinene, or a combination thereof.

[88]   In presently preferred embodiments, the terpenes are derived from *Cannabis* spp. or an extract thereof. The terpenes can be derived from any source and in certain embodiments, they can be present in steam distillate or an ethanolic extract of *Cannabis sativa.*

[89]   The compositions in certain embodiments include extracts of *Cannabis* spp, such as C. *sativa* as a source of cannabinoids and/ or terpenes. In various embodiments, the C. *sativa* comprises one or more of Ringo's Gift, Harle Tsu, ACDC, Charlotte's Web, The Gift, or Pineberry strains. In other embodiments, the C. *sativa* comprises the proprietary Apollon Formularies strain, APM.

[90]   In various embodiments, the compositions, or one or more components thereof, may be solubilized, micronized, provided as, for example. extracts, powders, lyophilized powders, concentrates, tinctures, essential oils, aqueous or lipid suspensions, emulsions,

microemulsions, or nano-emulsions, or in whole or part as liposomal, vesicular, or other delivery systems. As described below, the compounding or formulation of any of the compositions provided herein may be optimized for the intended delivery route.

[91]   The compositions may be administered and delivered as pharmaceuticals, however, it is also contemplated that one or more of the compositions may be formulated for administration and delivery by oral routes that include as food and beverages, including solid, semisolid, and liquid foods, such as smoothies, shakes, pudding, broths, teas, and soups. The food and or beverage compositions can also include hot, cold, or even frozen foods (such as frozen desserts).

[92]   In another aspect of the disclosure, provided are methods of treating a subject suffering from cancer. The methods generally comprise administering a therapeutically effective dose of a composition to the subject. The composition typically comprises one or more edible or medicinal mushrooms or an extract, fraction, or isolate thereof, one or more cannabinoids, one or more terpenes, and optionally, one or more flavonoids.

[93]   The cancer in various embodiments is a common cancer such as bladder cancer, brain tumors, breast cancer, cervical cancer, colorectal cancer, endometrial cancer, esophageal cancer, gastric cancer, head and neck cancers, Kaposi sarcoma, kidney (renal cell) cancer, leukemia, liver cancer, lung cancer, lymphoma, melanoma, non-Hodgkin lymphoma, neuroblastoma, ovarian cancer, osteosarcoma and other bone cancers, pancreatic cancer, pituitary tumors, prostate cancer, retinoblastoma, skin cancer, testicular cancer, thyroid cancer, or uterine cancer.

[94]   The composition for use in the methods is generally as described above for the first aspect. The description of the compositions above is incorporated by reference herein for purposes of the present methods.

[95]   The terpenes for use with the compositions can comprise one or more monoterpenes, one or more sesquiterpenes, or a combination thereof. In various embodiments, the terpenes comprise one or more of alpha bisabolol, alpha pinene, beta caryophyllene, beta pinene, borneol, camphor, camphene, caryophyllene oxide, cineole, delta-3 carene, eucalyptol, farnesenes, farnesol, fenchol, fenchone, geraniol, guaiol, humulene, isopulegol, limonene, linalool, menthol, myrcene, nerol, nerolidol, ocimene, pinene, phytol, pulegone, terpinene, terpineol, terpinolene, or valencene. In certain preferred embodiments, the terpenes are derived from *Cannabis* spp. or an extract thereof. The terpenes can be present in steam distillate or an ethanolic extract of *Cannabis sativa,* and can comprise limonene, myrcene, beta-

caryophyllene, linalool, alpha pinene, or a combination thereof, in some embodiments. The *Cannabis sativa* in one embodiment includes one or more of Ringo's Gift, Harle Tsu, ACDC, Charlotte's Web, The Gift, or Pineberry strains.

[96]   Preferably the additional administering steps are performed on a periodic basis of any frequency or schedule. For example, the administration or dosing can conveniently be on e.g. a daily, thrice weekly, twice weekly, weekly, biweekly, monthly, bimonthly, quarterly, semi-annual, or annual basis. The administration need not be the same over every period of time. By way of nonlimiting example, administration could be daily for a week, then weekly for a month. Or the administration could be every 4 months for a year, then every 6 months thereafter. Similarly, the actual amount of the composition or dosage administration can vary. For example, a monthly dosage schedule could feature a dose of x for the first dosage each quarter, and a dose of 0.lx for the remaining months in each quarter.

[97]   Just as the composition can be 'personalized', so can the administration or dosing schedule. Thus, in various embodiments, the methods further comprise the step of periodically assessing one or more of the subject's medication levels, enzyme levels, or other indicators of physiological health or status, genetic markers or antigen presence in the cancer cells, or the like, in order to determine the periodic basis for administration.

[98]   The methods provide for administration of the compositions via any useful route, including parenteral (intravenous, intra-arterial, intramuscular, intraperitoneal, or subcutaneous), oral, nasal, ocular, transmucosal (buccal, vaginal, or rectal), transdermal, or via inhalation.

[99]   It should be noted that the route of dosing or administration of compositions can vary over the course of treating a subject or patient with multiple steps of treatment, as well as from subject to subject, or with different types of cancer. For example, administration via one route may be useful when administering a larger dose and a different route may be useful for smaller doses. Or, administration via a particular route may be appropriate initially, with subsequent doses conveniently administered through another route.

[100]   In one embodiment, the method further comprises a step of providing to the subject additional treatment of the cancer comprising:

[101]   i)   one or more doses of a chemotherapeutic agent;

[102]   ii)   one or more treatments with ionizing radiation;

[103]   iii)   one or more doses of an immunotherapeutic;

[104] iv)   one or more targeted treatments of the cancer;

[105] v)   one or more other treatments specifically provided to treat the cancer; or any combination of any of the foregoing.

[106] Methods that further comprise a step of providing to the subject an additional composition are also provided. The additional composition may be administered in between doses of the base compositions, or may be provided on a separate and independent periodic basis. The addition compositions generally comprise any combination of less than three of the following:

[107] i)   one or more edible or medical mushrooms or an extract, fraction, or isolate thereof;

[108] ii)   one or more cannabinoids;

[109] iii)   one or more terpenes; or

[110] iv)   one or more flavonoids.

[111] The additional compositions further optionally comprise any combination of one or more of S-adenosylmethionine, methylfolate, omega-3 fatty acids, or a B vitamin, vitamin D, or a compound providing a biologically-available form thereof.

[112] In various embodiments of the methods;

[113] i)   the one or more edible or medical mushrooms comprise *Agaricus, Auricularia, Clitocybe, Ganoderma, Grifola, Hericium, Lentinus, Leucopaxillus, Phellinus, Pleurotus, Sarcodona, Trametes, Albatrellus, Antrodia, Calvatia, Cordyceps, Flammulina, Fames, Funlia, Inocybe, Inonotus, Lactarius, Russula, Schizophyllum, Suillus, or Xerocomus;*

[114] ii)   the cannabinoids comprise one or more of cannabidiol (CBD), cannabinol (CBN), cannabigerol (CBG), or tetrahydrocannabinol (THC);

[115] iii)   the terpenes comprise one or more of alpha bisabolol, alpha pinene, beta caryophyllene, beta pinene, borneol, camphor, camphene, caryophyllene oxide, cineole, delta-3 carene, eucalyptol, farnesenes, farnesol, fenchol, fenchone, geraniol, guaiol, humulene, isopulegol, limonene, linalool, menthol, myrcene, nerol, nerolidol, ocimene, pinene, phytol, pulegone, terpinene, terpineol, terpinolene, or valencene; and

[116] iv)   the one or more flavonoids comprise chalcones, flavones, isoflavonoids, flavanones, anthoxanthins, anthocyanins, flavonols, or glucosides or other biologically active derivatives or analogs thereof.

[117] The cancer can comprise any metastatic or neoplastic disease such as bladder cancer, breast cancer, colorectal cancer, endometrial, kidney (renal) cancer, leukemia, lung cancer, non-

Hodgkin's lymphoma, pancreatic cancer, prostate cancer, skin cancers, stomach cancer, or thyroid cancer.

[118]   The skilled artisan will appreciate that the methods are flexible as set forth herein, an aspect which is particularly useful given the varied and nature of the psychological disorders which they are intended to treat.

[119]   In yet another aspect of the disclosure, methods of optimizing a composition for use in treatment of a subject suffering from cancer using artificial intelligence are provided herein. The methods generally comprise, for each cancer of interest, or for a subject in need of therapeutic compositions for such cancer:

[120]   a)   providing data on the therapeutic effect on the cancer of each of:

[121]   i)   a plurality of edible or medicinal mushrooms, mushroom extracts, or components thereof;

[122]   ii)   a plurality of cannabinoids or combinations thereof;

[123]   iii)   a plurality of terpenes or combinations thereof;

[124]   b)   using an artificial intelligence algorithm to analyze the data for the mushrooms or extracts, cannabinoids, terpenes, and flavonoids; and

[125]   c)   generating one or more compositions optimized for therapeutic treatment of the cancer

[126]   In certain embodiments, the cancer comprises bladder cancer, brain tumors, breast cancer, cervical cancer, colorectal cancer, endometrial cancer, esophageal cancer, gastric cancer, head and neck cancers, Kaposi sarcoma, kidney (renal cell) cancer, leukemia, liver cancer, lung cancer, lymphoma, melanoma, non-Hodgkin lymphoma, neuroblastoma, ovarian cancer, osteosarcoma and other bone cancers, pancreatic cancer, pituitary tumors, prostate cancer, retinoblastoma, skin cancer, testicular cancer, thyroid cancer, or uterine cancer. Generally, the data for the method are obtained from original experiments and/ or reviews of the relevant scientific literature.

[127]   The artificial intelligence algorithm can comprise any useful software or algorithm approach capable of making the distinctions required. In various embodiments, the algorithm comprises a classification algorithm, a regression algorithm, a clustering algorithm, or a combination thereof .

[128]   In one embodiment, the methods comprise a classification algorithm that is a naive Bayes algorithm, decision tree, random forest algorithm, Support Vector Machines, or K Nearest Neighbor algorithm.

[129]   In another embodiment, the methods comprise a regression algorithm that is a liner regression, lasso regression, logistic regression, or multivariate regression.

[130]   In yet another embodiment, the methods comprise a clustering algorithm that is a K-means clustering, fuzzy C-means algorithm, expectation-maximization algorithm, or hierarchical clustering algorithm.

[131]   The skilled artisan will appreciate that the methods are designed to optimize the compositions, and that such optimization as set forth above can be with respect to each particular cancer or even each particular variant of a cancer. However, the compositions can also be optimized for, and a profile of relevant compositions generated for each particular subject, e.g. for a 'personalized medicine' approach.

[132]   Thus, also provided herein are the methods comprising the additional step of providing subject-specific data comprising, e.g. initial or subsequent blood work, enzyme test results, bioinformatic data (including measurements of e.g. the genome, transcriptome, proteome, metabolome, or any portion thereof, for a subject), specific symptomology, or the like. The artificial intelligence algorithm is then used to further optimize the composition based on those data in addition to the disorder-specific data. The optimized formulation of the compositions may also be changed based on data from the results from an initial treatment, subsequent treatment, or based on subsequent tests of the subject.

[133]   In certain embodiments, the cancer comprises a prevalent form of cancer such as bladder cancer, breast cancer, colorectal cancer, endometrial, kidney (renal) cancer, leukemia, lung cancer, non-Hodgkin's lymphoma, pancreatic cancer, prostate cancer, skin cancers, stomach cancer, or thyroid cancer.

[134]   The plurality of edible or medicinal mushrooms, mushroom extracts, or components for which data are analyzed generally comprise one or more of the species *Agaricus blazei, Albatrellus confluens, Antrodia camphorate, Boletus badius, Clitocybe maxima, Cordyceps militaris, Flanulina velutipes, Fames fomentarius, Funalia trogii, Ganoderma lucidum, Grifoloa fondosa, Hericium erinaceus, Inocybe umbrinella, Ionatus olbiquus, Lactarius flavidulus, Lentinula edodes, Phellinus linteus, Pleurotus ostreatus, Schizophyllum commune, Suillus placidus, Trametes versicolor, Cordyceps sinensis,* C. *liangshanensis,* C. *gunnii, or* C. *cicadicola* .

[135]   A further aspect of the invention provides methods for the treatment of cancer. The methods generally comprise the step of administering to a patient in need thereof a composition comprising at least one edible or medicinal mushroom or extract thereof, in combination with at least one cannabinoid, at least one terpene, and at least one flavonoid. In one embodiment the at least one cannabinoid, at least one terpene, and at least one flavonoid are administered separately from, sequentially to, or simultaneously with the edible or medicinal mushroom or extract thereof. In another embodiment, the at least one edible or medicinal mushroom or extract thereof is also administered separately from, sequentially to, or simultaneously with the cannabinoid, terpene, and flavonoid.

[136]   Again, the composition with respect to this aspect of the disclosure can comprise any of the compositions described hereinabove. In one embodiment of the methods:

[137]   i)   the at least one edible or medicinal mushroom or extract comprises *Agaricus blazei, Albatrellus confluens, Antrodia camphorate, Boletus badiis, Clitaocyte maxima, Caordyceps militaris, Flanuline velutipes, Fomes fomentarius, Funalia trogii, Ganoderma lucidum, Grifoloa fondosa, Hericium erinaceus, Inocybe umbrinella, Ionatus olbiquus, Lactarius flavidulus, Lentinula edodes, Phellinus linteus, Pleurotus ostreatus, Schizophyllum commune, Suillus placidus, Trametes versicolor, Cordyceps sinensis, C. liangshanensis, C. gunnii, or C. cicadicola;*

[138]   ii)   the at least one cannabinoid comprises CBD, CBG, CBN, and THC extracted from *Cannabis sativa* Ringo's Gift strain or APM strain.

[139]   iii)   the at least one terpene comprises limonene, myrcene, beta- caryophyllene, linalool, alpha pinene, or a combination thereof; and

[140]   iv)   the at least one flavonoid comprises a chalcone, flavone, isoflavonoid, flavanone, anthoxanthin, anthocyanin, flavonol, or glucoside or other biologically active derivatives or analogs thereof.

[141]   In various embodiments of the methods, one or more of the components have one more of the following functions:

[142]   i)   induces apoptosis of cancer cells;

[143]   ii)   inhibits the VEGF pathway and/ or prevent angiogenesis of cancer cells;

[144]   iii)   disrupts one or more aspect of cell growth of cancer cells;

[145]   iv)   restores normal differentiation of cancer cells, or restores normal cell cycle in cancer cells; or

[146]   v)   inhibits one of more of migration, adhesion, or invasion of cancer cells.

[147]   In certain embodiments the compositions may be provided in completely customized or personalized formulations for each person being treated - i.e. as personalized medicines. In such cases, the compositions may be adjusted based on initial or subsequent blood work, enzyme test results, bioinformatic data (including measurements of e.g. the genome, transcriptome, proteome, metabolome, or any portion thereof, for a subject), the type and stage of cancer, specific markers, antigen, or receptors of the cancer, specific symptomology, or the like. The formulation of the compositions may also be changed based on the results from an initial treatment, subsequent treatment, or based on subsequent tests.

[148]   In another embodiment, the composition still further comprises an extract or fraction from one or more traditional Jamaican medicinal plants other than *Cannabis* spp. Any of the traditional Jamaican or Caribbean medicinal plants may be useful herein. In one embodiment, the traditional medicinal plants comprise guinea hen weed *(Petiveria alliacea)*, and/ or soursop *(Annona murata)*. In a presently preferred embodiments, the compositions include a whole plant extract, or an extract from any parts or portion thereof including but not limited to leaves, stems, flowers, roots, fruit, seeds, or the like.

[149]   In terms of compounding the compositions, the skilled artisan will appreciate that methods of maximizing the efficacy of the composition such as by enhancing the bioavailability of one or more components, or by providing the components in optimized ratios, for example one component to another with which it interacts, or each component to the others in ratio(s) that optimize the absorption into the gut or bloodstream, or enhance the therapeutic effect of the composition. The skilled artisan will also understand that some information useful in improving the compounding may be obtained empirically.

[150]   In various embodiments, the compositions, or one or more components thereof, may be solubilized, micronized, provided as, for example, extracts, powders, lyophilized powders, concentrates, tinctures, essential oils, aqueous or lipid suspensions, emulsions, microemulsions, or nano-emulsions, or in whole or part as liposomal, vesicular, or other delivery systems. As described below, the compounding or formulation of any of the compositions provided herein may be optimized for the intended delivery route. Presently preferred forms include micro- and nanoemulsions.

[151]   The compositions may be administered and delivered as pharmaceuticals, however, it is also contemplated that one or more of the compositions may be formulated for administration and delivery by oral routes that include as food and beverages, including solid, semisolid, and liquid foods, such as smoothies, shakes, pudding, broths, teas, and soups. The food and or

beverage compositions can also include hot, cold, or even frozen foods (such as frozen desserts).

[152]   The cancer in various embodiments is a common cancer such as bladder cancer, brain tumors, breast cancer, cervical cancer, colorectal cancer, endometrial cancer, esophageal cancer, gastric cancer, head and neck cancers, Kaposi sarcoma, kidney (renal cell) cancer, leukemia, liver cancer, lung cancer, lymphoma, melanoma, non-Hodgkin lymphoma, neuroblastoma, ovarian cancer, osteosarcoma and other bone cancers, pancreatic cancer, pituitary tumors, prostate cancer, retinoblastoma, skin cancer, testicular cancer, thyroid cancer, or uterine cancer.

[153]   The composition for use in the methods is generally as described above for the first aspect. The description of the compositions above is incorporated by reference herein for purposes of the present methods.

[154]   In various embodiments, the compositions for use in the methods further comprise one or more optional ingredients comprising S-adenosylmethionine, methylfolate, omega-3 fatty acids, or a B vitamin, vitamin D or a compound providing a biologically-available form thereof.

[155]   The terpenes for use with the compositions can comprise one or more monoterpenes, one or more sesquiterpenes, or a combination thereof. In various embodiments, the terpenes comprise one or more of alpha bisabolol, alpha pinene, beta caryophyllene, beta pinene, borneol, camphor, camphene, caryophyllene oxide, cineole, delta-3 carene, eucalyptol, farnesenes, farnesol, fenchol, fenchone, geraniol, guaiol, humulene, isopulegol, limonene, linalool, menthol, myrecene, nerol, nerolidol, ocimene, pinene, phytol, puregone, terpinene, terpineol, terpineolene, or valencene. In certain preferred embodiments, the terpenes are derived from *Cannabis* spp. or an extract thereof. The terpenes can be present in steam distillate or an ethanolic extract of *Cannabis sativa,* and can comprise limonene, myrcene, beta-caryophyllene, linalool, alpha pinene, or a combination thereof, in some embodiments. The *Cannabis sativa* in one embodiment includes one or more of Ringo's Gift, Harle Tsu, ACDC, Charlotte's Web, The Gift, or Pineberry strains, or APM.

[156]   The compositions for use with the methods may also comprise one or more flavonoids that can be chalcones, flavones, isoflavonoids, flavanones, anthoxanthins, anthocyanins, flavonols, or glucosides or other biologically active derivatives or analogs thereof, and preferably they are from a plant, or other natural source.

[157]   Presently preferred flavonoids include cannaflavin A, cannaflavin B, or cannaflavin C, vitexin, isovitexin, apigenin, kaempferol, quercetin, orientin, luteolin, a catechin found in *Cannabis,* or a combination of any of the foregoing.

[158]   In certain embodiments, the methods further comprise one or more steps of administering an additional therapeutically effective dose of the composition.

[159]   Preferably the additional administering steps are performed on a periodic basis of any frequency or schedule. For example, the administration or dosing can conveniently be on e.g. a daily, thrice weekly, twice weekly, weekly, biweekly, monthly, bimonthly, quarterly, semi-annual, or annual basis. The administration need not be the same over every period of time. By way of nonlimiting example, administration could be daily for a week, then weekly for a month. Or the administration could be every 4 months for a year, then every 6 months thereafter. Similarly, the actual amount of the composition or dosage administration can vary. For example, a monthly dosage schedule could feature a dose of x for the first dosage each quarter, and a dose of 0.lx for the remaining months in each quarter.

[160]   Just as the composition can be 'personalized', so can the administration or dosing schedule. Thus, in various embodiments, the methods further comprise the step of periodically assessing one or more of the subject's medication levels, enzyme levels, or other indicators of physiological health or status, genetic markers or antigen presence in the cancer cells, or the like, in order to determine the periodic basis for administration.

[161]   The methods provide for administration of the compositions via any useful route, including parenteral (intravenous, intra-arterial, intramuscular, intraperitoneal, or subcutaneous), oral, nasal, ocular, transmucosal (buccal, vaginal, or rectal), transdermal, or via inhalation.

[162]   It should be noted that the route of dosing or administration of compositions can vary over the course of treating a subject or patient with multiple steps of treatment, as well as from subject to subject, or with different types of cancer. For example, administration via one route may be useful when administering a larger dose and a different route may be useful for smaller doses. Or, administration via a particular route may be appropriate initially, with subsequent doses conveniently administered through another route.

[163]   In one embodiment, the method further comprises a step of providing to the subject additional treatment of the cancer comprising:

[164]   i)   one or more doses of a chemotherapeutic agent;

[165] ii)   one or more treatments with ionizing radiation;

[166] iii)   one or more doses of an immunotherapeutic;

[167] iv)   one or more targeted treatments of the cancer;

[168] v)   one or more other treatments specifically provided to treat the cancer; or any combination of any of the foregoing.

[169] Methods that further comprise a step of providing to the subject an additional composition are also provided. The additional composition may be administered in between doses of the base compositions, or may be provided on a separate and independent periodic basis. The addition compositions generally comprise any combination of less than three of the following:

[170] i)   one or more *Cannabis* extracts, fractions, or isolates thereof;

[171] ii)   one or more cannabinoids;

[172] iii)   one or more terpenes; or

[173] iv)   one or more triterpenes.

[174] In various embodiments of the methods:

[175] i)   the cannabinoids comprise one or more of cannabidiol (CBD), cannabinol (CBN), cannabigerol (CBG), or tetrahydrocannabinol (THC);

[176] ii)   the terpenes comprise one or more of alpha bisabolol, alpha pinene, beta caryophyllene, beta pinene, borneol, camphor, camphene, caryophyllene oxide, cineole, delta-3 carene, eucalyptol, farnesenes, farnesol, fenchol, fenchone, geraniol, guaiol, humulene, isopulegol, limonene, linalool, menthol, myrcene, nerol, nerolidol, ocimene, pinene, phytol, pulegone, terpinene, terpineol, terpinolene, or valencene; and

[177] iii)   the one or more flavonoids comprise chalcones, flavones, isoflavonoids, flavanones, anthoxanthins, anthocyanins, flavonols, or glucosides or other biologically active derivatives or analogs thereof.

[178] The cancer can comprise any metastatic or neoplastic disease such as bladder cancer, breast cancer, colorectal cancer, endometrial, kidney (renal) cancer, leukemia, lung cancer, non-Hodgkin's lymphoma, pancreatic cancer, prostate cancer, skin cancers, stomach cancer, or thyroid cancer.

[179] In one embodiment of the methods, the composition further comprises an extract or fraction from one or more traditional Jamaican medicinal plants other than *Cannabis* spp. The traditional medicinal plants comprise guinea hen weed *(Petiveria alliacea),* or soursop *(Annona murata)* in certain embodiments.

[180]   The skilled artisan will appreciate that the methods are flexible as set forth herein, an aspect which is particularly useful given the varied and nature of the psychological disorders which they are intended to treat.

[181]   In yet another aspect of the disclosure, methods of optimizing a composition for use in treatment of a subject suffering from cancer using artificial intelligence are provided herein. The methods generally comprise, for each cancer of interest, or for a subject in need of therapeutic compositions for such cancer:

[182]   a)   providing data on the therapeutic effect on the cancer of each of:

[183]   i)   a plurality of cannabis extracts, or components thereof;

[184]   ii)   a plurality of cannabinoids or combinations thereof;

[185]   iii)   a plurality of terpenes or combinations thereof;

[186]   iv)   a plurality of flavonoids or combinations thereof; and optionally,

[187]   v)   a plurality of combinations of compositions comprising one or more of cannabinoids, terpenes, and flavonoid; or

[188]   vi)   a plurality of optional ingredients comprising S-adenosylmethionine, methylfolate, omega-3 fatty acids, or a B vitamin or vitamin D or a compound providing a biologically-available form thereof, or combinations thereof;

[189]   b)   using an artificial intelligence algorithm to analyze the data for the cannabinoids, terpenes, and flavonoids; and

[190]   c)   generating one or more base profiles of compositions optimized for therapeutic treatment of the cancer;

[191]   d)   optionally, using the artificial intelligence algorithm to analyze the data for the combination compositions and the optional ingredients, and

[192]   e)   generating one or more complete profiles of compositions with and without the optional ingredients.

[193]   In certain embodiments, the cancer comprises bladder cancer, brain tumors, breast cancer, cervical cancer, colorectal cancer, endometrial cancer, esophageal cancer, gastric cancer, head and neck cancers, Kaposi sarcoma, kidney (renal cell) cancer, leukemia, liver cancer, lung cancer, lymphoma, melanoma, non-Hodgkin lymphoma, neuroblastoma, ovarian cancer, osteosarcoma and other bone cancers, pancreatic cancer, pituitary tumors, prostate cancer, retinoblastoma, skin cancer, testicular cancer, thyroid cancer, or uterine cancer. Generally, the

data for the method are obtained from original experiments and/ or reviews of the relevant scientific literature.

[194] The artificial intelligence algorithm can comprise any useful software or algorithm approach capable of making the distinctions required. In various embodiments, the algorithm comprises a classification algorithm, a regression algorithm, a clustering algorithm, or a combination thereof.

[195] In one embodiment, the methods comprise a classification algorithm that is a na'ive Bayes algorithm, decision tree, random forest algorithm, Support Vector Machines, or K Nearest Neighbor algorithm.

[196] In another embodiment, the methods comprise a regression algorithm that is a liner regression, lasso regression, logistic regression, or multivariate regression.

[197] In yet another embodiment, the methods comprise a clustering algorithm that is a K-means clustering, fuzzy C-means algorithm, expectation-maximization algorithm, or hierarchical clustering algorithm.

[198] The skilled artisan will appreciate that the methods are designed to optimize the compositions, and that such optimization as set forth above can be with respect to each particular cancer or even each particular variant of a cancer. However, the compositions can also be optimized for, and a profile of relevant compositions generated for each particular subject, e.g. for a 'personalized medicine' approach.

[199] Thus, also provided herein are the methods comprising the additional step of providing subject-specific data comprising, e.g. initial or subsequent blood work, enzyme test results, bioinformatic data (including measurements of e.g. the genome, transcriptome, proteome, metabolome, or any portion thereof, for a subject), specific symptomology, or the like. The artificial intelligence algorithm is then used to further optimize the composition based on those data in addition to the disorder-specific data. The optimized formulation of the compositions may also be changed based on data from the results from an initial treatment, subsequent treatment, or based on subsequent tests of the subject.

[200] In certain embodiments, the cancer comprises a prevalent form of cancer such as bladder cancer, breast cancer, colorectal cancer, endometrial, kidney (renal) cancer, leukemia, lung cancer, non-Hodgkin's lymphoma, pancreatic cancer, prostate cancer, skin cancers, stomach cancer, or thyroid cancer.

[201] A further aspect of the invention provides methods for the treatment of cancer. The methods generally comprise the step of administering to a patient in need thereof a

composition comprising at least one *Cannabis* extract, in combination with at least one cannabinoid, at least one terpene, and at least one flavonoid. In one embodiment the at least one cannabinoid, at least one terpene, and at least one flavonoid are administered separately from, sequentially to, or simultaneously with the *Cannabis* extract or the like. In another embodiment, the at least one Cannabis extract or component thereof is also administered separately from, sequentially to, or simultaneously with the cannabinoid, terpene, and flavonoid.

[202] Again, the composition with respect to this aspect of the disclosure can comprise any of the compositions described hereinabove. In one embodiment of the methods:

[203] i)   the at least one cannabinoid comprises CBD, CBG, CBN, and THC extracted from *Cannabis sativa* Ringo's Gift strain or the APM strain;

[204] ii)   the at least one terpene comprises limonene, myrcene, beta- caryophyllene, linalool, alpha pinene, or a combination thereof; and

[205] iii)   the at least one flavonoid comprises a chalcone, flavone, isoflavonoid, flavanone, anthoxanthin, anthocyanin, flavonol, or glucoside or other biologically active derivatives or analogs thereof.

[206] In various embodiments of the methods, one or more of the components have one more of the following functions:

[207] i)   induces apoptosis of cancer cells;

[208] ii)   inhibits the VEGF pathway and/ or prevent angiogenesis of cancer cells;

[209] iii)   disrupts one or more aspect of cell growth of cancer cells;

[210] iv)   restores normal differentiation of cancer cells, or restores normal cell cycle in cancer cells; or

[211] v)   inhibits one of more of migration, adhesion, or invasion of cancer cells.

[212] In yet another embodiment of the methods provided in this aspect of the disclosure, the composition further comprises an extract or fraction from one or more traditional Jamaican medicinal plants other than *Cannabis* spp. Traditional medicinal plants comprise guinea hen weed *(Petiveria alliacea),* or soursop *(Annona murata)* are contemplated as useful herein.

[213]   EXAMPLES

[214]   <u>Example 1: 3D Cell Proliferation Screen</u>

[215]   This study tested the efficacy of mushroom compositions with and without cannabis against 10 cancerous cell lines (see Tables 1 and 2 below), at 9 concentrations in a 3D-format screening panel.

[216]   Experimental Methods: 3D Cell Proliferation Assay

[217]   Cells were seeded at 1000 cells/well in media in 384-well Corning® Spheroid microplates. The microplates were incubated at 37C in 5% CO_2 incubator to allow for spheroid formation.

[218]   Aliquots of each sample were vortexed and centrifuged at 3,000 rpm (800 g) for 5 minutes at room temperature. Supernatants (500-fold stocks) were stored under sterile conditions at 4C.

[219]   On the day of the assay, a 9-point dose-response of each 500-fold stock was prepared and added to the spheroid plates to provide final assay concentrations ranging from le -04 to 1-fold, in 0.2% DMSO. Final assay conditions for each sample are shown on Table 1.

[220]   Assay plates were incubated at 37C in an atmosphere containing 5% CO2 for 5 days. Staurosporine, a compound known to induce cellular apotosis, was used a positive control for terminated cell proliferation. Cell viability was determined at 5 days post-compound addition. Briefly, 3D-CellTiter Glow™ reagent (Promega) was added to assay plates per the manufacturer's recommendations. Plates were shaken until spheroids were lysed and luminescence was read at room temperature.

[221]   The data were analyzed using R statistical software. The data for the results of the 3D Cell Proliferation assays are provided and shown in graph form in Annex 1 hereto.

[222]   Table 1: Testing Details:

*approximate volume

Sample ID	μL stock*/vial	Vehicle	Stock Conc.	Unit	Top Conc. in Assay Plate	Unit	Bottom Conc. in Assay Plate	Unit	Serial Dilution
f1	5,000	DMSO	500	fold	1	fold	1E-04	fold	half-log
f2	5,000	DMSO	500	fold	1	fold	1E-04	fold	half-log
f3	5,000	DMSO	500	fold	1	fold	1E-04	fold	half-log
f4	5,000	DMSO	500	fold	1	fold	1E-04	fold	half-log
f5	5,000	DMSO	500	fold	1	fold	1E-04	fold	half-log
f6	5,000	DMSO	500	fold	1	fold	1E-04	fold	half-log
f7	5,000	DMSO	500	fold	1	fold	1E-04	fold	half-log
f8	5,000	DMSO	500	fold	1	fold	1E-04	fold	half-log
f9	5,000	DMSO	500	fold	1	fold	1E-04	fold	half-log
m1	5,000	DMSO	500	fold	1	fold	1E-04	fold	half-log
m2	5,000	DMSO	500	fold	1	fold	1E-04	fold	half-log
m3	5,000	DMSO	500	fold	1	fold	1E-04	fold	half-log
m4	5,000	DMSO	500	fold	1	fold	1E-04	fold	half-log
m5	5,000	DMSO	500	fold	1	fold	1E-04	fold	half-log
m6	5,000	DMSO	500	fold	1	fold	1E-04	fold	half-log
Staurosporine	200	DMSO	1	mM	2	μM	2E-04	μM	half-log

[223]   Table 2: Human Cell Lines

Cell Line	Origen
22Rv1	Prostate
A-431	Skin
A-549	Lung
BT-474	Breast
HT-29	Colon
MDA-MB-231	Breast
NCI-H460	Lung
PC-3	Prostate
T24	Bladder
T-47D	Breast

[224]   <u>Data Analysis</u>

[225]   Cell proliferation end point was analyzed as Percent of Control (POC) using the following formula:

$$POC = \frac{relative\ cell\ count\ (compound\ wells)}{relative\ cell\ count\ (vehicle\ control\ wells)} \times 100\%$$

[226]   A relative cell count EC50 is the concentration of the test compound that produces a response half-way between the maximum and baseline. Relative cell count IC50 is the concentration of the test compound that produces 50% of the cell proliferation inhibitory response or 50% cytotoxicity level. GI50 is the concentration of the test compound that produces 50% reduction in proliferation of cancer cells relative to T0. The output of each biomarker is fold-increase over vehicle control background normalized to the relative cell count in each well.

[227]   EC50, IC50 and GI50 values were calculated using nonlinear regression to fit data to a sigmoidal 4-point, 4-parameter log-logistic dose response model:

$$y = c + \frac{d-c}{1+\exp\ (b(\log(x)-\log(e)))}$$

[228]

[229]   Curve-fitting and EC50 calculations were performed using the **R** statistical software package with R's drc library.

[230]   <u>Results:</u>

[231]   The direct cytotoxicity data for each mushroom composition tested in the Cell Proliferation Screen, sorted by composition and cell line (in alphabetic order), are presented in Tables 3a – 3e.

[232]   Table 3a: Cell Proliferation Screen Results for Mushroom Compositions

Key	Agent	Cell Line	EC50 (Fold)	IC50 (Fold)	GI50 (Fold)
101	f1	22Rv-1	>1	>1	>1
102	f1	A-431	0.122	>1	0.064
103	f1	A-549	>1	>1	>1
104	f1	BT-474	>1	>1	>1
105	f1	HT-29	>1	>1	>1
106	f1	MDA-MB-231	0.348	>1	>1
107	f1	NCI-H460	0.317	>1	>1
108	f1	PC-3	>1	>1	>1
109	f1	T-47D	>1	>1	>1
110	f1	T24	>1	>1	>1
111	f2	22Rv-1	0.884	0.884	0.725
112	f2	A-431	0.002	>1	0.006
113	f2	A-549	>1	>1	>1
114	f2	BT-474	>1	>1	>1
115	f2	HT-29	>1	>1	>1
116	f2	MDA-MB-231	>1	>1	>1
117	f2	NCI-H460	>1	>1	>1
118	f2	PC-3	>1	>1	>1
119	f2	T-47D	>1	>1	>1
120	f2	T24	>1	>1	>1
121	f3	22Rv-1	0.919	0.919	0.677
122	f3	A-431	0.063	>1	0.031
123	f3	A-549	>1	>1	>1
124	f3	BT-474	>1	>1	>1
125	f3	HT-29	0.263	>1	>1
126	f3	MDA-MB-231	>1	>1	>1
127	f3	NCI-H460	>1	>1	>1
128	f3	PC-3	0.301	>1	>1
129	f3	T-47D	>1	>1	>1
130	f3	T24	>1	>1	>1
131	f4	22Rv-1	>1	>1	>1
132	f4	A-431	0.618	0.618	0.189
133	f4	A-549	>1	>1	>1
134	f4	BT-474	>1	>1	>1
135	f4	HT-29	>1	>1	>1

[233] Table 3b: Cell Proliferation Screen Results for Mushroom Compositions

Key	Agent	Cell.Line	EC50 (Fold)	IC50 (Fold)	GI50 (Fold)
136	f4	MDA-MB-231	0.128	>1	>1
137	f4	NCI-H460	>1	>1	>1
138	f4	PC-3	>1	>1	>1
139	f4	T-47D	>1	>1	>1
140	f4	T24	>1	>1	>1
141	f5	22Rv-1	>1	>1	>1
142	f5	A-431	0.051	0.189	0.013
143	f5	A-549	>1	>1	>1
144	f5	BT-474	>1	>1	>1
145	f5	HT-29	0.325	>1	>1
146	f5	MDA-MB-231	>1	>1	>1
147	f5	NCI-H460	0.027	>1	>1
148	f5	PC-3	>1	>1	>1
149	f5	T-47D	0.028	>1	>1
150	f5	T24	0.308	>1	0.318
151	f6	22Rv-1	>1	>1	>1
152	f6	A-431	0.525	>1	>1
153	f6	A-549	0.185	>1	>1
154	f6	BT-474	>1	>1	>1
155	f6	HT-29	>1	>1	>1
156	f6	MDA-MB-231	>1	>1	>1
157	f6	NCI-H460	>1	>1	>1
158	f6	PC-3	>1	>1	>1
159	f6	T-47D	>1	>1	>1
160	f6	T24	>1	>1	>1
161	f7	22Rv-1	>1	>1	>1
162	f7	A-431	0.004	0.062	0.002
163	f7	A-549	0.049	0.196	0.070
164	f7	BT-474	0.672	>1	0.507
165	f7	HT-29	0.396	0.396	0.354
166	f7	MDA-MB-231	0.368	0.440	0.361
167	f7	NCI-H460	0.414	0.428	0.419
168	f7	PC-3	0.610	0.610	0.163
169	f7	T-47D	>1	>1	0.517
170	f7	T24	>1	>1	>1

[234]   Table 3c: Cell Proliferation Screen Results for Mushroom Compositions

Key	Agent	Cell.Line	EC50 (Fold)	IC50 (Fold)	GI50 (Fold)
171	f8	22Rv-1	0.470	0.470	0.444
172	f8	A-431	0.599	0.599	0.429
173	f8	A-549	0.829	0.829	0.809
174	f8	BT-474	0.539	0.539	0.379
175	f8	HT-29	0.166	0.171	0.158
176	f8	MDA-MB-231	0.340	0.340	0.259
177	f8	NCI-H460	0.483	0.483	0.463
178	f8	PC-3	0.562	0.562	0.405
179	f8	T-47D	0.954	0.954	0.469
180	f8	T24	0.932	0.932	0.821
181	f9	22Rv-1	>1	>1	>1
182	f9	A-431	>1	>1	0.331
183	f9	A-549	>1	>1	>1
184	f9	BT-474	>1	>1	>1
185	f9	HT-29	>1	>1	>1
186	f9	MDA-MB-231	0.341	>1	>1
187	f9	NCI-H460	0.011	>1	>1
188	f9	PC-3	>1	>1	>1
189	f9	T-47D	>1	>1	>1
190	f9	T24	0.004	>1	>1
191	m1	22Rv-1	0.032	0.032	0.025
192	m1	A-431	0.050	0.050	0.012
193	m1	A-549	0.181	0.181	0.155
194	m1	BT-474	0.032	0.036	0.025
195	m1	HT-29	0.079	0.080	0.073
196	m1	MDA-MB-231	0.057	0.058	0.048
197	m1	NCI-H460	0.037	0.037	0.032
198	m1	PC-3	0.059	0.059	0.037
199	m1	T-47D	0.055	0.055	0.029
200	m1	T24	0.160	0.160	0.124

[235]   Table 3d: Cell Proliferation Screen Results for Mushroom Compositions

Key	Agent	Cell.Line	EC50 (Fold)	IC50 (Fold)	GI50 (Fold)
201	m2	22Rv-1	0.031	0.031	0.025
202	m2	A-431	0.038	0.038	0.021
203	m2	A-549	0.066	0.071	0.056
204	m2	BT-474	0.024	0.032	0.015
205	m2	HT-29	0.080	0.083	0.075
206	m2	MDA-MB-231	0.044	0.045	0.038
207	m2	NCI-H460	0.042	0.042	0.038
208	m2	PC-3	0.058	0.059	0.038
209	m2	T-47D	0.037	0.037	0.022
210	m2	T24	0.179	0.179	0.070
211	m3	22Rv-1	0.050	0.050	0.049
212	m3	A-431	0.089	0.089	0.021
213	m3	A-549	0.091	0.094	0.092
214	m3	BT-474	0.040	0.053	0.028
215	m3	HT-29	0.101	0.111	0.101
216	m3	MDA-MB-231	0.087	0.087	0.069
217	m3	NCI-H460	0.046	0.046	0.042
218	m3	PC-3	0.072	0.083	0.055
219	m3	T-47D	0.092	0.092	0.050
220	m3	T24	0.195	0.195	0.057
221	m4	22Rv-1	0.035	0.035	0.031
222	m4	A-431	0.020	0.020	0.006
223	m4	A-549	0.050	0.050	0.036
224	m4	BT-474	0.023	0.027	0.018
225	m4	HT-29	0.049	0.050	0.046
226	m4	MDA-MB-231	0.047	0.048	0.041
227	m4	NCI-H460	0.049	0.049	0.047
228	m4	PC-3	0.050	0.050	0.037
229	m4	T-47D	0.027	0.027	0.017
230	m4	T24	0.099	0.099	0.090

[236]   Table 3e: Cell Proliferation Screen Results for Mushroom Compositions

Key	Agent	Cell Line	EC50 (Fold)	IC50 (Fold)	GI50 (Fold)
231	m5	22Rv-1	0.018	0.018	0.013
232	m5	A-431	0.021	0.021	0.005
233	m5	A-549	0.059	0.059	0.047
234	m5	BT-474	0.015	0.019	0.011
235	m5	HT-29	0.029	0.029	0.027
236	m5	MDA-MB-231	0.031	0.031	0.025
237	m5	NCI-H460	0.043	0.043	0.041
238	m5	PC-3	0.047	0.047	0.036
239	m5	T-47D	0.026	0.026	0.017
240	m5	T24	0.096	0.097	0.059
241	m6	22Rv-1	0.014	0.014	0.012
242	m6	A-431	0.031	0.032	0.028
243	m6	A-549	0.069	0.072	0.061
244	m6	BT-474	0.033	0.038	0.020
245	m6	HT-29	0.031	0.031	0.031
246	m6	MDA-MB-231	0.019	0.019	0.019
247	m6	NCI-H460	0.052	0.052	0.047
248	m6	PC-3	0.018	0.018	0.012
249	m6	T-47D	0.028	0.029	0.028
250	m6	T24	0.039	0.039	0.029
251	Staurosporine	22Rv-1	0.019	0.020	0.013
252	Staurosporine	A-431	0.001	0.001	0.000
253	Staurosporine	A-549	0.017	0.020	0.011
254	Staurosporine	BT-474	0.044	0.044	0.003
255	Staurosporine	HT-29	0.009	0.009	0.007
256	Staurosporine	MDA-MB-231	0.001	0.001	0.001
257	Staurosporine	NCI-H460	0.020	0.021	0.017
258	Staurosporine	PC-3	0.002	0.004	0.001
259	Staurosporine	T-47D	0.101	0.101	0.038
260	Staurosporine	T24	0.013	0.013	0.001

[237]   Table 4: Cell Proliferation Screen Results for Mushroom Compositions

	Lion's Mane	Cordyceps	Shiitake	Turkey Tail	Reishi	Maitake	Chaga	Reishi Terpenes	Cubensis
Skin	±	±	±	±	±	−	±	+	±
Breast HER2+	−	−	±	−	−	−	±	+	−
Breast ER+ /PR+	−	±	−	±	−	±	±	+	−
Breast Trip Neg.	−	±	±	−	−	−	+	+	−
Colon	−	−	−	−	−	−	+	+	−
Prostate 22rv1	±	±	±	±	−	−	±	+	+
Prostate PC3	−	−	−	−	−	±	±	+	−
Lung Epithel	−	−	−	−	±	−	±	+	−
Lung Large	−	−	−	−	−	−	+	+	−
Bladder	−	−	−	−	−	−	−	+	−

[238]

[239]   <u>Example 2: ADCC Screen</u>

[240]   This study tested the efficacy of nine mushroom compositions against one cancer cell line (see Tables 5 and 6 below) over six concentrations in an antibody- dependent cellular cytotoxicity (ADCC) screening panel (2D-format).

[241]   <u>Experimental Materials and Methods</u>

[242]   ADCC Assay Protocol:

[243]   HER2-expressing BT-474 cell line cells (target cells) were labeled with CellBrite™ Green dye reagent (Biotium). Cell staining was performed according to the manufacturer's instructions. The DMSO stock solutions were diluted in pre-warmed (37°C) phosphate-buffered saline (PBS). The cells were gently re suspended in the staining solutions, and incubated at 37C for about 15 minutes (while protected from light). Cells were washed before the assay start.

[244]   Aliquots of each sample were vortexed and centrifuged at 3,000 rpm (800 g) for five minutes at room temperature. Supernatants (500-fold stocks) were stored under sterile conditions at 4C.

[245]   On the day of the assay, a 6-point dose-response of each 500-fold stock was prepared (Table 5). Samples were serially diluted (half-log) and added to the assay plates, containing fluorescence-labeled BT-474 cells (target cells) with or without 20 ng/ mL trastuzumab or isotype IgG control in RPMI 1640 media for 1 hour.

[246]   PBMC cells (effector cells) were added to the assay plates at a 1:9 ratio, and the assay plates were then incubated overnight at 37C with 5% CO_2.

[247]   After the incubation, dead dye reagent (Biotium) was added to the assay plates according to the manufacturer's instructions. After two hours, cells were fixed with 2% formalin for 10 minutes, washed with PBS buffer, and read using a Lionheart Automated Microscope.

[248]   Green-labeled BT-474 cells co-localized with red-dye were identified. The percentage of ADCC was calculated as the population of specific dead-BT-474 cells minus the background (percent of dead BT-474 /PBMC).

[249]   All tests were performed in triplicate, and the results were expressed as% ADCC (mean± standard deviation (STDEV)).

[250]   <u>Data Analysis</u>

[251]   ADCC endpoint data were analyzed as Percent of ADCC (% ADCC) using the following formula:

$$\% \; ADCC = \left(\frac{relative \; dead \; BT474 \; cell \; count \; (agent \; wells)}{relative \; total \; BT474 \; cell \; count \; (agent \; wells)} \times 100\% \right) - \left(\frac{relative \; dead \; BT474 \; cell \; count \; (vehicle \; control \; wells)}{relative \; total \; BT474 \; cell \; count \; (vehicle \; control \; wells)} \times 100\% \right)$$

[252]   The% ADCC was normalized as the fraction of dead BT-474 cells with agent, minus background (BT474 with PBMC and vehicle control). The output of each well was expressed as fold-increase over background, normalized to the relative cell count in each well.

[253]   Table 5: Testing Details

Compound ID	µL stock/vial	Vehicle	Stock Conc.	Unit	Top Conc. in Assay Plate	Unit	Bottom Conc. in Assay Plate	Unit	Serial Dilution
f1	5,000	DMSO	500	fold	1	fold	3E-03	fold	half-log
f2	5,000	DMSO	500	fold	1	fold	3E-03	fold	half-log
f3	5,000	DMSO	500	fold	1	fold	3E-03	fold	half-log
f4	5,000	DMSO	500	fold	1	fold	3E-03	fold	half-log
f5	5,000	DMSO	500	fold	1	fold	3E-03	fold	half-log
f6	5,000	DMSO	500	fold	1	fold	3E-03	fold	half-log
f7	5,000	DMSO	500	fold	1	fold	3E-03	fold	half-log
f8	5,000	DMSO	500	fold	1	fold	3E-03	fold	half-log
f9	5,000	DMSO	500	fold	1	fold	3E-03	fold	half-log

[254]   Table 6: Human Cell Line

Cell Line	Origen
BT-474	Breast

[255]   Results:

[256]   ADCC results for mushroom compositions with and without added 20 ng/ml trastuzamab against HER2+ human breast cancer cells are shown in Table 7 (a-e).

[257]   Table 7a ADCC Results

BT-474_ADCC   f1
% ADCC

fold	R1	R2	R3	MEAN	STDEV
0.003	4.7	4.9	7.8	5.8	1.6
0.010	1.2	7.2	6.2	4.9	3.2
0.032	2.9	2.5	4.3	3.2	1.0
0.100	3.4	1.0	4.7	3.0	1.9
0.316	3.5	0.2	3.4	2.4	1.9
1.000	-1.2	-5.8	-5.8	-4.3	2.6
0.000	-1.7	-0.6	2.3	0.0	2.1
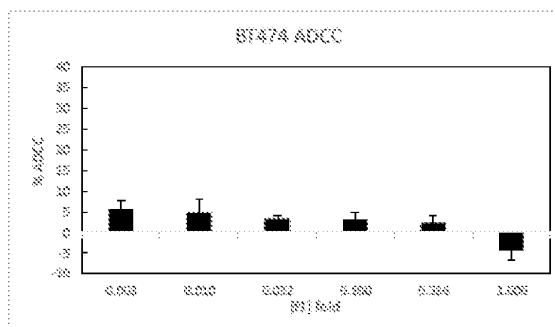
BT-474_ADCC   f1 + 20 ng/mL trastuzumab
% ADCC

fold	R1	R2	R3	MEAN	STDEV
0.003	11.1	12.4	13.6	12.4	1.2
0.010	15.9	15.3	20.6	17.3	2.9
0.032	15.2	17.1	18.2	16.8	1.5
0.100	14.8	16.0	16.0	15.6	0.7
0.316	17.6	16.3	18.5	17.5	1.1
1.000	15.2	19.7	18.7	17.9	2.4
0.000	19.6	22.3	19.0	20.3	1.8
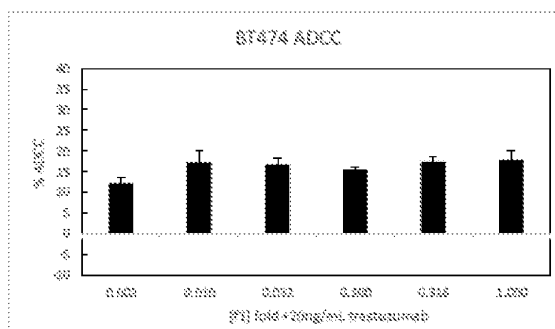
BT-474_ADCC   f2
% ADCC

fold	R1	R2	R3	MEAN	STDEV
0.003	-0.2	-0.6	1.3	0.2	1.0
0.010	-1.8	4.1	3.2	1.8	3.2
0.032	1.6	1.8	4.8	2.7	1.8
0.100	1.8	5.0	1.6	2.8	1.9
0.316	-1.3	0.2	2.3	0.4	1.8
1.000	-6.3	-5.9	-4.8	-5.7	0.7
0.000	0.4	-2.8	1.6	0.0	1.9
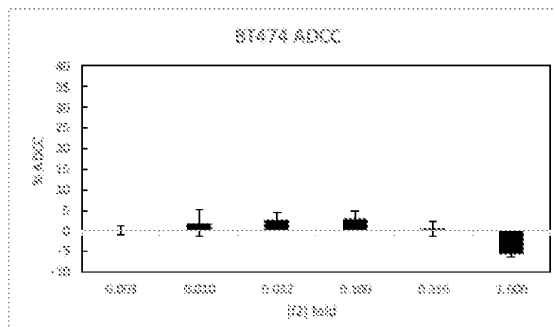
BT-474_ADCC   f2 + 20 ng/mL trastuzumab
% ADCC

fold	R1	R2	R3	MEAN	STDEV
0.003	15.4	20.9	20.7	19.0	3.1
0.010	15.3	13.6	19.0	16.0	2.7
0.032	17.1	13.1	14.3	14.8	2.1
0.100	19.8	18.8	24.6	21.1	3.1
0.316	17.9	17.6	22.4	19.3	2.7
1.000	15.2	12.4	18.4	15.3	3.0
0.000	17.9	17.7	17.7	17.8	0.1
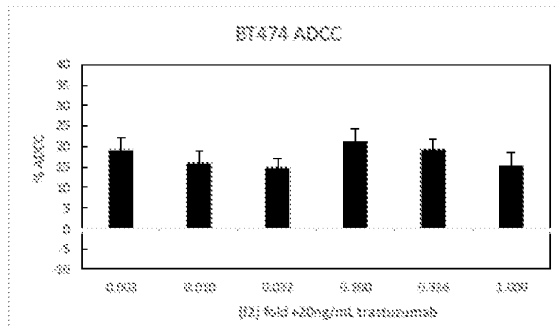
[258]

[259]   Table 7b ADCC Results

BT-474_ADCC   f3
% ADCC

fold	R1	R2	R3	MEAN	STDEV
0.003	-2.6	-2.7	-0.8	-2.0	1.2
0.010	-0.1	-1.7	0.8	-0.6	1.0
0.032	-1.9	-0.6	-1.3	-1.3	0.6
0.100	0.1	1.2	0.4	0.6	0.5
0.316	2.1	2.4	4.3	2.9	1.2
1.000	0.8	-1.6	-3.6	-1.5	2.2
0.000	-3.6	0.8	2.2	0.0	2.7
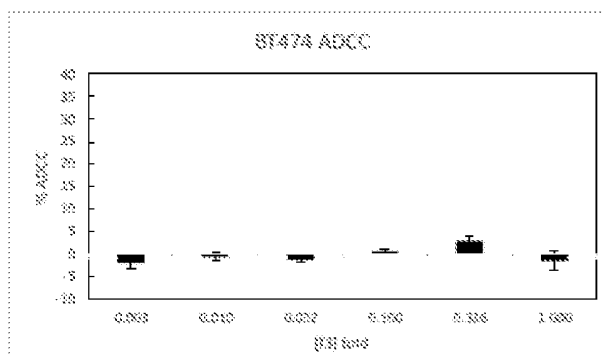
BT-474_ADCC   f3 + 20 ng/mL trastuzumab
% ADCC

fold	R1	R2	R3	MEAN	STDEV
0.003	13.6	17.4	18.0	16.3	2.4
0.010	19.3	19.5	18.0	18.9	0.8
0.032	15.3	17.2	20.3	17.6	2.5
0.100	20.2	18.4	22.0	20.2	1.8
0.316	19.0	17.1	21.4	19.1	2.2
1.000	20.6	15.8	21.5	19.3	3.1
0.000	15.5	18.4	16.4	16.8	1.5
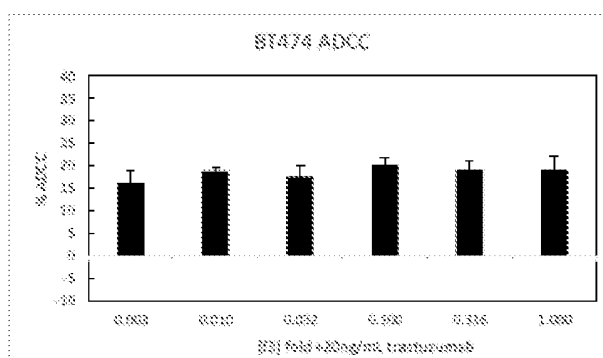
BT-474_ADCC   f4
% ADCC

fold	R1	R2	R3	MEAN	STDEV
0.003	-2.5	-4.4	-3.1	-3.3	1.0
0.010	1.3	2.5	3.9	2.6	1.3
0.032	-1.0	1.3	-0.8	-0.1	1.2
0.100	2.9	3.5	2.1	2.8	0.7
0.316	5.0	2.1	2.1	3.0	1.7
1.000	3.6	5.8	7.8	5.8	2.1
0.000	0.2	-1.8	1.6	0.0	1.7
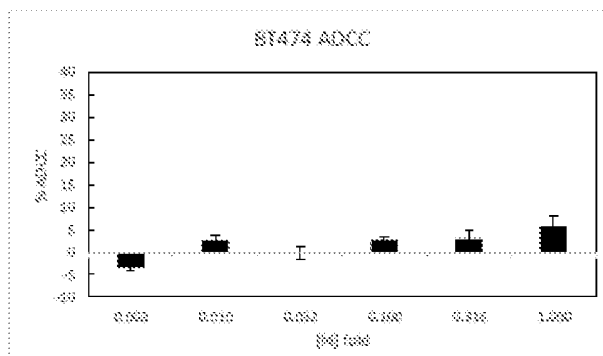
BT-474_ADCC   f4 + 20 ng/mL trastuzumab
% ADCC

fold	R1	R2	R3	MEAN	STDEV
0.003	15.9	14.8	19.3	16.7	2.4
0.010	15.5	18.4	20.3	17.4	2.5
0.032	16.7	16.8	16.3	16.6	0.3
0.100	19.0	20.6	20.0	19.9	0.8
0.316	15.1	19.0	23.5	19.2	4.2
1.000	22.4	19.8	19.8	20.7	1.5
0.000	18.5	16.6	16.0	17.0	1.3
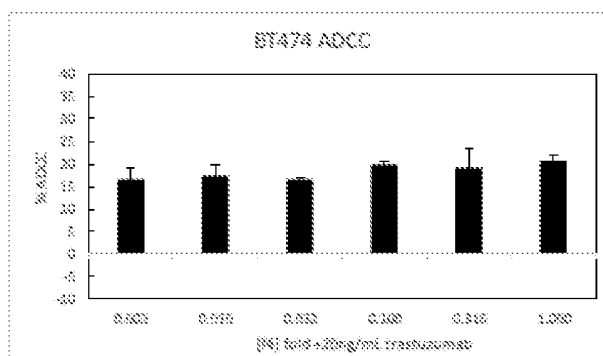
[260]　Table 7c ADCC Results

BT-474_ADCC　　　　　f5
% ADCC

fold	R1	R2	R3	MEAN	STDEV
0.003	2.3	3.1	2.1	2.4	0.5
0.010	-0.3	6.9	6.9	2.5	3.9
0.032	-2.5	2.9	3.8	1.3	3.3
0.100	3.3	3.3	0.6	2.4	1.5
0.316	4.9	10.3	8.4	7.6	3.2
1.000	-6.8	-8.1	-6.2	-7.0	1.0
0.000	-0.9	0.0	0.9	0.0	0.9
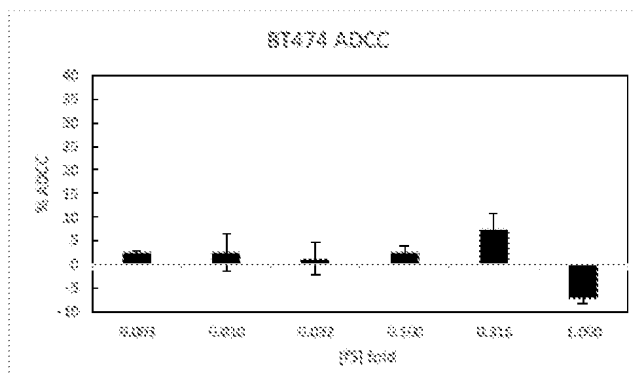
BT-474_ADCC　　　f5 + 20 ng/mL trastuzumab
% ADCC

fold	R1	R2	R3	MEAN	STDEV
0.003	19.8	22.6	21.4	21.4	1.5
0.010	16.6	17.1	19.6	17.7	1.6
0.030	17.5	22.9	18.0	19.5	2.9
0.010	20.9	27.6	19.8	22.8	4.2
0.300	27.6	26.2	28.6	27.5	1.2
1.000	6.3	6.4	5.9	6.2	0.2
0.000	19.5	20.4	20.7	20.2	0.7
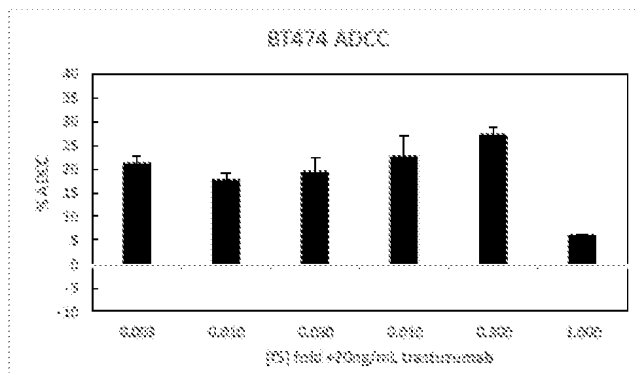
BT-474_ADCC　　　　　f6
% ADCC

fold	R1	R2	R3	MEAN	STDEV
0.003	-2.7	-0.7	0.5	-1.0	1.6
0.010	-1.4	-1.8	2.1	-0.4	2.1
0.032	-4.5	-0.6	2.4	-0.9	3.4
0.100	-1.0	-2.0	-4.6	-2.6	1.8
0.316	-13.3	-16.3	-13.2	-14.3	1.8
1.000	-17.6	-19.5	-21.0	-19.4	1.7
0.000	-3.6	1.8	1.8	0.0	3.1
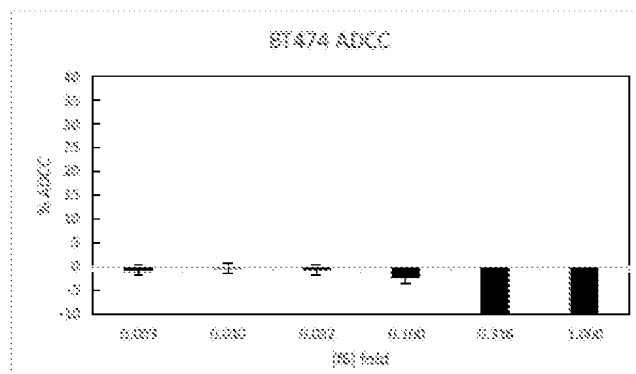
BT-474_ADCC　　　f6 + 20 ng/mL trastuzumab
% ADCC

fold	R1	R2	R3	MEAN	STDEV
0.003	17.6	17.6	15.3	16.8	1.3
0.010	19.5	18.6	14.8	17.6	2.6
0.032	17.5	18.9	19.4	18.6	1.0
0.100	15.6	18.6	17.4	17.2	1.5
0.316	2.3	2.6	4.8	3.0	0.9
1.000	-11.1	-12.6	-11.8	-11.5	0.8
0.000	18.7	19.5	19.1	19.1	0.4
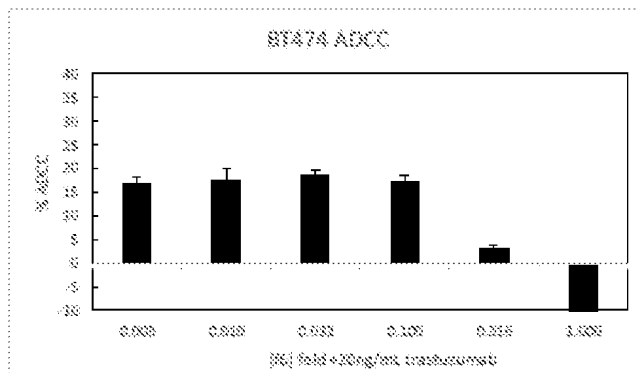
[261]   Table 7d ADCC Results

BT-474_ADCC   f7
                  % ADCC

fold	R1	R2	R3	MEAN	STDEV
0.003	8.6	7.6	12.8	9.7	2.8
0.010	16.0	11.7	15.7	14.5	2.4
0.032	11.9	12.8	10.0	11.6	1.5
0.100	2.7	-0.2	-1.2	0.5	2.0
0.316	-24.1	-24.0	-20.2	-22.8	2.2
1.000	-35.7	-38.8	-38.4	-37.6	1.7
0.000	-2.5	-2.6	5.1	0.0	4.4
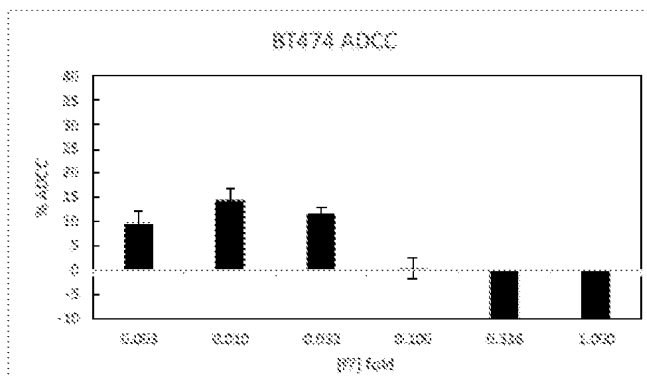
BT-474_ADCC   f7 + 28 ng/mL trastuzumab
                  % ADCC

fold	R1	R2	R3	MEAN	STDEV
0.003	26.4	29.6	27.1	27.6	1.6
0.010	33.1	32.1	30.7	32.0	1.2
0.032	32.9	32.5	35.5	33.7	1.6
0.100	23.0	27.7	25.7	25.4	2.4
0.316	-10.4	-3.5	-13.3	-9.1	5.0
1.000	-37.4	-37.2	-38.6	-37.7	0.8
0.000	19.7	29.8	19.5	23.0	5.9
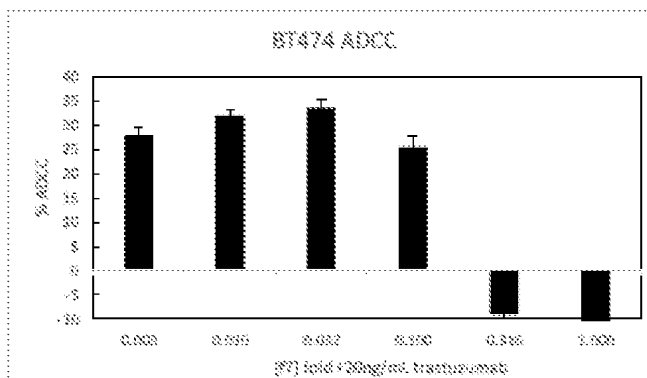
BT-474_ADCC   f8
                  % ADCC

fold	R1	R2	R3	MEAN	STDEV
0.003	-1.2	1.3	1.2	0.4	1.4
0.010	1.5	5.3	3.7	3.5	1.9
0.032	0.8	2.1	4.2	2.4	1.7
0.100	0.7	-4.0	-1.8	-1.7	2.3
0.316	-8.6	-6.1	-2.6	-6.1	3.5
1.000	-2.1	0.3	-1.1	-1.0	1.2
0.000	-1.4	-2.2	3.6	0.0	3.2
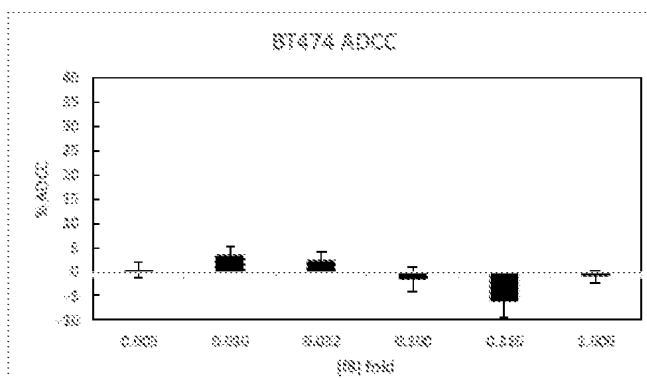
BT-474_ADCC   f8 + 28 ng/mL trastuzumab
                  % ADCC

fold	R1	R2	R3	MEAN	STDEV
0.003	14.5	16.7	13.2	14.8	1.8
0.010	19.6	15.8	13.3	16.2	3.2
0.032	16.6	19.5	15.8	17.3	2.0
0.100	15.0	17.7	17.4	16.7	1.5
0.316	9.6	17.1	15.3	14.0	3.9
1.000	3.1	1.2	-4.0	0.1	3.7
0.000	15.7	12.9	13.2	13.9	1.5
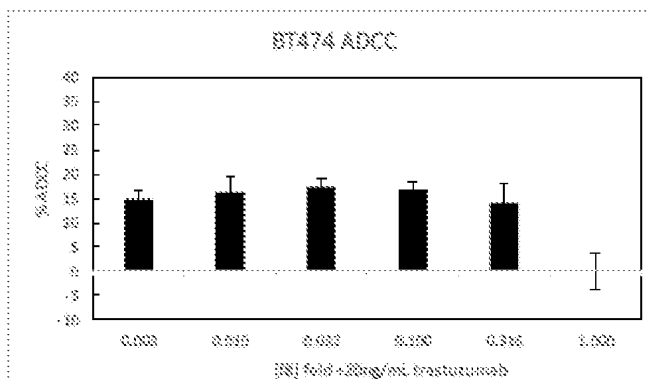
[262] Table 7e ADCC Results

BT-474_ADCC   f9   % ADCC

fold	R1	R2	R3	MEAN	STDEV
0.003	8.1	3.7	6.4	6.1	2.2
0.010	8.2	5.0	6.1	6.4	1.6
0.032	7.0	7.0	10.0	8.0	1.8
0.100	10.5	5.5	8.1	8.1	2.5
0.316	12.3	9.8	11.1	11.1	1.3
1.000	17.0	15.2	17.7	16.6	1.3
0.000	-0.3	-0.5	0.8	0.0	0.7
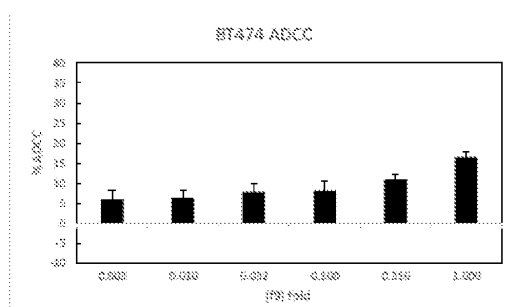
BT-474_ADCC   f9 + 20 ng/mL trastuzumab   % ADCC

fold	R1	R2	R3	MEAN	STDEV
0.003	21.8	22.9	21.9	22.2	0.6
0.010	24.1	22.0	23.6	23.2	1.1
0.032	22.5	25.1	27.5	25.0	2.5
0.100	27.8	25.8	23.6	25.7	2.1
0.316	27.9	30.3	31.2	29.8	1.7
1.000	28.9	30.6	35.2	31.6	3.3
0.000	17.3	21.0	19.5	19.3	1.8
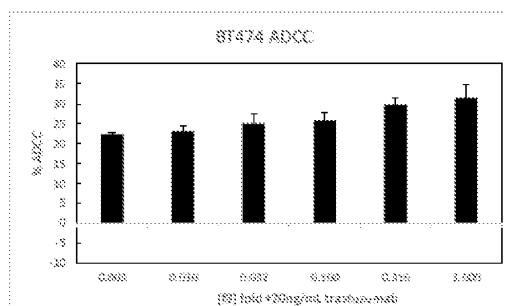
[263] <u>Example 3: ADCP Screen</u>

[264] This study tested the efficacy of nine mushroom compositions against one HER2+ breast cancer cell line (see Tables 5 and 6) over six concentrations in an antibody-dependent cellular phagocytosis (ADCP) screening panel (2D-format).

[265] <u>Experimental Materials and Methods</u> ADCP Assay Protocol:

[266] . Negatively selected human monocytes were differentiated into primary monocyte-derived macrophages (effector cells) after six days of incubation in 10% FBS RPMI media plus 50 ng/mL hM-CSF at 37C with 5% CO_2.

[267] On the day of the assay, HER2-expressing BT-474 cell line cells (target cells) were labeled with pHrodo™ Red dye (ThermoFisher Scientific). Primary (ThermoFisher Scientific). Cell staining was performed according to the manufacturer's instructions.

[268] Aliquots of each sample were vortexed and centrifuged at 3,000 rpm (800 g) for five minutes at room temperature. Supernatants (500-fold stocks) were stored under sterile conditions at 4C.

[269] On the day of the assay, a 6-point dose-response of each 500-fold stock was prepared. Samples were serially diluted (half-log) and added to the assay plates (Table 7), containing fluorescence-labeled BT-474 cells (target cells) with or without 50 ng/ mL trastuzumab or

isotype lgG control in RPMI 1640 media for 1 hour. Labeled primary monocyte-derived macrophages (effector cells) were added to the assay plates at a ratio of 1:2.

[270]   Assay plates were incubated for two hours at 37C with 5% CO_2. The plates were subsequently fixed with 2% formalin for 10 minutes, washed with PBS buffer, and read using a Lionheart™ FX Automated Microscope.

[271]   Phagocytized BT-474 cells (red) were co-localized with macrophages (blue). The phagocytosis index percentage was calculated as the specific population of phagocytized BT-474 cells, minus the background(% phagocytized control). All tests were performed in triplicate, and the results were expressed as% Phagocytosis Index (mean± standard deviation (STDEV)).

[272]   <u>Data Analysis</u>

[273]   ADCP endpoint was analyzed as Percent Phagocytosis Index(% Phagocytosis Index) using the following formula:

$$\% \text{ Phagocytosis Index} = \left(\frac{\text{relative phagocytized macrophage count (agent wells)}}{\text{relative total macrophage count (agent wells)}} \times 100\% \right) - \left(\frac{\text{relative phagocytized macrophage count (vehicle control wells)}}{\text{relative total macrophage cell count (vehicle control wells)}} \times 100\% \right)$$

[274]

[275]   The % Phagocytosis Index was normalized as the fraction of phagocytic macrophages, minus background (phagocytic macrophages with vehicle control). The output of each well was expressed as fold-increase over background, normalized to the relative cell count in each well.

[276]   Results:

[277]   ADCP results for mushroom compositions with and without added 50 ng/ml trastuzumab against HER2+ human breast cancer cells are shown in Tables 8 (a- e).

[278]

[279] Table 8a: ADCP Results

BT-474_ADCP   f1
% Phagocytosis Index

fold	R1	R2	R3	MEAN	STDEV
0.003	-0.1	-2.3	1.8	-0.2	2.0
0.010	-0.2	-0.6	1.1	0.1	0.9
0.031	4.0	0.5	3.4	2.6	1.9
0.100	3.6	2.4	2.3	2.8	0.7
0.316	2.1	0.6	2.5	1.7	1.0
1.000	4.0	3.6	3.5	3.8	0.2
0.000	-1.5	0.2	3.3	0.7	2.4
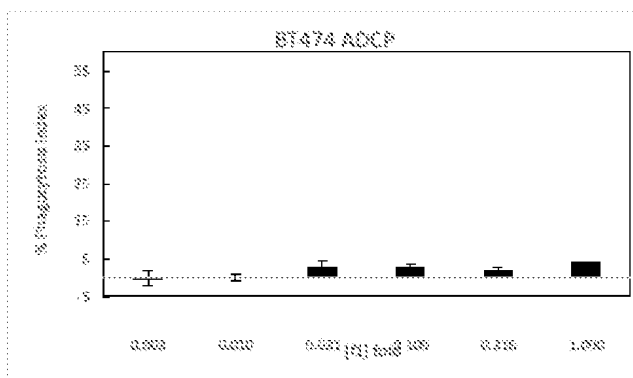
BT-474_ADCP   f1 + 50 ng/mL trastuzumab
% Phagocytosis Index

fold	R1	R2	R3	MEAN	STDEV
0.003	22.6	22.3	21.3	22.1	0.7
0.010	25.3	25.9	24.1	25.1	0.9
0.031	26.4	24.5	26.8	25.9	1.2
0.100	25.4	24.6	22.2	24.1	1.7
0.316	24.9	28.8	22.5	25.4	3.2
1.000	28.8	22.4	23.8	34.5	3.0
0.000	28.8	22.2	22.3	24.4	3.7
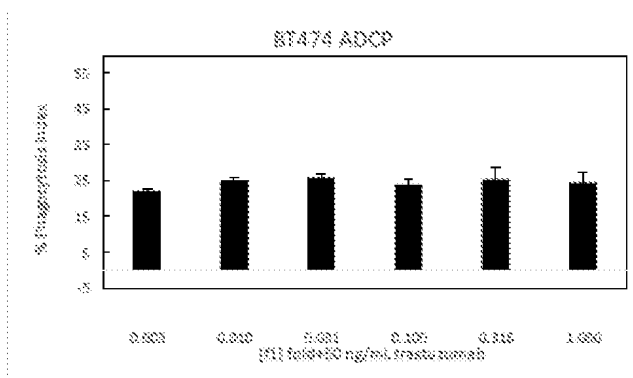
BT-474_ADCP   f2
% Phagocytosis Index

fold	R1	R2	R3	MEAN	STDEV
0.003	0.9	-0.2	0.4	0.4	0.6
0.010	2.8	0.9	3.7	2.4	1.4
0.031	1.7	2.5	3.8	2.3	0.6
0.100	1.5	1.3	4.7	2.5	1.9
0.316	2.3	2.6	1.7	2.2	0.6
1.000	6.3	5.3	8.7	6.8	1.8
0.000	-1.5	0.2	3.3	0.7	2.4
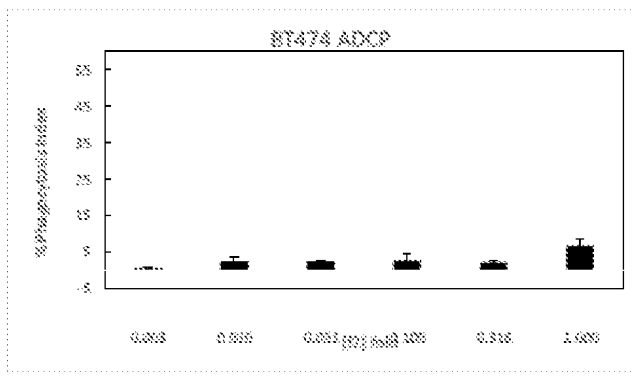
BT-474_ADCP   f2 + 50 ng/mL trastuzumab
% Phagocytosis Index

fold	R1	R2	R3	MEAN	STDEV
0.003	22.5	21.4	17.5	20.5	2.6
0.010	24.8	25.6	29.1	26.5	2.3
0.031	25.8	26.9	24.4	25.4	1.3
0.100	26.1	24.9	24.9	25.3	0.7
0.316	24.9	29.1	26.8	26.9	2.1
1.000	30.6	32.9	33.6	32.4	1.5
0.000	28.6	22.2	22.3	24.4	3.7
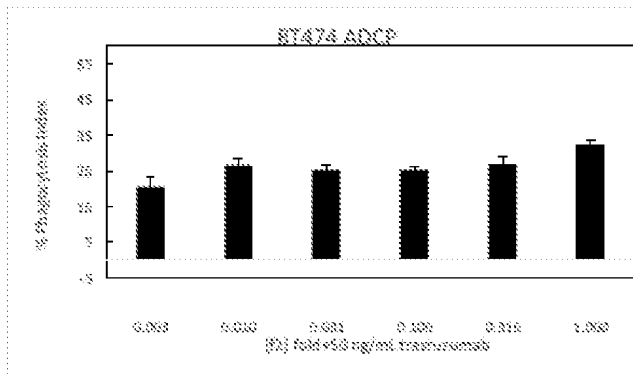
[280] Table 8b: ADCP Results

BT-474_ADCP   f3
% Phagocytosis Index

fold	R1	R2	R3	MEAN	STDEV
0.003	1.1	-0.1	1.5	0.8	0.8
0.010	2.0	2.8	5.4	3.4	1.6
0.031	2.1	3.2	3.4	2.9	0.7
0.100	2.8	4.9	3.3	3.7	1.1
0.316	0.7	-0.4	2.3	0.8	1.4
1.000	6.1	3.6	5.7	5.1	1.3
0.000	-0.9	-2.0	0.0	-1.0	1.0
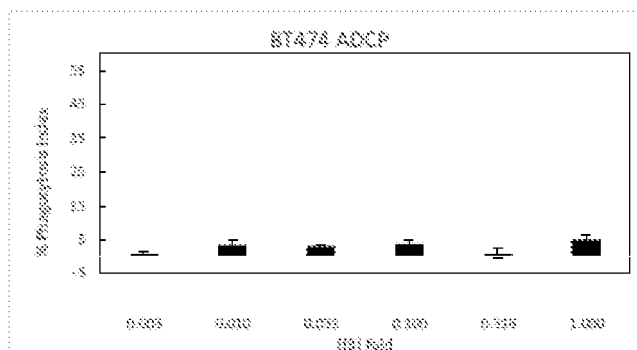
BT-474_ADCP   f3 + 50 ng/mL trastuzumab
% Phagocytosis Index

fold	R1	R2	R3	MEAN	STDEV
0.003	24.0	25.3	26.6	25.3	1.3
0.010	24.5	23.7	26.7	25.0	1.6
0.031	21.7	28.6	28.1	26.1	3.8
0.100	26.3	29.5	24.7	26.8	2.4
0.316	25.2	25.7	24.8	25.2	0.4
1.000	19.3	21.1	20.8	20.4	0.9
0.000	25.6	34.0	23.1	27.5	5.7
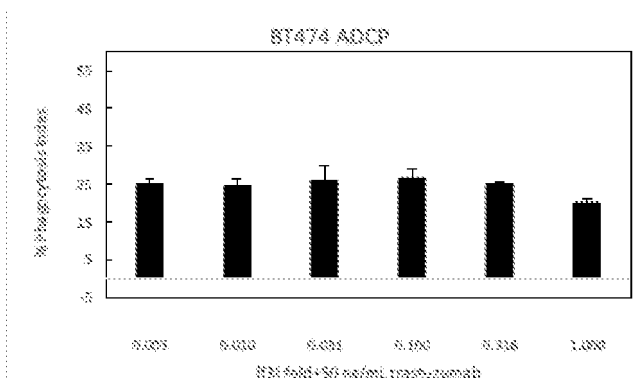
BT-474_ADCP   f4
% Phagocytosis Index

fold	R1	R2	R3	MEAN	STDEV
0.003	-2.3	-1.9	1.7	-0.8	2.2
0.010	0.8	2.3	3.1	2.1	1.2
0.031	0.7	-0.6	2.8	1.0	1.7
0.100	1.5	2.4	4.7	2.8	1.7
0.316	1.4	0.0	-0.5	0.3	1.0
1.000	9.4	4.2	9.8	7.8	3.1
0.000	-0.9	-2.0	0.0	-1.0	1.0
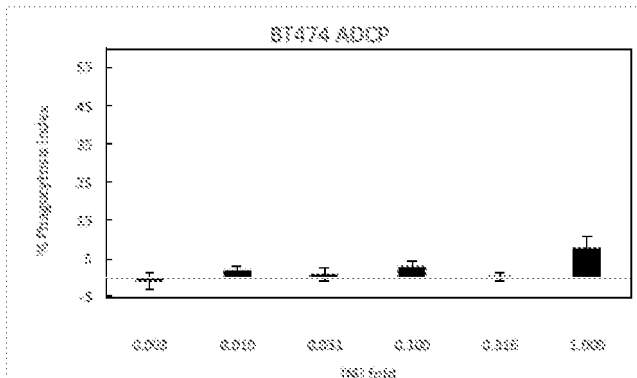
BT-474_ADCP   f4 + 50 ng/mL trastuzumab
% Phagocytosis Index

fold	R1	R2	R3	MEAN	STDEV
0.003	28.9	28.4	31.4	29.5	1.6
0.010	26.2	20.6	11.1	20.0	6.6
0.031	27.7	27.8	26.7	27.4	0.6
0.100	28.0	24.2	28.7	27.0	2.4
0.316	22.8	24.1	26.6	24.5	2.2
1.000	30.5	31.5	30.7	30.9	0.5
0.000	25.6	32.1	21.2	26.3	5.5
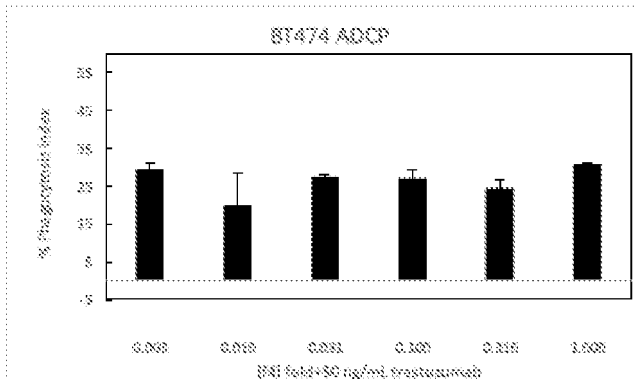
[281] Table 8c: ADCP Results

BT-474_ADCP   f5
% Phagocytosis Index

fold	R1	R2	R3	MEAN	STDEV
0.003	-1.4	3.9	3.2	1.9	2.9
0.010	0.4	2.1	1.2	1.2	0.9
0.031	2.9	2.2	4.2	3.1	1.0
0.100	6.0	7.6	5.1	6.0	1.0
0.316	7.6	8.6	7.9	7.9	0.9
1.000	9.8	16.6	7.7	11.4	4.7
0.000	-0.4	1.3	1.3	0.6	1.0
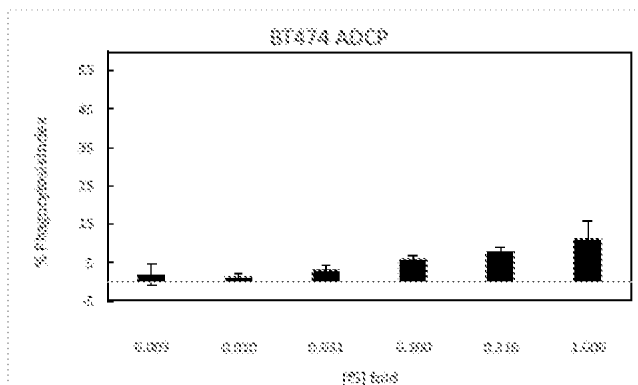
BT-474_ADCP   f5 + 50 ng/mL trastuzumab
% Phagocytosis Index

fold	R1	R2	R3	MEAN	STDEV
0.003	26.7	22.8	27.1	25.5	2.3
0.010	30.0	32.6	29.7	30.8	1.6
0.031	26.1	26.3	23.8	26.1	3.2
0.100	24.0	25.3	29.2	26.1	2.7
0.316	18.4	21.2	16.5	18.7	2.4
1.000	28.4	21.8	22.4	24.2	3.7
0.000	29.5	26.8	27.6	28.0	1.4
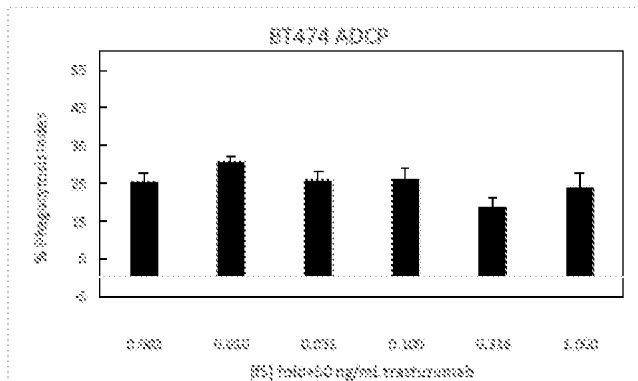
BT-474_ADCP   f6
% Phagocytosis Index

fold	R1	R2	R3	MEAN	STDEV
0.003	1.9	3.9	2.2	2.7	1.1
0.010	2.9	2.7	7.4	4.3	2.6
0.031	4.8	2.2	2.9	3.0	0.9
0.100	4.8	2.4	6.0	4.4	1.9
0.316	3.6	2.9	5.5	3.7	1.8
1.000	17.2	15.6	11.3	14.7	3.1
0.000	-0.4	1.4	1.3	0.7	1.0
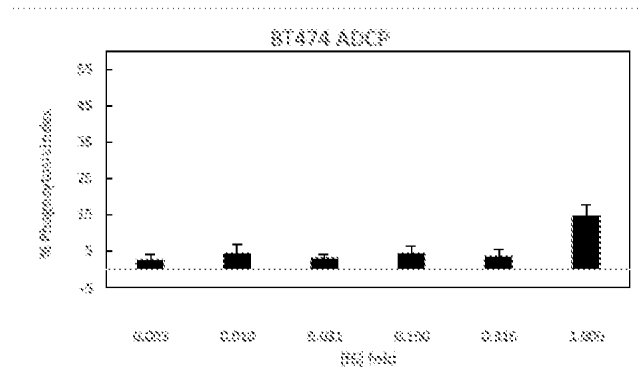
BT-474_ADCP   f6 + 50 ng/mL trastuzumab
% Phagocytosis Index

fold	R1	R2	R3	MEAN	STDEV
0.003	26.9	28.4	26.1	27.2	1.2
0.010	26.8	32.1	30.9	29.9	2.8
0.031	23.8	24.7	24.4	24.3	0.5
0.100	29.1	31.8	26.9	29.3	2.4
0.316	27.8	28.9	30.7	29.2	1.5
1.000	47.8	45.5	45.0	45.8	1.1
0.000	29.5	26.8	27.6	28.0	1.4

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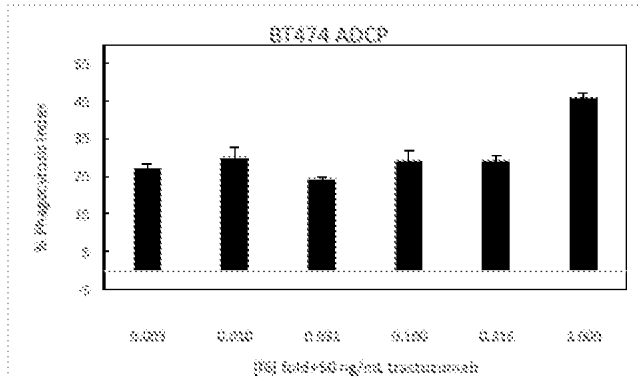
[282]   Table 8d: ADCP Results

BT-474_ADCP   f7
% Phagocytosis Index

fold	R1	R2	R3	MEAN	STDEV
0.003	-2.7	-2.6	0.6	-1.5	1.8
0.010	-3.1	-0.2	-0.3	-1.2	1.6
0.031	-3.0	-1.3	-2.9	-2.4	0.9
0.100	-5.2	-3.3	-2.3	-3.6	1.5
0.316	-2.7	-3.9	4.6	-0.7	4.6
1.000	3.8	9.9	7.4	7.0	3.1
0.000	-2.1	0.4	0.6	-0.4	1.5
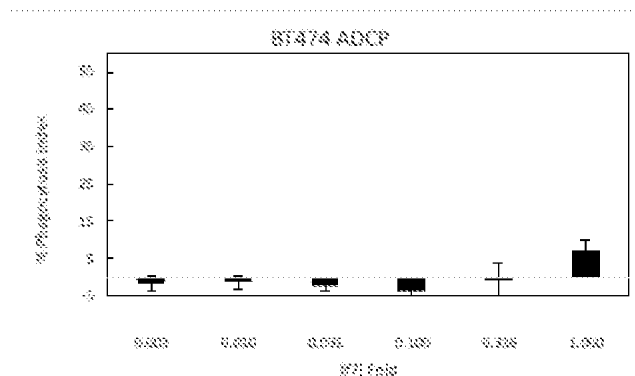
BT474 ADCP

BT-474_ADCP   f7 + 50 ng/mL trastuzumab
% Phagocytosis Index

fold	R1	R2	R3	MEAN	STDEV
0.003	35.0	35.6	32.7	34.4	1.5
0.010	21.7	19.9	24.0	21.9	2.1
0.031	3.0	1.9	0.6	1.8	1.2
0.100	-3.0	2.2	0.7	-0.1	2.7
0.316	-2.8	3.1	-0.8	-0.2	3.0
1.000	6.0	4.2	5.8	5.3	1.0
0.000	24.0	31.9	33.3	29.7	5.0
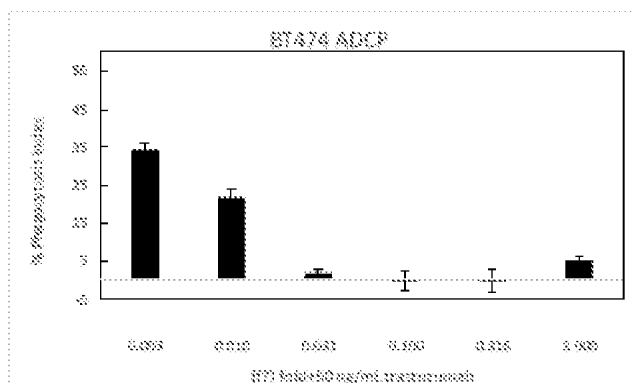
BT474 ADCP

BT-474_ADCP   f8
% Phagocytosis Index

fold	R1	R2	R3	MEAN	STDEV
0.003	1.0	0.1	1.4	0.8	0.7
0.010	0.1	3.3	-0.7	0.9	2.1
0.031	2.4	3.8	3.1	3.1	0.7
0.100	6.6	7.9	6.8	7.5	0.9
0.316	9.3	9.1	11.3	9.9	1.2
1.000	11.6	9.3	10.2	10.4	1.1
0.000	-2.1	0.4	0.6	-0.4	1.5
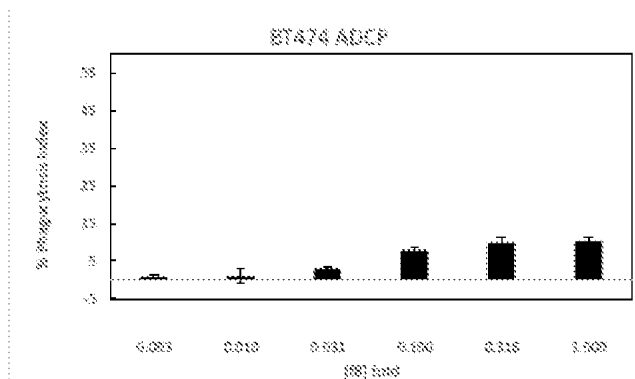
BT474 ADCP

BT-474_ADCP   f8 + 50 ng/mL trastuzumab
% Phagocytosis Index

fold	R1	R2	R3	MEAN	STDEV
0.003	33.1	34.0	37.0	34.7	2.0
0.010	34.3	34.3	37.2	35.3	1.6
0.031	33.7	37.2	41.7	37.5	4.0
0.100	42.8	47.7	48.5	46.4	3.1
0.316	41.8	39.4	44.5	41.9	2.5
1.000	15.7	14.7	16.9	15.8	1.1
0.000	24.0	31.9	33.3	29.7	5.0
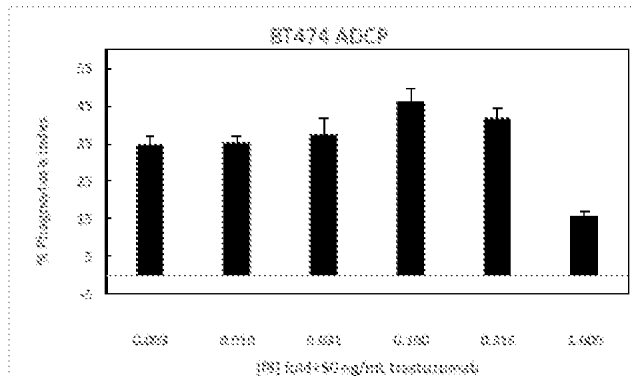
BT474 ADCP

[283]　Table 8e:　ADCP Results

BT-474_ADCP　　　　f9
% Phagocytosis Index

fold	R1	R2	R3	MEAN	STDEV
0.003	-3.3	-1.7	0.8	-1.4	2.1
0.010	1.1	2.1	-1.4	5.6	1.8
0.031	-2.3	0.0	1.1	-0.4	1.7
0.100	-0.8	-2.0	-0.9	-1.2	0.6
0.316	-2.2	-2.8	0.1	-1.6	1.5
1.000	4.9	1.8	3.5	3.4	1.6
0.000	0.5	-2.5	0.7	-0.4	1.8
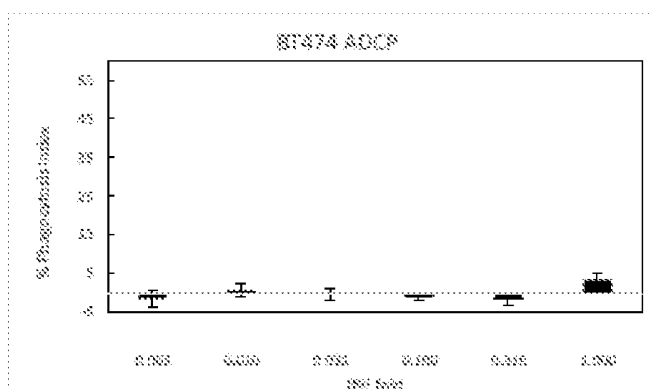
BT-474_ADCP　　　　f9 + 50 ng/mL trastuzumab
% Phagocytosis Index

fold	R1	R2	R3	MEAN	STDEV
0.003	27.5	31.1	30.4	29.6	1.9
0.010	28.1	28.4	32.5	29.7	2.5
0.031	23.3	28.7	29.8	26.9	3.4
0.100	27.0	25.1	26.5	26.2	1.0
0.316	19.9	25.8	23.5	23.1	2.9
1.000	29.8	33.6	33.4	32.3	2.2
0.000	32.7	31.1	19.3	27.7	7.3
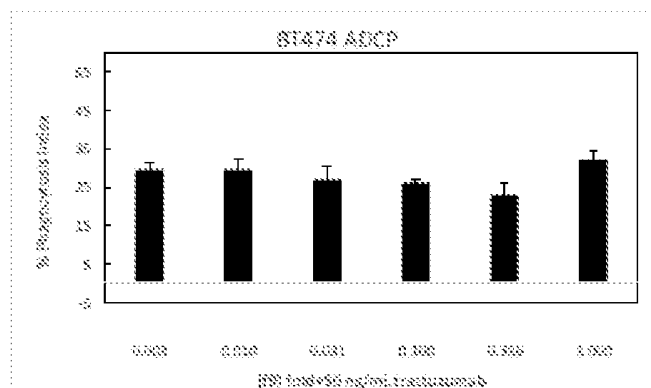
[284]　The scope of the invention is set forth in the claims appended hereto, subject, for example, to the limits of language. Although specific terms are employed to describe the invention, those terms are used in a generic and descriptive sense and not for purposes of limitation. Moreover, while certain presently preferred embodiments of the claimed invention have been described herein, those skilled in the art will appreciate that such embodiments are provided by way of example only. In view of the teachings provided herein, certain variations, modifications, and substitutions will occur to those skilled in the art. It is therefore to be understood that the invention may be practiced otherwise than as specifically described, and such ways of practicing the invention are either within the scope of the claims, or equivalent to that which is claimed, and do not depart from the scope and spirit of the invention as claimed.

WHAT IS CLAIMED IS:

1.   A method of treating a cancer in a human subject comprising the step of treating a human needing such treatment with a composition comprising a mushroom extract, fraction, isolate, or component thereof, in an amount effective to treat the cancer;

     wherein the composition comprises Lions Mane, Cordyceps, Shitake, Turkey Tail, Maitake, Chaga, Reishi, and Cubensis mushrooms and optionally, 1 or more cannabinoids.

2.   The method of claim 1 wherein the composition further comprises one or more terpenes, flavonoids, triterpenes, or other biologically-active phytochemical, in an amount that enhances the treatment.

6.   The method of claim 1 wherein the composition functions to induce apoptosis of cancer cells, inhibit the VEGF pathway, prevent angiogenesis of cancer cells, disrupt one or more aspect of cell growth of cancer cells, restore normal differentiation of cancer cells, restore normal cell cycle in cancer cells; or inhibit one of more of migration, adhesion, or invasion of cancer cells.

7.   The method of claim 6 wherein the composition is optimized for one or more said functions via an artificial intelligence algorithm.

8.   The method of claim 6 wherein said functions are detectable in the human subject.

9.   The method of claim 1 wherein the composition furhter comprises an extract, fraction, or isolate from a *Cannabis* strain that is Ringo's Gift, Harle Tsu, ACDC, Charlotte's Web, The Gift, or Pineberry, or Apollon Medical ("APM").

10.   The method of claim 1 wherein the composition is micro- or nano- emulsified.

11.   The method of claim 1 wherein the composition works in conjunction with a standard of care medical treatment, or a monoclonal antibody directed against a cancer antigen.

Atty. Docket. No. AIP-009PCT   PCT

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ABSTRACT OF THE DISCLOSURE

Compositions and methods are provided for the treatment of a variety of human cancers. The compositions generally comprise at least one mushroom extract, with or without an additional cannabinoid or extract of *Cannabis* spp., terpene, and/or at least one flavonoid. Methods for optimizing compositions using artificial intelligence algorithms are also provided.