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(54) Title: PHARMACEUTICAL COMPOSITION COMPRISING ALBUMIN-LIPID HYBRID NANOPARTICLES

(57) Abstract: Disclosed herein are pharmaceutical compositions comprising albumin-lipid hybrid nanoparticles having average particle size of less than about 500 nm and polydispersity index of less than about 0.6, wherein the nanoparticles contain (i) a therapeutically effective amount of an anti-cancer agent, (ii) human serum albumin, (iii) a lipid component comprising cholesterol and lecithin, and optionally, (iv) a targeting ligand; wherein the molar ratio of the human serum albumin to the lipid component ranges from about 1:1 to about 1:20. The methods of treating cancer using such compositions are also disclosed.



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PHARMACEUTICAL COMPOSITION COMPRISING ALBUMIN-LIPID HYBRID NANOPARTICLES

PRIORITY DOCUMENTS

- 5 This application claims priority from the Indian Provisional Patent Application Nos. IN 201721036146 and IN 201721036147; filed on Oct 11, 2017, the contents of which are incorporated herein in its entirety.

TECHNICAL FIELD

- 10 The invention relates to a pharmaceutical composition comprising albumin-lipid hybrid nanoparticles having average particle size of less than about 500 nm and polydispersity index of less than about 0.6, wherein the nanoparticles contain (i) a therapeutically effective amount of an anti-cancer agent, (ii) human serum albumin, (iii) a lipid component comprising cholesterol and lecithin, and optionally, (iv) a targeting ligand; wherein the molar ratio of the human serum
15 albumin to the lipid component ranges from about 1:1 to about 1:20.

BACKGROUND

- Nanotechnology has been extensively explored in the past decade to develop a myriad of functional nanostructures to facilitate the delivery of therapeutic and imaging agents for various
20 medical applications. Liposomes and polymeric nanoparticles represent two primary delivery vehicles that are currently under investigation. While many advantages of these two particle platforms have been disclosed, some intrinsic limitations remain to limit their applications at certain extent. Recently, a new type of nanoparticle platform, named lipid polymer hybrid nanoparticle, has been developed that combines the positive attributes of both liposomes and
25 polymeric nanoparticles while excluding some of their shortages. This new nanoparticle consists of a hydrophobic polymeric core, a lipid shell surrounding the polymeric core, and a hydrophilic polymer stealth layer outside the lipid shell.

- The physicochemical properties of the hybrid nanoparticles such as particle size, surface charge,
30 PEG chain density will affect their in vivo pharmacokinetics. Further, existing types of nanoparticles have some limitations: limited drug loading, visible cytotoxicity of certain polymers, used for nanoparticle manufacturing, poor compatibility with incorporated components and difficulties in regulation or delivery rate from polymeric nanoparticles, low physical stability of solid lipid nanoparticles and liposomal formulations.

Biodegradable or biocompatible polymeric nanoparticles are widely used as delivery systems for different applications such as targeted drug delivery, sustained release of incorporated materials, vaccination and immunization, imaging and other applications.

5 Etoposide is a semi-synthetic product derived from podophyllotoxin. The material is identified by the chemical name 4'-demethylepipodophyllotoxin-9-[4,6-O-(R)-ethylidene- β -D-glucopyranoside]. It is approved by the FDA for use in the treatment of refractory testicular cancer and small cell lung cancer and is currently being marketed under the trade name VePesid[®] as an injection solution or Etopophos[®] injection. Etoposide is sparingly soluble in water, its solubility in water
10 being about 0.1 mg/ml. Etoposide has also been administered to patients via the oral route, either in capsules or as a solution; however, etoposide oral bioavailability is only about 50% of that found after intravenous administration.

Eliana B. Souto et. al. "Lipid Nanocarriers in Cancer Diagnosis and Therapy" discloses lipid
15 nanoparticles are useful for the improvement of the pharmacokinetics and biodistribution profiles of anti-cancer drugs.

US Publication Number 20160145314 discloses fusion polypeptide comprising a human serum albumin and a p53-peptide wherein fusion polypeptide further comprises one or more anticancer
20 agent.

US Patent Number 8911786 discloses nanoparticles comprising rapamycin, chemotherapeutic agent and a carrier protein

25 PCT Publication Number 2016015522 discloses the preparation of lyophilized nanoparticles by using fatty acid binding albumin.

US Publication Number 20120021036 discloses nanostructures or products of manufacture for use as ex vivo or in vivo composition delivery vehicles.

30 Drug delivery across the physiological barriers of the brain is the bottleneck for the treatment of central nervous system (CNS) disorders and brain tumors. The delivery of most drugs to the CNS is limited by the anatomical structure at the blood-brain barrier and the blood-cerebrospinal fluid (CSF) barrier. It is therefore critical to search for alternative method to achieve effective drug
35 concentrations in the brain. In fact, it has been recognized that there is already an on-going shift from the traditional CNS therapy toward CNS targeting therapies.

Several drug delivery systems had been applied to improve the passage of an anticancer agent across blood brain barrier but still a novel technique having higher encapsulation efficiency, convenient production methods, and improved safety is needed in tumor therapy.

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SUMMARY

In one general aspect, there is provided a pharmaceutical composition comprising albumin-lipid hybrid nanoparticles having average particle size of less than about 500 nm and polydispersity index of less than about 0.6, wherein the nanoparticles contain (i) a therapeutically effective amount of an anti-cancer agent, (ii) human serum albumin, (iii) a lipid component comprising
10 cholesterol and lecithin, and optionally, (iv) a targeting ligand; wherein the molar ratio of the human serum albumin to the lipid component ranges from about 1:1 to about 1:20.

In an embodiment, the anti-cancer agent comprises alkylating agents, antimetabolites, antibiotics, microtubule inhibitors, hormones and their antagonists, and cytotoxic antineoplastic agents. In
15 another embodiment the anti-cancer agent comprises melphalan, hexamethyl melamine, etoposide, carmustine, lomustine, temozolomide, dianhydrogalactitol, veliparib, eflornithine, thiotepa, cytarabin, idatrexate, trimetrexate, dacarbazine, L-asparaginase, camptothecin, topotecan, bicalutamide, flutamide, leuprolide, sorafenib, sunitinib, erlotinib, imatinib, lapatinib, gefitinib, brivanib, raltitrexed, doxorubicin, fluorouracil, paclitaxel, docetaxel, vinblastin, vindesine,
20 vinorelbine, carboplatin, oxaloplatin, cyclophosphamide, mechlorethamine, chlorambucil, amsacrine, teniposide, irinotecan, bleomycin, mitomycin, plicamycin, busulfan, fosfamide, methotrexate, 6-mercaptopurine, 6-thioguanine, 5-fluorodeoxyuridine, fludarabine, 2-chlorodeoxyadenosine, 2-deoxycoformycin, genecitabine, dactinomycin, daunorubicin, mitoxantrone, chlorotrianisene, estramustine, toremifene, zoladex, and canertinib.

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In another embodiment, the nanoparticles have average particle size of less than about 400 nm. In another embodiment, the nanoparticles have average particle size of less than about 300 nm, or less than about 200 nm. In another embodiment, the nanoparticles have a polydispersity index of less than about 0.5, or less than about 0.4, or less than about 0.3. In another embodiment, the molar
30 ratio of the human serum albumin to the lipid component in the nanoparticles ranges from about 1:2 to about 1:15, or from about 1:4 to about 1:12. In another embodiment, the nanoparticles have anti-cancer agent entrapment efficiency of more than about 50%, or more than about 60 %.

In another embodiment, the lecithin comprises egg phospholipids, soybean phospholipids, hydrogenated phospholipids, synthetic phospholipids, and PEGylated phospholipids. In another
35 embodiment, the composition further comprises at least one pharmaceutically acceptable excipient selected from a surfactant, cryoprotectant or combination thereof.

In the context of present invention, the lipid comprises cholesterol, lipoid E80, lipoid S75 or combinations thereof. Preferably, the lipid comprises cholesterol. The lecithin used in the context of present invention includes egg phospholipids, soybean phospholipids, hydrogenated phospholipids, synthetic phospholipids, and PEGylated phospholipids. Preferably, the lecithin comprises a mixture of egg phospholipids (e.g., Lipoid E80), soybean phospholipids (e.g., Lipoid S75).

In another aspect of the invention, the pharmaceutical composition has a pH ranging from about 5 to about 8 or from about 5.5 to about 7.

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In another aspect, there is provided a pharmaceutical composition comprising albumin-lipid hybrid nanoparticles having average particle size of less than about 300 nm and polydispersity index of less than about 0.5, wherein the nanoparticles contain (i) a therapeutically effective amount of etoposide, (ii) human serum albumin, (iii) a lipid component comprising cholesterol and lecithin, and optionally, (iv) a targeting ligand; wherein the molar ratio of the human serum albumin to the lipid component ranges from about 1:2 to about 1:15. In another embodiment, the pharmaceutical composition is for use in the treatment of a cancer in a subject.

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In another aspect of the invention, there is provided a process of preparing a pharmaceutical composition of albumin-lipid hybrid nanoparticles having average particle size of less than about 500 nm, said process comprising the steps of: (i) preparing an aqueous solution containing etoposide and human serum albumin, (ii) preparing an ethanolic solution of lipid component, (iii) adding drop-wise the ethanolic solution of lipid component to the aqueous solution of step (i) under stirring to obtain a suspension, and (iv) subjecting the suspension of step (iii) to high-pressure homogenization till the nanoparticles having average particle size of less than about 500 nm and polydispersity index of less than about 0.6 are obtained.

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In another aspect of the invention, there is provided a process of preparing a pharmaceutical composition of albumin-lipid hybrid nanoparticles having average particle size of less than about 500 nm, said process comprising the steps of: (i) preparing an aqueous solution containing etoposide, human serum albumin, polyethylene glycol and polysorbate (ii) preparing an ethanolic solution of lipid component comprising cholesterol, lipoid E80, and lipoid S75, (iii) adding drop-wise the ethanolic solution of lipid component to the aqueous solution of step (i) under stirring, followed by heating the mixture to about 60 °C to about 70 °C to obtain a suspension, (iv) subjecting the suspension of step (iii) to high-pressure homogenization at about 10 °C to about 30 °C temperature, and a pressure of about 2000 psi and 8000 psi till the nanoparticles having average

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particle size of less than about 200 nm and polydispersity index of less than about 0.4 are obtained, and optionally (v) lyophilizing the nanoparticles in a freeze dryer.

In another general aspect, there is provided a method of treating cancer in a subject in need thereof, said method comprising administering parenterally to the subject a pharmaceutical composition comprising albumin-lipid hybrid nanoparticles having average particle size of less than about 300 nm and polydispersity index of less than about 0.5, wherein the nanoparticles contain (i) a therapeutically effective amount of an anti-cancer agent, (ii) human serum albumin, (iii) a lipid component comprising cholesterol and lecithin and optionally, (iv) a targeting ligand, wherein the molar ratio of the human serum albumin to the lipid component ranges from about 1:2 to about 1:20. In another embodiment, the parenteral administration comprises intravenous, intra-arterial, intramuscular, subcutaneous, intra-theal, and intra-cerebral injection. In another embodiment, the cancer comprises lung cancer, prostate cancer, colorectal cancer, stomach cancer, brain cancer, lymphoma, leukemia, neuroblastoma, and ovarian cancer. In another embodiment, the brain cancer comprises astrocytoma, glioblastoma, glioma, ependymoma, papilloma, neurofibroma, meningioma, glioblastoma, sarcoma, lipoma, histocytoma, gangliocytoma, neurocytoma, medulloblastoma, medulloepithelioma, melanoma, melanocytoma, lymphoma, atypical tumors and metastatic tumors.

In another general aspect there is provided a method of treating brain cancer in a subject in need thereof, said method comprising administering parenterally to the subject a pharmaceutical composition comprising albumin-lipid hybrid nanoparticles having average particle size of less than about 300 nm and polydispersity index of less than about 0.5, wherein the nanoparticles contain (i) a therapeutically effective amount of an anti-cancer agent, (ii) human serum albumin, (iii) a lipid component comprising cholesterol and lecithin, and (iv) a targeting ligand; wherein the molar ratio of the human serum albumin to the lipid component ranges from about 1:2 to about 1:20. In another embodiment, the targeting ligand is tagged on surface of the nanoparticles for targeting the anticancer agent to its site of action. In another embodiment, the ligand comprises monoclonal antibody such as bevacizumab, nivolumab or durvalumab, antibody fragments, growth factors, transferrin, peptides such as octreotide and tLyp-1 peptide, aptamers, polysaccharides, and small biomolecules such as folic acid, galactose, bisphosphonates, and biotin.

DETAILED DESCRIPTION

In one general embodiment, there is provided a pharmaceutical composition comprising albumin-lipid hybrid nanoparticles having average particle size of less than about 500 nm and polydispersity index of less than about 0.6, wherein the nanoparticles contain (i) a therapeutically effective amount of an anti-cancer agent, (ii) human serum albumin, (iii) a lipid component

comprising cholesterol and lecithin, and optionally, (iv) a targeting ligand; wherein the molar ratio of the human serum albumin to the lipid component ranges from about 1:1 to about 1:20.

In an embodiment, the anti-cancer agent comprises alkylating agents, antimetabolites, antibiotics,
5 microtubule inhibitors, hormones and their antagonists, and cytotoxic antineoplastic agents.

In another embodiment the anti-cancer agent comprises melphalan, hexamethyl melamine, etoposide, carmustine, lomustine, temozolomide, dianhydrogalactitol, veliparib, eflornithine, thiotepa, cytarabin, idatrexate, trimetrexate, dacarbazine, L-asparaginase, camptothecin, topotecan, bicalutamide, flutamide, leuprolide, sorafenib, sunitinib, erlotinib, imatinib, lapatinib, gefitinib,
10 brivanib, raltitrexed, doxorubicin, fluorouracil, paclitaxel, docetaxel, vinblastin, vindesine, vinorelbine, carboplatin, oxaloplatin, cyclophosphamide, mechlorethamine, chlorambucil, amsacrine, teniposide, irinotecan, bleomycin, mitomycin, plicamycin, busulfan, fosfamide, methotrexate, 6-mercaptopumme, 6-thioguanme, 5-fluorodeoxyuridine, fludarabine, 2-chlorodeoxyadenosine, 2-deoxycoformycin, genicitabine, dactinomycin, daunorubicin,
15 mitoxantrone, chlorotrianisene, estramustine, toremifene, zoladex, and canertinib.

In the context of present invention, the albumin-lipid hybrid nanoparticles described herein has average particle size less than about 400 nm, or less than about 300 nm, or less than about 200 nm. For example, the albumin-lipid hybrid nanoparticles has average particle size of about 350 nm, or
20 about 300 nm, or about 250 nm, or about 200 nm, or about 150 nm, or about 100 nm.

In an embodiment, the albumin-lipid hybrid nanoparticles typically has a polydispersity index of less than about 0.6, or less than about 0.5, or less than about 0.4, or less than about 0.3. For example, the albumin-lipid hybrid nanoparticles has polydispersity index of about 0.55, or about
25 0.5, or about 0.45, or about 0.4, or about 0.35, or about 0.3, or about 0.25.

In another embodiment, the molar ratio of the human serum albumin to the lipid component to in the albumin-lipid hybrid nanoparticles ranges from about 1:1 to about 1:20, or from about 1:4 to about 1:18, or from about 1:6 to about 1:16, or from about 1:8 to about 1:14. For example, the
30 molar ratio of the human serum albumin to the lipid component in the nanoparticle composition is about 1:2, or about 1:3, or about 1:4, or about 1:5, or about 1:6, or about 1:7, or about 1:8, or about 1:9, or about 1:10, or about 1:11, or about 1:12, or about 1:13, or about 1:14, or about 1:15, or about 1:16, or about 1:17, or about 1:18, or about 1:19, or about 1:20. In specific embodiment, the molar ratio of the human serum albumin to the lipid component in the nanoparticles ranges from
35 about 1:2 to about 1:15 or about 1:4 to about 1:12.

In another embodiment, the nanoparticles have anti-cancer agent entrapment efficiency of more than about 50%, or more than about 60%, or more than about 70%, or more than about 80%.

In the context of present invention, the lipid comprises cholesterol, lipid E80, lipid S75 or combinations thereof. Preferably, the lipid comprises cholesterol. The lecithin used in the context
5 of present invention includes egg phospholipids, soybean phospholipids, hydrogenated phospholipids, synthetic phospholipids, and PEGylated phospholipids. Preferably, the lecithin comprises a mixture of egg phospholipids (e.g., Lipoid E80), soybean phospholipids (e.g., Lipoid S75).

10 In another embodiment, the albumin-lipid hybrid nanoparticles further comprises at least one pharmaceutically acceptable excipient selected from the group of surfactant, cryoprotectant, solubilizer or combination thereof.

In another embodiment, the surfactants may be selected from anionic, cationic, non-ionic or
15 amphoteric type surfactants include but not limited to poloxamer, Cremophor EL, Solutol HS 15, polysorbate.

In another embodiment, the preferred surfactant is Tween 80 and preferred solubilizer is PEG 400.

In another embodiment, the cryoprotectant such as sucrose may be added to the nanoparticles prior
20 to freezing to maintain the integrity of the albumin-lipid hybrid nanoparticle composition.

In another embodiment, the sucrose is used as a cryoprotectant preferably from about 1% to about 15%, preferably from 5% to 9%, more preferably 7%.

In another embodiment, the albumin-lipid hybrid nanoparticle composition may further include
25 biocompatible polymer, stabilizers, rheology modifiers, antioxidants and preservatives.

The biocompatible polymer may be selected from polyacrylates, polycyanoacrylates, polylactic acid, polyglycolic acid, lactide-glycolide copolymers, lactide-glycolide polyethylene glycol copolymers, polyorthoesters, polyanhydrides, biodegradable block-copolymers, poly(caprolactone), poly(butyrolactone), poly(valerolactone) and other polylactones and their
30 copolymers.

Ethanol was used as dissolving agent and solvent for lipids. Ethanol concentration starting from 10 ml, 15ml, 20 ml, 25 ml, 30ml, 35 ml, 40 ml, 45 ml, 50 ml to 55ml were tested in coordination with the crosslinking temperature (30-70°C). It was found that the amount of ethanol affected the
35 crosslinking temperature of human serum albumin. Increase in ethanol amount led to decrease in crosslinking temperature.

In another embodiment, the pharmaceutical composition further comprises solvent such as ethanol. The preferred amount of ethanol ranges from 10 ml to 30 ml, more preferably 20 ml.

In another embodiment, the albumin-lipid hybrid nanoparticle composition have a pH of about 5.0 to about 8, preferably about 5.5 to about 7.

In another embodiment, there is provided a pharmaceutical composition comprising albumin-lipid hybrid nanoparticles having average particle size of less than about 300 nm and polydispersity index of less than about 0.5, wherein the nanoparticles contain (i) a therapeutically effective amount of etoposide, (ii) human serum albumin, (iii) a lipid component comprising cholesterol and lecithin, and optionally, (iv) a targeting ligand; wherein the molar ratio of the human serum albumin to the lipid component ranges from about 1:2 to about 1:15. In another embodiment, the pharmaceutical composition is for use in the treatment of a cancer in a subject.

In another embodiment, there is provided a pharmaceutical composition comprising albumin-lipid hybrid nanoparticles having average particle size of less than about 300 nm and polydispersity index of less than about 0.5, wherein the nanoparticles contain (i) 100 mg to 110 mg etoposide, (ii) 5 to 6 mL human serum albumin, (iii) a lipid component comprising 18 mg to 22 mg cholesterol, 70 mg to 80 mg of egg lecithin, and 45 mg to 50 mg of soybean lecithin; wherein the molar ratio of the human serum albumin to the lipid component ranges from about 1:2 to about 1:15.

In another embodiment, there is provided a pharmaceutical composition comprising albumin-lipid hybrid nanoparticles having average particle size of less than about 300 nm and polydispersity index of less than about 0.5, wherein the nanoparticles contain (i) 100 mg etoposide, (ii) 5 mL human serum albumin, (iii) a lipid component comprising 18 mg cholesterol, 70 mg egg lecithin, and 42 mg soybean lecithin and (iv) a targeting ligand comprising 62 mg of bevacizumab; wherein the molar ratio of the human serum albumin to the lipid component ranges from about 1:2 to about 1:15.

In another embodiment of the invention, there is provided a process of preparing a pharmaceutical composition of albumin-lipid hybrid nanoparticles having average particle size of less than about 500 nm, said process comprising the steps of: (i) preparing an aqueous solution containing etoposide and human serum albumin, (ii) preparing an ethanolic solution of lipid component, (iii) adding drop-wise the ethanolic solution of lipid component to the aqueous solution of step (i) under stirring to obtain a suspension, and (iv) subjecting the suspension of step (iii) to high-pressure homogenization till the nanoparticles having average particle size of less than about 500 nm and polydispersity index of less than about 0.5 are obtained.

In another embodiment of the invention, there is provided a process of preparing a pharmaceutical composition of albumin-lipid hybrid nanoparticles having average particle size of less than about 500 nm, said process comprising the steps of: (i) preparing an aqueous solution containing etoposide, human serum albumin, polyethylene glycol and polysorbate (ii) preparing an ethanolic solution of lipid component comprising cholesterol, lipoid E80, and lipoid S75, (iii) adding drop-wise the ethanolic solution of lipid component to the aqueous solution of step (i) under stirring, followed by heating the mixture to about 60 °C to about 70 °C to obtain a suspension, (iv) subjecting the suspension of step (iii) to high-pressure homogenization at about 10 °C to about 30 °C temperature, and a pressure of about 2000 psi and 8000 psi till the nanoparticles having average particle size of less than about 200 nm and polydispersity index of less than about 0.4 are obtained, and optionally (v) lyophilizing the nanoparticles in a freeze dryer.

The term “anticancer agent” includes any known agents (e.g., chemotherapeutic compound and/or molecular therapeutic compound) that are useful for the treatment of cancer including alkylating agents, antimetabolites, antibiotics, microtubule inhibitors, hormones and their antagonists, and cytotoxic antineoplastic agents. Non-limiting examples of the anti-cancer agent include melphalan, hexamethyl melamine, etoposide, carmustine, lomustine, temozolomide, dianhydrogalactitol, veliparib, eflornithine, thiotepa, cytarabin, idatrexate, trimetrexate, dacarbazine, L-asparaginase, camptothecin, topotecan, bicalutamide, flutamide, leuprolide, sorafenib, sunitinib, erlotinib, imatinib, lapatinib, gefitinib, brivanib, raltitrexed, doxorubicin, fluorouracil, paclitaxel, docetaxel, vinblastin, vindesine, vinorelbine, carboplatin, oxaloplatin, cyclophosphamide, mechlorethamine, chlorambucil, amsacrine, teniposide, irinotecan, bleomycin, mitomycin, plicamycin, busulfan, fosfamide, methotrexate, 6-mercaptopurine, 6-thioguanine, 5-fluorodeoxyuridine, fludarabine, 2-chlorodeoxyadenosine, 2-deoxycoformycin, genicitabine, dactinomycin, daunorubicin, mitoxantrone, chlorotrianisene, estramustine, toremifene, zoladex, and canertinib.

As used herein, the term “subject” encompasses all mammalian species, including a human patient. The term "brain cancer" includes a variety of cancers. There can be actual brain tumors which arise from the brain itself, known as primary brain cancers of which there are several. The term "brain cancer" refers to malignant tumors i.e., tumors that grow and spread aggressively, overpowering healthy cells by taking up their space, blood, and nutrients. In the context of present invention, the brain cancer comprises astrocytoma, glioblastoma, glioma, ependymoma, papilloma, neurofibroma, meningioma, glioblastoma, sarcoma, lipoma, histiocytoma, gangliocytoma, neurocytoma, medulloblastoma, medulloepithelioma, melanoma, melanocytoma, lymphoma, atypical tumors and metastatic tumors.

As used herein, the term “albumin-lipid hybrid nanoparticles” can exhibit the characteristics of both lipids and polymeric nanoparticles. It can be refer to nanoparticles comprising a therapeutically effective amount of drug, human serum albumin and a lipid component that results controllable particle size and surface functionality, high drug loading yield, sustained drug release
5 profile, and excellent in vitro and in vivo stability.

The term “nanoparticle,” as used herein, can refer to nano-scale particle between 1 and 500 nanometers (nm) in size. It is a small object that behaves as a whole unit with respect to its transport and properties. Average particle size of nanoparticle drug as defined herein is less than
10 about 500 nm.

As used herein, “therapeutically effective amount” means a dose which a particular pharmacological response obtained when the doses were administered to a large number of subjects in need of treatment. For example, with respect to the treatment of cancer, a
15 therapeutically effective amount can refer to the amount of an anticancer agent that decreases the rate of tumor growth, decreases tumor mass, decreases the number of metastases, increases time to tumor progression, increase tumor cell apoptosis, or increases survival time by at least 5% to 10%, at least 15% to 20%, at least 25% to 30%, at least 35% to 40%, at least 45% to 50%, at least 55% to 60%, at least 65% to 70%, at least 75% to 80%, at least 85% to 90%, at least 95% to 100%.

As used herein, “treatment refers to any of (i) the prevention of a pathogen or disorder in question (e.g. cancer or a pathogenic infection, as in a traditional vaccine), (ii) the reduction or elimination of symptoms, (iii) the substantial or complete elimination of the pathogen or disorder in question and (iv) approach for obtaining beneficial or desired results including clinical results. Treatment
20 may be effected prophylactically (prior to arrival of the pathogen or disorder in question) or therapeutically (following arrival of the same).

As used herein, by “pharmaceutically acceptable” is meant a material that is not biologically or otherwise undesirable, e.g., the material may be incorporated into a pharmaceutical composition administered to a patient without causing any significant undesirable biological effects or
30 interacting in a deleterious manner with any of the other components of the composition in which it is contained. Pharmaceutically acceptable carriers or excipients have preferably met the required standards of toxicological and manufacturing testing and/or are included on the Inactive Ingredient Guide prepared by the U.S. FDA. The term “excipient” refers to any essentially accessory
35 substance that may be present in the pharmaceutical composition.

The term “lipid” as used herein, refers to any lipophilic compound. Non-limiting examples of lipid compounds include fatty acids and their derivatives, including straight chain, branched chain, saturated and unsaturated fatty acids, carotenoids, terpenes, bile acids, and steroids, natural or synthetic lipids, fats, mono-, di- and triglycerides, fatty alcohols, waxes, cholesterol and cholesterol derivatives, aliphatic and aromatic esters. Further examples of lipids includes but not limited to Fatty acids such as Dodecanoic acid, Myristic acid, Palmitic acid, Stearic acid, Monoglycerides such as Glyceryl monostearate, Glyceryl hydroxystearate, Glyceryl behenate, Diglycerides such as Glyceryl palmitostearate, Glyceryl dibehenate, Triglycerides, Waxes, Liquid lipids, Cationic lipids, Egg phosphatidylcholine (Lipoid E PC S), Soy phosphatidylcholine (Lipoid S 100, Lipoid S PC), Hydrogenated egg phosphatidylcholine (Lipoid E PC-3), Hydrogenated soy phosphatidylcholine (Lipoid S PC-3, Phospholipon 80 H, Phospholipon 90 H), Egg phospholipid (Lipoid E 80, Lipoid E 80 S) or Soy phospholipid (Lipoid S 75).

Useful polymers for forming the nanoparticless described herein include homopolymers, copolymers and polymer blends, both natural and synthetic. Such polymers may be derived, for example, from the following: polyhydroxybutyric acid (also known as polyhydroxybutyrate); polyhydroxy valeric acid (also known as polyhydroxyvalerate); polyglycolic acid (PGA) (also known as polyglycolide); polylactic acid (PLA) (also known as polylactide); polydioxanone; polycaprolactone; polyorthoester; polycyanoacrylates, polyanhydrides; and combinations thereof. More typical are poly(α -hydroxy acids), such as poly(L-lactide), poly(D,L-lactide) (both referred to as PLA herein), poly(hydroxybutyrates), copolymers of lactide and glycolide, such as poly(D,L-lactide-co-glycolides) or copolymers of D,L-lactide and caprolactone.

Other agents used in the preparation of lipid nanoparticles include surface modifiers, counterions such as organic salts, ionic polymers. Examples are Dipalmitoyl-phosphatidyl-ethanolamine conjugated with polyethylene glycol 2000 (DPPE-PEG2000), Distearoyl-phosphatidyl-ethanolamine-N-poly(ethylene glycol) 2000 (DSPE-PEG2000), Stearic acid-PEG 2000 (SA-PEG2000), α -methoxy-PEG 2000-carboxylic acid- α -lipoamino acids (mPEG2000-C-LAA18), α -methoxy-PEG 5000-carboxylic acid- α -lipoamino acids (mPEG5000-C-LAA18), Mono-octyl phosphate, Mono-hexadecyl phosphate, Mono-decyl phosphate, Sodium hexadecyl phosphate.

The term “monoclonal antibody” refers to an antibody that is derived from a single cell clone, including any eukaryotic or prokaryotic cell clone, or a phage clone, and not the method by which it is produced. Thus, the term “monoclonal antibody” as used herein is not limited to antibodies produced through hybridoma technology. Non-limiting examples of monoclonal antibody include RITUXAN® (Rituximab) AVASTIN™ (Bevacizumab), HERCEPTIN® (Trastumab) cetuximab, panitumumab, infliximab, adalimumab, efalizumab, ipilimumab, tremelimumab, adecatumumab,

alemtuzumab, ranibizumab, tositumomab, ibritumomab tiuxetan, infliximab, figitumumab, nivolumab and durvalumab.

5 The term “targeting ligand” refers to an agent, which when tagged or attached to the surface of the nanoparticles, helps or directs the nanoparticles reach to its site of action in the brain of the subject. In the context of present invention, the targeting ligand includes monoclonal antibody (e.g., bevacizumab, nivolumab or durvalumab), antibody fragments, growth factors, transferrin, peptides (e.g., octreotide and tLyp-1 peptide), aptamers, polysaccharides (e.g., hyaluronic acid), and small biomolecules (e.g., folic acid, galactose, bisphosphonates, and biotin). Preferably, the
10 targeting ligand is a monoclonal antibody such as bevacizumab.

The term “linker” as used herein, refers to any linker moieties that are capable of conjugating two fragments or molecules together under various conditions. Particularly, N-γ-maleimidobutyryl-oxy succinimide ester or 2-Iminothiolane or combination thereof used as a linker. Conventional
15 polymers applied in conjugates include poly(ethylene glycol) (PEG), polyethylenimine (PEI), dextran, hyaluronic acid, poly(acrylic acid) (PAA), poly(e-caprolactone) (PCL), polylactide (PLA) and poly(lactide-co-glycolide) (PLGA).

The term “surfactant” comes from the phrase “surface active agent”. Surfactants accumulate at
20 interfaces (e.g., at liquid-liquid, liquid-solid and/or liquid-gas interfaces) and change the properties of that interface. As used herein, surfactants include Ionic surfactants, amphoteric surfactants, Non-ionic surfactants or Co-surfactants. Examples of surfactant includes but not limited to Tween 20, Tween 80, Span 20, Span 80, Span 85, Tyloxapol, Poloxamer 188, Poloxamer 407, Poloxamine 908, Brij78, Tego care 450, Solutol HS15.

25 As used herein, a “cryoprotective agent” is an agent that protects a composition from experiencing adverse effects upon freezing and thawing. For example, in the present invention, cryoprotective agents may be added to prevent substantial nanoparticle agglomeration from occurring when the lyophilized compositions of the invention are resuspended.

30 As used herein, “entrapment efficiency” meant a % drug that is successfully entrapped/adsorbed into nanoparticles. It is calculated as follows: % entrapment efficiency = [(Drug added - Free "unentrapped drug")/Drug added] *100. Example: If the % entrapment efficiency is 50%, it means that 50% of your drug is entrapped into the nanoparticles.

35 The term “polydispersity index” used herein refers to a measure of the distribution of molecular mass in a given polymer sample or the distribution of particle sizes in a particulate sample.

Particle size and polydispersity index for the developed nanoparticles were performed using Malvern zetasizer instrument 90S (Malvern Instruments Ltd., United Kingdom). Particle size indicated the average size acquired by the nanoparticles when dispersed in water whereas polydispersity index depicted the homogeneity in distribution of these particles. Nanoparticle suspension was placed in a polystyrene cuvette. The cuvette was then placed in the pathway of scattered light to record the fluctuations in the intensity of the light due to the presence of the particles. The intensity of the scattered light was measured at 900 using Malvern Software to give the hydrodynamic diameter and the polydispersity index.

Zeta potential measurements were made to determine surface charge acquired by the particles in solution. Zeta potential is the electric potential in the interfacial double layer [DL] at the location of the splitting plane versus a point in the bulk fluid away from the interface. Zeta potential is the potential difference between the dispersion medium and the stationary layer of fluid attached to the dispersed particle. The significance of zeta potential is that its value can be related to the stability of colloidal dispersions. The zeta potential indicates the degree of repulsion between adjacent, similarly charged particles in dispersion. Measurements were carried out using Malvern zetasizer.

The composition may be for any route of drug administration, e.g. oral, rectal, buccal, nasal, vaginal, transdermal (e.g. patch technology); parenteral, intravenous, intramuscular or subcutaneous injection; intracisternal, intravaginal, intraperitoneal, local (powders, ointments or drops) or as a buccal or nasal spray. Preferably the composition is an injectable composition or injectable formulation. Injectable composition can be supplied in any suitable container, e.g. ampoule, vial, pre-filled syringe, injection device (e.g. single use injection device such as that sold under the mark Uniject by Becton Dickinson), injection cartridge, ampoule, (multi-) dose pen and the like. Preferably the composition is for intramuscular administration (e.g. intramuscular injection) or intravenous administration (e.g., i.v. injection).

In another general embodiment, there is provided a method of treating cancer in a subject in need thereof, said method comprising administering parenterally to the subject a pharmaceutical composition comprising albumin-lipid hybrid nanoparticles having average particle size of less than about 300 nm and polydispersity index of less than about 0.5, wherein the nanoparticles contain (i) a therapeutically effective amount of an anti-cancer agent, (ii) human serum albumin, (iii) a lipid component comprising cholesterol and lecithin and optionally, (iv) a targeting ligand, wherein the molar ratio of the human serum albumin to the lipid component ranges from about 1:2 to about 1:20. In another embodiment, the parenteral administration comprises intravenous, intra-arterial, intramuscular, subcutaneous, intra-theal, and intra-cerebral injection. In another embodiment, the cancer comprises lung cancer, prostate cancer, colorectal cancer, stomach cancer,

brain cancer, lymphoma, leukemia, neuroblastoma, and ovarian cancer. In another embodiment, the brain cancer comprises astrocytoma, glioblastoma, glioma, ependymoma, papilloma, neurofibroma, meningioma, glioblastoma, sarcoma, lipoma, histocytoma, gangliocytoma, neurocytoma, medulloblastoma, medulloepithelioma, melanoma, melanocytoma, lymphoma,
5 atypical tumors and metastatic tumors.

In another general embodiment there is provided a method of treating brain cancer in a subject in need thereof, said method comprising administering parenterally to the subject a pharmaceutical composition comprising albumin-lipid hybrid nanoparticles having average particle size of less
10 than about 300 nm and polydispersity index of less than about 0.5, wherein the nanoparticles contain (i) a therapeutically effective amount of an anti-cancer agent, (ii) human serum albumin, (iii) a lipid component comprising cholesterol and lecithin, and (iv) a targeting ligand; wherein the molar ratio of the human serum albumin to the lipid component ranges from about 1:2 to about 1:20. In another embodiment, the targeting ligand is tagged on surface of the nanoparticles for
15 targeting the anticancer agent to its site of action. In another embodiment, the ligand comprises monoclonal antibody such as bevacizumab, nivolumab or durvalumab, antibody fragments, growth factors, transferrin, peptides such as octreotide and tLyp-1 peptide, aptamers, polysaccharides, and small biomolecules such as folic acid, galactose, bisphosphonates, and biotin.

EXAPMLES

EXAMPLE 1: Pharmaceutical Composition

Table 1

Sr. No.	Ingredients	Quantity
1	Etoposide	105 mg
2	Polyethylene glycol 400	5 ml
3	Tween 80	5 g
4	Human serum albumin	6 ml
5	Distilled water	98 ml
6	Ethanol	20 ml
7	Cholesterol	20 mg
8	Egg Lecithin	75 mg
9	Soybean Lecithin	46 mg
10	Sucrose	8%

5 Manufacturing Procedure:

Etoposide and PEG 400 were added and mixed well then Tween 80 was added until transparent solution was obtained. Human serum albumin was added and mixed well to form gel. Water was added in this solution and sonicated for 15-20 minutes. Ethanolic solution of lipids were prepared and added to etoposide containing aqueous solution of drop wise using syringe under magnetic stirrer and this mixture was kept for 30 min under magnetic stirring. After mixing, the solution was heated in boiling water bath till it reaches 66 °C. Temperature control at this step is very critical should not exceed the said temperature. The solution was then subjected to high-pressure homogenisation for reducing the particle size at 20 °C throughout homogenization process. Sucrose was added and nanoparticles were then lyophilized in freeze dryer.

15

EXAMPLES 2-4: Pharmaceutical Compositions

Table 2

Sr. No.	Ingredients	Quantity		
		Example 2	Example 3	Example 4
1	Etoposide	100 mg	100 mg	100 mg
2	Polyethylene glycol 400	3-6 ml	4 mL	4 mL
3	Tween 80	2-6 gm	-	-
4	Poloxamer	-	10 gm	-
5	Solutol HS15	-	-	8 gm
6	Human serum albumin	4-5 ml	5 mL	5 mL

7	Distilled water	90-100 ml	98 mL	98 mL
8	Ethanol	10-30 ml	20 mL	20 mL
9	Cholesterol	16-18 mg	20 mg	20 mg
10	Egg Lecithin	60-80 mg	75 mg	75 mg
11	Soybean Lecithin	40-48 mg	44 mg	44 mg
12	Sucrose	6-9 %	7%	7%

Manufacturing Procedure: Manufacturing procedure is similar as described for Example 1.

EXAMPLES 5-6: Pharmaceutical Compositions

5 Table 3

Sr. No.	Ingredients	Quantity	
		Example 5	Example 6
1	Etoposide	110 mg	100 mg
2	Polyethylene glycol 400	3-4 ml	4 mL
3	Tween 20	3-5 g	4 g
4	Human serum albumin	5-6 ml	5 mL
5	Distilled water	95-98 ml	95 mL
6	Ethanol	15-25 ml	20 mL
7	Cholesterol	18-22 mg	18 mg
8	Egg lecithin	70-80 mg	70 mg
9	Soybean lecithin	45-50 mg	42 mg
10	Sucrose	6-7%	7%
Surface modification of nanoparticles by tagging a targeting ligand			
11	Bevacizumab	64 mg	62.66 mg
12	N- γ -maleimidobutyryl-oxysuccinimide ester	5 mg	4.66 mg
13	2-Iminothiolane	28 mg	25 mg

Manufacturing Procedure: Etoposide and PEG 400 were added and mixed well then Tween 80 was added until transparent solution was obtained. Human serum albumin was added and mixed well to form gel. Water was added in this solution and sonicated for 15-20 minutes. Ethanolic solution of lipids were prepared and added to etoposide containing aqueous solution of drop wise using syringe under magnetic stirrer and this mixture was kept for 30 min under magnetic stirring. After mixing, the solution was heated in boiling water bath till it reaches 66 °C. Temperature control at this step is very critical should not exceed the said temperature. The solution was then subjected to

high-pressure homogenisation for reducing the particle size at 20 °C throughout homogenization process. Sucrose was added and nanoparticles were then lyophilized in freeze dryer. Surface modification of lyophilized nanoparticles was done by bevacizumab and further lyophilized in freeze dryer for better stability.

5

Table 4: Table 4 shows particle size, polydispersity index, zeta potential, and entrapment efficiency of pharmaceutical composition exemplified in Examples 1 to 5.

Table 4

Example	Particle size (nm)	Polydispersity index	Zeta potential (mV)	Entrapment efficiency (%)
Example 1	111.90	0.268	-7.12	77.38
Example 2	108.8	0.275	-6.78	75.93
Example 3	106.4	0.264	-6.80	76.79
Example 4	97.39	0.243	22.8	80.54
Example 5	118.76	0.236	11.36	73.66

10 Table 5 shows in-vitro release data of pharmaceutical composition of Example 2 and Example 5

Table 5

Time in hours	% Drug released Example 2	% Drug released Example 5
5	66.5	55.2
10	85.3	85.1
25	91.7	87.6
35	96.3	92.6

EXAMPLE 7: Comparative analysis of different molar ratio of human serum albumin: lipid

Effect of HSA: Lipid molar ratio on particle size and polydispersity index of nanoparticles before and after lyophilisation was studied. Different HSA:Lipid molar ratio were tried for optimization were 1:1, 1:3, 1:6, 1:12, 1:24 and 1:48.

15

Table 6

Molar ratio HSA:Lipid	Particle Size		Polydispersity Index	
	Before lyophilisation	After lyophilisation	Before lyophilisation	After lyophilisation
1:1	101	102	0.24	0.25
1:3	103	100	0.24	0.24

1:6	95	90	0.25	0.29
1:12	100	98	0.25	0.28
1:24	94	210	0.26	0.47
1:48	100	240	0.3	0.33

The molar ratio of HSA: Lipid 1:1 to 1:12 retained particle size and polydispersity index after lyophilisation however HSA: Lipid ratio greater than 1:12 i.e. 1:24 and 1:48 led to increase in particle size and polydispersity index after lyophilisation. It was observed that HSA: Lipid ratio higher than 1:12 M led to increased particle size after lyophilisation.

EXAMPLE 8: In-vitro cell lines studies and in-vitro permeability studies

Cytotoxicity can be defined as the adverse effects deriving from reactions with structures and/or processes crucial for cell maintenance such as proliferation, survival, and normal biochemical/physiology. The assay was performed to assess in-vitro efficacy of nanoparticles in treating glioblastoma. In this study, two cell lines including U87MG (likely glioblastoma cell line) and SVGp12 (normal human brain cell lines) were used to evaluate toxicity and safety of etoposide loaded nanoparticles.

The cell viability was measured by Presto blue assay. The U87MG and SVGp12 cells were seeded in 96 well plate at a density of 2500 cells per well. After 24 h, the medium was substituted with drug solution and nanoparticles with different concentration of etoposide in the media, followed by incubation for 24h, 48h and 72h. The cell viability was measured using 10 μ l of presto blue in each well and reading plate at 535nm excitation and 615 nm emission wavelength.

Table 7 shows in-vitro cytotoxicity on A] U87MG cells; IC₅₀ values and B] SVGp12 cells; IC₅₀ values at different time points.

Table 7

A] U87MG cells			
Formulations	24 hr	48 hr	72 hr
Etoposide solution	Above 50 μ g/ml	8.5 μ g/ml	1 μ g/ml
Etoposide Nanoparticles (ENP)	Above 50 μ g/ml	38 μ g/ml	13 μ g/ml
Etoposide Nanoparticles tagged with bevacizumab (BENP)	Above 50 μ g/ml	22 μ g/ml	1.75 μ g/ml
B] SVGp12 cells			
Etoposide solution	Above 50 μ g/ml	7.5 μ g/ml	1 μ g/ml
Etoposide Nanoparticles (ENP)	Above 50 μ g/ml	42 μ g/ml	16 μ g/ml
Etoposide Nanoparticles tagged with bevacizumab (BENP)	Above 50 μ g/ml	24 μ g/ml	6 μ g/ml

EXAMPLE 9: In-vitro cellular uptake studies

This study was conducted to demonstrate uptake of nanoparticles in different cell lines. Cellular uptake studies were conducted on four cell lines U87MG (glioblastoma cell line), SVGp12 (normal human brain cell line), HBMEC (human brain microvasculature endothelial cells), RAW macrophage cell line. Cells were seeded in 6 well plates at a density of 30×10^4 cells per well and cultured for 24h. The cells were then incubated with the Rh123 labeled nanoparticles for the period of 30 min, 1h, 4h and 24h. At the experimental end point, the cells were washed with PBS three times, and trypsinised to detach from surface. The cells were then collected, centrifuged at 840 rpm for 5 min. Supernatant was removed and cell pellet were re-suspended in 300 μ l PBS and incubated with 30 μ l of propidium iodide (50 μ g/ml) and analyzed by flow cytometer.

Table 8 shows bevacizumab tagged etoposide loaded lipid-albumin hybrid nanoparticles (BENP) were up taken in U87MG (cancer cell lines) more than SVGp12 (normal brain cells) and RAW (macrophages) cell lines demonstrating their targetability

Table 8

Formulation	Time	U87MG	SVGp12	RAW
		Mean Fluorescence integrity (AU)		
Etoposide Nanoparticles (ENP)	1hr	741	614	382
	4hr	3580	3460	1186
	24hr	8526	7222	1691
Etoposide Nanoparticles tagged with bevacizumab (BENP)	1hr	850	539	486
	4hr	5044	2655	1217
	24hr	13075	8664	1764

Table 9 shows nanoparticles were up taken in U87MG more than HBMEC cell line.

Table 9

Formulation	Time	U87MG	HBMEC cells (Endothelial cells)
		Mean Fluorescence integrity (AU)	
Etoposide Nanoparticles (ENP)	30 min	168	120
	1 hr	316	227
	4 hr	1074	884
	24 hr	3446	2961
Etoposide Nanoparticles tagged with bevacizumab (BENP)	30 min	218	138
	1 hr	610	255
	4 hr	1672	1079
	24 hr	4269	3338

Table 10 shows higher uptake of BENP than ENP in U87MG cells again demonstrating target ability of BENP.

Table 10

Formulation	Time	Uptake on U87MG cells (Mean Fluorescence integrity (AU))
Etoposide Nanoparticles (ENP)	30 min	316
	1 hr	741
	4 hr	3580
	24 hr	8526
Etoposide Nanoparticles tagged with bevacizumab (BENP)	30 min	385
	1 hr	850
	4 hr	5044
	24 hr	13075

5

EXAMPLE 10: Confirmation study for cellular uptake

U87MG cells were seeded in 6 well plates at a density of 30×10^4 cells per well and cultured for 24h. The cells were then treated with inhibitors and nanoparticles. Briefly, Nystatin (30 μ g/ml) for caveolae dependent, sucrose (0.45M) for clathrin dependent, Cytochalasin B (5 μ g/ml) for phagocytosis, phlorentine (non-specific fatty acid uptake inhibitor) and bevacizumab (VEGF inhibitor). After 30 min of respective inhibitor treatment, cells were exposed to Rh123 tagged nanoparticles for 1h. To inhibit endocytosis process, cells were incubated at 4 $^{\circ}$ C for 30 min instead of 37 $^{\circ}$ C before nanoparticle treatment. Both nanoparticles etoposide nanoparticles (ENP) and BENP showed lower uptake at 4 $^{\circ}$ C and in presence of nystatin and sucrose as inhibitor, indicating that nanoparticles were up taken by energy dependent active process and by caveolae and clathrin dependent endocytosis process (Table 5). In presence of Bevacizumab, BENP showed fewer uptakes than ENP. These results showed that BENP were also up taken by receptor mediated endocytosis.

10

15

Table 11

Inhibitors	ENP Relative Cellular Uptake (%)	BENP Relative Cellular Uptake (%)
None	100	100
4 $^{\circ}$ C	31	28
Nystatin	43	42
Sucrose	34	58
Cytochalasin B	84	96

Phlorentine	109	105
Bevacizumab	93	58

EXAMPLE 11: In-vitro permeability studies using BBB model

TEER values after nanoparticle incubation were compared with control where tri-culture was incubated with media. While control TEER values were stable throughout experiment from 0 h to 24 hr. There was decrease in TEER values up to 1.5 h when treated with nanoparticles and then TEER values were restored back to initial value after 2h and then were maintained till 24 hr. The decrease in TEER value indicated opening of tight junctions in BBB because of nanoparticles. This allowed nanoparticle passage by passive diffusion. The transient opening of BBB was then restored and maintained till 24 hr.

Table 12 shows TEER values at different time points after addition of nanoparticles in the apical side of the BBB model (A to B) and comparison with control

Table 12

Time (h)	Control A to B	ETP NP A to B
TEER Values		
0	263	240
0.25	268	174
0.5	266	169
0.75	264	165
1	259	161
1.5	254	182
2	253	219
4	239	243
6	226	221
8	233	229
16	231	227
24	214	213

Table 13 shows TEER values at different time points after addition of nanoparticles in the basolateral side of the BBB model (B to A) and comparison with control

Table 13

Time (hr)	Control B to A	ETP NP B to A
	TEER Values	
0	232	257
0.25	223	170
0.5	237	169
0.75	237	169
1	240	168
1.5	265	178
2	244	225
4	245	225
6	243	222
8	222	222
16	241	234
24	228	215

EXAMPLE 12: % Permeability Data

Determination of permeability of nanoparticles by Florescence of Rh 123 tagged nanoparticles and etoposide content by HPLC method gave same results confirming the ruggedness of results.

Table 14 shows the % permeability of ENP by fluorescence and HPLC analysis and % permeability of etoposide by HPLC analysis at different time points.

Table 14

Time (h)	ETP NP (Fluorescence)	ETP NP (HPLC)	Etoposide (HPLC)
0	0	0	0
2	24.38	29.46	5.37
4	35.00	35.68	10.85
6	39.46	38.51	16.01
8	49.16	59.55	28.10
24	56.14	54.26	28.03

Table 15 shows the Papp ($\times 10^{-6}$ cm/sec) values of ENP by fluorescence and HPLC analysis and Papp values of etoposide by HPLC analysis at different time points.

Table 15

Time (hr)	ETP NP (Fluorescence)	ETP NP (HPLC)	Etoposide (HPLC)
0	0	0	0

2	21	22.3	2.99
4	15.1	16.0	3.02
6	11.2	12.1	2.97
8	10.5	11.3	3.9
24	3.92	4.9	0.60

Etoposide free drug showed markedly less permeability. ENP showed significantly higher permeability than etoposide (ETP). There was 7.45 times increased in permeability of drug due to encapsulation in nanoparticles.

- 5 Table 16 shows the comparison of Papp ($\times 10^{-6}$ cm/sec) value of etoposide pure drug and Etoposide nanoparticles at 2hr.

Table 16

Parameters	Etoposide Pure drug	Etoposide Nanoparticles
Papp (A to B) ($\times 10^{-6}$ cm/sec) at 2 hr	2.99 $\pm$ 0.11	22.3 $\pm$ 0.43

- 10 Table 17 shows that BENP have more permeability from A to B than B to A and there was no active efflux.

Table 17

Time (h)	Avg. B to A	Avg. A to B	Efflux ratio
0	0	0	0
0.25	1.78	1.093	1.6285
0.5	3.57	1.4159	2.5213
0.75	4.39	1.887	2.3264
1	3.89	3.896	0.9984
1.5	7.02	7.02	0.9998
2	7.62	19.6	0.3887
4	8.9	10.2	0.8725
6	4.96	6.27	0.7910
8	4.38	6.612	0.6624
16	5.66	5.884	0.9619
24	4.01	4.332	0.9256

Nanoparticles showed more permeability than etoposide solution. Functionalization of nanoparticles ENP with bevacizumab did not much reduce its permeability though was lower than

ENP. Table 18 shows the comparison of Papp values of etoposide solution, ENP and BENP at 2 hr.

Table 18

Papp values	Etoposide Solution	ENP	BENP
Papp ($\times 10^{-6}$ cm/sec)	2.99	22.3	19.6

CLAIMS

We claim:

1. A pharmaceutical composition comprising albumin-lipid hybrid nanoparticles having average particle size of less than about 500 nm and polydispersity index of less than about 0.6, wherein the nanoparticles contain (i) a therapeutically effective amount of an anti-cancer agent, (ii) human serum albumin, (iii) a lipid component comprising cholesterol and lecithin, and optionally, (iv) a targeting ligand; wherein the molar ratio of the human serum albumin to the lipid component ranges from about 1:1 to about 1:20.
2. The pharmaceutical composition of claim 1, wherein the anti-cancer agent comprises alkylating agents, antimetabolites, antibiotics, microtubule inhibitors, hormones and their antagonists, and cytotoxic antineoplastic agents.
3. The pharmaceutical composition of claim 1, wherein the anti-cancer agent comprises melphalan, hexamethyl melamine, etoposide, carmustine, lomustine, temozolomide, dianhydrogalactitol, veliparib, eflornithine, thiotepa, cytarabin, idatrexate, trimetrexate, dacarbazine, L-asparaginase, camptothecin, topotecan, bicalutamide, flutamide, leuprolide, sorafenib, sunitinib, erlotinib, imatinib, lapatinib, gefitinib, brivanib, raltitrexed, doxorubicin, fluorouracil, paclitaxel, docetaxel, vinblastin, vindesine, vinorelbine, carboplatin, oxaloplatin, cyclophosphamide, mechlorethamine, chlorambucil, amsacrine, teniposide, irinotecan, bleomycin, mitomycin, plicamycin, busulfan, fosfamide, methotrexate, 6-mercaptopurine, 6-thioguanine, 5-fluorodeoxyuridine, fludarabine, 2-chlorodeoxyadenosine, 2-deoxycoformycin, genicitabine, dactinomycin, daunorubicin, mitoxantrone, chlorotrianisene, estramustine, toremifene, zoladex, and canertinib.
4. The pharmaceutical composition of claim 1, wherein the nanoparticles have average particle size of less than about 400 nm.
5. The pharmaceutical composition of claim 1, wherein the nanoparticles have average particle size of less than about 300 nm.
6. The pharmaceutical composition of claim 1, wherein the nanoparticles have a polydispersity index of less than about 0.5.
7. The pharmaceutical composition of claim 1, wherein the molar ratio of the human serum albumin to the lipid component in the nanoparticles ranges from about 1:2 to about 1:15.
8. The pharmaceutical composition of claim 1, wherein the molar ratio of the human serum albumin to the lipid component in the nanoparticles ranges from about 1:4 to about 1:12.
9. The pharmaceutical composition of claim 1, wherein the nanoparticles have anti-cancer agent entrapment efficiency of more than about 50%.
10. The pharmaceutical composition of claim 1, wherein the lecithin comprises egg phospholipids, soybean phospholipids, hydrogenated phospholipids, synthetic phospholipids, and PEGylated phospholipids.

11. The pharmaceutical composition of claim 1, wherein the composition further comprises at least one pharmaceutically acceptable excipient selected from a surfactant, cryoprotectant or combination thereof.
12. A pharmaceutical composition comprising albumin-lipid hybrid nanoparticles having
5 average particle size of less than about 300 nm and polydispersity index of less than about 0.5, wherein the nanoparticles contain (i) a therapeutically effective amount of etoposide, (ii) human serum albumin, (iii) a lipid component comprising cholesterol and lecithin, and optionally, (iv) a targeting ligand; wherein the molar ratio of the human serum albumin to the lipid component ranges from about 1:2 to about 1:15.
- 10 13. The pharmaceutical composition of claim 1 or 12 for use in the treatment of a cancer in a subject.
14. A method of treating cancer in a subject in need thereof, said method comprising administering parenterally to the subject a pharmaceutical composition comprising albumin-lipid hybrid nanoparticles having average particle size of less than about 300 nm
15 and polydispersity index of less than about 0.5, wherein the nanoparticles contain (i) a therapeutically effective amount of an anti-cancer agent, (ii) human serum albumin, (iii) a lipid component comprising cholesterol and lecithin and optionally, (iv) a targeting ligand, wherein the molar ratio of the human serum albumin to the lipid component ranges from about 1:2 to about 1:20.
- 20 15. The method of claim 14, wherein the parenteral administration comprises intravenous, intra-arterial, intramuscular, subcutaneous, intra-theal, and intra-cerebral injection.
16. The method of claim 14, wherein the cancer comprises lung cancer, prostate cancer, colorectal cancer, stomach cancer, brain cancer, lymphoma, leukemia, neuroblastoma, and ovarian cancer.
- 25 17. The method of claim 16, wherein the brain cancer comprises astrocytoma, glioblastoma, glioma, ependymoma, papilloma, neurofibroma, meningioma, glioblastoma, sarcoma, lipoma, histiocytoma, gangliocytoma, neurocytoma, medulloblastoma, medulloepithelioma, melanoma, melanocytoma, lymphoma, atypical tumors and metastatic tumors.
- 30 18. A method of treating brain cancer in a subject in need thereof, said method comprising administering parenterally to the subject a pharmaceutical composition comprising albumin-lipid hybrid nanoparticles having average particle size of less than about 300 nm and polydispersity index of less than about 0.5, wherein the nanoparticles contain (i) a therapeutically effective amount of an anti-cancer agent, (ii) human serum albumin, (iii) a
35 lipid component comprising cholesterol and lecithin, and (iv) a targeting ligand; wherein the molar ratio of the human serum albumin to the lipid component ranges from about 1:2 to about 1:15.

19. The method of claim 18, wherein the targeting ligand is tagged on surface of the nanoparticles for targeting the anticancer agent to its site of action.
20. The method of claim 19, wherein the ligand comprises monoclonal antibody such as bevacizumab, nivolumab or durvalumab, antibody fragments, growth factors, transferrin,
5 peptides such as octreotide and tLyp-1 peptide, aptamers, polysaccharides, and small biomolecules such as folic acid, galactose, bisphosphonates, and biotin.

INTERNATIONAL SEARCH REPORT

International application No
PCT/IB2018/057801

A. CLASSIFICATION OF SUBJECT MATTER

INV. A61K9/107 A61K9/19 A61K9/51 A61K31/7048
ADD.

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

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)

EP0-Internal, BIOSIS, WPI Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

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X	US 2011/166214 A1 (DESU HARI R [IN] ET AL) 7 July 2011 (2011-07-07) paragraphs [0019], [0025], [0045], [0054], [0056], [0058], [0059]; figure 1A; example 2; table 2.1 -----	1-11, 13-20
X	US 2004/082521 A1 (SINGH CHANDRA U [US]) 29 April 2004 (2004-04-29) paragraphs [0048], [0052], [0053], [0071]; examples 2,7 -----	1-11, 13-20
X	EP 2 243 495 A1 (UNIV SHENYANG PHARMACEUTICAL [CN]; HANGZHOU YUHONG PHARMACEUTICAL [CN]) 27 October 2010 (2010-10-27)	1-11, 13-20
Y	paragraphs [0024], [0034], [0035], [0038] - [0044], [0050]; examples 30,31 ----- -/--	1-20



Further documents are listed in the continuation of Box C.



See patent family annex.

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C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
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