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(54) **Title:** METHODS OF USING CROCETIN IN TREATING SOLID TUMORS

(57) **Abstract:** Disclosed herein is the novel use of crocetin. In particular, it relates to methods of treating solid tumors, such as glioblastoma, by administering to a mammal in need thereof a pharmaceutically effective amount of crocetin, prior to, during, or after a therapy that is radiotherapy, chemotherapy, immunotherapy or a combination thereof. It also relates to the use of crocetin in composition for treating solid tumors, such as glioblastoma.

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METHODS OF USING CROCETIN IN TREATING SOLID TUMORS

Technical Field

The present disclosure relates to crocetin that has therapeutic uses in treating solid tumors. For example, the present disclosure relates to methods of treating solid tumors, especially glioblastoma, by administering to a mammal in need thereof a pharmaceutically effective amount of crocetin, prior to, during, or after a therapy that is radiotherapy, chemotherapy, immunotherapy or a combination thereof. The present disclosure also relates to the use of crocetin in the composition for treating solid tumors, especially glioblastoma.

Background Art

Malignant cancers, such as hematological neoplasms and solid tumors, severely threaten human health. Treatments of cancers have traditionally been accomplished through one of, or a combination of, surgery, chemotherapy, radiotherapy, immunotherapy, and hormone therapy, among others. With the emergence of new anticancer drugs, especially the rise of immunotherapy, the efficacy of hematological neoplasms has recently been improved greatly, while radiotherapy and chemotherapy remain the major treatments after surgery in treatment of solid tumors.

However, radiotherapy and chemotherapy have harm or toxicity on normal tissues and organs, lead to decreased self-immunity, and can result in certain side effects, e.g., hair loss, nausea, vomit, loss of appetite, compromised immune system, myelosuppression, etc. These side effects may jeopardize the efficacy of chemotherapy and radiotherapy, and even discontinue chemotherapy and radiotherapy entirely. Unfortunately, attempts to develop chemotherapeutic drugs capable of targeting a specific molecule or cancer-associated signaling pathway with reduction in side effects have often failed to yield the expected improvements in patient prognoses. This is largely due to the ability of cancer cells to utilize a combination of many different cellular mechanisms to enhance viability. In many cases, cancer cells are able to circumvent apoptosis induced from targeted therapies by simply activating other survival pathways after the initial treatment. Meanwhile, due to the primary and acquired drug resistance of tumor cells and other factors, the therapeutic effect of chemotherapy is still unsatisfactory.

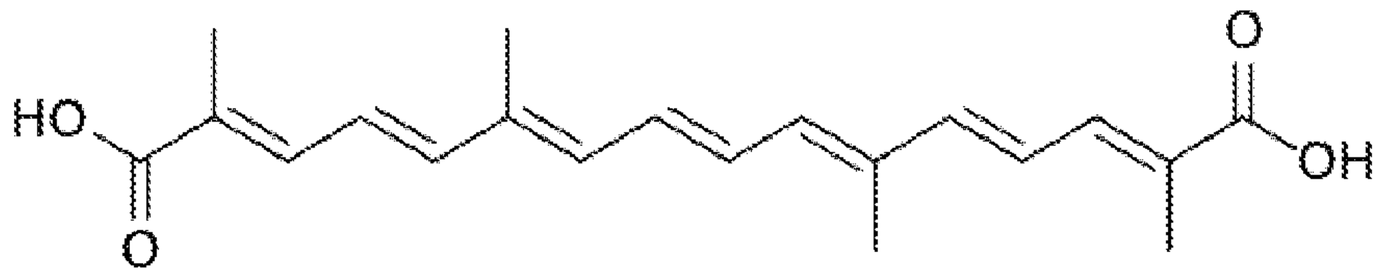
Glioblastoma is the most common primary intracranial tumor, caused by the canceration of brain and spinal glial cells, and characterized by high incidence, high recurrence rate, high mortality, and low cure rate. It does not significantly differentiate from normal brain tissues due to its infiltrative growth. And in most cases, it is not limited to one cerebral lobe, penetrating beyond brain tissues in fingerlike pattern and thus damaging brain tissues.

Glioblastoma accounts for approximately 12% to 15% of all brain tumors (which account for 85% to 90% of all primary central nervous system (CNS) tumors). The number of new cases for CNS tumors in the U.S. is approximately 18,800 (6.6 per 100,000 persons) per year, with around 12,800 (4.7 per 100,000 persons) deaths. This type of cancer accounts for approximately 1.3% of all cancers and 2.2% of all cancer-related deaths in the U.S. In China, there are more than 100,000 new cases every year. Worldwide, there are approximately 176,000 new cases of brain and other CNS tumors per year, with an estimated mortality of 128,000. In general, the incidence of primary brain tumors is higher in whites than in blacks, and the mortality is higher in males than in females. The peak incidence occurs between the ages of 45 and 70 years old, and it has the highest incidence in children. Glioblastoma multiforme (GBM) is the commonest and deadliest type, and remains an incurable disease; patients with GBM only have an average life expectancy of 12-15 months even when treated under the most aggressive therapeutic regimens available.

The current standard treatment for glioblastoma is maximum surgical resection, followed by radiotherapy together with temozolomide (TMZ) chemotherapy (concurrently with or after radiotherapy). Specifically, patients with glioblastoma receive fractionated focal irradiation in daily fractions of 2 Gy given 5 days per week for 6 weeks (total 60 Gy) plus continuous daily temozolomide 75 mg/m2/day, followed by six cycles of adjuvant temozolomide (150 to 200 mg/m^2/day for 5 days during each 28-day cycle) after one-month break. However, patients treated by the standard treatment still have low two-year survival rate. Thus, it is urgent to develop new effective therapeutic agents and/or effective radiotherapy/chemotherapy sensitizers for treating glioblastoma.

Natural products are a historically successful source of medicinally active compounds with fewer unwanted side effects, especially in regard to chemotherapeutics. In fact, 63% of cancer drugs used between 1981 and 2006 were natural products, were inspired by natural products, or were synthesized from a natural pharmacophore. Medicinally active compounds derived from natural materials have the potential to provide targeted cytotoxic and immune modulating responses while limiting the side effects associated with currently utilized cancer treatments. The use of natural products attempts to balance a robust ability to target numerous pathways simultaneously with a historical record of safe human consumption and benign side effects.

Crocetin ($C_{20}H_{24}O_4$, CAS: 27876-94-4) is a naturally-existed 8,8′-diapo-ψ,ψ’-carotenedioic acid with extensive physiological activities. As a brick-red crystal with a melting point of 285 °C, it is insoluble in water and most organic solvents. Crocetin has the following chemical structure:



As a minor compound in Saffron Yellow or Gardenia Yellow, colorants extracted from the flower of Saffron (*Crocus salivus*) or the fruits of Gardenia (*Gardenia jasminoides*) respectively, crocetin has been used for more than 1,000 years. Natural crocetin are scare in saffron and gardenia. Commercially, crocetin is mainly obtained through crocin hydrolyzation and glycosyl removal. Now it can also be obtained via chemical synthesis.

It has been proved that crocetin can effectively increase the oxygen diffusion and oxygen consumption in mammal tissues. Crocetin is sparingly soluble in water and common solvents, limiting its applications in food, beverage, drugs, and nutritional supplements. Trans-sodium crocetinate (TSC) was obtained by reacting crocetin with sodium hydroxide to increase its solubility in water (*see* U.S. Patent No. 6,060,511). It is found that TSC could treat a series of diseases, e.g., hypertension, atherosclerosis, cardiovascular disease, peripheral vascular disease, cerebral apoplexy, vascular thrombosis, cerebral ischemia, ischemic osteonecrosis, chronic eye disease, macular degeneration, and diabetic retinopathy.

Crocetin was reported to be not beneficial when treating more severe (55%-60%) blood losses. In contrast, TSC worked well in the more severe hemorrhagic shock model (see, Gainer, *Expert Opinion on Investigational Drugs*, 17:6, 917-924 (2008)). In a study regarding the effect of TSC on cerebral infarction, a U-shaped reduction in infarct volume was observed: the reductions in infarct volume achieved statistical significance at dosages ranging from 23 to 229 μg/kg, with a maximal protective effect was achieved at a dosage of 92 μg/kg. (See, Manabe et al, *J Neurosurg*. 113(4):802-809 (2010)). Comparatively, in studies on the efficacy enhancement of radiotherapy and chemo-radiation therapy for the survival rate of animal glioblastoma model by TSC, it was discovered that peak efficacy was achieved at a dose around 100 μg/kg (see, Sheehan et al, *J Neurosurg*. 108:972-978 (2008); *J Neurosurg*. 113:234-239 (2010)). Then a clinical trial was conducted to determine the effect of adding TSC to radiotherapy sessions (hereinafter referred to as "TSC + radiotherapy") for treating GBM in the U.S. The Phase-II clinical results were published in 2017, wherein Kaplan-Meier analysis showed that 36% of patients treated with TSC + radiotherapy were alive at 2 years, compared with historical 2-year survival rate ranging from 27% to 30% for the standard of care. Therefore, the results strongly suggested that adding TSC during radiotherapy is beneficial for the treatment of GBM. (See, Gainer et al., *J Neurosurg*. 126:460-466 (2017)).

There remain unmet medical needs to develop novel therapeutic drugs and/or sensitizers

for radiotherapy and chemotherapy in the treatment of solid tumors, especially glioblastoma. The present inventors have surprisingly found that, by administering crocetin prior to radiotherapy or chemotherapy or in combination thereof, it is possible to significantly enhance the efficacy of radiotherapy and chemotherapy, and thereby effectively treat solid tumors and prevent tumor recurrence and metastasis.

Summary of the Invention

In one aspect, the present disclosure provides a method of treating a solid tumor in a mammal in need thereof comprising administering to the mammal a pharmaceutically effective amount of crocetin, prior to, during, or after a therapy that is radiotherapy, chemotherapy, immunotherapy or a combination thereof.

In some embodiments, the mammal is human.

In some embodiments, the radiotherapy is selected from the form of electromagnetic waves, such as X-rays or gamma rays, or charged particles or neutral particles.

In some embodiments, the radiotherapy is administered by external beam, an interstitial implant, or a combination thereof.

In some embodiments, the radiotherapy is given at a dose of about 60-70 Gy over 4-7 weeks.

In some embodiments, the solid tumor disclosed herein is selected from glioblastoma, squamous cell carcinoma, skin cancer-related tumors, breast cancer, head and neck cancer, gynecological cancer, urinary and male genital cancer, bladder cancer, prostate cancer, bone cancer, endocrine adenocarcinoma, digestive tract cancer, breast cancer, major digestive/organ cancer, central nervous system cancer, and lung cancer.

In a further embodiment, the solid tumor is glioblastoma.

In some embodiments, the chemotherapy is selected from administering the drugs with temozolomide, cisplatin, methotrexate, or paclitaxel.

In a further embodiment, the chemotherapy is a therapy with temozolomide.

In one embodiment, the solid tumor is glioblastoma, and the chemotherapy is a therapy with temozolomide.

In some embodiments, crocetin is used as a sensitizer.

In some embodiments, the method further comprises administering with at least one anticancer entity.

The anticancer entity disclosed herein is, for example, selected from an additional sensitizer in a cancer therapy, a targeted therapeutic agent, and an immunotherapeutic agent.

In a further embodiment, the anticancer entity is selected from anticancer alkylating agents, anticancer antimetabolites, anticancer antibiotics, plant-derived anticancer agents,

anticancer platinum coordination compounds, anticancer camptothecin derivatives, anticancer tyrosine kinase inhibitors, monoclonal antibodies, and biological response modifiers.

In some embodiments, the pharmaceutically effective amount of crocetin is administered prior to a therapy that is radiotherapy, chemotherapy, immunotherapy or a combination thereof.

In another aspect, the present disclosure provides a composition comprising crocetin and at least one pharmaceutically acceptable carrier or auxiliary for use in treatment of a solid tumor.

In some embodiments, the composition is in the form of injection, tablet, capsule, pill, suppository, aerosol, oral liquid preparation, granule, powder, sustained release preparation, nano preparation, syrup, vina, tincture, lotion, film or a combination thereof.

In some embodiments, the composition is in the form of liposomal formulation.

In some embodiments, the composition is administered by orally, injection, implant, spray, inhalation or a combination thereof.

In another aspect, the present disclosure provides a method of sensitizing a mammal in need thereof to a therapy that is radiotherapy, chemotherapy, or a combination thereof, comprising administering to the mammal with a pharmaceutically effective amount of crocetin.

In some embodiments, the mammal is human.

Brief Description of the Drawings

Figure 1 shows the change curves of the HCT116 subcutaneous tumor relative volume (Vt/V0) of BALB/c nude mice with the number of observation days.

Figure 2 shows the change curves of the Hela subcutaneous tumor relative volume (Vt/V0) of BALB/c nude mice with the number of observation days.

Figure 3 shows the change curves of the HepG2 subcutaneous tumor relative volume (Vt/V0) of BALB/c nude mice with the number of observation days.

Figure 4 shows Kaplan-Meier survival curves of BALB/c nude mice bearing orthotopic C6 glioblastoma and the statistical variance among various groups of mice.

Figure 5 shows Kaplan-Meier survival curves of BALB/c nude mice bearing orthotopic C6 glioblastoma and the statistical variance among various groups of mice.

Figure 6 shows Kaplan-Meier survival curves of BALB/c nude mice bearing orthotopic C6 glioblastoma and the statistical variance among various groups of mice.

Figure 7 shows Kaplan-Meier survival curves of C57BL/6 mice bearing syngeneic orthotopic GL261 glioblastoma and the statistical variance among various groups of mice.

Figure 8 shows Kaplan-Meier survival curves of C57BL/6 mice bearing syngeneic

orthotopic GL261 glioblastoma and the statistical variance among various groups of mice.

Detailed Description

The following terms, unless otherwise indicated, shall be understood to have the following meanings:

As used herein, including the claims, the singular forms of words, such as "a," "an," and "the," include their corresponding plural references unless the context clearly dictates otherwise.

The term “or a combination thereof” as used herein refers to all permutations and combinations of the listed items preceding the term. For example, “A, B, C, or combinations thereof” is intended to include at least one of: A, B, C, AB, AC, BC, or ABC, and if order is important in a particular context, also BA, CA, CB, CBA, BCA, ACB, BAC, or CAB. Continuing with this example, expressly included are combinations that contain repeats of one or more item or term, such as BB, AAA, AB, BBC, AAABCCCC, CBBAAA, CABABB, and so forth. The skilled artisan will understand that typically there is no limit on the number of items or terms in any combination, unless otherwise apparent from the context.

As used herein, the term “cancer” refers to a proliferative disorder disease caused or characterized by the proliferation of cells which have lost susceptibility to normal growth control. The term “cancer,” as used in the present application, includes tumors and any other proliferative disorders. Cancers of the same tissue type originate in the same tissue, and may be divided into different subtypes based on their biological characteristics. The cancer may be selected, for example, from glioblastoma, squamous cell carcinoma, skin cancer-related tumors, breast cancer, head and neck cancer, gynecological cancer, urinary and male genital cancer, bladder cancer, prostate cancer, bone cancer, endocrine adenocarcinoma, digestive tract cancer, major digestive/organ cancer, central nervous system cancer, and lung cancer.

Glioblastoma, also known as glioblastoma multiforme, may develop from a diffuse astrocytoma or an anaplastic astrocytoma but more commonly presents de novo without evidence of a less malignant precursor. Histologically, this tumor is an anaplastic, cellular glioma composed of poorly differentiated, often pleomorphic astrocytic tumor cells with marked nuclear atypia and brisk mitotic activity. Glioblastoma primarily affects the cerebral hemispheres. Central nervous system tumors are, for example, associated with characteristic patterns of altered oncogenes, altered tumor-suppressor genes, and chromosomal abnormalities.

Skin cancer related tumors include, for example, squamous cell carcinoma, Kaposi's sarcoma, malignant melanoma, Merkel cell skin cancer, and non-melanoma skin cancer. Head-and-neck cancers include, for example, laryngeal, hypopharyngeal, nasopharyngeal,

oropharyngeal cancer, lip and oral cavity cancer and squamous cell. Lymphomas include, for example, AIDS-related lymphoma, non-Hodgkin's lymphoma, cutaneous T-cell lymphoma, Burkitt lymphoma, Hodgkin's disease, and lymphoma of the central nervous system.

Examples of breast cancer include, but are not limited to, invasive ductal carcinoma, invasive lobular carcinoma, ductal carcinoma in situ, and lobular carcinoma in situ. Examples of cancers of the respiratory tract include, but are not limited to, small-cell and non-small-cell lung carcinoma, as well as bronchial adenoma and pleuropulmonary blastoma.

Examples of urinary and male genital cancers include, but are not limited to, bladder, penile, kidney, renal pelvis, ureter, urethral, testicular and human papillary renal cancers.

Examples of gynecological cancers include, but are not limited to, endometrial, cervical, ovarian, vaginal, and vulvar cancer, as well as sarcoma of the uterus.

Endocrine adenocarcinoma can be named by reference to the hormone that they produce, e.g., gastrinomas (which produce gastrin), insulinomas (which produce insulin), somatostatinomas (which produce somatostatin), VIPomas (which produce VIP) and glucagonomas (which produce glucagon).

Examples of digestive tract cancers include, but are not limited to, anal, colon, colorectal, esophageal, gallbladder, gastric, pancreatic, rectal, small-intestine, and salivary gland cancers.

Examples of lung cancers include, but are not limited to, small-cell lung carcinoma and non-small-cell lung carcinoma comprising squamous cell lung carcinoma, adenocarcinoma and large cell lung carcinoma.

These disorders have been well characterized in humans, but also exist with a similar etiology in other mammals, and can be treated by administering pharmaceutical compositions disclosed herein.

As used herein, the term “sensitize,” “sensitizing,” or “sensitizer” refers to an increased sensitivity or reduce the resistance of a cancer sample or a mammal responding to a therapeutic treatment. An increased sensitivity or a reduced sensitivity to a therapeutic treatment is measured according to a known method in the art for the particular treatment and methods described herein below, including, for example, cell proliferative assays (Tanigawa N, Kern D H, Kikasa Y, Morton D L, *Cancer Res*, 42: 2159-2164 (1982)), cell death assays (Weisenthal L M, Shoemaker R H, Marsden J A, Dill P L, Baker J A, Moran E M, *Cancer Res*, 94: 161-173 (1984); Weisenthal L M, Lippman M E, *Cancer Treat Rep*, 69: 615-632 (1985); Weisenthal L M, In: Kaspers G J L, Pieters R, Twentyman P R, Weisenthal L M, Veerman A J P, eds. *Drug Resistance in Leukemia and Lymphoma.* Langhorne, PA: Harwood Academic Publishers, 415-432 (1993); Weisenthal L M, *Contrib Gynecol Obstet*, 19: 82-90 (1984)). The sensitivity or resistance may also be measured in animals by measuring the tumor size

reduction over a period of time, for example, 6 months for human and 4-6 weeks for mouse. A composition or a method sensitizes response to a therapeutic treatment if the increase in treatment sensitivity or the reduction in resistance is 25% or more, for example, 30%, 40%, 50%, 60%, 70%, 80%, or more, to 2-fold, 3-fold, 4-fold, 5-fold, 10-fold, 15-fold, 20-fold or more, compared to treatment sensitivity or resistance in the absence of such composition or method. The determination of sensitivity or resistance to a therapeutic treatment is routine in the art and within the skill of an ordinarily skilled clinician.

As used herein, a "cancer therapy sensitizer" refers to a compound or a composition containing at least one compound that can sensitize the cancer therapy. For example, it refers to a compound or a composition containing an effective amount of at least one compound and a pharmaceutically acceptable carrier, diluent, excipient or a combination thereof. For example, the aforementioned compound or composition can be applied before, during or both before and during the cancer therapy to improve or enhance the effect of one or more therapeutically active compositions upon a cancer or a tumor in an individual in need, and then achieve the goal of eliminating, inhibiting, improving, comforting or preventing a cancer and its symptoms; retarding, prohibiting, reversing the rate of tumor proliferation; or the medical effects similar to the foregoing goals.

As used herein, the term "chemotherapy" refers to the use of chemical agents to destroy cancer cells. Exemplary chemotherapy agents include, but are not limited to, actinomycin D, adriamycin, altretamine, asparaginase, bleomycin, busulphan, capecitabine, carboplatin, carmustine, chlorambucil, cisplatin, CPT-11, cyclophosphamide, cytarabine, dacarbazine, daunorubicin, doxorubicin, epirubicin, etoposide, fludarabine, fluorouracil, gemcitabine, hydroxyurea, idarubicin, fosfamide, irinotecan, liposomal doxorubicin, lomustine, melphalan, mercaptopurine, methotrexate, mitomycin, mitozantrone, oxaliplatin, procarbazine, steroids, streptozocin, taxol, taxotere, taxotere, temozolomide, thioguanine, thiotepa, tomudex, topotecan, treosulfan, UFT (Uracil-Tegufur), vinblastine, vincristine, vindesine, and vinorelbine. Chemotherapy may be used alone or in combination to treat some types of cancers. Sometimes it can be used together with other types of treatment such as surgery, radiotherapy, immunotherapy, or a combination thereof.

As used herein, "radiotherapy," also called "radiation therapy," refers to the treatment of cancer and other diseases with ionizing radiation. Ionizing radiation deposits energy that injures or destroys cells in the area being treated (i.e., the "target tissue") by damaging their genetic material, making it impossible for these cells to continue to grow. Although radiation damages both cancer and normal cells, the normal cells are able to repair themselves and function properly. Radiotherapy may be used to treat localized solid tumors, such as cancers of the skin, tongue, larynx, brain, breast, or uterine cervix. It can also be used to treat

leukemia and lymphoma (cancers of the blood-forming cells and lymphatic system, respectively). Exemplary radiotherapy may be selected from the forms of electromagnetic waves, such as X-rays or gamma rays, or charged particles or neutral particles. The radiotherapy may be administered by external beam, an interstitial implant, or a combination thereof. A course of radiotherapy consisting of 60-70 Gy for the majority of tumors over 4-7 weeks.

The radiation and chemotherapy sensitizer disclosed herein is a pharmaceutical compound or composition that can be used prior to, or simultaneously with, radiotherapy as well as chemotherapy, to strengthen the effect that radiotherapy and chemotherapy has on the tumor.

As used herein, the term “administer” or “administering” refers to introduce by any means a compound or composition (e.g., a therapeutic agent) into the body of a mammal in order to prevent or treat a disease or condition (e.g., cancer).

As used herein, the terms “treating,” “treatment,” “therapy,” and “therapeutic treatment” as used herein refer to curative therapy, prophylactic therapy, or preventative therapy. These terms also describe the management and care of a mammal for the purpose of combating a disease, or related condition, and include the administration of a composition to alleviate the symptoms, side effects, or other complications of the disease or condition. Therapeutic treatment for cancer includes, for example, surgery, chemotherapy, radiation therapy, gene therapy, and immunotherapy.

As used herein, the term “mammal” refers to human or other animals, such as farm animals or laboratory animals (e.g. guinea pig or mice). In some embodiments, the mammal is human. It may be a human who has been diagnosed as in need of treatment for a disease or disorder disclosed herein.

“Pharmaceutically effective amount” encompasses an amount sufficient to ameliorate or prevent a symptom or sign of the medical condition. An effective amount for a particular patient or a veterinary subject may vary depending on factors, such as the condition being treated, the overall health of the patient, the method route and dose of administration and the severity of side effects. A pharmaceutically effective amount can be the maximal dose or dosing protocol that avoids significant side effects or toxic effects. The effect will result in an improvement of a diagnostic measure or parameter by at least 5%, such as by at least 10%, further such as at least 20%, further such as at least 30%, further such as at least 40%, further such as at least 50%, further such as at least 60%, further such as at least 70%, further such as at least 80%, and even further such as at least 90%, wherein 100% is defined as the diagnostic parameter shown by a normal subject. A pharmaceutically effective amount of crocetin would be an amount that is, for example, sufficient to reduce a tumor volume, inhibit tumor growth,

or prevent or reduce metastasis, prior to, during, or after a cancer therapy.

The term "pharmaceutically acceptable" refers to those compounds, materials, compositions, and/or dosage forms, which are suitable for use in contact with the tissues of human beings or animals without excessive toxicity, irritation, allergic response, or other problem or complication, commensurate with a reasonable benefit/risk ratio.

The pharmaceutical compositions disclosed herein may be tableted with conventional tablet bases such as lactose, sucrose and cornstarch in combination with binders such as acacia, corn starch or gelatin, disintegrating agents intended to assist the break-up and dissolution of the tablet following administration such as potato starch, alginic acid, corn starch, and guar gum, gum tragacanth, acacia, lubricants intended to improve the flow of tablet granulation and to prevent the adhesion of tablet material to the surfaces of the tablet dies and punches, for example, talc, stearic acid, or magnesium, calcium or zinc stearate, dyes, coloring agents, and flavoring agents such as peppermint, oil of wintergreen, or cherry flavoring, intended to enhance the aesthetic qualities of the tablets and make them more acceptable to the patient.

Suitable excipients for use in oral liquid dosage forms include, for example, dicalcium phosphate and diluents such as water and alcohols, for example, ethanol, benzyl alcohol, and polyethylene alcohols, either with or without the addition of a pharmaceutically acceptable surfactant, suspending agent or emulsifying agent. Various other materials may be present as coatings or to otherwise modify the physical form of the dosage unit. For instance, tablets, pills or capsules may be coated with shellac, sugar or both. Dispersible powders and granules are suitable for the preparation of an aqueous suspension. They provide the active ingredient in admixture with a dispersing or wetting agent, a suspending agent and one or more preservatives. Suitable dispersing or wetting agents and suspending agents are exemplified by those already mentioned above. Additional excipients, for example, those sweetening, flavoring and coloring agents described above, may also be present.

The pharmaceutical compositions disclosed herein may also be in the form of oil-in-water emulsions. The oily phase may be a vegetable oil such as liquid paraffin or a mixture of vegetable oils. Suitable emulsifying agents may, for example, be chosen from (1) naturally occurring gums, such as gum acacia and gum tragacanth, (2) naturally occurring phosphatides, such as soy bean and lecithin, (3) esters or partial esters derived from fatty acids and hexitol anhydrides, for example, sorbitan monooleate, (4) condensation products of the partial esters with ethylene oxide, for example, polyoxyethylene sorbitan monooleate. The emulsions may also contain, for example, sweetening and flavoring agents.

Oily suspensions may be formulated by suspending the active ingredient in a vegetable oil, such as arachis oil, olive oil, sesame oil or coconut oil, or in a mineral oil such as liquid

paraffin. The oily suspensions may contain a thickening agent, such as beeswax, hard paraffin, or cetyl alcohol. The suspensions may also contain one or more preservatives, for example, ethyl or n-propyl p-hydroxybenzoate; one or more coloring agents; one or more flavoring agents; and one or more sweetening agents such as sucrose or saccharin.

Syrups and elixirs may be formulated with sweetening agents, such as glycerol, propylene glycol, sorbitol or sucrose. Such formulations may also contain, for example, at least one entity chosen from demulcents, preservatives, such as methyl and propyl parabens, and flavoring and coloring agents.

The combinations disclosed herein may also be administered parenterally, that is, subcutaneously, intravenously, intraocularly, intramuscularly, or interperitoneally, as injectable dosages of the crocetin in, for example, a physiologically acceptable diluent with a pharmaceutical carrier that can be a sterile liquid or mixture of liquids such as water, saline, aqueous dextrose and related sugar solutions, an alcohol such as ethanol, isopropanol, or hexadecyl alcohol, glycols such as propylene glycol or polyethylene glycol, glycerol ketals such as 2,2-dimethyl-1,1-dioxolane-4-methanol, ethers such as polyethylene glycol) 400, an oil, a fatty acid, a fatty acid ester or, a fatty acid glyceride, or an acetylated fatty acid glyceride, with or without the addition of a pharmaceutically acceptable surfactant such as a soap or a detergent, suspending agent such as pectin, carbomers, methylcellulose, hydroxypropylmethylcellulose, or carboxymethylcellulose, or emulsifying agent and other pharmaceutical adjuvants.

Illustrative of the oils that can be used in the parenteral formulations disclosed herein are those of petroleum, animal, vegetable, or synthetic origin, chosen, for example, from peanut oil, soybean oil, sesame oil, cottonseed oil, corn oil, olive oil, petrolatum and mineral oil. Suitable fatty acids include oleic acid, stearic acid, isostearic acid, and myristic acid. Suitable fatty acid esters are, for example, ethyl oleate and isopropyl myristate. Suitable soaps include fatty acid alkali metal, ammonium, and triethanolamine salts and suitable detergents include cationic detergents, for example dimethyl dialkyl ammonium halides, aIkyI pyridinium halides, and alkylamine acetates; anionic detergents, for example, alkyl, aryl, and olefin sulfonates, alkyl, olefin, ether, and monoglyceride sulfates, and sulfosuccinates; non-ionic detergents, for example, fatty amine oxides, fatty acid alkanolamides, and poly(oxyethylene-oxypropylene)s or ethylene oxide or propylene oxide copolymers; and amphoteric detergents, for example, alkyl-beta-aminopropionates, and 2-alkylimidazoline quaternary ammonium salts, as well as mixtures.

Illustrative of the surfactants used in parenteral formulations are the class of polyethylene sorbitan fatty acid esters, for example, sorbitan monooleate and the high molecular weight adducts of ethylene oxide with a hydrophobic base, formed by the

condensation of propylene oxide with propylene glycol.

The pharmaceutical compositions disclosed herein may be in the form of sterile injectable aqueous suspensions. Such suspensions may be formulated according to known methods using suitable dispersing or wetting agents and suspending agents, such as, sodium carboxymethylcellulose, methylcellulose, hydroxypropyl methyl cellulose, sodium alginate, polyvinylpyrrolidone, gum tragacanth and gum acacia; dispersing or wetting agents that may be a naturally occurring phosphatide such as lecithin, a condensation product of an alkylene oxide with a fatty acid, for example, polyoxyethylene stearate, a condensation product of ethylene oxide with a long chain aliphatic alcohol, for example, heptadeca-ethyleneoxycetanol, a condensation product of ethylene oxide with a partial ester derived from a fatty acid and a hexitol such as polyoxyethylene sorbitol monooleate, or a condensation product of an ethylene oxide with a partial ester derived from a fatty acid and a hexitol anhydride, for example polyoxyethylene sorbitan monooleate.

The sterile injectable preparation disclosed herein may also be, for example, a sterile injectable solution or suspension in a non-toxic parenterally acceptable diluent or solvent. Diluents and solvents that may be employed are, for example, water, Ringer's solution, isotonic sodium chloride solutions and isotonic glucose solutions. In addition, sterile fixed oils are conventionally employed as solvents or suspending media. For this purpose, any bland, fixed oil may be employed including synthetic mono- or diglycerides. In addition, fatty acids such as oleic acid can be used in the preparation of injectables.

The pharmaceutical composition disclosed herein may also be administered in the form of suppositories for rectal administration of the drug. These compositions can be prepared, for example, by mixing the drug with a suitable non-irritation excipient that is solid at the room temperature but liquid at the rectal temperature and will therefore melt in the rectum to release the drug. Such materials are chosen, for example, from cocoa butter and polyethylene glycol.

Controlled release formulations for parenteral administration include, for example, liposomal, polymeric microsphere and polymeric gel formulations that are known in the art.

It may be desirable or necessary to introduce the pharmaceutical composition disclosed herein to the patient via a mechanical delivery device. The construction and use of mechanical delivery devices for the delivery of pharmaceutical agents is well known in the art. Direct techniques for, for example, administering a drug directly to the brain usually involve placement of a drug delivery catheter into the patient's ventricular system to bypass the blood-brain barrier. One such implantable delivery system, used for the transport of agents to specific anatomical regions of the body, is described in U.S. Patent No. 5,011,472.

The pharmaceutical compositions disclosed herein can also contain other conventional pharmaceutically acceptable ingredients, generally referred to as carriers, diluents, or

auxiliaries, as necessary or desired. Conventional procedures for preparing such compositions in appropriate dosage forms can be utilized. Such ingredients and procedures include those described in the following references: Powell, M.F. et al, "Compendium of Excipients for Parenteral Formulations", *PDA Journal of Pharmaceutical Science ft Technology* 52(5), 238-311 (1998); Strickley, R.G "Parenteral Formulations of Small Molecule Therapeutics Marketed in the United States (1999)-Part-1" *PDA Journal of Pharmaceutical Science & Technology* 53(6), 324-349 (1999); and Nema, S. et al, "Excipients and Their Use in Injectable Products" *PDA Journal of Pharmaceutical Science Et Technology*, 51 (4), 166-171 (1997). Commonly used pharmaceutical ingredients that can be used as appropriate to formulate the composition disclosed herein for its intended route of administration include, for example, acidifying agents (examples include, but are not limited to, acetic acid, citric acid, fumaric acid, hydrochloric acid, and nitric acid); alkalinizing agents (examples include, but are not limited to, ammonia solution, ammonium carbonate, diethanolamine, monoethanolamine, potassium hydroxide, sodium borate, sodium carbonate, sodium hydroxide, and triethanolamine, trolamine); adsorbents (examples include, but are not limited to, powdered cellulose and activated charcoal); aerosol propellants (examples include, but are not limited to, carbon dioxide, chlorofluorocarbon such as Freon-11 (CCl_3F), Freon-13 ($CClF_3$) and Freon-114 (mostly $CClF_2$-$CClF_2$)); air displacement agents (examples include, but are not limited to, nitrogen and argon); antifungal preservatives (examples include, but are not limited to, benzoic acid, butylparaben, ethylparaben, methylparaben, propylparaben, and sodium benzoate); antimicrobial preservatives (examples include, but are not limited to, benzalkonium chloride, benzethonium chloride, benzyl alcohol, cetylpyridinium chloride, chlorobutanol, phenol, phenylethyl alcohol, phenylmercuric nitrate and thimerosal); antioxidants (examples include, but are not limited to, ascorbic acid, ascorbyl palmitate, butylated hydroxyanisole, butylated hydroxytoluene, hypophosphorus acid, monothioglycerol, propyl gallate, sodium ascorbate, sodium bisulfite, sodium formaldehyde sulfoxylate, and sodium metabisulfite); binding materials (examples include, but are not limited to, block polymers, natural and synthetic rubber, polyacrylates, polyurethanes, silicones, polysiloxanes and styrene-butadiene copolymers); buffering agents (examples include, but are not limited to, potassium metaphosphate, dipotassium phosphate, sodium acetate, sodium citrate anhydrous, and sodium citrate dihydrate); carrying agents (examples include, but are not limited to, acacia syrup, aromatic syrup, aromatic elixir, cherry syrup, cocoa syrup, orange syrup, syrup, corn oil, mineral oil, peanut oil, sesame oil, bacteriostatic sodium chloride for injection, and bacteriostatic water for injection); chelating agents (examples include, but are not limited to, edetate disodium and edetic acid); colorants (examples include, but are not limited to, FD&C Red No. 3, FD&C Red No. 20, FD&C Yellow No. 6, FD&C Blue No. 2, D&C Green No. 5,

D&C Orange No. 5, D&C Red No. 8, caramel and ferric oxide red); clarifying agents (examples include, but are not limited to, bentonite); emulsifying agents (examples include, but are not limited to, acacia, cetomacrogol, cetyl alcohol, glyceryl monostearate, lecithin, sorbitan monooleate, and polyoxyethylene 50 monostearate); encapsulating agents (examples include, but are not limited to, gelatin and cellulose acetate phthalate); flavorants (examples include, but are not limited to, anise oil, cinnamon oil, cocoa, menthol, orange oil, peppermint oil, and vanillin); humectants (examples include, but are not limited to, glycerol, propylene glycol, and sorbitol); levigating agents (examples include, but are not limited to, mineral oil and glycerin); oils (examples include, but are not limited to, arachis oil, mineral oil, olive oil, peanut oil, sesame oil, and vegetable oil); ointment bases (examples include, but are not limited to, lanolin, hydrophilic ointment, polyethylene glycol ointment, petrolatum, hydrophilic petrolatum, white ointment, yellow ointment, and rose water ointment); penetration enhancers for, for example, transdermal delivery (examples include, but are not limited to, monohydroxy or polyhydroxy alcohols, mono-or polyvalent alcohols, saturated or unsaturated fatty alcohols, saturated or unsaturated fatty esters, saturated or unsaturated dicarboxylic acids, essential oils, phosphatidyl derivatives, cephalin, terpenes, amides, ethers, ketones and urea); plasticizers (examples include, but are not limited to, diethyl phthalate and glycerol); solvents (examples include, but are not limited to, ethanol, corn oil, cottonseed oil, glycerol, isopropanol, mineral oil, oleic acid, peanut oil, purified water, and sterile water for injection; stiffening agents (examples include, but are not limited to, cetyl alcohol, cetyl esters wax, microcrystalline wax, paraffin, stearyl alcohol, white wax, and yellow wax); suppository bases (examples include, but are not limited to, cocoa butter and polyethylene glycols); surfactants (examples include, but are not limited to, benzalkonium chloride, nonoxynol 10, oxtoxynol 9, polysorbate 80, sodium lauryl sulfate, and sorbitan mono-palmitate); suspending agents (examples include, but are not limited to, agar, bentonite, carbomers, carboxymethylcellulose sodium, hydroxyethyl cellulose, hydroxypropyl cellulose, hydroxypropyl methylcellulose, kaolin, methylcellulose, tragacanth, and veegum); sweetening agents (examples include, but are not limited to, aspartame, dextrose, glycerol, mannitol, propylene glycol, saccharin sodium, sorbitol, and sucrose); tablet anti-adherents (examples include, but are not limited to, magnesium stearate and talc); tablet binders (examples include, but are not limited to, acacia, alginic acid, carboxymethylcellulose sodium, compressible sugar, ethylcellulose, gelatin, liquid glucose, methylcellulose, non-crosslinked polyvinyl pyrrolidone, and pregelatinized starch); tablet and capsule diluents (examples include, but are not limited to, dibasic calcium phosphate, kaolin, lactose, mannitol, microcrystalline cellulose, powdered cellulose, precipitated calcium carbonate, sodium carbonate, sodium phosphate, sorbitol, and starch); tablet coating agents (examples include, but are not limited to, liquid

glucose, hydroxyethyl cellulose, hydroxypropyl cellulose, hydroxypropyl methylcellulose, methylcellulose, ethylcellulose, cellulose acetate phthalate, and shellac); tablet direct compression excipients (examples include, but are not limited to, dibasic calcium phosphate); tablet disintegrants (examples include, but are not limited to, alginic acid, carboxymethylcellulose calcium, microcrystalline cellulose, polacrillin potassium, cross-linked polyvinylpyrrolidone, sodium alginate, sodium starch glycollate, and starch); tablet glidants (examples include, but are not limited to, colloidal silica, corn starch, and talc); tablet lubricants (examples include, but are not limited to, calcium stearate, magnesium stearate, mineral oil, stearic acid, and zinc stearate); tablet/capsule opaquants (examples include, but are not limited to, titanium dioxide); tablet polishing agents (examples include, but are not limited to, carnuba wax and white wax); thickening agents (examples include, but are not limited to, beeswax, cetyl alcohol and paraffin); tonicity agents (examples include, but are not limited to, dextrose and sodium chloride); viscosity increasing agents (examples include, but are not limited to, alginic acid, bentonite, carbomers, carboxymethylcellulose sodium, methylcellulose, polyvinyl pyrrolidone, sodium alginate, and tragacanth); and wetting agents (examples include, but are not limited to, heptadecaethylene oxycetanol, lecithins, sorbitol monooleate, polyoxyethylene sorbitol monooleate, and polyoxyethylene stearate).

When the sensitizer liquid or gel disclosed herein is an injection form, the injection can be directly intratumorally injected using, for example, a syringe, or indirectly injected via, for example, an angiography catheter into the tumor area at time of the irradiation. In this case, the sensitizer liquid or gel disclosed herein can be injected into the target tumor area using, for example, a syringe or angiography catheter, before, after or at the same time as the administration of the anti-cancer agent. For example, a syringe having a needle of about 21 gauge can be used to perform the intratumoral injection guided by ultrasonographic examination while observing the state of permeation of the sensitizer into the tissue. The sensitizer can be delivered widely to the tissue under ultrasonographic guidance to modify the depth and direction of the injection needle. The dose of sensitizer administered to the tumor area differs depending on the size of the tumor and the administration method.

Anti-cancer agents used herein include, for example, alkylating agents such as cyclophosphamide, ifosfamide, busulfan, melphalan, bendamustine hydrochloride, nimustine hydrochloride, ranimustine, dacarbazine, procarbazine hydrochloride, and temozolomide; antimetabolites such as methotrexate, pemetrexed sodium, fluorouracil, doxifluridine, capecitabine, tegafur, cytarabine, cytarabine ocfosfate hydrate, enocitabine, gemcitabine hydrochloride, mercaptopurine hydrate, fludarabine phosphate, nelarabine, pentostatin, cladribine, levofolinate calcium, calcium folinate, hydroxycarbamide, L-asparaginase, and azacitidine; antitumor antibiotics such as doxorubicin hydrochloride, daunorubicin

hydrochloride, pirarubicin, epirubicin hydrochloride, idarubicin hydrochloride, aclarubicin hydrochloride, amrubicin hydrochloride, mitoxantrone hydrochloride, mitomycin C, actinomycin D, bleomycin, peplomycin sulfate, and zinostatin stimalamer; microtubule inhibitors such as vincristine sulfate, vinblastine sulfate, vindesine sulfate, vinorelbine tartrate, paclitaxel, docetaxel hydrate, and eribulin mesylate; hormonal agents such as anastrozole, exemestane, letrozole, tamoxifen citrate, toremifene citrate, fulvestrant, flutamide, bicalutamide, medroxyprogesterone acetate, estramustine phosphate sodium hydrate, and leuprolide acetate; platinum drugs such as cisplatin, miriplatin hydrate, carboplatin, nedaplatin, and oxaliplasin; topoisomerase I inhibitors such as irinotecan hydrochloride hydrate and nogitecan hydrochloride; topoisomerase II inhibitors such as etoposide and sobuzoxane; cytokines such as interferon γ1a, teceleukin, and celmoleukin; antibody drugs such as trastusumab, rituximab, gemtuzumab ozogamicine, bevacizumab, and cetuximab; radioimmunotherapeutic agents such as ibritumomab tiuxetan; molecular target drugs such as gefitinib, imatinib mesylate, bortezomib, erlotinib hydrochloride, sorafenib tosylate, sunitinib malate, thalidomide, nilotinib hydrochloride hydrate, dasatinib hydrate, lapatinib tosylate hydrate, everolimus, lenalidomide hydrate, dexamethasone, temsirolimus, vorinostat, tretinoin, and tamibarotene; non-specific immune stimulants such as OK-432, dry BCG, Coriolus versicolor polysaccharide formulation, lentinan, and ubenimex. Other examples of the anti-cancer agents include aceglatone, porfimer sodium, talaporfin sodium, ethanol, and arsenic trioxide.

Examples of the anti-cancer agents include anthracycline anti-cancer agents such as doxorubicin hydrochloride, daunorubicin hydrochloride, pirarubicin, epirubicin hydrochloride, idarubicin hydrochloride, aclarubicin hydrochloride, amrubicin hydrochloride, and mitoxantrone hydrochloride; platinum anti-cancer agents such as cisplatin, miriplatin hydrate, carboplatin, nedaplatin, and oxaliplatin; and pyrimidine antimetabolite-based anti-cancer agents such as fluorouracil, doxifluridine, capecitabine, tegafur, cytarabine, cytarabine ocfostate hydrate, enocitabine, and gemcitabine hydrochloride.

The present disclosure, via the examples below, has demonstrated that Crocetin can successfully be employed as, for example, a chemotherapy and/or radiotherapy sensitizer, for various solid tumors that were grafted onto mice. It can synergize with radiotherapy and/or chemotherapy to prolong the survival of tumor-bearing mice. In addition, compared with the TSC that is currently in phase III clinical trials, crocetin can have the following advantages: (1) the overall survival of tumor-bearing mice treated with crocetin in combination with chemo-radiation therapy can be significantly longer than those treated with TSC in combination with chemo-radiation therapy at the same dose; (2) crocetin can not only enhance the sensitivity of tumor cells to radiotherapy, but also enhance the sensitivity of tumor cells to

chemotherapy; (3) the optimal dose of TSC achieving the maximum synergistic effect with chemo-radiation therapy is 100 μg/kg, followed by a dose-dependent decrease, while the synergistic effect of crocetin with chemo-radiation therapy can be dose-dependently increasing in the range of 100-400 μg/kg.

The scope of the present disclosure is best understood with reference to the following examples, which are not intended to limit the present disclosure to the specific embodiments.

Examples

1. Process of preparing crocetin-carrying liposome

13 mg of Lecithin, 5 mg of cholesterol, and 2 mg of PEG-DSPE2000 were dissolved in 2 ml of chloroform, followed by adding 3 mg of crocetin (Shaoxing Tiankang Biotechnology Co., Ltd.) dissolved in 30 μl of DMSO to form a mixture. The mixture was added to an eggplant-shaped flask under pressure to recover chloroform and form a liposome film. 2 ml of deionized water was added to the flask for rotational hydration, and the drug-carrying liposome preparations were obtained.

2. Crocetin enhances radiotherapy to suppress the growth rates of subcutaneous solid tumors in BALB/c nude mice

1. Materials

Hela human cervical cancer cell line, HCT116 human colonic cancer cell line, and HepG2 human hepatic carcinoma cell line (ATCC).

Crocetin: brick-red powder, HPLC purity greater than 98% (Shaoxing Tiankang Biotechnology Co., Ltd.).

Intravenous injection of crocetin: the liposome preparation was prepared according to Example 1 and diluted to certain concentration with saline; intragastric administration of crocetin: a crocetin suspension with certain concentration was formulated by use of 0.5% sodium carboxymethyl cellulose.

TSC was prepared from crocetin according to the method disclosed in U.S. Patent No. 6,060,511, with an HPLC purity of greater than 98%.

2. Methods

2.1 Subcutaneous solid tumors in BALB/c nude mice

Male BALB/c nude mice of 4-8 weeks in age, weighing about 18±2 g, were provided by, and maintained in a SPF facility under sterile atmosphere at the animal facility of the Center of Laboratory Animals, Zhejiang Academy of Medical Sciences. 2×10^6 Hela, HCT116, and HepG2 cells were re-suspended in 200 μl PBS and injected subcutaneously into the right flank of the mice, respectively. The inoculated sites and the number of tumor cells inoculated

were the same for each mouse, and each cell line in four mice (12 in total). After tumor volume increased to 0.3 cm^3 in size, the tumor tissues with good growth (no degeneration or necrosis) and reddish color were selected for next inoculation, and the inoculated tumor tissue was about 2 mm in diameter for each mouse. Each type of tumor tissue was inoculated into 25 mice.

2.2 Treatment protocol

The treatment was initiated 2-3 weeks after tumor inoculation, when the tumor volume range was between 0.25-0.35 cm^3. 25 mice bearing same type of tumor were randomly assigned to one of five groups:

(1) Control group (Control);

(2) Radiotherapy group (RT);

(3) Radiotherapy + TSC i.v. group (RT + TSC i.v. (100 μg/kg));

(4) Radiotherapy + crocetin i.v. group (RT + crocetin i.v. (100 μg/kg));

(5) Radiotherapy + crocetin i.g. group (RT + crocetin i.g. (200 μg/kg)).

All mice were treated for consecutive five days. Each mouse in groups 2-5 received a single dose (5 Gy) of local irradiation (RS-2000-PRO-225 (RAD SOURCE, Simmens)) within 30 minutes after administration of test compounds on day 3 (Figure 1, 2 and 3).

2.3 Follow-Up of the Animal

Tumor volumes were measured using caliper two or three times a week until 4-fold of the volume at the beginning of treatment. The tumor volume was calculated by the formula: 0.5 * length * $width^2$; and the relative tumor volume = tumor volume measured on that day (Vt) / tumor volume on the first day of administration (V0) * 100%.

2.4 Statistical Analysis

The relative tumor volume-time curve was plotted. The statistical difference between different groups was analyzed by one-way ANOVA. A $P<0.05$ was considered to be statistically significant.

3. Results

Figure 1 shows the inhibitory effects of the treatment of crocetin or TSC together with radiation against HCT116 subcutaneous tumor in mice. Figure 2 shows the inhibitory effects of the treatment of crocetin or TSC together with radiation against Hela subcutaneous tumor in mice. Figure 3 shows the inhibitory effects of the treatment of crocetin or TSC together with radiation against HepG2 subcutaneous tumor in mice. Compared with the control group, all other groups could effectively inhibit the growth of subcutaneous tumors ($p<0.05$). Compared with the radiation-only group, TSC or crocetin together with radiation group significantly delay the growth of subcutaneous tumors ($P<0.01$). Furthermore, the growth of subcutaneous tumors in crocetin together with radiation group is significantly slower than that

in TSC together with radiation group ($P<0.01$). Thus, the inhibitory effect of crocetin, intravenous injection or intragastric administration, in combination with radiation on subcutaneous human tumor models in mice had statistically significant advantages over the other treatment groups.

4. Conclusions

Accordingly, crocetin in the present disclosure could significantly enhance radiotherapy to suppress the growth of a plurality of solid tumors.

3. Crocetin synergizes with chemotherapy (temozolomide) to enhance survival in BALB/c nude mice bearing orthotopic C6 glioblastoma

1. Materials

C6 glioma cell line (ATCC).

Crocetin: brick-red powder, HPLC purity greater than 98% (Shaoxing Tiankang Biotechnology Co., Ltd.).

Intravenous injection of crocetin: the liposome preparation was prepared according to Example 1 and diluted to certain concentration with saline; intragastric administration of crocetin: a crocetin suspension with certain concentration was formulated by use of 0.5% sodium carboxymethyl cellulose.

TSC was prepared from crocetin according to the method disclosed in U.S. Patent No. 6,060,511, with an HPLC purity of greater than 98%.

Temozolomide (TMZ) has a purity greater than 99% (from Guangzhou Tomums Life Science Co., Ltd.).

2. Methods

2.1 Orthotopic C6 glioblastoma in BALB/c nude mice

Male BALB/c nude mice of 6-8 weeks in age, weighing about 18±2 g, were provided by, and maintained in a SPF facility under sterile atmosphere at the animal facility of the Center of Laboratory Animals, Zhejiang Academy of Medical Sciences. With antiseptic technique, C6 glioma cells were prepared for suspensions concentrated at 2×10^6 cells/10 μl in PBS. Each mouse was intracranially inoculated with 2.5 μl of cell suspension (5×10^5 cells). The inoculated sites and the number of tumor cells inoculated were the same for each mouse, and a total of 50 mice were inoculated.

2.2 Treatment protocol

After inoculation for 7 days, 50 mice were randomly assigned to one of the six groups, and each group has 8-9 mice;

(1) Control group (Control);

(2) TMZ group (TMZ);

(3) TMZ + TSC i.v. group (TMZ + TSC (100 μg/kg));

(4) TMZ + crocetin i.v. group (TMZ + crocetin (100 μg/kg));

(5) TSC i.v. group (TSC (100 μg/kg));

(6) Crocetin i.v. group (Crocetin (100 μg/kg)).

From Days 10 to 14, all mice were intravenously injected with TSC, crocetin or saline (control) for consecutive 5 days, followed by intraperitoneal administration of temozolomide (50 mg/kg, 149 mg/m^2) 20 minutes later.

2.3 Follow-Up of the Animal

Animals were examined daily for alertness, feeding ability, external appearance, focal motor deficits, fecal traits and response to external stimuli. The body weight of each mouse during the survival was continuously weighed, and the survival duration of each mouse was recorded. The survival rate of each group was calculated by formula: number of mice remaining in each group / total number of mice in each group at the beginning.

2.4 Statistical Analysis

The survival curve was plotted using Kaplan-Meier methodology. Log-Rank test (Mantel-Cox) was used to determine the difference between the survival distributions of different groups. A $P < 0.05$ was considered to be statistically significant.

3. Results

Figure 4 shows the Kaplan-Meier survival curves of mice and the statistical variance among survival curves of mice in various groups. Treatment with TSC or crocetin alone could not prolong the survival relative to untreated mice. Treatment with TMZ improved overall survival relative to untreated mice, adding 4 days to the median survival time (25 vs. 21 days, $P<0.01$). Crocetin could further enhance such effect of TMZ, adding another 4 days to the median survival time (29 vs. 25 days, $P<0.01$), while TSC did not have such effect.

4. Conclusions

Accordingly, crocetin in the present disclosure could significantly enhance the inhibitory effects of TMZ against the C6 orthotopic glioblastoma in BALB/c nude mice, therefore extend the survival of orthotopic tumor-planted mice.

4. Crocetin synergizes with chemo-radiation therapy to enhance survival in BALB/c nude mice bearing orthotopic C6 glioblastoma

1. Materials

C6 glioma cell line (ATCC).

Crocetin: brick-red powder, HPLC purity greater than 98% (Shaoxing Tiankang Biotechnology Co., Ltd.).

Intravenous injection of crocetin: the liposome preparation was prepared according to

Example 1 and diluted to certain concentration with saline; intragastric administration of crocetin: a crocetin suspension with certain concentration was formulated by use of 0.5% sodium carboxymethyl cellulose.

TSC was prepared from crocetin according to the method disclosed in U.S. Patent No. 6,060,511, with an HPLC purity of greater than 98%.

Temozolomide (TMZ) has a purity greater than 99% (from Guangzhou Tomums Life Science Co., Ltd.).

2. Methods

2.1 Orthotopic C6 glioblastoma in BALB/c nude mice

Male BALB/c nude mice of 4-8 weeks in age, weighing about 18±2 g, were provided by, and maintained in a SPF facility under sterile atmosphere at the animal facility of the Center of Laboratory Animals, Zhejiang Academy of Medical Sciences. With antiseptic technique, C6 glioma cells were prepared for suspensions concentrated at 2×10^6 cells/10 μl in PBS. Each mouse was intracranially inoculated with 2.5 μl of cell suspension (5×10^5 cells). The inoculated sites and the number of tumor cells inoculated were the same for each mouse, and a total of 50 mice were inoculated.

2.2 Treatment protocol

After inoculation for 7 days, 50 mice were randomly assigned to one of the six groups, and each group has 8-9 mice;

(1) Control group (Control);

(2) TMZ + radiotherapy group (TMZ + RT);

(3) TMZ + radiotherapy + TSC i.v. group (TMZ + RT + TSC (100 μg/kg));

(4) TMZ + radiotherapy + crocetin i.v. group (TMZ + RT + TK10 i.v. (100 μg/kg));

(5) TMZ + radiotherapy + crocetin i.v. group (TMZ + RT + TK10 i.v. (200 μg/kg));

(6) TMZ + radiotherapy + crocetin i.g. group (TMZ + RT + TK10 i.g. (200 μg/kg).

From Days 8 to 12, all mice were intravenously injected with TSC, crocetin or saline (control) for consecutive 5 days, followed by intraperitoneal administration of temozolomide (50 mg/kg, 149 mg/m^2) 20 minutes later. Mice in Groups 2-6 received 8 Gy local irradiation (RS-2000-PRO-225 (RAD SOURCE)) within 30 minutes after administration of test compounds on Day 12

2.3 Follow-Up of the Animal

Animals were examined daily for alertness, feeding ability, external appearance, focal motor deficits, fecal traits and response to external stimuli. The body weight of each mouse during the survival was continuously weighed, and the survival duration of each mouse was recorded. The survival rate of each group was calculated by formula: number of mice remaining in each group / total number of mice in each group at the beginning.

2.4 Statistical Analysis

The survival curve was plotted using Kaplan-Meier methodology. Log-Rank test (Mantel-Cox) was used to determine the difference between the survival distributions of different groups. A $P < 0.05$ was considered to be statistically significant.

3. Results

Figure 5 shows the survival curves of each group of mice changing with the number of observation days, and the statistical variance among the survival curves of various groups of mice. Treatment with TMZ + Radiation improved overall survival relative to untreated mice, adding 7 days to the median survival time (31 vs. 24 days, $P<0.01$). Intravenous injection or intragastric administration of crocetin, as well as Intravenous injection of TSC could further enhance such effect of TMZ, extend 6-8 days to the median survival time, respectively (39, 38, 37 vs. 31 days, respectively, $P<0.01$). Noteworthy, there are significant difference (39 vs. 37 days, $p<0.5$) in overall survival between mice treated with crocetin (i.v. 200 μg/kg) + TMZ + Radiation and mice treated with TSC (i.v. 100 μg/kg, which is reported as the optimal dose) +TMZ + Radiation.

4. Conclusions

Accordingly, crocetin in the present disclosure could significantly enhance the inhibitory effects of chemo-radiation therapy against the C6 orthotopic glioblastoma, therefore extend the survival of orthotopic tumor-bearing mice.

5. Crocetin synergizes with chemo-radiation therapy plus chemotherapy to enhance survival in BALB/c nude mice with orthotopic C6 glioblastoma

1. Materials

C6 glioma cell line (ATCC).

Crocetin: brick-red powder, HPLC purity greater than 98% (Shaoxing Tiankang Biotechnology Co., Ltd.).

Intravenous injection of crocetin: the liposome preparation was prepared according to Example 1 and diluted to certain concentration with saline; Intragastric administration of crocetin: a crocetin suspension with certain concentration was formulated by use of 0.5% sodium carboxymethyl cellulose.

TSC was prepared from crocetin according to the method disclosed in U.S. Patent No. 6,060,511, with an HPLC purity of greater than 98%.

Temozolomide (TMZ), with purity greater than 99% (from Guangzhou Tomums Life Science Co., Ltd.).

2. Methods

2.1 Orthotopic C6 glioblastoma in BALB/c nude mice

Male BALB/c nude mice of 6-8 weeks in age, weighing about 18±2 g, were provided by, and maintained in a SPF facility under sterile atmosphere at the animal facility of the Center of Laboratory Animals, Zhejiang Academy of Medical Sciences. With antiseptic technique, C6 glioma cells were prepared for suspensions concentrated at 2×10^6 cells/10 μl in PBS. Each mouse was intracranially inoculated with 2.5 μl of cell suspension (5×10^5 cells).

2.2 Treatment protocol

After inoculation for 6 days, 41 mice were randomly assigned to one of five groups, and each group has 8-9 mice;

(1) Control group (Control)

(2) TMZ + radiotherapy group (TMZ + RT);

(3) TMZ + radiotherapy + TSC i.v. group (TMZ + RT + TSC (100 μg/kg));

(4) TMZ + radiotherapy + crocetin i.v. group (TMZ + RT + TK10 i.v. (100 μg/kg));

(5) TMZ + radiotherapy + crocetin i.g. group (TMZ + RT + TK10 i.g. (200 μg/kg)).

To simulate the mainstays of clinical regimen that includes 6 weeks of chemo-radiation therapy, one-month break, followed by 6 cycles of chemotherapy (five-time treatment every 28 days), mice bearing tumors were treated with two phases: chemo-radiation therapy and chemotherapy.

Administration in Phase 1: from Days 7 to 11, all mice were intravenously (or intragastrically) injected with TSC, crocetin or saline (control) for consecutive 5 days, followed by intraperitoneal administration of temozolomide (50 mg/kg, 149 mg/m^2) 20 minutes later. Mice in groups 2-5 received 8 Gy local irradiation (RS-2000-PRO-225 (RAD SOURCE)) within 30 minutes after dosing on Day 11.

Administration in Phase 2: from Days 16 to 20, all mice were intravenously (or intragastrically) injected with TSC, crocetin or saline (control) for consecutive 5 days, followed by intraperitoneal administration of temozolomide (50 mg/kg, 149 mg/m^2) 20 minutes later.

2.3 Follow-Up of the Animal

Animals were examined daily for alertness, feeding ability, external appearance, focal motor deficits, fecal traits and response to external stimuli. The body weight of each mouse during the survival was continuously weighed, and the survival duration of each mouse was recorded. The survival rate of each group was calculated by formula: number of mice remaining in each group / total number of mice in each group at the beginning.

2.4 Statistical Analysis

The survival curve was plotted using Kaplan-Meier methodology. Log-Rank test (Mantel-Cox) was used to determine the difference between the survival distributions of different groups. A $P < 0.05$ was considered to be statistically significant.

3. Results

Figure 6 shows the survival curves of each group of mice changing with the number of observation days, and the statistical variance among the survival curves of various groups of mice. Treatment with chemo-radiation therapy plus chemotherapy improved overall survival relative to untreated mice, adding 14 days to the median survival time (36 vs. 22 days, $P<0.01$). Intravenous injection of TSC could enhance such effect of chemo-radiation therapy plus chemotherapy, extend 5 days to the median survival time (41 vs. 36, $P<0.01$). Intravenous injection or intragastric administration of crocetin could further enhance the effect of chemo-radiation therapy plus chemotherapy, extend 9 days to the median survival time (45 vs. 36 days, respectively, $P<0.01$). Noteworthy, there are statistically significant difference (45 vs. 41 days, $p<0.01$) of overall survival between mice treated with crocetin + TMZ + radiation and mice treated with TSC + TMZ + radiation (45 vs. 41 days, $p<0.01$).

4. Conclusions

Accordingly, crocetin in the present disclosure could significantly enhance the inhibitory effects of chemo-radiation therapy plus chemotherapy against the C6 orthotopic glioblastoma, therefore extend the survival of orthotopic tumor-bearing mice.

6. Crocetin synergizes with chemo-radiation therapy to enhance survival in C57BL/6 mice bearing syngeneic orthotopic GL261 glioblastoma

1. Materials

GL261 glioblastoma cell line (ATCC).

Crocetin: brick-red powder, HPLC purity greater than 98% (Shaoxing Tiankang Biotechnology Co., Ltd.).

Intravenous injection of crocetin: the liposome preparation was prepared according to Example 1 and diluted to certain concentration with saline; Intragastric administration of crocetin: a crocetin suspension with certain concentration was formulated by use of 0.5% sodium carboxymethyl cellulose.

TSC was prepared from crocetin according to the method disclosed in U.S. Patent No. 6,060,511, with an HPLC purity of greater than 98%.

Temozolomide (TMZ) has a purity greater than 99% (from Guangzhou Tomums Life Science Co., Ltd.).

2. Methods

2.1 Orthotopic GL261 glioblastoma in C57BL/6 mice

Male C57BL/6 mice of 4-8 weeks in age, weighing about 18 ± 2 g, were provided by, and maintained in a SPF facility under sterile atmosphere at the animal facility of the Center of Laboratory Animals, Zhejiang Academy of Medical Sciences. With antiseptic technique,

GL261 glioblastoma cells were prepared for suspensions concentrated at 2×10^6 cells/10 μl in PBS. Each mouse was intracranially inoculated with 2.5 μl of cell suspension (5×10^5 cells).

2.2 Treatment protocol

After inoculation for 9 days, 49 tumor-bearing mice were randomly assigned to one of six groups, and each group has 8-9 mice;

(1) Control group (Control);

(2) TMZ + radiotherapy group (TMZ + RT);

(3) TMZ + radiotherapy + TSC i.v. group (TMZ + RT + TSC (100 μg/kg));

(4) TMZ + radiotherapy + crocetin i.v. group (TMZ + RT + TK10 i.v. (100 μg/kg));

(5) TMZ + radiotherapy + crocetin i.v. group (TMZ + RT + TK10 i.v. (300 μg/kg));

(6) TMZ + radiotherapy + crocetin i.g. group (TMZ + RT + TK10 i.g. (200 μg/kg)).

From Days 9 to 11, all mice were intravenously or intragastically injected with TSC, crocetin or physiological saline (control) for consecutive 3 days, followed by single intraperitoneal administration of temozolomide (100 mg/kg, 298 mg/m^2) 20 minutes later on day 10. Mice in Groups 2-6 received 5 Gy local irradiation (RS-2000-PRO-225 (RAD SOURCE)) within 30 minutes after administration of test compounds on Day 11

2.3 Follow-Up of the Animal

Animals were examined daily for alertness, feeding ability, external appearance, focal motor deficits, fecal traits and response to external stimuli. The body weight of each mice during the survival was continuously weighed, and the survival duration of each mouse was recorded. The survival rate of each group was calculated by formula: number of mice remaining in each group / total number of mice in each group at the beginning.

2.4 Statistical Analysis

The survival curve was plotted using Kaplan-Meier methodology. Log-Rank test (Mantel-Cox) was used to determine the difference between the survival distributions of different groups. A $P < 0.05$ was considered to be statistically significant.

3. Results

Figure 7 shows the Kaplan-Meier survival curves of mice and the statistical variance among survival curves of mice in various groups. Compared with the control group, the other treatment groups could all effectively extend the survival of tumor-bearing mice ($P<0.05$), with their median survival increasing from 25 days to over 36 days. Compared with TMZ + RT group, the efficacy of TMZ + RT combining with TSC or crocetin improved ($P<0.01$), with their median survival time increasing from 36 days to over 45 days. Compared with TSC + TMZ + RT group, the efficacy of crocetin + TMZ + RT group (i.g. or i.v.) was superior ($P<0.05$), with their median survival time increasing from 45 days to 47-51 days, respectively. Therefore, the mice treated by crocetin + TMZ + RT had a statistically significant survival

advantage over mice in the other treatment groups ($P<0.01$).

4. Conclusion

Accordingly, crocetin in the present disclosure could significantly enhance the inhibitory effects of chemo-radiation therapy against the syngeneic orthotopic GL261 glioblastoma model of C57BL/6 mice, therefore to extend the survival of tumor-bearing mice.

7. Crocetin synergizes with chemo-radiation therapy to dose-dependently enhance survival in C57BL/6 mice with syngeneic orthotopic GL261 glioblastoma

1. Materials

GL261 glioblastoma cell line (ATCC).

Crocetin: brick-red powder, HPLC purity greater than 98% (Shaoxing Tiankang Biotechnology Co., Ltd.).

Intravenous injection of crocetin: the liposome preparation was prepared according to Example 1 and diluted to certain concentration with saline; Intragastric administration of crocetin: a crocetin suspension with certain concentration was formulated by use of 0.5% sodium carboxymethyl cellulose.

TSC was prepared from crocetin according to the method disclosed in U.S. Patent No. 6,060,511, with an HPLC purity of greater than 98%.

Temozolomide (TMZ) has a purity greater than 99% (from Guangzhou Tomums Life Science Co., Ltd.).

2. Methods

2.1 Orthotopic GL261 glioblastoma in C57BL/6 mice

Male C57BL/6 mice of 6-8 weeks in age, weighing about 18±2 g, were provided by, and maintained in a SPF facility under sterile atmosphere at the animal facility of the Center of Laboratory Animals, Zhejiang Academy of Medical Sciences. With antiseptic technique, GL261 glioblastoma cells were prepared for suspensions concentrated at 2×10^6 cells/10 μl in PBS. Each mouse was intracranially inoculated with 2.5 μl of cell suspension (5×10^5 cells).

2.2 Treatment protocol

After inoculation for 11 days, 42 tumor-bearing mice were randomly assigned to one of five groups, and each group has 8-9 mice;

(1) Control group (Control);

(2) TMZ + radiotherapy group (TMZ + RT);

(3) TMZ + radiotherapy + crocetin i.v. group (TMZ + RT + crocetin i.v. (100 μg/kg));

(4) TMZ + radiotherapy + crocetin i.v. group (TMZ + RT + crocetin i.v. (200 μg/kg));

(5) TMZ + radiotherapy + crocetin i.v. group (TMZ + RT + crocetin i.v. (400 μg/kg));

From Days 11 to 13, all mice were administered for three consecutive days. They were

intravenously injected with crocetin or saline (control) on Day 11. On Day 12, mice were intravenously injected with crocetin or saline, followed by single intraperitoneal administration of temozolomide (100 mg/kg, 298 mg/m^2) 20 minutes later. On Day 13, mice in groups 2-5 were intravenously injected with crocetin or saline first, and received 5 Gy local irradiation (RS-2000-PRO-225 (RAD SOURCE)) within 30 minutes.

2.3 Follow-Up of the Animal

Animals were examined daily for alertness, feeding ability, external appearance, focal motor deficits, fecal traits and response to external stimuli. The body weight of each mouse during the survival was continuously weighed, and the survival duration of each mouse was recorded. The survival rate of each group was calculated by formula: number of mice remaining in each group / total number of mice in each group at the beginning.

2.4 Statistical Analysis

The survival curve was plotted using Kaplan-Meier methodology. Log-Rank test (Mantel-Cox) was used to determine the difference between the survival distributions of different groups. A $P < 0.05$ was considered to be statistically significant.

3. Results

Figure 8 shows the Kaplan-Meier survival curves of mice and the statistical variance among survival curves of mice in various groups. Compared with the control group, other treatment groups could all effectively extend the survival time of tumor-bearing mice ($P<0.01$), with their median survival period increased from 24 days to over 35 days. Compared with TMZ + RT group, the efficacy of TMZ + RT combining with different c doses of crocetin improved ($P<0.01$), with their median survival period increasing from 35 days to over 44 days. And significant difference exists among three groups of different doses of crocetin in combination with TMZ+RT ($P<0.05$), which showed that the higher the dose of crocetin, the longer the median survival, i.e. from 44 to 49 days.

4. Conclusion

Accordingly, crocetin in the present disclosure can significantly enhance the inhibitory effects of chemo-radiation therapy against the syngeneic orthotopic GL261 glioblastoma model of C57BL/6 mice, therefore to extend the survival of tumor-bearing mice in a dose-dependent manner.

What is claimed is:

1. A method of treating a solid tumor in a mammal in need thereof comprising administering to the mammal a pharmaceutically effective amount of crocetin, prior to, during, or after a therapy that is radiotherapy, chemotherapy, immunotherapy or a combination thereof.

2. The method of claim 1, wherein the mammal is human.

3. The method of claim 1, wherein the radiotherapy is selected from the form of electromagnetic waves, charged particles or neutral particles.

4. The method of claim 1, wherein the radiotherapy is given at a dose of about 60-70 Gy over 4-7 weeks.

5. The method of claim 1, wherein the solid tumor is selected from glioblastoma, squamous cell carcinoma, skin cancer-related tumors, breast cancer, head and neck cancer, gynecological cancer, urinary and male genital cancer, bladder cancer, prostate cancer, bone cancer, endocrine adenocarcinoma, digestive tract cancer, major digestive/organ cancer, central nervous system cancer, and lung cancer.

6. The method of claim 5, wherein the solid tumor is glioblastoma.

7. The method of claim 1, wherein the solid tumor is glioblastoma, and the chemotherapy is a therapy with temozolomide.

8. The method of claim 1, wherein crocetin is used as a sensitizer.

9. The method of claim 1, further comprising administering with at least one anticancer entity.

10. The method of claim 9, wherein the at least one anticancer entity is selected from an additional sensitizer in a cancer therapy, a targeted therapeutic agent, and an immunotherapeutic agent.

11. The method of claim 9, wherein the at least one anticancer entity is selected from anticancer alkylating agents, anticancer antimetabolites, anticancer antibiotics, plant-derived anticancer agents, anticancer platinum coordination compounds, anticancer camptothecin derivatives, anticancer tyrosine kinase inhibitors, monoclonal antibodies, biological response modifiers, and other anticancer agents.

12. A method as in claim 1 wherein the effective amount of crocetin is administering before said radiotherapy or chemotherapy or immunotherapy or a combination thereof.

13. A composition comprising crocetin and pharmaceutically acceptable carriers or

auxiliaries for use in treatment of a solid tumor.

14. The composition of claim 13, wherein the composition is in the form of injection, tablet, capsule, pill, suppository, aerosol, oral liquid preparation, granule, powder, sustained release preparation, nano preparation, syrup, vina, tincture, lotion, film or a combination thereof.

15. The composition of claim 13, wherein the composition is in the form of liposomal formulation.

16. The composition of claim 13, wherein the composition is administered by orally, injection, implant, spray, inhalation or a combination thereof.

17. A method of sensitizing a mammal in need thereof to a therapy that is radiotherapy, chemotherapy, or a combination thereof, comprising administering to the mammal with a pharmaceutically effective amount of crocetin.

18. A method of claim 17, wherein the mammal is human.

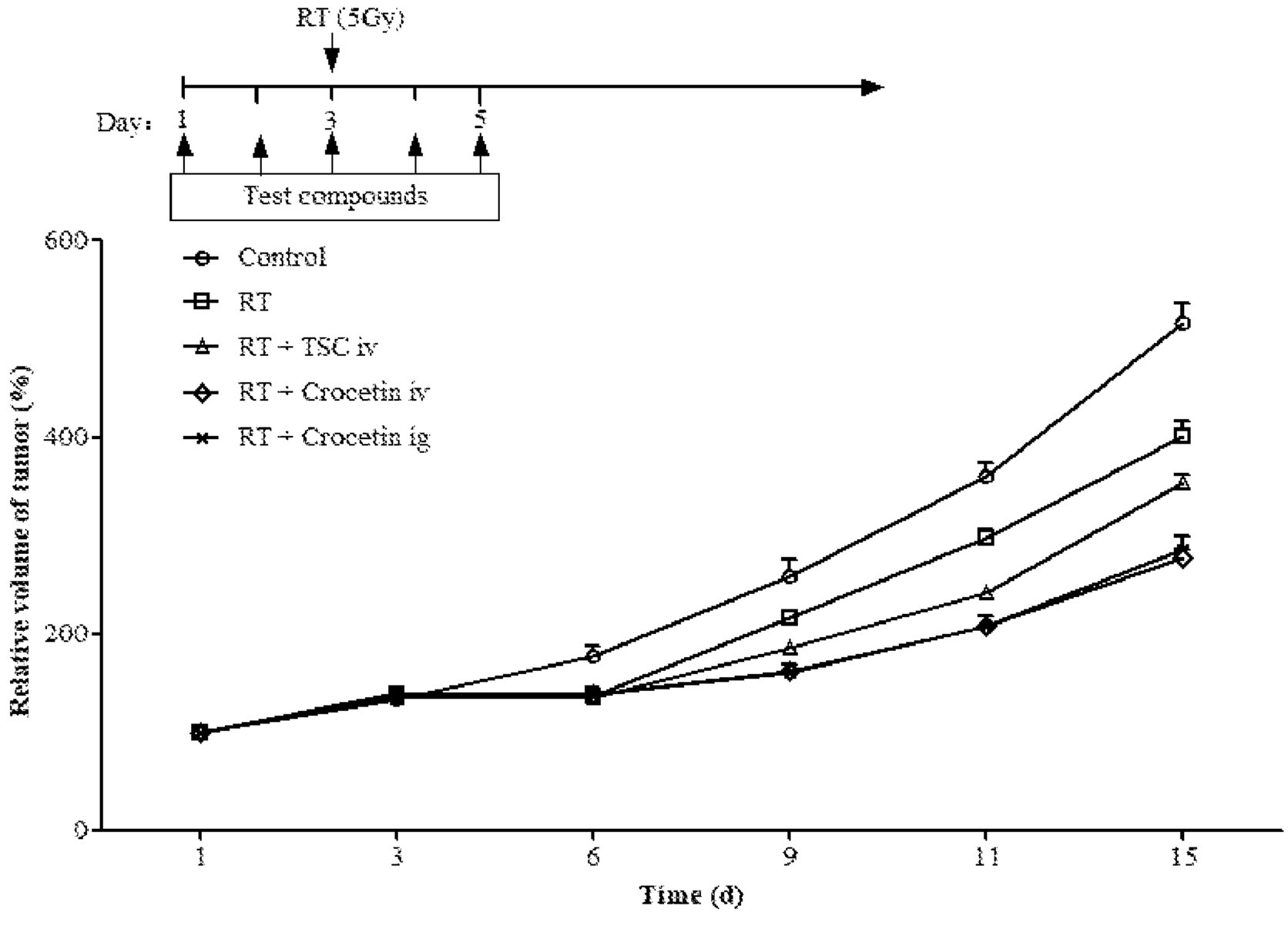


Figure 1

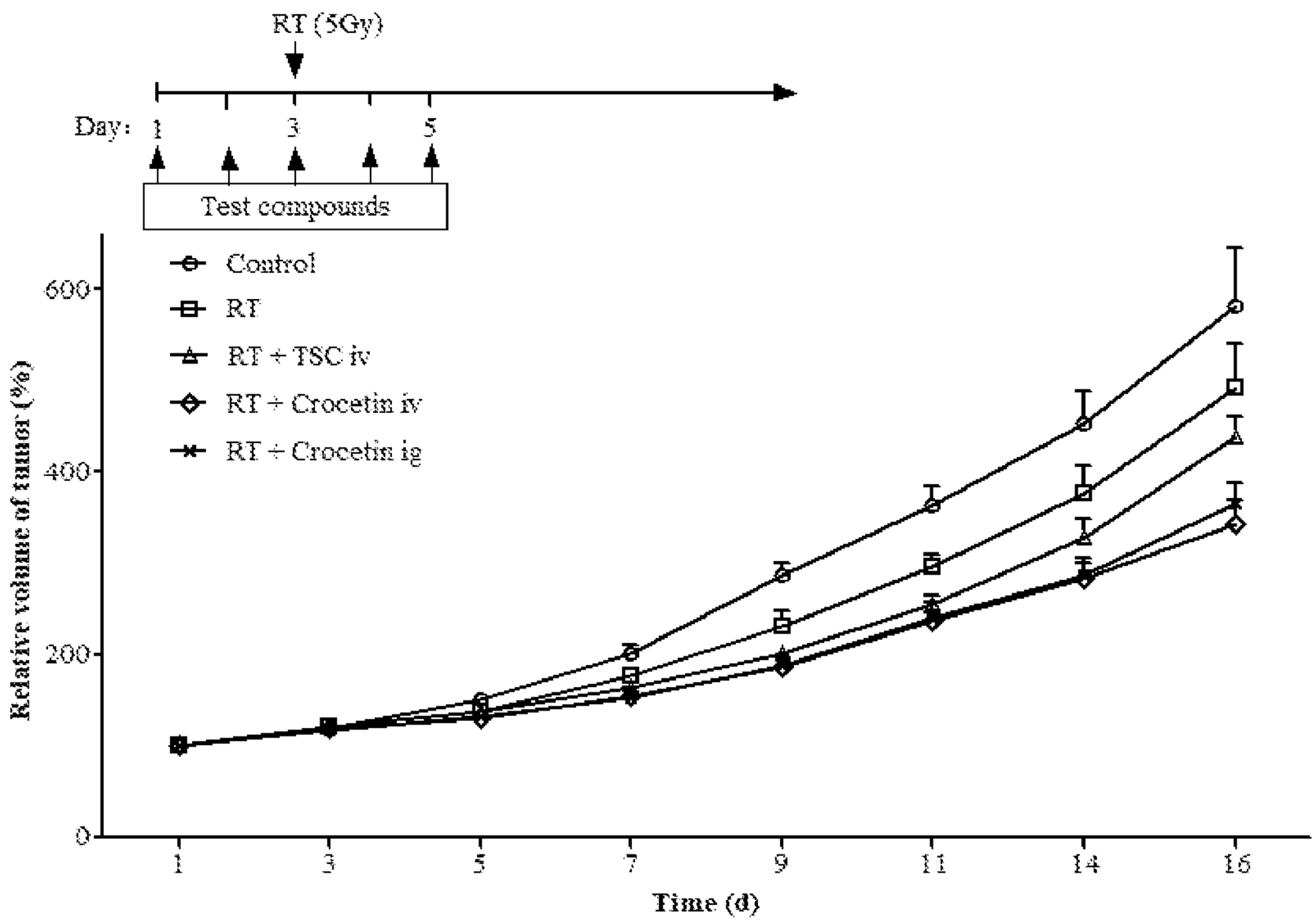


Figure 2

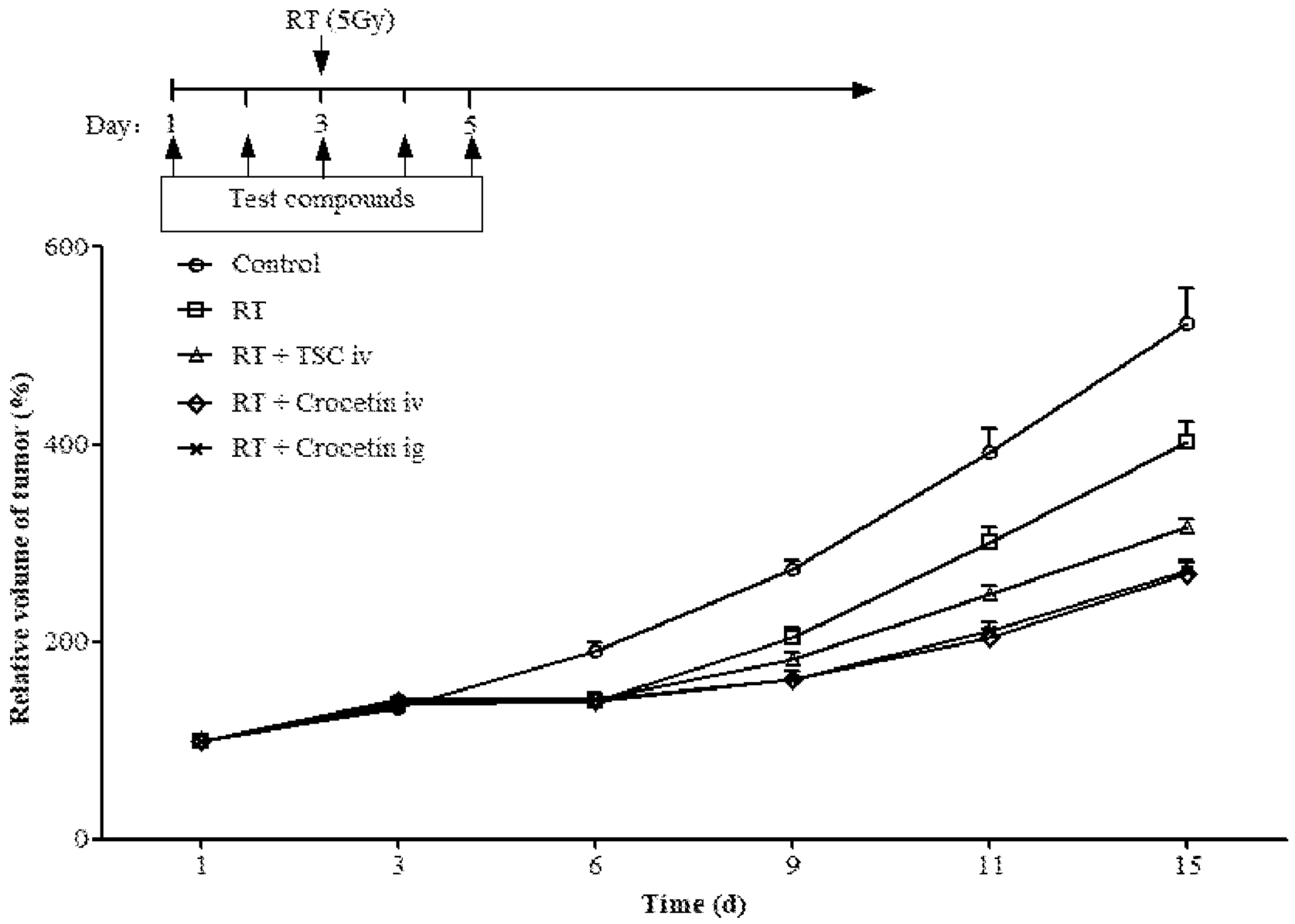
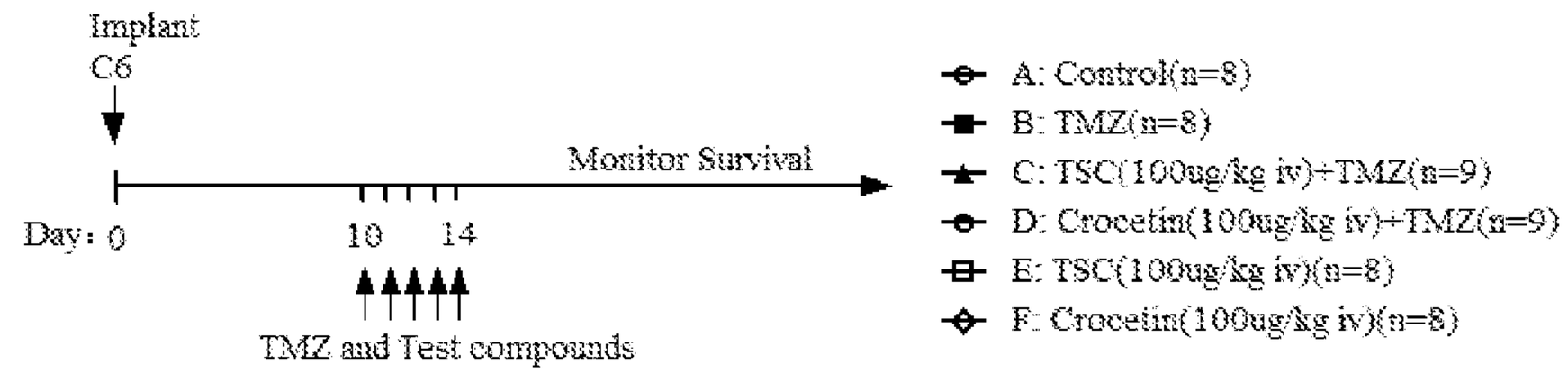


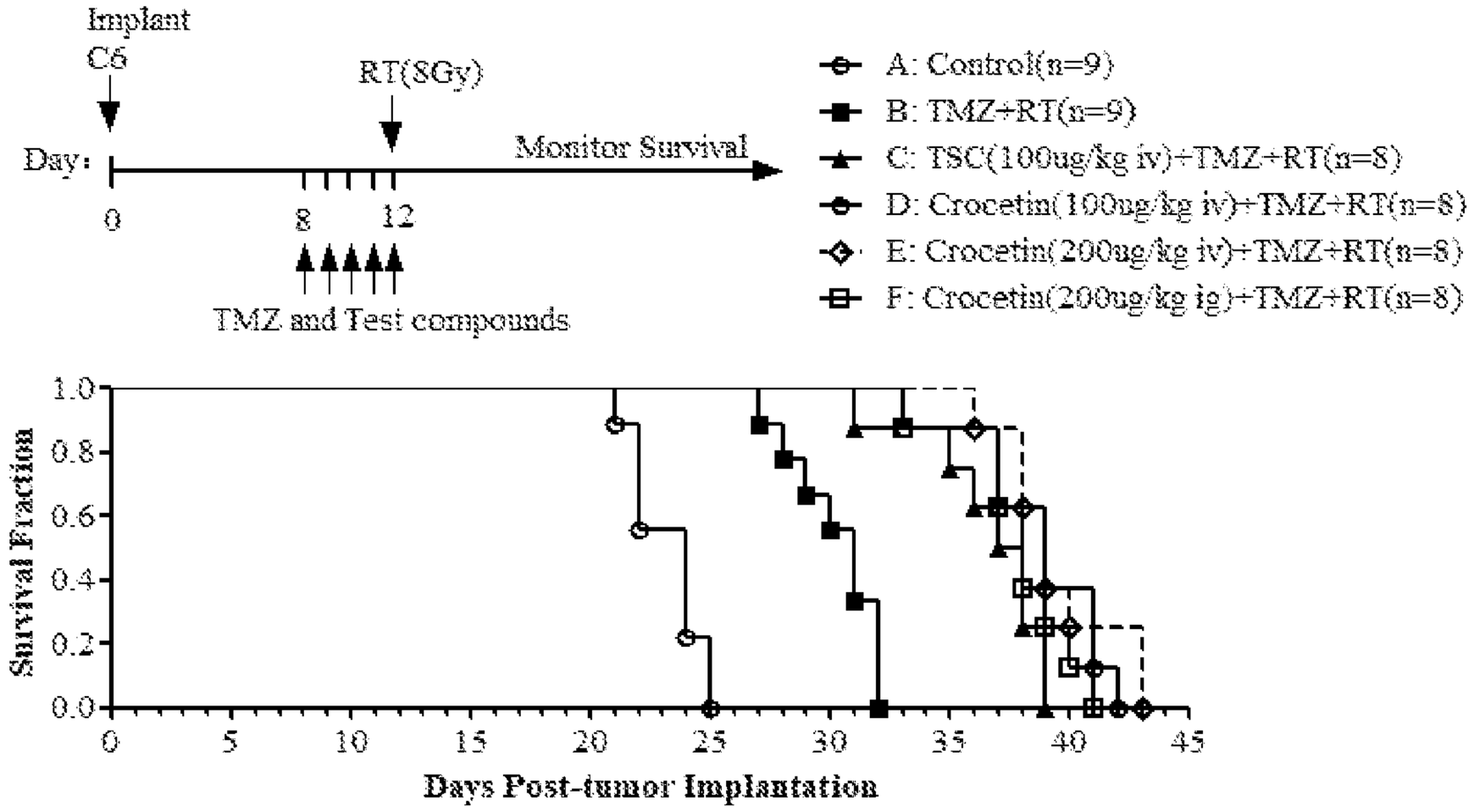
Figure 3



Pvalue	A	B	C	D	E	F
B	0.001**					
C	0.001**	0.582				
D	0.001**	0.001**	0.001**			
E	0.644	0.001**	0.001**	0.001**		
F	0.150	0.001**	0.001**	0.001**	0.253	
Median	21	25	26	29	21	21
SE	0.306	0.398	0.354	0.416	0.456	0.566

* P<0.05, ** P<0.01

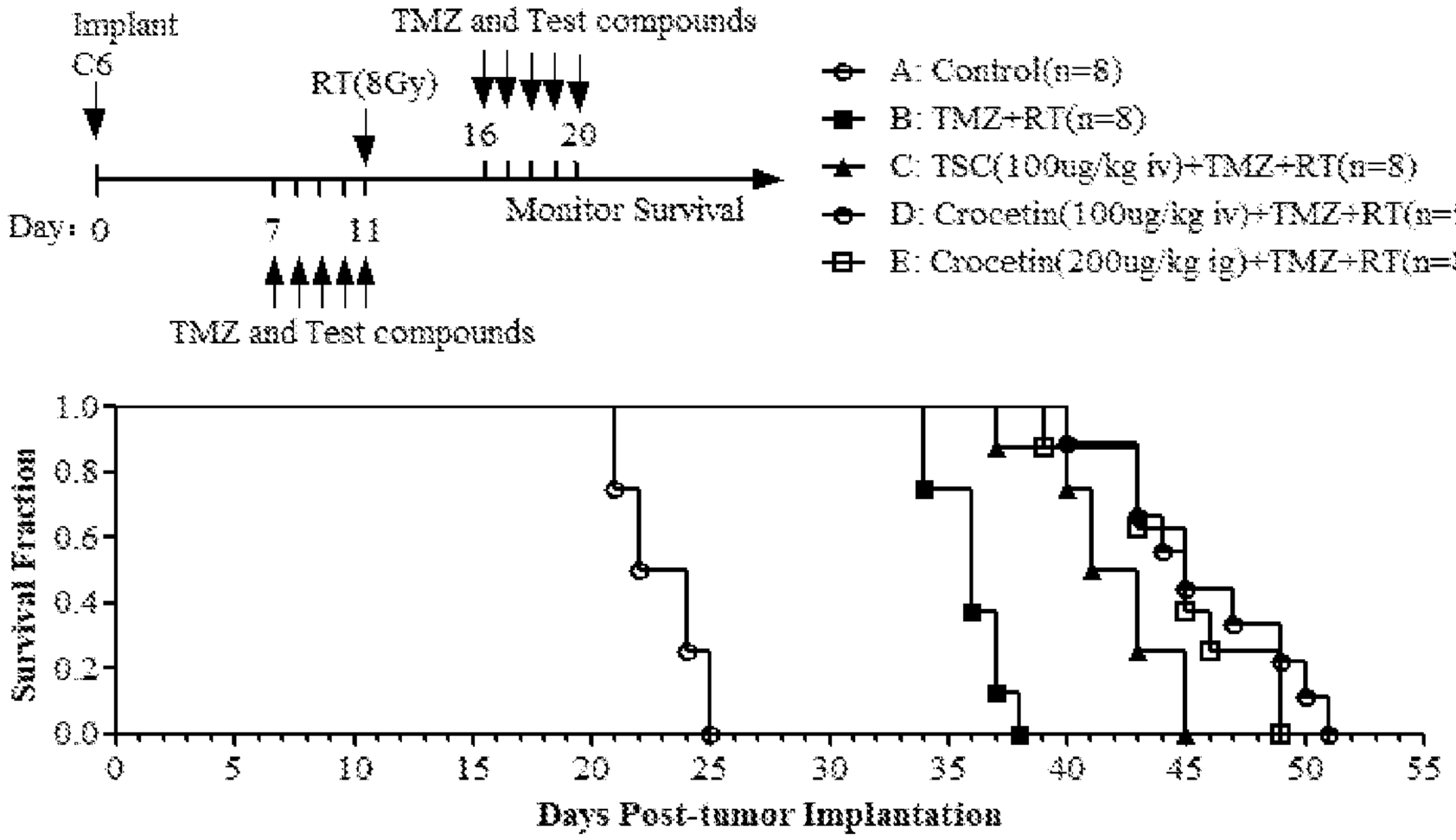
Figure 4



Pvalue	A	B	C	D	E	F
B	0.001**					
C	0.001**	0.001**				
D	0.001**	0.001**	0.074			
E	0.001**	0.001**	0.035*	0.538		
F	0.001**	0.001**	0.250	0.286	0.254	
Median	24	31	37	39	39	38
SE	0.831	0.707	0.943	1.369	0.685	0.685

* P<0.05, ** P<0.01

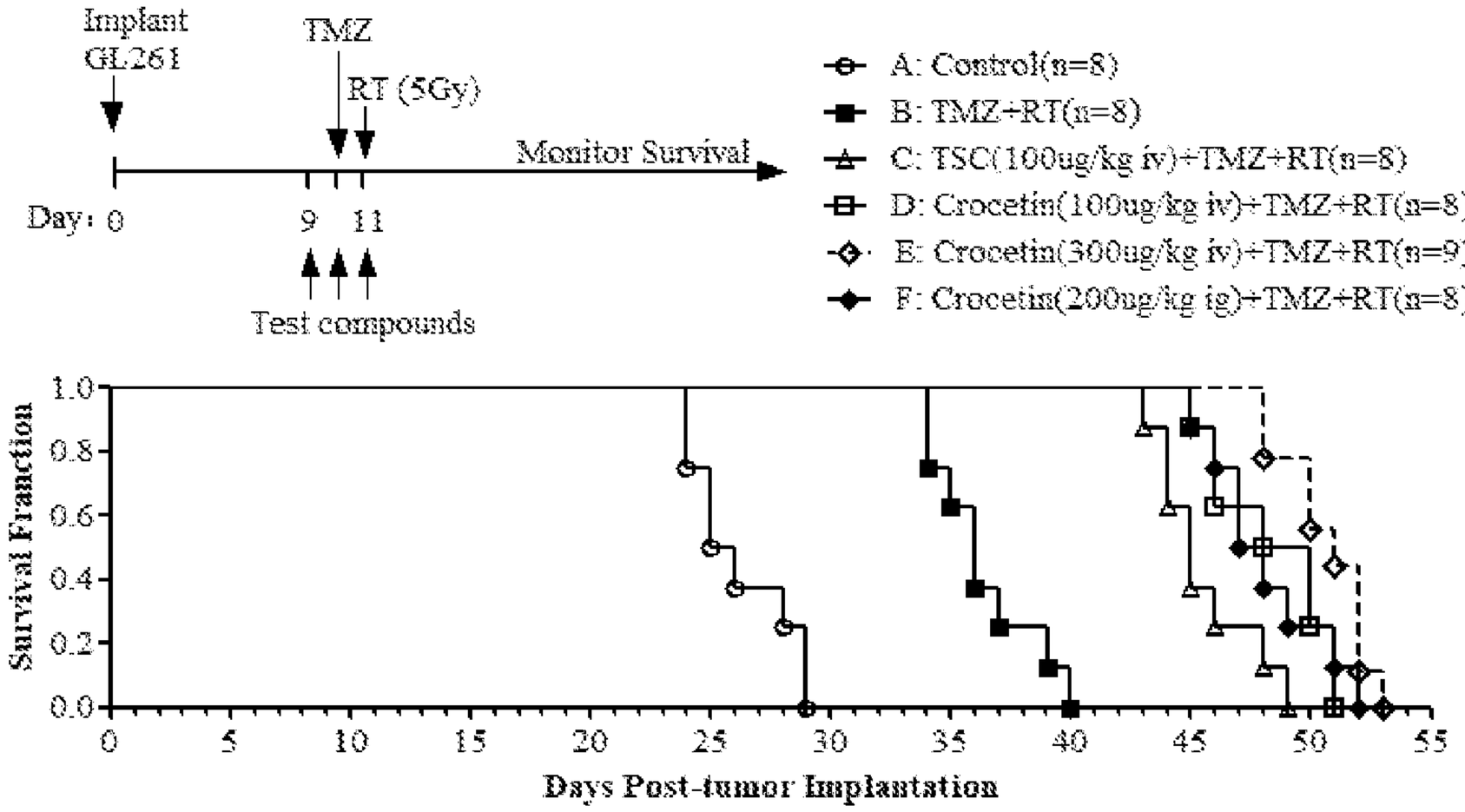
Figure 5



Pvalue	A	B	C	D	E
B	0.001**				
C	0.001**	0.001**			
D	0.001**	0.001**	0.030*		
E	0.001**	0.001**	0.045*	0.445	
Median	22	36	41	45	45
SE	1.061	0.913	1.061	1.491	1.309

* P<0.05, ** P<0.01

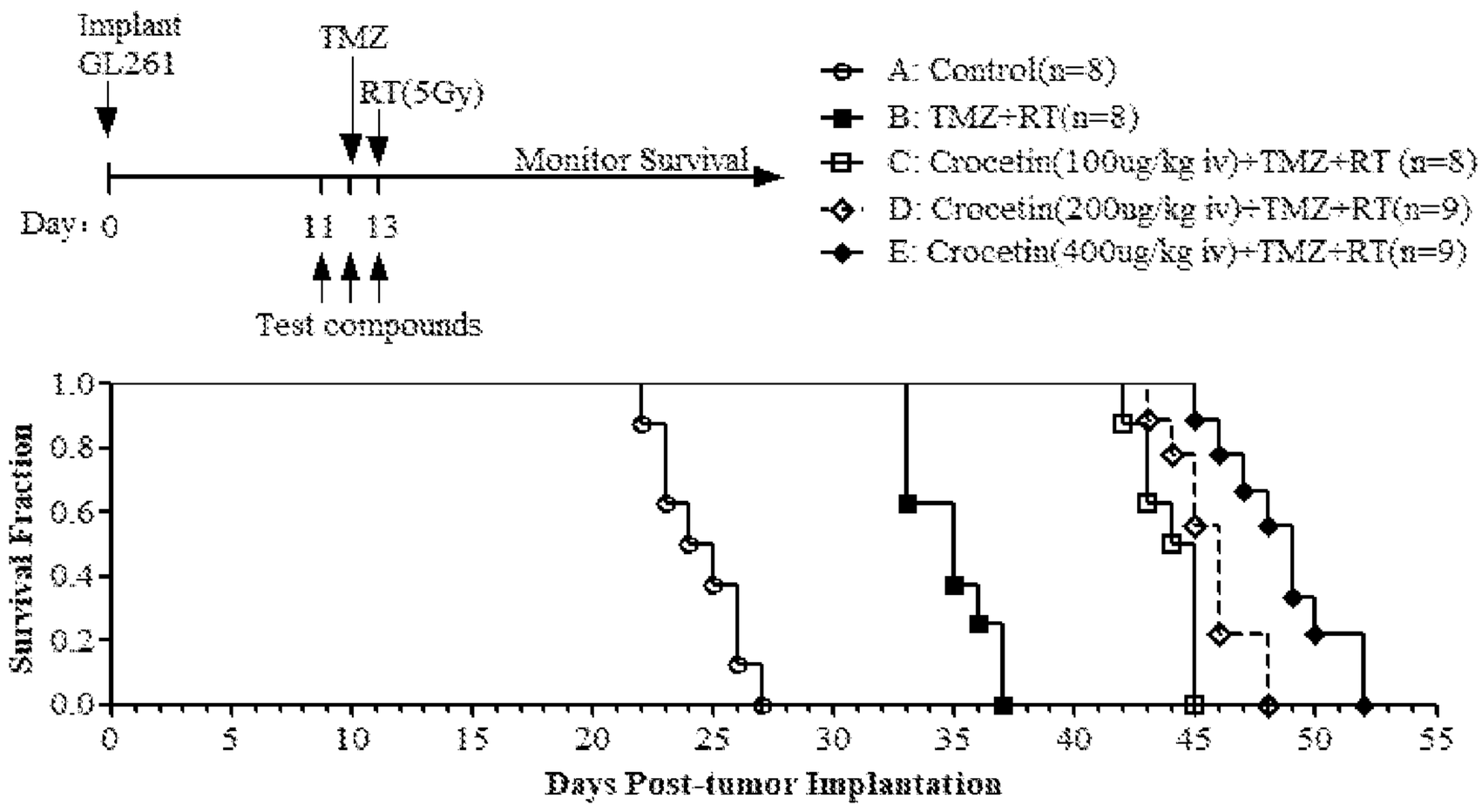
Figure 6



P value	A	B	C	D	E	F
B	0.001**					
C	0.001**	0.001**				
D	0.001**	0.001**	0.027*			
E	0.001**	0.001**	0.003**	0.033*		
F	0.001**	0.001**	0.041*	0.964	0.039*	
Median	25	36	45	48	51	47
SE	0.943	0.685	0.685	1.886	1.491	0.943

*P<0.05, **P<0.01

Figure 7



Pvalue	A	B	C	D	E
B	0.001**				
C	0.001**	0.001**			
D	0.001**	0.001**	0.021*		
E	0.001**	0.001**	0.001**	0.007**	
Median	24	35	44	46	49
SE	1.414	1.369	0.566	0.416	0.707

* P<0.05, ** P<0.01

Figure 8

INTERNATIONAL SEARCH REPORT

International application No.
PCT/CN2019/124307

A. CLASSIFICATION OF SUBJECT MATTER

A61K 31/202(2006.01)i; A61P 35/00(2006.01)i

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

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

A61K; A61P

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

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

CNABS, CNTXT, VEN, DWPI, USTXT, EPTXT, CNKI, PUBMED, STN, crocetin, crocetinate, tumor, tumour, cancer, carcinoma, glioblastoma, glioma, sensitizer, 27876-94-4

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 2014141082 A1 (GAO Song) 22 May 2014 (2014-05-22) the abstract, claims 34-36, description paragraphs [0021], [0086]-[0097]	1-18
X	WO 2012060854 A1 (US DEPARTMENT OF VETERANS AFFAIRS et al.) 10 May 2012 (2012-05-10) description paragraphs [0012]-[0023]	1-18
X	WO 2004078695 A1 (RIKEN VITAMIN CO., LTD.) 16 September 2004 (2004-09-16) description page 2 lines 1-5, page 13 line 1- page 14 line 12	1-18
X	US 2010210572 A1 (EIDENBERGER Thomas) 19 August 2010 (2010-08-19) description paragraphs [0017]-[0023], [0080]-[0097]	1-18
X	CN 101157645 A (JIANGNAN UNIVERSITY) 09 April 2008 (2008-04-09) the abstract, description page 1 lines 4-7	1-18
X	CN 101863754 A (HANGZHOU FAHE CHEM. CO., LTD.) 20 October 2010 (2010-10-20) description paragraph [0002]	1-18
X	CN 109091458 A (JIAWENLI FUJIAN COSMETICS CO., LTD.) 28 December 2018 (2018-12-28) the abstract	1-5, 8-18

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

* Special categories of cited documents:
"A" document defining the general state of the art which is not considered to be of particular relevance
"E" earlier application or patent but published on or after the international filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed

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

Date of the actual completion of the international search	Date of mailing of the international search report
28 August 2020	**15 September 2020**
Name and mailing address of the ISA/CN **National Intellectual Property Administration, PRC** **6, Xitucheng Rd., Jimen Bridge, Haidian District, Beijing 100088** **China** Facsimile No. **(86-10)62019451**	Authorized officer **YU,Li** Telephone No. **86-(10)-53961883**

INTERNATIONAL SEARCH REPORT

International application No.
PCT/CN2019/124307

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	JP 2011098902 A (理研ビタミン株式会社) 19 May 2011 (2011-05-19) the abstract	1-5，8-18

INTERNATIONAL SEARCH REPORT

International application No.
PCT/CN2019/124307

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☑ Claims Nos.: **1-12,17-18**
because they relate to subject matter not required to be searched by this Authority, namely:

[1] Claims 1-12,17-18 relate to a method of treating a solid tumor or sensitizing a mammal to a therapy and do not meet the criteria set out in PCT Rules 39.1(iv). The search report is made on the basis of the following subject matter of claims 1-12,17-18: Use of a therapeutically effective amount of crocetin in manufacturing of a medicament for treating a solid tumor or sensitizing a mammal to a therapy.

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

INTERNATIONAL SEARCH REPORT
Information on patent family members

International application No.
PCT/CN2019/124307

Patent document cited in search report			Publication date (day/month/year)	Patent family member(s)			Publication date (day/month/year)
US	2014141082	A1	22 May 2014	US	9211298	B2	15 December 2015
				US	2020016184	A9	16 January 2020
				US	2016199398	A1	14 July 2016
WO	2012060854	A1	10 May 2012	US	10155715	B2	18 December 2018
				US	2013231300	A1	05 September 2013
WO	2004078695	A1	16 September 2004	JP	2004269663	A	30 September 2004
US	2010210572	A1	19 August 2010	JP	2010195779	A	09 September 2010
				US	8569247	B2	29 October 2013
				WO	2010094745	A1	26 August 2010
				CN	102802636	A	28 November 2012
CN	101157645	A	09 April 2008	CN	101157645	B	02 June 2010
CN	101863754	A	20 October 2010		None		
CN	109091458	A	28 December 2018		None		
JP	2011098902	A	19 May 2011	JP	5491133	B2	14 May 2014