The attending clinician, in judging whether treatment is effective at the dosage administered, will consider the general well-being of the subject as well as more definite signs such as relief of disease-related symptoms, inhibition of tumor growth, actual shrinkage of the tumor, or inhibition of metastasis. Size of the tumor can be measured by standard methods such as radiological studies, e.g., CAT or MRI scan, and successive measurements can be used to judge whether or not growth of the tumor has been retarded or even reversed. Relief of disease-related symptoms such as pain, and improvement in overall condition can also be used to help judge effectiveness of treatment.

Cardiovascular disease

The disclosed methods may be useful in the prevention and treatment of cardiovascular disease. Cardiovascular diseases that can be treated or prevented using polymer-agent conjugates, particles, compositions and methods described herein include cardiomyopathy or myocarditis; such as idiopathic cardiomyopathy, metabolic cardiomyopathy, alcoholic cardiomyopathy, drug-induced cardiomyopathy, ischemic cardiomyopathy, and hypertensive cardiomyopathy. Also treatable or preventable using polymer-agent conjugates, particles, compositions and methods described herein are atheromatous disorders of the major blood vessels (macrovascular disease) such as the aorta, the coronary arteries, the carotid arteries, the cerebrovascular arteries, the renal arteries, the iliac arteries, the femoral arteries, and the popliteal arteries. Other vascular diseases that can be treated or prevented include those related to platelet aggregation, the retinal arterioles, the glomerular arterioles, the vasa nervorum, cardiac arterioles, and associated capillary beds of the eye, the kidney, the heart, and the central and peripheral nervous systems. The polymer-agent conjugates, particles, compositions and methods described herein may also be used for increasing HDL levels in plasma of an individual.

Yet other disorders that may be treated with polymer-agent conjugates, particles, compositions and methods described herein include restenosis, e.g., following coronary intervention, and disorders relating to an abnormal level of high density and low density cholesterol.

The polymer-agent conjugate, particle or composition can be administered to a subject undergoing or who has undergone angioplasty. In one embodiment, the polymer-agent conjugate, particle or composition is administered to a subject undergoing or who has undergone angioplasty with a stent placement. In some embodiments, the polymer-agent conjugate, particle or composition can be used as a strut of a stent or a coating for a stent.

The polymer-agent conjugates, particles or compositions can be used during the implantation of a stent, e.g., as a separate intravenous administration, as coating for a stent or as the strut of a stent.

Stent

The polymer-agent conjugates, particles or compositions described herein can be used as or be part of a stent. As used herein, the term "stent" refers to a man-made 'tube' inserted into a natural passage or conduit in the body to prevent or counteract localized flow constriction. Types of stents include, e.g., coronary stent, urinary tract stent, urethral/prostatic stent, vascular stent (e.g., peripheral vascular stent, or stent graft), esophageal stent, duodenal stent, colonic stent, biliary stent, and pancreatic stent. Types of stents that can be used in coronary arteries include, e.g., bare-metal stent (BMS) and drug-eluting stent (DES). A coronary stent can be placed within the coronary artery during an angioplasty procedure.

Bare-metal stent (BMS)

In one embodiment, the polymer-agent conjugate, particle or composition can be used in combination with a BMS. As used herein, BMS refers to a stent without a coating that is made or a metal or combination of metals. BMS can be made from, e.g., stainless steel (e.g., BxVelocity™ stent, Express2 ™ stent, R stent™, and Matrix® coronary stent), cobalt-chromium alloy (e.g., Driver® coronary stent, ML Vision® stent, and Coronnium® stent), or nickel titanium (Nitinol® stent). A polymer-agent conjugate, particle or composition described herein can be used as a coating of a BMS, e.g., to coat the luminal and/or abluminal surface of a BMS.

Drug-eluting stent (DES)

In one embodiment, the polymer-agent conjugate, particle or composition can be a DES or can be part of a DES. As used herein, DES refers to a stent placed into a natural passage or conduit of the body (e.g., a narrowed coronary artery) that releases (e.g., slowly releases) one or more agents to treat one or more symptoms associated with the constricted flow to the passage or conduit and/or one or more effect caused by or associated with the stent. For example, the DES can release one (or more) agent that reduces or inhibits the migration and/or proliferation of vascular smooth muscle cells (SMCs), that promotes or increases epithelialization, that reduces or inhibits a hypersensitivity reaction, that reduces or inhibits inflammation, that reduces or inhibits thrombosis, that reduces the risk of restenosis, and/or that reduces or inhibits other unwanted effects due to the stent.

One type of DES includes a stent strut and a polymer, on which an agent is loaded. Thus, in one embodiment, a polymer-agent conjugate, particle or composition described herein can be used in combination with other polymeric struts (e.g., other biocompatible or bioasorbable polymers). For example, a polymer-agent conjugate, particle or composition described herein can be coated on a polymeric strut, e.g., on the luminal and/or abluminal surface of a polymeric strut.

In another embodiment, the polymer-agent conjugates, particles and compositions described herein can be used as a polymeric strut, with out without an additional polymer and/or agent.

In one embodiment, the rate of major adverse cardiac events (MACE) of a subject having a stent made of a polymer-agent conjugate, particle or composition described herein or a strut coated with a polymer-agent conjugate, particle or composition described herein is reduced by at least 10, 20, 30, 40, 50, 60, 70, 80, 90, 95% or more, as compared to the rate of MACE of a subject having a stent made of a different material (e.g., a metal or polymer) or a stent not coated or coated with a polymer and/or agent other than the polymer-agent conjugate, particle or composition. In another embodiment, the need for target vessel revascularization (TVR) of a subject having a stent made of a polymer-agent conjugate, particle or composition described herein or a strut coated with a polymer-agent conjugate, particle or composition described herein is reduced by at least 10, 20, 30, 40, 50, 60, 70, 80, 90,

95% or more, compared to the TVR of a subject having a stent made of a different material (e.g., a metal or polymer) or a stent not coated or coated with a polymer and/or agent other than the polymer-agent conjugate, particle or composition. In yet another embodiment, the rate for target lesion revascularization (TLR) of a subject having a stent made of a polymer-agent conjugate, particle or composition described herein or a strut coated with a polymer-agent conjugate, particle or composition described herein is reduced by at least 10, 20, 30, 40, 50, 60, 70, 80, 90, 95% or more, compared to the TLR of a subject having a stent made of a different material (e.g., a metal or polymer) or a stent not coated or coated with a polymer and/or agent other than the polymer-agent conjugate, particle or composition.

Agents

Agents that can be loaded onto a DES include, for example, antiproliferative agents, e.g., anticancer agents (e.g., a taxane (e.g., docetaxel, paclitaxel, larotaxel and cabazitaxel) and an anthracycline (e.g., doxorubicin); pro-endothelial cell agents, anti-restenotic agents; anti-inflammatory agents; statins (e.g., simovastatin); immunosuppresants (e.g., mycophenolic acid); somatostatin receptor agonists (e.g., angiopeptin); and dimethyl sulfoxide.

Exemplary anti-proliferative agents include, e.g., an anticancer agent, e.g., a taxane (e.g., docetaxel, paclitaxel, larotaxel and cabazitaxel) and an anthracycline (e.g., doxorubicin); and an immunosuppressive agent, e.g., a rapamycin analogue (e.g., everolimus, zotarolimus, biolimus), pimecrolimus, or tacrolimus.

One or more of the pro-endothelial agents can be loaded on the stents, e.g., to promote, accelerate or increase endothelial healing. Exemplary pro-endothelial agents include, e.g., agents that diminish platelet adhesion and/or fibrinogen binding (e.g., titanium-nitride-oxide or titanium-nitride), agents that capture endothelial progenitor cells (EPCs) (e.g., antibodies (e.g., anti-CD34 antibody) or peptides (e.g., integrin-binding cyclic Arg-Gly-Asp peptide)), or estradiol.

One or more of anti-restenotic agent can also be loaded on or in the stents, e.g., anti-inflammatory agents (e.g., dexamethasone), immunosuppressive agents (e.g., mycophenolic acid), antisense agents (e.g., an advanced six-ring morpholino

backbone c-myc antisense (AVI-4126)), inhibitors of vascular smooth muscle cell proliferation and/or tissue factor expression (e.g., 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA)-reductase-inhibitors (statins), simvastatin, angiopeptin or dimethyl sulfoxide (DMSO)), or anti-hyperlipidemic agents (e.g., probucol).

In one embodiment, the agent (or agents) is loaded on the luminal side of the stent. In another embodiment, the agent (or agents) is loaded on the abluminal side of the stent. In yet another embodiment, the agent (or agents) is loaded on both the luminal and abluminal sides of the stent. In another embodiment, an agent (or agents) is loaded on the luminal side of the stent and a different agent (or combination of agents) is loaded on the abluminal side of the stent. Thus, different agents (e.g., an anti-proliferation agent and a pro-endothelial agent) can be loaded on different sides (luminal or abluminal) of the stent, e.g., to allow for differential agent elution, or different agents can be loaded on the same side (luminal or abluminal side) of the stent, e.g., to allow for dual local agent elution.

In one embodiment, the agent is present at a concentration of at least about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 50, or 100 μg/mm. In one embodiment, more than about 50, 60, 70, 80, 90, 95, 99% of the agent is released over a period of one month. In one embodiment, the release of the agent (e.g., a pro-endothelial agent) is delayed for at least about 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 days. In one embodiment, the release of the agent sustains for at least 7, 14, 21, 28, 35, or 42 days.

Polymeric Stents

Stents described herein can be made of biocompatible and/or bioabsorbable polymers. A polymer-agent conjugate, particle or composition described herein can be the stent, the strut of a stent or the poly-agent conjugate, particle or composition can coat a strut made of a polymeric material.

An example of a biocompatible stent is the Endeavor Rsolute® stent. This system is composed of three elements: one hydrophobic polymer ('C10') to retain the drug and control drug release, another polymer ('C19') to provide improved biocompatibility, and finally (on the outer-most side of the stent) a polyvinyl pyrrolidinone (PVP) hydrophilic polymer which increases the initial drug burst and

further enhances biocompatibility. Thus, in one embodiment, the polymer-agent conjugate, particle or composition can be coated on an Endeavor Rsolute® stent. In other embodiments, a polymer-agent conjugate, particle or composition described herein can replace one or more of the elements of the Endeavor Rsolute® stent.

Bioabsorbable polymers (e.g., inert bioabsorbable polymer) can also be used in a DES, e.g., to reduce prothrombogenic potential and/or allow non-invasive imaging. In some embodiments, the bioabsorbable polymer has a degradation time of at least about 14, 21, 28, 35, 42, 49, 56, 63, 70 days.

Exemplary bioasorbable stents include, e.g., a polymeric stent (e.g., a poly-L-lactide stent, a tyrosine poly(desaminotyrosyl-tyrosine ethyl ester) carbonate stent, and a poly(anhydride ester) salicyclic acid stent). For example, Igaki-Tamai stent is constructed from a poly-L-lactic acid polymer and contains either the tyrosine kinase antagonist ST638 or paclitaxel. REVA® stent is a tyrosine poly(desaminotyrosyl-tyrosine ethyl ester) carbonate stent. It is radio-opaque and has slide and lock mechanism designed to allow for substantial reductions in stent-strut thickness. IDEAL™ stent is a poly(anhydride ester) salicyclic acid stent. Infinnium® stent is composed of two biodegradable polymers with different paclitaxel-release kinetics. Other exemplary bioasorbable stents include, e.g., BVS®, Sahajanand®, Infinnium®, BioMATRIX®, Champion®, and Infinnium®. In one embodiment, a polymer-agent conjugate, particle or composition described herein can be coated onto any of these bioabsorbable stents. In other embodiments, a polymer-agent conjugate, particle or composition described herein can replace one or more elements of one of these bioabsorbable stents.

Biosorbable Metallic Stents

The polymer-agent conjugates, particles and compositions described herein can be used to coat a bioabsorbable metallic stent. An exemplary bioabsorbable stent is the Absorbable Metal Stent (AMS®) which is an alloy stent made of 93% magnesium and 7% rare-earth metals.

Reservoir stents

As described herein, reservoir stents can be used, e.g., to decrease the "thickness" of the stent or reduce the unwanted effect due to microfragmentation of the polymer and/or the agent. For example, the drug can be loaded in one or more reservoirs or wells in the stent, compared to, e.g., more or less uniformly spread over the stent.

In one embodiment, a polymer-agent conjugate, particle or composition described herein is loaded in the reservoirs or wells located on the stent, e.g., the polymer-agent conjugate, particle or composition described herein is loaded in the reservoirs or wells located on the luminal side or the abluminal side of the stent. In yet another embodiment, the polymer-agent conjugate, particle or composition described herein is loaded in the reservoirs or wells located on both the luminal and abluminal sides of the stent.

In one embodiment, different agents (e.g., an anti-proliferation agent and a pro-endothelial agent) can be loaded into the reservoirs or wells on different sides (luminal or abluminal) of the stent, e.g., to allow for differential agent elution. In another embodiment, different agents can be loaded into adjacent reservoirs or wells of the same side (luminal or abluminal side) of the stent, e.g., to allow for dual local drug elution.

Strut

In one embodiment, the strut thickness is at least about 25, 50, 100, 150, 200, 250 μm. In another embodiment, the strut wideness is at least about 0.002, 0.004, 0.006, 0.008, or 0.01 inch. In yet another embodiment, the number of struts is at least about 4, 8, 12, 16, or 18 in its cross-section.

Various shapes of struts such as a zig zag coil, a ratchet log design, circumferential loops, etc. are known in the art and can be employed in the stents described herein.

In one embodiment, the strut can be made of a polymer-agent conjugate particle or composition described herein.

Combination therapy

In one embodiment, a polymer-agent conjugate, particle or composition described herein may be administered as part of a combination therapeutic with another cardiovascular agent including, for example, an anti-arrhythmic agent, an antihypertensive agent, a calcium channel blocker, a cardioplegic solution, a cardiotonic agent, a fibrinolytic agent, a sclerosing solution, a vasoconstrictor agent, a vasodilator agent, a nitric oxide donor, a potassium channel blocker, a sodium channel blocker, statins, or a naturiuretic agent.

In one embodiment, a polymer-agent conjugate, particle or composition may be administered as part of a combination therapeutic with an anti-arrhythmia agent. Anti-arrhythmia agents are often organized into four main groups according to their mechanism of action: type I, sodium channel blockade; type II, beta-adrenergic blockade; type III, repolarization prolongation; and type IV, calcium channel blockade. Type I anti-arrhythmic agents include lidocaine, moricizine, mexiletine, tocainide, procainamide, encainide, flecanide, tocainide, phenytoin, propafenone, quinidine, disopyramide, and flecainide. Type II anti-arrhythmic agents include propranolol and esmolol. Type III includes agents that act by prolonging the duration of the action potential, such as amiodarone, artilide, bretylium, clofilium, isobutilide, sotalol, azimilide, dofetilide, dronedarone, ersentilide, ibutilide, tedisamil, and trecetilide. Type IV anti- arrhythmic agents include verapamil, diltiazem, digitalis, adenosine, nickel chloride, and magnesium ions.

In another embodiment, a polymer-agent conjugate, particle or composition may be administered as part of a combination therapeutic with another cardiovascular agent. Examples of cardiovascular agents include vasodilators, for example, hydralazine; angiotensin converting enzyme inhibitors, for example, captopril; anti-anginal agents, for example, isosorbide nitrate, glyceryl trinitrate and pentaerythritol tetranitrate; antiarrhythmic agents, for example, quinidine, procainaltide and lignocaine; cardioglycosides, for example, digoxin and digitoxin; calcium antagonists, for example, verapamil and nifedipine; diuretics, such as thiazides and related compounds, for example, bendrofluazide, chlorothiazide, chlorothalidone, hydrochlorothiazide and other diuretics, for example, fursemide and triamterene, and sedatives, for example, nitrazepam, flurazepam and diazepam.

Other exemplary cardiovascular agents include, for example, a cyclooxygenase inhibitor such as aspirin or indomethacin, a platelet aggregation inhibitor such as clopidogrel, ticlopidene or aspirin, fibrinogen antagonists or a diuretic such as chlorothiazide, hydrochlorothiazide, flumethiazide, hydroflumethiazide, bendroflumethiazide, methylchlorthiazide, trichloromethiazide, polythiazide or benzthiazide as well as ethacrynic acid tricrynafen, chlorthalidone, furosemide, musolimine, bumetanide, triamterene, amiloride and spironolactone and salts of such compounds, angiotensin converting enzyme inhibitors such as captopril, zofenopril, fosinopril, enalapril, ceranopril, cilazopril, delapril, pentopril, quinapril, ramipril, lisinopril, and salts of such compounds, angiotensin II antagonists such as losartan, irbesartan or valsartan, thrombolytic agents such as tissue plasminogen activator (tPA), recombinant tPA, streptokinase, urokinase, prourokinase, and anisoylated plasminogen streptokinase activator complex, or animal salivary gland plasminogen activators, calcium channel blocking agents such as verapamil, nifedipine or diltiazem, thromboxane receptor antagonists such as ifetroban, prostacyclin mimetics, or phosphodiesterase inhibitors. Such combination products if formulated as a fixed dose employ the compounds of this invention within the dose range described above and the other pharmaceutically active agent within its approved dose range.

Yet other exemplary cardiovascular agents include, for example, vasodilators, e.g., bencyclane, cinnarizine, citicoline, cyclandelate, cyclonicate, ebumamonine, phenoxezyl, fiunarizine, ibudilast, ifenprodil, lomerizine, naphlole, nikamate, nosergoline, nimodipine, papaverine, pentifylline, nofedoline, vincamin, vinpocetine, vichizyl, pentoxifylline, prostacyclin derivatives (such as prostaglandin El and prostaglandin 12), an endothelin receptor blocking drug (such as bosentan), diltiazem, nicorandil, and nitroglycerin. Examples of cerebral protecting drugs include radical scavengers (such as edaravone, vitamin E, and vitamin C), glutamate antagonists, AMPA antagonists, kainate antagonists, NMDA antagonists, GABA agonists, growth factors, opioid antagonists, phosphatidylcholine precursors, serotonin agonists, Na^+/Ca^{2+} channel inhibitory drugs, and K^+ channel opening drugs. Examples of brain metabolic stimulants include amantadine, tiapride, and gamma-aminobutyric acid.

Examples of anticoagulants include heparins (such as heparin sodium, heparin potassium, dalteparin sodium, dalteparin calcium, heparin calcium, parnaparin sodium, reviparin sodium, and danaparoid sodium), warfarin, enoxaparin, argatroban, batroxobin, and sodium citrate. Examples of antiplatelet drugs include ticlopidine hydrochloride, dipyridamole, cilostazol, ethyl icosapentate, sarpogrelate hydrochloride, dilazep hydrochloride, trapidil, a nonsteroidal anti-inflammatory agent (such as aspirin), beraprostsodium, iloprost, and indobufene.

Examples of thrombolytic drugs include urokinase, tissue-type plasminogen activators (such as alteplase, tisokinase, nateplase, pamiteplase, monteplase, and rateplase), and nasaruplase. Examples of antihypertensive drugs include angiotensin converting enzyme inhibitors (such as captopril, alacepril, lisinopril, imidapril, quinapril, temocapril, delapril, benazepril, cilazapril, trandolapril, enalapril, ceronapril, fosinopril, imadapril, mobertpril, perindopril, ramipril, spirapril, and randolapril), angiotensin II antagonists (such as losartan, candesartan, valsartan, eprosartan, and irbesartan), calcium channel blocking drugs (such as aranidipine, efonidipine, nicardipine, bamidipine, benidipine, manidipine, cilnidipine, nisoldipine, nitrendipine, nifedipine, nilvadipine, felodipine, amlodipine, diltiazem, bepridil, clentiazem, phendilin, galopamil, mibefradil, prenylamine, semotiadil, terodiline, verapamil, cilnidipine, elgodipine, isradipine, lacidipine, lercanidipine, nimodipine, cinnarizine, flunarizine, lidoflazine, lomerizine, bencyclane, etafenone, and perhexiline), β-adrenaline receptor blocking drugs (propranolol, pindolol, indenolol, carteolol, bunitrolol, atenolol, acebutolol, metoprolol, timolol, nipradilol, penbutolol, nadolol, tilisolol, carvedilol, bisoprolol, betaxolol, celiprolol, bopindolol, bevantolol, labetalol, alprenolol, amosulalol, arotinolol, befunolol, bucumolol, bufetolol, buferalol, buprandolol, butylidine, butofilolol, carazolol, cetamolol, cloranolol, dilevalol, epanolol, levobunolol, mepindolol, metipranolol, moprolol, nadoxolol, nevibolol, oxprenolol, practol, pronetalol, sotalol, sufinalol, talindolol, tertalol, toliprolol, xybenolol, and esmolol), α-receptor blocking drugs (such as amosulalol, prazosin, terazosin, doxazosin, bunazosin, urapidil, phentolamine, arotinolol, dapiprazole, fenspiride, indoramin, labetalol, naftopidil, nicergoline, tamsulosin, tolazoline, trimazosin, and yohimbine), sympathetic nerve inhibitors (such as

clonidine, guanfacine, guanabenz, methyldopa, and reserpine), hydralazine, todralazine, budralazine, and cadralazine.

Examples of antianginal drugs include nitrate drugs (such as amyl nitrite, nitroglycerin, and isosorbide), β-adrenaline receptor blocking drugs (such as propranolol, pindolol, indenolol, carteolol, bunitrolol, atenolol, acebutolol, metoprolol, timolol, nipradilol, penbutolol, nadolol, tilisolol, carvedilol, bisoprolol, betaxolol, celiprolol, bopindolol, bevantolol, labetalol, alprenolol, amosulalol, arotinolol, befunolol, bucumolol, bufetolol, buferalol, buprandolol, butylidine, butofilolol, carazolol, cetamolol, cloranolol, dilevalol, epanolol, levobunolol, mepindolol, metipranolol, moprolol, nadoxolol, nevibolol, oxprenolol, practol, pronetalol, sotalol, sufinalol, talindolol, tertalol, toliprolol, andxybenolol), calcium channel blocking drugs (such as aranidipine, efonidipine, nicardipine, bamidipine, benidipine, manidipine, cilnidipine, nisoldipine, nitrendipine, nifedipine, nilvadipine, felodipine, amlodipine, diltiazem, bepridil, clentiazem, phendiline, galopamil, mibefradil, prenylamine, semotiadil, terodiline, verapamil, cilnidipine, elgodipine, isradipine, lacidipine, lercanidipine, nimodipine, cinnarizine, flunarizine, lidoflazine, lomerizine, bencyclane, etafenone, and perhexiline) trimetazidine, dipyridamole, etafenone, dilazep, trapidil, nicorandil, enoxaparin, and aspirin.

Examples of diuretics include thiazide diuretics (such as hydrochlorothiazide, methyclothiazide, trichlormethiazide, benzylhydrochlorothiazide, and penflutizide), loop diuretics (such as furosemide, etacrynic acid, bumetanide, piretanide, azosemide, and torasemide), K^+ sparing diuretics (spironolactone, triamterene, andpotassiumcanrenoate), osmotic diuretics (such as isosorbide, D-mannitol, and glycerin), nonthiazide diuretics (such as meticrane, tripamide, chlorthalidone, and mefruside), and acetazolamide. Examples of cardiotonics include digitalis formulations (such as digitoxin, digoxin, methyldigoxin, deslanoside, vesnarinone, lanatoside C, and proscillaridin), xanthine formulations (such as aminophylline, choline theophylline, diprophylline, and proxyphylline), catecholamine formulations (such as dopamine, dobutamine, and docarpamine), PDE III inhibitors (such as amrinone, olprinone, and milrinone), denopamine, ubidecarenone, pimobendan, levosimendan, aminoethylsulfonic acid, vesnarinone, carperitide, and colforsin

daropate. Examples of antiarrhythmic drugs include ajmaline, pirmenol, procainamide, cibenzoline, disopyramide, quinidine, aprindine, mexiletine, lidocaine, phenyloin, pilsicainide, propafenone, flecainide, atenolol, acebutolol, sotalol, propranolol, metoprolol, pindolol, amiodarone, nifekalant, diltiazem, bepridil, and verapamil. Examples of antihyperlipidemic drugs include atorvastatin, simvastatin, pravastatin sodium, fluvastatin sodium, clinofibrate, clofibrate, simfibrate, fenofibrate, bezafibrate, colestimide, and colestyramine.

Yet other exemplary cardiovascular agents include, for example, anti-angiogenic agents and vascular disrupting agents.

Inflammation and Autoimmune Disease

The polymer-agent conjugates, particles, compositions and methods described herein may be used to treat or prevent a disease or disorder associated with inflammation. A polymer-agent conjugate, particle or composition described herein may be administered prior to the onset of, at, or after the initiation of inflammation. When used prophylactically, the polymer-agent conjugate, particle or composition is preferably provided in advance of any inflammatory response or symptom. Administration of the polymer-agent conjugate, particle or composition may prevent or attenuate inflammatory responses or symptoms. Exemplary inflammatory conditions include, for example, multiple sclerosis, rheumatoid arthritis, psoriatic arthritis, degenerative joint disease, spondouloarthropathies, gouty arthritis, systemic lupus erythematosus, juvenile arthritis, rheumatoid arthritis, osteoarthritis, osteoporosis, diabetes (e.g., insulin dependent diabetes mellitus or juvenile onset diabetes), menstrual cramps, cystic fibrosis, inflammatory bowel disease, irritable bowel syndrome, Crohn's disease, mucous colitis, ulcerative colitis, gastritis, esophagitis, pancreatitis, peritonitis, Alzheimer's disease, shock, ankylosing spondylitis, gastritis, conjunctivitis, pancreatis (acute or chronic), multiple organ injury syndrome (e.g., secondary to septicemia or trauma), myocardial infarction, atherosclerosis, stroke, reperfusion injury (e.g., due to cardiopulmonary bypass or kidney dialysis), acute glomerulonephritis, vasculitis, thermal injury (i.e., sunburn), necrotizing enterocolitis, granulocyte transfusion associated syndrome, and/or

Sjogren's syndrome. Exemplary inflammatory conditions of the skin include, for example, eczema, atopic dermatitis, contact dermatitis, urticaria, schleroderma, psoriasis, and dermatosis with acute inflammatory components.

In another embodiment, a polymer-agent conjugate, particle, composition or method described herein may be used to treat or prevent allergies and respiratory conditions, including asthma, bronchitis, pulmonary fibrosis, allergic rhinitis, oxygen toxicity, emphysema, chronic bronchitis, acute respiratory distress syndrome, and any chronic obstructive pulmonary disease (COPD). The polymer-agent conjugate, particle or composition may be used to treat chronic hepatitis infection, including hepatitis B and hepatitis C.

Additionally, a polymer-agent conjugate, particle, composition or method described herein may be used to treat autoimmune diseases and/or inflammation associated with autoimmune diseases such as organ-tissue autoimmune diseases (e.g., Raynaud's syndrome), scleroderma, myasthenia gravis, transplant rejection, endotoxin shock, sepsis, psoriasis, eczema, dermatitis, multiple sclerosis, autoimmune thyroiditis, uveitis, systemic lupus erythematosis, Addison's disease, autoimmune polyglandular disease (also known as autoimmune polyglandular syndrome), and Grave's disease.

Combination therapy

In certain embodiments, a polymer-agent conjugate, particle or composition described herein may be administered alone or in combination with other compounds useful for treating or preventing inflammation. Exemplary anti-inflammatory agents include, for example, steroids (e.g., Cortisol, cortisone, fludrocortisone, prednisone, 6[alpha]-methylprednisone, triamcinolone, betamethasone or dexamethasone), nonsteroidal anti-inflammatory drugs (NSAIDS (e.g., aspirin, acetaminophen, tolmetin, ibuprofen, mefenamic acid, piroxicam, nabumetone, rofecoxib, celecoxib, etodolac or nimesulide). In another embodiment, the other therapeutic agent is an antibiotic (e.g., vancomycin, penicillin, amoxicillin, ampicillin, cefotaxime, ceftriaxone, cefixime, rifampinmetronidazole, doxycycline or streptomycin). In another embodiment, the other therapeutic agent is a PDE4 inhibitor (e.g., roflumilast or rolipram). In another embodiment, the other therapeutic agent is an antihistamine

(e.g., cyclizine, hydroxyzine, promethazine or diphenhydramine). In another embodiment, the other therapeutic agent is an anti-malarial (e.g., artemisinin, artemether, artsunate, chloroquine phosphate, mefloquine hydrochloride, doxycycline hyclate, proguanil hydrochloride, atovaquone or halofantrine). In one embodiment, the other therapeutic agent is drotrecogin alfa.

Further examples of anti-inflammatory agents include, for example, aceclofenac, acemetacin, e-acetamidocaproic acid, acetaminophen, acetaminosalol, acetanilide, acetylsalicylic acid, S-adenosylmethionine, alclofenac, alclometasone, alfentanil, algestone, allylprodine, alminoprofen, aloxiprin, alphaprodine, aluminum bis(acetylsalicylate), amcinonide, amfenac, aminochlorthenoxazin, 3-amino-4-hydroxybutyric acid, 2-amino-4-picoline, aminopropylon, aminopyrine, amixetrine, ammonium salicylate, ampiroxicam, amtolmetin guacil, anileridine, antipyrine, antrafenine, apazone, beclomethasone, bendazac, benorylate, benoxaprofen, benzpiperylon, benzydamine, benzylmorphine, bermoprofen, betamethasone, betamethasone- 17-valerate, bezitramide, [alpha]-bisabolol, bromfenac, p-bromoacetanilide, 5-bromosalicylic acid acetate, bromosaligenin, bucetin, bucloxic acid, bucolome, budesonide, bufexamac, bumadizon, buprenorphine, butacetin, butibufen, butorphanol, carbamazepine, carbiphene, caiprofen, carsalam, chlorobutanol, chloroprednisone, chlorthenoxazin, choline salicylate, cinchophen, cinmetacin, ciramadol, clidanac, clobetasol, clocortolone, clometacin, clonitazene, clonixin, clopirac, cloprednol, clove, codeine, codeine methyl bromide, codeine phosphate, codeine sulfate, cortisone, cortivazol, cropropamide, crotethamide and cyclazocine.

Further examples of anti-inflammatory agents include deflazacort, dehydrotestosterone, desomorphine, desonide, desoximetasone, dexamethasone, dexamethasone-21- isonicotinate, dexoxadrol, dextromoramide, dextropropoxyphene, deoxycorticosterone, dezocine, diampromide, diamorphone, diclofenac, difenamizole, difenpiramide, diflorasone, diflucortolone, diflunisal, difluprednate, dihydrocodeine, dihydrocodeinone enol acetate, dihydromorphine, dihydroxyaluminum acetylsalicylate, dimenoxadol, dimepheptanol, dimethylthiambutene, dioxaphetyl butyrate, dipipanone, diprocetyl, dipyrone, ditazol, droxicam, emorfazone, enfenamic

acid, enoxolone, epirizole, eptazocine, etersalate, ethenzamide, ethoheptazine, ethoxazene, ethylmethylthiambutene, ethylmorphine, etodolac, etofenamate, etonitazene, eugenol, felbinac, fenbufen, fenclozic acid, fendosal, fenoprofen, fentanyl, fentiazac, fepradinol, feprazone, floctafenine, fluazacort, flucloronide, flufenamic acid, flumethasone, flunisolide, flunixin, flunoxaprofen, fluocinolone acetonide, fluocinonide, fluocinolone acetonide, fluocortin butyl, fluocoitolone, fluoresone, fluorometholone, fluperolone, flupirtine, fluprednidene, fluprednisolone, fluproquazone, flurandrenolide, flurbiprofen, fluticasone, formocortal and fosfosal.

Further examples of anti-inflammatory agents include gentisic acid, glafenine, glucametacin, glycol salicylate, guaiazulene, halcinonide, halobetasol, halometasone, haloprednone, heroin, hydrocodone, hydro cortamate, hydrocortisone, hydrocortisone acetate, hydrocortisone succinate, hydrocortisone hemisuccinate, hydrocortisone 21-lysinate, hydrocortisone cypionate, hydromorphone, hydroxypethidine, ibufenac, ibuprofen, ibuproxam, imidazole salicylate, indomethacin, indoprofen, isofezolac, isoflupredone, isoflupredone acetate, isoladol, isomethadone, isonixin, isoxepac, isoxicam, ketobemidone, ketoprofen, ketorolac, p- lactophenetide, lefetamine, levallorphan, levorphanol, levophenacyl-morphan, lofentanil, lonazolac, lornoxicam, loxoprofen, lysine acetylsalicylate, mazipredone, meclofenamic acid, medrysone, mefenamic acid, meloxicam, meperidine, meprednisone, meptazinol, mesalamine, metazocine, methadone, methotrimeprazine, methylprednisolone, methylprednisolone acetate, methylprednisolone sodium succinate, methylprednisolone suleptnate, metiazinic acid, metofoline, metopon, mofebutazone, mofezolac, mometasone, morazone, morphine, morphine hydrochloride, morphine sulfate, morpholine salicylate and myrophine.

Further examples of anti-inflammatory agents include nabumetone, nalbuphine, nalorphine, 1-naphthyl salicylate, naproxen, narceine, nefopam, nicomorphine, nifenazone, niflumic acid, nimesulide, 5'-nitro-2'-propoxyacetanilide,norlevorphanol, normethadone, normorphine, norpipanone, olsalazine, opium, oxaceprol, oxametacine, oxaprozin, oxycodone, oxymorphone, oxyphenbutazone, papaveretum, paramethasone, paranyline, parsalmide, pentazocine, perisoxal, phenacetin, phenadoxone, phenazocine, phenazopyridine hydrochloride,

phenocoll, phenoperidine, phenopyrazone, phenomorphan, phenyl acetylsalicylate, phenylbutazone, phenyl salicylate, phenyramidol, piketoprofen, piminodine, pipebuzone, piperylone, pirazolac, piritramide, piroxicam, pirprofen, pranoprofen, prednicarbate, prednisolone, prednisone, prednival, prednylidene, proglumetacin, proheptazine, promedol, propacetamol, properidine, propiram, propoxyphene, propyphenazone, proquazone, protizinic acid, proxazole, ramifenazone, remifentanil, rimazolium metilsulfate, salacetamide, salicin, salicylamide, salicylamide o-acetic acid, salicylic acid, salicylsulfuric acid, salsalate, salverine, simetride, sufentanil, sulfasalazine, sulindac, superoxide dismutase, suprofen, suxibuzone, talniflumate, tenidap, tenoxicam, terofenamate, tetrandrine, thiazolinobutazone, tiaprofenic acid, tiaramide, tilidine, tinoridine, tixocortol, tolfenamic acid, tolmetin, tramadol, triamcinolone, triamcinolone acetonide, tropesin, viminol, xenbucin, ximoprofen, zaltoprofen and zomepirac.

In one embodiment, a polymer-agent conjugate, particle or composition described herein may be administered with a selective COX-2 inhibitor for treating or preventing inflammation. Exemplary selective COX-2 inhibitors include, for example, deracoxib, parecoxib, celecoxib, valdecoxib, rofecoxib, etoricoxib, and lumiracoxib.

Having thus described several aspects of at least one embodiment of this invention, it is to be appreciated various alterations, modifications, and improvements will readily occur to those skilled in the art. Such alterations, modifications, and improvements are intended to be part of this disclosure, and are intended to be within the spirit and scope of the invention. Accordingly, the foregoing description and drawings are by way of example only.

Unless otherwise defined, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this invention belongs. All publications, patent applications, patents, and other references mentioned herein are incorporated by reference in their entirety. In case of conflict, the present specification, including definitions, will control. In addition, the materials, methods, and examples are illustrative only and not intended to be limiting.

EXAMPLES

Example 1. Purification and characterization of 5050 PLGA.

Step A: A 3-L round-bottom flask equipped with a mechanical stirrer was charged with 5050PLGA (300 g, Mw: 7.8 KDa; Mn: 2.7 KDa) and acetone (900 mL). The mixture was stirred for 1 h at ambient temperature to form a clear yellowish solution.

Step B: A 22-L jacket reactor with a bottom-outlet valve equipped with a mechanical stirrer was charged with MTBE (9.0 L, 30 vol. to the mass of 5050 PLGA). Celite® (795 g) was added to the solution with overhead stirring at ~200 rpm to produce a suspension. To this suspension was slowly added the solution from Step A over 1 h. The mixture was agitated for an additional one hour after addition of the polymer solution and filtered through a polypropylene filter. The filter cake was washed with MTBE (3 × 300 mL), conditioned for 0.5 h, air-dried at ambient temperature (typically 12 h) until residual MTBE was ≤ 5 wt% (as determined by 1H NMR analysis.

Step C: A 12-L jacket reactor with a bottom-outlet valve equipped with a mechanical stirrer was charged with acetone (2.1 L, 7 vol. to the mass of 5050 PLGA). The polymer/Celite® complex from Step B was charged into the reactor with overhead stirring at ~200 rpm to produce a suspension. The suspension was stirred at ambient temperature for an additional 1 h and filtered through a polypropylene filter. The filter cake was washed with acetone (3 × 300 mL) and the combined filtrates were clarified through a 0.45 mM in-line filter to produce a clear solution. This solution was concentrated to ~1000 mL.

Step D: A 22-L jacket reactor with a bottom-outlet valve equipped with a mechanical stirrer was charged with water (9.0 L, 30 vol.) and was cooled down to 0 - 5 °C using a chiller. The solution from Step C was slowly added over 2 h with overhead stirring

at ~ 200 rpm. The mixture was stirred for an additional one hour after addition of the solution and filtered through a polypropylene filter. The filter cake was conditioned for 1 h, air-dried for 1 day at ambient temperature, and then vacuum-dried for 3 days to produce the purified 5050 PLGA as a white powder [258 g, 86%]. The ^{1}H NMR analysis was consistent with that of the desired product and Karl Fisher analysis showed 0.52 wt% of water. The product was analyzed by HPLC (AUC, 230 nm) and GPC (AUC, 230 nm). The process produced a more narrow polymer polydispersity, i.e. Mw: 8.8 kDa and Mn: 5.8 kDa.

Example 2. Purification and characterization of 5050 PLGA lauryl ester.

A 12-L round-bottom flask equipped with a mechanical stirrer was charged with MTBE (4 L) and heptanes (0.8 L). The mixture was agitated at ~300 rpm, to which a solution of 5050 PLGA lauryl ester (65 g) in acetone (300 mL) was added dropwise. Gummy solids were formed over time and finally clumped up on the bottom of the flask. The supernatant was decanted off and the solid was dried under vacuum at 25 °C for 24 h to afford 40 g of purified 5050 PLGA lauryl ester as a white powder [yield: 61.5%]. ^{1}H NMR ($CDCl_3$, 300 MHz): δ 5.25 – 5.16 (m, 53H), 4.86 – 4.68 (m, 93H), 4.18 (m, 7H), 1.69 – 1.50 (m, 179H), 1.26 (bs, 37H), 0.88 (t, J = 6.9 Hz, 6H). The ^{1}H NMR analysis was consistent with that of the desired product. GPC (AUC, 230 nm): 6.02 – 9.9 min, t_R = 7.91 min.

Example 3. Purification and characterization of 7525 PLGA.

A 22-L round-bottom flask equipped with a mechanical stirrer was charged with 12 L of MTBE, to which a solution of 7525 PLGA (150 g, approximately 6.6 kD) in dichloromethane (DCM, 750 mL) was added dropwise over an hour with an agitation of ~300 rpm, resulting in a gummy solid. The supernatant was decanted off and the gummy solid was dissolved in DCM (3 L). The solution was transferred to a round-bottom flask and concentrated to a residue, which was dried under vacuum at 25 °C for 40 h to afford 94 g of purified 7525 PLGA as a white foam [yield: 62.7%,]. ^{1}H NMR ($CDCl_3$, 300 MHz): δ 5.24 – 5.15 (m, 68H), 4.91 – 4.68 (m, 56H), 3.22 (s, 2.3H, MTBE), 1.60 – 1.55 (m, 206H), 1.19 (s, 6.6H, MTBE). The ^{1}H NMR analysis

was consistent with that of the desired product. GPC (AUC, 230 nm): 6.02 – 9.9 min, t_R = 7.37 min.

Example 4. Synthesis, purification and characterization of O-acetyl-5050-PLGA.

A 2000-mL, round-bottom flask equipped with an overhead stirrer was charged with purified 5050 PLGA [220 g, Mn of 5700] and DCM (660 mL). The mixture was stirred for 10 min to form a clear solution. Ac2O (11.0 mL, 116 mmol) and pyridine (9.4 mL, 116 mmol) were added to the solution, resulting in a minor exotherm of ~ 0.5 °C. The reaction was stirred at ambient temperature for 3 h and concentrated to ~600 mL. The solution was added to a suspension of Celite® (660 g) in MTBE (6.6 L, 30 vol.) over 1 h with overhead stirring at ~200 rpm. The suspension was filtered through a polypropylene filter and the filter cake was air-dried at ambient temperature for 1 day. It was suspended in acetone (1.6 L, ~ 8 vol) with overhead stirring for 1 h. The slurry was filtered though a fritted funnel (coarse) and the filter cake was washed with acetone (3 × 300 mL). The combined filtrates were clarified though a Celite pad to afford a clear solution. It was concentrated to ~700 mL and added to cold water (7.0 L, 0 - 5 °C) with overhead stirring at 200 rpm over 2 h. The suspension was filtered though a polypropylene filter. The filter cake was washed with water (3 × 500 mL), and conditioned for 1 h to afford 543 g of wet cake. It was transferred to two glass trays and air-dried at ambient temperature overnight to afford 338 g of wet product, which was then vacuum-dried at 25 °C for 2 days to constant weight to afford 201 g of product as a white powder [yield: 91%]. The 1H NMR analysis was consistent with that of the desired product. The product was analyzed by HPLC (AUC, 230 nm) and GPC (Mw: 9.0 kDa and Mn: 6.3 kDa).

Example 5. Synthesis, purification and characterization of doxorubicin 5050 PLGA amide

A 1000-ml round-bottom flask with a magnetic stirrer was charged with purified 5050 PLGA [55.0 g, 10.4 mmol, 1.0 equiv.], doxorubicin•HCl (6.7 g, 11.4 mmol, 1.1 equiv, 2-chloro-N-methyl pyridinium iodide (3.45 g, 13.5 mmol, 1.3 equiv, and DMF (250 mL, anhydrous) under N_2. The suspension was stirred for 15 min and

triethylamine (4.6 mL, 32.2 mmol, 3.15 equiv.) was added dropwise over 10 min. The reaction mixture became a dark red solution after the addition of TEA and an exotherm from 23.2 °C to 26.2 °C was observed. The reaction was complete after 1.5 h as indicated by HPLC analysis. The mixture was filtered through a 0.5 μM PTFE membrane and the filtrate was added dropwise into water (5.50 L) containing 11 mL of AcOH over 20 min via addition funnels. The suspension was stirred for 1 h (pH ~3 – 4), filtered over 30 min, and the filter cake was washed with water (3 × 300 mL). The solid was suspended in water (3.0 L) containing 0.1 vol% of AcOH and 5 vol% of acetone, stirred for 1 h, and filtered (pH ~4 – 5) to afford 201.9 g of wet doxorubicin 5050 PLGA amide. The wet doxorubicin 5050 PLGA amide sample was transferred into a glass tray and dried under vacuum with nitrogen bleeding at 25 °C for 16 h to afford 162.9 g of semi-dry solid. The ^{1}H NMR analysis indicated ~1.0 wt% of residual DMF. This sample was suspended in H_2O (3 L) containing 3 mL of AcOH and 15 mL of acetone and stirred for 6 h, filtered, washed with H_2O (0.5 L), and held for 0.5 h to afford 163.3 g of wet doxorubicin 5050 PLGA amide. The wet doxorubicin 5050 PLGA amide (155.8 g) was dried under vacuum with N_2 bleeding at 25 °C for 16 h to afford 120.3 g of semi-dry product, which was dried at ambient temperature with N_2 purge for 16 h to afford 54.4 g of doxorubicin 5050 PLGA amide [yield: 93%]. ^{1}H NMR ($CDCl_3$, 300 MHz): δ 14.00 (s, 1H), 13.27 (s, 1H), 8.05 (d, J = 7.8 Hz, 1H), 7.80 (t, J = 7.8 Hz, 1H), 7.40 (d, J = 8.4 Hz, 1H), 6.44 (bs, 0.8H), 5.51 (bs, 1.2H), 5.22 – 5.17 (m, 40H), 4.91 – 4.72 (m, 81H), 4.31 – 4.08 (m, 7H), 3.64 (bs, 0.9H), 3.30 (d, J = 20.4, 1H), 3.04 (d, J = 18.9 Hz, 1H), 2.94 (s, 0.1H, DMF), 2.89 (s, 0.1H, DMF), 2.36 (d, J = 14.4 Hz, 1H), 2.17(d, J = 14.1 Hz, 1H), 1.84 (bs, 5H), 1.60 – 1.55 (m, 120H), 1.28 (d, J = 6.6 Hz). The ^{1}H NMR analysis was consistent with that of the desired product. HPLC (AUC, 480 nm): 13.00 – 17.80 min, t_R 16.8 min. GPC (AUC, 480 nm): 5.2 – 8.6 min, t_R 6.51 min. The product may also include free 5050 PLGA and/or a trace amount of doxorubicin.

Example 6. Synthesis, purification and characterization of doxorubicin 7525 PLGA amide

2-chloro-N-methyl pyridinium iodide (1.95 g, 7.63 mmol) and TEA (3.15 mL, 22.6 mmol) were added to a mixture of purified 7525 PLGA [25.0 g, 3.80 mmol] and doxorubicin•HCl (3.08 g, 5.32 mmol) in DMF (125 mL, anhydrous) and stirred at ambient temperature. After 1 h, the reaction was complete by HPLC (0.4% doxorubicin remaining); however, there was 5.2% of an impurity at 12.0 min by HPLC analysis. The mixture was added into 2.50 L of water (25 mL of acetone wash) and 5.0 mL of acetic acid was added (pH = 4 – 5). The resulting slurry was stirred for 30 min and filtered (250 mL water wash). The isolated wet cake was found to have only 1.7% of the 12.0 min impurity by HPLC analysis. The wet cake was slurried in water (1.25 L) and 1.3 mL of acetic acid was added. The mixture was stirred for 45 min, filtered (washed with 250 mL of water), and dried under vacuum for 44 h to afford 25.2 g of doxorubicin 7525 PLGA amide as a red solid [Yield: 93%]. ^{1}H NMR ($CDCl_3$, 300 MHz): δ 13.99 (s, 1H), 13.26 (s, 1H), 8.04 (d, J = 7.8 Hz, 1.2H), 7.79 (t, J = 7.8 Hz, 1.1H), 7.40 (d, J = 8.4 Hz, 1.1H), 6.44 (bs, 0.8H), 5.50 (bs, 1.3H), 5.22 – 5.17 (m, 60H), 4.91 – 4.72 (m, 53H), 4.31 – 4.08 (m, 8H), 3.64 (bs, 1.1H), 3.30 (d, J = 20.4, 1.0H), 3.04 (d, J = 18.9 Hz, 1.2H), 2.94 (s, ~1.0H, DMF), 2.89 (s, 1.1H, DMF), 2.36 (d, J = 14.4 Hz, 1.8H), 2.17(m, 3.4H), 1.84 (bs, 3H), 1.60 – 1.55 (m, 184H), 1.28 (d, J = 4.6 Hz, 6.6H). The ^{1}H NMR analysis was consistent with that of the desired product. HPLC (AUC, 480 nm): 13.15 – 18.50 min, t_R 17.6 min. GPC (AUC, 480 nm): 5.2 – 8.5 min, t_R 6.29 min. The product may also include free 7525 PLGA and/or a trace amount of doxorubicin.

Example 7. Synthesis, purification and characterization of paclitaxel-5050 PLGA-O-acetyl

A 250-mL round-bottom flask equipped with an overhead stirrer was charged with 5050 PLGA-O-acetyl [20 g, 2.6 mmol], paclitaxel (1.85 g, 2.1 mmol, 0.8 equiv., *N*,*N*′-dicyclohexyl-carbodiimide (DCC, 0.66 g, 3.2 mmol, 1.3 equiv.), 4-dimethylaminopyridine (DMAP, 0.39 g, 3.2 mmol, 1.3 equiv.), and DCM (100 mL, 5 vol). The mixture was agitated at 20 ºC for 16 h and filtered to remove the dicyclohexylurea (DCU). The filtrate was concentrated to a residue and the residue was dissolved in acetone (100 mL), resulting in a cloudy suspension. It was filtered

to remove residual DCU byproduct. The filtrate was added dropwise to 5:1 MTBE/heptanes (1.2 L) with vigorously stirring. The white precipitates formed a gum shortly after precipitation. The supernatant was decanted off and the gummy solid was isolated. The precipitation was repeated twice and the gummy solid was dried under vacuum at 25 °C for 16 h to afford 15.7 g of paclitaxel-5050 PLGA-O-acetyl [yield: 72%] ^{1}H NMR ($CDCl_3$, 300 MHz): δ 8.15 (d, J = 7.5 Hz, 1H), 7.75 (d, J = 6.6 Hz, 1H), 7.54 – 7.38 (m, 6H), 6.29 – 6.24 (a singlet overlaps with a triplet, 1H), 6.06 (bs, 0.5H), 5.69 (d, J = 6.9 Hz, 0.4H), 5.58 (bs, 0.5H), 5.26 – 5.17 (m, 40H), 4.93 (d, J = 7.8 Hz, 0.5H), 4.90 – 4.72 (m, 85H), 4.43 (t, J = 3.9 Hz, 1 H), 4.31 (d, J = 8.1 Hz, 0.5H), 4.21 (d, J = 8.1 Hz, 0.5H), 3.81 (d, J = 6.6 Hz, 0.5H), 2.44 (bs, 2.5H), 2.23 (s, 1.5H), 2.17 (s, 19H, acetone), 1.8 – 1.7 (bs, 15H), 1.68 (s, 1.5H), 1.60 – 1.55 (m, 124H), 1.22 (bs, 2.5H), 1.14 (s, 1.5H). The ^{1}H NMR analysis was consistent with that of the desired product. HPLC (AUC, 230 nm): 13.00 – 16.50 min, t_R 15.60 min. GPC (AUC, 230 nm): 6.0 – 9.7 min, t_R = 7.35 min. The major product is paclitaxel-2'-5050 PLGA-O-acetyl (wherein paclitaxel is attached to 5050 PLGA-O-acetyl via the 2' hydroxyl group); the product may also include free 5050 PLGA-O-acetyl, 7 paclitaxel-conjugate, 1 paclitaxel-conjugate, product in which two or more polymer chains are linked to paclitaxel (e.g., via the 2' and 7 positions) and/or a trace amount of paclitaxel.

Example 8. Synthesis, purification and characterization of docetaxel-5050 PLGA-O-acetyl

A 250-mL round-bottom flask equipped with an overhead stirrer was charged with O-acetyl-5050 PLGA (16 g, 2.6 mmol), docetaxel (1.8 g, 2.1 mmol, 0.8 equiv.), DCC (0.66 g, 3.2 mmol, 1.3 equiv.), 4-dimethylaminopyridine (DMAP, 0.35 g, 3.2 mmol, 1.3 equiv.), and EtOAc (80 mL, 5 vol). The mixture was agitated at 20 °C for 2.5 h and an additional 0.5 equivalents of DCC (0.27 g) and DMAP (0.16 g) were added. The reaction was stirred at ambient temperature for 16 h and filtered to remove the dicyclohexylurea (DCU). The filtrate was diluted with EtOAc to 250 mL. It was washed with 1% HCl (2 × 60 mL) and brine (60 mL). The organic layer was

separated, dried over Na_2SO_4, and filtered. The filtrate was concentrated to a residue and the residue was dissolved in acetone (100 mL), resulting in a cloudy suspension. It was filtered to remove residual DCU byproduct. The filtrate was added dropwise to 5:1 MTBE/heptanes (600 mL) with vigorously stirring. The white precipitates formed a gum shortly after precipitation. The supernatant was decanted off and the gummy solid was isolated. The precipitation was repeated three more times and the gummy solid was dissolved in acetone (300 mL). The solution was concentrated to a residue, which was dried under vacuum at 25 °C for 64 h to afford 14 g of docetaxel-5050 PLGA-O-acetyl [yield: 78%]. ^{1}H NMR ($CDCl_3$, 300 MHz): δ 8.11 (d, J = 6.9 Hz, 1H), 7.61 (m, 0.6H), 7.50 (t, J = 7.2 Hz, 6H), 7.39 (m, 1.3H), 6.22 (bs, 0.5H), 6.68 (d, J = 7.5 Hz, 5.69 – 5.67 (m, 2.2H), 5.49 – 5.17 (m, 49H), 4.90 – 4.72 (m, 102H), 4.43 (m, 1.2 H), 3.92 (d, J = 5.7 Hz, 0.5H), 2.42 (bs, 2.1H), 2.17 (s, 29.3H, acetone), 1.90 (s, 3H), 1.80 (bs, 3H), 1.72 (s, 2H), 1.64 – 1.55 (m, 164H), 1.34 (s, 7H), 1.22 (m, 4H), 1.12 (s, 2.4H). The ^{1}H NMR analysis was consistent with that of the desired product. HPLC (AUC, 230 nm): 15.50 – 18.00 min, t_R 17.34 min. GPC (AUC, 230 nm): 6.0 – 9.7 min, t_R = 7.35 min. The major product is docetaxel-2'-5050 PLGA-O-acetyl (wherein docetaxel is attached to 5050 PLGA-O-acetyl via the 2' hydroxyl group); the product may also include free 5050 PLGA-O-acetyl, 7 docetaxel-conjugate, 10 docetaxel-conjugate, 1 docetaxel-conjugate, product in which two or more polymer chains are linked to docetaxel (e.g., via the 2' and 7 positions) and/or a trace amount of docetaxel.

Example 9. Synthesis, purification and characterization of bis(docetaxel) glutamate-5050 PLGA-O-acetyl

A 500-mL, round-bottom flask was charged with 5050 PLGA-O-acetyl [40 g, 5.88 mmol], dibenzyl glutamate (3.74 g, 7.35 mmol), and DMF (120 mL, 3 vol.) and allowed to mix for 10 min to afford a clear solution. CMPI (2.1 g, 8.23 mmol) and TEA (2.52 mL) were added and the solution was stirred at ambient temperature for 3 h. The yellowish solution was added to a suspension of Celite (120 g) in MTBE (2.0 L) over 0.5 h with overhead stirring. The solid was filtered, washed with MTBE (300 mL), and vacuum-dried at 25 °C for 16 h. The solid was then suspended in acetone

(400 mL, 10 vol), stirred for 0.5 h, filtered and the filter cake was washed with acetone (3 × 100 mL). The combined filtrates were concentrated to 150 mL and added to cold water (3.0 L, 0 – 5 °C) over 0.5 h with overhead stirring. The resulting suspension was stirred for 2 h and filtered through a PP filter. The filter cake was air-dried for 3 h and then vacuum-dried at 28 °C for 16 h to afford the product, dibenzylglutamate 5050 PLGA-O-acetyl [40 g, yield: 95%]. The ^{1}H NMR analysis indicated that the ratio of benzyl aromatic protons to methine protons of lactide was 10:46. HPLC analysis indicated 96% purity (AUC, 227 nm) and GPC analysis showed Mw: 8.9 kDa and Mn: 6.5 kDa.

Dibenzylglutamate 5050 PLGA-O-acetyl (40 g) was dissolved in ethyl acetate (400 mL) to afford a yellowish solution. Charcoal (10 g) was added to the mixture and stirred for 1 h at ambient temperature. The solution was filtered through a pad of Celite (60 mL) to afford a colorless filtrate. The filter cake was washed with ethyl acetate (3 × 50 mL) and the combined filtrates were concentrated to 400 mL. Palladium on activated carbon (Pd/C, 5 wt%, 4.0 g) was added, the mixture was evacuated for 1 min, filled up with H_2 using a balloon and the reaction was stirred at ambient temperature for 3 h. The solution was filtered through a Celite pad (100 mL) and the filter cake was washed with acetone (3 × 50 mL). The combined filtrates had a grey color and were concentrated to 200 mL. The solution was added to a suspension of Celite (120 g) in MTBE (2.0 L) over 0.5 h with overhead stirring. The suspension was stirred at ambient temperature for 1 h and filtered through a PP filter. The filter cake was dried at ambient temperature for 16 h, suspended in acetone (400 mL), and stirred for 0.5 h. The solution was filtered through a PP filter and the filter cake was washed with acetone (3 × 50 mL). To remove any residual Pd, macroporous polystyrene-2,4,6-trimercaptotriazine resin (MP-TMT, 2.0 g, Biotage, capacity: 0.68 mmol/g) was added at ambient temperature for 16 h with overhead stirring. The solution was filtered through a Celite pad to afford a light grey solution. The solution was concentrated to 200 mL and added to cold water (3.0 L, 0 – 5 °C) over 0.5 h with overhead stirring. The resulting suspension was stirred at <5 °C for 1 h and filtered through a PP filter. The filter cake was air-dried for 12 h and vacuum-dried for 2 days

to afford a semi-glassy solid [glutamic acid-PLGA5050-O-acetyl, 38 g, yield: 95%]. HPLC analysis showed 99.6% purity (AUC, 227 nm) and GPC analysis indicated Mw: 8.8 kDa and Mn: 6.6 kDa.

To remove any residual water, the glutamic acid-PLGA5050-O-acetyl [38 g] was dissolved in acetonitrile (150 mL) and concentrated to dryness. The residue was vacuum-dried at ambient temperature for 16 h to afford the desired product as a light grey powder [36 g]. A 1000-mL, round-bottom flask equipped with a magnetic stirrer was charged with glutamic acid-PLGA5050-O-acetyl [30 g, 4.5 mmol, Mn: 6.6 kDa], docetaxel (4.3 g, 2.9 mmol, 1.2 equiv), DMF (60 mL), and DCM (60 mL). The mixture was stirred for 10 min to afford a light brown solution. The first portion of EDC•HCl (1.6 g, 8.3 mmol) and DMAP (1.0 g, 8.3 mmol) was added and stirred at ambient temperature to yield a dark brown solution. After 2 h, a second portion of EDC•HCl (0.8 g, 4.2 mmol) and DMAP (0.50 g, 4.2 mmol) was added and stirred for an additional 2 to produce a darker solution. A third portion of EDC•HCl (0.3 g, 1.6 mmol) and DMAP (0.2 g, 1.6 mmol) was added. An additional portion of EDC•HCl (0.3 g, 1.6 mmol) and DMAP (0.2 g, 1.6 mmol) was added and stirred at ambient temperature for 2 h. The reaction mixture was added to a suspension of Celite (100 g) in MTBE (3.0 L) over 0.5 h with overhead stirring. The suspension was filtered through a PP filter and the filter cake was dried under vacuum at 25 °C for 12 h. The solid was suspended in acetone (250 mL) for 0.5 h with overhead stirring. The suspension was filtered and the filter cake was washed with acetone (3 × 60 mL). The combined filtrates were concentrated to 200 mL and added to cold water (3 L, 0°C) over 0.5 h with overhead stirring. The suspension was filtered through a PP filter; the filter cake was washed with water (3 × 100 mL) and the solid was dried under vacuum at 25 °C for 16 h to afford a crude product [33 g]. To reduce any possible residual docetaxel, a second MTBE purification was conducted. The crude product was dissolved in acetone (150 mL) and added to a suspension of Celite (100 g) in MTBE (3 L). The suspension was filtered; the solid was vacuum-dried for 3 h, and suspended in acetone (500 mL) with overhead stirring. The suspension was filtered and the filter cake was washed with acetone (3 × 100 mL). The combined filtrates were concentrated to 200 mL and co-evaporated with acetonitrile (100 mL) to

dryness. The residue was dissolved in acetone (200 mL) and the solution was precipitated into a suspension of Celite® (100 g)/MTBE (3 L) a third time. The mixture was stirred at ambient temperature for 1 h and filtered. The filter cake was washed with MTBE (2 × 200 mL) and vacuum-dried at ambient temperature overnight. The bis(docetaxel) glutamate-5050 PLGA-O-acetyl /Celite complex was suspended in acetone (300 mL) with overhead stirring. The suspension was filtered and added to cold water (3 L) over 0.5 h with overhead stirring. The suspension was stirred at <5 °C for 1 h and filtered through a PP filter. The filter cake was washed with water (3 × 200 mL); the filter cake was conditioned for 0.5 h and vacuum-dried for 2 days to afford the desired product as an off-white powder [30 g, yield: 88%;]. This product was purified by another MTBE precipitation without using Celite. The product was dissolved in acetone to afford a solution (200 mL) and added to cold MTBE (2 L, 0 °C) over 1 h with overhead stirring. The resulting suspension was filtered and the filter cake was vacuum-dried at 25 °C for 16 h to afford a product with a tan color [34 g]. This sample was further dried for another 24 h and the residual MTBE was not reduced. To remove the residual MTBE, the product was precipitated into water. The isolated solid was vacuum-dried for 2 days to constant weight to afford the desired product as an off-white powder [bis(docetaxel) glutamate-5050 PLGA-O-acetyl, 28.5 g, yield: 84%]. The ^{1}H NMR analysis indicated that the docetaxel loading was 10% and HPLC analysis showed >99.5% purity (AUC, 227 nm). GPC analysis indicated Mw: 9.9 kDa and Mn: 6.1 kDa. The major product is bis(2'-docetaxel) glutamate-5050 PLGA-O-acetyl (wherein each docetaxel is attached to the glutamate linker via the 2' hydroxyl group); the product may also include free 5050 PLGA-O-acetyl, mono(2'-docetaxel) glutamate-5050 PLGA-O-acetyl, mono(7-docetaxel) glutamate-5050 PLGA-O-acetyl, mono(10-docetaxel) glutamate-5050 PLGA-O-acetyl, mono(1-docetaxel) glutamate-5050 PLGA-O-acetyl, (2'-docetaxel)(7-docetaxel) glutamate-5050 PLGA-O-acetyl, (2'-docetaxel)(10-docetaxel) glutamate-5050 PLGA-O-acetyl, (2'-docetaxel)(1-docetaxel) glutamate-5050 PLGA-O-acetyl, (7-docetaxel)(10-docetaxel) glutamate-5050 PLGA-O-acetyl, (7-docetaxel)(1-docetaxel) glutamate-5050 PLGA-O-acetyl, (10-docetaxel)(1-docetaxel) glutamate-5050 PLGA-O-acetyl, and/or a trace amount of docetaxel.

Example 10. Synthesis, purification and characterization of tetra-(docetaxel) triglutamate-5050 PLGA-O-acetyl

A 250-mL, round-bottom flask equipped with a magnetic stirrer was charged with *N*-(*tert*-butoxycarbonyl)-L-glutamic acid (20 g, 40 mmol), (S)-dibenzyl 2-aminopentanedioate (4.85 g, 19.5 mmol), and DMF (100 mL). The mixture was stirred for 5 min to afford a clear solution. EDC•HCl (8.5 g, 44.3 mmol) and DMAP (9.8 g, 80 mmol) were added. The reaction was stirred at ambient temperature for 3 h, at which time HPLC analysis indicated completion of the reaction. The reaction was concentrated to a syrup (~75 g) and EtOAc (250 mL) was added with overhead stirring. The resulting suspension was filtered to remove the *N*,*N*-dimethyl pyridinium *p*-toluenesulfonate. The filtrate was concentrated to a yellowish oil and water (200 mL) was added with vigorous stirring. White solid was gradually formed and the suspension was filtered. The solid was washed with water (2 × 50 mL) and dried under vacuum for 24 h to afford the N-Boc-tetrabenzyl-triglutamate product as a white powder [16.5 g, yield: 95%]. The 1H NMR analysis showed the desired product and HPLC analysis indicated a 92% purity (AUC, 254 nm). This crude product was further purified by recrystallization as follows. N-Boc-tetrabenzyl-triglutamate (15 g) was dissolved in hot IPAc (15 mL, 1 vol) and the solution was allowed to cool down to ambient temperature. A hydrogel like solid was formed and it was slurried in MTBE (200 mL) for 1 h, filtered. The filtration was slow owing to the hydrogel-like particles. The hydrogel solid was vacuum-dried at ambient temperature to afford product as a white powder [12.5 g, recovery yield: 83%]. The 1H NMR analysis showed the desired product and HPLC analysis indicated ~100% purity (AUC, 254 nm).

A 250-mL, round bottom flask was charged with N-tert-butyloxycarbonyl-tetrabenzyl-triglutamate [N-t-BOC-tetrabenzyl-triglutamate, 11 g, 12.7 mmol] and DCM (25 mL) to afford a clear solution. Trifluoroacetic acid (TFA, 25 mL) was added to the solution and the reaction was stirred at ambient temperature. The solution was concentrated to a residue, dissolved in DCM (200 mL) and washed with saturated sodium bicarbonate ($NaHCO_3$, 2 × 25 mL) and brine (30 mL). The organic

layer was separated and dried over sodium sulfate (Na_2SO_4, 15 g). The solution was filtered and the filtrate was concentrated to a residue and vacuum-dried at ambient temperature for 16 h to afford the desired product (NH_2-tetrabenzyl-triglutamate) as a wax-like semi-solid product [9.3 g, yield: 96%]. HPLC analysis indicated a 97% purity (AUC, 254 nm).

A 1000-mL, round-bottom flask equipped with a magnetic stirrer was charged with NH_2-tetrabenzyl-triglutamate [4.0 g, 5.3 mmol], o-acetyl PLGA 5050 [30 g, 4.4 mmol, Mn: 6.8 kDa,], and DMF (100 mL). The mixture was stirred for a few minutes to afford a clear solution. 1-chloro-4-methylpyridinium iodide (CMPI, 1.7 g, 6.6 mmol) and trifluoroacetic acid (TEA, 1.3 mL, 8.8 mmol) were added and the reaction was stirred at ambient temperature for 3 h. The reaction mixture was added into cold water (2 L) over 1 h with overhead stirring. The generated suspension was filtered through a PP filter. The filter cake was washed with water (3 × 300 mL) and air-dried at ambient temperature for 16 h to afford a crude product. It was dissolved in acetonitrile (200 mL) and the solution concentrated to dryness. The residue was dissolved in acetone (100 mL) and the solution was added to cold MTBE (0 °C, 2 L) over 0.5 h with overhead stirring to afford a suspension. It was filtered through a PP filter and the filter cake was vacuum-dried for 16 h to afford the product (tetrabenzyl-triglutamate-PLGA 5050-O-acetyl [30 g, yield: 88%]. The ^{1}H NMR analysis indicated the ratio of benzyl aromatic protons over methine protons of lactide was 20:45. HPLC analysis showed > 95% purity (AUC, 227 nm) and GPC analysis indicated a Mw: 8.9 kDa and a Mn: 6.7 kDa.

The tetrabenzyl-triglutamate-PLGA 5050-O-acetyl [30 g, 1.5 mmol] was dissolved in ethyl acetate (300 mL) to afford a pale yellowish solution. Charcoal (10 g) was added and the mixture was stirred at ambient temperature for 1 h and filtered through a Celite pad (100 mL). The filtrate became colorless and was transferred to a 1000-mL, round bottom flask equipped with a magnetic stirrer. Palladium on activated carbon (Pd/C, 5 wt.%, 4.0 g) was added, the mixture was evacuated for 1 min, filled up with H_2 using a balloon and stirred at ambient temperature for 3 h. It was filtered through a Celite pad (100 mL) and the filter cake was washed with

acetone (3 × 50 mL). The combined filtrates had a grey color and were filtered through multiple 0.45 μM polytetrafluoroethylene (PTFE) filters. The filtrate was concentrated to 150 mL and added to cold water (1.5 L, 0 – 5 °C) over 0.5 h with overhead stirring. The suspension was filtered and the filter cake was washed with water (3 × 100 mL), conditioned for 0.5 h, and vacuum-dried for 24 h to afford a white powder [triglutamate-PLGA5050-O-acetyl, 21 g, yield: 72%]. HPLC analysis indicated a 100% purity (AUC, 227 nm) and. GPC analysis showed a Mw: 9.2 kDa and Mn: 6.9 kDa.

A 1000-mL, round-bottom flask equipped with a magnetic stirrer was charged with triglutamate-PLGA5050-O-acetyl [20 g, 2.9 mmol, Mn 6.9 kDa,], docetaxel (5.7 g, 7.0 mmol, 2.4 equiv.), and DMF (75 mL). The mixture was stirred for 5 min to afford a clear solution. EDC•HCl (1.08 g, 5.6 mmol) and DMAP (0.72 g, 5.6 mmol) were added and the reaction was stirred at ambient temperature for 3 h. A second portion EDC•HCl (0.54 g, 2.8 mmol), and DMAP (0.54 g, 2.8 mmol) was added and the reaction was stirred for an additional 3 h. A third portion of EDC•HCl (0.36 g, 1.9 mmol) and DMAP (0.24 g, 1.9 mmol) was added and the reaction was stirred for 14 h. An additional portion of EDC•HCl (0.36 g, 1.9 mmol) and DMAP (0.24 g, 1.9 mmol) was added and the reaction was stirred for another 4 h. The reaction mixture was added to a suspension of Celite (60 g) in MTBE (2.0 L) over 0.5 h with overhead stirring. The suspension was filtered through a PP filter and the crude product/Celite complex was dried under vacuum at 25 °C for 12 h. The product/complex was suspended in acetone (200 mL) for 0.5 h with overhead stirring and filtered. The filter cake was washed with acetone (3 × 60 mL). The combined filtrates were concentrated to 100 mL. A second Celite/MTBE precipitation was conducted; the filtrate from the acetone extraction was concentrated to 100 mL, added to cold water (1.0 L, 0 – 5 °C) with overhead stirring and filtered. The solid was vacuum-dried for 2 days to afford crude product as a white powder [24 g]. The crude product was dissolved in acetone (120 mL) and added to a suspension of Celite (70 g, Aldrich, standard supercell, acid washed) in MTBE (2.0 L) at ambient temperature with overhead stirring. The suspension was stirred for 2 h and filtered through a fritted funnel. The filter cake was washed with MTBE (2 × 200 mL) and vacuum-dried at

ambient temperature overnight. The solid was suspended in acetone (200 mL) with overhead stirring for 1 h. The suspension was filtered through a fritted funnel and the filter cake was rinsed with acetone (3 × 100 mL). The combined filtrates were concentrated to ~150 mL and precipitated into Celite/MTBE a fourth time. To facilitate the purification, the filtrate was concentrated to ~120 mL and added to MTBE (2.0 L) at ambient temperature with vigorous stirring. The suspension was filtered through a fritted funnel and the filter cake was vacuum-dried for 16 h to afford a crude product as a white powder containing ~30 wt% of residual MTBE [30 g, > 100% yield,]. The crude product was dissolved in acetone (120 mL) and the solution was precipitated into MTBE (2.0 L). The resultant suspension was stirred at ambient temperature for 3 h and filtered through a fritted funnel. The filter cake was vacuum-dried for 12 h to afford a white solid [30 g]. At this point, a third water precipitation was conducted to isolate the product and reduce the residual MTBE. The above crude product was dissolved in acetone (100 mL) and the solution was added to cold water (1.5 L, 0 – 5 °C) over 0.5 h with overhead stirring. The suspension was filtered through a fritted funnel. The filter cake was washed with water (3 × 200 mL), conditioned for 2 h, and vacuum-dried for 2 days to afford the desired product (tetra-(docetaxel) triglutamate-5050 PLGA-O-acetyl) as a white powder [20 g, yield: 78%;]. HPLC analysis showed a 99.5% purity along with 0.5% of residual docetaxel. GPC analysis indicated a Mw: 10.8 kDa and Mn: 6.6 kDa.

The major product is tetra(2'-docetaxel) triglutamate-5050 PLGA-O-acetyl (wherein each docetaxel is attached to the triglutamate linker via the 2' hydroxyl group); the product may also include free 5050 PLGA-O-acetyl, monofunctionalized polymers (e.g., mono(2'-docetaxel) triglutamate-5050 PLGA-O-acetyl or monosubstituted products attached via the 7, 10 or 1 hydroxyl groups), difunctionalized polymers (e.g., bis(2'-docetaxel)triglutamate-5050 PLGA-O-acetyl, or disubstituted products with docetaxel molecules attached via other hydroxyl groups or mixtures thereof), trifunctionalized polymers (e.g., tris(2'-docetaxel)triglutamate-5050 PLGA-O-acetyl, or trisubstituted products with docetaxel molecules attached via other hydroxyl groups or mixtures thereof), and/or a trace amount of docetaxel.

Example 11. Synthesis, purification and characterization of folate-PEG-PLGA-lauryl ester

The synthesis of folate-PEG-PLGA-lauryl ester involves the direct coupling of folic acid to PEG bisamine (Sigma-Aldrich, n=75, MW 3350 Da). PEG bisamine was purified due to the possibility that small molecular weight amines were present in the product. 4.9 g of PEG bisamine was dissolved in DCM (25 mL, 5 vol) and then transferred into MTBE (250 mL, 50 vol) with vigorous agitation. The polymer precipitated as white powder. The mixture was then filtered and the solid was dried under vacuum to afford 4.5 g of the product [92%]. The ^{1}H NMR analysis of the solid gave a clean spectrum; however, not all alcohol groups were converted to amines based on the integration of α-methylene to the amine group (63% bisamine, 37% monoamine).

Folate-(γ)CO-NH-PEG-NH_2 was synthesized using the purified PEG bisamine. Folic acid (100 mg, 1.0 equiv.) was dissolved in hot DMSO (4.5 mL, 3 vol to PEG bisamine). The solution was cooled to ambient temperature and (2-(7-Aza-1H-benzotriazole-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate) (HATU, 104 mg, 1.2 equiv.) and N,N- Diisopropylethylamine (DIEA, 80 μL, 2.0 equiv.) were added. The resulting yellow solution was stirred for 30 minutes and PEG bisamine (1.5 g, 2 equiv.) in DMSO (3 mL, 2 vol) was added. Excess PEG bisamine was used to avoid the possible formation of di-adduct of PEG bisamine and to improve the conversion of folic acid. The reaction was stirred at 20 °C for 16 h and directly purified by CombiFlash using a C18 column (RediSep, 43 g, C18). The fractions containing the product were combined and the CH_3CN was removed under vacuum. The remaining water solution (~200 mL) was extracted with chloroform (200 mL × 2). The combined chloroform phases were concentrated to approximately 10 mL and transferred into MTBE to precipitate the product as a yellow powder. In order to completely remove any unreacted PEG bisamine in the material, the yellow powder was washed with acetone (200 mL) three times. The remaining solid was dried under vacuum to afford a yellow semi-solid product (120 mg). HPLC analysis indicated a purity of 97% and the ^{1}H NMR analysis showed that the product was clean.

Folate-(γ)CO-NH-PEG-NH2 was reacted with *p*-nitrophenyl-COO-PLGA-CO_2-lauryl to provide folic acid-PEG-PLGA-lauryl ester. To prepare *p*-nitrophenyl-COO-PLGA-CO_2-lauryl, PLGA 5050 (lauryl ester) [10.0 g, 1.0 equiv.] and *p*-nitrophenyl chloroformate (0.79 g, 2.0 equiv.) were dissolved in DCM. To the dissolved polymer solution, one portion of TEA (3.0 equiv.) was added. The resulting solution was stirred at 20 °C for 2 h and the ^{1}H NMR analysis indicated complete conversion. The reaction solution was then transferred into a solvent mixture of 4:1 MTBE/heptanes (50 vol). The product precipitated and gummed up. The supernatant was decanted off and the solid was dissolved in acetone (20 vol). The resulting acetone suspension was filtered and the filtrate was concentrated to dryness to produce the product as a white foam [7.75 g, 78%, Mn = 4648 based on GPC]. The ^{1}H NMR analysis indicated a clean product with no detectable *p*-nitrophenol.

Folate-(γ)CO-NH-PEG-NH2 (120 mg, 1.0 equiv.) was dissolved in DMSO (5 mL) and TEA (3.0 equiv.) was added. The pH of the reaction mixture was 8 – 9. *p*-nitrophenyl-COO-PLGA-CO_2-lauryl (158 mg, 1.0 equiv.) in DMSO (1 mL) was added and the reaction was monitored by HPLC. A new peak at 16.1 min (~40%, AUC, 280 nm) was observed from the HPLC chromatogram in 1 h. A small sample of the reaction mixture was treated with excess 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) and the color instantly changed to dark yellow. HPLC analysis of this sample indicated complete disappearance of *p*-nitrophenyl-COO-PLGA-CO_2-lauryl and the 16.1 min peak. Instead, a peak on the right side of folate-(γ)CO-NH-PEG-NH2 appeared. It can be concluded that the *p*-nitrophenyl-COO-PLGA-CO_2-lauryl and the possible product were not stable under strong basic conditions. In order to identify the new peak at 16.1 min, ~1/3 of the reaction mixture was purified by CombiFlash. The material was finally eluted with a solvent mixture of 1:4 DMSO/CH_3CN. It was observed that this material was yellow which could have indicated folate content. Due to the large amount of DMSO present, this material was not isolated from the solution. The fractions containing unreacted folate-(γ)CO-NH-PEG-NH2 was combined and concentrated to a residue. A ninhydrin test of this residue gave a

negative result, which may imply the lack of amine group at the end of the PEG. This observation can also explain the incomplete conversion of the reaction.

The rest of reaction solution was purified by CombiFlash. Similarly to the previous purification, the suspected yellow product was retained by the column. MeOH containing 0.5% TFA was used to elute the material. The fractions containing the possible product were combined and concentrated to dryness. The ^{1}H NMR analysis of this sample indicated the existence of folate, PEG and lauryl-PLGA and the integration of these segments was close to the desired value of 1:1:1 ratio of all three components. High purities were observed from both HPLC and GPC analyses. The Mn based on GPC was 8.7 kDa. The sample in DMSO was recovered by precipitation into MTBE.

Example 12. Synthesis and purification of docetaxel-2'-hexanoate-5050 PLGA-O-acetyl

A 500-mL round-bottom flask equipped with a magnetic stirrer was charged with 6-(carbobenzyloxyamino) caproic acid (4.13 g, 15.5 mmol), docetaxel (12.0 g, 14.8 mmol), and dichloromethane (240 mL). The mixture was stirred for 5 min to afford a clear solution, to which 1-ethyl-3-(3-dimethyllaminopropyl)carbodiimide hydrochloride (EDC•HCl) (3.40 g, 17.6 mmol) and 4 dimethylaminopyridine (DMAP) (2.15 g, 17.6 mmol) were added. The mixture was stirred at ambient temperature for 3 h at which time, IPC analysis showed a 57% conversion along with 34% residual docetaxel. An additional 0.2 equivalents of EDC•HCl and DMAP were added and the reaction was stirred for 3 h, at which time IPC analysis showed 63% conversion. An additional 0.1 equivalents of 6-(carbobenzyloxyamino) caproic acid along with 0.2 equivalents of EDC•HCl and DMAP were added. The reaction was stirred for 12 h and IPC analysis indicated 74% conversion and 12% residual docetaxel. To further increase the conversion, an additional 0.1 equivalents of 6-(carbobenzyloxyamino) caproic acid and 0.2 equivalents of EDC•HCl and DMAP were added. The reaction was continued for another 3 h at which time, IPC analysis revealed 82% conversion and the residual docetaxel dropped to 3%. The reaction was

diluted with DCM (200 mL) and washed with 0.01% HCl (2× 150 mL) and brine (150 mL). The organic layer was separated, dried over sodium sulfate, and filtered. The filtrate was concentrated to a residue and dissolved in ethyl acetate (25 mL). The solution was divided into two portions, each of which was passed through a 120-g silica column (Biotage F40). The flow rate was adjusted to 20 mL/min and 2000 mL of 55:45 ethyl acetate/heptanes was consumed for each of the column purifications. The fractions containing minor impurities were combined, concentrated, and passed through a column a third time. The fractions containing product (shown as a single spot by TLC analysis) from all three column purifications were combined, concentrated to a residue, vacuum-dried at ambient temperature for 16 h to afford the product, H_2N-$(CH_2)_5$CO-O-2'-docetaxel as a white powder [10 g, yield: 64%]. The ^1H NMR analysis was consistent with the assigned structure of the desired product; however, HPLC analysis (AUC, 227 nm) indicated only a 97% purity along with 3% of bis-adducts. To purify the H_2N-$(CH_2)_5$CO-O-2'-docetaxel product, ethyl acetate (20 mL) was added to dissolve the batch to produce a clear solution. The solution was divided into two portions, each of which was passed through a 120-g silica column. The fractions containing product were combined, concentrated to a residue, vacuum-dried at ambient temperature for 16 h to afford the desired product (CBZ-NH-$(CH_2)_5$CO-O-2'-docetaxel) as a white powder [8.6 g, recovery yield: 86%]. HPLC analysis (AUC, 227 nm) indicated >99% purity.

A 1000-mL round-bottom flask equipped with a magnetic stirrer was charged with CBZ-NH-$(CH_2)_5$CO-O-2'-docetaxel product [5.3 g, 5.02 mmol] and THF (250 mL). To the resultant clear solution, MeOH (2.5 mL) and 5% Pd/C (1.8 g, 10 mol% of Pd) were added. The mixture was cooled to 0 °C and methanesulfonic acid (316 μL, 4.79 mmol) was added. The flask was evacuated for 10 seconds and filled with hydrogen using a balloon. After 3 h, IPC analysis indicated 62% conversion. The ice-bath was removed and the reaction was allowed to warm up to ambient temperature. After an additional 3 h, IPC analysis indicated that the reaction was complete. The solution was filtered through a Celite® pad and the filtrate was black in appearance. To remove the possible residual Pd, charcoal (5 g, Aldrich, Darco®) was added and the mixture was placed in a fridge overnight and filtered through a

Celite® pad to produce a clear colorless solution. This was concentrated at < 20°C under reduced pressure to a volume of ~100 mL, to which methyl tert-butyl ether (MTBE) (100 mL) was added. The resultant solution was added to a solution of cold MTBE (1500 mL) with vigorous stirring over 0.5 h. The suspension was left at ambient temperature for 16 h, the upper clear supernatant was decanted off and the bottom layer was filtered through a 0.45 μm filter membrane. The filter cake was vacuum-dried at ambient temperature for 16 h to afford the desired product (H_2N-$(CH_2)_5CO$-O-2'-docetaxel) as a white solid [4.2 g, yield: 82%]. HPLC analysis indicated >99% purity and the ^1H NMR analysis indicated the desired product.

A 100-mL round-bottom flask equipped with a magnetic stirrer was charged with 5050 PLGA-O-acetyl (5.0 g, 0.7 mmol), H_2N-$(CH_2)_5CO$-O-2'-docetaxel [0.85 g, 0.84 mmol, GAO-G-28(3)], DCM (5 mL), and DMF (20 mL). The mixture was stirred for 5 min to produce a clear solution. EDC•HCl (0.2 g, 1.05 mmol) and DMAP (0.21 g, 1.75 mmol) were added and the reaction was stirred for 3h, at which time IPC analysis indicated 79% conversion along with 18% of H_2N-$(CH_2)_5CO$-O-2'-docetaxel. Two small impurities were observed at 11.6 min and 11.7 min (2.8%, AUC, 227 nm). An additional portion of EDC•HCl (0.1 g, 0.5 mmol) and DMAP (0.15 g, 1.2 mmol) was added and the reaction was stirred overnight. IPC analysis showed 92% conversion along with 6% of H_2N-$(CH_2)_5CO$-O-2'-docetaxel; the level of the two impurities did not change. To increase the conversion, an additional amount of 5050 PLGA-O-acetyl (0.5 g) along with EDC•HCl (0.1 g) and DMAP (0.15 g) was added and the reaction was stirred at ambient temperature for 3 h. IPC analysis showed a 95.6% conversion along with 3.0% of H_2N-$(CH_2)_5CO$-O-2'-docetaxel; the two impurities were about 1.3%. The reaction was combined with a previously prepared product and added to a suspension of Celite® (20 g) in MTBE (600 mL) with mechanical stirring over 30 min. The suspension was stirred at ambient temperature for 0.5 h and filtered. The filter cake was air-dried for 30 min and then vacuum-dried such that the residual MTBE contained no more than 5 wt%. The polymer/Celite® complex was then suspended in acetone (50 mL) and the slurry was stirred for 30 min, filtered through a Celite pad. The filter cake was washed with acetone (3 × 30 mL). The combined filtrates were concentrated to ~25 mL and this

solution was analyzed by HPLC showing that the level of H_2N-$(CH_2)_5CO$-O-2'-docetaxel or the impurities was identical to these prior to MTBE precipitation. The solution was added to cold water (500 mL) containing 0.05% acetic acid over 30 min. The suspension was stirred at 0 °C for 1 h and filtered through a PP filter. The filter cake was washed with water (3 × 50 mL), conditioned for 30 min, vacuum-dried at ambient temperature for 48 h to produce docetaxel-2'-hexanoate-5050 PLGA-O-acetyl as a white powder [6.3 g, 85%]. The ^{1}H NMR analysis indicated 10.5 wt% of loading. No DMAP or DMF was observed. GPC analysis indicated a Mw of 8.2 kDa and a Mn of 5.7 kDa. HPLC analysis indicated a purity of 98.6% (AUC, 230 nm) and a 0.75% of H_2N-$(CH_2)_5CO$-O-2'-docetaxel. The two impurities totaled ≤ 0.5% (AUC, 230 nm).

Example 13. Synthesis, purification and characterization of O-acetyl-5050-PLGA-(2'-β-alanine glycolate)-docetaxel

A 1000 mL round-bottom flask equipped with a magnetic stirrer was charged with carbobenzyloxy-β-alanine (Cbz-β-alanine, 15.0 g, 67.3 mmol), tert-butyl bromoacetate (13.1 g, 67.3 mmol), acetone (300 mL), and potassium carbonate (14 g, 100 mmol). The mixture was heated to reflux at 60 °C for 16 h, cooled to ambient temperature and then the solid was removed by filtration. The filtrate was concentrated to a residue, dissolved in ethyl acetate (EtOAc, 300 mL), and washed with 100 mL of water (three times) and 100 mL of brine. The organic layer was separated, dried over sodium sulfate and filtered. The filtrate was concentrated to clear oil [22.2 g, yield: 99%]. HPLC analysis showed 97.4% purity (AUC, 227 nm) and ^{1}H NMR analysis confirmed the desired intermediate product, t-butyl (carbobenzyloxy-β-alanine) glycolate.

To prepare the intermediate product, carbobenzyloxy-β-alanine glycolic acid (Cbz-β-alanine glycolic acid), a 100 mL round-bottom flask equipped with a magnetic stirrer was charged with t-butyl (Cbz-β-alanine) glycolate [7.5 g, 22.2 mmol] and formic acid (15 mL, 2 vol). The mixture was stirred at ambient temperature for 3 h to give a red-wine color and HPLC analysis showed 63% conversion. The reaction was

continued stirring for an additional 2 h, at which point HPLC analysis indicated 80% conversion. An additional portion of formic acid (20 mL, 5 vol in total) was added and the reaction was stirred overnight, at which time HPLC analysis showed that the reaction was complete. The reaction was concentrated under vacuum to a residue and redissolved in ethyl acetate (7.5 mL, 1 vol.). The solution was added to the solvent heptanes (150 mL, 20 vol.) and this resulted in the slow formation of the product in the form of a white suspension. The mixture was filtered and the filter cake was vacuum-dried at ambient temperature for 24 h to afford the desired product, Cbz-β-alanine glycolic acid as a white powder [5.0 g, yield: 80%]. HPLC analysis showed 98% purity. The ^{1}H NMR analysis in DMSO-d6 was consistent with the assigned structure of Cbz-β-alanine glycolic acid [δ 10.16 (s, 1H), 7.32 (bs, 5H), 5.57 (bs, 1H), 5.14 (s, 2H), 4.65 (s, 2H), 3.45 (m, 2H), 2.64 (m, 2H)].

To prepare the intermediate, docetaxel-2'-carbobenzyloxy-β-alanine glycolate (docetaxel-2'-Cbz- β-alanine glycolate), a 250-mL round-bottom flask equipped with a magnetic stirrer was charged with docetaxel (5.03 g, 6.25 mmol), Cbz-β-alanine glycolic acid [1.35 g, 4.80 mmol] and dichloromethane (DCM, 100 mL). The mixture was stirred for 5 min to produce a clear solution, to which N-(3-Dimethylaminopropyl)-N′-ethylcarbodiimide hydrochloride (EDC•HCl, 1.00 g, 5.23 mmol) and 4-(dimethylamino)pyridine (DMAP, 0.63 g, 5.23 mmol) were added. The mixture was stirred at ambient temperature for 3 h, at which point HPLC analysis showed 48% conversion along with 46% of residual docetaxel. A second portion of Cbz-β-alanine glycolic acid (0.68 g, 2.39 mmol), EDC•HCl (0.50 g, 1.04 mmol) and DMAP (0.13 g, 1.06 mmol) were added and the reaction was allowed to stirred overnight. At this point, HPLC analysis showed 69% conversion along with 12% of residual docetaxel. The solution was diluted to 200 mL with DCM and then washed with 80 mL of water (twice) and 80 mL of brine. The organic layer was separated, dried over sodium sulfate, and then filtered. The filtrate was concentrated to a residue, re-dissolved in 10 mL of chloroform, and purified using a silica gel column. The fractions containing product (shown as a single spot by TLC analysis) were combined, concentrated to a residue, vacuum-dried at ambient temperature for 16 h to produce docetaxel-2'-Cbz- β-alanine glycolate as a white powder [3.5 g, yield: 52%].

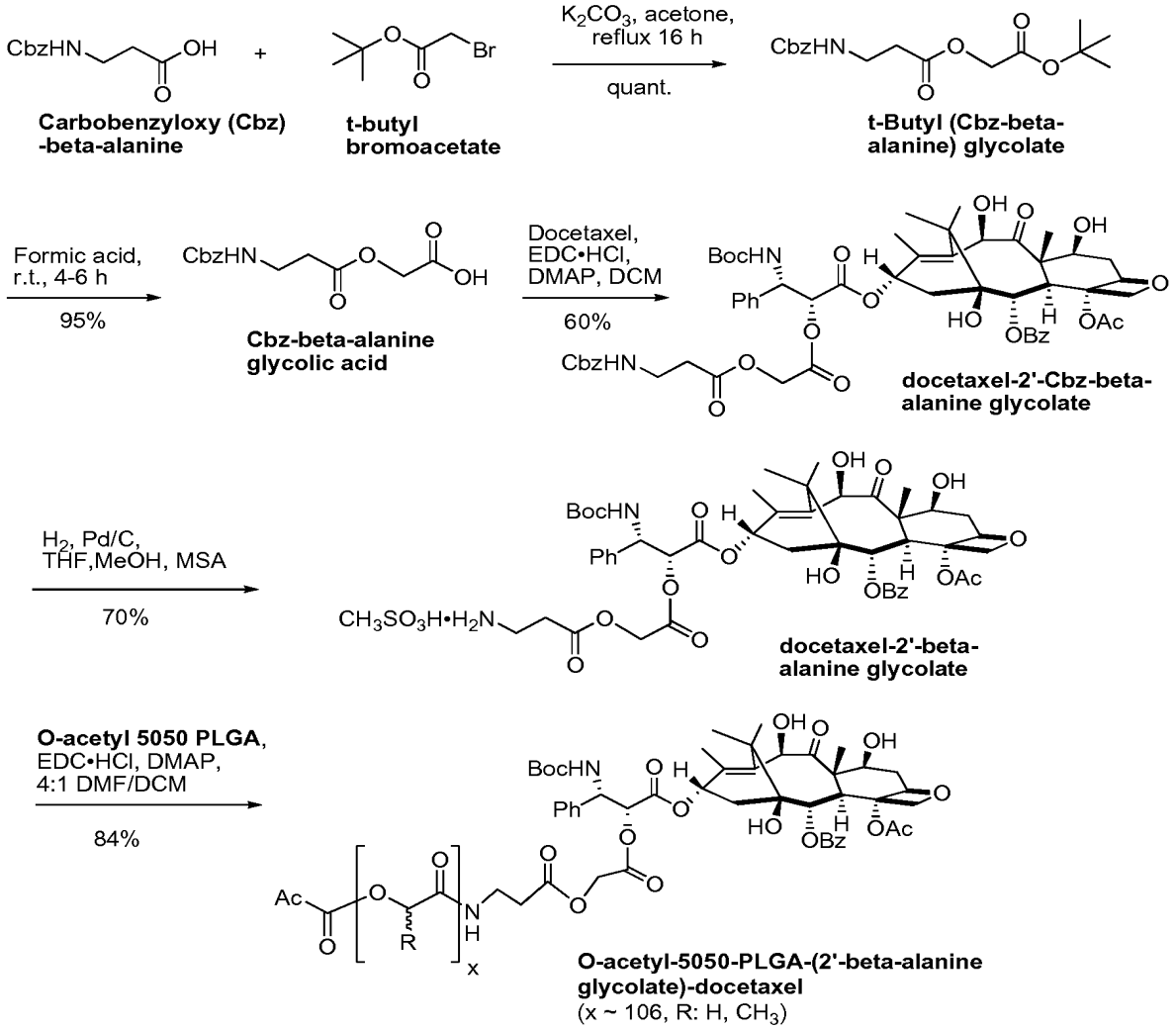
HPLC analysis (AUC, 227 nm) indicated > 99.5% purity. The ^{1}H NMR analysis confirmed the corresponding peaks.

To prepare the intermediate, docetaxel-2'-β-alanine glycolate, a 250 mL round-bottom flask equipped with a magnetic stirrer was charged with docetaxel-2'-Cbz-β-alanine glycolate [3.1 g, 2.9 mmol] and tetrahydrofuran (THF, 100 mL). To the clear solution methanol (MeOH, 4 mL), methanesulfonic acid (172 μL, 2.6 mmol), and 5% palladium on activated carbon (Pd/C, 1.06 g, 10 mol% of Pd) were added. The mixture was evacuated for 15 seconds and filled with hydrogen using a balloon. After 3 h, HPLC analysis indicated that the reaction was complete. Charcoal (3 g, Aldrich, Darco®#175) was then added and the mixture was stirred for 15 min and filtered through a Celite® pad to produce a clear colorless solution. It was concentrated under reduced pressure at < 20°C to ~5 mL, to which 100 mL of heptanes was added slowly resulting in the formation of a white gummy solid. The supernatant was decanted and the gummy solid was vacuum-dried for 0.5 h to produce a white solid. A volume of 100 mL of heptanes were added and the mixture was triturated for 10 min and filtered. The filter cake was vacuum-dried at ambient temperature for 16 h to produce docetaxel-2'-β-alanine glycolate as a white powder [2.5 g, yield: 83%]. The HPLC analysis indicated >99% purity (AUC, 230 nm). MS analysis revealed the correct molecular mass (m/z: 936.5).

A 100 mL round bottom equipped with a magnetic stirrer was charged with O-acetyl-5050-PLGA [5.0 g, 0.7 mmol], docetaxel-2'-β-alanine glycolate [0.80 g, 0.78 mmol], dichloromethane (DCM, 5 mL) and dimethylformamide (DMF, 20 mL). The mixture was stirred for 5 min to produce a clear solution. EDC•HCl (0.22 g, 1.15 mmol) and DMAP (0.22 g, 1.80 mmol) were added to the mixture and the reaction was stirred for 3h, at which time HPLC analysis indicated completion of the reaction. The reaction was concentrated under vacuum to remove DCM and then DCM was twice exchanged with 10 mL of acetone. The residue was diluted with acetone to 30 mL and precipitated in cold water containing 600 mL of 0.1% acetic acid. The resulting suspension was filtered and the filter cake was vacuum-dried for 24 h to afford a crude product as a white powder [yield = 5.0 g]. The ^{1}H NMR analysis indicated the presence of trace amounts of DMF and DMAP. The docetaxel loading

was estimated to be approximately 10 wt% and HPLC analysis indicated > 99% purity (AUC, 230 nm). To purify the crude product, it was dissolved in 20 mL of acetone and precipitated in 500 mL of cold water. The suspension was filtered through a polypropylene (PP) filter and the filter cake was vacuum-dried for 48 h to produce O-acetyl-5050-PLGA-(2'-β-alanine glycolate)-docetaxel as a white powder [4.8 g, yield: 84%]. GPC analysis showed that Mw = 7.4 kDa, Mn = 5.0 kDa and PDI = 1.48. ^{1}H NMR analysis indicated a docetaxel loading of 10.7 wt% and HPLC analysis showed > 99% purity (AUC, 230 nm).

Synthetic scheme of O-acetyl-5050-PLGA-(2'-β-alanine glycolate)-docetaxel



Example 14. Synthesis of lauryl-polylactide (PLA)-O-CO-O-docetaxel

To prepare lauryl-PLA-O-CO-O-docetaxel, PLA-lauryl ester (inherent viscosity: 1-2 dL/g) was first purified. A mass of 25 g of PLA lauryl ester was dissolved in a 1:1 MTBE/heptanes mixture (100 vol.) with mechanical stirring at ambient temperature. The entire solution was concentrated to dryness and further dried under vacuum at ambient temperature to afford a white powder (18 g). The ^{1}H NMR analysis indicated 1.44 equivalents of lauryl segment. GPC analysis indicated a Mn and Mw of 8.5 kDa and 10.7 kDa respectively.

A 250-mL round-bottom flask was charged with purified PLA-lauryl ester (10.0 g, 1.18 mmol] and anhydrous DCM (50 mL) under nitrogen. The mixture was stirred for 10 min to afford a clear solution. p-Nitrophenyl chloroformate (0.5 g, 2.4 mmol) was added to the solution and the mixture was stirred for an additional 10 min. A solution of TEA (0.5 mL) was then added dropwise and the reaction was stirred at ambient temperature for 6 h. An additional one equivalent of p-nitrophenyl chloroformate (0.25 g, 1.2 mmol) and TEA (0.25 mL) were added and the reaction was stirred for 12 h. IPC analysis (^{1}H NMR) indicated completion of the reaction. The solution was concentrated to a residue and dissolved in acetone (20 mL), resulting in a cloudy mixture. This mixture was filtered to remove TEA•HCl and the filtrate was precipitated into a solution of 2:1 MTBE/heptanes (1000 mL). The resulting gummy solid was dissolved in acetone (20 mL) and concentrated to a residue, which was dried under vacuum at ambient temperature for 24 h to afford 5.6 g of p-NO_2-phenyl-COO-PLA-CO_2-lauryl [yield: ~50%]. The ^{1}H NMR analysis confirmed the desired product and GPC analysis showed a Mn and Mw of 9.3 and 11.1 kDa respectively.

A 100-mL round-bottom flask was charged with p-NO_2-phenyl-COO-PLA-CO_2-lauryl [2.5 g, 0.28 mmol], docetaxel (0.20 g, 0.25 mmol) and 1:1 DCM/EtOAc (15 mL). The entire mixture was stirred for 10 min. A catalyst, dialkylaminopyridine (DMAP, 61 mg, 0.5 mmol) was added to the mixture and allowed to stir at ambient temperature under N_2 for 6 h. The reaction was stirred for another 10 h to reach completion as confirmed by IPC analysis (^{1}H NMR). The

reaction was then filtered through a 0.45 μM PTFE membrane and the filtrate was added dropwise into 2:1 MTBE/heptanes (600 mL) with vigorous agitation, resulting in a suspension. The milky supernatant was decanted off and the gummy solid was dissolved in acetone (15 mL). The solution was then added dropwise into an ice-cold solution of 0.1% sodium bicarbonate (300 mL) with agitation. The resulting suspension was filtered and the solid was dried under vacuum at ambient temperature for 24 h to afford 1.34 g of lauryl- PLA-O-CO-O-docetaxel [yield: 51%]. The ^{1}H NMR analysis indicated 9.3 wt% of docetaxel loading. GPC analysis showed a Mn and Mw of 12.4 and 14.3 kDa respectively.

Example 15. Synthesis of PLGA-PEG-PLGA

The triblock copolymer PLGA-PEG-PLGA will be synthesized using a method developed by Zentner et al., Journal of Controlled Release, 72, 2001, 203-215. The molecular weight of PLGA obtained using this method would be ~3 kDa. A similar method reported by Chen et al., International Journal of Pharmaceutics, 288, 2005, 207-218 will be used to synthesize PLGA molecular weights ranging from 1-7 kDa. The LA/GA ratio would typically be, but not limited to a ratio of 1:1. The minimum PEG molecular weight would be 2 kDa with an upper limit of 30 kDa. The preferred range of PEG would be 3-12 kDa. The PLGA molecular weight would be a minimum value of 4 kDa and a maximum of 30 kDa. The preferred range of PLGA would be 7-20 kDa. Any drug (e.g. docetaxel, paclitaxel, doxorubicin, etc.) could be conjugated to the PLGA through an appropriate linker (i.e. as listed in the previous examples) to form a polymer-drug conjugate. In addition, the same drug or a different drug could be attached to the other PLGA to form a dual drug polymer conjugate with two same drugs or two different drugs. Nanoparticles could be formed from either the PLGA-PEG-PLGA alone or from a single drug or dual polymer conjugate composed of this triblock copolymer.

Example 16. Synthesis of polycaprolactone-poly(ethylene glycol)-polycaprolactone (PCL-PEG-PCL)

The triblock PCL-PEG-PCL will be synthesized using a ring open polymerization method in the presence of a catalyst (i.e. stannous octoate) as reported in Hu et al., Journal of Controlled Release, 118, 2007, 7-17. The molecular weights of PCL obtained from this synthesis range from 2 to 22 kDa. A non-catalyst method shown in the article by Ge et al. Journal of Pharmaceutical Sciences, 91, 2002, 1463-1473 will also be used to synthesize PCL-PEG-PCL. The molecular weights of PCL that could be obtained from this particular synthesis range from 9 to 48 kDa. Similarly, another catalyst free method developed by Cerrai et al., Polymer, 30, 1989, 338-343 will be used to synthesize the triblock copolymer with molecular weights of PCL ranging from 1-9 kDa. The minimum PEG molecular weight would be 2 kDa with an upper limit of 30 kDa. The preferred range of PEG would be 3-12 kDa. The PCL molecular weight would be a minimum value of 4 kDa and a maximum of 30 kDa. The preferred range of PCL would be 7-20 kDa. Any drug (e.g. docetaxel, paclitaxel, doxorubicin, etc.) could be conjugated to the PCL through an appropriate linker (i.e. as listed in the previous examples) to form a polymer-drug conjugate. In addition, the same drug or a different drug could be attached to the other PCL to form a dual drug polymer conjugate with two same drugs or two different drugs. Nanoparticles could be formed from either the PCL-PEG-PCL alone or from a single drug or dual polymer conjugate composed of this triblock copolymer.

Example 17. Synthesis of polylactide- poly(ethylene glycol)-polylactide (PLA-PEG-PLA)

The triblock PLA-PEG-PLA copolymer will be synthesized using a ring opening polymerization using a catalyst (i.e. stannous octoate) reported in Chen et al., Polymers for Advanced Technologies, 14, 2003, 245-253. The molecular weights of PLA that can be formed range from 6 to 46 kDa. A lower molecular weight range (i.e. 1-8 kDa) could be achieved by using the method shown by Zhu et al., Journal of Applied Polymer Science, 39, 1990, 1-9. The minimum PEG molecular weight would be 2 kDa with an upper limit of 30 kDa. The preferred range of PEG would be 3-12 kDa. The PCL molecular weight would be a minimum value of 4 kDa and a maximum of 30 kDa. The preferred range of PCL would be 7-20 kDa. Any drug

(e.g. docetaxel, paclitaxel, doxorubicin, etc.) could be conjugated to the PLA through an appropriate linker (i.e. as listed in the previous examples) to form a polymer-drug conjugate. In addition, the same drug or a different drug could be attached to the other PLA to form a dual drug polymer conjugate with two same drugs or two different drugs. Nanoparticles could be formed from either the PLA-PEG-PLA alone or from a single drug or dual polymer conjugate composed of this triblock copolymer.

Example 18. Synthesis of p-dioxanone-co-lactide-poly(ethylene glycol)-p-dioxanone-co-lactide (PDO-PEG-PDO)

The triblock PDO-PEG-PDO will be synthesized in the presence of a catalyst (stannous 2-ethylhexanoate) using a method developed by Bhattari et al., Polymer International, 52, 2003, 6-14. The molecular weight of PDO obtained from this method ranges from 2-19 kDa. The minimum PEG molecular weight would be 2 kDa with an upper limit of 30 kDa. The preferred range of PEG would be 3-12 kDa. The PDO molecular weight would be a minimum value of 4 kDa and a maximum of 30 kDa. The preferred range of PDO would be 7-20 kDa. Any drug (e.g. docetaxel, paclitaxel, doxorubicin, etc.) could be conjugated to the PDO through an appropriate linker (i.e. as listed in the previous examples) to form a polymer-drug conjugate. In addition, the same drug or a different drug could be attached to the other PDO to form a dual drug polymer conjugate with two same drugs or two different drugs. Nanoparticles could be formed from either the PDO-PEG-PDO alone or from a single drug or dual polymer conjugate composed of this triblock copolymer.

Example 19. Formulation of Docetaxel-PLGA particles via nanoprecipitation using PVA as surfactant

Docetaxel-5050 PLGA-O-acetyl (700 mg, 70 wt% or 600 mg, 60 wt%,) and mPEG-PLGA (300 mg, 30 wt% or 400 mg, 40 wt%, Mw 12.9 kDa) were dissolved to form a total concentration of 1.0% polymer in acetone. In a separate solution, 0.5% w/v PVA (80% hydrolyzed, Mw 9-10 kDa) in water was prepared. The polymer acetone solution was added using a syringe pump at a rate of 1 mL/min to the aqueous solution (v/v ratio of organic to aqueous phase = 1:10), with stirring at 500 rpm.

Acetone was removed by stirring the solution for 2-3 hours. The nanoparticles were then washed with 10 volumes of water and concentrated using a tangential flow filtration system (300 kDa MW cutoff, membrane area = 50 cm^2). The solution was then passed through a 0.22 μm filter, and adjusted to a final concentration of 10% sucrose. The nanoparticles could be lyophilized into powder form. The nanoparticles contain about half the initial amount of mPEG-PLGA, and 15-30% PVA.

Particle properties, evaluated by using the resulting plurality of particles made in the method above: (prior to passing through 0.22 μm filter):

	Docetaxel-5050 PLGA-O-acetyl/ mPEG-PLGA Starting amt:(70/30 wt%)	Docetaxel-5050 PLGA-O-acetyl/ mPEG-PLGA Starting amt:(60/40 wt%)
Z-average (nm)	93	84
Particle PDI	0.09	0.06
Dv50 (nm)	76	71
Dv90 (nm)	124	109

Example 20. Formulation of PEGylated docetaxel-5050 PLGA-O-acetyl nanoparticles via Nanoprecipitation using polysorbate 80 as the surfactant

Docetaxel-5050 PLGA-O-acetyl (672 mg, 84 wt%) and mPEG-PLGA (128 mg, 16 wt%, Mw 12.9 kDa,) were dissolved to form a total concentration of 2.0% polymer in acetone. In a separate solution, 0.5% w/v polysorbate 80 in water was prepared. The polymer acetone solution was added using a syringe pump at a rate of 1 mL/min to the aqueous solution (v/v ratio of organic to aqueous phase = 1:10), with stirring at 500 rpm. Acetone was removed by stirring the solution for 2-3 hours. The nanoparticles were then washed with 10 volumes of 0.5% w/v polysorbate 80 and concentrated using a tangential flow filtration system (300 kDa MW cutoff, membrane area = 50 cm^2). The solution was then passed through a 0.22 μm Nylon filter, and adjusted to a final concentration of 10% sucrose. The nanoparticles could

be lyophilized into powder form. The nanoparticles contain about half the initial amount of mPEG-PLGA, and 5-15% surfactant.

Particle properties, evaluated by using the resulting plurality of particles made in the method above:

Zavg = 107 nm

Particle PDI = 0.112

Dv50 = 89 nm

Dv90 = 150 nm

Example 21. Formulation of PEGylated Docetaxel-5050 PLGA-O-acetyl nanoparticles via Nanoprecipitation using Solutol® HS 15 as the surfactant

Docetaxel-5050 PLGA-O-acetyl (672 mg, 84 wt%) and mPEG-PLGA (128 mg, 16 wt%, Mw 12.9 kDa,) were dissolved to form a total concentration of 2.0% polymer in acetone. In a separate solution, 0.5% w/v Solutol® HS 15 in water was prepared. The polymer acetone solution was added using a syringe pump at a rate of 1 mL/min to the aqueous solution (v/v ratio of organic to aqueous phase = 1:10), with stirring at 500 rpm. Acetone was removed by stirring the solution for 2-3 hours. The nanoparticles were then washed with 10 volumes of 0.5% w/v Solutol® HS 15 and concentrated using a tangential flow filtration system (300 kDa MW cutoff, membrane area = 50 cm^2). The solution was then passed through a 0.22 μm Nylon filter, and adjusted to a final concentration of 10% sucrose. The nanoparticles could be lyophilized into powder form. The nanoparticles contain about half the initial amount of mPEG-PLGA, and 5-15% surfactant.

Particle properties, evaluated by using the resulting plurality of particles made in the method above:

Zavg = 106 nm

Particle PDI = 0.093

Dv50 = 91 nm

Dv90 = 147 nm

Example 22. Formulation of PEGylated Docetaxel-5050 PLGA-O-acetyl/ Doxorubicin 5050 PLGA amide nanoparticles via Nanoprecipitation using PVA as the surfactant

Docetaxel-5050 PLGA-O-acetyl (400 mg, 59 wt%), doxorubicin 5050 PLGA amide (200 mg, 8.9 wt%) and mPEG-PLGA (40 mg, 6.25 wt%, Mwt. 8232 Da) were dissolved to form a total concentration of 1.0% polymer in acetone. In a separate solution, 0.5% w/v PVA (viscosity 2.5-3.5 cp) in water was prepared. The polymer acetone solution was added using a syringe pump at a rate of 1 mL/min to the aqueous solution (v/v ratio of organic to aqueous phase = 1:10), with stirring at 500 rpm. Acetone was removed by stirring the solution for 2-3 hours. The nanoparticles were then washed with 10 volumes of water and concentrated using a tangential flow filtration system (300 kDa MW cutoff, membrane area = 50 cm^2). The nanoparticle solution was adjusted to a final concentration of 10% sucrose. The nanoparticles could be lyophilized into powder form.

Particle properties, evaluated by using the resulting plurality of particles made in the method above:

Zavg = 146.6 nm

Particle PDI = 0.146

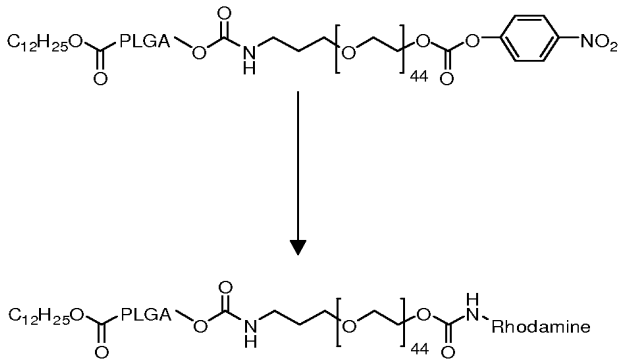
Dv50 = 137 nm

Dv90 = 211 nm

Example 23. Synthesis and Formulation of Rhodamine labeled PEGylated Docetaxel-5050 PLGA-O-acetyl via nanoprecipitation using PVA as the surfactant

Para-nitrophenyl protected PEG-PLGA 5050-lauryl ester (150 mg, 1.36×10^{-5} moles) was added to rhodamine B ethylene diamine (8 mg, 1.36×10^{-5} moles) in *N,N* dimethylformamide (DMF) in the presence of triethylamine (4 uL, 2.72×10^{-5} moles). The reaction mixture was stirred at room temperature overnight. DMF was removed from the reaction mixture under vacuum. Purification of the product was obtained

through 3 times precipitation of the crude product dissolved in dichloromethane in methyl tert-butyl ether. The product was then dried under vacuum overnight.



Docetaxel-5050 PLGA-O-acetyl (120 mg, 59 wt%), mPEG-PLGA (18 mg, 8.9 wt%, Mw 12.9 kDa), Rhodamine B-labeled-PEG-PLGA-lauryl ester (4 mg, 1.9 wt%) and purified PLGA (60 mg, 30 wt%) were dissolved to form a total concentration of 1.0% polymer in acetone. In a separate solution, 0.5% w/v PVA (viscosity 2.5-3.5 cp) in water was prepared. The polymer acetone solution was added using a syringe pump at a rate of 1 mL/min to the aqueous solution (v/v ratio of organic to aqueous phase = 1:10), with stirring at 500 rpm. Acetone was removed by stirring the solution for 2-3 hours. The nanoparticles were then washed with 10 volumes of water and concentrated using a tangential flow filtration system (300 kDa MW cutoff, membrane area = 50 cm^2). The nanoparticle solution was adjusted to a final concentration of 10% sucrose. The nanoparticles could be lyophilized into powder form.

Example 24. Formulation of Docetaxel-5050 PLGA-O-acetyl nanoparticles via Micro-Mixer using PVA as the surfactant

5050 purified PLGA (211 mg, 32 μmol), docetaxel-5050 PLGA-O-acetyl (633 mg, 71 μmol) and mPEG-PLGA (Mw 8.3 kDa, 5 wt% total polymer) were combined at a total concentration of 1.0 % polymer in acetone.

A separate solution of 0.5% polyvinylalcohol (80% hydrolyzed, Mw 9-10 kDa) in water was prepared. The organic and aqueous solutions were then blended

using a Caterpillar MicroMixer (CPMM-v1.2-R300), using flow rates of 5 mL/min and 15 mL/min respectively.

The acetone was removed from the resulting nanoparticle dispersion by rotary evaporation. The aqueous nanoparticle dispersion was washed with 10 volumes of water using a tangential flow filtration system (300 kDa MW cutoff, membrane area = 50 cm^2). The dispersion was then concentrated using a tangential flow filtration system (300 kDa MW cutoff, membrane area = 50 cm^2). The solution was then passed through a 0.22 μm filter, and adjusted to a final concentration of 10% sucrose. The solution was then lyophilized to provide the particles. The nanoparticles contain half the initial amount of mPEG-PLGA, and 15-30% PVA.

Particle properties:

Zavg = 133.9 nm

Particle PDI = 0.199

Dv50 = 110 nm

Dv90 = 237 nm

Example 25. Formulation of Doxorubicin 5050 PLGA amide nanoparticles via emulsion using PVA as the surfactant

Doxorubicin 5050 PLGA amide (100 mg, 100 wt%) was dissolved to form a total concentration of 1.0% polymer in dichloromethane. In a separate solution, 0.5% w/v PVA (viscosity 2.5-3.5 cp) in water was prepared. The dissolved polymer solution in dichloromethane was mixed with the aqueous PVA solution and emulsified through a microfluidizer processor for three cycles at a pressure of 8500 psi. Dichloromethane was removed by stirring the solution for 12 hours. The nanoparticles were then washed with 10 volumes of water and concentrated using a tangential flow filtration system (300 kDa MW cutoff, membrane area = 50 cm^2). The nanoparticle solution was adjusted to a final concentration of 10% sucrose. The nanoparticles could be lyophilized into powder form and were prepared for purposes of comparison.

Particle properties:

Zavg = 91.19 nm

Particle PDI = 0.135

Dv50 = 70.5 nm

Dv90 = 120 nm

Example 26. Formulation of Embedded Docetaxel/Paclitaxel in Docetaxel-5050 PLGA-O-acetyl nanoparticles via emulsion using PVA as the surfactant

Docetaxel-5050 PLGA-O-acetyl (90 wt%), mPEG-PLGA (10 wt%) and either docetaxel or paclitaxel (30 mg) were dissolved in dichloromethane (DCM, 14 mL). A separate solution of 0.5% polyvinylalcohol (PVA, 80% hydrolyzed, Mw 9-10 kDa) in water was prepared. The dissolved polymer-drug solution was transferred with a syringe into a beaker containing the 0.5% PVA (96 mL, v/v ratio of organic to aqueous phase = ~1:7) and sonicated using a micro-tip horn (tip diameter = ½ inch) for 5 minutes to form an emulsion. The emulsion is then transferred to a microfluidizer processor and passed through seven times with processing pressures ranging from 13,000-16,100 psi.

The DCM was removed from the resulting nanoparticle dispersion by rotary evaporation. The aqueous nanoparticle dispersion was washed with 10-20 times volumes of water and concentrated using a tangential flow filtration system (300 kDa MW cutoff, membrane area = 50 cm^2). The solution was passed through a 0.22 μm filter, and for lyoprotection, 10% sucrose was added. The nanoparticles were lyophilized to form a white powder.

Particle properties:

	Docetaxel	Paclitaxel
Zavg (nm)	94	102
Particle PDI	0.107	0.103
Dv50 (nm)	75	82
Dv90 (nm)	128	142

Embedded drug (% w/w)	1.9	4.5
Conjugate docetaxel (% w/w)	4.0	4.1

Example 27. Formulation of docetaxel-2'-hexanoate-5050 PLGA-O-acetyl nanoparticles

One could prepare nanoparticles by combining docetaxel-2'-hexanoate-5050 PLGA-O-acetyl and mPEG-PLGA at a weight ratio ranging from 84-60/16-40 wt% with a total concentration of 1% polymer in acetone. In a separate solution, 0.5% w/v PVA (viscosity 2.5-3.5 cp) in water could be prepared. The polymer acetone solution could be added using a syringe pump at a rate of 1 mL/min to the aqueous solution (v/v ratio of organic to aqueous phase = 1:10), with stirring at 500 rpm. Acetone could be removed by stirring the solution for 2-3 hours. The nanoparticles could be then washed with 10 volumes of water and concentrated using a tangential flow filtration system (300 kDa MW cutoff, membrane area = 50 cm^2). For lyoprotection, standard lyoprotectants could be used (e.g. sucrose) and the nanoparticles could be lyophilized into powder form.

Example 28. Formulation of PEGylated O-acetyl-5050-PLGA-(2'-β-alanine glycolate)-docetaxel nanoparticles

O-acetyl-5050-PLGA-(2'-β-alanine glycolate)-docetaxel (600 mg, 60 wt%) and mPEG-PLGA (400 mg, 40 wt%) were dissolved to form a total concentration of 1.0% polymer in acetone. In a separate solution, 0.5% w/v PVA (viscosity 2.5-3.5 cp) in water was prepared. The organic and aqueous solutions were then mixed together using a nanoprecipitation method at an organic to aqueous ratio of 1:10. Acetone was removed from the resulting nanoparticle dispersion by passive evaporation. The nanoparticles were then washed with 12 volumes of water and concentrated using a tangential flow filtration system (300 kDa MW cutoff, membrane area = 50 cm^2). The nanoparticle solution was adjusted to a final concentration of 10%

sucrose. The nanoparticles could be lyophilized into powder form. The nanoparticles contain half the initial amount of mPEG-PLGA, and 15-30% PVA.

Particle properties:

Zavg = 74.3 nm

Particle PDI = 0.097

Dv50 = 57.5 nm

Dv90 = 94.4 nm

Example 29. Formulation of PEGylated bis(docetaxel) glutamate-5050 PLGA-O-acetyl nanoparticles

Bis(docetaxel) glutamate-5050 PLGA-O-acetyl (600 mg, 60 wt%) and mPEG-PLGA (400 mg, 40 wt%) were dissolved to form a total concentration of 1.0% polymer in acetone. In a separate solution, 0.5% w/v PVA (viscosity 2.5-3.5 cp) in water was prepared. The organic and aqueous solutions were then mixed together using a nanoprecipitation method at an organic to aqueous ratio of 1:10. Acetone was removed from the resulting nanoparticle dispersion by passive evaporation. The nanoparticles were then washed with 12 volumes of water and concentrated using a tangential flow filtration system (300 kDa MW cutoff, membrane area = 50 cm^2). The nanoparticle solution was adjusted to a final concentration of 10% sucrose. The nanoparticles could be lyophilized into powder form. The nanoparticles contain half the initial amount of mPEG-PLGA, and 15-30% PVA.

Particle properties:

Zavg = 68.6 nm

Particle PDI = 0.082

Dv50 = 55.9 nm

Dv90 = 87.2 nm

Example 30. Formulation of PEGylated O-acetyl-5050-PLGA-(2'-β-alanine glycolate)-docetaxel/ docetaxel-2'5050 PLGA-o-acetyl nanoparticles

O-acetyl-5050-PLGA-(2'-β-alanine glycolate)-docetaxel, docetaxel-5050 PLGA-o-acetyl and mPEG-PLGA could be combined at a weight ratio of 84-60/16-40 wt% (polymer drug conjugates/mPEG-PLGA) with a total concentration of 1% polymer in acetone. In a separate solution, 0.5% w/v PVA (viscosity 2.5-3.5 cp) in water could be prepared. The polymer drug conjugates could vary from a ratio of 10:1 to 1:10. The organic and aqueous solutions could then be mixed together using a nanoprecipitation method at an organic to aqueous ratio of 1:10. The acetone could be removed from the resulting nanoparticle dispersion by passive evaporation. The nanoparticles could be washed with 15 volumes of water and concentrated using a tangential flow filtration system (300 kDa MW cutoff, membrane area = 50 cm^2). The nanoparticle solution could be adjusted to a final concentration of 10% sucrose. The nanoparticles could be lyophilized into powder form. This particular nanoparticle configuration could allow for different release rates of docetaxel.

Example 31. Preparation of Docetaxel-PLGA Nanoparticles samples for Imaging using Cryo Scanning Electron Microscopy (Cryo-SEM)

Lyophilized samples of docetaxel-PLGA nanoparticles containing PVA were reconstituted and fixed in 0.5% osmium tetroxide (OsO_4) in water for *ca.* 15 min prior to centrifugation and washing with water. Sample droplets were placed into a rivet holder, which was fast frozen in liquid nitrogen slush (*ca.* -210 ^oC) A vacuum was pulled and the sample was transferred to a Gatan Alto 2500-pre chamber (cooled to *ca.* -160^oC). The sample was fractured, sublimated at -90^oC for 7-10 minutes and coated with platinum using a sputter coating for 120 sec. Finally the samples were transferred to the microscope cryostage which is maintained at -130^oC. The samples were imaged with an FEI NOVA nanoSEM field emission scanning electron microscope operating at an accelerating velocity of 5 kV.

The cryo-SEM images showed that the docetaxel-PLGA nanoparticles containing PVA were spherical and no apparent surface structure was evident. The particle sizes ranged from 50-75 nm.

Example 32. Preparation of Docetaxel-PLGA Nanoparticles samples for Imaging using Transmission Electron Microscopy (TEM)

Carbon coated formvar grids (400 mesh) were glow-discharged prior to use. A droplet sample of docetaxel-PLGA nanoparticles containing PVA was added to the carbon grids and allowed to sit for *ca.* 2 min. The grids were then quickly touched to droplets for 2% uranyl acetate. The excess stain was removed with filter paper and allowed to dry. The samples were imaged with a Phillips CM-100 transmission electron microscope operating at an accelerating velocity of 80 kV.

The TEM images showed that the docetaxel-PLGA nanoparticles containing PVA were spherical and relatively uniform in size. The particle size from the TEM micrograph were typically less than 150 nm.

Example 33. Synthesis, Purification and Characterization of Doxorubicin Tosylate

In a 250-mL round-bottom flask equipped with a magnetic bar and a thermocouple, doxorubicin•HCl (NetQem, 1.43 g, 2.46 mmol) was suspended in anhydrous THF (143 mL, 100 vol). The mixture was evacuated for 15 seconds while being stirred and filled up with nitrogen (1 atm). 1 M potassium tert-butoxide (KOtBu)/THF solution (2.7 mL, 2.70 mmol) was added dropwise with stirring within 10 min. The solution turned a purple color and a slight exotherm was observed. The reaction temperature rose from 19°C to 21.7°C within 15 min and then slightly climbed up to a maximum of 22.4°C in half hour. The mixture was stirred for another hour at 22.4°C and then p-Toluenesulfonic acid (p-TSA, 0.70 g, 3.96 mmol) was added in one portion. The solution immediately turned a red color along with the precipitation of fine particles. The mixture was stirred for an additional half hour at ambient temperature and then cooled to 5 °C and stirred for 1 h. The resulting red suspension was filtered under nitrogen. The filter cake was washed with THF (3 × 10

mL) and dried under vacuum at 25°C for 16 h to produce doxorubicin tosylate [1.73 g, 97% yield)]. HPLC analysis indicated a 97% purity (AUC, 480 nm).

To remove the excess p-TSA, the product was slurried in 5:1 MTBE/MeOH (60 mL) at ambient temperature for 3 h. The filtered solid was dried under vacuum at 25°C for 16 h to afford 1.32 g of product. HPLC analysis indicated 99% purity (AUC, 480 nm); however, the ^{1}H NMR analysis showed that the equivalents of *p*-TSA were still ~1.2. DSC analysis of doxorubicin tosylate showed a sharp peak with a melting range of 188.5-196.5°C.

Example 34. Synthesis and Characterization of Doxorubicin Octanesulfonate

In a 250 mL round-bottom flask equipped with a magnetic stirrer, 1-octanesulfonic acid sodium salt monohydrate (0.44 g, 1.86 mmol) was dissolved in water (50 mL). The mixture was stirred for 10 min to afford a clear solution, to which doxorubicin•HCl (1.08 g, 1.86 mmol) was added in one portion. The solution became a dark red color after being stirred for a few minutes. After about 30 min, an orange powder formed. The mixture was stirred at ambient temperature for 2 h. The suspension was stored in fridge for 16 h and filtered through a Sharkskin® filter paper. The filtrate had a slightly red color and contained trace amounts of doxorubicin as evidenced by HPLC analysis. The presence of chloride in the filtrate was confirmed by the silver nitrate test. The filter cake was dried under vacuum at 28°C for 16 h to afford doxorubicin octanesulfonate [1.16 g, yield: 85%] as an orange powder. The ^{1}H NMR analysis indicated the desired product and HPLC analysis indicated >99.5% purity. DSC analysis of doxorubicin octanesulfonate showed a sharp peak with a melting range of 198.7 – 202.0°C.

Example 35. Synthesis, Purification and Characterization of Doxorubicin Naphthalene-2-Sulfonate

A 250-mL round-bottom flask equipped with a magnetic bar and a thermocouple was charged with doxorubicin•HCl (NetQem, 1.47 g, 2.53 mmol) and anhydrous THF (150 mL, 100 vol). The suspension was evacuated for 15 seconds with stirring and filled up with nitrogen (1 atm). 1 M (KOtBu)/THF solution (2.7 mL,

2.70 mmol) was added dropwise with stirring over 10 min. The mixture turned a purple color and a slight exotherm was observed, causing the reaction temperature to rise from 20.2 °C to 21.4 °C within 15 min. The solution was stirred at 21.1 °C for one hour and 2-naphthalenesulfonic acid (0.63 g, 3.04 mmol) was added in one portion. The mixture immediately turned to a red color and the precipitation of fine particles was observed. The solution was stirred for an hour at ambient temperature and then filtered under nitrogen. The filtration was slow and took about 1 h. The filter cake was washed with THF (3 × 10 mL) and dried under vacuum at 25 °C for 16 h to afford 2.1 g of doxorubicin naphthalene-2-sulfonate as a dark red solid [yield: >100%]. HPLC analysis indicated a 98% purity (AUC, 480 nm). The ^{1}H NMR analysis showed that the ratio of 2-naphthalenesulfonic acid to doxorubicin was ~1.08.

To remove residual 2-naphthalenesulfonic acid, the doxorubicin naphthalene-2-sulfonate was slurried in 5:1 MTBE/MeOH (60 mL) for 3 h. The suspension was filtered and the filter cake was dried under vacuum at 25°C for 24 h to afford 1.90 g of the product as a fine red powder [yield: 100%]. The ^{1}H NMR analysis indicated a clean product with a 1:1 ratio of doxorubicin to 2-naphthalenesulfonic acid. HPLC analysis showed >98% purity (AUC, 480 nm). The physical appearance of the product was similar to doxorubicin•HCl. DSC analysis of doxorubicin naphthalene-2-sulfonate showed a sharp peak with a melting range of 203.1 – 207.4 °C.

Example 36. Cytotoxicity of nanoparticles formed from polymer drug conjugates

To measure the cytotoxic effect of nanoparticles formed from doxorubicin 5050 PLGA amide, paclitaxel-5050 PLGA-O-acetyl, docetaxel-5050 PLGA-O-acetyl or bis(docetaxel) glutamate-5050 PLGA-O-acetyl, the CellTiter-Glo® Luminescent Cell Viability Assay (CTG) (Promega) was used. Briefly, ATP and oxygen in viable cells reduce luciferin to oxyluciferin in the presence of luciferase to produce energy in the form of light. B16.F10 cells, grown to 85-90% confluency in 150 cm^2 flasks (passage <30), were resuspended in media (MEM-alpha, 10% HI-FBS, 1X antibiotic-

antimycotic) and added to 96-well opaque-clear bottom plates at a concentration of 1500 cells/well in 200 μL/well. The cells were incubated at 37°C with 5% CO_2 for 24 hours. The following day, serial dilutions of 2X concentrated particles and 2X concentrated free drug were made in 12-well reservoirs with media to specified concentrations. The media in the plates was replaced with 100 μL of fresh media and 100 μL of the corresponding serially diluted drug. Three sets of plates were prepared with duplicate treatments. Following 24, 48 and 72 hours of incubation at 37°C with 5% CO_2, the media in the plates was replaced with 100 μL of fresh media and 100 μL of CTG solution, and then incubated for 5 minutes on a plate shaker at room temperature set to 450 rpm and allowed to rest for 15 minutes. Viable cells were measured by luminescence using a microtiter plate reader. The data was plotted as % viability vs. concentration and standardized to untreated cells. The doxorubicin 5050 PLGA amide, paclitaxel-5050 PLGA-O-acetyl, docetaxel-5050 PLGA-O-acetyl and bis(docetaxel) glutamate-5050 PLGA-O-acetyl polymer drug conjugates inhibited the growth of B16.F10 cells in a dose and time dependent manner. Also, in comparison to the corresponding free drug, the polymer drug conjugates exhibited a slower release profile.

IC_{50} on Day 3:

Group	IC_{50} (μM)
Free doxorubicin	14
Doxorubicin 5050 PLGA amide nanoparticles	2.9
Free paclitaxel	7
Paclitaxel-5050 PLGA-O-acetyl nanoparticles	480
Free docetaxel	0.13
Docetaxel-5050 PLGA-O-acetyl nanoparticles	20
bis(docetaxel) glutamate-5050 PLGA-O-acetyl nanoparticles	25

Example 37. Bioburden test for contamination of nanoparticles formed from polymer drug conjugate

To measure the formulation sterility for PEGylated docetaxel-5050 PLGA-O-acetyl nanoparticles, the spot colony forming units per gram (CFU) assay, a modified plate count method, was used. A positive control was prepared by inoculating 10 mL of trypticase soy broth (TSB) with an isolated colony from an in house bacterial stock and grown at 37°C in a shaking incubator at 350 rpm for 24 hours. A subculture (1:100) was then prepared and grown at 37°C in a shaking incubator (350 rpm for 3 hours). The bacteria were then pelleted, washed with PBS and resuspended with fresh TSB. A 0.5 McFarland standard bacterial solution (equal to 1.5×10^6 CFU/mL based on turbidity measurement) was then prepared. An aliquot of 100 μL was sampled from each of the following solutions: a *ca.* 1.5 mg/ml nanoparticle solution (4-5 mL batch size), a positive control and TSB, as well as a negative control. These were each mixed with 400 μL of TSB in a 1.5 mL microcentrifuge tube and cultured in a shaking incubator at 37°C (450 rpm for 3 days). On days 0 and 3, 50 μL of each sample were removed from the sample mix and serially diluted at a ratio of 1:10 with TSB in a 96-well plate. The diluted samples (6 μL) were spotted onto pre-dried trypticase soy agar (TSA) plates using a multichannel pipet. The spots were allowed to dry and the plates were incubated at 37°C for 24 hours. After 24 hours, the isolated colonies were counted and the CFU/mL calculated. To detect very low concentrations of contaminants, 200 μL of each sample mix were spread onto agar plates on day 3 and incubated at 37°C for 24 hours. The tests were carried out over an open flame.

Colony forming units per gram

Description	T_0 Spot CFU CFU/mL	T_{72} Spot CFU CFU/mL	T_{72} Plate CFU CFU/mL
PEGylated docetaxel-5050 PLGA-O-acetyl nanoparticles, Filtered with 0.22 μm Steriflip	0	0	0
PEGylated docetaxel-5050 PLGA-O-acetyl nanoparticles, Filtered with 0.45 μm Steriflip	0	0	0

Positive control, 1.5 x 10^6 CFU/mL standardized stock solution in TSB	6.67×10^5	3.80×10^{11}	Lawn
Negative control, TSB	0	0	0

Example 38. In vivo efficacy of PEGylated Doxorubicin 5050 PLGA amide nanoparticles in a B16.F10 mouse model of melanoma

B16.F10 cells were grown in culture to 85-90% confluency in MEM-α medium supplemented with 10% FBS and 1% penicillin/streptomycin (passage = 4) and then resuspended in PBS. B16.F10 cells (density = 5×10^6 cells/mL) were implanted subcutaneously (SC) into the right flank of male C57BL/6 mice (20-22 g on day 1.

The five treatment groups that were administered to the mice were: 1) 0.9% NaCl solution; 2) Doxil (liposomal formulation of doxorubicin HCl containing 2mg/mL doxorubicin HCl, Ortho Biotech) at 1 mg/kg dose; 3) three PEGylated doxorubicin 5050 PLGA amide nanoparticles with 1, 2 and 3 mg/kg doxorubicin equivalent doses.

The treatments were administered IV into the tail vein of the mouse at a dose volume of 6mL/kg, beginning on day 5 post-implantation, when the mean tumor volume was 50 mm^3. The treatments were administered on days 5, 8, and 12 (biweekly × 3 injections) post tumor implantation. Health status of the animals was monitored and the tumor was measured three times a week. On day-17 post-tumor implantation, mice were euthanized by CO_2 inhalation according to the IUCAC procedure guideline. Tumor from each animal was dissected and tumor volume as well as tumor growth inhibition (TGI) was measured. Tumor volume was calculated using the formula: (width × width × length) / 2 mm^3. TGI represented as % was calculated using the formula: (1 – (treated tumor volume/control tumor volume)) × 100.

Tumor growth inhibition (TGI)

The treatment groups of Doxil and all the PEGylated doxorubicin 5050 PLGA amide nanoparticles showed inhibition of tumor growth on day-17. A dose-dependent tumor growth inhibition was seen with PEGylated doxorubicin 5050 PLGA amide nanoparticles; 37% TGI at 1mg/kg, 48% TGI at 2mg/kg and 57% TGI at 3mg/kg. Doxil at 1mg/kg exhibited 60% TGI on day 17.

Tumor growth inhibition (n=4)

Group	Dose mg/kg	Day-17 TGI, %
0.9% NaCl control	---	---
Doxil	1	60%
PEGylated doxorubicin 5050 PLGA amide nanoparticles	1	37%
PEGylated doxorubicin 5050 PLGA amide nanoparticles	2	48%
PEGylated doxorubicin 5050 PLGA amide nanoparticles	3	58%

Example 39. In vivo efficacy of PEGylated paclitaxel-5050 PLGA-O-acetyl nanoparticles in a B16.F10 mouse model of melanoma

B16.F10 cells were grown in culture to 85-90% confluency in MEM-α medium supplemented with 10% FBS and 1% penicillin/streptomycin (passage = 4) and then resuspended in PBS. B16.F10 cells (density = 5×10^6 cells/mL) were implanted subcutaneously (SC) into the right flank of male C57BL/6 mice (20-22 g on day 1.

The four treatment groups that were administered to the mice were: 1) 0.9% NaCl solution; 2) Abraxane® (Abraxis) at 1.5, 6 and 15 mg/kg dose; 3) free paclitaxel at doses of 1.5, 6 and 15 mg/kg and 4) PEGylated paclitaxel-5050 PLGA-O-acetyl nanoparticles at doses of 1.5, 3, 6, 9, and 15 mg/kg paclitaxel equivalent.

The treatments were administered IV into the tail vein at a dose volume of 6mL/kg, beginning on day-5 post-implantation, when the mean tumor volume was $55mm^3$. The treatments were administered on days 5, 8, and 12 (biweekly × 3

injections) post tumor implantation. Health status of the animals was monitored and tumor size was measured three times a week. On day17, post-tumor implantation, mice were euthanized by CO_2 inhalation according to the IUCAC procedure guideline. Tumors from each animal were dissected and tumor size was measured. Tumor volume was calculated using the formula: (width × width × length) / 2 mm^3. TGI represented as % was calculated using the formula: (1 – (treated tumor volume/control tumor volume)) × 100.

Tumor growth inhibition

Abraxane®, free paclitaxel and all PEGylated paclitaxel-5050 PLGA-O-acetyl nanoparticles groups showed inhibition of tumor growth on day 17. A dose-dependent TGI was seen with the free paclitaxel treated groups; 37% TGI at 1.5mg/kg, 57% % TGI at 6mg/kg and 83% TGI at 15mg/kg doses. Abraxane® showed a 36% TGI at 1.5mg/kg, 13% % TGI at 6mg/kg and 70% TGI at 15mg/kg doses. At the lowest dose of 1.5mg/kg, PEGylated paclitaxel-5050 PLGA-O-acetyl nanoparticles exhibited a 42% TGI, which is similar to free paclitaxel and Abraxane® treated groups at the same dose. However, PEGylated paclitaxel-5050 PLGA-O-acetyl nanoparticles showed a 42% TGI at 1.5mg/kg, 40% TGI at 3mg/kg, 46% TGI at 6mg/kg, 61% TGI at 9mg/kg and 58% TGI at 15mg/kg doses.

Tumor growth inhibition (n=4)

Group	Dose mg/kg	Day-17 TGI, %
0.9% NaCl control	---	---
Abraxane®	1.5	36%
Abraxane®	6	13%
Abraxane®	15	70%
Free paclitaxel	1.5	37%
Free paclitaxel	6	57%
Free paclitaxel	15	83%

PEGylated paclitaxel-5050 PLGA-O-acetyl nanoparticles	1.5	42%
PEGylated paclitaxel-5050 PLGA-O-acetyl nanoparticles	3	40%
PEGylated paclitaxel-5050 PLGA-O-acetyl nanoparticles	6	46%
PEGylated paclitaxel-5050 PLGA-O-acetyl nanoparticles	9	61%
PEGylated paclitaxel-5050 PLGA-O-acetyl nanoparticles	15	58%

Example 40. Tolerability and in vivo efficacy of PEGylated docetaxel-5050 PLGA-O-acetyl nanoparticles in a B16.F10 mouse model of melanoma

B16F10 cells were grown in culture to 85% confluency in MEM-α medium containing 10% FBS and 1% penicillin/streptomycin (passage = 4) and then resuspended in PBS. B1610 cells (density = 10×10^6 cells) were implanted subcutaneously (SC) into the right flank of male C57BL/6 mice on Day 1. On Day 5 following tumor inoculations, animals were assigned to different treatment groups according to the tumor size.

The three treatment groups that were administered to the mice included: 1) a docetaxel vehicle formulation consisting of a 10 mg/mL stock solution (prepared with 20 mg of docetaxel, 0.2 mL ethanol, 0.5 mL polysorbate 80 and 1.3 mL water, added in that specific order and vortexed to ensure proper mixing). The stock solution was diluted further with PBS to 0.6 and 1.5 mg/mL (for a corresponding dose of 6 and 15 mg/kg) so that all the groups received the same amount of ethanol, polysorbate 80, water and PBS. 2) PEGylated (10 mol%) docetaxel-5050 PLGA-O-acetyl nanoparticles at doses of 6, 15 and 30 mg/kg). 3) Docetaxel vehicle.

Animals were treated with different concentrations of docetaxel and PEGylated docetaxel-5050 PLGA-O-acetyl nanoparticles as per the schedule (on Days 5, 8 and 12 following inoculation). The schedule consisted of 3 injections biweekly. The animals were monitored three times a week for health status and adverse effects from tumor cell inoculation to the end of the study. The body weight and tumor volume were also measured three times a week to evaluate the effect of the treatment.

Tumor growth inhibition

On Day 17, the PEGylated (10 mol%) docetaxel-5050 PLGA-O-acetyl nanoparticles showed dose-dependent TGI. At 6, 15 and 30 mg/kg, the TGI was 53%, 88% and 93% after biweekly × 3 injections.

Example 41. Tolerability and maximum tolerated dose of PEGylated bis(docetaxel) glutamate-5050 PLGA-O-acetyl nanoparticles in a B16.F10 mouse model of melanoma

B16F10 cells were grown in culture to confluency in MEM-α medium containing 10% FBS and 1% penicillin/streptomycin (passage = 4) and then resuspended in PBS. B1610 cells (density = 1×10^6 cells/mL in a 0.1 mL volume) were subcutaneously (SC) implanted into the right flank of male C57BL/6 mice on Day 1.

The five treatment groups that were administered to the mice included: 1) a docetaxel vehicle formulation consisting of a 10 mg/mL stock solution (prepared with 20 mg of docetaxel, 0.2 mL ethanol, 0.5 mL polysorbate 80 and 1.3 mL water, added in that specific order and vortexed to ensure proper mixing). The stock solution was diluted further with PBS to 0.6, 1.5, 3, 4.5 and 6 mg/mL (for a corresponding dose of 6, 15, 30, 45 and 60 mg/kg) so that all the groups received the same amount of ethanol, polysorbate 80, water and PBS. 2) PEGylated bis(docetaxel) glutamate-5050 PLGA-O-acetyl nanoparticles at doses of 6, 15, 30, 45 and 60 mg/kg. 3) Docetaxel vehicle at the highest concentration of 6 mg/mL consisting of 6% ethanol/ 15% polysorbate 80/ 39% water and 40% PBS. 4) Sucrose vehicle (100 mg/kg). 5) PEGylated O-acetyl-5050-PLGA nanoparticle vehicle at the highest concentration of 6 mg/mL.

The treatments were administered IV into the tail vein at a dose volume of 10 mL/kg, beginning on post-implantation Day 5, when the mean tumor volume was 55 mm^3. The treatments were administered 4 times, on Days 5, 8, 12 and 15 (biweekly ×

4 injections). On Day 17 post-tumor implantation, mice were euthanized by CO_2 inhalation according to the procedure guideline. Blood was collected by cardiac puncture and put into ethylenediaminetetraacetic acid (EDTA) or serum separation blood collection tubes. Whole blood was analyzed on the day of collection for CBC analyses. After the blood clotted and was centrifuged, serum was frozen immediately on dry ice for serum chemistry analyses. The tumors were removed by dissection, frozen immediately on dry ice and stored at -80°C, in which they were later analyzed for bis(docetaxel) glutamate-5050 PLGA-O-acetyl and free docetaxel levels.

Tolerability was determined by changes in body weight, expressed as a percent of the initial body weight on post-implantation Day 5. The criterion at which a group was removed from the study was a mean of 20% body weight loss. Health monitoring was conducted daily, but no mice warranted removal due to indications of lethargy, tremors, hypothermia, etc. The maximum tolerated dose (MTD) was determined as the highest dose that did not cause a 20% body weight loss. Other indices of toxicity, complete blood count (CBC) and serum chemistry were determined from blood collected from animals that were euthanized on Day 17 by CO_2 inhalation, according to the procedure guideline.

Body weight changes

The groups administered 6, 15, 30 and 45 mg/kg of PEGylated bis(docetaxel) glutamate-5050 PLGA-O-acetyl nanoparticles all gained weight on Day 17, a mean of 111%, 112%, 106% and 106%, 112% of the initial body weight was observed respectively. For the 60 mg/kg, at Day 17, a mean of 91% of the initial body weight was observed. In comparison, the three vehicle-treated groups all gained weight similarly, i.e. the docetaxel vehicle treatment gained 14.8%, the sucrose vehicle gained 13.8% and the PEGylated O-acetyl-5050-PLGA vehicle gained 16.2%. In contrast, there was a dose-related decline in body weights of mice administered docetaxel, i.e., the higher doses (e.g. 45 and 60 mg/kg) caused a mean 20% of body weight loss earlier (Day 15) compared to the lower doses (e.g. 30 mg/kg occurred at Day 17). The 6 and 15 mg/kg of docetaxel groups caused a mean of 4 and 8% body weight respectively by Day 17.

Tumor growth and tumor growth inhibition

On Day 17, all PEGylated bis(docetaxel) glutamate-5050 PLGA-O-acetyl nanoparticles groups showed inhibition of tumor growth. The lower 2 doses, 6 and 15 mg/kg caused similar inhibition of tumor growth, 49% and 48% TGI, respectively. For 30, 45 and 60 mg/kg, a 73%, 83% and 93% TGI was shown. The TGI was directly related to the tumor docetaxel content, r >0.9. In comparison, for the docetaxel control, at 6 and 15 mg/kg, a 78% and 94% TGI, respectively was observed. In contrast, there was no effect by any vehicle on tumor growth, compared to the other vehicle-treated groups.

Complete blood count

PEGylated bis(docetaxel) glutamate-5050 PLGA-O-acetyl nanoparticles showed a trend for a decline in the white blood cell (WBC) number, lymphocyte number and neutrophil number. However, there was no significant effect on either the WBC number (ranged from 10.8-6.2 × 1000 cells/μL for 6-60 mg/kg doses), lymphocyte number (ranged from 6221-4317 cells/μL for 6-60 mg/kg doses) or neutrophil number (ranged from 4404-1889 cells/μL for 6-60 mg/kg doses). In addition, other CBC parameters were not affected by PEGylated bis(docetaxel) glutamate-5050 PLGA-O-acetyl nanoparticles at doses up to 60 mg/kg. In comparison, for the 3 vehicle treated groups (sucrose, docetaxel, O-acetyl-5050-PLGA PEGylated nanoparticle), the WBC (ranged from 11.4-14.1 × 1000 cells/μL), lymphocyte number (7592-10222 cells/μL) and neutrophil number (3524-4557 cells/μL) all were within the normal range for mice.

Serum chemistry

The PEGylated bis(docetaxel) glutamate-5050 PLGA-O-acetyl nanoparticles did not affect any serum chemistry parameter at doses up to 15 mg/kg and 60 mg/kg respectively. In comparison, docetaxel did not affect any serum chemistry parameter at doses up to 30 mg/kg. The vehicle formulations did not affect any serum chemistry

parameter. (Serum chemistry parameters determined were alkaline phosphatase, ALT, AST, CPK, albumin, total protein, total bilirubin, direct bilirubin, BUN, creatinine, cholesterol, glucose, calcium, bicarbonate and A/G ratio.)

Maximum tolerated dose

The maximum tolerated dose (MTD) of PEGylated bis(docetaxel) glutamate-5050 PLGA-O-acetyl nanoparticles was 60 mg/kg at the 4-dose treatment schedule administered, 4-fold greater than free docetaxel (MTD = 15 mg/kg when administered IV biweekly for 2 weeks).

Tumor growth inhibition of B16F10 tumor-bearing mice administered treatments.

Group	Dose mg/kg	Day 17 Tumor Growth Inhibition, %
Sucrose Vehicle control	0	-----
PNP Vehicle	0	107%
Free docetaxel	6	78%
Free docetaxel	15	96%
Free docetaxel	30	95%
bis(docetaxel) glutamate-5050 PLGA-O-acetyl nanoparticles	6	49%
bis(docetaxel) glutamate-5050 PLGA-O-acetyl nanoparticles	15	48%
bis(docetaxel) glutamate-5050 PLGA-O-acetyl nanoparticles	30	73%
bis(docetaxel) glutamate-5050 PLGA-O-acetyl nanoparticles	45	83%
bis(docetaxel) glutamate-5050 PLGA-O-acetyl nanoparticles	60	93%

Example 42. In vivo efficacy of PEGylated docetaxel-5050 PLGA-O-acetyl nanoparticles in a A2780 ovarian human xenograft model

A2780 cells were grown in culture in RPMI-1640 containing 10% FBS and 1% penicillin/streptomycin (passage = 2). When confluent, the cells were removed using 0.05% trypsin and suspended in 1:1 mixture of RPMI-1640/Matrigel at a

density of 50×10^6 cells/mL. The tumors were implanted SC by injecting 5×10^6 A2780 cells in a 0.1 mL volume into the mammary fat pad of female CD-1 nude mice that were 6-8 weeks old.

The three treatment groups that were administered to the mice consisted of: 1) a docetaxel vehicle formulation consisting of a 10 mg/mL stock solution (prepared with 20 mg of docetaxel, 0.2 mL ethanol, 0.5 mL polysorbate 80 and 1.3 mL water, added in that specific order and vortexed to ensure proper mixing). The stock solution was diluted further with PBS to 1.5 mg/mL (for a dose of 15 mg/kg at 10 mL/kg and 30 mg/kg at 20 mL/kg). This formulation was made within 30 minutes of administration to mice. 2) Filtered PEGylated O-acetyl-5050-PLGA nanoparticles at a dose of 30 mg/kg, 3) docetaxel vehicle at the highest concentration of 1.5 mg/mL consisting of 1.5% ethanol, 3.8% polysorbate 80, 9.8% water and 85% PBS.

The treatments were administered IV into the tail vein at a dose volume of 10 mL/kg for the 15 mg/kg group and 20 mL/kg for the other groups, beginning on post-implantation Day 8, when the mean tumor volume was 128 mm^3. The treatments were administered 2 times, on Day 8 and Day 15 (weekly × 2 injections) for n = 8 mice per group. The study endpoint for the vehicle-treated and the docetaxel 15 mg/kg groups was a group mean tumor size of 1000 mm^3. The study endpoint for the docetaxel 30 mg/kg and the nanoparticles groups was an individual mouse tumor size of 1000 mm^3. On Day 50, the study was ended for all remaining mice. When removed from the study, mice were euthanized by CO_2 inhalation.

Body weight changes

On Day 8, the PEGylated O-acetyl-5050-PLGA nanoparticles (dose= 30 mg/kg) treatment group had a mean body weight of 27.6 ±1.0 g. On Day 29, this group had a mean body weight of 26.1 ±1.1 g, representing a maximum body weight loss of 5 ±3%. On the last day in the study (i.e. Day 50), the mean body weight was 27.2 ±1.7 g. The mice were regaining weight, to 97 ±3% of this group's initial body weight. The formulation administered as a treatment to the mice was shown to be sterile using a bioburden assay.

The initial mean body weight of the docetaxel vehicle treated group was 26.3 ±1.9 g on Day 8. When this group was removed from the study on Day 25, the mean body weight was 27.8 ±2.3 g. This represented a 106±2% of the initial mean body weight. In comparison for the mice administered with docetaxel, on Day 8, the mean body weight of the docetaxel administered 15 mg/kg group was 27.3 ±2.3 g. On Day 22, this group decreased in body weight to 25.3 ±1.7 g, representing a maximum of 7% body weight loss. On Day 36, when the docetaxel administered 15 mg/kg group was removed from the study, the mean body weight was 30.7 ±2.5 g, representing a 113 ±11% of the initial body weight. Similarly, on Day 8, the mean body weight of the docetaxel administered 30 mg/kg group was 26.3 ±1.3g. On Day 22, the mean body weight decreased to 23.7 ±1.9g, representing a maximum of 10% body weight loss. On Day 36, this group weighed 30.7 ±2.5g, representing a 105±9% of the initial body weight. Overall, there was a dose-related decline in body weights of mice administered with docetaxel.

Tumor growth inhibition and tumor growth delay (TGD)

Tumor growth delay (TGD) is calculated by the difference between the day when the treatment group tumor size reached the maximum tumor volume of 3000mm^3 and the day when the vehicle treated group reached a tumor volume of 3000mm^3.

For the PEGylated O-acetyl-5050-PLGA nanoparticles administered at a dose of 30 mg/kg, on Day 25, the tumor volume was 110 ±135 mm^3 (range 30-408 mm^3), with a TGI of 91%. The group mean tumor volume did not reach the endpoint during the duration of the study. One individual mouse reached 1000 mm^3 on Day 29, however 6 mice remained in the study on Day 50. The TGD could not be calculated, but is estimated to be greater than 25 days.

For the docetaxel treatment group, on Day 25, the tumor volume of the 15 mg/kg group was 349 ± 470 mm^3 (range 68-1481 mm^3), with a TGI of 71%. This group surpassed the endpoint on Day 32 with a tumor volume of 1477 ± 1730 mm^3 (range 165-5692 mm^3). No difference in the slope of the growth curve was apparent. The TGD was determined to be 5 days for the docetaxel treatment group (15 mg/kg)

by extrapolating to when the tumor growth curve crossed 1000 mm^3. On Day 25, the tumor volume of the 30 mg/kg group was 63 ±68 mm^3 (range 7-218 mm^3), with a TGI of 95%. This group reached the endpoint on Day 39 with a tumor volume of 950 ±1239 (0-3803 mm^3). Individual mice reached 1000 mm^3 on Day 32 (1 mouse), Day 39 (1 mouse), Day 42 (3 mice) and Day 46 (1 mouse). On Day 50, 2 mice still remained in the study. No difference in the slope of the growth curve was apparent. The TGD was calculated to be 14 days. There was a dose-related inhibition of tumor growth of mice administered with the docetaxel treatment groups.

In contrast, on Day 25, the mean tumor volume was 1000 mm^3 for the docetaxel vehicle treatment group and the tumor doubling time was 4 days. There was no effect by the docetaxel vehicle on tumor growth, compared to the other treatment groups. The PEGylated O-acetyl-5050-PLGA nanoparticles administered at a dose of 30 mg/kg showed improved efficacy and a greater TGD, compared to docetaxel, at the same dose and schedule.

Tumor growth inhibition and tumor growth delay of A2780 tumor-bearing mice administered treatments.

Group	Dose (mg/kg)	Day 25 Tumor Growth Inhibition (%)	Tumor Growth Delay (day)
Docetaxel Vehicle control	0	-----	
Free docetaxel	15	71	5
Free docetaxel	30	95	14
PEGylated O-acetyl-5050-PLGA nanoparticles	30	91	>25

In the following examples when reference is made to "mPEG(Xk)-PLGA Y wt%", Xk indicates the weight average molecular weight of the mPEG portion of the mPEG-PLGA polymer (e.g., mPEG(2k) indicates that 2 kDa mPEG is conjugated to PLGA), and Y indicates the weight percentage of mPEG-PLGA as compared to the PLGA-drug conjugate in the initial mixture used to make the nanoparticles. For example, 16 wt% indicates that an 84:16 weight ratio of PLGA-drug conjugate to mPEG-PLGA

was prepared and added to surfactant in order to prepare the nanoparticles. Typically, approximately half of the mPEG-PLGA used in the reaction is incorporated in to the product nanoparticles. Thus the approximate components of the nanoparticles in the following examples are as follows:

mPEG(2k)-PLGA 16 wt% = In the particle: mPEG(2k)-PLGA ~8 wt%, PVA ~23wt%, Docetaxel-5050 PLGA-O-acetyl ~69wt%
mPEG(2k)-PLGA 30 wt% = In the particle: mPEG(2k)-PLGA ~17 wt%, PVA ~23wt%, Docetaxel-5050 PLGA-O-acetyl ~60wt%
mPEG(2k)-PLGA 40 wt% = In the particle: mPEG(2k)-PLGA ~23 wt%, PVA ~26wt%, Docetaxel-5050 PLGA-O-acetyl ~51wt%
mPEG(5k)-PLGA 16 wt% = In the particle: mPEG(5k)-PLGA ~8 wt%, PVA ~22%, Docetaxel-5050 PLGA-O-acetyl ~70%
mPEG(5k)-PLGA 30 wt% = In the particle: mPEG(5k)-PLGA ~16 wt%, PVA ~24%, Docetaxel-5050 PLGA-O-acetyl ~60%
mPEG(5k)-PLGA 40 wt% = In the particle: mPEG(5k)-PLGA ~18 wt%, PVA ~24%, Docetaxel-5050 PLGA-O-acetyl ~58%

Example 43. Efficacy and tolerability of PEGylated docetaxel-5050 PLGA-O-acetyl nanoparticles in a B16.F10 murine melanoma model

B16.F10 cells were grown in culture to confluency in MEM-α medium supplemented with 10% fetal bovine serum (FBS, passage 4) and 1% penicillin/streptomycin and then resuspended in PBS. A volume of 0.1 mL containing 1×10^6 cells was subcutaneously implanted into the right flank of male C57BL/6 mice on day-1.

The seven treatment groups that were administered to the mice included: 1) A docetaxel formulation prepared at 10 mg/mL stock solution (with 20 mg of docetaxel, 0.2 mL ethanol, 0.5 mL polysorbate 80 and 1.3 mL water, added in that specific order and vortexed to ensure proper mixing) diluted further with PBS to 1.5 and 3 mg/mL for a corresponding dose of 15 and 30 mg/kg. For a 60 mg/kg dose, a 20 mL/kg

injection volume of a concentration of 3 mg/mL docetaxel formulation was administered. 2) PEGylated docetaxel-5050 PLGA-O-acetyl nanoparticles (mPEG(2k)-PLGA at 16 wt%) administered at doses of 15 and 30 mg/kg. 3) PEGylated docetaxel-5050 PLGA-O-acetyl nanoparticles (mPEG(2k)-PLGA at 30 wt%) administered at doses of 15, 30 and 60 mg/kg. 4) PEGylated docetaxel-5050 PLGA-O-acetyl nanoparticles (mPEG(2k)-PLGA at 40 wt%)) administered at doses of 15 and 30 mg/kg. 5) PEGylated docetaxel-5050 PLGA-O-acetyl nanoparticles (mPEG(5k)-PLGA at 16 wt%) administered at a dose of 15 mg/kg. 6) PEGylated docetaxel-5050 PLGA-O-acetyl nanoparticles (mPEG(5k)-PLGA at 30 wt%) administered at doses of 15 and 30 mg/kg. 7) PEGylated docetaxel-5050 PLGA-O-acetyl nanoparticles (mPEG(5k)-PLGA at 40 wt%) administered at a dose of 15 mg/kg. Refer to table for detailed description of formulations.

The treatments were administered IV into the tail vein at a dose volume of 10 or 20 mL/kg depending on the treatment group, beginning on post-implantation day 5, when the mean tumor volume was approximately 55 mm^3. Animals were monitored for any morbidity and adverse effect three times a week. Body weight and tumor volume were also measured three times a week.

Tumor volume was calculated with the following equation: (width × width × length) / 2 mm^3. Efficacy was determined by tumor growth inhibition (TGI), tumor growth delay (TGD) and survival. TGI was represented as % and calculated as follows: (1- (treated tumor volume/control tumor volume)) × 100 when the control group mean tumor volume reached ≥ 3000 mm^3. Tolerability was determined by changes in body weight, expressed as a percent of the initial body weight on post-implantation day-5. Health monitoring was conducted three times a week to evaluate lethargy, tremors, hypothermia, ataxia, hind limb paralysis etc. The criteria at which a mouse was removed from the study were > 20% body weight loss or severe morbidity or hind limb paralysis.

PEGylated nanoparticles (mPEG(2k)-PLGA at 16 wt%)- q3dq4d

The docetaxel control group and the PEGylated nanoparticles were administered three times over a two week schedule at a dose of 15 mg/kg and 30

mg/kg respectively. The docetaxel group showed a TGI of 90% in comparison to the PEGylated nanoparticles, which had a TGI of 84%. The docetaxel group exhibited a similar TGD of 12 days compared to 13 days for the PEGylated nanoparticles. The PEGylated nanoparticles did not cause any body weight loss and was better tolerated than the docetaxel group which caused a 12% maximum body weight loss.

PEGylated nanoparticles (mPEG(2k)-PLGA at 30 wt%)- q3dq4d

The docetaxel control group and the PEGylated nanoparticles were administered three times over a two week schedule at a dose of 15 mg/kg. Both the PEGylated nanoparticles and the docetaxel groups were equally efficacious. The TGI of the docetaxel and PEGylated groups were 90% and 86% respectively. Similarly both groups exhibited the same TGD of 11 days. The PEGylated nanoparticles did not show any body weight loss and was better tolerated than docetaxel, which caused a 11% maximum body weight loss.

PEGylated nanoparticles (mPEG(2k)-PLGA at 30 wt%)- q7d

Both the docetaxel control group and the PEGylated nanoparticles were administered three times, once every week at a dose of 30 mg/kg. The TGI for the docetaxel and PEGylated nanoparticles group was 90% and 96% respectively. The PEGylated nanoparticles showed a greater TGD (25 days) and survival compared to the docetaxel group (17 days). In addition, the PEGylated nanoparticles were better tolerated and caused no body weight loss, whereas the docetaxel group had a maximum body weight loss of 11%.

PEGylated nanoparticles (mPEG(2k)-PLGA at 30 wt%)- q14d

Both the docetaxel control group and the PEGylated nanoparticles were administered two times, once every two weeks at a dose of 60 mg/kg. The TGI for the PEGylated nanoparticles group was greater (i.e. 97%) than that of the docetaxel group (i.e. 71%). The PNP also exhibited an increased TGD and survival compared to docetaxel. The docetaxel group reached the tumor volume end point on day 29 and showed a TGD of 11 days. In the case of the PEGylated nanoparticles group, the

average tumor volume was 118 mm^3 on day 42. A TGD for the PEGylated nanoparticles could not be determined because at the time of measurement, the group still had not reached the tumor volume end point (i.e. on day 56, the average tumor volume was 840 mm^3). In addition, the PEGylated nanoparticles were well tolerated and caused only 8% maximum body weight loss. The control group docetaxel did not show any body weight loss.

PEGylated nanoparticles (mPEG(2k)-PLGA at 40 wt%)- q7d

Both the docetaxel control group and the PEGylated nanoparticles were administered three times, once every week at a dose of 15 mg/kg. The TGI of the docetaxel group and the PEGylated nanoparticles was shown to be similar (approximately 90%). The TGD of the free docetaxel and the PEGylated nanoparticles was 11 and 13 days respectively. There was no body weight loss associated with the PEGylated nanoparticles; in contrast, the docetaxel group showed a maximum body weight loss of 11%.

PEGylated nanoparticles (mPEG(5k)-PLGA at 16 wt%)-q3dq4d

The docetaxel and the PEGylated nanoparticles groups were administered three times over a two week schedule at a dose of 15 mg/kg. The docetaxel group had a TGI of 90% compared to the PEGylated nanoparticles group which had a TGI of 71%. The TGD of the docetaxel and PEGylated nanoparticles groups were 11 and 7 days respectively. The PEGylated nanoparticles were better tolerated and showed no body weight loss compared to the docetaxel group, which exhibited an 11% maximum body weight loss.

PEGylated nanoparticles (mPEG(5k)-PLGA at 30 wt%)-q3dq4d

The docetaxel and the PEGylated nanoparticles groups were administered three times over a two week schedule at a dose of 15 mg/kg. The docetaxel and PEGylated nanoparticles groups showed a similar TGI (i.e. 90%). In terms of the TGD, the docetaxel group showed 11 days compared to the PEGylated nanoparticles (i.e. 13 days). The PEGylated nanoparticles were better tolerated than the docetaxel

control group. Also, the docetaxel group exhibited a maximum body weight loss of 11% compared to no body weight loss shown by the PEGylated nanoparticles group.

PEGylated nanoparticles (mPEG(5k)-PLGA at 30 wt%)-q7d

Both the docetaxel and PEGylated nanoparticles groups were administered three times, once a week at a dose of 30 mg/kg. The TGI of the docetaxel and PEGylated nanoparticles groups were 90% and 97% respectively. The TGD of the docetaxel group was determined to be 17 days as the average tumor volume reached the end point of 3000 mm^3 at day 37. A TGD for the PEGylated nanoparticles could not be determined because at the time of measurement, the group still had not reached the tumor volume end point (i.e. on day 47, the average tumor volume was 2100 mm^3). The PEGylated nanoparticles did not cause any body weight loss and was better tolerated than free docetaxel which caused a 11% body weight loss.

PEGylated nanoparticles (mPEG(5k)-PLGA at 40 wt%)-q4dq3d

The docetaxel and PEGylated nanoparticles groups were administered three times over a two week schedule at a dose of 15 mg/kg. The TGI for both groups was similar (approximately 90-92%). The TGD for the PEGylated nanoparticles (i.e. 15 days) was greater than that for the docetaxel group (i.e. 11 days). The PEGylated nanoparticles did not cause any body weight loss to the mice and were better tolerated compared to the docetaxel group which resulted in a 11% maximum body weight loss.

Comparison of efficacy and tolerability of different PEGylated nanoparticles (2k) formulation and the control docetaxel treatment group

Formulation	Schedule	Dose (mg/kg)	Tumor growth inhibition (TGI) (%)	Tumor growth delay (TGD) (days)	Maximum body weight loss (%)
Docetaxel	q3dq4dx3	15	90	12	12

PEGylated nps (mPEG(2k)-PLGA 16 wt%)	q3dq4dx3	30	84	13	0
Docetaxel	q3dq4dx3	15	90	11	11
PEGylated nps (mPEG(2k)-PLGA 30 wt%)	q3dq4dx3	15	86	11	0
Docetaxel	q7dx3	30	90	17	11
PEGylated nps (mPEG(2k)-PLGA 30 wt%)	q7dx3	30	96	25	0
Docetaxel	q14dx2	60	71	11	0
PEGylated nps (mPEG(2k)-PLGA 30 wt%)	q14dx2	60	97	>38	8
Docetaxel	q3dq4dx3	15	90	11	11
PEGylated nps (mPEG(2k)-PLGA 40 wt%)	q3dq4dx3	15	89	13	0

* q3dq4dx3- three injections administered over 2 weeks (3 days in between 1st and 2nd injection, 4 days in between 2nd and 3rd injection).

* q7dx3- three injections seven days apart.

* q14dx2- two injections 14 days apart.

Comparison of efficacy and tolerability of different PEGylated nanoparticles (5k) formulation and the control docetaxel treatment group

Formulation	Schedule	Dose (mg/kg)	Tumor growth inhibition (TGI) (%)	Tumor growth delay (TGD) (days)	Maximum body weight loss (%)
Docetaxel	q3dq4dx3	15	90	11	11
PEGylated nps (PEG(5k)-PLGA 16	q3dq4dx3	15	71	7	0

wt%)					
Docetaxel	q3dq4dx3	15	90	11	11
PEGylated nps (PEG(5k)-PLGA 30 wt%)	q3dq4dx3	15	90	13	0
Docetaxel	q7dx3	30	90	17	11
PEGylated nps (PEG(5k)-PLGA 30 wt%)	q7dx3	30	97	>38	0
Docetaxel	q4dq3dx3	15	90	11	11
PEGylated nps (PEG(5k)-PLGA 40 wt%)	q4dq3dx3	15	92	15	0

* q3dq4dx3- three injections administered over 2 weeks (3 days in between 1st and 2nd injection, 4 days in between 2nd and 3rd injection).

* q4dq3dx3- three injections administered over 2 weeks (4 days in between 1st and 2nd injection, 3 days in between 2nd and 3rd injection).

* q7dx3- three injections seven days apart.

Example 44. In vivo efficacy of PEGylated docetaxel-5050 PLGA-O-acetyl nanoparticles in a HCT-116 colon xenograft model

HCT-116 cells were grown in culture to confluency in McCoy's 5a medium containing 10% FBS and 1% penicillin/streptomycin and then resuspended in McCoy's 5a (passage 4). This suspension of HCT-116 cells (density = 3.7×10^6 cells/mL) was implanted subcutaneously above the right hind leg of male CD-1 nude mice on day 1.

The three treatment groups that were administered to HCT-116 tumor bearing mice (n = 6-7 per group) included: 1) a docetaxel vehicle formulation consisting of 1.5% ethanol/ 3.75% polysorbate 80/ 9.75% water/ 85% PBS at 20 mL/kg; 2) 10 mg/mL docetaxel stock solution (prepared with 20 mg of docetaxel, 0.2 mL ethanol, 0.5 mL polysorbate 80 and 1.3 mL water, added in that specific order and vortexed to ensure proper mixing) diluted in PBS to 1.5 mg/mL for a corresponding dose of 30 mg/kg at an injection volume of 20 mL/kg respectively; 3) PEGylated docetaxel-5050

PLGA-O-acetyl nanoparticle formulation (mPEG(2k)-PLGA with initial amount of 16 wt%) at a docetaxel equivalent concentration of 1.5 mg/mL for a corresponding dose of 30 mg/kg at an injection volume of 20 mL/kg

The treatments were administered IV into the tail vein at the respective dose volumes (refer to previous paragraph), beginning on post-implantation Day 13, when the mean tumor volume was 131 mm^3. The vehicle and docetaxel treatments were administered two times, on Days 13 and 20 (weekly × two injections).

The mice that were administered docetaxel at a dose of 30 mg/kg lost a maximum body weight of 14%. In comparison, the PEGylated formulation administered at a dose of 30 mg/kg, did not lose any weight during the study.

Tumor growth inhibition

The tumor growth inhibition (TGI) of the mice treated with docetaxel at a dose of 30 mg/kg was 88%. Extrapolating to where the tumor growth curve reached the end point at a tumor volume of 1000 mm^3, the TGD was calculated to be 22 days. For the PEGylated nanoparticles at a dose of 30 mg/kg, the TGI was 77%. The TGD was determined to be 21 days.

Example 45. In vivo efficacy of PEGylated docetaxel-5050 PLGA-O-acetyl nanoparticles in a SK-OV-3 ovarian human xenograft model

SK-OV-3 cells were grown in culture to confluency in RPMI medium containing 10% FBS and 1% penicillin/streptomycin and then resuspended in RPMI (passage 4) for implantation into mice. This suspension of SK-OV-3 cells (density = 30×10^6 cells/mL) was implanted into the mammary gland of female CD-1 nude mice on Day 1.

Treatment groups that were administered to SK-OV-3 tumor-bearing mice (n = 4-5 per group) included: 1) a docetaxel vehicle formulation consisting of 1.5% ethanol/ 3.75% polysorbate 80/ 9.75% water/ 85% PBS at 20 mL/kg; 2) 10 mg/mL docetaxel stock solution (prepared with 20 mg of docetaxel, 0.2 mL ethanol, 0.5 mL polysorbate 80 and 1.3 mL water, added in that specific order and vortexed to ensure

proper mixing) diluted in PBS to A) 1.5 mg/mL for a corresponding dose of 15 mg/kg and 30 mg/kg at an injection volume of 10 mL/kg and 20 mL/kg respectively, and B) 3 mg/mL for a dose of 60 mg/kg at an injection volume of 20 mL/kg; 3) PEGylated docetaxel-5050 PLGA-O-acetyl nanoparticle formulation (mPEG(2k)-PLGA with initial amount of 16 wt%) at a docetaxel equivalent concentration of 2.9 mg/mL for a corresponding dose of 60 mg/kg at an injection volume of 21 mL/kg.

The treatments were administered IV into the tail vein at the dose volumes stated above, beginning on post-implantation Day 51, when the mean tumor volume was 232 mm^3. The vehicle and docetaxel treatments were administered two times, on Days 51 and 58 (weekly × two injections). The PEGylated nanoparticles treatment was administered once, on Day 51.

The high dose of docetaxel, 60 mg/kg, caused greater than 20% body weight loss. Ataxia, which is defined as the inability to coordinate voluntary muscular movements that is symptomatic of some CNS disorders and injuries and not due to muscle weakness, was observed in all the mice four days after the second treatment of docetaxel. This group was removed 18 days after the second treatment, despite supportive measures (fluid replacement, easier access to food), due to the ataxia becoming more severe and affecting the forelimbs. The lower dose of docetaxel, 30 mg/kg, did not cause ataxia. Maximum body weight loss in the group administered docetaxel 30 mg/kg was 13%. The group administered the PEGylated nanoparticles at a dose of 60 mg/kg was only administered that treatment one time. No ataxia developed in this group, but this could not be compared to the high dose of docetaxel because of the different numbers of treatments. Maximum body weight loss in the group administered the PEGylated nanoparticles at 60 mg/kg was 11%, equivalent to the free drug (i.e. docetaxel) at 30 mg/kg.

Tumor growth inhibition

All treatments inhibited tumor growth. The tumor growth delay (TGD) for docetaxel at a dose of 15 mg/kg was 18 days. The TGD for docetaxel at a dose of 30 mg/kg was 42 days. At this time, this group had a large variation, with two mice >1000 mm^3 and three mice <50 mm^3. The TGD for PEGylated nanoparticles at 60

mg/kg was 94 days, with a large intragroup variation with two mice > 1000 mm^3 and three mice < 325 mm^3, a similar pattern to free drug at a dose of 30 mg/kg, but delayed approximately 54 days relative to free drug.

Example 46. In vivo efficacy of PEGylated docetaxel-5050 PLGA-O-acetyl nanoparticles in a MDA-MB-435 melanoma human xenograft model

MDA-MB-435 cells were grown in culture to confluency in RPMI medium containing 10% FBS and 1% penicillin/streptomycin and then resuspended in RPMI (passage 4) for implantation into mice. A volume of 0.1 mL containing 4.0×10^6 cells MDA-MB-435 cells were implanted into the mammary gland of female CD-1 nude mice on Day 1.

Treatments that were administered to the mice (n = 6-7/group) included: 1) a docetaxel vehicle formulation consisting of 1.5% ethanol/ 3.75% polysorbate 80/ 9.75% water/ 85% PBS at 20 mL/kg; 2) 10 mg/mL docetaxel stock solution (prepared with 20 mg of docetaxel, 0.2 mL ethanol, 0.5 mL polysorbate 80 and 1.3 mL water, added in that specific order and vortexed to ensure proper mixing) diluted in PBS to A) 1.5 mg/mL for a corresponding dose of 15 and 30 mg/kg at an injection volume of 10 mL/kg and 20 mL/kg, respectively, B) 3.0 mg/mL for a dose of 60 mg/kg at an injection volume of 20 mL/kg; 3) PEGylated docetaxel-5050 PLGA-O-acetyl nanoparticle formulation (mPEG(2k)-PLGA with initial amount of 16 wt%) made at a docetaxel equivalent concentration of 1.1 mg/mL for a corresponding dose of 30 mg/kg at an injection volume of 26 mL/kg; 4) PEGylated docetaxel-5050 PLGA-O-acetyl nanoparticle formulation (mPEG(2k)-PLGA with initial amount of 30 wt%) made at a docetaxel equivalent of 1.5 and 2.85 mg/mL for corresponding doses of A) 15 mg/kg at an injection volume of 10 mL/kg, B) 30 and 60 mg/kg at an injection volume of 11 mL/kg and 21 mL/kg, respectively.

The treatments were administered IV into the tail vein at the dose volumes stated above, beginning on post-implantation Day 21, when the mean tumor volume was 150 mm^3 or, for one group, on Day 37, when the mean tumor volume for that

group was 433 mm^3. The treatments were administered two times, on Days 21 and 28 (weekly × two injections) for the vehicle, docetaxel and PEGylated nanoparticles groups and on Days 37 and 44 for a group that was administered PEGylated nanoparticles when the tumors were at a larger tumor volume (i.e. 433 mm^3).

For groups administered the free docetaxel, the high dose, 60 mg/kg, caused greater than 20% body weight loss. Ataxia was observed four days after the second treatment. This group was removed nine days after the second treatment, despite supportive measures (fluid replacement, easier access to food), due to severe ataxia. The docetaxel group administered at a dose of 30 mg/kg did not cause ataxia. Maximum body weight loss in the docetaxel dosed at 30 mg/kg group was 14% and in the case of the 15 mg/kg group, it was 10% of initial body weight.

Groups administered PEGylated nanoparticles had different responses depending on the wt% and dose. The PEGylated nanoparticles (PEG at initial amount of 16 wt%) administered at a dose of 30 mg/kg did not show any weight loss. The PEGylated nanoparticles (PEG at initial amount of 30 wt%) administered at a dose of 15 mg/kg also did not show any weight loss. At a higher dose (30 mg/kg), the PEGylated nanoparticles treatment group lost 6% of its initial body weight. At an even higher dosage (60 mg/kg), the treatment group receiving PEGylated nanoparticles administered starting on Day 21 (i.e. when the mean tumor size was 150 mm^3) lost 11% body weight, which was equivalent to the free drug at a dose of 30 mg/kg. The treatment group receiving same PEGylated nanoparticles at a dose of 60 mg/kg were also administered on Day 37 (i.e. when the mean tumor size was 433 mm^3) lost 19% body weight. This exaggerated weight loss was likely due to undetermined necrotic factors released from a relatively large amount of dead tumor tissue. One mouse in this latter group was found dead on Day 64 despite supportive measures (fluid replacement, easier access to food). The other mice in that group almost fully recovered their lost body weight and do not appear to be at any health risk at this time (Day 76).

Ataxia

Mice administered docetaxel at a dose of 60 mg/kg developed ataxia. The entire group showed abnormal gait and lack of coordination of the front limbs nine days after the second treatment. No other doses of docetaxel were observed to cause ataxia. In contrast to docetaxel, none of the mice administered PEGylated nanoparticles at any dose developed ataxia.

Tumor growth inhibition

All treatments groups resulted in tumor growth inhibition. The mean tumor volume of vehicle-treated group reached the endpoint of 1000 mm^3 on Day 58 post-tumor implantation. As of Day 76, it appears that the treatment at a dose of 15 mg/kg resulted in the same TGI for free docetaxel and PEGylated nanoparticles. At a dose of 30 mg/kg, the TGI for free docetaxel was greater than that for PEGylated nanoparticles (mPEG-PLGA initial amount of 30 wt% > mPEG-PLGA initial amount of 16 wt%). At a dose of 60 mg/kg, free docetaxel was equivalent to PEGylated nanoparticles until the free drug group was removed from the study. As the study continues, docetaxel at a dose of 30 mg/kg is equivalent to PEGylated nanoparticles at a dose of 60 mg/kg.

Example 47. Tolerability of the free drug docetaxel and PEGylated docetaxel-5050 PLGA-O-acetyl nanoparticles in normal male C57BL/6 non-tumor-bearing mice.

Treatments that were administered to the male C57BL/6 mice (n = 5/group) included: 1) a docetaxel vehicle formulation consisting of 1.5% ethanol/ 3.75% polysorbate 80/ 9.75% water/ 85% PBS at 20 mL/kg; 2) 10 mg/mL docetaxel stock solution (prepared with 20 mg of docetaxel, 0.2 mL ethanol, 0.5 mL polysorbate 80 and 1.3 mL water, added in that specific order and vortexed to ensure proper mixing) diluted in PBS to 1.5, 2.25 and 3 mg/mL for a corresponding dose of 30, 45 and 60 mg/kg at an injection volume of 20 mL/kg; 3) PEGylated docetaxel-5050 PLGA-O-acetyl nanoparticles formulation (mPEG(2k)-PLGA initial amount of 30 wt%) at a

docetaxel equivalent of 2.85 mg/mL for a dose of 60 mg/kg at an injection volume of 21 mL/kg.

Treatments were administered intravenously on a q7dx2 schedule, i.e., two treatments seven days apart (the first treatment was on Day one). The study ended on Day 14, six days after the 2nd treatment. Blood was collected for complete blood count (CBC) and serum chemistry. Leg muscles were collected so that nerve degeneration could be assessed from the sciatic nerve.

The vehicle-treated group gained 23% of its initial body weight by the end of the study. Docetaxel administered at doses of 30 and 45 mg/kg gained weight, up to 7% at the second treatment, weighing 3% and 2% respectively more than the initial on Day 14. The group administered docetaxel at a dose of 60 mg/kg did not gain weight after the first treatment and lost weight (19%) after the second treatment, by the end of the study. The group administered PEGylated nanoparticles at a dose of 60 mg/kg did not gain weight after the first treatment and lost weight (16%) after the second treatment, by the end of the study.

Complete blood count

From the table below, the CBC analyses showed that the white blood cell number, neutrophil number and lymphocyte number were lower in the groups administered docetaxel and PNP at a dose of 60 mg/kg. The white blood cells are expressed in units of × 1000 cells/μL, the neutrophils and lymphocytes are expressed in units of cells/μL.

Treatment	WBC #		Neutrophil		Lymphocyte	
	mean	SD	mean	SD	mean	SD
Docetaxel vehicle group	8.3	1.0	1474	390	6563	757
Docetaxel, 30 mg/kg	5.1	1.7	556	254	4350	1394
Docetaxel, 45 mg/kg	7.8	1.7	752	266	6780	1855
Docetaxel, 60 mg/kg	6.2	1.0	470	159	5590	938
PEGylated docetaxel-5050 PLGA-O-	4.6	0.9	488	162	3958	1001

acetyl nanoparticles (mPEG(2k)-PLGA initial amount of 30 wt%)						

Serum chemistry

Both the free docetaxel group and the PEGylated docetaxel-5050 PLGA-O-acetyl nanoparticles formulation (mPEG(2k)-PLGA initial amount of 30 wt%) did not affect any serum chemistry parameter at doses up to 60 mg/kg.

Sciatic nerve histopathology assessment

Mice administered the free docetaxel was observed to develop ataxia during the study with a dose-related effect. Specifically, no mice in the 30 mg/kg group were seen to develop ataxia or any overt signs of nerve damage. One mouse in the 45 mg/kg group was observed to develop ataxia on Day 14, while the others in that group had a normal gait. Five out of five mice in the 60 mg/kg group was observed to develop ataxia – one on Day 12, all on Day 14. None of the mice in the group administered PEGylated nanoparticles at a dose of 60 mg/kg was shown to develop ataxia. Refer to the table below for results.

Group	Dose (mg/kg)	Ataxia (%)
Docetaxel vehicle control	0	---
Free docetaxel	30	0
Free docetaxel	45	20
Free docetaxel	60	100
PEGylated docetaxel-5050 PLGA-O-acetyl nanoparticles (mPEG(2k)-PLGA initial amount of 30 wt%)	60	0

These data showed that, contrary to the MDA-MB-435 study described above and historical data, free docetaxel and PEGylated docetaxel-5050 PLGA-O-acetyl nanoparticles (mPEG(2k)-PLGA initial amount of 30 wt%) at a dose of 60 mg/kg

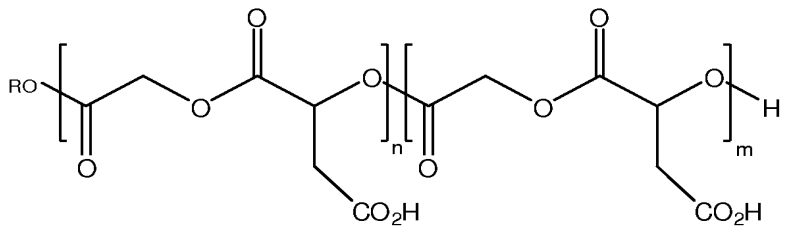
q7dx2 (i.e. two treatments seven days apart) are equivalent regarding body weight loss. Further, and also contrary to historical data, these treatments were similar regarding effects on the CBC.

A pathologist's assessment of the sciatic nerve histology found no treatment effects in any animals. Since ataxia was observed to be severe in the docetaxel group at a dose of 60 mg/kg, and damage by taxanes of the sciatic nerve at the level of the muscle was shown previously in published studies, it was suggested by the pathologist that the section of sciatic nerve that was examined was too far from the spinal chord, and damage did not yet develop in that part of the sciatic nerve at the time of tissue collection.

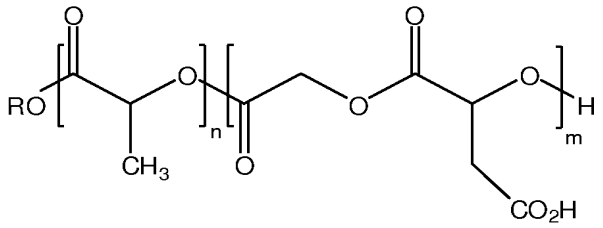
Example 48. Synthesis of Polyfunctionalized PLGA/PLA based polymers

One could synthesize a PLGA/PLA related polymer with functional groups that are dispersed throughout the polymer chain that is readily biodegradable and whose components are all bioacceptable components (i.e. known to be safe in humans). Specifically, PLGA/PLA related polymers derived from 3-S-[benxyloxycarbonyl)methyl]-1,4-dioxane-2,5-dione (BMD) could be synthesized (see structures below). (The structures below are intended to represent random copolymers of the monomeric units shown in brackets.)

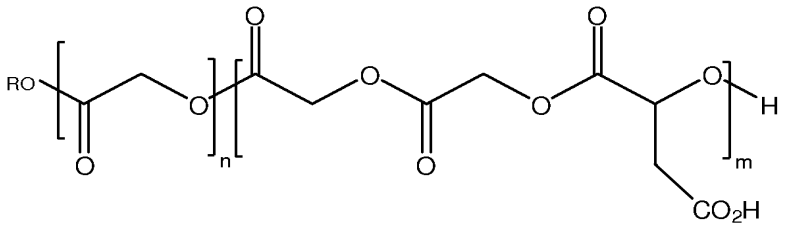
1. PLGA/PLA related polymer derived from BMD



2. PLGA/PLA related polymer with BMD and 3,5-dimethyl-1,4-dioxane-2,5-dione (bis-DL-lactic acid cyclic diester)



3. PLGA/PLA related polymer with BMD and 1,4-dioxane-2,5-dione (bis-glycolic acid cyclic diester



In a preferred embodiment, PLGA/PLA polymers derived from BMD and bis-DL-lactic acid cyclic diester will be prepared with a number of different pendent functional groups by varying the ratio of BMD and lactide. For reference, if it is assumed that each polymer has a number average molecular weight (Mn) of 8 kDa, then a polymer that is 100 wt % derived from BMD has approximately 46 pendant carboxylic acid groups (1 acid group per 0.174 kDa). Similarly, a polymer that is 25 wt% derived from BMD and 75 wt% derived from 3,5-dimethyl-1,4-dioxane-2,5-dione (bis-DL-lactic acid cyclic diester) has approximately 11 pendant carboxylic acid groups (1 acid group per 0.35 kDa). This compares to just 1 acid group for an 8 kDa PLGA polymer that is not functionalized and 1 acid group/2 kDa if there are 4 sites added during functionalization of the terminal groups of a linear PLGA/PLA polymer or 1 acid group/1 kDa if a 4 kDa molecule has four functional groups attached.

Specifically, the PLGA/PLA related polymers derived from BMD will be developed using a method by Kimura et al., Macromolecules, 21, 1988, 3338-3340. This polymer would have repeating units of glycolic and malic acid with a pendant carboxylic acid group on each unit $[RO(COCH_2OCOCHR_1O)_nH$ where R is H, or alkyl or PEG unit etc. and R_1 is $CO_2H]$. There is one pendant carboxylic acid group for each 174 mass units. The molecular weight of the polymer and the polymer polydispersity can vary with different reaction conditions (i.e. type of initiator,

temperature, processing condition). The Mn could range from 2 to 21 kDa. Also, there will be a pendant carboxylic acid group for every two monomer components in the polymer. Based on the reference previously sited, NMR analysis showed no detectable amount of the β-malate polymer was produced by ester exchange or other mechanisms.

Another type of PLGA/PLA related polymer derived from BMD and 3,5-dimethyl-1,4-dioxane-2,5-dione (bis-DL-lactic acid cyclic diester) will be synthesized using a method developed by Kimura et al., Polymer, 1993, 34, 1741-1748. They showed that the highest BMD ratio utilized was 15 mol% and this translated into a polymer containing 14 mol% (16.7 wt%) of BMD-derived units. This level of BMD incorporation represents approximately 8 carboxylic acid residues per 8 kDa polymer (1 carboxylic acid residue/kDa of polymer). Similarly to the use of BMD alone, no β-malate derived polymer was detected. Also, Kimura et al. reported that the glass transition temperatures (T_g) were in the low 20°C's despite the use of high polymer molecular weights (36-67 kDa). The T_g's were in the 20-23°C for these polymers whether the carboxylic acid was free or still a benzyl group. The inclusion of more rigidifying elements (i.e. carboxylic acids which can form strong hydrogen bonds) should increase the T_g. Possible prevention of aggregation of any nanoparticles formed from a polymer drug conjugate derived from this specific polymer will have to be evaluated due to possible lower T_g values.

Another method for synthesizing a PLA-PEG polymer that contains varying amounts of glycolic acid malic acid benzyl ester involves the polymerization of BMD in the presence of 3,5-dimethyl-1,4-dioxane-2,5-dione (bis-DL-lactic acid cyclic diester), reported by Lee et al., Journal of Controlled Release, 94, 2004, 323-335. They reported that the synthesized polymers contained 1.3-3.7 carboxylic acid units in a PLA chain of approximately 5-8 kDa (total polymer weight was approximately 11-13 kDa with PEG being 5 kDa) depending on the quantity of BMD used in the polymerization. In one polymer there were 3.7 carboxylic acid units/hydrophobic block in which the BMD represents approximately 19 wt% of the weight of the hydrophobic block. The ratio of BMD to lactide was similar to that observed by

Kimura et al., Polymer, 1993, 34, 1741-1748 and the acid residues were similar in the resulting polymers (approximately 1 acid unit/kDa of hydrophobic polymer).

Polymers functionalized with BMD that are more readily hydrolysable will be prepared using the method developed by Kimura et al., International Journal of Biological Macromolecules, 25, 1999, 265-271. They reported that the rate of hydrolysis was related to the number of free acid groups present (with polymers with more acid groups hydrolyzing faster). The polymers had approximately 5 or 10 mol% BMD content. Also, in the reference by Lee et al., Journal of Controlled Release, 94, 2004, 323-335, the rate of hydrolysis of the polymer was fastest with the highest concentration of pendent acid groups (6 days for polymer containing 19.5 wt% of BMD and 20 days for polymer containing 0 wt% of BMD.

A drug (e.g. docetaxel, paclitaxel, doxorubicin, etc.) could be conjugated to a PLGA/PLA related polymer with BMD (refer to previous examples above). Similarly, a nanoparticle could be prepared from such a polymer drug conjugate.

Example 49. Synthesis of polymers prepared using β-lactone of malic acid benzyl esters

One could prepare a polymer by polymerizing MePEGOH with RS-β-benzyl malolactonate (a β-lactone) with DL-lactide (cyclic diester of lactic acid) to afford a polymer containing MePEG (lactic acid) (malic acid) Me(OCH2CH2O)[OCCCH(CH3)O]m[COCH2CH(CO2H)O]. as developed by Wang et al., Colloid Polymer Sci., 2006, 285, 273-281. These polymers would potentially degrade faster because they contain higher levels of acidic groups. It should be noted that the use of β -lactones generate a different polymer from that obtained using 3-[(benzyloxycarbonyl)methyl]-1,4-dioxane-2,5-dione. In these polymers, the carboxylic acid group is directly attached to the polymer chain without a methylene spacer.

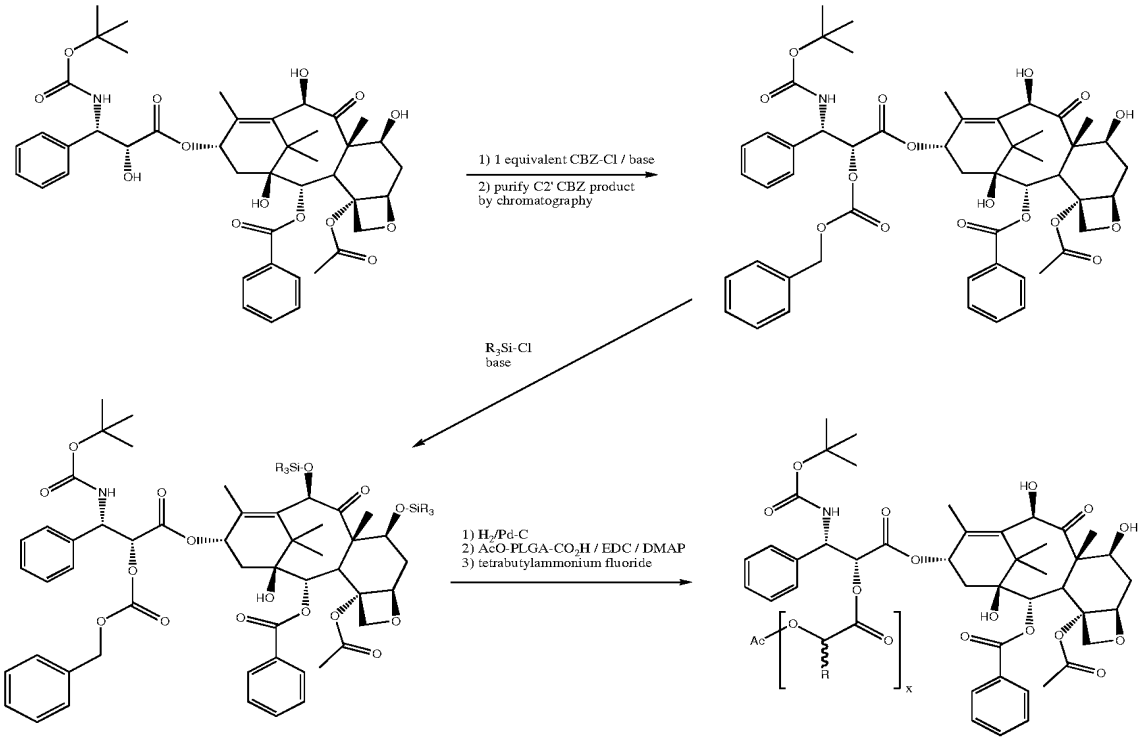
Another polymer that could be prepared directly from a β-lactone was reported by Ouhib et al., Ch. Des. Monoeres. Polym, 2005, 1, 25. The resulting polymer (i.e. poly-3,3-dimethylmalic acid) is water soluble as the free acid, has pendant carboxylic

acid groups on each unit of the polymer chain and as well it has been reported that 3,3-dimethylmalic acid is a nontoxic molecule.

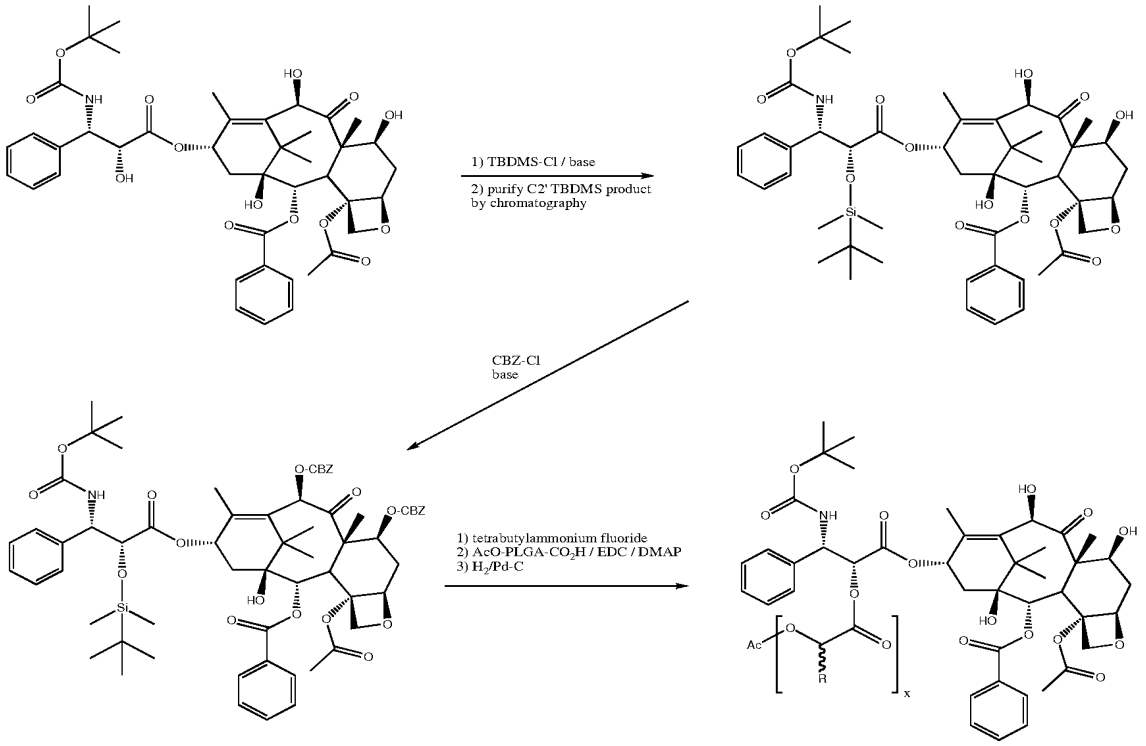
One could polymerize 4-benzyloxycarbonyl-,3,3-dimethyl-2-oxetanone in the presence of 3,5-dimethyl-1,4-dioxane-2,5-dione (DDD) and β-butyrolactone to generate a block copolymer with pendant carboxylic acid groups as shown by Coulembier et al., Macromolecules, 2006, 39, 4001-4008. This polymerization reaction was carried out with a carbene catalyst in the presence of ethylene glycol. The catalyst used was a triazole carbene catalyst which leads to polymers with narrow polydispersities.

Example 50. Regioselective synthesis of docetaxel-2'-5050 PLGA-O-acetyl

Docetaxel-2'-5050 PLGA-O-acetyl could be regioselectively prepared as illustrated in the following scheme. The 2' hydroxyl group of docetaxel is first protected using benzylchloroformate. Following purification of the 2' Cbz-protected docetaxel, the product may be orthogonally protected on the 7 and 10 hydroxyl groups using a silyl chloride (e.g., tert-butyldimethylsilyl chloride). The Cbz group may then be removed using hydrogenation over Pd/C, followed by coupling of PLGA-O-acetyl using EDC and DMAP. Final deprotection of the silyl protecting groups using TBAF would produce the docetaxel-2'-5050 PLGA-O-acetyl selectively coupled via the 2' hydroxyl group.



Alternatively, docetaxel-2'-5050 PLGA-O-acetyl could be regioselectively prepared as illustrated in the scheme below. The 2' hydroxyl group of docetaxel is first protected using tert-butyldimethylsilyl chloride. Following purification of the 2' TBDMS-protected docetaxel, the product may be orthogonally protected on the 7 and 10 hydroxyl groups using a benzylchloroformate. The TBDMS group may then be removed using TBAF, followed by coupling of PLGA-O-acetyl using EDC and DMAP. Final deprotection of the Cbz protecting groups via hydrogenation over Pd/C would produce the docetaxel-2'-5050 PLGA-O-acetyl selectively coupled via the 2' hydroxyl group.



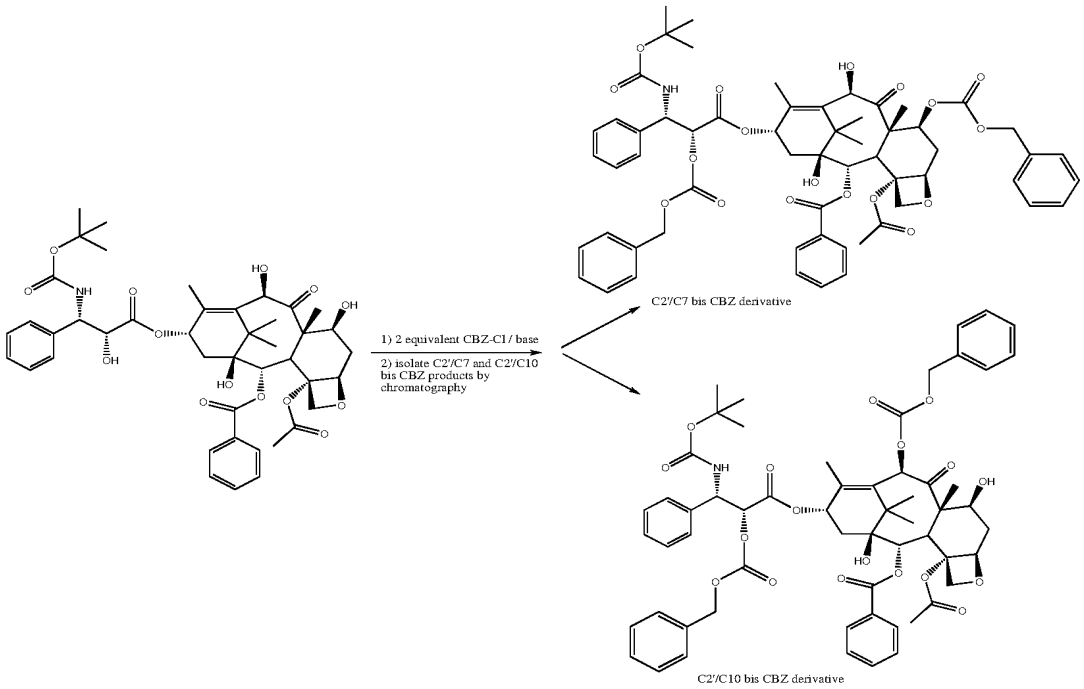
Example 51. Regioselective synthesis of docetaxel-7-5050 PLGA-O-acetyl and docetaxel-10-5050 PLGA-O-acetyl

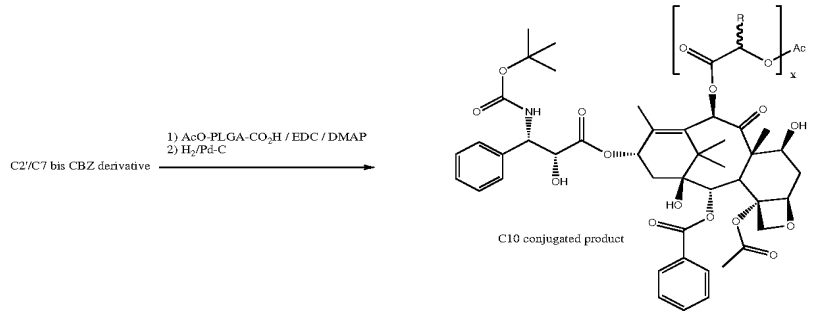
Docetaxel-7-5050 PLGA-O-acetyl and docetaxel-10-5050 PLGA-O-acetyl could be regioselectively prepared as illustrated in the following scheme. Docetaxel is first protected using two equivalents of benzylchloroformate, yielding a mixture of products. Two products, C2'/C7-bis-Cbz-docetaxel, and C2'/C10-bis-Cbz-docetaxel, can each be selectively purified.

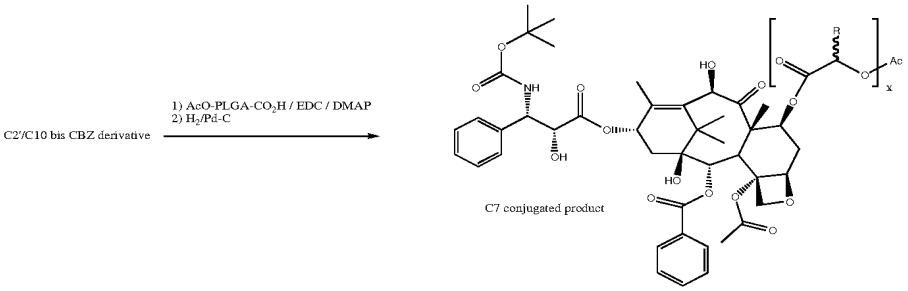
C2'/C7-bis-Cbz-docetaxel can then be coupled to PLGA-O-acetyl using EDC and DMAP, which would result in attachment of PLGA-O-acetyl to the hydroxyl group at the 10-position of docetaxel. Deprotection of the Cbz protecting groups via hydrogenation over Pd/C would produce the docetaxel-10-5050 PLGA-O-acetyl selectively coupled via the 10 hydroxyl group.

C2'/C10-bis-Cbz-docetaxel can then be coupled to PLGA-O-acetyl using EDC and DMAP, which would result in attachment of PLGA-O-acetyl to the hydroxyl group at the 7-position of docetaxel. Deprotection of the Cbz protecting groups via

hydrogenation over Pd/C would produce the docetaxel-7-5050 PLGA-O-acetyl selectively coupled via the 7 hydroxyl group.







What is claimed is:

CLAIMS

1. A particle comprising:

a) a plurality of hydrophobic polymer-agent conjugates, wherein

i) each hydrophobic polymer-agent conjugate of said plurality comprises a hydrophobic polymer attached to an agent;

b) a plurality of hydrophilic-hydrophobic polymers, wherein

i) each of said hydrophilic-hydrophobic polymers of said plurality comprises a hydrophilic portion attached to a hydrophobic portion,

c) a surfactant.

2. The particle of claim 1, comprising:

a) a plurality of hydrophobic polymer-agent conjugates, wherein

i) each hydrophobic polymer-agent conjugate of said plurality comprises a hydrophobic polymer attached to an agent,

ii) said hydrophobic polymer attached to agent can be a homopolymer or a polymer made up of more than one kind of monomeric subunit,

iii) said hydrophobic polymer attached to said agent has a weight average molecular weight of about 4-15 kD,

iv) said agent is about 1-30 weight % of said particle and

v) said plurality of hydrophobic-agent conjugates is about 25-80 weight % of said particle;

b) a plurality of hydrophilic-hydrophobic polymers, wherein

i) each of said hydrophilic-hydrophobic polymers of said plurality comprises a hydrophilic portion attached to a hydrophobic portion,

ii) said hydrophilic portion has a weight average molecular weight of about 1-6 kD (e.g., 2-6 kD), and

iii) said plurality of hydrophilic-hydrophobic polymers is about 5-30 weight % of said particle;

and

c) a surfactant, wherein said surfactant is about 15-35 weight % of said particle; and

wherein:

the diameter of said particle is less than about 200nm.

3. The particle of claim 2, wherein a)iii) said hydrophobic polymer attached to said agent has a weight average molecular weight of about 4-8 kD.

4. The particle of claim 2, comprising:

a) a plurality of hydrophobic polymer-agent conjugates, wherein

i) each hydrophobic polymer-agent conjugate of said plurality comprises a hydrophobic polymer attached to an agent,

ii) said hydrophobic polymer attached to agent can be a homopolymer or a polymer made up of more than one kind of monomeric subunit,

iii) said hydrophobic polymer attached to said agent has a weight average molecular weight of about 4-15 kD,

iv) said agent is about 1-30 weight % of said particle, and

v) said plurality of hydrophobic-agent conjugates is about 25-80 weight % of said particle;

b) a plurality of hydrophilic-hydrophobic polymers, wherein

i) each of said hydrophilic-hydrophobic polymers of said plurality comprises a hydrophilic portion attached to a hydrophobic portion,

ii) said hydrophilic portion has a weight average molecular weight of about 1-6 kD (e.g., 2-6 kD), wherein

if the weight average molecular weight of said hydrophilic portion is about 1-3 kD, e.g., about 2 kD, the ratio of the weight average molecular weight of said hydrophilic portion to the weight average molecular weight of said hydrophobic portion is between 1:3-1:7, and if the weight average molecular weight of said hydrophilic portion is about 4-6 kD, e.g., about 5 kD,

the ratio of the weight average molecular weight of said hydrophilic portion to the weight average molecular weight of said hydrophobic portion is between 1:1-1:4; and

iii) said plurality of hydrophilic-hydrophobic polymers is about 5-30 weight % of said particle;

and

c) a surfactant, wherein

said surfactant is about 15-35 weight % of said particle; and

wherein:

the diameter of said particle is less than about 200nm.

5. The particle of claim 4, wherein a)iii) said hydrophobic polymer attached to said agent has a weight average molecular weight of about 4-8 kD.

6. The particle of claim 2, comprising:

a) a plurality of hydrophobic-agent conjugates, wherein

i) each hydrophobic-agent conjugate of said plurality comprises a hydrophobic polymer attached to an agent,

ii) said hydrophobic polymer attached to said agent can be a homopolymer or a polymer made up of more than one kind of monomeric subunit,

iii) said hydrophobic polymer attached to said agent has a weight average molecular weight of about 4-15 kD,

iv) said agent is about 1-30 weight % of said particle and

v) said plurality of hydrophobic-agent conjugates is about 35-80 weight % of said particle;

b) a plurality of hydrophilic-hydrophobic polymers, wherein

i) each of said hydrophilic-hydrophobic polymers of said plurality comprises a hydrophilic portion attached to a hydrophobic portion, and

ii) said hydrophilic portion has a weight average molecular weight of about 2-6 kD and said hydrophobic portion has a weight average molecular weight of between about 8-13 kD,

iii) said plurality of hydrophilic-hydrophobic polymers is about 10-25 weight % of said particle;

iv) said hydrophilic portion of said hydrophilic-hydrophobic polymer terminates in an OMe,

and

c) a surfactant, wherein said surfactant is about 15-35 weight % of said particle;

wherein:

said particle further comprises a hydrophobic polymer having a terminal acyl moiety;

and

the diameter of said particle is less than about 200nm.

7. The particle of claim 6, wherein a)iii) said hydrophobic polymer attached to said agent has a weight average molecular weight of about 4-8 kD.

8. A method of making the particle of claim 2, comprising:

providing an organic solution comprising:

a) a plurality of hydrophobic-agent conjugates, wherein

i) each hydrophobic-agent conjugate of said plurality comprises a hydrophobic polymer attached to an agent,

ii) said hydrophobic polymer attached to said agent can be a homopolymer or a polymer made up of more than one kind of monomeric subunit,

iii) said hydrophobic polymer attached to said agent has a weight average molecular weight of about 4-15 kD,

iv) said agent is about 1-30 weight % of said particle and

v) said plurality of hydrophobic-agent conjugates is about 25-80 weight % of said particle;

b) a plurality of hydrophilic-hydrophobic polymers, wherein

i) each of said hydrophilic-hydrophobic polymers of said plurality comprises a hydrophilic portion attached to a hydrophobic portion,

ii) said hydrophilic portion has a weight average molecular weight of about 1-6 kD (e.g., 2-6 kD), and

iii) said plurality of hydrophilic-hydrophobic polymers is about 5-30 weight % of said particle;

and

combining said organic solution with an aqueous solution comprising a solvent to provide said particles.

9. The particle of claim 8, wherein a)iii) said hydrophobic polymer attached to said agent has a weight average molecular weight of about 4-8 kD.

10. A pharmaceutically acceptable composition comprising a plurality of particles of claim 2 and an additional component.

11. A kit comprising a plurality of particles of claim 2.

12. A single dosage unit comprising a plurality of particles of claim 2.

13. A method of treating a subject having a disorder comprising administering to said subject an effective amount of particles of claim 2.

14. The particle of claim 2 comprising:

a) a plurality of hydrophobic polymer-agent conjugates, wherein

i) each hydrophobic polymer-agent conjugate of said plurality comprises a hydrophobic polymer attached to an agent,

ii) said hydrophobic polymer attached to said agent can be a homopolymer or a polymer made up of more than one kind of monomeric subunit,

iii) said hydrophobic polymer attached to said agent has a weight average molecular weight of about 4-15 kD,

iv) said agent is about 1-30 weight % of said particle and

v) said plurality of hydrophobic-agent conjugates is about 25-80 weight % of said particle;

b) a plurality of PEG-hydrophobic polymers, wherein

i) each of said PEG-hydrophobic polymers of said plurality comprises a PEG portion attached to a hydrophobic portion,

ii) said PEG portion has a weight average molecular weight of about 1-6 kD (e.g., 2-6 kD), and

iii) said plurality of PEG-hydrophobic polymers is about 5-30 weight % of said particle;

and

c) PVA, wherein

said PVA has a weight average molecular weight of about 5-45 kD and is about 15-35 weight % of said particle; and

wherein:

the diameter of said particle is less than about 200nm.

15. The particle of claim 14, wherein a)iii) said hydrophobic polymer attached to said agent has a weight average molecular weight of about 4-8 kD.

16. The particle of claim 14, comprising:

a) a plurality of hydrophobic polymer-agent conjugates, wherein

i) each hydrophobic polymer-agent conjugate of said plurality comprises a hydrophobic polymer attached to an agent,

ii) the hydrophobic polymer is made up of a first and a second type of monomeric subunit, and the ratio of the first to second type of

monomeric subunit in said hydrophobic polymer attached to said agent is from about 25:75 to about 75:25,

iii) said hydrophobic polymer attached to said agent has a weight average molecular weight of about 4-15 kD,

iv) said agent is about 1-30 weight % of said particle and

v) said plurality of hydrophobic-agent conjugates is about 25-80 weight % of said particle;

b) a plurality of PEG-hydrophobic polymers, wherein

i) each of said PEG-hydrophobic polymers of said plurality comprises a PEG portion attached to a hydrophobic portion,

ii) said PEG portion has a weight average molecular weight of about 1-6 kD (e.g., 2-6 kD), wherein

if the weight average molecular weight of said PEG portion is about 1-3 kD, e.g., about 2 kD, the ratio of the weight average molecular weight of said PEG portion to the weight average molecular weight of said hydrophobic portion is between 1:3-1:7, and if the weight average molecular weight of said PEG portion is about 4-6 kD, e.g., about 5 kD, the ratio of the weight average molecular weight of said PEG portion to the weight average molecular weight of said hydrophobic portion is between 1:1-1:4; and

iii) said plurality of PEG-hydrophobic polymers is about 5-30 weight % of said particle;

and

c) PVA, wherein

said PVA has a weight average molecular weight of about 5-45 kD and is about 15-35 weight % of said particle; and

wherein:

the diameter of said particle is less than about 200nm

17. The particle of claim 16, wherein a)iii) said hydrophobic polymer attached to said agent has a weight average molecular weight of about 4-8 kD.

18. The particle of claim 14, comprising:

a) a plurality of hydrophobic polymer-agent conjugates, wherein

i) each hydrophobic polymer-agent conjugate of said plurality comprises a hydrophobic polymer attached to an agent,

ii) the hydrophobic polymer is made up of a first and a second type of monomeric subunit, and the ratio of the first to second type of monomeric subunit in said hydrophobic polymer attached to said agent is from about 25:75 to about 75:25,

iii) said hydrophobic polymer attached to said agent has a weight average molecular weight of about 4-15 kD,

iv) said agent is about 1-30 weight % of said particle and

v) said plurality of hydrophobic polymer-agent conjugates is about 35-80 weight % of said particle;

b) a plurality of PEG-hydrophobic polymers, wherein

i) each of said PEG-hydrophobic polymers of said plurality comprises a PEG portion attached to a hydrophobic portion, and

ii) said PEG portion has a weight average molecular weight of about 2-6 kD and said hydrophobic portion has a weight average molecular weight of between about 8-13 kD,

iii) said plurality of PEG-hydrophobic polymers is about 10-25 weight % of said particle;

iv) said PEG portion of said PEG-hydrophobic polymer terminates in an OMe,

and

c) PVA, wherein said PVA has a weight average molecular weight of about 23-26 kD and is about 15-35 weight % of said particle;

wherein:

the particle further comprises a hydrophobic polymer having a terminal acyl moiety;

and

the diameter of said particle is less than about 200nm.

19. The particle of claim 18, wherein a)iii) said hydrophobic polymer attached to said agent has a weight average molecular weight of about 4-8 kD.

20. A method of making the particle of claim 14, comprising:

providing an organic solution comprising:

a) a plurality of hydrophobic polymer-agent conjugates, wherein

i) each hydrophobic polymer-agent conjugate of said plurality comprises a hydrophobic polymer attached to an agent,

ii) the hydrophobic polymer is made up of a first and a second type of monomeric subunit, and the ratio of the first to second type of monomeric subunit in said hydrophobic polymer attached to said agent is from about 25:75 to about 75:25,

iii) said hydrophobic polymer attached to said agent has a weight average molecular weight of about 4-15 kD,

iv) said agent is about 1-30 weight % of said particle and

v) said plurality of hydrophobic polymer-agent conjugates is about 25-80 weight % of said particle;

b) a plurality of PEG-hydrophobic polymers, wherein

i) each of said PEG-hydrophobic polymers of said plurality comprises a PEG portion attached to a hydrophobic portion,

ii) said PEG portion has a weight average molecular weight of about 1-6 kD (e.g., 2-6 kD), and

iii) said plurality of PEG-hydrophobic polymers is about 5-30 weight % of said particle; and

combining the organic solution with an aqueous solution comprising PVA to provide said particles.

21. The particle of claim 20, wherein a)iii) said hydrophobic polymer attached to said agent has a weight average molecular weight of about 4-8 kD.

22. A pharmaceutically acceptable composition comprising a plurality of particles of claim 14 and an additional component.

23. A kit comprising a plurality of particles of claim 14.

24. A single dosage unit comprising a plurality of particles of claim 14.

25. A method of treating a subject having a disorder comprising administering to said subject an effective amount of the particle of claim 14.

26. The particle of claim 2 comprising:

a) a plurality of PLGA-agent (e.g., therapeutic or diagnostic agent) conjugates, wherein

i) each PLGA-agent conjugate of said plurality comprises a PLGA polymer attached to an agent,

ii) the ratio of lactic acid to glycolic acid in said PLGA polymer attached to said agent is from about 25:75 to about 75:25,

iii) said PLGA polymer attached to said agent has a weight average molecular weight of about 4-15 kD,

iv) said agent is about 1-30 weight % of said particle and

v) said plurality of PLGA-agent conjugates is about 25-80 weight % of said particle;

b) a plurality of PEG-PLGA polymers, wherein

i) each of said PEG-PLGA polymers of said plurality comprises a PEG portion attached to a PLGA portion,

ii) said PEG portion has a weight average molecular weight of about 1-6 kD (e.g., 2-6 kD), and

iii) said plurality of PEG-PLGA polymers is about 5-30 weight % of said particle;

and

c) PVA, wherein

said PVA has a weight average molecular weight of about 5-45 kD and is about 15-35 weight % of said particle; and

wherein:

the diameter of said particle is less than about 200nm.

27. The particle of claim 26, wherein a)iii) said PLGA polymer attached to said agent has a weight average molecular weight of about 4-8 kD.

28. The particle of 26, comprising:

a) a plurality of PLGA-agent (e.g., therapeutic or diagnostic agent) conjugates, wherein

i) each PLGA-agent conjugate of said plurality comprises a PLGA polymer attached to an agent,

ii) the ratio of lactic acid to glycolic acid in said PLGA polymer attached to said agent is from about 25:75 to about 75:25,

iii) said PLGA polymer attached to said agent has a weight average molecular weight of about 4-15 kD,

iv) said agent is about 1-30 weight % of said particle and

v) said plurality of PLGA-agent conjugates is about 25-80 weight % of said particle;

b) a plurality of PEG-PLGA polymers, wherein

i) each of said PEG-PLGA polymers of said plurality comprises a PEG portion attached to a PLGA portion,

ii) said PEG portion has a weight average molecular weight of about 1-6 kD (e.g., 2-6 kD), wherein

if the weight average molecular weight of said PEG portion is about 1-3 kD, e.g., about 2 kD, the ratio of the

weight average molecular weight of said PEG portion to the weight average molecular weight of said PLGA portion is between 1:3-1:7, and if the weight average molecular weight of said PEG portion is about 4-6 kD, e.g., about 5 kD, the ratio of the weight average molecular weight of said PEG portion to the weight average molecular weight of said PLGA portion is between 1:1-1:4; and

iii) said plurality of PEG-PLGA polymers is about 5-30 weight % of said particle;

and

c) PVA, wherein

said PVA has a weight average molecular weight of about 5-45 kD and is about 15-35 weight % of said particle; and

wherein:

the diameter of said particle is less than about 200nm.

29. The particle of claim 28, wherein a)iii) said PLGA polymer attached to said agent has a weight average molecular weight of about 4-8 kD.

30. The particle of claim 26, comprising:

a) a plurality of PLGA-agent conjugates, wherein

i) each PLGA-agent conjugate of said plurality comprises a PLGA polymer attached to an agent,

ii) the ratio of lactic acid to glycolic acid in said PLGA polymer attached to said agent is from about 25:75 to about 75:25,

iii) said PLGA polymer attached to said agent has a weight average molecular weight of about 4-15 kD,

iv) said agent is about 1-30 weight % of said particle and

v) said plurality of PLGA-agent conjugates is about 35-80 weight % of said particle;

b) a plurality of PEG-PLGA polymers, wherein

i) each of said PEG-PLGA polymers of said plurality comprises a PEG portion attached to a PLGA portion, and

ii) said PEG portion has a weight average molecular weight of about 2-6 kD and said PLGA portion has a weight average molecular weight of between about 8-13 kD,

iii) said plurality of PEG-PLGA polymers is about 10-25 weight % of said particle;

iv) said PEG portion of said PEG-PLGA polymer terminates in an OMe,

and

c) PVA, wherein said PVA has a weight average molecular weight of about 23-26 kD and is about 15-35 weight % of said particle;

wherein:

said particle further comprises PLGA having a terminal acyl moiety;

and

the diameter of said particle is less than about 200nm.

31. The particle of claim 30, wherein iii) said PLGA polymer attached to said agent has a weight average molecular weight of about 4-8 kD.

32. A method of making the particle of claim 26, comprising:

providing an organic solution comprising:

a) a plurality of PLGA-agent conjugates, wherein

i) each PLGA-agent conjugate of said plurality comprises a PLGA polymer attached to an agent,

ii) the ratio of lactic acid to glycolic acid in said PLGA polymer attached to said agent is from about 25:75 to about 75:25,

iii) said PLGA polymer attached to said agent has a weight average molecular weight of about 4-15 kD,

iv) said agent is about 1-30 weight % of said particle and

v) said plurality of PLGA-agent conjugates is about 25-80 weight % of said particle;

b) a plurality of PEG-PLGA polymers, wherein

i) each of said PEG-PLGA polymers of said plurality comprises a PEG portion attached to a PLGA portion,

ii) said PEG portion has a weight average molecular weight of about 1-6 kD (e.g., 2-6 kD), and

iii) said plurality of PEG-PLGA polymers is about 5-30 weight % of said particle; and

combining said organic solution with an aqueous solution comprising PVA to provide said particles.

33. The particle of claim 32, wherein a)iii) said PLGA polymer attached to said agent has a weight average molecular weight of about 4-8 kD.

34. A pharmaceutically acceptable composition comprising a plurality of particles of claim 26 and an additional component.

35. A kit comprising a plurality of particles of claim 26.

36. A single dosage unit comprising a plurality of particles of claim 26.

37. A method of treating a subject having a disorder comprising administering to said subject an effective amount of the particles of claim 26.

38. The particle of any of claims 1-12, 14-24, or 26-36, wherein said agent is a diagnostic agent.

39. The particle of any of claims 1-12, 14-24, or 26-36, wherein said agent is a therapeutic agent.

40. The particle of any of claims 1-12, 14-24, or 26-36, wherein said therapeutic agent is an anti-inflammatory agent or an agent for treatment of a cardiovascular disease.

41. The particle of any of claims 1-12, 14-24, or 26-36, wherein said therapeutic agent is an anti-cancer agent.

42. The particle of any of claims 1-12, 14-24, or 26-36, wherein said therapeutic agent is an alkylating agent, a vascular disrupting agent, a taxane, an anthracycline, a vinca alkaloid, a platinum-based agent, a topoisomerase inhibitor, an anti-angiogenic agent or an anti-metabolite.

43. The particle of any of claims 1-12, 14-24, or 26-36, wherein said therapeutic agent a taxane.

44. The particle of any of claims 1-12, 14-24, or 26-36, wherein said therapeutic agent is paclitaxel.

45. The particle of any of claims 1-12, 14-24, or 26-36, wherein said therapeutic agent is larotaxel.

46. The particle of any of claims 1-12, 14-24, or 26-36, wherein said therapeutic agent is cabazitaxel.

47. The particle of any of claims 1-12, 14-24, or 26-36, wherein said therapeutic agent is an anthracycline.

48. The particle of any of claims 1-12, 14-24, or 26-36, wherein said therapeutic agent is an doxorubicin.

49. The particle of any of claims 1-12, 14-24, or 26-36, wherein said therapeutic agent is a platinum-based agent.

50. The particle of claim any of claims 1-12, 14-24, or 26-36, wherein said therapeutic agent is selected from cisplatin, carboplatin and oxaliplatin.

51. The particle of any of claims 1-12, 14-24, or 26-36, wherein said therapeutic agent is a pyrimidine analog.

52. The particle of any of claims 1-12, 14-24, or 26-36, wherein said therapeutic agent is gemcitabine.

53. The pharmaceutically acceptable composition of any of claims 10, 22 or 34, wherein said additional component is a lyoprotectant.

54. The pharmaceutically acceptable composition of any of claims 10, 22 or 34, wherein said additional component is a carbohydrate.

55. The pharmaceutically acceptable composition of any of claims 10, 22 or 34, wherein said additional component is a cyclodextrin.

56. The pharmaceutically acceptable composition of any of claims 10, 22 or 34, wherein said additional component is a 2-hydroxypropyl-beta-cyclodextrin.

57. The kit of any of claims 11, 23, or 35, further comprising a liquid resistant container in which said plurality of particles is disposed.

58. The kit of any of claims 11, 23, or 35, further comprising a diluent.

59. The method of any of claims 13, 25, or 37, wherein said disorder is a disorder characterized by an unwanted proliferation of cells.

60. The method of any of claims 13, 25, or 37, wherein said disorder is an inflammatory disorder.

61. The method of any of claims 13, 25, or 37, wherein said disorder is a cardiovascular disorder.

62. The method of any of claims 13, 25, or 37, wherein the disorder is cancer.

63. The method of claim 62, wherein the cancer is breast cancer.

64. The method of claim 63, wherein the breast cancer is locally advanced breast cancer.

65. The method of claim 63, wherein the breast cancer is metastatic.

66. The method of claim 62, wherein the cancer is non small cell lung cancer.

67. The method of claim 66, wherein the cancer is refractory, relapsed or resistant to a platinum-based agent and is unresectable, locally advanced or metastatic.

68. The method of claims 62, wherein the cancer is prostate cancer.

69. The method of claim 68, wherein the cancer is hormone refractory.

70. The method of claim 68, wherein the cancer is metastatic.

71. The method of claim 62, wherein the cancer is an unresectable cancer.

72. The method of claim 62, wherein the cancer is a chemotherapeutic sensitive cancer.

73. The method of claim 62, wherein the cancer is a chemotherapeutic refractory cancer.

74. The method of claim 62, wherein the cancer is a chemotherapeutic resistant cancer.

75. The method of claim 62, wherein the cancer is a relapsed cancer

76. The method of claim 62, wherein said plurality of particles is administered as adjunctive therapy with another therapy, e.g., radiation or surgery.

77. The particle of claim 2 comprising:

a) a plurality of hydrophobic polymer-docetaxel conjugates, wherein

i) each hydrophobic polymer-docetaxel conjugate of said plurality comprises a hydrophobic polymer attached to docetaxel,

ii) said hydrophobic polymer attached to said docetaxel can be a homopolymer or a polymer made up of more than one kind of monomeric subunit,

iii) said hydrophobic polymer attached to said docetaxel has a weight average molecular weight of about 4-15 kD,

iv) said docetaxel is about 1-30 weight % of said particle and

v) said plurality of hydrophobic polymer-docetaxel conjugates is about 25-80 weight % of said particle;

b) a plurality of hydrophilic-hydrophobic polymers, wherein

i) each of said hydrophilic-hydrophobic polymers of said plurality comprises a hydrophilic portion attached to a hydrophobic portion,

ii) said hydrophilic portion has a weight average molecular weight of about 1-6 kD (e.g., 2-6 kD), and

iii) said plurality of hydrophilic-hydrophobic polymers is about 5-30 weight % of said particle;

and

c) a surfactant, wherein said surfactant is about 15-35 weight % of said particle; and

wherein:

the diameter of said particle is less than about 200nm.

78. The particle of claim 77, wherein a)iii) said hydrophobic polymer attached to said docetaxel has a weight average molecular weight of about 4-8 kD.

79. The particle of claim 77, comprising:

a) a plurality of hydrophobic polymer-docetaxel conjugates, wherein

i) each hydrophobic polymer-docetaxel conjugate of said plurality comprises a hydrophobic polymer attached to docetaxel,

ii) said hydrophobic polymer attached to said docetaxel can be a homopolymer or a polymer made up of more than one kind of monomeric subunit,

iii) said hydrophobic polymer attached to said docetaxel has a weight average molecular weight of about 4-15 kD,

iv) said docetaxel is about 1-30 weight % of said particle and

v) said plurality of hydrophobic polymer-docetaxel conjugates is about 25-80 weight % of said particle;

b) a plurality of hydrophilic-hydrophobic polymers, wherein

i) each of said hydrophilic-hydrophobic polymers of said plurality comprises a hydrophilic portion attached to a hydrophobic portion,

ii) said hydrophilic portion has a weight average molecular weight of about 1-6 kD (e.g., 2-6 kD), wherein

if the weight average molecular weight of said hydrophilic portion is about 1-3 kD, e.g., about 2 kD, the ratio of the weight average molecular weight of said hydrophilic portion to the weight average molecular weight of said hydrophobic portion is between 1:3-1:7,

and if the weight average molecular weight of said hydrophilic portion is about 4-6 kD, e.g., about 5 kD, the ratio of the weight average molecular weight of said hydrophilic portion to the weight average molecular weight of said hydrophobic portion is between 1:1-1:4; and

iii) said plurality of hydrophilic-hydrophobic polymers is about 5-30 weight % of said particle; and

c) a surfactant, wherein said surfactant is about 15-35 weight % of said particle; and

wherein:

the diameter of said particle is less than about 200nm

80. The particle of claim 79, wherein a)iii) said hydrophobic polymer attached to said docetaxel has a weight average molecular weight of about 4-8 kD.

81. The particle of claim 77, comprising:

a) a plurality of hydrophobic polymer-docetaxel conjugates, wherein

i) each hydrophobic polymer-docetaxel conjugate of said plurality comprises a

hydrophobic polymer attached to docetaxel,

ii) said hydrophobic polymer attached to said docetaxel can be a homopolymer or a polymer made up of more than one kind of monomeric subunit,

iii) said hydrophobic polymer attached to said docetaxel has a weight average molecular weight of about 4-15 kD,

iv) said docetaxel is about 1-30 weight % of said particle and

v) said plurality of hydrophobic polymer-docetaxel conjugates is about 35-80 weight % of said particle;

b) a plurality of hydrophilic-hydrophobic polymers, wherein

i) each of said hydrophilic-hydrophobic polymers of said plurality comprises a hydrophilic portion attached to a hydrophobic portion, and

ii) said hydrophilic portion has a weight average molecular weight of about 2-6 kD and said hydrophobic portion has a weight average molecular weight of between about 8-13 kD,

iii) said plurality of hydrophilic-hydrophobic polymers is about 10-25 weight % of said particle;

iv) said hydrophilic portion of said hydrophilic-hydrophobic polymer terminates in an OMe,

and

c) a surfactant, wherein said surfactant is about 15-35 weight % of said particle;

wherein:

said particle further comprises a hydrophobic polymer having a terminal acyl moiety;

and

the diameter of said particle is less than about 200nm.

82. The particle of claim 81, wherein a)iii) said hydrophobic polymer attached to said docetaxel has a weight average molecular weight of about 4-8 kD.

83. A method of making the particle of claim 77, comprising:

providing an organic solution comprising:

a) a plurality of hydrophobic polymer-docetaxel conjugates, wherein

i) each hydrophobic polymer-docetaxel conjugate of said plurality comprises a hydrophobic polymer attached to docetaxel,

ii) said hydrophobic polymer attached to said docetaxel can be a homopolymer or a polymer made up of more than one kind of monomeric subunit,

iii) said hydrophobic polymer attached to said docetaxel has a weight average molecular weight of about 4-15 kD,

iv) said docetaxel is about 1-30 weight % of said particle and

v) said plurality of hydrophobic polymer-docetaxel conjugates is about 25-80 weight % of said particle;

b) a plurality of hydrophilic-hydrophobic polymers, wherein

i) each of said hydrophilic-hydrophobic polymers of said plurality comprises a hydrophilic portion attached to a hydrophobic portion,

ii) said hydrophilic portion has a weight average molecular weight of about 1-6 kD (e.g., 2-6 kD), and

iii) said plurality of hydrophilic-hydrophobic polymers is about 5-30 weight % of said particle; and

combining said organic solution with an aqueous solution comprising a surfactant, to provide said particles.

84. The particle of claim 83, wherein a)iii) said hydrophobic polymer attached to said docetaxel has a weight average molecular weight of about 4-8 kD.

85. A pharmaceutically acceptable composition comprising a plurality of particles of claim 77 and an additional component.

86. A kit comprising a plurality of particles of claim 77.

87. A single dosage unit comprising a plurality of particles of claim 77.

88. A method of treating a subject having a disorder comprising administering to said subject an effective amount of particles of claim 77.

89. The particle of claim 2 comprising:

a) a plurality of hydrophobic polymer-agent conjugates, wherein

i) each hydrophobic polymer-docetaxel conjugate of said plurality comprises a hydrophobic polymer attached to docetaxel

ii) said hydrophobic polymer attached to said docetaxel can be a homopolymer or a polymer made up of more than one kind of monomeric subunit,

iii) said hydrophobic polymer attached to said docetaxel has a weight average molecular weight of about 4-15 kD,

iv) said docetaxel is about 1-30 weight % of said particle and

v) said plurality of hydrophobic polymer-docetaxel conjugates is about 25-80 weight % of said particle;

b) a plurality of PEG-hydrophobic polymers, wherein

i) each of said PEG-hydrophobic polymers of said plurality comprises a PEG portion attached to a hydrophobic portion,

ii) said PEG portion has a weight average molecular weight of about 1-6 kD (e.g., 2-6 kD), and

iii) said plurality of PEG-hydrophobic polymers is about 5-30 weight % of said particle;

and

c) PVA, wherein

said PVA has a weight average molecular weight of about 5-45 kD and is about 15-35 weight of said particle; and

wherein:

the diameter of said particle is less than about 200nm.

90. The particle of claim 89, wherein a)iii) said hydrophobic polymer attached to said docetaxel has a weight average molecular weight of about 4-8 kD.

91. The particle of claim 89, comprising:

a) a plurality of hydrophobic polymer-docetaxel conjugates, wherein

i) each hydrophobic polymer-docetaxel conjugate of said plurality comprises a hydrophobic polymer attached to docetaxel,

ii) the hydrophobic polymer is made up of a first and a second type of monomeric subunit, and the ratio of the first to second type of

monomeric subunit in said hydrophobic polymer attached to said agent is from about 25:75 to about 75:25,

iii) said hydrophobic polymer attached to said docetaxel has a weight average molecular weight of about 4-15 kD,

iv) said docetaxel is about 1-30 weight % of said particle and

v) said plurality of hydrophobic polymer-docetaxel conjugates is about 25-80 weight % of said particle;

b) a plurality of PEG-hydrophobic polymers, wherein

i) each of said PEG-hydrophobic polymers of said plurality comprises a PEG portion attached to a hydrophobic portion,

ii) said PEG portion has a weight average molecular weight of about 1-6 kD (e.g., 2-6 kD), wherein

if the weight average molecular weight of said PEG portion is about 1-3 kD, e.g., about 2 kD, the ratio of the weight average molecular weight of said PEG portion to the weight average molecular weight of said hydrophobic portion is between 1:3-1:7, and if the weight average molecular weight of said PEG portion is about 4-6 kD, e.g., about 5 kD, the ratio of the weight average molecular weight of said PEG portion to the weight average molecular weight of said hydrophobic portion is between 1:1-1:4; and

iii) said plurality of PEG-hydrophobic polymers is about 5-30 weight % of said particle;

and

c) PVA, wherein

said PVA has a weight average molecular weight of about 5-45 kD and is about 15-35 weight % of said particle; and

wherein:

the diameter of said particle is less than about 200nm

92. The particle of claim 91, wherein a)iii) said hydrophobic polymer attached to said docetaxel has a weight average molecular weight of about 4-8 kD.

93. The particle of claim 89, comprising:

a) a plurality of hydrophobic polymer-docetaxel conjugates, wherein

i) each hydrophobic polymer-docetaxel conjugate of said plurality comprises a hydrophobic polymer attached to docetaxel,

ii) the hydrophobic polymer is made up of a first and a second type of monomeric subunit, and the ratio of the first to second type of monomeric subunit in said hydrophobic polymer attached to said agent is from about 25:75 to about 75:25,

iii) said hydrophobic polymer attached to said docetaxel has a weight average molecular weight of about 4-15 kD,

iv) said docetaxel is about 1-30 weight % of said particle and

v) said plurality of hydrophobic polymer-docetaxel conjugates is about 35-80 weight % of said particle;

b) a plurality of PEG-hydrophobic polymers, wherein

i) each of said PEG-hydrophobic polymers of said plurality comprises a PEG portion attached to a hydrophobic portion, and

ii) said PEG portion has a weight average molecular weight of about 2-6 kD and said hydrophobic portion has a weight average molecular weight of between about 8-13 kD,

iii) said plurality of PEG-hydrophobic polymers is about 10-25 weight % of said particle;

iv) said PEG portion of said PEG-hydrophobic polymer terminates in an OMe,

and

c) PVA, wherein said PVA has a weight average molecular weight of about 23-26 kD and is about 15-35 weight % of said particle;

wherein:

said particle further comprises a hydrophobic polymer having a terminal acyl moiety;

and

the diameter of said particle is less than about 200nm.

94. The particle of claim 93, wherein a)iii) said hydrophobic polymer attached to said docetaxel has a weight average molecular weight of about 4-8 kD.

95. A method of making the particle of claim 89, comprising:

providing an organic solution comprising:

a) a plurality of hydrophobic polymer-docetaxel conjugates, wherein

i) each hydrophobic polymer-docetaxel conjugate of said plurality comprises a hydrophobic polymer attached to docetaxel,

ii) the hydrophobic polymer is made up of a first and a second type of monomeric subunit, and the ratio of the first to second type of monomeric subunit in said hydrophobic polymer attached to said agent is from about 25:75 to about 75:25,

iii) said hydrophobic polymer attached to said docetaxel has a weight average molecular weight of about 4-15 kD,

iv) said docetaxel is about 1-30 weight % of said particle and

v) said plurality of hydrophobic polymer-docetaxel conjugates is about 25-80 weight % of said particle;

b) a plurality of PEG-hydrophobic polymers, wherein

i) each of said PEG-hydrophobic polymers of said plurality comprises a PEG portion attached to a hydrophobic portion,

ii) said PEG portion has a weight average molecular weight of about 1-6 kD (e.g., 2-6 kD), and

iii) said plurality of PEG-hydrophobic polymers is about 5-30 weight % of said particle; and

combining the organic solution with an aqueous solution comprising PVA to provide said particles.

96. The particle of claim 95, wherein iii) said hydrophobic polymer attached to said docetaxel has a weight average molecular weight of about 4-8 kD.

97. A pharmaceutically acceptable composition comprising a plurality of particles of claim 89 and an additional component.

98. A kit comprising a plurality of particles of claim 89.

99. A method of treating a subject having a disorder comprising administering to said subject an effective amount of the particles of claim 89.

100. The pharmaceutically acceptable composition of any of claims 85 or 97, wherein said additional component is a lyoprotectant.

101. The pharmaceutically acceptable composition of any of claims 85 or 97, wherein said additional component is a carbohydrate.

102. The pharmaceutically acceptable composition of any of claims 85 or 97, wherein said additional component is a cyclodextrin.

103. The pharmaceutically acceptable composition of any of claims 85 or 97, wherein said additional component is a 2-hydroxypropyl-beta-cyclodextrin.

104. The kit of any of claims 86 or 98, further comprising a liquid resistant container in which said plurality of particles is disposed.

105. The kit of any of claims 86 or 98, further comprising a diluent.

106. The method of any of claims 85 or 99, wherein said disorder is a disorder characterized by an unwanted proliferation of cells.

107. The method of any of claims 85 or 99, wherein said disorder is an inflammatory disorder.

108. The method of any of claims 85 or 99, wherein said disorder is a cardiovascular disorder.

109. The method of any of claims 85 or 99, wherein the disorder is cancer.

110. The method of any of claims 109, wherein the cancer is breast cancer.

111. The method of claim 110, wherein the breast cancer is locally advanced breast cancer.

112. The method of claim 110, wherein the breast cancer is metastatic.

113. The method of claim 109, wherein the cancer is non small cell lung cancer.

114. The method of claim 113, wherein the cancer is refractory, relapsed or resistant to a platinum-based agent and is unresectable, locally advanced or metastatic.

115. The method of claim 109, wherein the cancer is prostate cancer.

116. The method of claim 115, wherein the cancer is hormone refractory.

117. The method of claim 115, wherein the cancer is metastatic.

118. The method of any of claims 109, wherein the cancer is an unresectable cancer.

119. The method of any of claims 109, wherein the cancer is a chemotherapeutic sensitive cancer.

120. The method of any of claims 109, wherein the cancer is a chemotherapeutic refractory cancer.

121. The method of any of claims 109, wherein the cancer is a chemotherapeutic resistant cancer.

122. The method of any of claims 109, wherein the cancer is a relapsed cancer.

123. The method of any of claims 109, wherein said plurality of particles is administered as adjunctive therapy with another therapy, e.g., radiation or surgery.

124. The particle of claim 2 comprising:

a) a plurality of PLGA-docetaxel conjugates, wherein

i) each PLGA-docetaxel conjugate of said plurality comprises a PLGA polymer attached to docetaxel,

ii) the ratio of lactic acid to glycolic acid in said PLGA polymer attached to said docetaxel is from about 25:75 to about 75:25,

iii) said PLGA polymer attached to said docetaxel has a weight average molecular weight of about 4-15 kD,

iv) said docetaxel is about 1-30 weight % of said particle and

v) said plurality of PLGA-docetaxel conjugates is about 25-80 weight % of said particle;

b) a plurality of PEG-PLGA polymers, wherein

i) each of said PEG-PLGA polymers of said plurality comprises a PEG portion attached to a PLGA portion,

ii) said PEG portion has a weight average molecular weight of about 1-6 kD (e.g., 2-6 kD), and

iii) said plurality of PEG-PLGA polymers is about 5-30 weight % of said particle;

and

c) PVA, wherein said PVA has a weight average molecular weight of about 5-45 kD and is about 15-35 weight % of said particle; and

wherein:

the diameter of said particle is less than about 200nm.

125. The particle of claim 124, wherein a)iii) said PLGA polymer attached to said docetaxel has a weight average molecular weight of about 4-8 kD.

126. The particle of claim 124, comprising:

a) a plurality of PLGA-docetaxel conjugates, wherein

i) each PLGA-docetaxel conjugate of said plurality comprises a PLGA polymer attached to docetaxel,

ii) the ratio of lactic acid to glycolic acid in said PLGA polymer attached to said docetaxel is from about 25:75 to about 75:25,

iii) said PLGA polymer attached to said docetaxel has a weight average molecular weight of about 4-15 kD,

iv) said docetaxel is about 1-30 weight % of said particle and

v) said plurality of PLGA-docetaxel conjugates is about 25-80 weight % of said particle;

b) a plurality of PEG-PLGA polymers, wherein

i) each of said PEG-PLGA polymers of said plurality comprises a PEG portion attached to a PLGA portion,

ii) said PEG portion has a weight average molecular weight of about 1-6 kD (e.g., 2-6 kD), wherein

if the weight average molecular weight of said PEG portion is about 1-3 kD, e.g., about 2 kD, the ratio of the weight average molecular weight of said PEG portion to the weight average molecular weight of said PLGA

portion is between 1:3-1:7, and if the weight average molecular weight of said PEG portion is about 4-6 kD, e.g., about 5 kD, the ratio of the weight average molecular weight of said PEG portion to the weight average molecular weight of said PLGA portion is between 1:1-1:4; and

iii) said plurality of PEG-PLGA polymers is about 5-30 weight % of said particle; and

c) PVA, wherein

said PVA has a weight average molecular weight of about 5-45 kD and is about 15-35 weight % of said particle; and

wherein:

the diameter of said particle is less than about 200nm

127. The particle of claim 126, wherein a)iii) said PLGA polymer attached to said docetaxel has a weight average molecular weight of about 4-8 kD.

128. The particle of claim 124, comprising:

a) a plurality of PLGA-docetaxel conjugates, wherein

i) each PLGA-docetaxel conjugate of said plurality comprises a PLGA polymer attached to docetaxel,

ii) the ratio of lactic acid to glycolic acid in said PLGA polymer attached to said docetaxel is from about 25:75 to about 75:25,

iii) said PLGA polymer attached to said docetaxel has a weight average molecular weight of about 4-15 kD,

iv) said docetaxel is about 1-30 weight % of said particle and

v) said plurality of PLGA-docetaxel conjugates is about 35-80 weight % of said particle;

b) a plurality of PEG-PLGA polymers, wherein

i) each of said PEG-PLGA polymers of said plurality comprises a PEG portion attached to a PLGA portion, and

ii) said PEG portion has a weight average molecular weight of about 2-6 kD and said PLGA portion has a weight average molecular weight of between about 8-13 kD,

iii) said plurality of PEG-PLGA polymers is about 10-25 weight % of said particle;

iv) said PEG portion of said PEG-PLGA polymer terminates in an OMe,

and

c) PVA, wherein said PVA has a weight average molecular weight of about 23-26 kD and is about 15-35 weight % of said particle;

wherein:

said particle further comprises PLGA having a terminal acyl moiety; and

the diameter of said particle is less than about 200nm.

129. The particle of claim 128, wherein a)iii) said PLGA polymer attached to said docetaxel has a weight average molecular weight of about 4-8 kD.

130. A method of making the particle of claim 124, comprising:

providing an organic solution comprising:

a) a plurality of PLGA-docetaxel conjugates, wherein

i) each PLGA-docetaxel conjugate of said plurality comprises a PLGA polymer attached to docetaxel,

ii) the ratio of lactic acid to glycolic acid in said PLGA polymer attached to said docetaxel is from about 25:75 to about 75:25,

iii) said PLGA polymer attached to said docetaxel has a weight average molecular weight of about 4-15 kD,

iv) said docetaxel is about 1-30 weight % of said particle and

v) said plurality of PLGA-docetaxel conjugates is about 25-80 weight % of said particle;

b) a plurality of PEG-PLGA polymers, wherein

i) each of said PEG-PLGA polymers of said plurality comprises a PEG portion attached to a PLGA portion,

ii) said PEG portion has a weight average molecular weight of about 1-6 kD (e.g., 2-6 kD), and

iii) said plurality of PEG-PLGA polymers is about 5-30 weight % of said particle; and

combining said organic solution with an aqueous solution comprising PVA to provide said particles.

131. The particle of claim 130, wherein a)iii) said PLGA polymer attached to said docetaxel has a weight average molecular weight of about 4-8 kD.

132. A pharmaceutically acceptable composition comprising a plurality of particles of claim 124 and an additional component.

133. The pharmaceutically acceptable composition of claim 124, wherein said additional component is a lyoprotectant.

134. The pharmaceutically acceptable composition of claim 124, wherein said additional component is a carbohydrate.

135. The pharmaceutically acceptable composition of claim 124, wherein said additional component is a cyclodextrin.

136. The pharmaceutically acceptable composition of claim 124, wherein said additional component is a 2-hydroxypropyl-beta-cyclodextrin.

137. A kit comprising a plurality of particles of claim 124.

138. The kit of claim 137, further comprising a liquid resistant container in which said plurality of particles is disposed.

139. The kit of claim 137, further comprising a diluent.

140. A single dosage unit comprising a plurality of particles of claim 124.

141. A method of treating a subject having a disorder comprising administering to said subject an effective amount of particles of claim 124.

142. The method of claim 141, wherein said disorder is a disorder characterized by an unwanted proliferation of cells.

143. The method of claim 141, wherein said disorder is an inflammatory disorder.

144. The method of claim 141, wherein said disorder is a cardiovascular disorder.

145. The method of claim 141, wherein the disorder is cancer.

146. The method of claims 145, wherein the cancer is breast cancer.

147. The method of claim 146, wherein the breast cancer is locally advanced breast cancer.

148. The method of claim 146, wherein the breast cancer is metastatic.

149. The method of claim 145, wherein the cancer is non small cell lung cancer.

150. The method of claim 149, wherein the cancer is refractory, relapsed or resistant to a platinum-based agent and is unresectable, locally advanced or metastatic.

151. The method of claim 145, wherein the cancer is prostate cancer.

152. The method of claim 151, wherein the cancer is hormone refractory.

153. The method of claim 151, wherein the cancer is metastatic.

154. The method of claim 145, wherein the cancer is an unresectable cancer.

155. The method of claim 145, wherein the cancer is a chemotherapeutic sensitive cancer.

156. The method of claim 145, wherein the cancer is a chemotherapeutic refractory cancer.

157. The method of claim 145, wherein the cancer is a chemotherapeutic resistant cancer.

158. The method of claim 145, wherein the cancer is a relapsed cancer

159. The method of claim 145, wherein the plurality of particles of claim 124 is administered as adjunctive therapy with another therapy, e.g., radiation or surgery.

160. The method of treating a subject of claim 13, wherein the particle is the particle of claim 4, the disorder is cancer, and the agent is a taxane.

161. The method of treating a subject of claim 160, wherein the agent is docetaxel.

162. The method of treating a subject of claim 161, wherein the disorder is breast cancer.

163. The method of claim 162, wherein the breast cancer is locally advanced breast cancer.

164. The method of claim 162, wherein the breast cancer is metastatic.

165. The method of claim 161, wherein the disorder is non small cell lung cancer.

166. The method of claim 165, wherein the cancer is refractory, relapsed or resistant to a platinum-based agent and is unresectable, locally advanced or metastatic.

167. The method of claim 161, wherein the cancer is prostate cancer.

168. The method of claim 167, wherein the cancer is hormone refractory.

169. The method of claim 167, wherein the cancer is metastatic.

170. The method of treating a subject of claim 25, wherein the particle is the particle of claim 16, the disorder is cancer, and the agent is a taxane.

171. The method of treating a subject of claim 170, wherein the agent is docetaxel.

172. The method of treating a subject of claim 171, wherein the disorder is breast cancer.

173. The method of claim 172, wherein the breast cancer is locally advanced breast cancer.

174. The method of claim 172, wherein the breast cancer is metastatic.

175. The method of claim 171, wherein the disorder is non small cell lung cancer.

176. The method of claim 175, wherein the cancer is refractory, relapsed or resistant to a platinum-based agent and is unresectable, locally advanced or metastatic.

177. The method of claim 171, wherein the cancer is prostate cancer.

178. The method of claim 177, wherein the cancer is hormone refractory.

179. The method of claim 177, wherein the cancer is metastatic.

180. The method of treating a subject of claim 37, wherein the particle is the particle of claim 28, the disorder is cancer, and the agent is a taxane.

181. The method of treating a subject of claim 180, wherein the agent is docetaxel.

182. The method of treating a subject of claim 181, wherein the disorder is breast cancer.

183. The method of claim 182, wherein the breast cancer is locally advanced breast cancer.

184. The method of claim 182, wherein the breast cancer is metastatic.

185. The method of claim 181 wherein the disorder is non small cell lung cancer.

186. The method of claim 185, wherein the cancer is refractory, relapsed or resistant to platinum-based agent and is unresectable, locally advanced or metastatic.

187. The method of claim 181, wherein the cancer is prostate cancer.

188. The method of claim 187, wherein the cancer is hormone refractory.

189. The method of claim 187, wherein the cancer is metastatic.

190. The method of treating a subject of claim 141, wherein the particle is the particle of claim 130 and the disorder is cancer.

191. The method of treating a subject of claim 190, wherein the disorder is breast cancer.

192. The method of claim 191, wherein the breast cancer is locally advanced breast cancer.

193. The method of claim 191, wherein the breast cancer is metastatic.

194. The method of claim 190, wherein the disorder is non small cell lung cancer.

195. The method of claim 194, wherein the cancer is refractory, relapsed or resistant to a platinum-based agent and is unresectable, locally advanced or metastatic.

196. The method of claim 190, wherein the cancer is prostate cancer.

197. The method of claim 196, wherein the cancer is hormone refractory.

198. The method of claim 196, wherein the cancer is metastatic.

199. The particle of claim 2, wherein a)iii) said hydrophobic polymer attached to said agent has a weight average molecular weight of about 9-12 kD.

200. The particle of claim 2, wherein a)iii) said hydrophobic polymer attached to said agent has a weight average molecular weight of about 8-13 kD.

201. The particle of claim 4, wherein a)iii) said hydrophobic polymer attached to said agent has a weight average molecular weight of about 9-12 kD.

202. The particle of claim 4, wherein a)iii) said hydrophobic polymer attached to said agent has a weight average molecular weight of about 8-13 kD.

203. The particle of claim 6, wherein a)iii) said hydrophobic polymer attached to said agent has a weight average molecular weight of about 9-12 kD.

204. The particle of claim 6, wherein a)iii) said hydrophobic polymer attached to said agent has a weight average molecular weight of about 8-13 kD.

205. The particle of claim 8, wherein a)iii) said hydrophobic polymer attached to said agent has a weight average molecular weight of about 9-12 kD.

206. The particle of claim 8, wherein a)iii) said hydrophobic polymer attached to said agent has a weight average molecular weight of about 8-13 kD.

207. The particle of claim 14, wherein a)iii) said hydrophobic polymer attached to said agent has a weight average molecular weight of about 9-12 kD.

208. The particle of claim 14, wherein a)iii) said hydrophobic polymer attached to said agent has a weight average molecular weight of about 8-13 kD.

209. The particle of claim 16, wherein a)iii) said hydrophobic polymer attached to said agent has a weight average molecular weight of about 9-12 kD.

210. The particle of claim 16, wherein a)iii) said hydrophobic polymer attached to said agent has a weight average molecular weight of about 8-13 kD.

211. The particle of claim 18, wherein a)iii) said hydrophobic polymer attached to said agent has a weight average molecular weight of about 9-12 kD.

212. The particle of claim 18, wherein a)iii) said hydrophobic polymer attached to said agent has a weight average molecular weight of about 8-13 kD.

213. The particle of claim 20, wherein a)iii) said hydrophobic polymer attached to said agent has a weight average molecular weight of about 9-12 kD.

214. The particle of claim 20, wherein a)iii) said hydrophobic polymer attached to said agent has a weight average molecular weight of about 8-13 kD.

215. The particle of claim 77, wherein a)iii) said hydrophobic polymer attached to said docetaxel has a weight average molecular weight of about 9-12 kD.

216. The particle of claim 77, wherein a)iii) said hydrophobic polymer attached to said docetaxel has a weight average molecular weight of about 8-13 kD.

217. The particle of claim 79, wherein a)iii) said hydrophobic polymer attached to said docetaxel has a weight average molecular weight of about 9-12 kD.

218. The particle of claim 79, wherein a)iii) said hydrophobic polymer attached to said docetaxel has a weight average molecular weight of about 8-13 kD.

219. The particle of claim 81, wherein a)iii) said hydrophobic polymer attached to said docetaxel has a weight average molecular weight of about 9-12 kD.

220. The particle of claim 81, wherein a)iii) said hydrophobic polymer attached to said docetaxel has a weight average molecular weight of about 8-13 kD.

221. The particle of claim 83, wherein a)iii) said hydrophobic polymer attached to said docetaxel has a weight average molecular weight of about 9-12 kD.

222. The particle of claim 83, wherein a)iii) said hydrophobic polymer attached to said docetaxel has a weight average molecular weight of about 8-13 kD.

223. The particle of claim 89, wherein a)iii) said hydrophobic polymer attached to said docetaxel has a weight average molecular weight of about 9-12 kD.

224. The particle of claim 89, wherein a)iii) said hydrophobic polymer attached to said docetaxel has a weight average molecular weight of about 8-13 kD.

225. The particle of claim 91, wherein a)iii) said hydrophobic polymer attached to said docetaxel has a weight average molecular weight of about 9-12 kD.

226. The particle of claim 91, wherein a)iii) said hydrophobic polymer attached to said docetaxel has a weight average molecular weight of about 8-13 kD.

227. The particle of claim 93, wherein a)iii) said hydrophobic polymer attached to said docetaxel has a weight average molecular weight of about 9-12 kD.

228. The particle of claim 93, wherein a)iii) said hydrophobic polymer attached to said docetaxel has a weight average molecular weight of about 8-13 kD.

229. The pharmaceutically acceptable composition of any of claims 10, 22 or 34, wherein said additional component is a polysaccharide.

230. A polymer-agent conjugate comprising:

a hydrophobic polymer, wherein said hydrophobic polymer includes a terminal protecting group; and

an agent attached to said polymer.

231. The polymer-agent conjugate of claim 230, wherein said hydrophobic polymer is PLGA.

232. The polymer-agent conjugate of claim 231, wherein said PLGA polymer has a weight average molecular weight of about 4-8 kD.

233. The polymer-agent conjugate of claim 231, wherein said PLGA polymer has a weight average molecular weight of about 8-13 kD.

234. The polymer-agent conjugate of claim 231, wherein said PLGA polymer has a weight average molecular weight of about 9-12 kD.

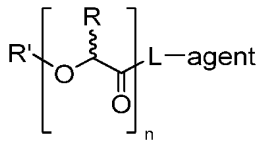
235. The polymer-agent conjugate of claim 230, wherein said hydrophobic polymer is PLA.

236. The polymer-agent conjugate of claim 230, wherein said hydrophobic polymer is PGA.

237. The polymer-agent conjugate of claim 230, wherein said terminal protecting group is an acyl group.

238. The polymer-agent conjugate of claim 230, wherein said terminal protecting group is an acetyl group.

239. The polymer-agent conjugate of claim 230, having the formula:



wherein:

L is selected from a bond or linker;

R is selected from hydrogen and methyl, wherein about 45% to about 55% of R substituents are hydrogen and about 45% to about 55% are methyl;

R' is selected from acyl and a hydroxy protecting group; and

n is an integer from about 15 to about 308.

240. The polymer-agent conjugate of claim 239, wherein R' is acyl.

241. The polymer-agent conjugate of claim 239, wherein R' is acetyl.

242. The polymer-agent conjugate of claim 239, wherein about 50% of R substituents are hydrogen and about 50% are methyl.

243. The polymer-agent conjugate of claim 10, wherein n is from about 77 to about 123.

244. The polymer-agent conjugate of claim 239, wherein n is from about 123 to about 200.

245. The polymer-agent conjugate of any of claims 230-244, wherein said agent is a therapeutic agent.

246. The polymer-agent conjugate of claim 245, wherein said therapeutic agent is an anti-cancer agent.

247. The polymer-agent conjugate of claim 246, wherein said anti-cancer agent is an alkylating agent, a vascular disrupting agent, a taxane, an anthracycline, a vinca alkaloid, a platinum-based agent, a topoisomerase inhibitor, an anti-angiogenic agent or an anti-metabolite.

248. The polymer-agent conjugate of claim 246, wherein said anti-cancer agent is a taxane.

249. The polymer-agent conjugate of claim 248, wherein said taxane is docetaxel.

250. The polymer-agent conjugate of claim 248, wherein said taxane is paclitaxel.

251. The polymer-agent conjugate of claim 248, wherein said taxane is larotaxel.

252. The polymer-agent conjugate of claim 248, wherein said taxane is cabazitaxel.

253. The polymer-agent conjugate of claim 248, wherein said anti-cancer agent is an anthracycline.

254. The polymer-agent conjugate of claim 248, wherein said anthracycline is doxorubicin.

Polymer[1]-ABX-Agent

Example	A	B	X	Drug	Hydroxy Protecting Groups	Process for Preparation	Final Product
1.	-	-	3-NH2	doxorubicin	None	1	doxorubicin attached to the polymer
2.	-	-NH(CH_2)$_5$CO-	3-NH2	doxorubicin	None	2	doxorubicin attached to the polymer
3.	-	-NH(CH_2)$_3$OCH_2CO-	3-NH2	doxorubicin	None	2	doxorubicin attached to the polymer
4.	-	-NHCH_2CH_2COOCH_2CO-	3-NH2	doxorubicin	None	2	doxorubicin attached to the polymer
5.	-	-NHCH_2CH_2SCH_2CH_2QCO- Q is O or NH	3-NH2	doxorubicin	None	2	doxorubicin attached to the polymer
6.	-NH(CH_2)$_2$S-	-S(CH_2)$_2$NHCO-	3-NH2	doxorubicin	None	3	doxorubicin attached to the polymer
7.	-NH(CH_2)$_2$S-	-S(CH_2)$_2$OCO-	3-NH2	doxorubicin	None	3	doxorubicin attached to the polymer
8.	-	-NH(CH_2)$_n$CO- n is 1, 2, or 3	3-NH2	doxorubicin	None	2	doxorubicin attached to the polymer
9.	-	-NHZCO- *	3-NH2	doxorubicin	None	2	doxorubicin attached to the polymer
10.	-	-	2'-OH	paclitaxel	-	4	2'-paclitaxel attached to polymer
11.	-	-	2'-OAcetyl, 7-OH	paclitaxel	Acetyl	5	2'-acetyl-7-paclitaxel attached to polymer
12.	-	-NH(CH_2)$_5$CO-	2'-OH	paclitaxel	None	6	2'- paclitaxel attached to polymer
13.	-	-NH(CH_2)$_3$OCH_2CO-	2'-OH	paclitaxel	None	6	2'- paclitaxel attached to polymer
14.	-	-NHCH_2CH_2COOCH_2CO-	2'-OH	paclitaxel	None	6	2' -paclitaxel attached to the polymer
15.	-	-NHCH_2CH_2SCH_2CH_2QCO- Q is O or NH	2'-OH	paclitaxel	None	6	2'- paclitaxel attached to the polymer
16.	-NH(CH_2)$_2$S-	-S(CH_2)$_2$NHCO-	2'-OH	paclitaxel	None	7	2'- paclitaxel attached to the polymer
17.	-NH(CH_2)$_2$S-	-S(CH_2)$_2$OCO-	2'-OH	paclitaxel	None	7	2'- paclitaxel attached to the polymer
18.	-	-NH(CH_2)$_n$CO- n is 1, 2, or 3	2'-OH	paclitaxel	None	6	2'- paclitaxel attached to the polymer
19.	-	-NHZCO- *	2'-OH	paclitaxel	None	6	2'- paclitaxel attached to the polymer
20.	-	-	2'-OH	docetaxel	-	8	2'-docetaxel attached to the polymer
21.	-	-	2'-OAcetyl, 7-OH	docetaxel	Acetyl	9	2'-acetyl-7-docetaxel attached to the polymer

* Z is a mono, di, or tripeptide or other peptide or derivative thereof where NH and CO represent the amino and acid terminus of the amino acid or peptide

[1]Polymer = AcO-PLGA-C(O)-

FIGURE 1

Example	A	B	X	Drug	Hydroxy Protecting Groups	Process for Preparation	Final Product
22.	-	$-NH(CH_2)_5CO-$	2'-OH	docetaxel	None	10	2'-docetaxel attached to the polymer
23.	-	$-NH(CH_2)_3OCH_2CO-$	2'-OH	docetacel	None	10	2'-docetaxel attached to the polymer
24.	-	$-NHCH_2CH_2COOCH_2CO-$	2'-OH	docetaxel	None	10	2'-docetaxel attached to the polymer
25.	-	$-NHCH_2CH_2SSCH_2CH_2QCO-$ Q is O or NH	2'-OH	docetaxel	None	10	2'-docetaxel attached to the polymer
26.	$-NH(CH_2)_2S-$	$-S(CH_2)_2NHCO-$	2'-OH	docetaxel	None	11	2'-docetaxel attached to the polymer
27.	$-NH(CH_2)_2S-$	$-S(CH_2)_2OCO-$	2'-OH	docetaxel	None	11	2'-docetaxel attached to the polymer
28.	-	$-NH(CH_2)_nCO-$ n is 1, 2, or 3	2'-OH	docetaxel	None	10	2'-docetaxel attached to the polymer
29.	-	-NHZCO- *	2'-OH	docetaxel	None	10	2'-docetaxel attached to the polymer

* Z is a mono, di, or tripeptide or other peptide or derivative thereof where NH and CO represent the amino and acid terminus of the amino acid or peptide

FIGURE 1 (continued)

Polymer[1]-ABX-Agent

Example	A	B	X	Drug	Hydroxy Protecting Groups	Process for Preparation	Final Product
1.	$-CO(CH_2)_2CO-$	-	2'-OH	paclitaxel	-	12	2'-paclitaxel attached to Polymer
2.	$-CO(CH_2)_2CO-$	-	2'-Ac, 7'-OH	paclitaxel	Acetyl or hexanoyl	13	2'-acetyl-7-paclitaxel attached to Polymer
3.	$-CO(CH_2)_2CO-$		7'-OH	paclitaxel	TBDMS (t-butyldimethylsilyl) or TROC	14	7'-paclitaxel attached to Polymer
4.	$-CO(CH_2)_2CO-$	$-NH(CH_2)_5CO-$	2'-OH	paclitaxel	-	15	2'-paclitaxel attached to Polymer
5.	$-CO(CH_2)_2CO-$	$-NH(CH_2)_5CO-$	2'-OH	paclitaxel	Acetyl or hexanoyl	16	2'-acetyl-7-paclitaxel attached to Polymer
6.	$-CO(CH_2)_2CO-$	$-NH(CH_2)_5CO-$	7'-OH	paclitaxel	TBDMS or TROC	17	7'-paclitaxel attached to Polymer
7.	-CO-	-	2'-OH	paclitaxel	-	12	2'-paclitaxel attached to Polymer
8.	-CO-	-	2'-Ac, 7'-OH	paclitaxel	Acetyl or hexanoyl	13	2'-acetyl-7-paclitaxel attached to Polymer
9.	-CO-		7'-OH	paclitaxel	TBDMS or TROC	14	7'-paclitaxel attached to Polymer
10.	-CO-	$-NH(CH_2)_5CO-$	2'-OH	paclitaxel	-	15	2'-paclitaxel attached to Polymer
11.	-CO-	$-NH(CH_2)_5CO-$	2'-OH	paclitaxel	Acetyl or hexanoyl	16	2'-acetyl-7-paclitaxel attached to Polymer
12.	-CO-	$-NH(CH_2)_5CO-$	7'-OH	paclitaxel	TBDMS or TROC	17	7'-paclitaxel attached to Polymer

[1]Polymer = ROOC-PLGA-O-, wherein R is H or alkyl, or HO-PLGA-Y-PLGA-O-, wherein Y is a diol

FIGURE 2

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 10/28770

A. CLASSIFICATION OF SUBJECT MATTER
IPC(8) - A61K 9/00 (2010.01)
USPC - 424/400
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)
USPC - 424/400

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched
USPC - 424/94.3, 194.1, 195.11, 424, 471, 490, 497, 499, 800; 435/7.23; 514/449 (text search - see search terms below)

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
PubWEST (PGPB, USPT, EPAB, JPAB); Google Patents; Google
Search terms used: polymer, conjugate, nanoparticle, PLGA, PEG, PVA, docetaxel, doxorubicin, platinum, cancer, breast cancer, non-small cell lung cancer, prostate cancer, metastatic, refractory, kit, lyoprotectant, hydroxypropyl cyclodextrin

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X --- Y	WO 2006/014626 A2 (MAYER et al.) 9 February 2006 (09.02.2006) para [0024]-[0026], [0039], [0044]-[0047], [0066], [0070]-[0072], [0076], [0079], [0081], [0086], [0092], [0095], [0108], [0110], [0115]-[0116]	1, 38-52 --- 2-37, 53-159, 161-169, 171-179, 181-189, 191-254
Y	US 2002/0164290 A1 (STEFELY et al.) 7 November 2002 (07.11.2002) para [0012]-[0013], [0054], [0111]	6-7, 18-19, 30-31, 81-82, 93-94, 128-129, 203-204, 211-212, 219-220, 227-228, 230-254
Y	US 2008/0248126 A1 (CHENG et al.) 9 October 2008 (09.10.2008) para [0008], [0032], [0073], [0101], [0124]-[0125], [0134]; Table 1; Fig. 2	2-37, 53-159, 161-169, 171-179, 181-189, 191-229
Y	US 2007/0071790 A1 (AMEER et al.) 29 March 2007 (29.03.2007) para [0010], [0012], [0079], [0092]	2-37, 53-159, 161-169, 171-179, 181-189, 191-229
Y	US 7,163,698 B2 (OH et al.) 16 January 2007 (16.01.2007) col 3, ln 50-53; col 4, ln 4-7; col 11, ln 5-6; col 12, ln 47-67	14-37, 89-99, 124-159, 171-179, 181-189, 191-198, 207-214, 223-228, 239-254

☒ Further documents are listed in the continuation of Box C. ☐

* 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
15 May 2010 (15.05.2010)	15 JUL 2010
Name and mailing address of the ISA/US Mail Stop PCT, Attn: ISA/US, Commissioner for Patents P.O. Box 1450, Alexandria, Virginia 22313-1450 Facsimile No. 571-273-3201	Authorized officer: Lee W. Young PCT Helpdesk: 571-272-4300 PCT OSP: 571-272-7774

Form PCT/ISA/210 (second sheet) (July 2009)

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 10/28770

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	US 2008/0081074 A1 (GU et al.) 3 April 2008 (03.04.2008) para [0012], [0015], [0030], [0125], [0127]	12, 24, 36, 58, 87, 105, 139-140
Y	US 2008/0107631 A1 (WU et al.) 8 May 2008 (08.05.2008) para [0018]	55-56, 102-103, 135-136, 229
Y	US 2008/0063724 A1 (DESAI et al.) 13 March 2008 (13.03.2008), para [0029]-[0030], [0055], [0078], [0160], [0238]; Table 1	63-76, 110-123, 146-159, 162-169, 172-179, 182-189, 191-198

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 10/28770

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.:
because they relate to subject matter not required to be searched by this Authority, namely:

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.: 160, 170, 180 and 190
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying additional fees, this Authority did not invite payment of additional fees.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.

☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.

☐ No protest accompanied the payment of additional search fees.

(12) DEMANDE INTERNATIONALE PUBLIÉE EN VERTU DU TRAITÉ DE COOPÉRATION EN MATIÈRE DE BREVETS (PCT)

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Bureau international

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(10) Numéro de publication internationale
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A61K 31/7068 (2006.01)

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(81) États désignés *(sauf indication contraire, pour tout titre de protection nationale disponible)* : AE, AG, AL, AM, AO, AT, AU, AZ, BA, BB, BG, BH, BR, BW, BY, BZ, CA, CH, CL, CN, CO, CR, CU, CZ, DE, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IS, JP, KE, KG, KM, KN, KP, KR, KZ, LA, LC, LK, LR, LS, LT, LU, LY, MA, MD, ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PE, PG, PH, PL, PT, RO, RS, RU, SC, SD, SE, SG, SK, SL, SM, ST, SV, SY, TH, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW.

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Publiée :

— *avec rapport de recherche internationale (Art. 21(3))*

WO 2010/128258 A1

(54) **Title** : ANTITUMOR COMBINATION INCLUDING CABAZITAXEL AND CAPECITABINE

(54) **Titre** : COMBINAISON ANTITUMORALE COMPRENANT LE CABAZITAXEL ET LA CAPECITABINE

(57) **Abstract** : The invention relates to a pharmaceutical antitumor combination including cabazitaxel and capecitabine, wherein both of said antitumor agents may be in the form of a base, in the form of a pharmaceutically acceptable acid salt or in the form of a hydrate or solvate, intended for the treatment of metastatic breast cancer in patients progressing after a previous treatment with anthracyclines and taxanes.

(57) **Abrégé** : L'invention est relative à une combinaison pharmaceutique antitumorale comprenant le cabazitaxel et la capecitabine, ces deux agents antitumoraux pouvant être sous forme de base, sous forme d'un sel d'un acide pharmaceutiquement acceptable ou sous forme d'un hydrate ou d'un solvat, destinée à traiter le cancer du sein métastatique chez les patientes progressant après un traitement antérieur par anthracyclines et taxanes.

COMBINAISON ANTITUMORALE COMPRENANT LE CABAZITAXEL ET LA CAPECITABINE

La présente invention concerne une combinaison antitumorale associant le cabazitaxel et la capecitabine dans le traitement du cancer du sein métastatique chez les patientes progressant après un traitement antérieur par anthracyclines et taxanes.

[Art antérieur et problème technique]

Le cancer du sein touche une large partie de la population féminine mondiale : 1,15 million de cas dans le monde en 2002 ; il est prévu qu'il touche 1,4 million de cas en 2010 (*CA cancer J.Clin.* **2005**, *55*, 74-108). Il s'agit du cancer le plus fréquent chez la femme.

Le cancer du sein métastatique (ou MBC en Anglais) est généralement traité par une chimiothérapie à base d'anthracyclines et de taxanes (« Concise Review for clinicians : advances in screening, diagnosis and treatment of breast cancer » *Mayo clinic proceedings* **2004**, *76*, 810-816).

Le cancer peut être devenu résistant aux agents utilisés, en particulier aux taxanes, ce qui limite les options de traitement possible. Plusieurs mécanismes de résistance aux taxanes ont été décrits (expression de la P-glycoprotéine P-gp, gène mdr-1, métabolisme modifié du taxane, mutation du gène de la tubuline,...) : voir *Drug Resistance Updates* **2001**, *4*(1), 3-8 ; *J.Clin.Onc.* **1999**, *17*(3), 1061-1070.

Pour les patientes dont le cancer a progressé après un traitement antérieur à base d'anthracyclines et/ou de taxanes (75% des patients développent une résistance à ce traitement), la capecitabine en monothérapie ou la combinaison associant la capecitabine et le docetaxel est indiquée (*J.Clin.Onc.* **2002**, *20*(12), 2812-2823).

Il a également été observé que le cabazitaxel (ou **XRP6258**) pouvait être efficace dans le traitement du cancer du sein métastatique résistant aux taxanes (« A multicenter phase II study of **XRP6258** administered as a 1-h i.v. infusion every 3 weeks in taxane-resistant metastatic breast cancer patients » *Ann.Oncol.* **2008**, *19*(9), 1547-1552).

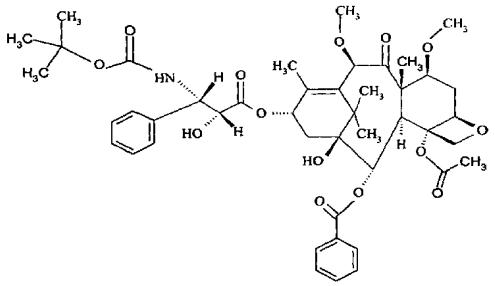
De plus, dans la conclusion de l'abstract intitulé « *In vitro* induction of Thymidine Phosphorylase by **XRP6258**, a new taxoid » présenté au Congrès de Société Française de Pharmacologie à Clermont-Ferrand du 9 au 11 avril 2008, il est précisé : « **XRP6258** induces TP expression, especially with MCF-7 breast carcinoma cells. This induction might be clinically relevant in the field of **XRP6258**/capecitabine combination, assessed in patients with breast cancer, as predictive of an increased cytotoxicity in the tumor cells for the combination. ».

Il existe toujours un besoin de trouver et d'optimiser de nouvelles options thérapeutiques chez les patientes progressant après un traitement antérieur par anthracyclines et taxanes.

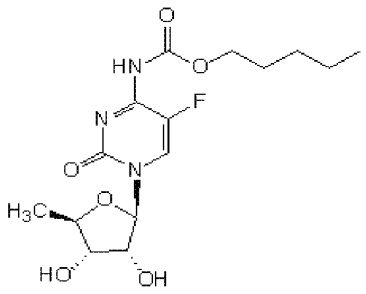
La présente invention répond à ce besoin en fournissant une nouvelle combinaison pharmaceutique antitumorale comprenant le cabazitaxel et la capecitabine pour laquelle il a fallu déterminer les doses de chaque drogue et le schéma d'administration convenable, de manière à obtenir une combinaison bien tolérée qui n'exacerbe pas la toxicité de chacun des deux agents antitumoraux et qui permette le traitement des patientes progressant après un traitement antérieur par anthracyclines et taxanes afin d'en evaluer l' activitité antitumorale.

[Brève description de l'invention]

L'invention est relative à une combinaison pharmaceutique antitumorale comprenant le cabazitaxel de formule



et la capecitabine de formule



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ces deux agents antitumoraux pouvant être sous forme de base, sous forme d'un sel d'un acide pharmaceutiquement acceptable ou sous forme d'un hydrate ou d'un solvat, destinée à traiter le cancer du sein métastatique chez les patientes progressant après un traitement antérieur par anthracyclines et taxanes.

Le cabazitaxel peut être notamment sous forme d'un solvat acétonique. Plus particulièrement, le solvat acétonique du cabazitaxel contient entre 5 et 8% en poids d'acétone, de préférence entre 5 et 7%.

La combinaison comprend une quantité efficace de cabazitaxel et une quantité efficace de capecitabine.

Le cabazitaxel peut être administré à une dose (définie pour chaque administration) comprise entre 15 et 25 mg/m².

La capecitabine peut être administrée deux fois par jour à une dose (définie pour chaque administration) comprise entre 675 et 1250 mg/m², plutôt entre 825 et 1000 mg/m².

Le cabazitaxel peut être administré par perfusion à une dose comprise entre 15 et 25 mg/m² et la capecitabine est administrée par voie orale deux fois par jour pendant 14 jours à une dose (définie pour chaque administration) comprise entre 675 et 1250 mg/m², plutôt entre 825 et 1000 mg/m², ce cycle d'administration des deux agents antitumoraux étant répétés avec un intervalle entre deux administrations de cabazitaxel de 3 semaines, pouvant être prolongé de 1 à 2 semaines en fonction de la tolérance à la précédente administration de cabazitaxel.

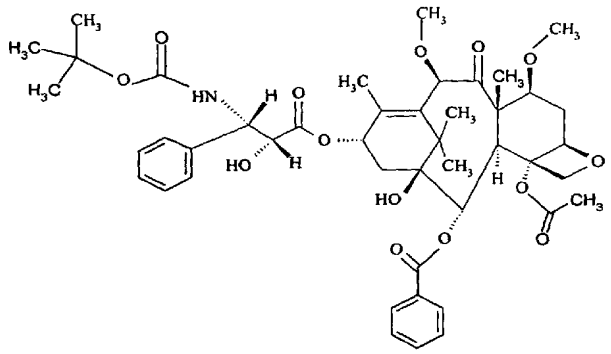
L'invention est aussi relative à l'utilisation du cabazitaxel et de la capecitabine de formule pour la préparation de la combinaison pharmaceutique antitumorale précitée.

[Description de l'invention]

définitions

- acide pharmaceutiquement acceptable : acide organique ou inorganique présentant une faible toxicité (voir Pharmaceutical salts *J.Pharm.Sci.* **1977**, *66*, 1-19) ;
- quantité efficace : quantité d'un composé pharmaceutique produisant un effet sur le cancer traité.

S'agissant du cabazitaxel, celui appartient à la famille des taxoïdes et a pour formule :



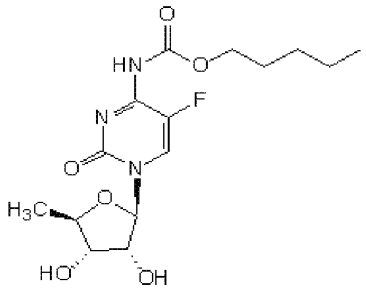
Il a pour nom chimique : 4α-acetoxy-2α-benzoyloxy-5β, 20-epoxy-1β-hydroxy-7β, 10β-dimethoxy-9-oxo-11-taxen-13α-yl (2R,3S)-3 tert-butoxycarbonylamino-2-hydroxy-3-phenylpropionate. Ce composé et un mode de préparation est décrit dans le document **WO 96/30355**. Le cabazitaxel peut être administré sous forme de base (cf. formule ci-dessus), sous forme d'un sel d'un acide pharmaceutiquement acceptable ou sous forme d'un hydrate. Il peut s'agir aussi d'un solvat c'est-à-dire d'un complexe moléculaire caractérisé par l'incorporation du solvant de cristallisation dans le cristal de la molécule du principe actif (voir à ce propos, en page 1276 de *J.Pharm.Sci.* **1975**, *64*(8), 1269-1288). En particulier, il pourra s'agir d'un solvat acétonique, plus particulièrement celui décrit dans **WO 2005/028462**. Il peut s'agir d'un solvat acétonique du cabazitaxel contenant entre 5 et 8% en poids d'acétone, de préférence entre 5 et 7% (% signifie teneur en acétone/teneur en acétone+cabazitaxel x100). Une valeur moyenne de la teneur en acétone est de 7%, ce qui représente à peu près la stoechiométrie en acétone qui est de 6,5% pour un solvat à une molécule d'acétone. Le mode opératoire décrit ci-dessous permet de préparer un solvat acétonique du cabazitaxel :

A une solution de 207 g de 4-acétoxy-2α-benzoyloxy-5β,20-époxy-1-hydroxy-7β,10β-diméthoxy-9-oxo-tax-11-ène-13α-yle, à environ 92 % en poids dans environ 2 litres d'acétone, on ajoute à 20 ± 5°C température ambiante, 940 ml d'eau purifiée puis on ensemence avec une suspension de 2 g de (2R,3S)-3-tert-butoxycarbonylamino-2-

hydroxy-3-phénylpropionate de 4-acétoxy-2α -benzoyloxy-5β,20-époxy-1-hydroxy-7β,10β-diméthoxy-9-oxo-tax-11-ène-13α-yle isolé dans acétone/eau dans un mélange de 20 ml d'eau et 20 ml d'acétone. On laisse agiter environ 10 à 22 heures et on additionne en 4 à 5 heures 1,5 litres d'eau purifiée. On laisse agiter 60 à 90 minutes puis la suspension est filtrée sous pression réduite. Le gâteau est lavé sur filtre avec une solution préparée à partir de 450 ml d'acétone et 550 ml d'eau purifiée puis séché en étuve à 55°C sous pression réduite (0,7 kPa 5 mmHg) pendant 4 heures. On obtient 197 g de (2R,3S)-3-tert-butoxycarbonylamino-2-hydroxy-3-phénylpropionate de 4-acétoxy-2 α-benzoyloxy-5β,20-époxy-1-hydroxy-7β,10β-diméthoxy-9-oxo-tax-11-ène-13α-yle, acétone contenant 0,1 % d'eau et 7,2 % d'acétone (théorie de 6,5 % pour un solvat stoéchiométrique).

Le cabazitaxel est administré par voie parentérale, telle que par administration intraveineuse, en bolus ou par perfusion. Une forme galénique du cabazitaxel adaptée à être administrée par perfusion est celle où le cabazitaxel est en solution dans l'eau en présence d'excipients choisis parmi les surfactants, les cosolvants, le glucose ou le chlorure de sodium,... Par exemple, une forme galénique du cabazitaxel peut être préparée par dilution d'une solution prémix de cabazitaxel contenue dans une ampoule stérile (80 mg de cabazitaxel + 2 ml de solvant + Polysorbate 80) avec une ampoule stérile contenant une solution de 6 ml d'eau et d'éthanol (13%poids d'éthanol 95%) afin d'obtenir 8 ml d'une solution prête à être rediluée dans une poche de perfusion. La concentration du cabazitaxel dans cette solution prête à être rediluée est d'environ 10 mg/ml. La perfusion est alors préparée en injectant la quantité idoine de cette solution prête à être rediluée dans la poche de perfusion contenant de l'eau et du glucose (environ 5%) ou du chlorure de sodium (environ 0,9%).

S'agissant de la capecitabine (CAS RN 154361-50-9), celle-ci est commercialisée par la société Roche sous la marque Xeloda®. Il s'agit d'une prodrug du 5-fluorouracyl :



et a pour nom chimique : 5'-Deoxy-5-fluoro-N4-(pentyloxycarbonyl)cytidine N-[1-(5-Deoxy-beta-D-ribofuranosyl)-5-fluoro-2-oxo-1,2-dihydropyrimidin-4-yl]carbamic acid pentyl ester. Ce composé est décrit dans **EP 0602454** ou **US 5472949**. La capecitabine

peut être administrée sous forme de base (cf. formule ci-dessus), sous forme d'un sel d'un acide pharmaceutiquement acceptable ou sous forme d'un hydrate ou d'un solvat.

Une forme galénique de la capecitabine adaptée à la voie orale est par exemple celle commercialisée sous la marque Xeloda® sous forme de comprimés contenant 150 ou 500 mg de capecitabine et du lactose anhydre comme excipient.

S'agissant de la combinaison, celle-ci consiste à associer sous forme de deux préparations pharmaceutiques distinctes le cabazitaxel et la capecitabine. La combinaison peut être utilisée dans le traitement du cancer du sein métastatique, notamment pour les patientes échappant à un traitement à base d'anthracyclines et/ou de taxanes.

La combinaison est administrée de façon répétée selon un protocole qui dépend de la patiente à soigner (surface corporelle, tolérance au précédent cycle…). Le cabazitaxel peut être administré par perfusion à la patiente selon un schéma intermittent avec un intervalle entre chaque administration de 3 semaines, pouvant être prolongé de 1 à 2 semaines en fonction de la tolérance de la précédente administration. La capecitabine peut quant à elle être administrée quotidiennement, par exemple sous forme de deux prises par jour, pendant une durée de 14 jours. Au cours d'un cycle, la 1ère prise de capecitabine peut coincider avec l'administration du cabazitaxel.

Un exemple de protocole est le suivant : le cabazitaxel est administré par perfusion sur une durée de 1 heure environ à un jour J1 donné (1er jour du cycle). La capecitabine est administrée par voie orale deux fois par jour, matin et soir, du jour J1 au jour J14 (du 1er au 14ème jour du cycle). Ce cycle consistant à administrer à la fois le cabazitaxel (à J1) et la capecitabine (de J1 à J14) est ensuite répété avec un intervalle de 3 semaines (prolongeable de 1 à 2 semaines).

Les doses de cabazitaxel et de capecitabine administrées à chaque fois à la patiente dépendent de différents paramètres : surface corporelle, tolérance au précédent cycle…. Le cabazitaxel peut être administré à une dose (définie pour chaque administration) comprise entre 15 et 25 mg/m². La capecitabine peut être administrée deux fois par jour à une dose (définie pour chaque administration) comprise entre 675 et 1250 mg/m², plutôt entre 825 et 1000 mg/m².

De préférence, la dose recommandée est de 20 mg/m^2 de cabazitaxel le premier jour de traitement et de 2x1000 mg/m^2/jour de capecitabine du premier au quatorzième jour, ce cycle d'administration des deux agents antitumoraux étant répété avec un intervalle entre deux administrations de cabazitaxel de 3 semaines, pouvant être prolongé de 1 à 2 semaines en fonction de la tolérance à la précédente administration de cabazitaxel.

[Example]

Une étude de phase I/II a été conduite dans quatre centres d'étude en Europe.

Partie 1 : la dose maximale administrée (Maximal Administered Dose an anglais - MAD) et la dose recommandée (Recommended Dose - RD) de cabazitaxel en association avec la capécitabine ont été déterminées.

Partie 2 : l'activité anti-tumorale et la caractérisation du profil de tolérance ont été déterminées à la dose recommandée

La pharmacocinétique (PK), incluant l'étude des interactions médicamenteuses, a été aussi étudiée.

Patientes

Critères d'inclusion principaux des patients

Les critères d'inclusion principaux ont été un âge supérieur ou égal à 18 ans, un cancer du sein métastatique ou localement récurrent et inopérable histologiquement prouvé, un indice Eastern Cooperative Oncology Group performance status (ECOG PS) de 0 à 2, une exposition antérieure aux taxanes et aux anthracyclines et un fonctionnement adéquat au niveau hématologique, rénal et hépatique. Pour la partie 2, les patientes sélectionnées devaient avoir au moins 1 lésion mesurable selon les directives RECIST (*J Natl Cancer Inst* **2000**, 92, 205-216).

Les critères d'exclusion principaux ont été un cancer simultané, plus d'un traitement de chimiothérapie pour cancer métastatique, l'exposition antérieure à la capécitabine ou des conditions co-morbides significatives non contrôlées.

Trente-trois patientes avec un cancer du sein métastatique ont été incluses dans l'étude, toutes précédemment traitées avec des anthracyclines et des taxanes: 15 patientes pour la partie 1 et 18 pour la partie 2.

Les caractéristiques relatives aux patientes traitées sont précisées dans le **Tableau I**.

Tableau I

nombre de patientes	33
âge médian [min-max] (doit être >=18)	55 [34-74]
diagnostic : - carcinome ductal infiltrant - carcinome lobulaire infiltrant - carcinome mixte infiltrant	 25 (76%) 4 (12%) 4 (12%)
temps médian depuis le diagnostic initial en années [min-max]	4,97 [1,2-21,5]
statut Her2 Neu par FISH négatif	 33 (100%)
récepteur hormonal ER+ et/ou PR+ Er- et PR-	 29 (88%) 4 (12%)
ECOG PS à l'entrée dans l'étude 0 1	 20 (61%) 13 (39%)
nombre médian d'organes métastatiques	3 (1-6)
statut de la maladie au début du traitement principaux organes envahis: os foie ganglions distants poumon plèvre	métastatique : 33 (100%) 27 (82%) 20 (61%) 13 (39%) 10 (30%) 7 (21%)

ganglions régionaux autres[a]	4 (12%) 6 (18%)
traitement antérieur Nb de lignes de chimiotherapie antérieure pour maladie avancée : 0 1	 5 (15%) 28 (85%)

[a] inclut surrénal, tissu mou, péritoine, épanchement péricardique.

ECOG PS: Eastern Cooperative Oncology Group performance status; ER: recepteur aux oestrogènes ; PgR: recepteur à la progesterone ; HER2: human epidermal growth factor receptor.

Pharmacocinétique:

Les paramètres de pharmacocinétique ont été calculés pour le cabazitaxel, la capecitabine, et leur métabolites (C_{max}, T_{max}, $AUC_{0\text{-}last}$, AUC, $t_{½\lambda}$).

Des prélèvements de sang ont été rassemblés à des moments divers dans la partie 1 de l'atude et testés par chromatographie liquide couplée à des méthodes de spectrométrie de masse.

L'analyse pharmacocinétique n'a pas révélé d'apparente interaction entre le cabazitaxel et la capecitabine; la pharmacocinétique du cabazitaxel et de son metabolite ne semble pas affectée par la co-administration de capecitabine et *vice versa*.

Partie 1 : détermination de la dose maximale administrée (Maximal Administered Dose an anglais - MAD) et de la dose recommandée (Recommended Dose - RD) de cabazitaxel en association avec la capécitabine

Les toxicités doses limitantes (DLTs) c'est-à-dire la liste des évènements à surveiller, qui permettent de guider l'escalade de dose, ont tout d'abord été prédéfinies dans le protocole en accord avec l'échelle de classification NCI-CTCAE version 3.

L'augmentation des doses a été étudiée par groupe de trois patientes tant qu'aucune DLT n'a été observée. Si une DLT a été constatée chez une patiente, le groupe a été étendu à six patientes, la MAD étant atteinte si au moins 2 patientes ont atteint une DLT. La RD a été défini comme la plus haute dose à laquelle moins de 33 % des patientes ont présenté une DLT.

Premier niveau de dose :

- 3 patientes - cabazitaxel 20 mg/m^2 (J1) et capecitabine 825 mg/m² deux fois par jour (J1-14)

- 1 DLT de type neutropénie grade 4 durant plus de 7 jours chez une patiente a été observée

- groupe étendu à 6 patientes sans nouvelles DLTs.

Second niveau de dose :

- 3 patientes - cabazitaxel 20 mg/m^2 (J1) et capecitabine 1000 mg/m² deux fois par jour (J1-14)

- pas de DLT observée.

Troisième niveau de dose :

- 3 patientes - cabazitaxel 25 mg/m^2 (J1) et capecitabine 1000 mg/m^2 deux fois par jour (J1-14)

- 1 DLT de type neutropénie grade 4 durant plus de 7 jours chez une patiente a été observée

- groupe étendu à 6 patientes – une seconde DLT du même type chez une autre patiente a été observée.

La MAD a par conséquent été définie comme étant : cabazitaxel 25 mg/m^2 et capecitabine 1000 mg/m^2.

La RD a par conséquent été définie comme étant : cabazitaxel 20 mg/m^2 et capecitabine 1000 mg/m².

Partie 2 : Etude de l'activité anti-tumorale et caractérisation du profil de tolérance à la dose recommandée.

Les patientes incluses dans la partie 2 de l'étude ont été traitées avec la RD.

Le critère d'évaluation d'efficacité principal a été le taux de réponse objective (Objective Response Rate en anglais – ORR).

Le taux de réponse objective est défini, selon les directives RECIST (*J Natl Cancer Inst* **2000**, 92, 205-216), comme la proportion de patientes avec une réponse complète

(Complete Response - CR) ou une réponse partielle (Partial Response - PR) confirmée divisée par le nombre total de patientes dans la population d'analyse.

Les critères secondaires d'évaluation d'efficacité ont été la durée de réponse au traitement (Duration of response en anglais - DR) et le temps de progression (Time To Progression - TTP).

Le temps jusqu'a progression (TTP) est défini, selon les directives RECIST (*J Natl Cancer Inst* **2000**, 92, 205-216), comme le temps écoulé entre la première date d'administration de la combinaison et la date de la première documentation d'une progression de la maladie.

La durée de réponse au traitement (DR) est définie, selon les directives RECIST (*J Natl Cancer Inst* **2000**, 92, 205-216), comme le temps écoulé entre la date de la première documentation d'une réponse objective (CR ou PR) et la date de la première documentation d'une progression de la maladie ou la survenue d'un décès.

La capecitabine est administrée par voie orale deux fois par jour, matin et soir, du jour J1 au jour J14 (du 1er au 14ème jour du cycle). Deux heures après l'administration du matin, le cabazitaxel est administré par perfusion (iv) sur une durée de 1 heure environ au jour J1 (1er jour du cycle). Ce cycle consistant à administrer à la fois le cabazitaxel (à J1) et la capecitabine (de J1 à J14) est ensuite répété toutes les trois semaines.

Le **tableau II** détaille un exemple concret d'un cycle.

Tableau II

heure	**jour J1**	**jours J2 à J14**
7:30	petit déjeuner	petit déjeuner
8 :00	capecitabine (voie orale)	capecitabine (voie orale)
10:00	cabazitaxel (perfusion)	
19:30	dîner	dîner
20:00	capecitabine (voie orale)	capecitabine (voie orale)

Les doses ont pu être réduites et la durée du cycle de traitement a pu être prolongée en cas d'évènement indésirable (Adverse Event en anglais - AE) sévère. Le traitement a été

continué jusqu'à la progression de maladie, la présence d'AE inacceptable ou le retrait de consentement de la patiente.

Un examen physique, des numérations globulaires, des formules leucocytaires complètes et des analyses de chimie sanguine ont été réalisés avant le recrutement des patientes et régulièrement pendant le traitement. Des évaluations tumorales par radiologie ont été réalisées au recrutement des patientes et toutes les 6 semaines. Les réponses ont été confirmées par 2 évaluations à au moins 4 semaines d'intervalle. Des données complémentaires ont été rassemblées toutes les 6 semaines jusqu'à la date de d'arrêt de l'étude.

Le **Tableau III** résume les caractéristiques de traitement des patients aux différents niveaux de dose testés.

Tableau III

dose		**DL1 (20 mg/m^2 cabazitaxel + 2x825 mg/m^2/jour capecitabine pendant 14 jours)**	**DL2 (20 mg/m^2 cabazitaxel + 2x1000 mg/m^2/jour capecitabine pendant 14 jours)**	**DL3 (25 mg/m^2 cabazitaxel + 2x1000 mg/m^2/jour capecitabine pendant 14 jours)**	**total**
nombre de patientes		6	21[a]	6	33
nombre de cycles		30	112	36	178
cabazitaxel	total médiane [min-max]	30 4,5 [2-12]	109 5 [2-13]	36 5,5 [4-9]	175 5 [2-13]
capecitabine	total médiane [min-max]	30 4,5 [2-12]	112 6 [2-13]	36 5,5 [4-9]	178 5 [2-13]
Nombre de patients avec un traitement retardé		1	10	5	16
nombre de patients avec réduction de dose	cabazitaxel	1	4	5	10
	capecitabine	0	3	4	7
dose intensité relative médiane	cabazitaxel	0,97 [0,86-1,01]	0,97 [0,69-1,00]	0,82 [0,74-0,91]	0,96 [0,69-1,01]
	capecitabine	0,92 [0,64-1,00]	0,89 [0,55-1,02]	0,83 [0,71-1,03]	0,87 [0,55-1,03]

[a] incluant 3 patientes de la partie 1 et 18 patients supplémentaires

Tolérance

Les évènements indésirables (AE) les plus fréquents ont été les troubles gastrointestinaux, la fatigue, le syndrome pied-main et la toxicité hématologique, ce qui correspond au profil généralement attendu d'une association taxane-capécitabine.

Le **Tableau IV** présente les résultats d'activité anti-tumorale aux différents niveaux de dose testés.

Tableau IV

Résultats nombre de patientes (%)	**DL1 (20 mg/m^2 cabazitaxel + 2x825 mg/m^2/jour capecitabine pendant 14 j)**	**DL2 (20 mg/m^2 cabazitaxel + 2x1000 mg/m^2/jour capecitabine pendant 14 j)**	**DL3 (25 mg/m^2 cabazitaxel + 2x1000 mg/m^2/jour capecitabine pendant 14 j)**	**total**
nombre de patientes	6	21[a]	6	33
réponse complète	0	1 (5)[c]	1 (17)	2 (6)
réponse partielle	0	4 (19)	1 (17)	5 (15)
stabilisation	5 (83)	11 (52)	4 (67)	20 (64)[b]
progression	1 (17)	5 (24)	0	6 (18)
ORR, % (95% CI)	-	24 (8-47)	-	-
RD moyenne, mois (intervalle)	-	3,06 (2,1-8.4)	4,42 (3,1-5,8)	3,06 (2,1-8,4)
TTP moyen, mois (95% CI)		4,9 (2,7-NA)	NA	4,9 (3,4-NA)

[a] incluant 3 patientes de la partie 1 et 18 patients supplémentaires
[b] incluant 7 patientes ayant une réponse partielle non confirmée (4 patientes avec cabazitaxel 20 mg/m² + capecitabine 825 mg/m^2; 2 patientes avec cabazitaxel 20 mg/m² + capecitabine 1000 mg/m^2 et 1 patiente avec cabazitaxel 25 mg/m² + capecitabine 1000 mg/m²).
[c] Patiente avec une carcinose péritonéale à l'entrée dans l'étude qui a présenté une CR confirmée.
CI : intervalle de confiance; NA : non disponible.

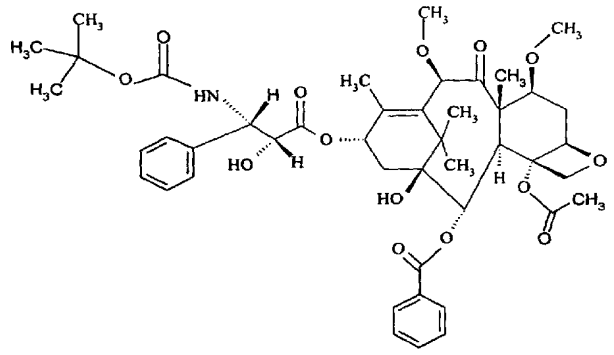
La partie 2 de l'étude a confirmé la faisabilité de la combinaison pharmaceutique antitumorale comprenant le cabazitaxel et la capecitabine à la dose recommandée puisqu'il a été constaté que la combinaison est bien tolérée et n'exacerbe pas la toxicité de chacun des deux agents antitumoraux. De plus des signaux d'activité anti-tumorale encourageants on été observés à cette dose.
Une activité anti-tumorale a par ailleurs été constatée à tous les niveaux de dose testés.

Au total 7 patientes ont présenté une réponse objective.

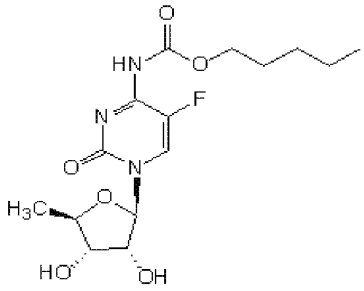
Il est remarquable de noter que 2 des 4 patientes qui avaient une progression de la maladie comme meilleure réponse sous la thérapie antérieure par taxane, ont vu leur cancer se stabiliser avec la combinaison cabazitaxel et capécitabine, tandis que 4 patientes qui avaient une stabilisation de leur maladie comme meilleure réponse sous la thérapie antérieure par taxane, ont obtenu une réponse objective au traitement.

REVENDICATIONS

1. Combinaison pharmaceutique antitumorale comprenant le cabazitaxel de formule



et la capecitabine de formule



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ces deux agents antitumoraux pouvant être sous forme de base, sous forme d'un sel d'un acide pharmaceutiquement acceptable ou sous forme d'un hydrate ou d'un solvat destinée à traiter le cancer du sein métastatique chez les patientes progressant après un traitement antérieur par anthracyclines et taxanes.

2. Combinaison selon la revendication 1 comprenant une quantité efficace de cabazitaxel et une quantité efficace de capecitabine.

3. Combinaison selon la revendication 1 ou 2 dans laquelle le cabazitaxel est sous forme d'un solvat acétonique.

4. Combinaison selon la revendication 3 dans laquelle le solvat acétonique du cabazitaxel contient entre 5 et 8% en poids d'acétone, de préférence entre 5 et 7%.

5. Combinaison selon la revendication 1 à 4 dans laquelle le cabazitaxel est administré à une dose (définie pour chaque administration) comprise entre 15 et 25 mg/m².

6. Combinaison selon la revendication 1 à 5 dans laquelle la capecitabine est administrée deux fois par jour à une dose (définie pour chaque administration) comprise entre 675 et 1250 mg/m².

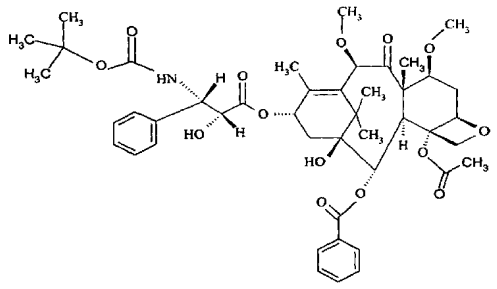
7. Combinaison selon la revendication 6 dans laquelle la capecitabine est administrée pendant 14 jours.

8. Combinaison selon la revendication 1 à 7 dans laquelle le cabazitaxel est administré par perfusion à une dose comprise entre 15 et 25 mg/m² et la capecitabine est administrée par voie orale deux fois par jour pendant 14 jours à une dose (définie pour chaque administration) comprise entre 675 et 1250 mg/m², plutôt entre 825 et 1000 mg/m², ce cycle d'administration des deux agents antitumoraux étant répété avec un intervalle entre deux administrations de cabazitaxel de 3 semaines, pouvant être prolongé de 1 à 2 semaines en fonction de la tolérance à la précédente administration de cabazitaxel.

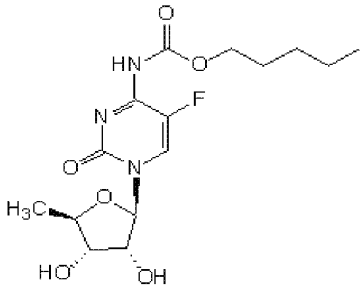
9. Combinaison selon la revendication 8 dans laquelle le cabazitaxel est administré à la dose de 20 mg/m^2 le premier jour de traitement et la capecitabine est administrée à la dose de 2x1000 mg/m^2/jour du premier au quatorzième jour de traitement.

10. Combinaison selon la revendication 8 ou 9 dans laquelle au cours d'un cycle, la 1ère prise de capecitabine coïncide avec l'administration du cabazitaxel.

11. Utilisation du cabazitaxel de formule



et de la capecitabine de formule



pour la préparation d'une combinaison pharmaceutique antitumorale telle que définie à l'une des revendications 1 à 10.

INTERNATIONAL SEARCH REPORT

International application No
PCT/FR2010/050873

A. CLASSIFICATION OF SUBJECT MATTER
INV. A61K31/337 A61K31/7068 A61P35/00
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 practical, search terms used)
EPO-Internal, EMBASE, BIOSIS, WPI Data, CHEM ABS Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	PIVOT X ET AL: "A multicenter phase II study of XRP6258 administered as a 1-h i.v. infusion every 3 weeks in taxane-resistant metastatic breast cancer patients." ANNALS OF ONCOLOGY : OFFICIAL JOURNAL OF THE EUROPEAN SOCIETY FOR MEDICAL ONCOLOGY / ESMO SEP 2008, vol. 19, no. 9, September 2008 (2008-09), pages 1547-1552, XP002551475 ISSN: 1569-8041 page 1551, passage "discussion"	1-11

-/--

[X] Further documents are listed in the continuation of Box C.

[X] 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

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"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
26 July 2010	03/08/2010

Name and mailing address of the ISA/	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Fax: (+31-70) 340-3016	Borst, Markus

Form PCT/ISA/210 (second sheet) (April 2005)

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	PEREZ E A: "Impact, mechanisms, and novel chemotherapy strategies for overcoming resistance to anthracyclines and taxanes in metastatic breast cancer" BREAST CANCER RESEARCH AND TREATMENT 200903 US, vol. 114, no. 2, March 2009 (2009-03), pages 195-201, XP002551476 page 197-198, passage "Treatment options for patients whose tumors are resistant to anthracyclines and taxanes"	1-11
A	ANONYMOUS: "View of NCT00327743 on 2009_04_11" INTERNET CITATION 11 April 2009 (2009-04-11), pages 1-3, XP002551477 Retrieved from the Internet: URL:http://clinicaltrials.gov/archive/NCT00327743/2009_04_11 [retrieved on 2009-10-20] the whole document	1-11
A	ANONYMOUS: "View of NCT00397761 on 2009_02_08" INTERNET CITATION 8 February 2009 (2009-02-08), pages 1-3, XP002551478 Retrieved from the Internet: URL:http://clinicaltrials.gov/archive/NCT00397761/2009_02_08 [retrieved on 2009-10-20] the whole document	1-11
A	WO 2005/028462 A (AVENTIS PHARMA SA [FR]) 31 March 2005 (2005-03-31)	3,4
X,P	BESSE-HAMMER T ET AL.: "A dose escalating study of XRP6258 in combination with capecitabine..." JOURNAL OF CLINICAL ONCOLOGY, [Online] vol. 27, no. 15S, 20 May 2009 (2009-05-20) , XP002551479 Retrieved from the Internet: URL:http://meeting.ascopubs.org/cgi/content/abstract/27/15S/1053> [retrieved on 2009-10-20] * abstract	1-11

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No
PCT/FR2010/050873

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 2005028462 A	31-03-2005	AU 2004274212 A1	31-03-2005
		BR PI0414492 A	14-11-2006
		CA 2539309 A1	31-03-2005
		CN 1849311 A	18-10-2006
		EP 1667986 A1	14-06-2006
		FR 2859996 A1	25-03-2005
		HK 1093340 A1	19-06-2009
		JP 2007505866 T	15-03-2007
		KR 20060072147 A	27-06-2006
		MX PA06002639 A	05-06-2006
		NZ 545835 A	30-10-2009
		PA 8612401 A1	04-08-2005
		RU 2342373 C2	27-12-2008
		ZA 200602255 A	25-07-2007

RAPPORT DE RECHERCHE INTERNATIONALE

Demande internationale n°
PCT/FR2010/050873

A. CLASSEMENT DE L'OBJET DE LA DEMANDE
INV. A61K31/337 A61K31/7068 A61P35/00
ADD.

Selon la classification internationale des brevets (CIB) ou à la fois selon la classification nationale et la CIB

B. DOMAINES SUR LESQUELS LA RECHERCHE A PORTE

Documentation minimale consultée (système de classification suivi des symboles de classement)
A61K

Documentation consultée autre que la documentation minimale dans la mesure où ces documents relèvent des domaines sur lesquels a porté la recherche

Base de données électronique consultée au cours de la recherche internationale (nom de la base de données, et si cela est réalisable, termes de recherche utilisés)
EPO-Internal, EMBASE, BIOSIS, WPI Data, CHEM ABS Data

C. DOCUMENTS CONSIDERES COMME PERTINENTS

Catégorie*	Identification des documents cités, avec, le cas échéant, l'indication des passages pertinents	no. des revendications visées
X	PIVOT X ET AL: "A multicenter phase II study of XRP6258 administered as a 1-h i.v. infusion every 3 weeks in taxane-resistant metastatic breast cancer patients." ANNALS OF ONCOLOGY : OFFICIAL JOURNAL OF THE EUROPEAN SOCIETY FOR MEDICAL ONCOLOGY / ESMO SEP 2008, vol. 19, no. 9, septembre 2008 (2008-09), pages 1547-1552, XP002551475 ISSN: 1569-8041 page 1551, passage "discussion"	1-11

-/--

[X] Voir la suite du cadre C pour la fin de la liste des documents

[X] Les documents de familles de brevets sont indiqués en annexe

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"&" document qui fait partie de la même famille de brevets

Date à laquelle la recherche internationale a été effectivement achevée	Date d'expédition du présent rapport de recherche internationale
26 juillet 2010	03/08/2010

Nom et adresse postale de l'administration chargée de la recherche internationale	Fonctionnaire autorisé
Office Européen des Brevets, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Fax: (+31-70) 340-3016	Borst, Markus

C(suite). DOCUMENTS CONSIDERES COMME PERTINENTS

Catégorie*	Identification des documents cités, avec, le cas échéant, l'indication des passages pertinents	no. des revendications visées
A	PEREZ E A: "Impact, mechanisms, and novel chemotherapy strategies for overcoming resistance to anthracyclines and taxanes in metastatic breast cancer" BREAST CANCER RESEARCH AND TREATMENT 200903 US, vol. 114, no. 2, mars 2009 (2009-03), pages 195-201, XP002551476 page 197-198, passage "Treatment options for patients whose tumors are resistant to anthracyclines and taxanes"	1-11
A	ANONYMOUS: "View of NCT00327743 on 2009_04_11" INTERNET CITATION 11 avril 2009 (2009-04-11), pages 1-3, XP002551477 Extrait de l'Internet: URL:http://clinicaltrials.gov/archive/NCT00327743/2009_04_11 [extrait le 2009-10-20] le document en entier	1-11
A	ANONYMOUS: "View of NCT00397761 on 2009_02_08" INTERNET CITATION 8 février 2009 (2009-02-08), pages 1-3, XP002551478 Extrait de l'Internet: URL:http://clinicaltrials.gov/archive/NCT00397761/2009_02_08 [extrait le 2009-10-20] le document en entier	1-11
A	WO 2005/028462 A (AVENTIS PHARMA SA [FR]) 31 mars 2005 (2005-03-31)	3,4
X,P	BESSE-HAMMER T ET AL.: "A dose escalating study of XRP6258 in combination with capecitabine..." JOURNAL OF CLINICAL ONCOLOGY, [Online] vol. 27, no. 15S, 20 mai 2009 (2009-05-20) , XP002551479 Extrait de l'Internet: URL:http://meeting.ascopubs.org/cgi/content/abstract/27/15S/1053> [extrait le 2009-10-20] * abrégé	1-11

RAPPORT DE RECHERCHE INTERNATIONALE

Renseignements relatifs aux membres de familles de brevets

Demande internationale n°
PCT/FR2010/050873

Document brevet cité au rapport de recherche	Date de publication	Membre(s) de la famille de brevet(s)	Date de publication
WO 2005028462 A	31-03-2005	AU 2004274212 A1	31-03-2005
		BR PI0414492 A	14-11-2006
		CA 2539309 A1	31-03-2005
		CN 1849311 A	18-10-2006
		EP 1667986 A1	14-06-2006
		FR 2859996 A1	25-03-2005
		HK 1093340 A1	19-06-2009
		JP 2007505866 T	15-03-2007
		KR 20060072147 A	27-06-2006
		MX PA06002639 A	05-06-2006
		NZ 545835 A	30-10-2009
		PA 8612401 A1	04-08-2005
		RU 2342373 C2	27-12-2008
		ZA 200602255 A	25-07-2007

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(19) World Intellectual Property Organization
International Bureau

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5 May 2011 (05.05.2011)

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WO 2011/051894 A1

(51) International Patent Classification:
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A61K 31/56 (2006.01) *A61P 35/00* (2006.01)

(21) International Application Number:
PCT/IB2010/054866

(22) International Filing Date:
27 October 2010 (27.10.2010)

(25) Filing Language: English

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(30) Priority Data:

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61/355,834	17 June 2010 (17.06.2010)	US
61/355,888	17 June 2010 (17.06.2010)	US
61/369,929	2 August 2010 (02.08.2010)	US
61/383,933	17 September 2010 (17.09.2010)	US
61/389,969	5 October 2010 (05.10.2010)	US

(71) Applicant *(for all designated States except US)*: **AVENTIS PHARMA S.A.** [FR/FR]; 20 avenue Raymond Aron, F-92160 Antony (FR).

(72) Inventor; and
(75) Inventor/Applicant *(for US only)*: **GUPTA, Sunil** [US/US]; c/o Sanofi-Aventis US. Inc., 1041 Routes 202-206 Mail Code: D-303A, Bridgewater, New Jersey 08807 (US).

(74) Agent: **GASLONDE, Aude**; c/o Sanofi-Aventis, Patent Department, 174 avenue de France, F-75013 Paris (FR).

(81) Designated States *(unless otherwise indicated, for every kind of national protection available)*: AE, AG, AL, AM, AO, AT, AU, AZ, BA, BB, BG, BH, BR, BW, BY, BZ, CA, CH, CL, CN, CO, CR, CU, CZ, DE, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IS, JP, KE, KG, KM, KN, KP, KR, KZ, LA, LC, LK, LR, LS, LT, LU, LY, MA, MD, ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PE, PG, PH, PL, PT, RO, RS, RU, SC, SD, SE, SG, SK, SL, SM, ST, SV, SY, TH, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW.

(84) Designated States *(unless otherwise indicated, for every kind of regional protection available)*: ARIPO (BW, GH, GM, KE, LR, LS, MW, MZ, NA, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European (AL, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV, MC, MK, MT, NL, NO, PL, PT, RO, RS, SE, SI, SK, SM, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

Declarations under Rule 4.17:

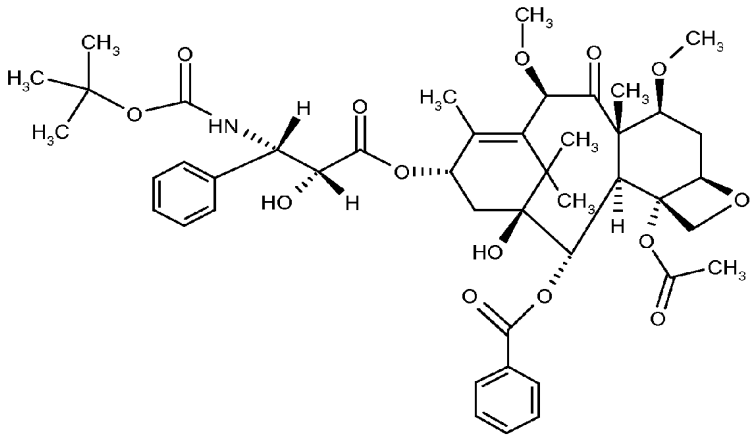
— *of inventorship (Rule 4.17(iv))*

Published:

— *with international search report (Art. 21(3))*

— *before the expiration of the time limit for amending the claims and to be republished in the event of receipt of amendments (Rule 48.2(h))*

(54) **Title**: NOVEL ANTITUMORAL USE OF CABAZITAXEL



(I)

(57) **Abstract**: The invention relates to a compound of Formula (I): which may be in base form or in the form of a hydrate or a solvate, in combination with prednisone or prednisolone, for its use as a medicament in the treatment of prostate cancer, particularly metastatic prostate cancer, especially for patients who are not catered for by a taxane-based treatment.

WO 2011/051894 A1

NOVEL ANTITUMORAL USE OF CABAZITAXEL

The present invention relates to a novel antitumoral use of cabazitaxel in the treatment of prostate cancer, which may be metastatic, especially for patients who are not catered for by a taxane-based treatment. In particular, the present invention relates to the use of cabazitaxel in the treatment of patients with castration resistant metastatic prostate cancer, who have been previously treated with a docetaxel based regimen, an unmet medical need.

[Technical problem]

Prostate cancer affects a large proportion of the male population worldwide: 680 000 cases worldwide in 2002; it is predicted that there will be 900 000 new cases per year up to 2010 (*CA Cancer J. Clin.,* **2005**, *55*, 74-108). It is the most frequently occurring cancer in men after lung cancer.

Prostate cancer is generally treated at the start by depriving the androgenic hormones, i.e. by surgical excision of the testicles **The Current State of Hormonal Therapy for Prostate Cancer** CA **Cancer** J. Clin., May 2002; 52: 154-179, or by radiotherapy treatment **External beam radiation therapy for prostate cancer** CA **Cancer** J. Clin., Nov. 2000; 50: 349-375. Treatments with antiandrogens or hormone manipulations are associated with responses of short duration and without any improvement in the survival time.

The use of cytotoxic chemotherapy is not a routine treatment, whereas its role in alleviating the symptoms and reducing the levels of PSA (prostate-specific antigen) is established. No monotherapy has obtained a degree of response of greater than 30%; combinations with an effect on PSA levels were tested. No effect on the survival time was seen and, what is more, the toxicity of these treatments, particularly on elderly patients, is problematic since, in addition to their tumour, they are generally suffering from related health problems and have a limited reserve of bone marrow.

Until recently, the chemotherapies used were limited to cyclophosphamide, anthracyclines (doxorubicin or mitoxantrone) and estramustine, and the effects of these treatments are relatively mediocre. Palliative effects were observed in patients following the administration of corticoids alone or of mitoxantrone with either prednisone or hydrocortisone. Following Phase II trials, the combination of mitoxantrone with corticoids was recognized as the reference treatment for hormone-resistant prostate cancer. More recently, treatments with docetaxel in combination with estramustine or prednisone have made it possible to treat cancers that are resistant to hormone deprivation **Advances in Prostate Cancer Chemotherapy: A New Era Begins** CA **Cancer** J. Clin., Sep. 2005; 55: 300-318, the survival was improved by 2.4 months.

It is generally accepted that the responses in advanced prostate cancers are difficult to evaluate on account of the heterogeneity of the disease and the lack of consensus regarding the treatment response criteria. Many patients with metastatic prostate cancer have no measurable disease, but have symptoms dominated by bone metastases. Measurement of the PSA level has been found to be a means for evaluating novel candidates and also the measurement of the tumour when this is possible, the measurement of bone tumours, the quality of life and the measurement of the pain.

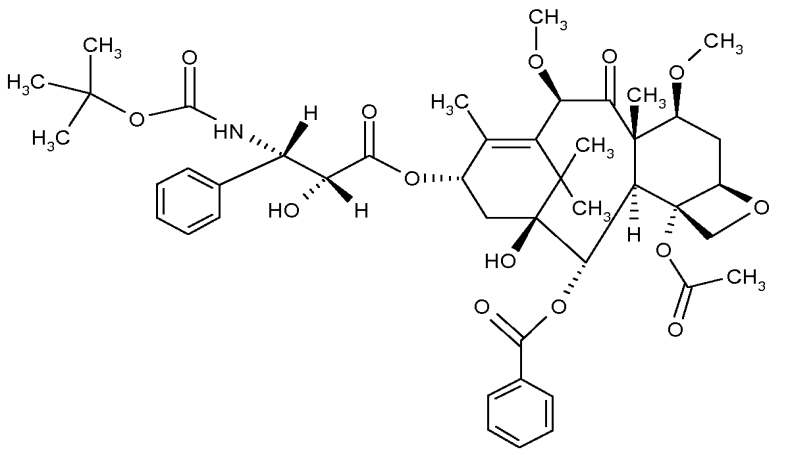
Furthermore, cancer may become resistant to the agents used, in particular to taxanes, which limits the possible treatment options. Several taxane resistance mechanisms have been described (expression of P-glycoprotein P-gp, *mdr-1* gene, modified metabolism of taxane, mutation of the tubulin gene, etc.): see *Drug Resistance Updates* **2001**, *4*(1), 3-8; J. Clin. Onc. **1999**, *17*(3), 1061-1070.

The technical problem that the invention intends to solve is that of providing a novel therapeutic option for treating prostate cancer, especially for patients who are not catered for by a taxane-based treatment, such as patients with castration resistant metastatic prostate cancer who have been previously treated with docetaxel (sold under the brand name Taxotere®) based regimen, an unmet medical need.

Four clinical trials on cabazitaxel are known since April 2006. Three monotherapy tests have made it possible to determine the maximum tolerated dose and the toxicities at the limit doses: these tests were performed on breast, sarcoma and prostate tumours. Doses of 10-30 mg/m^2 every three hours were used. A phase II trial was performed on patients with a breast cancer, who had previously received taxanes and anthracyclines as adjuvant (i.e. after a surgery) or as a first-line treatment. The response levels were 14.6% as adjuvant and 9.5% as second-line treatment.

[Brief description of the invention]

The invention relates to a novel antitumoral pharmaceutical therapeutic use comprising cabazitaxel of formula



The invention also relates to methods of treating patients with prostate cancer comprising administering an effective amount of the antitumoral agent cabazitaxel to said patient.

This antitumoral agent may be in the form of anhydrous base, a hydrate or a solvate, intended for treating prostate cancer, in particular for treating patients who are not catered for by a taxane-based treatment, such as patients who have been previously treated with a docetaxel-based regimen. This compound is preferably administered to a patient with advanced metastatic disease. In particular, the compound is administered to a patient with castration resistant prostate cancer. Cabazitaxel is preferably administered in combination with a corticoid chosen especially from prednisone and prednisolone. This corticoid is preferably administered at a daily dose of 10 mg orally.

In some aspects of the invention, cabazitaxel is administered in combination with prednisone for its use as a medicament in the treatment of patients with hormone-refractory prostate cancer who have been previously treated with docetaxel based regimen.

In some aspects of the invention, cabazitaxel is administered at a dose (defined for each administration) of between 20 and 25 mg/m^2. Cabazitaxel may be in the form of an acetone solvate. More particularly, the acetone solvate of cabazitaxel contains between 5% and 8% and preferably between 5% and 7% by weight of acetone.

In some aspects of the invention, cabazitaxel may be administered by intravenous infusion at a dose of between 15 and 25 mg/m^2, this administration cycle of the antitumour agent being repeated at an interval of 3 weeks between each cabazitaxel administration, which interval may be prolonged by 1 to 2 weeks depending on the tolerance to the preceding cabazitaxel administration.

In some embodiments, the effective amount of cabazitaxel produces at least one therapeutic effect selected from the group consisting of increase in overall survival, partial response, reduction in tumor size, reduction in metastasis, complete remission, partial remission, stable disease, or complete response.

The present invention also relates to a pharmaceutical composition that treats patients with prostate cancer comprising a clinically proven safe and effective amount of cabazitaxel.

Further embodiments of the invention comprise methods or using, treating, promoting, and providing cabazitaxel.

The present invention also relates to packages and articles of manufacture.

[Brief Description of the Drawings]

Figure 1 displays the Kaplan-Meier curves of the overall survival in a cabazitaxel study.

Figure 2 displays the Kaplan-Meier curves of progression-free survival in a cabazitaxel study.

Figure 3 shows an intention-to-treat analysis of overall survival in subgroups of patients defined by baseline characteristics. Hazard ratios <1 favor the cabazitaxel group, while those >1 favor the mitoxantrone group. CI denotes confidence intervals.

Figure 4 graphically depicts the proportion of patients with changes in ECOG performance status from baseline during treatment (safety population).

Figure 5 graphically depicts the proportion of patients with changes from baseline in the Present Pain Intensity score during treatment (ITT).

Figure 6 graphically presents the mean area under the curve for PPI and analgesic scores by treatment cycle.

Figure 7 graphically presents the mean AUC analgesic score.

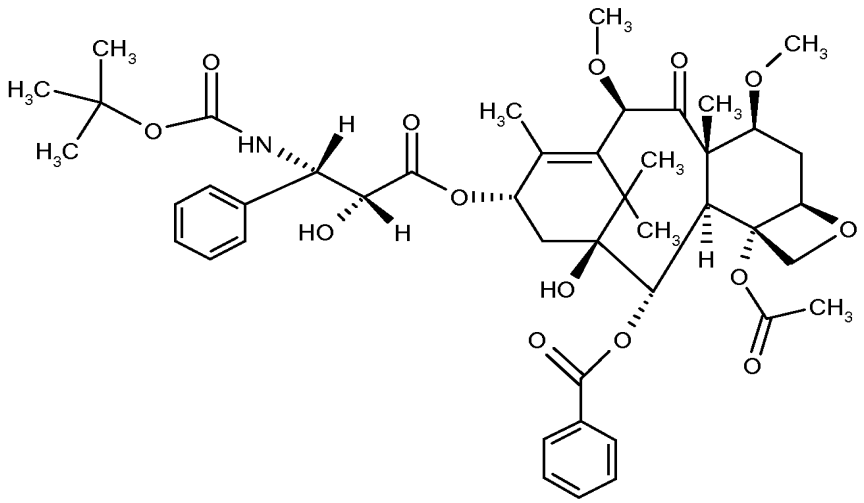
[Description of the invention]

Definitions

- Effective amount, as used herein, means an amount of a pharmaceutical compound, such as cabazitaxel, that produces an effect on the cancer to be treated.

- Clinically proven, as used herein, means clinical efficacy results that are sufficient to meet FDA approval standards.
- Castration resistant prostate cancer, as used herein, is synonymous with hormone-refractory prostate cancer.
- "Patient," as used herein, includes both human and animals. In one embodiment, a patient is a human.

Cabazitaxel belongs to the taxoid family and has the formula:



The chemical name of cabazitaxel is 4α-acetoxy-2α-benzoyloxy-5β,20-epoxy-1β-hydroxy-7β,10β-dimethoxy-9-oxo-11-taxen-13α-yl (2R,3S)-3-*tert*-butoxycarbonylamino-2-hydroxy-3-phenylpropionate. Cabazitaxel is synonymously known as (2α,5β,7β,10β,13α)-4-acetoxy-13-({(2R,3S)-3-[(tertbutoxycarbonyl)amino]-2-hydroxy-3-phenylpropanoyl}oxy)-1-hydroxy-7,10-dimethoxy-9-oxo-5,20-epoxytax-11-en-2-yl benzoate.

This compound and a preparative method thereof is described in **WO 96/30355, EP 0 817 779 B1** and **US 5 847 170**, which are hereby incorporated herein by reference. Cabazitaxel may be administered in base form (cf. above formula), or in the form of a hydrate. It may also be a solvate, i.e. a molecular complex characterized by the incorporation of the crystallization solvent into the crystal of the molecule of the active principle (see in this respect page 1276 of *J. Pharm. Sci.* **1975**, *64*(8), 1269-1288). In particular, it may be an acetone solvate, and, more particularly, may be the solvate described in **WO 2005/02846**. It may be an acetone solvate of cabazitaxel containing between 5% and 8% and preferably between 5% and 7% by weight of acetone (% means content of acetone/content of acetone+cabazitaxel × 100). An average value of the acetone content is 7%, which approximately represents the acetone stoichiometry, which is 6.5%

for a solvate containing one molecule of acetone. The procedure described below allows the preparation of an acetone solvate of cabazitaxel:

940 ml of purified water are added at 20±5°C (room temperature) to a solution of 207 g of 4α-acetoxy-2α-benzoyloxy-5β,20-epoxy-1β-hydroxy-7β,10β-dimethoxy-9-oxo-11-taxen-13α-yl (2R,3S)-3-*tert*-butoxycarbonylamino-2-hydroxy-3-phenylpropionate at about 92% by weight in about 2 litres of acetone, followed by seeding with a suspension of 2 g of 4α-acetoxy-2α-benzoyloxy-5β,20-epoxy-1β-hydroxy-7β,10β-dimethoxy-9-oxo-11-taxen-13α-yl (2R,3S)-3-*tert*-butoxycarbonylamino-2-hydroxy-3-phenylpropionate isolated from acetone/water in a mixture of 20 ml of water and 20 ml of acetone. The resulting mixture is stirred for about 10 to 22 hours, and 1.5 litres of purified water are added over 4 to 5 hours. This mixture is stirred for 60 to 90 minutes, and the suspension is then filtered under reduced pressure. The cake is washed on the filter with a solution prepared from 450 ml of acetone and 550 ml of purified water, and then oven-dried at 55°C under reduced pressure (0.7 kPa) for 4 hours. 197 g of 4α-acetoxy-2α-benzoyloxy-5β,20-epoxy-1β-hydroxy-7β,10β-dimethoxy-9-oxo-11-taxen-13α-yl (2R,3S)-3-*tert*-butoxycarbonylamino-2-hydroxy-3-phenylpropionate acetone containing 0.1% water and 7.2% acetone (theoretical amount: 6.5% for a stoichiometric solvate) are obtained.

Cabazitaxel may be administered parenterally, such as via intravenous administration. A galenical form of cabazitaxel suitable for administration by intravenous infusion is that in which the cabazitaxel is dissolved in water in the presence of excipients chosen from surfactants, cosolvents, glucose or sodium chloride, etc. For example, a galenical form of cabazitaxel may be prepared by diluting a premix solution of cabazitaxel contained in a sterile vial (80 mg of cabazitaxel + 2 ml of solvent + Polysorbate 80) with a sterile vial containing a solution of 6 ml of water and ethanol (13% by weight of 95% ethanol) in order to obtain 8 ml of a solution ready to be rediluted in a perfusion bag. The concentration of cabazitaxel in this ready-to-redilute solution is about 10 mg/ml. The perfusion is then prepared by injecting the appropriate amount of this ready-to-redilute solution into the perfusion bag containing water and glucose (about 5%) or sodium chloride (about 0.9%).

Cabazitaxel may be administered in combination with a corticoid, such as prednisone or prednisolone, as two distinct pharmaceutical preparations.

Accordingly, one aspect of the invention is a method of treating prostate cancer comprising administering to a patient in need thereof an effective amount of cabazitaxel in combination with a corticoid, such as prednisone or prednisolone.

The combination is administered repeatedly according to a protocol that depends on the patient to be treated (age, weight, treatment history, etc.), which can be determined by a skilled physician. In one aspect of the invention, cabazitaxel is administered by perfusion to the patient according to an intermittent program with an interval between each administration of 3 weeks, which may be prolonged by 1 to 2 weeks depending on the tolerance to the preceding administration. The median number of cycles is 6. The prednisone or prednisolone may be administered daily, for example in the form of one dosage intake per day, throughout the duration of the treatment. Examples of doses for the two antitumoral agents are given in the "Example" section. The currently recommended dose is 25 mg/m^2 of cabazitaxel administered as a on-hour infusion and 10 mg per day of prednisone or prednisolone administered orally.

In some aspects of the invention, the patient to be treated has prostate cancer that is resistant to hormone therapy (i.e., hormone refractory) and has previously been treated with docetaxel. In some aspects, the patient has prostate cancer that progressed during or after treatment with docetaxel. In some aspects, the patient was previously treated with at least 225 mg/m^2 cumulative dose of docetaxel. In a particular aspect, the patient showed progression of their disease in the six months following hormone therapy or during docetaxel treatment or after docetaxel treatment. In another particular aspect, the patient showed progression of their disease in the three months following hormone therapy or after docetaxel treatment.

In some aspects of the invention, the patient to be treated has a measurable tumour and may show progression of the disease via a metastatic lesion of the viscera or of a soft tissue of at least 1 cm determined by MRI or by an axial tomographic scan (CT scan).

In some aspects of the invention, the patient to be treated has an unmeasurable tumour and may show an increase in the PSA level with three measurements at a 1-week interval or the appearance of new lesions.

In some aspects of the invention, the patient to be treated has undergone castration by orchidectomy or with LHRH agonists, elimination of the androgens or monotherapy with estramustine.

In a preferred aspect, the life expectancy of the patient to be treated should be at least 2 months.

In some aspects, the treatment does not include patients who have previously received mitoxantrone, or who have received less than 225 mg/m^2 of docetaxel, or who have undergone a radiotherapy that has eliminated more than 40% of the marrow, who have received a treatment within the 4 weeks preceding the test, who have a neuropathy or a stomatitis, involving the brain

or the meninges, who have shown severe hypersensitivity to polysorbate or to prednisone, whose blood analysis shows an appreciable decrease in neutrophils, haemoglobin or platelets, an increase in bilirubin and/or liver enzymes and creatinine, or who have heart problems or an infection requiring antibiotics.

An aspect of the invention comprises increasing the survival of a patient with hormone refractory metastatic prostate cancer, comprising administering a clinically proven effective amount of cabazitaxel to the patient in combination with prednisone or prednisolone. In a particular aspect, the patient has previously been treated with a docetaxel-containing regimen.

Cabazitaxel may be administered in combination with a medication to prevent or control nausea and vomiting or to prevent or control hypersensitivity to the cabazitaxel treatment. Preferably, a patient is pre-medicated with the medication, for example, at least 30 minutes prior to administering each dose of cabazitaxel.

One aspect of the invention comprises a method of reducing the risk of a severe hypersensitivity reaction in a patient with prostate cancer being treated with cabazitaxel, comprising administering to the patient a medication to prevent hypersensitivity prior to the administration of cabazitaxel.

Severe hypersensitivity reactions to cabazitaxel can occur and may include generalized rash/erythema, hypotension and bronchospasm. Patients should be observed closely for hypersensitivity reactions, especially during the first and second infusions. Hypersensitivity reactions may occur within a few minutes following the initiation of the infusion of cabazitaxel, thus facilities and equipment for the treatment of hypotension and bronchospasm should be available. If severe hypersensitivity reaction occurs, cabazitaxel infusion should be immediately discontinued and appropriate therapy should be administered. Examples of medications which may be used to prevent hypersensitivity to the cabazitaxel treatment include antihistamines, such as dexchloropheniramine (for example 5 mg), and diphenhydramine (for example 25 mg) or equivalent antihistamines; and corticosteroids, such as dexamethasone (for example 8 mg) or an equivalent steroid.

Nevertheless, cabazitaxel should not be given to and may be contraindicated in patients who have a history of severe hypersensitivity reactions to cabazitaxel. Depending on the formulation administered, cabazitaxel may also be contraindicated in patients who have a history of hypersensitivity reactions to other drugs formulated with polysorbate 80.

One aspect of the invention comprises an article of manufacture comprising:

a) a packaging material;

b) cabazitaxel, and

c) a label or package insert contained within the packaging material indicating that severe hypersensitivity reactions can occur.

Gastrointestinal symptoms, such as, for example nausea, vomiting, and diarrhea, may occur with the treatment of cabazitaxel. Mortality related to diarrhea and electrolyte imbalance has been reported. Therefore, patients may also be rehydrated and treated with anti-diarrheal or anti-emetic medications as needed. Treatment delay or dosage reduction may be necessary if patients experience Grade ≥ 3 diarrhea.

Accordingly, the methods of the invention include administering a medication to prevent hypersensitivity or a medication to prevent or control nausea and vomiting in combination with cabazitaxel.

Examples of medications which may be used to prevent or control nausea and vomiting include histamine H_2 antagonists and antiemetics, such as ondansetron, granisetron and dolesetron.

A possible side effect of the treatment with cabazitaxel is neutropenia, which is characterized by a reduced number of neutrophils. Unfortunately, a number of neutropenia deaths have been reported. Therefore, frequent blood counts should be obtained or performed to monitor for neutropenia. If neutropenia occurs, cabazitaxel treatment may be discontinued, and restarted when neutrophil counts recover to a level of >1,500 /mm^3. Cabazitaxel should not be given to a patient with a neutrophil count ≤ 1,500 cells/mm^3.

The present invention therefore also relates to a method of treating prostate cancer with cabazitaxel comprising administering cabazitaxel to the patient, monitoring blood counts in the patient, and measuring neutrophil levels. In one aspect, the method further comprises discontinuing cabazitaxel treatment if neutropenia occurs, and optionally restarting cabazitaxel treatment when neutrophil counts recover to a level of >1,500 /mm^3. In one aspect, the monitoring comprises taking a blood sample from the patient.

Determining neutrophil counts can be performed according to procedures well know to those skilled in the art.

One aspect of the invention is a method of reducing the risk of neutropenia complications comprising administering cabazitaxel in combination with an agent useful for treating neutropenia. Such a neutropenia treatment agent is, for example, a hematopoietic growth factor which regulates the production and function of neutrophils such as a human granulocyte colony

stimulating factor, (G-CSF). In a particular aspect of the invention, the neutropenia is complicated neutropenia. Complicated neutropenia includes febrile neutropenia, prolonged neutropenia, or neutropenic infection. In a preferred embodiment, the neutropenia treatment agent is administered prior to the administration of cabazitaxel.

A particular aspect of the invention comprises a method of reducing the risk of neutropenia complications in a patient with prostate cancer being treated with cabazitaxel, comprising monitoring blood counts in the patient at regular intervals during treatment of the patient with cabazitaxel; reducing the dose of cabazitaxel if the patient experiences febrile neutropenia or prolonged neutropenia; discontinuing cabazitaxel treatment if the patient's neutrophil count is ≤ 1,500 cells/mm^3; and optionally restarting cabazitaxel treatment when the patient's neutrophil counts recover to a level ≥ 1,500 cells/mm^3.

In a particular aspect, primary prophylaxis with G-CSF should be considered in patients with high-risk clinical features (age > 65 years, poor performance status, previous episodes of febrile neutropenia, extensive prior radiation ports, poor nutritional status, or other serious co-morbidities) that predispose them to increased complications from prolonged neutropenia. Therapeutic use of G-CSF and secondary prophylaxis should be considered in all patients considered to be at increased risk for neutropenia complications.

In another aspect, the monitoring of complete blood counts is performed on a weekly basis during cycle 1 and before each treatment cycle thereafter so that the dose can be adjusted, if needed. Therefore, another aspect for reducing the risk of neutropenia complications comprises monitoring blood counts in the patient and adjusting the dose of cabazitaxel. An example of a dose modification is described in Example 2.

One aspect of the invention comprises an article of manufacture comprising:

a) a packaging material;

b) cabazitaxel, and

c) a label or package insert contained within the packaging material indicating that cabazitaxel should not be given to patients with neutrophil counts of ≤1,500 cells/mm^3.

Cases of renal failure should be indentified and managed aggressively, accordingly to procedures known to those skilled in the art. Renal failure may be associated with sepsis, dehydration, or obstructive uropathy. Furthermore, impaired hepatic function (e.g., total bilirubin ≥ ULN, or AST and/or ALT ≥ 1.5 x ULN) may increase cabazitaxel concentrations, and cabazitaxel should not be given to patients with hepatic impairment.

Cabazitaxel may cause fetal harm when administered to a pregnant woman.

Prednisone or prednisolone administered at 10 mg daily does not affect the pharmacokinetics of cabazitaxel.

Cabazitaxel is primarily metabolized through CYP3A. Concomitant administration of strong CYP3A inhibitors (for example, ketoconazole, itraconazole, clarithromycin, atazanavir, indinavir, nefazodone, nelfinavir, ritonavir, saquinavir, telithromycin, voriconazole) may increase cabazitaxel concentrations. Therefore co-administration of cabazitaxel with strong CYP3A inhibitors should be avoided. Caution should be exercised with concomitant use of moderate CYP3A inhibitors. One aspect of the invention is a method of treating a patient for prostate cancer comprising determining whether the patient is undergoing treatment with a CYP3A inhibitor, discontinuing treatment with a CYP3A inhibitor, and then administering cabazitaxel to the patient.

Concomitant administration of strong CYP3A inducer (e.g., phenytoin, carbamazepine, rifampin, rifabutin, rifapentin, phenobarbital) may decrease cabazitaxel concentrations. Therefore co-administration of cabazitaxel with strong CYP3A inducers should be avoided. Therefore, one aspect of the invention is a method of treating a patient for prostate cancer comprising determining whether the patient is undergoing treatment with a CYP3A inducer, discontinuing treatment with a CYP3A inducer, and administering cabazitaxel to the patient.

In addition, patients should also refrain from taking St. John's Wort.

In some aspects of the invention, the cabazitaxel is administered in an amount to provide an AUC of about 991 ng•h/mL (CV 34%).

In some aspects of the invention, the cabazitaxel is administered in an amount to provide an C_{max} of about 226 ng•h/mL (CV 107%).

In some aspects of the invention, the cabazitaxel is administered in an amount to provide a plasma clearance of 48.5 L/h (CV 39%).

One aspect of the invention is a package comprising cabazitaxel and a label, in a position which is visible to prospective purchasers, comprising a printed statement which informs prospective purchasers that the mean C_{max} of cabazitaxel in patients with metastatic prostate cancer was 226 ng/mL (CV 107%).

Another aspect of the invention is a package comprising cabazitaxel and a label, in a position which is visible to prospective purchasers, comprising a printed statement which informs prospective purchasers that the mean AUC of cabazitaxel in patients with metastatic prostate cancer was 991 ng•h/mL (CV 34%).

Another aspect of the invention is a package comprising cabazitaxel and a label, in a position which is visible to prospective purchasers, comprising a printed statement which informs prospective purchasers that cabazitaxel has a plasma clearance of 48.5 L/h (CV 39%).

A variety of educational materials may be employed to ensure proper prescribing, dispensing, and patient compliance according to the methods described herein. For example, a variety of literature and other materials, such as, for example, prescribing information, package inserts, medications guides, physician information sheets, healthcare professional information sheets, medical journal advertisements, and product websites may describe the risks and benefits of taking cabazitaxel.

The invention also concerns a package comprising cabazitaxel and a label, said label comprising one or more messages that:

a) the efficacy and safety of cabazitaxel in combination with prednisone were evaluated in patients with hormone refractory metastatic prostate cancer previously treated with a docetaxel containing regimen; or

b) a total of 755 patients were randomized to receive either cabazitaxel 25 mg/m^3 every 3 weeks for a maximum of 10 cycles with prednisone mg orally daily, or to receive mitoxantrone 12 mg/m^2 intravenously every 3 weeks for a maximum of 10 cycles with prednisone 10 mg orally daily; or

c) the median number of cycles was 6 in the cabazitaxel group and 4 in the mitoxantrone group.

The invention also concerns a package comprising cabazitaxel and a label, said label comprising one or more messages that:

a) neutropenic deaths have been reported; or

b) frequent blood counts should be obtained to monitor for neutropenia; or

c) cabazitaxel should not be given if neutrophil counts are ≤ 1,500 cells/mm^3.

The invention also concerns a method of promoting the use of cabazitaxel the method comprising the step of conveying to a recipient at least one message selected from:

a) neutropenic deaths have been reported; or

b) frequent blood counts should be obtained to monitor for neutropenia; or

c) cabazitaxel should not be given if neutrophil counts are ≤ 1,500 cells/mm^3;

d) severe hypersensitivity can occur; or

e) severe hypersensitivity can occur and may include generalized rash/erythema, hypotension and brochospasm; or

f) discontinue cabazitaxel immediately if severe reactions occur; or

g) discontinue cabazitaxel immediately if severe reactions occur and administer appropriate therapy; or

h) cabazitaxel is contraindicated in patients with a history of severe hypersensitivity reactions to cabazitaxel or drugs formulated with polysorbate 80.

The invention also concerns a method of providing cabazitaxel, wherein said cabazitaxel is provided along with information indicating that:

a) neutropenic deaths have been reported; or

b) frequent blood counts should be obtained to monitor for neutropenia; or

c) cabazitaxel should not be given if neutrophil counts are ≤ 1,500 cells/mm^3;

d) severe hypersensitivity can occur; or

e) severe hypersensitivity can occur and may include generalized rash/erythema, hypotension and brochospasm; or

f) discontinue cabazitaxel immediately if severe reactions occur; or

g) discontinue cabazitaxel immediately if severe reactions occur and administer appropriate therapy; or

h) cabazitaxel is contraindicated in patients with a history of severe hypersensitivity reactions to cabazitaxel or drugs formulated with polysorbate 80.

Example 1

A clinical study was performed wherein patients received either treatment with cabazitaxel or the reference treatment based on mitoxantrone each combined with prednisone or prednisolone.

More specifically, patients over 18 years of age with metastatic castration resistant metastatic prostate cancer either measurable by RECIST criteria or non-measurable disease with rising PSA levels or appearance of new lesions, ECOG (Eastern Cooperative Oncology Group) performance stage 0–2, and adequate organ function (patients had to have neutrophils >1,500 cells/mm^3, platelets >100,000 cells/mm^3, hemoglobin >10 g/dL, creatinine <1.5 x upper limit of normal (ULN), total bilirubin < 1xULN, AST <1.5 x ULN, and ALT <1.5 x ULN) who had had prior hormone therapy, chemotherapy, and radiotherapy, but had progressive during or after docetaxel treatment

(cumulative dose ≥225 mg/m^2) were randomized to 10 mg/day of prednisone with either mitoxantrone 12 mg/m^2 or cabazitaxel 25 mg/m^2, both administered every 3 weeks.

Patients with a history of congestive heart failure, or myocardial infarction within the last 6 months, or patients with uncontrolled cardiac arrhythmias, angina pectoris, and/or hypertension were not included in the study.

720 patients were planned to be included in the clinical study: 360 in each cabazitaxel + prednisone and mitoxantrone + prednisone group. Seven hundred and fifty-five patients (755) (median age 68; 84% white) were actually enrolled, 378 in the cabazitaxel and prednisone/prednisolone group and 377 in the mitoxantrone and prednisone/prednisolone group. The maximal number of treatment cycles was 10 for cabazitaxel and 10 for mitoxantrone. The median number of treatment cycles was 6 for cabazitaxel and 4 for mitoxantrone. The median prior dose of docetaxel treatment was 576 mg/m^2 for the cabazitaxel group and 529 mg/m^2 for the mitoxantrone group. Median follow-up was 12.8 months.

The measurements of the results are performed via the same tests as at inclusion. MRI and spiral computed tomographic (CT) scans are preferably used.

The results are evaluated according to the following criteria (cf RECIST guideline):

overall survival (OS): the time from inclusion to the study to the date of death

complete response (CR): disappearance of the lesions

partial response (PR): at least 30% reduction of the largest diameter of the lesion

progression (PD): at least 20% increase in the sum of the largest diameter of the lesion or appearance of one or more new lesions

stable disease (SD): reduction of the tumour insufficient to be included in PR and increase of the tumour insufficient to be included in PD.

The confirmations of the measurements are made at least 4 weeks after the response criterion has been established for the first time.

The progression-free survival (PFS) is the time from inclusion in the study and the date of progression or death when the progression is either an increase of the PSA, or of the tumour, or of the pain.

It was found that the combination of cabazitaxel and prednisone is a well-tolerated combination with the safety profile of taxanes. At the dose investigated in this trial (LD2: 25 mg/m^2 cabazitaxel + 10 mg/m^2/day prednisone), patients receiving cabazitaxel demonstrated statistically significant

longer overall survival (OS) compared to mitoxantrone (p<0.0001). The hazard ratio was 0.70 (95%CI. 0.59, 0.83) in favor of cabazitaxel corresponding to a 30% reduction in risk of death. The median survival for patients in the cabazitaxel group was 15.1 months in comparison to 12.7 months in the mitoxantrone group. Notably, the extension of survival was observed irrespective of ECOG performance status, number of prior chemotherapy regimens and age. Benefit was also seen in the third of patients who were docetaxel-refractory and had progressed during docetaxel therapy.

The data related to the treated patients are given in Table 1:

Table 1: Efficacy analysis (intention-to-treat)

		CbzP N=378 Median (months)	MP N=377 Median (months)
Overall survival Median (months)		15.1	12.7
	Hazard ratio (95% CI)	0.70 (0.59; 0.83)	
	p-value[1]	0.0001	
PFS	Median (months)	2.8	1.4
	Hazard ratio (95% CI)	0.74 (0.64 - 0.86)	
	p-value[1]	0.0001	
Tumor response rate		14.4%	4.4%
p-value[2]		0.0005	
Time to Tumor Progression Median (months)		8.8	5.4
	p-value	<0.001	
PSA Response rate		39.2%	17.8%
p-value[2]		0.0002	
PSA PFS	Median (months)	6.4	3.1
	Hazard ratio (95% CI)	0.75 (0.63 - 0.90)	
	p-value[1]	0.0010	
Pain Response rate		9.2%	7.8%
p-value[2]		0.6526	
Pain PFS	Median (months)	Not reached	11.1
	Hazard ratio (95% CI)	0.91 (0.69 - 1.19)	
	p-value[1]	0.5192	

[1]Log-rank test, [2]Chi-square test

CbzP : cabazitaxel with prednisone
MP: mitoxantrone with prednisone

Progression free survival (PFS) defined as the earliest progression in tumor, PSA or pain was also statistically significantly longer in the cabazitaxel group compared to the mitoxantrone group (p<0.0001, hazard ratio = 0.74 (95%CI, 0.64, 0.86), and the median progression-free survival was 2.8 months versus 1.4 months. Response rates and PFS for PSA and tumor assessments were statistically significant in favor of cabazitaxel, while response rate and PFS for pain did not show a statistically significant difference.

The most frequent Grade 3/4 toxicities were neutropenia observed with a higher frequency in the cabazitaxel group with 81.7% compared to the mitoxantrone group with 58.0%. Rates of febrile neutropenia were 7.5% in the cabazitaxel group and 1.3% in the mitoxantrone group.

The most common (≥ 20%) grade 1-4 adverse reactions were anemia, leukopenia, neutropenia, thrombocytopenia, diarrhea, fatigue, nausea, vomiting, asthenia, and constipation.

The most common (≥ 5%) grade 3-4 adverse reactions in patients who received cabazitaxel were neutropenia, leukopenia, anemia, febrile neutropenia, diarrhea, fatigue, and asthenia.

Subgroup analyses by risk factors and a multivariate analysis showed that OS outcomes were consistent and robust in favor of cabazitaxel as shown in the herebelow table:

Table 2

	MP		CbzP		CbzP vs MP
	N (%)	Median OS (mos)	N (%)	Median OS (mos)	HR (95%CI)
ITT	377 (100)	12.7	378 (100)	15.1	0.70 (0.59–0.83)
PD while on D	103 (27)	12.0	113 (30)	14.2	0.65 (0.47–0.90)
PD after last D dose, ≤3 mos	180 (48)	10.3	158 (42)	13.9	0.70 (0.54–0.90)
PD after last D dose, >3 mos	91 (24)	17.7	103 (27)	17.5	0.78 (0.53–1.14)

mos = months
D = Docetaxel

Table 3 – Incidence of Reported Adverse Reactions[1] and Hematologic Abnormalities in ≥ 5% of Patients Receiving cabazitaxel in Combination with Prednisone or Mitoxantrone in Combination with Prednisone

	Cabazitaxel 25 mg/m^2 every 3 weeks with prednisone 10 mg daily n=371		**Mitoxantrone 12 mg/m^2 every 3 weeks with prednisone 10 mg daily n=371**	
	Grade 1-4 n (%)	**Grade 3-4 n (%)**	**Grade 1-4 n (%)**	**Grade 3-4 n (%)**
Any Adverse Reaction				
Blood and Lymphatic System Disorders				
Neutropenia[2]	347 (94%)	303 (82%)	325 (87%)	215 (58%)
Febrile Neutropenia	27 (7%)	27 (7%)	5 (1%)	5 (1%)
Anemia[2]	361 (98%)	39 (11%)	302 (82%)	18 (5%)
Leukopenia[2]	355 (96%)	253 (69%)	343 (93%)	157 (42%)
Thrombocytopenia[2]	176 (48%)	15 (4%)	160 (43%)	6 (2%)
Cardiac Disorders				
Arrhythmia[3]	18 (5%)	4 (1%)	6 (2%)	1 (< 1%)
Gastrointestinal Disorders				
Diarrhea	173 (47%)	23 (6%)	39 (11%)	1 (< 1%)
Nausea	127 (34%)	7 (2%)	85 (23%)	1 (< 1%)
Vomiting	83 (22%)	6 (2%)	38 (10%)	0
Constipation	76 (20%)	4 (1%)	57 (15%)	2 (< 1%)
Abdominal Pain[4]	64 (17%)	7 (2%)	23 (6%)	0
Dyspepsia[5]	36 (10%)	0	9 (2%)	0
General Disorders and Administration Site Conditions				
Fatigue	136 (37%)	18 (5%)	102 (27%)	11 (3%)
Asthenia	76 (20%)	17 (5%)	46 (12%)	9 (2%)
Pyrexia	45 (12%)	4 (1%)	23 (6%)	1 (< 1%)
Peripheral Edema	34 (9%)	2 (< 1%)	34 (9%)	2 (< 1%)
Mucosal Inflammation	22 (6%)	1 (< 1%)	10 (3%)	1 (< 1%)
Pain	20 (5%)	4 (1%)	18 (5%)	7 (2%)
Infections and				

	Cabazitaxel 25 mg/m^2 every 3 weeks with prednisone 10 mg daily n=371		Mitoxantrone 12 mg/m^2 every 3 weeks with prednisone 10 mg daily n=371	
	Grade 1-4 n (%)	**Grade 3-4 n (%)**	**Grade 1-4 n (%)**	**Grade 3-4 n (%)**
Infestations				
Urinary Tract Infection[6]	29 (8%)	6 (2%)	12 (3%)	4 (1%)
Pneumonia[7]	12 (3%)	9 (2%)	4 (1%)	3 (< 1%)
Investigations				
Weight Decreased	32 (9%)	0	28 (8%)	1 (< 1%)
Metabolism and Nutrition Disorders				
Anorexia	59 (16%)	3 (< 1%)	39 (11%)	3 (< 1%)
Dehydration	18 (5%)	8 (2%)	10 (3%)	3 (< 1%)
Musculoskeletal and Connective Tissue Disorders				
Back Pain	60 (16%)	14 (4%)	45 (12%)	11 (3%)
Arthralgia	39 (11%)	4 (1%)	31 (8%)	4 (1%)
Pain in Extremity	30 (8%)	6 (2%)	27 (7%)	4 (1%)
Muscle Spasms	27 (7%)	0	10 (3%)	0
Bone Pain	19 (5%)	3 (< 1%)	19 (5%)	9 (2%)
Musculoskeletal Pain	18 (5%)	2 (< 1%)	20 (5%)	3 (< 1%)
Nervous System Disorders				
Peripheral Neuropathy[8]	50 (13%)	3 (< 1%)	12 (3.2%)	3 (< 1%)
Dysgeusia	41 (11%)	0	15 (4%)	0
Dizziness	30 (8%)	0	21 (6%)	2 (< 1%)
Headache	28 (8%)	0	19 (5%)	0
Renal and Urinary Tract Disorders				
Hematuria	62 (17%)	7 (2%)	13 (4%)	1 (< 1%)
Dysuria	25 (7%)	0	5 (1%)	0
Respiratory, Thoracic and Mediastinal Disorders				
Dyspnea	43 (12%)	4 (1%)	16 (4%)	2 (< 1%)
Cough	40 (11%)	0	22 (6%)	0
Skin and Subcutaneous Tissue Disorders				
Alopecia	37 (10%)	0	18 (5%)	0
Vascular Disorders				
Hypotension	20 (5%)	2 (<1 %)	9 (2%)	1 (< 1%)
Median Duration of Treatment	6 cycles		4 cycles	

[1]Graded using NCI CTCAE version 3
[2]Based on laboratory values, cabazitaxel: n =369, mitoxantrone: n = 370.
[3]Includes atrial fibrillation, atrial flutter, atrial tachycardia, atrioventricular block complete, bradycardia, palpitations, supraventricular tachycardia, tachyarrhythmia, and tachycardia.
[4]Includes abdominal discomfort, abdominal pain lower, abdominal pain upper, abdominal tenderness, and GI pain.
[5]Includes gastroesophageal reflux disease and reflux gastritis.
[6]Includes urinary tract infection enterococcal and urinary tract infection fungal.
[7]Includes bronchopneumonia, lobar pneumonia, and pneumonia klebsiella.
[8]Includes peripheral motor neuropathy and peripheral sensory neuropathy.

Table 4: Patient Characteristics

	MP (n=377)	CBZP (n=378)
Age (years)		
Median [range]	67 [47–89]	68 [46–92]
≥65 (%)	57.0	64.9
ECOG PS (%)		
0, 1	91.2	92.6
2	8.8	7.4
PSA* (ng/mL)		
Median [range]	127.5 [2–11220]	143.9 [2–7842]
Measurability of disease (%)		
Measurable	54.1	53.2
Non-measurable	45.9	46.8
Disease site (%)		
Bone	87.0	80.2
Lymph node	44.8	45.0
Visceral	24.9	24.9
Pain at Baseline, no. (%)	168 (44.6)	174 (46.0)
Previous Therapy, no. (%)		
Hormonal	375 (99.5)	375 (99.2)
No. of Chemotherapy Regimens		
1	268 (71.1)	260 (68.8)
2	79 (21.0)	94 (24.9)
> 2	30 (8.0)	24 (6.3)
Radiation	222 (58.9)	232 (61.4)
Surgery	205 (54.4)	198 (52.4)
Biologic Agent	36 (9.5)	26 (6.9)
Previous docetaxel regimens, n (%)		
1	327 (86.7)	316 (83.6)
2	43 (11.4)	53 (14.0)
>2	7 (1.9)	9 (2.4)
Median total previous docetaxel dose (mg/m^2)	529.2	576.6
Disease progression relative to docetaxel administration, n (%)		
During treatment	104 (27.6)	115 (30.4)
<3 months from last dose	181 (48.0)	158 (41.8)
≥3 months from last dose	90 (23.9)	102 (27.0)
Unknown	2 (0.5)	3 (0.8)
Median time from last docetaxel dose to disease progression (months)	0.7	0.8

The primary reason for treatment discontinuation in both groups was disease progression **(Table 5).** The median delivered relative dose intensity was 96.1% in the cabazitaxel group and 97.3% in the mitoxantrone group. In the cabazitaxel group, >75% of patients received >90% of the planned dose intensity. Overall, 5.1% of mitoxantrone treatment courses were dose reduced compared with 9.8% of cabazitaxel treatment courses; 6.3 and 7% of all treatment courses were delayed by 9 days or less, and 1.6 and 2.3% of courses were delayed by more than 9 days for mitoxantrone and cabazitaxel respectively (See Table 5).

Table 5. Treatment Received and Reasons for Discontinuation in the Intention-to-Treat Population.*

	Mitoxantrone (N=377)	**Cabazitaxel (N=378)**
Patients receiving study treatment, no. (%)	371 (98.4)	371 (98.1)
Patients completing planned ten cycles of study treatment, no. (%)	46 (12.2)	105 (27.8)
Discontinuation of study treatment, no. (%)	325 (86.2)	266 (70.4)
Reasons for discontinuation of study treatment, no. (%)		
Disease progression	267 (70.8)	180 (47.6)
Adverse event	32 (8.5)	67 (17.7)
Non-compliance with protocol	0	1 (0.3)
Lost to follow-up	2 (0.5)	0
Patient's request	17 (4.5)	8 (2.1)
Other	7 (1.9)	10 (2.7)
No. of treatment cycles, median (range)†	4 (1–10)	6 (1–10)
Relative dose intensity, median % (range)†	97.3 (42.5–106.0)	96.1 (49.0–108.2)
Treatment delays, no. of cycles (%) ‡		
≤ 9 days	110 (6.3)	157 (7.0)
> 9 days	28 (1.6)	51 (2.3)
Dose reductions, no. of cycles (%) ‡	88 (5.1)	221 (9.8)

The results of this study are further illustrated to Figures 1, 2, and 3.

Example 2

Table 6 illustrates an example of a dosage modification for adverse reactions in patients treated with cabazitaxel

Table 6

Toxicity	Dosage Modification
Prolonged grade ≥3 neutropenia (greater than 1 week) despite appropriate medication including G-CSF	Delay treatment until neutrophil count is >1,500 cells/mm^3, then reduce dosage of cabazitaxel to 20 mg/m^2. Use G-CSF for secondary prophylaxis.
Febrile neutropenia	Delay treatment until improvement or resolution, and until neutrophil count is >1,500 cells/mm^3, then reduce dosage of cabazitaxel to 20 mg/m^2. Use G-CSF for secondary prophylaxis.
Grade ≥3 diarrhea or persisting diarrhea despite appropriate medication, fluid and electrolytes replacement	Delay treatment until improvement or resolution, then reduce dosage of cabazitaxel to 20 mg/m^2.

Discontinue cabazitaxel treatment if a patient continues to experience any of these reactions at 20 mg/m^2.

Example 3

Performance Status and Pain Scores during Treatment

Methods

- ECOG PS, pain measures, and analgesic consumption were assessed prior to every treatment cycle and at the end of study treatment.

- Pain assessments: Present Pain Intensity (PPI) scale from the McGill-Melzack questionnaire (Melzack R. Pain 1975;1:277−99). Mean Analgesic Score (AS) derived from analgesic consumption (in morphine equivalents) was calculated for the one-week period prior to each evaluation. Area under the curve (AUC) of PPI and AS was calculated by the trapezoid formula. Cumulative AUC of PPI and AS was calculated up to the last cycle of data available for each patient. Average AUC of the treatment groups was compared from Cycle 1 to Cycle 10.

Results

- Performance status remained stable in most patients during the treatment period and was similar between groups. See Figure 4.

- Overall, PPI scores were comparable; improving from baseline in 21.3% of men in the CbzP group and 18.2% in the MP group. See Figure 5.

- The CbzP group had a lower mean area under the curve (AUC) of PPI, suggesting less severe pain especially during cycles 7–10. See Figure 6.

- Analgesic use was comparable between the groups (lower mean AUC of AS means lower pain medication use). See Figure 7.

Conclusion

Despite longer treatment with CbzP no worsening in ECOG PS was seen.

Present Pain Intensity score improved in 21% of men in CbzP vs. 18% in MP arm. Assessment of pain scores suggested less severe pain in the CbzP group during treatment.

Pain medication use was similar between groups.

Example 4

A population pharmacokinetic analysis was conducted in 170 patients with solid tumors at doses ranging from 10 to 30 mg/m^2 weekly or every 3 weeks.

Based on the population pharmacokinetic analysis, after an intravenous dose of cabazitaxel 25 mg/m^2 every 3 weeks, the mean C_{max} in patients with metastatic prostate cancer was 226 ng/mL (CV 107%) and was reached at the end of the 1-hour infusion (T_{max}). The mean AUC in patients with metastatic prostate cancer was 991 ng•h/mL (CV 34%). No major deviation from the dose proportionality was observed from 10 to 30 mg/m^2 in patients with advanced solid tumors. The volume of distribution (Vss) was 4,864 L (2,643 L/m^2 for a patient with a median BSA of 1.84 m^2) at steady state.

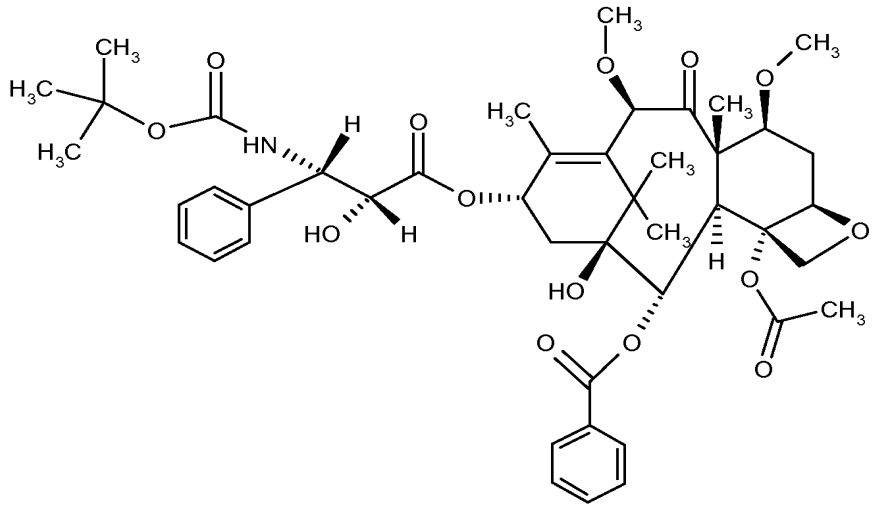
Based on the population pharmacokinetic analysis, cabazitaxel has a plasma clearance of 48.5 L/h (CV 39%; 26.4 L/h/m² for a patient with a median BSA of 1.84 m²) in patients with metastatic

prostate cancer. Following a 1-hour intravenous infusion, plasma concentrations of cabazitaxel can be described by a 3-compartment PK model with α-, β-, and γ- half-lives of 4 minutes, 2 hours, and 95 hours, respectively.

CLAIMS

What is claimed is:

1. Compound of formula



which may be in base form or in the form of a hydrate or a solvate,
in combination with prednisone or prednisolone,
for its use as a medicament in the treatment of prostate cancer.

2. Compound according to claim 1, where the treated patients are not catered for by a taxane-based treatment.

3. Compound according to claim 1 or 2, where the patients treated have been previously treated with a docetaxel-based regimen.

4. Compound according to any one of claims 1 to 3, where the prostate cancer is an advanced metastatic disease.

5. Compound according to any one of claims 1 to 4, where the prostate cancer is a castration resistant prostate cancer or hormone-refractory prostate cancer.

6. Compound according to any one of claims 1 to 5, in the form of an acetone solvate.

7. Compound according to Claim 6, in which the acetone solvate contains between 5% and 8% and preferably between 5% and 7% by weight of acetone.

8. Compound according to any one of claims 1 to 7, administered at a dose of between 15 and 25 mg/m^2, the prednisone or prednisolone being administered at a dose of 10 mg/day.

9. Compound according to claim 8, administered at a dose of 25 mg/m^2.

10. Compound according to any one of claims 1 to 9, comprising repeating the administration of such compound as a new cycle every 3 weeks.

11. Compound according to claim 10, wherein the median number of cycles is 6.

12. Compound according to any one of claims 1 to 11, in combination with prednisone for its use as a medicament in the treatment of patients with castration resistant metastatic prostate cancer or hormone-refractory prostate cancer who have been previously treated with docetaxel based regimen.

13. Compound according to claim 12, which is cabazitaxel.

14. Compound according to any one of claims 1 to 13, wherein said compound is administered in an amount to provide an AUC of about 991 ng•h/mL (CV 34%).

15. Compound according to any one of claims 1 to 14, wherein said compound is administered in an amount to provide an C_{max} of about 226 ng•h/mL (CV 107%).

16. Compound according to any one of claims 1 to 15 wherein said compound is administered in an amount to provide a plasma clearance of 48.5 L/h (CV 39%).

17. Compound according to any one of Claims 1 to 16, further comprising monitoring blood counts and measuring neutrophil levels in the patient.

18. Compound according to Claim 17, wherein said monitoring comprises taking a blood sample from the patient.

19. Compound according to Claim 18, further comprising discontinuing cabazitaxel treatment in a patient with a neutrophil count of ≤1,500 cells/mm^3.

20. A pharmaceutical composition comprising a compound as defined in claims 1 to 19.

21. A method of reducing the risk of neutropenia complications in a patient with prostate cancer being treated with a compound as defined in any one of claims 1 to 16, comprising monitoring blood counts in the patient at regular intervals during treatment of the patient; reducing the dose of the compound if the patient experiences febrile neutropenia or prolonged neutropenia; discontinuing the treatment if the patient's neutrophil count is ≤ 1,500 cells/mm^3; and optionally restarting thetreatment when the patient's neutrophil counts recover to a level ≥ 1,500 cells/mm^3.

22. The method according to claim 21, further comprising administering G-CSF to a patient prior to the compound administration, wherein said patient is considered to be at increased risk for neutropenia complications.

23. A method of reducing the risk of a severe hypersensitivity reaction in a patient with prostate cancer being treated with a compound as defined in any one of claims 1 to 16, comprising administering to the patient a medication to prevent hypersensitivity prior to the administration of cabazitaxel.

24. A method of increasing the survival of a patient with hormone refractory metastatic prostate cancer, comprising administering a clinically proven effective amount of a compound as defined in any one of claims 1 to 16 to the patient in combination with prednisone or prednisolone.

25. An article of manufacture comprising:

a) a packaging material;

b) cabazitaxel, and

c) a label or package insert contained within the packaging material indicating that cabazitaxel should not be given to patients with neutrophil counts of ≤1,500 cells/mm^3.

26. An article of manufacture comprising:

a) a packaging material;

b) cabazitaxel, and

c) a label or package insert contained within the packaging material indicating that severe hypersensitivity reactions can occur.

27. A package comprising cabazitaxel and a label, said label comprising one or more messages that:

a) the efficacy and safety of cabazitaxel in combination with prednisone were evaluated in patients with hormone refractory metastatic prostate cancer previously treated with a docetaxel containing regimen;

b) a total of 755 patients were randomized to receive either cabazitaxel 25 mg/m^3 every 3 weeks for a maximum of 10 cycles with prednisone mg orally daily, or to receive mitoxantrone 12 mg/m^2 intravenously every 3 weeks for a maximum of 10 cycles with prednisone 10 mg orally daily; or

c) the median number of cycles was 6 in the cabazitaxel group and 4 in the mitoxantrone group.

28. A package comprising cabazitaxel and a label, said label comprising one or more messages that:

a) neutropenic deaths have been reported;

b) frequent blood counts should be obtained to monitor for neutropenia; or

c) cabazitaxel should not be given if neutrophil counts are ≤ 1,500 cells/mm^3.

29. A package comprising cabazitaxel and a label said label comprising a printed statement which informs a reader that the mean C_{max} of cabazitaxel in patients with metastatic prostate cancer was 226 ng/mL(CV 107%).

30. A package comprising cabazitaxel and a label, said label comprising a printed statement which informs a reader that the mean AUC of cabazitaxel in patients with metastatic prostate cancer was 991 ng•h/mL (CV 34%).

31. A package comprising cabazitaxel and a label, said label comprising a printed statement which informs a reader that cabazitaxel has a plasma clearance of 48.5 L/h (CV 39%).

32. A method of promoting the use of cabazitaxel, the method comprising the step of conveying to a recipient at least one message selected from:

a) neutropenic deaths have been reported;

b) frequent blood counts should be obtained to monitor for neutropenia;

c) cabazitaxel should not be given if neutrophil counts are ≤ 1,500 cells/mm^3;

d) severe hypersensitivity can occur;

e) severe hypersensitivity can occur and may include generalized rash/erythema, hypotension and brochospasm;

f) discontinue cabazitaxel immediately if severe reactions occur;

g) discontinue cabazitaxel immediately if severe reactions occur and administer appropriate therapy; or

h) cabazitaxel is contraindicated in patients with a history of severe hypersensitivity reactions to cabazitaxel or drugs formulated with polysorbate 80.

33. A method of providing cabazitaxel, wherein said cabazitaxel is provided along with information indicating that:

a) neutropenic deaths have been reported;

b) frequent blood counts should be obtained to monitor for neutropenia;

c) cabazitaxel should not be given if neutrophil counts are ≤ 1,500 cells/mm^3;

d) severe hypersensitivity can occur;

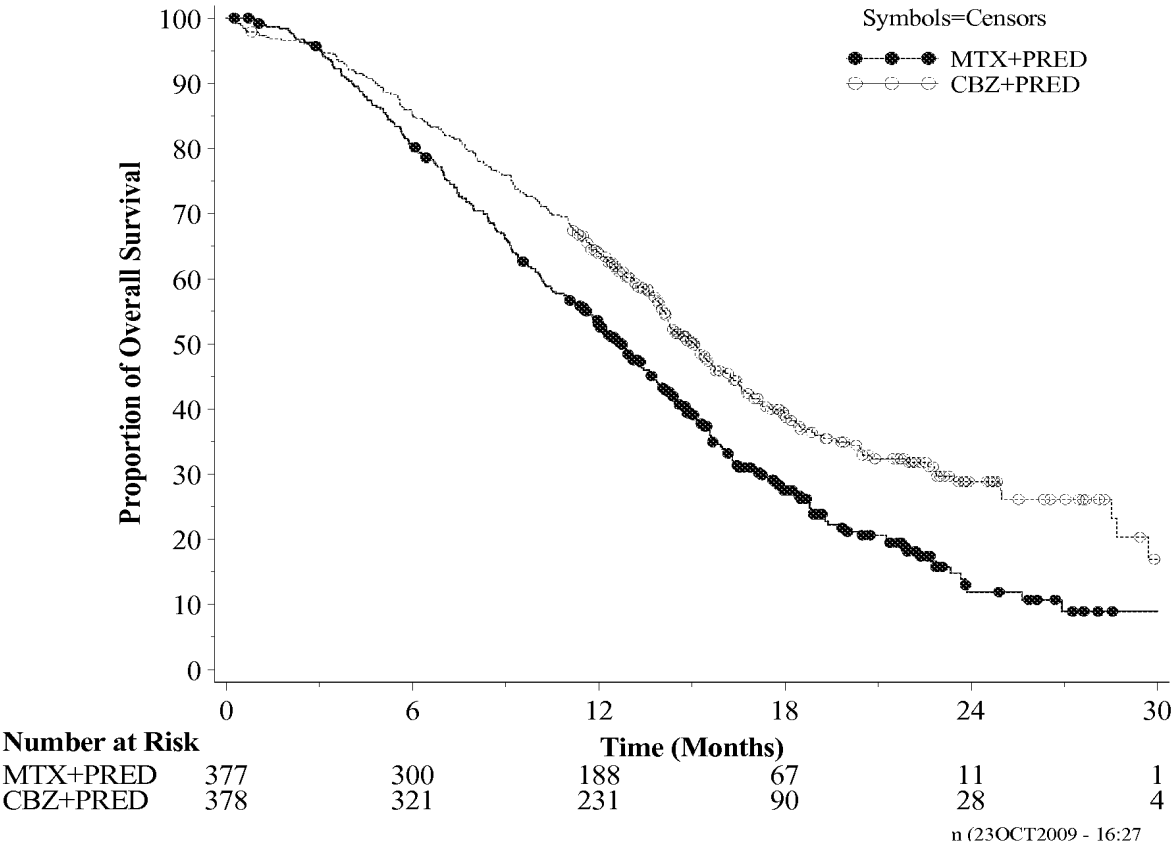
e) severe hypersensitivity can occur and may include generalized rash/erythema, hypotension and brochospasm;

f) discontinue cabazitaxel immediately if severe reactions occur;

g) discontinue cabazitaxel immediately if severe reactions occur and administer appropriate therapy; or

h) cabazitaxel is contraindicated in patients with a history of severe hypersensitivity reactions to cabazitaxel or drugs formulated with polysorbate 80.

FIGURE 1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CBZ+PRED
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90
80
70
60
50
40
30
20
10
0
0
6
12
18
24
30
Time (Months)
Number at Risk
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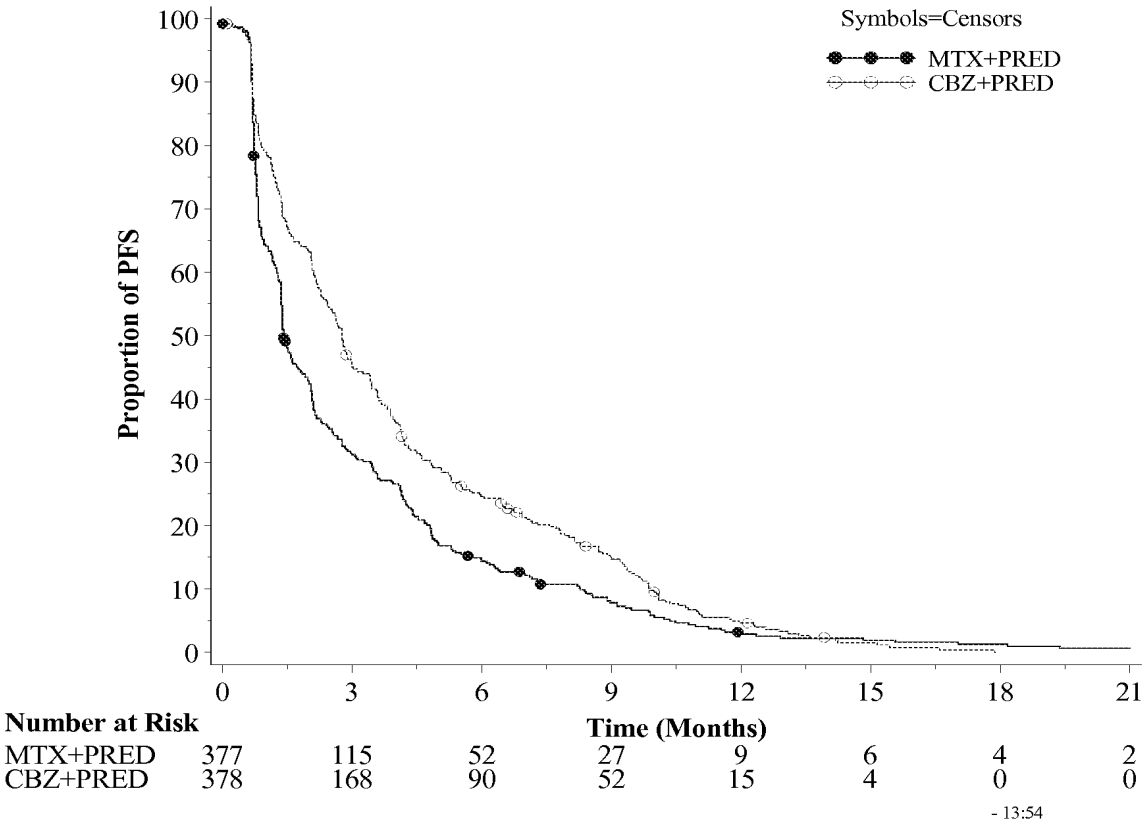
CBZ+PRED 378 321 231 90 28 4
n (23OCT2009 - 16:27

FIGURE 2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MTX+PRED
CBZ+PRED
Proportion of PFS
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90
80
70
60
50
40
30
20
10
0
0
3
6
9
12
15
18
21
Time (Months)
Number at Risk
MTX+PRED 377 115 52 27 9 6 4 2
CBZ+PRED 378 168 90 52 15 4 0 0
- 13:54

FIGURE 3

Factor	Patient number	Hazard ratio (95% CI)
All randomized patients	**755**	**0.70 (0.59–0.83)**
ECOG status: 0,1	694	0.68 (0.57–0.82)
ECOG status: 2	61	0.81 (0.48–1.38)
Measurable disease: No	350	0.72 (0.55–0.93)
Measurable disease: Yes	405	0.68 (0.54–0.85)
No. of prior chemotherapies: 1	528	0.67 (0.55–0.83)
No. of prior chemotherapies: ≥2	227	0.75 (0.55–1.02)
Age: <65 years	295	0.81 (0.61–1.08)
Age: ≥65 years	460	0.62 (0.50–0.78)
Rising PSA at baseline: No	159	0.88 (0.61–1.26)
Rising PSA at baseline: Yes	583	0.65 (0.53–0.80)
Total docetaxel dose: <225 mg/m²	59	0.96 (0.49–1.86)
Total docetaxel dose: ≥225 to 450 mg/m²	206	0.60 (0.43–0.84)
Total docetaxel dose: ≥450 to 675 mg/m²	217	0.83 (0.60–1.16)
Total docetaxel dose: ≥675 to 900 mg/m²	131	0.73 (0.48–1.10)
Total docetaxel dose: ≥900 mg/m²	134	0.51 (0.33–0.79)
Progression during docetaxel treatment	219	0.65 (0.47–0.90)
Progression <3 months after docetaxel	339	0.70 (0.55–0.91)
Progression ≥3 months after docetaxel	192	0.75 (0.51–1.11)

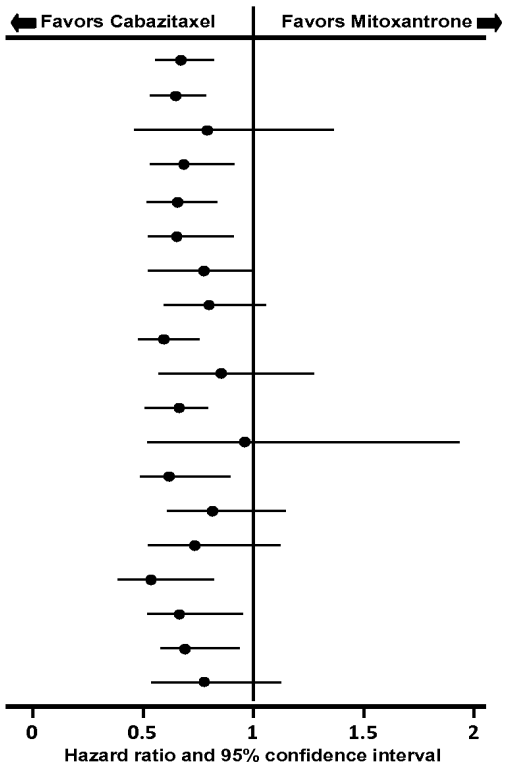


FIGURE 4

Proportion of patients with changes in ECOG performance status from baseline during treatment (safety population)

Improved: PS2 at baseline changed to 0 or 1 during treatment

Stable: no change

Worse: PS2 at baseline and changed to ≥3, or 0 or 1 at baseline changed to ≥2 during treatment

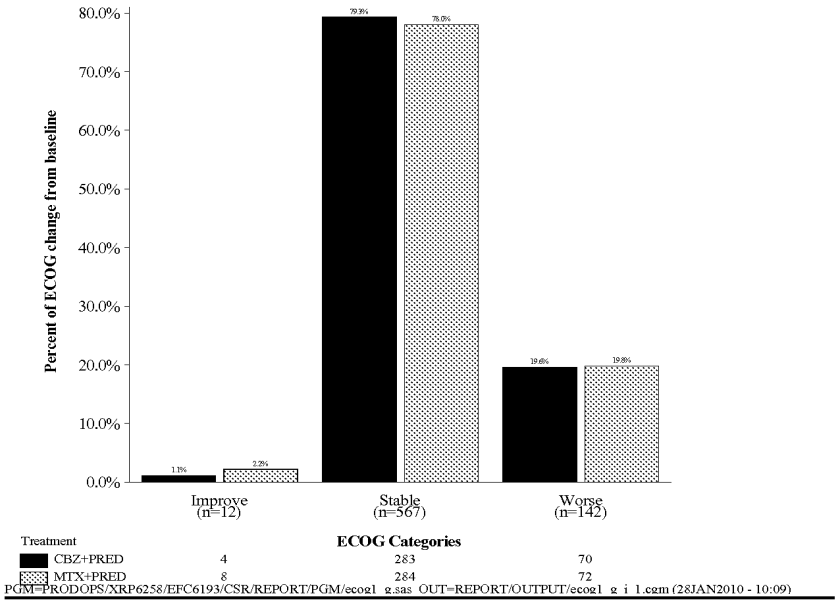


FIGURE 5

Proportion of patients with changes from baseline in the Present Pain Intensity score during treatment (ITT)

Improved: PPI score during treatment was lower vs baseline

Stable: no change

Worse: >1 unit increase in PPI score during treatment vs baseline

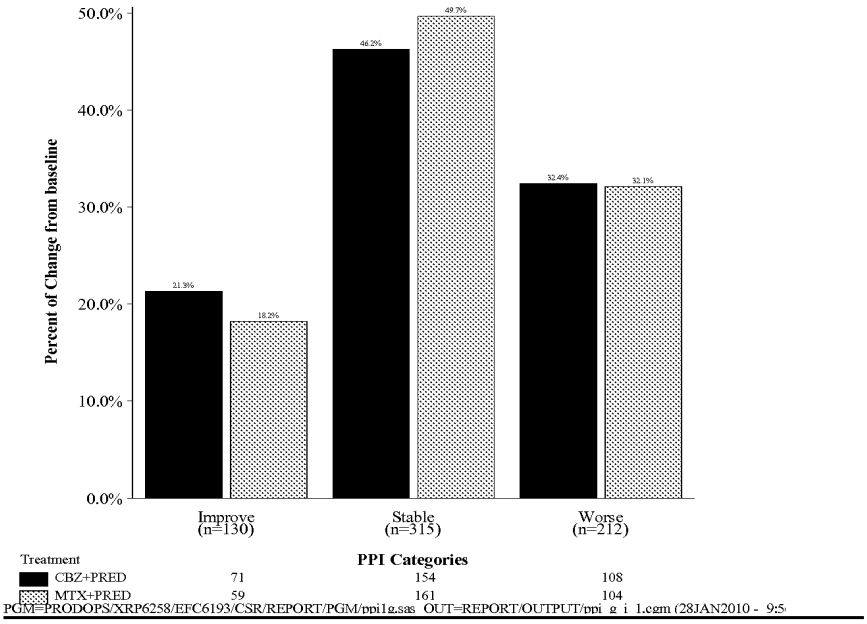


FIGURE 6

Mean area under the curve for PPI and analgesic scores by treatment cycle

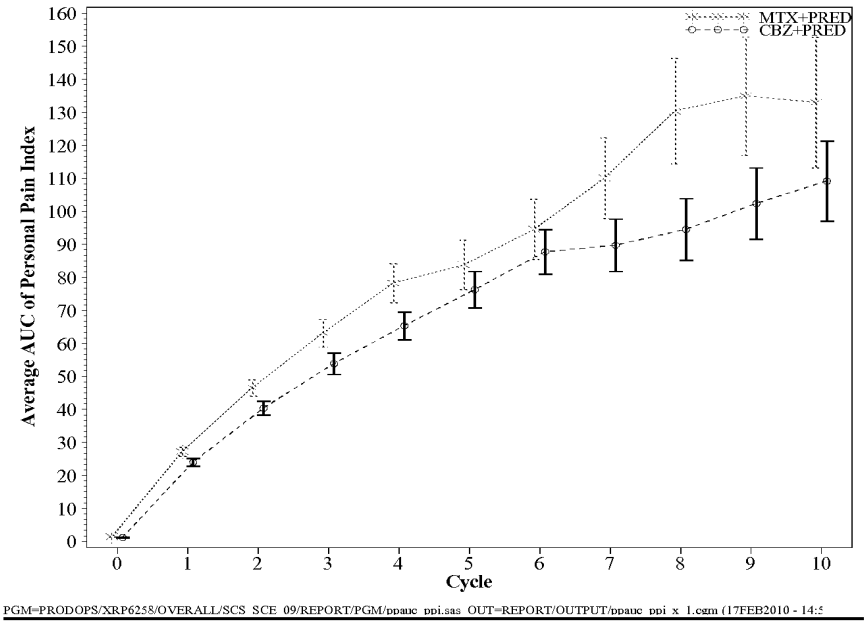
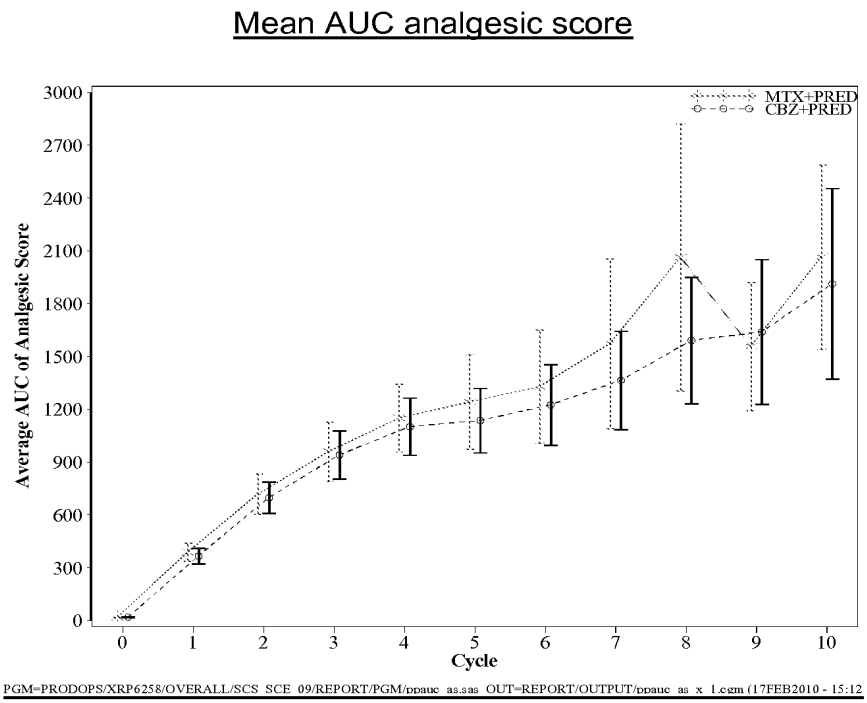


FIGURE 7


Mean AUC analgesic score
MTX+PRED
CBZ+PRED
Average AUC of Analgesic Score
3000
2700
2400
2100
1800
1500
1200
900
600
300
0
0
1
2
3
4
5
6
7
8
9
10
Cycle
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INTERNATIONAL SEARCH REPORT

International application No
PCT/IB2010/054866

A. CLASSIFICATION OF SUBJECT MATTER
INV. A61K31/164 A61K31/56 A61K45/06 A61P35/00
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 practical, search terms used)
EPO-Internal, BIOSIS, CHEM ABS Data, EMBASE, WPI Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	MITA, ALAIN C. ET AL: "Phase I and Pharmacokinetic Study of XRP6258 (RPR 116258A), a Novel Taxane, Administered as a 1-Hour Infusion Every 3 Weeks in Patients with Advanced Solid Tumors", CLINICAL CANCER RESEARCH , 15(2), 723-730 CODEN: CCREF4; ISSN: 1078-0432, 2009, XP002623316,	2-31,33
Y	the whole document	1
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[X] Further documents are listed in the continuation of Box C.

[X] 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 document but published on or after the international filing date

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"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
3 March 2011	14/03/2011

Name and mailing address of the ISA/	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Fax: (+31-70) 340-3016	Damiani, Federica

INTERNATIONAL SEARCH REPORT

International application No
PCT/IB2010/054866

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	TANNOCK IAN F ET AL: "Docetaxel plus prednisone or mitoxantrone plus prednisone for advanced prostate cancer.", THE NEW ENGLAND JOURNAL OF MEDICINE 7 OCT 2004 LNKD- PUBMED:15470213, vol. 351, no. 15, 7 October 2004 (2004-10-07), pages 1502-1512, XP002623317, ISSN: 1533-4406 * abstract	1-33
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(54) Title: METHODS FOR PREDICTING OR MONITORING WHETHER A PATIENT AFFECTED BY A CANCER IS RESPONSIVE TO A TREATMENT WITH A MOLECULE OF THE TAXOID FAMILY

(57) Abstract: The present invention concerns *in vitro* methods for predicting or monitoring whether a patient affected by a cancer is responsive to a treatment with a molecule of the taxoid family based on a resistance expression signature, kits for performing the methods, and methods for screening or identifying a compound suitable for improving the treatment of a cancer with a molecule of the taxoid family or for reducing the resistance development during the treatment of a cancer with the molecule of the taxoid family.

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METHODS FOR PREDICTING OR MONITORING WHETHER A PATIENT AFFECTED BY A CANCER IS RESPONSIVE TO A TREATMENT WITH A MOLECULE OF THE TAXOID FAMILY

FIELD OF THE INVENTION

The present invention relates to method for predicting the response to a treatment with a molecule of the taxoid family, kits and method for screening compounds useful for improve the treatment with the molecule.

BACKGROUND OF THE INVENTION

Prostate cancer became, based on frequency and in Western countries, the first cancer in men, behind the lung cancer. This disease is the second cause of cancer death in men. Since 2005, more than 60,000 men are touched by prostate cancer (PCa) each year and 10,000 men died of this disease. The efficiency of docetaxel chemotherapy (Taxotere®) in prostate cancer (CaP) has been demonstrated for the first time in 2004 in two clinical trials, i.e. TAX 327 and SWOG 99-16, with an increase in survival. Accordingly, docetaxel became today a treatment of choice of metastatic hormone-refractory prostate cancers and phase III clinical trials are ongoing to assess its efficacy for the treatment of high-risk localized prostate cancer. Taxotere® is currently approved in 5 different cancer types in Europe and the US: Prostate cancer, breast cancer, lung cancer, gastric cancer and head and neck cancer. However, in spite of the survival benefit provided by this molecule, docetaxel has a great toxicity and almost half of the patients treated with docetaxel develop a resistance to the chemotherapy either from the beginning, or in a secondary way. Moreover, docetaxel is not effective on all the types of cancer. For instance, in case of breast cancer, only 30 to 50% of the metastatic tumours respond to docetaxel. Resistance to taxanes is common and there is an increasing need to try and identify those patients who will respond to treatment.

A genomic analysis was performed with two cell lines (PC3 and DU145) resistant to a docetaxel dose of 11 nM (Patterson et al, *Oncogene*, 2006, 25: 6113-6122). The article discloses an expression signature of 30 genes. The authors also demonstrated the effect of STAT1 and Clusterin in an *in vitro* model for the docetaxel resistance. However, the validation of the expression of these two genes in the docetaxel-resistance has not been performed on tumours. The authors further demonstrated that resveratrol leads to a decreased expression of clusterin in docetaxel resistant cells and, then to an increase of apoptosis (Sallman et al, *Mol. Can. Ther.*, 2007, 6 : 2938-2947). Other groups used docetaxel resistance cell lines (PC3-R) in their research

(Lo Nigro et al, *BJU Int.*, 2008, 102 : 622-7). Some other groups used prostate cancer cell lines treated during a short period (24-72 h) with docetaxel for studying the role of genes in the docetaxel response.

In addition, a patent application WO 2006/062811 concerns a method for measuring resistance or sensitivity to docetaxel.

Therefore, there is still a strong need of a diagnostic method for predicting responsiveness to docetaxel and avoiding useless treatments. Indeed, before the initiation of the treatment, it is currently impossible to identify the patients who will respond to or who will have a resistance to docetaxel.

SUMMARY OF THE INVENTION

The present invention provides an expression signature specific of the docetaxel resistance in human prostate cancer. Based on this signature, the present invention provides a method for predicting or monitoring whether a patient affected by a cancer is responsive to a treatment with a molecule of the taxoid family.

Accordingly, the present invention concerns an *in vitro* method for predicting or monitoring whether a patient affected by a cancer is responsive to a treatment with a molecule of the taxoid family, wherein the method comprises: 1) providing a biological sample from said subject; 2) determining in the biological sample the expression level of the following genes JAM3, PCDH7, DCDC2, KHDRBS2, MFAP5, AUTS2, C2orf55, SLC3A1, AKAP12, ZNF649, RNASET2, NCF2, DLC1, CXCR4, CR594735, TRIM6 and MBNL3.

Preferably, the expression level is compared to a reference expression level, for instance the expression level of the genes in cell-lines or patients sensitive to the treatment by the molecule of the taxoid family. In particular, the over-expression of genes from Tables 1 and 3 and/or the under-expression of genes from Tables 2 and 4 are indicative of a resistance to the treatment by the molecule of the taxoid family. More preferably, the over-expression of genes selected from the group consisting of PCDH7, KHDRBS2, AUTS2, and C2orf55 and/or the under-expression of genes selected from the group consisting of JAM3, DCDC2, MFAP5, SLC3A1, AKAP12, ZNF649, RNASET2, NCF2, DLC1, CXCR4, CR594735, TRIM6, and MBNL3 are indicative of a resistance to the treatment by the molecule of the taxoid family. The expression level of genes can be determined by the quantity of protein or mRNA encoded by said genes. Preferably, the biological sample is a cancer sample.

In a preferred embodiment, the molecule of the taxoid family is selected from the group consisting of docetaxel, larotaxel, cabazitaxel (XRP6258), BMS-184476, BMS-188797, BMS-

275183, ortataxel, RPR 109881A, RPR 116258, NBT-287, PG-paclitaxel, ABRAXANE®, Tesetaxel, IDN 5390, Taxoprexin, DHA-paclitaxel, and MAC-321. More preferably, the molecule of the taxoid family is docetaxel.

Optionally, the method further comprises determining the expression level of at least one gene selected from the group consisting of FBN2, HIST2H2AA4, WDR31, FBXO15, THAP2, BF207040, HIST1H2BK, UNC13A, FAM27E3, LOC728613, FAM27E1, NPDC1, HIST1H2BL, UBE2J1, TJP2, HAVCR1, ZBTB24, CDKAL1, COQ3, TMCC3, ZFPM2, SLC3A2, LIMCH1, EPB41L2, B4GALT4, BX281122 and TPD52L1. Alternatively, the method further comprises determining the expression level of the genes FBN2, HIST2H2AA4, WDR31, FBXO15, THAP2, BF207040, HIST1H2BK, UNC13A, FAM27E3, LOC728613, FAM27E1, NPDC1, HIST1H2BL, UBE2J1, TJP2, HAVCR1, ZBTB24, CDKAL1, COQ3, TMCC3, ZFPM2, SLC3A2, LIMCH1, EPB41L2, B4GALT4, BX281122 and TPD52L1.

Optionally, the method further comprises determining the expression level of at least one gene selected from the group consisting of the genes listed in Tables 1-4.

Preferably, the biological sample is a cancer sample.

Preferably, the cancer is selected from the group consisting of the breast cancer, the lung cancer, the prostate cancer, the gastric cancer and the head and neck cancer, more preferably a prostate cancer.

The present invention also concerns kits and DNA chips suitable for this method. Accordingly, the present invention concerns a kit for predicting or monitoring whether a patient affected by a cancer is responsive to a treatment with a molecule of the taxoid family, wherein the kit comprises detection means selected from the group consisting of a pair of primers, a probe and an antibody specific to the genes JAM3, PCDH7, DCDC2, KHDRBS2, MFAP5, AUTS2, C2orf55, SLC3A1, AKAP12, ZNF649, RNASET2, NCF2, DLC1, CXCR4, CR594735, TRIM6 and MBNL3 or a DNA chip comprises a solid support which carries nucleic acids that are specific to the genes JAM3, PCDH7, DCDC2, KHDRBS2, MFAP5, AUTS2, C2orf55, SLC3A1, AKAP12, ZNF649, RNASET2, NCF2, DLC1, CXCR4, CR594735, TRIM6 and MBNL3. Optionally, the kit further comprises detection means selected from the group consisting of a pair of primers, a probe and an antibody specific to at least one gene selected from the group consisting of the genes the kit or DNA chip further comprises detection means for at least one gene selected from the group consisting of FBN2, HIST2H2AA4, WDR31, FBXO15, THAP2, BF207040, HIST1H2BK, UNC13A, FAM27E3, LOC728613, FAM27E1, NPDC1, HIST1H2BL, UBE2J1, TJP2, HAVCR1, ZBTB24, CDKAL1, COQ3, TMCC3, ZFPM2, SLC3A2, LIMCH1, EPB41L2, B4GALT4, BX281122 and TPD52L1. Optionally, the

DNA chip further carries nucleic acids that are specific to at least one gene selected from the group consisting of the genes the kit or DNA chip further comprises detection means for at least one gene selected from the group consisting of FBN2, HIST2H2AA4, WDR31, FBXO15, THAP2, BF207040, HIST1H2BK, UNC13A, FAM27E3, LOC728613, FAM27E1, NPDC1, HIST1H2BL, UBE2J1, TJP2, HAVCR1, ZBTB24, CDKAL1, COQ3, TMCC3, ZFPM2, SLC3A2, LIMCH1, EPB41L2, B4GALT4, BX281122 and TPD52L1.

The present invention further concerns methods for screening or identifying a compound suitable for improving the treatment of a cancer with a molecule of the taxoid family or for reducing the resistance development during the treatment of a cancer with a molecule of the taxoid family. In a first embodiment, the method comprises: 1) providing a cell-line with the genes PCDH7, KHDRBS2, AUTS2, and C2orf55 being over-expressed and the genes JAM3, DCDC2, MFAP5, SLC3A1, AKAP12, ZNF649, RNASET2, NCF2, DLC1, CXCR4, CR594735, TRIM6, and MBNL3 being under-expressed; 2) contacting said cell-line with a test compound; 3) determining the expression level of said genes; and, 4) selecting the compound which decreases the expression level of one or several of the over-expressed genes and increases the expression level of one or several of the under-expressed genes. In a second embodiment, the method comprises: 1) providing a cell-line sensitive to the molecule of the taxoid family; 2) contacting said cell-line with a test compound and the molecule of the taxoid family; 3) determining the expression level of the genes JAM3, PCDH7, DCDC2, KHDRBS2, MFAP5, AUTS2, C2orf55, SLC3A1, AKAP12, ZNF649, RNASET2, NCF2, DLC1, CXCR4, CR594735, TRIM6, and MBNL3; and, 4) selecting the compound which inhibits the appearance of an over-expression of the genes PCDH7, KHDRBS2, AUTS2, and C2orf55 and/or an under-expression of the genes JAM3, DCDC2, MFAP5, SLC3A1, AKAP12, ZNF649, RNASET2, NCF2, DLC1, CXCR4, CR594735, TRIM6, and MBNL3.

DETAILED DESCRIPTION OF THE INVENTION

The present invention provides the identification of protein coding genes involved in the mechanism of docetaxel resistance in prostate cancer treatment.

The inventors prepared *in vitro* cellular models of docetaxel resistant prostate cancer by selecting cell clones by pharmaceutical pressure from a cellular model of prostate cancer i.e. IGR-CaP1 cell line. Cell lines became resistant to increasing doses of docetaxel (5nM ; 12nM ; 25nM, 50nM ; 100nM ; 200nM). The prostate cancer cell line IGR-CaP1 is described in CNCM deposit number I-4126 on February 10, 2009.

A micro-array genomic analysis was performed by comparing sensitive and resistant IGR-CaP1 cell line at six docetaxel concentrations (5; 12; 25, 50, 100 and 200 nM), as detailed in the experimental section. This analysis led to the identification of 1081 genes associated to the resistance to increasing doses of docetaxel (with a *P* value ≤ 1e-3 and a fold change between the first and the last doses of docetaxel (between 5 and 200 nM) ≥ 2). In this signature, 772 genes are over-expressed and 309 genes are under-expressed. These genes are presented in Tables 1 to 4.

The inventors realized a second microarray experiment in which the same IGR-CaP1 resistant cell lines have been used. Contrasting with the first analysis, the different resistant cell lines were cultured in the total absence of docetaxel for 2 passages before RNA extraction and microarray analysis, to retain only irreversible resistance mechanisms. To enhance the robustness of bioinformatics analysis, this analysis was generated from biological duplicates.

The second microarray genomic analysis was performed by comparing sensitive and resistant IGR-CaP1 cell line at six docetaxel concentrations (5; 12; 25, 50, 100 and 200 nM), as detailed in the experimental section. This analysis led to the identification of 486 genes associated to the resistance to increasing doses of docetaxel (with a *P* value ≤ 1e-5 and a fold change between the first and the last doses of docetaxel (between 5 and 200 nM) ≥ 2). Table 5 showed the 44 genes which were found in common in the two microarray analyses. Among these genes, a final list of 17 genes was selected with 4 genes that were overexpressed in a dose-dependent manner and 13 genes that were under-expressed in a dose-dependent manner (Table 6).

On this basis, the inventors identified a set of genes whose combined expression profiles allow to distinguish patients between responder and non-responder to a treatment with a molecule of the taxoid family. A "responder" or "responsive" patient refers to a patient who shows or will show a clinically significant recovery when treated in the cancer when treated with a molecule of the taxoid family. In particular, the size of the tumor will no more increase, decrease or the tumor will disappear.

Therefore, the present invention discloses an expression signature useful for *in vitro* method for predicting whether a patient suffering of a cancer would be responsive to a treatment with a molecule of the taxoid family.

In a preferred embodiment of the invention, the method comprises determining the expression level of genes of Table 6, namely JAM3, PCDH7, DCDC2, KHDRBS2, MFAP5, AUTS2, C2orf55, SLC3A1, AKAP12, ZNF649, RNASET2, NCF2, DLC1, CXCR4, CR594735, TRIM6, and MBNL3. In particular, when comparing with the expression level of the genes in cell-lines or patients sensitive to the treatment by the molecule of the taxoid family, the over-

expression of genes PCDH7, KHDRBS2, AUTS2, and C2orf55, and/or the under-expression of genes JAM3, DCDC2, MFAP5, SLC3A1, AKAP12, ZNF649, RNASET2, NCF2, DLC1, CXCR4, CR594735, TRIM6, and MBNL3 are indicative of a resistance to the treatment by the molecule of the taxoid family. In addition, the expression level of one or several genes of Table 5 may further be determined, namely genes selected from the group consisting of FBN2, HIST2H2AA4, WDR31, FBXO15, THAP2, BF207040, HIST1H2BK, UNC13A, FAM27E3, LOC728613, FAM27E1, NPDC1, HIST1H2BL, UBE2J1, TJP2, HAVCR1, ZBTB24, CDKAL1, COQ3, TMCC3, ZFPM2, SLC3A2, LIMCH1, EPB41L2, B4GALT4, BX281122 and TPD52L1. More particularly, when comparing with the expression level of the genes in cell-lines or patients sensitive to the treatment by the molecule of the taxoid family, the over-expression of one or several genes selected among FBN2, HIST2H2AA4, WDR31, FBXO15, THAP2, BF207040, HIST1H2BK, UNC13A, FAM27E3, LOC728613, FAM27E1, NPDC1 and HIST1H2BL, and/or the under-expression of one or several genes selected among UBE2J1, TJP2, HAVCR1, ZBTB24, CDKAL1, COQ3, TMCC3, ZFPM2, SLC3A2, LIMCH1, EPB41L2, B4GALT4, BX281122 and TPD52L1 may be indicative of a resistance to the treatment by the molecule of the taxoid family. Optionally, the expression level of at least 6, 7, 8, 9, 10, 15, 20, 25, 30, 40 or all genes of Table 5 are determined. Optionally, the expression level of at least 6, 7, 8, 9, 10, 15, 20, 25, 30, 40, 50, 60, 70, 80, 90, 100, 150, 200, 300, 500 or 1000 additional genes of Tables 1-4 may also be determined. However, in a preferred embodiment, the method does not comprise the determination of the expression level of more than 200, 100 or 50 genes, more preferably no more than 50, 40, 30 or 20 genes.

Alternatively, the method comprises determining the expression level of genes from the present expression signature (see Tables 1 to 4 or Tables 1-6) in a biological sample of said patient. In particular, the method comprises determining the expression level of at least 5 genes selected from the group consisting of the genes listed in Tables 1-6 or in Tables 1 and 2, in a biological sample of said patient. Preferably, the method comprises determining the expression level of at least 6, 7, 8, 9, 10, 15, 20, 25, 30, 40, 50, 60, 70, 80, 90, 100, 150, 200, 300, 500 or 1000 genes of Tables 1-6 or Tables 1 and 2. Optionally, the method further comprises determining the expression level of at least one gene selected from the group consisting of the genes listed in Tables 3 and 4. Optionally, the method further comprises determining the expression level of at least one gene selected from the group consisting of the genes listed in Tables 5 and 6. Preferably, the expression level of at least 2, 3, 5, 10, 15 or 20 genes selected from the group consisting of the genes listed in Tables 3-6 or Tables 3 and 4, is determined. Preferably, the expression level of at least 2, 3, 5, 10, 15 or 20 genes selected from the group

consisting of the genes listed in Tables 5 and 6, is also determined. Alternatively, the method comprises determining the expression level of 5 to 1081 genes of Tables 1 to 4, optionally of 10 to 500, 20 to 300, 30 to 250, 50 to 250, 20 to 250, 30 to 200, 40 to 150, 50 to 100, 60 to 90 or 70 to 80. However, in a preferred embodiment, the method does not comprise the determination of the expression level of more than 200, 100 or 50 genes, more preferably no more than 50, 40, 30 or 20 genes.

By "predicting" or "prediction" is intended herein the likelihood that a patient will respond or not to a molecule of the taxoid family and also the extent of the response. Predictive methods of the invention can be used clinically to make treatment decisions by choosing the most appropriate treatment modalities for any particular patient.

Therefore, the present invention also concerns a method for selecting a patient suffering of a cancer for a treatment with a molecule of the taxoid family, comprising determining the expression level of genes of Table 6, namely JAM3, PCDH7, DCDC2, KHDRBS2, MFAP5, AUTS2, C2orf55, SLC3A1, AKAP12, ZNF649, RNASET2, NCF2, DLC1, CXCR4, CR594735, TRIM6, and MBNL3 in a biological sample of said patient and selecting the patient predicted to be responsive to a treatment with a molecule of the taxoid family. In particular, when comparing with the expression level of the genes in cell-lines or patients sensitive to the treatment by the molecule of the taxoid family, the over-expression of genes PCDH7, KHDRBS2, AUTS2, and C2orf55, and/or the under-expression of genes JAM3, DCDC2, MFAP5, SLC3A1, AKAP12, ZNF649, RNASET2, NCF2, DLC1, CXCR4, CR594735, TRIM6, and MBNL3 are indicative of a resistance to the treatment by the molecule of the taxoid family. Optionally, the method further comprises determining the expression level of at least one gene selected from the group consisting of genes of Table 5, namely FBN2, HIST2H2AA4, WDR31, FBXO15, THAP2, BF207040, HIST1H2BK, UNC13A, FAM27E3, LOC728613, FAM27E1, NPDC1, HIST1H2BL, UBE2J1, TJP2, HAVCR1, ZBTB24, CDKAL1, COQ3, TMCC3, ZFPM2, SLC3A2, LIMCH1, EPB41L2, B4GALT4, BX281122 and TPD52L1. More particularly, when comparing with the expression level of the genes in cell-lines or patients sensitive to the treatment by the molecule of the taxoid family, the over-expression of one or several genes selected among FBN2, HIST2H2AA4, WDR31, FBXO15, THAP2, BF207040, HIST1H2BK, UNC13A, FAM27E3, LOC728613, FAM27E1, NPDC1 and HIST1H2BL, and/or the under-expression of one or several genes selected among UBE2J1, TJP2, HAVCR1, ZBTB24, CDKAL1, COQ3, TMCC3, ZFPM2, SLC3A2, LIMCH1, EPB41L2, B4GALT4, BX281122 and TPD52L1 may be indicative of a resistance to the treatment by the molecule of the taxoid family. Optionally, the expression level of at least 6, 7, 8, 9, 10, 15, 20, 25, 30, 40 or all genes of Table 5

are determined. Optionally, the expression level of at least 6, 7, 8, 9, 10, 15, 20, 25, 30, 40, 50, 60, 70, 80, 90, 100, 150, 200, 300, 500 or 1000 additional genes of Tables 1-4 may also be determined. However, in a preferred embodiment, the method does not comprise the determination of the expression level of more than 200, 100 or 50 genes, more preferably no more than 50, 40, 30 or 20 genes.

In addition, the present invention also concerns a method for selecting a patient suffering of a cancer for a treatment with a molecule of the taxoid family, comprising determining the expression level of at least 5, 6, 7, 8, 9, 10, 15, 20, 25, 30, 40, 50, 60, 70, 80, 90, 100, 150, 200, 300, 500 or 1000 genes selected from the group consisting of the genes listed in Tables 1-6 or Tables 1 and 2, in a biological sample of said patient and selecting the patient predicted to be responsive to a treatment with a molecule of the taxoid family. Optionally, the method further comprises determining the expression level of at least one gene selected from the group consisting of the genes listed in Tables 3-6 or Tables 3 and 4.

In an embodiment, the genes, preferably the additional genes, are selected from Tables 1 and 2 on the criteria of "fold change". Accordingly, the genes with the greatest fold change (in absolute value) between 5nM of docetaxel and 200nM of docetaxel are chosen. For instance, the genes associated with a fold change greater (in absolute value) than 3, preferably than 4, 5, 6, 7, 8, 9 or 10, are selected. In a particular embodiment, the genes are selected from the group consisting of ENST00000399723, BI836406, C10orf79, AK022962, TMTC1, LOC728295, SUSD5, WNT6, BC044624, AY358241, ZNF251, ST6GAL2, LOC643401, NOV, CLGN, PROM1, SPEF2, FLRT2, RGS2, FOXP2, TRIM55, PKD2L1, RP4-692D3.1, CB985069, ARL14, AY831680, XRN1, THAP5, ZNF248, BC016022, PLAG1, THC2724353, THC2488083, C5orf41, BMS1P5, BMS1, THC2627008, PLA2G4A, DPY19L2, VCX2, PPP1R1C, GLT25D2, KIAA1841, IFIT2, ZNF596, TSPAN19, BC029907, C10orf107, ZNF594, AMPD1, C21orf88, THC2694827, HSPC105, IFI44, THC2662262, FAM84A, DNAH7, KHDRBS2, NANP, AK091357, N4BP2L1, FAM105A, CA941346, CCDC68, CASC1, FAM90A12, PBX1, THC2739159, KCNQ2, ANXA1, AL122040, THC2655194, ENST00000342608, DSC2, ENOX1, IL13, BG571904, BX455216, LOC729085, BG188151, LOC729409, C1orf103, PPP1R14C, NAIP, C13orf31, GOLGA8E, AK022848, CXorf22, KIF5C, LRRCC1, FAM81B, ID2, CMYA5, C1orf194, TTC18, tcag7.1314, ZNF385B, ADAMTS6, RHOU, ENST00000378850, C2orf55, GPR83, LRRIQ1, WDR31, DEFB126, ARMETL1, LOC642826, LOC129881, C2orf13, THC2553512, ACVR1C, ZNF207, ANTXR1, CHD9, THC2526838, ABCA12, TncRNA, FKTN, PTPRG, ZNF233, ENST00000370378, FANK1, PCM1, SERPINI1, ARID4B, KIAA1377, FGF7, CV339166, LINCR, DA834198, CFH, SCG2, ARHGEF10, DA093175, GOLGA8A, AK021467, LOC283666, FLJ35767, THC2725553, ZNF430, CCDC141, MAP3K13, CCDC66, THC2727226, THC2528990, THC2718728, THC2507829, AK123972, EDEM3, DB304731, TPD52L1,

MFAP5, EHF, NCF2, TRIM6, PERLD1, ATXN1, INHBB, CR627122, JAM3, CXCL14, CR594735, FLJ11235, C15orf52, LIMCH1, LOH11CR2A, BX281122, GPR110, ARNT2, ATP6V0A4, PDGFRB, ELA3B, NEDD9, MYH6, SLC35F2, HAS3, COLEC12, SLC3A2, AW993939, RUNX2, SUSD3, PLAU, SLC22A3, FCRL4, DOCK2, SOX3, THC2616558, RNASET2, LOC100130360, IL1R2, MGAT5B, TCF7L1, AF222857, AHNAK, HOXB8, S100A16, INSIG1 and DCDC2. Preferably, the genes are selected from the group consisting of ENST00000399723, BI836406, C10orf79, AK022962, TMTC1, LOC728295, SUSD5, WNT6, BC044624, AY358241, ZNF251, ST6GAL2, LOC643401, NOV, CLGN, PROM1, SPEF2, FLRT2, RGS2, FOXP2, TRIM55, PKD2L1, RP4-692D3.1, CB985069, ARL14, AY831680, XRN1, THAP5, ZNF248, BC016022, PLAG1, THC2724353, THC2488083, C5orf41, BMS1P5, BMS1, THC2627008, PLA2G4A, DPY19L2, VCX2, PPP1R1C, GLT25D2, KIAA1841, IFIT2, ZNF596, TSPAN19, BC029907, C10orf107, ZNF594, AMPD1, C21orf88, THC2694827, HSPC105, IFI44, THC2662262, FAM84A, DNAH7, KHDRBS2, NANP, AK091357, N4BP2L1, FAM105A, CA941346, CCDC68, CASC1, FAM90A12, PBX1, THC2739159, KCNQ2, ANXA1, AL122040, THC2655194, ENST00000342608, DSC2, ENOX1, IL13, BG571904, BX455216, LOC729085, BG188151, LOC729409, C1orf103, PPP1R14C, NAIP, C13orf31, GOLGA8E, AK022848, CXorf22, KIF5C, TPD52L1, MFAP5, EHF, NCF2, TRIM6, PERLD1, ATXN1, INHBB, CR627122, JAM3, CXCL14, CR594735, FLJ11235, C15orf52, LIMCH1, LOH11CR2A, BX281122, GPR110, ARNT2, ATP6V0A4, PDGFRB, ELA3B, NEDD9, MYH6, SLC35F2, HAS3, COLEC12, SLC3A2, AW993939, RUNX2 and SUSD3. More preferably, the genes are selected from the group consisting of ENST00000399723, BI836406, C10orf79, AK022962, TMTC1, LOC728295, SUSD5, WNT6, BC044624, AY358241, ZNF251, ST6GAL2, LOC643401, NOV, CLGN, PROM1, SPEF2, FLRT2, RGS2, FOXP2, TRIM55, PKD2L1, RP4-692D3.1, TPD52L1, MFAP5, EHF, NCF2, TRIM6, PERLD1, ATXN1, INHBB, CR627122, JAM3, CXCL14 and CR594735. In the most preferred embodiment, the genes are selected from the group consisting of ENST00000399723, BI836406, C10orf79, AK022962, TMTC1, LOC728295, SUSD5, WNT6, BC044624, TPD52L1, MFAP5, EHF, NCF2, TRIM6, PERLD1, ATXN1, INHBB and CR627122.

In another embodiment, the genes, preferably the additional genes, are selected from Tables 1 on the criteria of "fold change". Accordingly, the over-expressed genes with the greatest fold change (in absolute value) are chosen. For instance, the over-expressed genes associated with a fold change greater (in absolute value) than 3, preferably than 4, 5, 6, 7, 8, 9 or 10, are selected. In a particular embodiment, the genes are selected from the group consisting of ENST00000399723, BI836406, C10orf79, AK022962, TMTC1, LOC728295, SUSD5, WNT6, BC044624, AY358241, ZNF251, ST6GAL2, LOC643401, NOV, CLGN, PROM1, SPEF2, FLRT2, RGS2, FOXP2, TRIM55, PKD2L1, RP4-692D3.1, CB985069, ARL14, AY831680, XRN1, THAP5, ZNF248, BC016022, PLAG1, THC2724353, THC2488083, C5orf41, BMS1P5, BMS1, THC2627008, PLA2G4A, DPY19L2, VCX2, PPP1R1C, GLT25D2, KIAA1841, IFIT2, ZNF596, TSPAN19,

BC029907, C10orf107, ZNF594, AMPD1, C21orf88, THC2694827, HSPC105, IFI44, THC2662262, FAM84A, DNAH7, KHDRBS2, NANP, AK091357, N4BP2L1, FAM105A, CA941346, CCDC68, CASC1, FAM90A12, PBX1, THC2739159, KCNQ2, ANXA1, AL122040, THC2655194, ENST00000342608, DSC2, ENOX1, IL13, BG571904, BX455216, LOC729085, BG188151, LOC729409, C1orf103, PPP1R14C, NAIP, C13orf31, GOLGA8E, AK022848, CXorf22, KIF5C, LRRCC1, FAM81B, ID2, CMYA5, C1orf194, TTC18, tcag7.1314, ZNF385B, ADAMTS6, RHOU, ENST00000378850, C2orf55, GPR83, LRRIQ1, WDR31, DEFB126, ARMETL1, LOC642826, LOC129881, C2orf13, THC2553512, ACVR1C, ZNF207, ANTXR1, CHD9, THC2526838, ABCA12, TncRNA, FKTN, PTPRG, ZNF233, ENST00000370378, FANK1, PCM1, SERPINI1, ARID4B, KIAA1377, FGF7, CV339166, LINCR, DA834198, CFH, SCG2, ARHGEF10, DA093175, GOLGA8A, AK021467, LOC283666, FLJ35767, THC2725553, ZNF430, CCDC141, MAP3K13, CCDC66, THC2727226, THC2528990, THC2718728, THC2507829, AK123972, EDEM3 and DB304731. Preferably, the genes are selected from the group consisting of ENST00000399723, BI836406, C10orf79, AK022962, TMTC1, LOC728295, SUSD5, WNT6, BC044624, AY358241, ZNF251, ST6GAL2, LOC643401, NOV, CLGN, PROM1, SPEF2, FLRT2, RGS2, FOXP2, TRIM55, PKD2L1, RP4-692D3.1, CB985069, ARL14, AY831680, XRN1, THAP5, ZNF248, BC016022, PLAG1, THC2724353, THC2488083, C5orf41, BMS1P5, BMS1, THC2627008, PLA2G4A, DPY19L2, VCX2, PPP1R1C, GLT25D2, KIAA1841, IFIT2, ZNF596, TSPAN19, BC029907, C10orf107, ZNF594, AMPD1, C21orf88, THC2694827, HSPC105, IFI44, THC2662262, FAM84A, DNAH7, KHDRBS2, NANP, AK091357, N4BP2L1, FAM105A, CA941346, CCDC68, CASC1, FAM90A12, PBX1, THC2739159, KCNQ2, ANXA1, AL122040, THC2655194, ENST00000342608, DSC2, ENOX1, IL13, BG571904, BX455216, LOC729085, BG188151, LOC729409, C1orf103, PPP1R14C, NAIP, C13orf31, GOLGA8E, AK022848, CXorf22 and KIF5C. More preferably, the genes are selected from the group consisting of ENST00000399723, BI836406, C10orf79, AK022962, TMTC1, LOC728295, SUSD5, WNT6, BC044624, AY358241, ZNF251, ST6GAL2, LOC643401, NOV, CLGN, PROM1, SPEF2, FLRT2, RGS2, FOXP2, TRIM55, PKD2L1 and RP4-692D3.1. In the most preferred embodiment, the genes are selected from the group consisting of ENST00000399723, BI836406, C10orf79, AK022962, TMTC1, LOC728295, SUSD5, WNT6 and BC044624.

In a further embodiment, the genes, preferably the additional genes are selected from Tables 2 on the criteria of "fold change". Accordingly, the under-expressed genes with the greatest fold change (in absolute value) are chosen. For instance, the under-expressed genes associated with a fold change greater (in absolute value) than 3, preferably than 4, 5, 6, 7, 8, 9 or 10, are selected. In a particular embodiment, the genes are selected from the group consisting of TPD52L1, MFAP5, EHF, NCF2, TRIM6, PERLD1, ATXN1, INHBB, CR627122, JAM3, CXCL14, CR594735, FLJ11235, C15orf52, LIMCH1, LOH11CR2A, BX281122, GPR110, ARNT2, ATP6V0A4, PDGFRB, ELA3B, NEDD9, MYH6, SLC35F2, HAS3, COLEC12, SLC3A2, AW993939, RUNX2,

SUSD3, PLAU, SLC22A3, FCRL4, DOCK2, SOX3, THC2616558, RNASET2, LOC100130360, IL1R2, MGAT5B, TCF7L1, AF222857, AHNAK, HOXB8, S100A16, INSIG1, DCDC2. Preferably, the genes are selected from the group consisting of TPD52L1, MFAP5, EHF, NCF2, TRIM6, PERLD1, ATXN1, INHBB, CR627122, JAM3, CXCL14, CR594735, FLJ11235, C15orf52, LIMCH1, LOH11CR2A, BX281122, GPR110, ARNT2, ATP6V0A4, PDGFRB, ELA3B, NEDD9, MYH6, SLC35F2, HAS3, COLEC12, SLC3A2, AW993939, RUNX2 and SUSD3. More preferably, the genes are selected from the group consisting of TPD52L1, MFAP5, EHF, NCF2, TRIM6, PERLD1, ATXN1, INHBB, CR627122, JAM3, CXCL14 and CR594735. In the most preferred embodiment, the genes are selected from the group consisting of TPD52L1, MFAP5, EHF, NCF2, TRIM6, PERLD1, ATXN1, INHBB and CR627122.

Optionally, the method of the invention further comprises determining the expression level of at least one gene, preferably at least one additional gene, selected from the group consisting of the genes listed in Table 3 and 4, preferably TFPI2, PCDH7, SMAD9, AK090762, RAB39B, BF831953, AL050204, VCX, ITGA2, CXCR4, SLC16A10, PDE1A, MAL, KRT80, FXYD2 and AK3L1, more preferably TFPI2, PCDH7, SMAD9, AK090762, RAB39B, BF831953, AL050204, VCX, CXCR4, SLC16A10, PDE1A, MAL, and the most preferably TFPI2, PCDH7, SMAD9, CXCR4 and SLC16A10. In particular, the expression level of at least 2, 3, 4, 5, 6, 7, 8, 9 or 10 genes was determined. In an embodiment, the method of the invention further comprises determining the expression level of at least one gene, preferably at least one additional gene, selected from the group consisting of TFPI2, PCDH7, SMAD9, AK090762, RAB39B, BF831953, AL050204, VCX, ITGA2, AKAP9, AUTS2, CEP152, SLITRK6, CCPG1, MANEAL, THC2733296, CD55, ANKRD18A, LAT2, BRCA2, LRP2BP, LPHN2 and ITGB8, preferably TFPI2, PCDH7, SMAD9, AK090762, RAB39B, BF831953, AL050204, VCX and ITGA2, more preferably TFPI2, PCDH7, SMAD9, AK090762, RAB39B, BF831953, AL050204 and VCX, the most preferably TFPI2, PCDH7 and SMAD9. In another embodiment, the method of the invention further comprises determining the expression level of at least one gene, preferably at least one additional gene, selected from the group consisting of CXCR4, SLC16A10, PDE1A, MAL, KRT80, FXYD2, AK3L1, LIN7A, GPR177, TNF, WNT2B, CGNL1, RPS6KA2, SUNC1, DIAPH2, AKAP12, NRG1, PDE4DIP, IL1R1, LZTS1, SLC3A1, MGST1, ACOT9, SLC12A3, ASRGL1 and HRG, preferably CXCR4, SLC16A10, PDE1A, MAL, KRT80, FXYD2 and AK3L1, more preferably CXCR4, SLC16A10, PDE1A and MAL, and the most preferably CXCR4 and SLC16A10.

The method can comprise the step of comparing the expression levels of the genes determined in the sample to reference or control expression levels. The reference or control expression levels are determined with a sample of cells, preferably cancer cells, which are

sensitive to the molecule of the taxoid family. Alternatively, reference or control expression levels are determined with a sample of patients or subjects sensitive to the treatment with the molecule of the taxoid family. Hence, an over-expressed gene herein refers to a gene having an increased expression in comparison to the expression level of this gene in a sensitive cell, and an under-expressed gene herein refers to a gene having a decreased expression in comparison to the expression level of this gene in a sensitive cell. However, the man skilled in art understands that other references can be used. For instance, the invention also contemplates a reference level corresponding to the expression level in a cell resistant to the molecule of the taxoid family.

In particular, when the genes selected from Table 1 (and optionally from Table 3) are over-expressed, one can predict that the patient would be resistant to a treatment with a molecule of the taxoid family. On the contrary, when the genes selected from Table 1 (and optionally from Table 3) are not over-expressed, one can predict that the patient would be responsive to a treatment with a molecule of the taxoid family. The same rules applied to the over-expressed genes in Tables 5 and 6 (with a positive fold change (FC)). At the opposite, when the genes selected from Table 2 (and optionally from Table 4) are under-expressed, one can predict that the patient would be resistant to a treatment with a molecule of the taxoid family. On the contrary, when the genes selected from Table 2 (and optionally from Table 4) are not under-expressed, one can predict that the patient would be responsive to a treatment with a molecule of the taxoid family. The same rules applied to the under-expressed genes in Tables 5 and 6 (with a negative fold change (FC)).

In addition, the genes can be selected in such a way that they comprise some over-expressed genes and some under-expressed ones. In this embodiment, the selected genes can comprise at least 5, 6, 7, 8, 9, 10, 15, 20, 25, 30, 40, 50, 60, 70, 80, 90, 100, or 150 genes of Tables 1 and 3 and overexpressed genes of Tables 5 and 6, and at least 5, 6, 7, 8, 9, 10, 15, 20, 25, 30, 40, 50, 60, 70, 80, 90, 100, or 150 genes of Tables 2 and 4 and under-expressed genes of Tables 5 and 6. In a particular embodiment, the genes are selected in Tables 1 and 3 for overexpressed genes and in Tables 2 and 4 for the under-expressed genes. Alternatively, they can be selected in such a way that they comprise only over-expressed or under-expressed genes. In a preferred embodiment, the genes are selected among the genes having the greatest fold change.

In addition to the genes selected from Tables 1 to 6, optionally 1 to 4, the method can also comprise the determination of the expression level for control genes. The control genes are chosen among the genes known to have a constant expression level, in particular between sensitive and resistant cells to a molecule of the taxoid family. In addition, the expression level

of at least one control gene is determined in order to normalize the result. For instance, the control gene can be GAPDH, 18S RNA, beta-actine or lamin.

The molecule of the taxoid family refers to a class of anti-tumoral drugs belonging to the taxane family. It can be selected from the group consisting of paclitaxel, docetaxel and analogs, prodrugs or formulations thereof. In particular, analogs, prodrugs or formulations thereof can be for instance selected in the group consisting of larotaxel (also called XRP9881; Sanofi-Aventis), cabazitaxel (XRP6258) (Sanofi-Aventis), BMS-184476 (Bristol-Meyer-Squibb), BMS-188797 (Bristol-Meyer-Squibb), BMS-275183 (Bristol-Meyer-Squibb), ortataxel (also called IDN 5109, BAY 59-8862 or SB-T-101131 ; Bristol-Meyer-Squibb), RPR 109881A (Bristol-Meyer-Squibb), RPR 116258 (Bristol-Meyer-Squibb), NBT-287 (TAPESTRY), PG-paclitaxel (also called CT-2103, PPX, paclitaxel poliglumex, paclitaxel polyglutamate or Xyotax^{TM}), ABRAXANE® (also called Nab-Paclitaxel ; ABRAXIS BIOSCIENCE), Tesetaxel (also called DJ-927), IDN 5390 (INDENA), Taxoprexin (also called docosahexanoic acid-paclitaxel ; PROTARGA), DHA-paclitaxel (also called Taxoprexin®), and MAC-321 (WYETH). Also see the review of Hennenfent & Govindan (2006, *Annals of Oncology*, 17, 735-749). In a preferred embodiment of the present invention, the molecule of the taxoid family is the docetaxel.

The expression level of the selected genes can be determined by measuring the amounts of RNA, in particular mRNA, DNA, in particular cDNA, or protein using a variety of techniques well-known by the man skilled in art. In a particular embodiment, the under-expression of a gene can be indirectly assessed through the determination of the methylation status of its promoter. Indeed, a methylated promoter is indicative of an expression repression, and therefore of an under-expression. At the opposite, an unmethylated promoter is indicative of a normal expression. The methylation state of a promoter can be assessed by any method known by the one skilled in the art, for instance by the methods disclosed in the following documents: Frommer et al (Proc Natl Acad Sci U S A. 1992;89:1827-31) and Boyd et al (Anal Biochem. 2004;326:278-80).

The cancer can be selected from the group consisting of the breast cancer, the lung cancer, the prostate cancer, the gastric cancer and the head and neck cancer. In a preferred embodiment, the cancer is the prostate cancer.

The term "biological sample" means any biological sample derived from a patient, preferably a sample which contains nucleic acids or proteins. Examples of such samples include fluids, tissues, cell samples, organs, biopsies, etc. Most preferred samples are cancer tissue samples, in particular breast, lung, prostate, stomach, ovary or head and neck tumor samples. Blood, plasma, saliva, urine, seminal fluid, etc, may also be used. Cancer cells obtain form blood

as circulating tumor cells may also be used. The biological sample may be treated prior to its use, *e.g.* in order to render nucleic acids or proteins available. Techniques of cell lysis, concentration or dilution of nucleic acids or proteins, are known by the skilled person.

Generally, the expression level as determined is a relative expression level (mRNA or protein).

More preferably, the determination comprises contacting the sample with selective reagents such as probes, primers or ligands, and thereby detecting the presence, or measuring the amount, of proteins or nucleic acids of interest originally in the sample. Contacting may be performed in any suitable device, such as a plate, microtiter dish, test tube, well, glass, column, and so forth. In specific embodiments, the contacting is performed on a substrate coated with the reagent, such as a nucleic acid array or chip or a specific ligand array. The substrate may be a solid or semi-solid substrate such as any suitable support comprising glass, plastic, nylon, paper, metal, polymers and the like. The substrate may be of various forms and sizes, such as a slide, a membrane, a bead, a column, a gel, etc. The contacting may be made under any condition suitable for a detectable complex, such as a nucleic acid hybrid or an antibody-antigen complex, to be formed between the reagent and the nucleic acids or proteins of the sample.

In a preferred embodiment, the expression level may be determined by determining the quantity of mRNA.

Methods for determining the quantity of mRNA are well known in the art. For example the nucleic acid contained in the samples (*e.g.,* cell or tissue prepared from the patient) is first extracted according to standard methods, for example using lytic enzymes or chemical solutions or extracted by nucleic-acid-binding resins following the manufacturer's instructions. The extracted mRNA is then detected by hybridization (*e. g.,* Northern blot analysis) and/or amplification (*e.g.,* RT-PCR). Preferably quantitative or semi-quantitative RT-PCR is preferred. Real-time quantitative or semi-quantitative RT-PCR is particularly advantageous.

Other methods of Amplification include ligase chain reaction (LCR), transcription-mediated amplification (TMA), strand displacement amplification (SDA) and nucleic acid sequence based amplification (NASBA).

Nucleic acids having at least 10 nucleotides and exhibiting sequence complementarity or homology to the mRNA of interest herein find utility as hybridization probes or amplification primers. It is understood that such nucleic acids need not be identical, but are typically at least about 80% identical to the homologous region of comparable size, more preferably 85% identical and even more preferably 90-95% identical. In certain embodiments, it will be advantageous to use nucleic acids in combination with appropriate means, such as a detectable