

LIPOSOMAL COMPOSITION OF INTRA-ARTICULAR CORTICOSTEROID FOR PAIN CONTROL

CROSS REFERENCE TO RELATED APPLICATIONS

The present application claims priority to U.S. Provisional Application No.

63/547,217, filed on November 3, 2023, and U.S. Provisional Application No.

63/633,336, filed on April 12, 2024, the disclosures of which are incorporated herein in their entireties.

FIELD

[0001] The disclosure relates to a method of treating pain or inflammation in long term with a lipid-based delivery system for delivering a therapeutic agent to an osteoarthritic patient. The disclosure also relates to a dosing regimen for administering a liposomal composition to an osteoarthritic patient in need of joint pain treatment in long term.

BACKGROUND

[0002] Osteoarthritis (OA) is a common degenerative joint disorder of the articular cartilage associated with hypertrophic bone changes. Treatment for OA primarily involves a non-pharmacological combination of exercise and lifestyle modification, pharmacological treatment (e.g., nonsteroidal anti-inflammatory drugs, intra-articular corticosteroids), supplementation with glucosamine or chondroitin sulphate, and surgical joint replacement.

[0003] Intra-articular (IA) corticosteroid injection is a current treatment recommendation for individuals with osteoarthritis (OA) by Osteoarthritis Research Society International (OARSI) guidelines in 2019 (Bannuru RR et al. *Osteoarthritis*

Cartilage. 2019;27(11): 1578-1589). Although IA injection of corticosteroid was known to relieve pain associated with osteoarthritis, reduce inflammation, and improve mobility, the mean duration of benefit is usually short, lasting only for 2 to 4 weeks (Jüni P. et al. *Cochrane Database Syst Rev*. 2015;(10) Art. No.: CD005328). Considering OA is a chronic and often progressive disease that can make the joint unusable and cause long-term pain, patients are almost always expected to receive a long term treatment with several doses to maintain their quality of life.

[0004] Repeated injections may be given in the same joint, but there is limited data on the safety and duration of the effectiveness of this procedure. It has been reported that repeated injections of corticosteroids, particularly for long periods of time, may increase the risk of local and systemic side effects, including pericapsular calcification, tendon rupture, skin atrophy/depigmentation, steroid arthropathy, joint infection, facial flushing, hypersensitivity, and hyperglycemia. For example, chronic use of corticosteroid may lead to suppression of the hypothalamic-pituitary-adrenal axis (HPA axis) through negative feedback. This may further lead to secondary adrenal insufficiency. Therefore, it is a common practice to use IA corticosteroids for no more than once every 3 months, however, this may lead to gaps in therapeutic effect between IA injections and worsen the arthritis symptoms.

[0005] Even though sustained-release lipid-based formulation of dexamethasone sodium phosphate (DSP) was designed for IA injection in the treatment of pain from knee osteoarthritis (International Publication No. WO2020/056399 A1), there remains an unmet need for establishing the robustness of the efficacy response to IA corticosteroids injection in the long term treatment with reduced or minimum side effects considering potential risks associated with repeat dosing.

SUMMARY

[0006] According to some embodiments, the disclosure provides a method for

treating joint pain in a human subject with osteoarthritis in long term, comprising intra-articularly administering to the human subject with repeat doses of liposomal composition, each dose comprising an effective amount of intra-articular corticosteroid (IACS) or a pharmaceutically acceptable salt thereof and a lipid mixture, wherein administration of the repeat doses of liposomal composition does not trigger signs and/or symptoms of adrenal insufficiency in the human subject.

[0007] In some embodiments, provided is a dosing regimen for joint pain treatment in long term, comprising administering intra-articularly in a joint of an osteoarthritic patient with repeat doses of liposomal composition, each dose comprising an effective amount of intra-articular corticosteroid and a lipid mixture, wherein the dosing regimen does not trigger signs and/or symptoms of adrenal insufficiency in the osteoarthritic patient.

[0008] In some embodiments, provided is a method for treating joint pain of an osteoarthritic patient in long term, comprising intra-articularly administering to the osteoarthritic patient with repeat doses of intra-articular corticosteroid, wherein at least one dose of intra-articular corticosteroid is made in a liposomal form or a liposomal composition containing an intra-articular corticosteroid or a pharmaceutically acceptable salt thereof and a lipid mixture, and the effective amount of intra-articular corticosteroid of the liposomal composition ranges from about 6 mg to about 18 mg.

[0009] In some embodiments, the repeat doses may be administered with a dosing interval selected from a group consisting of 3 weeks, 4 weeks, 5 weeks, 6 weeks, 7 weeks, 8 weeks, 9 weeks, 10 weeks, 11 weeks, 12 weeks, 13 weeks, 14 weeks, 15 weeks, 16 weeks, 17 weeks, 18 weeks, 19 weeks, 20 weeks, 21 weeks, 22 weeks, 23 weeks, 24 weeks, 25 weeks, 26 weeks, 27 weeks, 28 weeks, 29 weeks, 30 weeks, 31 weeks, 32 weeks, 33 weeks, 34 weeks, 35 weeks, and 36 weeks.

[0010] In some embodiments, the intra-articular corticosteroid administered intra-articularly in repeat dose is released over a duration selected from a group consisting of 52 weeks, 53 weeks, 54 weeks, 55 weeks, 56 weeks, 57 weeks, 58 weeks, 59 weeks, 60 weeks, 61 weeks, 62 weeks, 63 weeks, 64 weeks, 65 weeks, 66 weeks, 67 weeks, 68 weeks, 69 weeks, 70 weeks, 71 weeks, 72 weeks, 73 weeks, 74 weeks, 75 weeks, 76 weeks, 77 weeks, 78 weeks, 79 weeks and 80 weeks.

[0011] In some embodiments, the osteoarthritis is classified as a Kellgren-Lawrence Grade 2 or Grade 3. In some embodiments, the severity of the osteoarthritis is evaluated by radiography or magnetic resonance imaging of a joint of the human subject, with multiple osteophytes observed in the joint of the human subject.

[0012] In some embodiments, the human subject is female. In some embodiments, the human subject is at an age between 50 to 65 or over 65. In other embodiments, the human subject has body mass index (BMI) above, about, or no less than, 30.

[0013] In some embodiments, the human subject has unilateral osteoarthritis pain or bilateral osteoarthritis pain. In some embodiments, the joint pain is knee pain.

[0014] In some embodiments, the human subject is at an average daily pain (ADP) score of 5-9.

[0015] In some embodiments, the intra-articular corticosteroid or a pharmaceutically acceptable salt thereof is released at a rate that does not adversely suppress the hypothalamic-pituitary-adrenal axis (HPA axis). In other embodiments, the intra-articular corticosteroid or a pharmaceutically acceptable salt thereof is released at a rate that does not adversely suppress cortisol levels in the human subject. In other embodiments, the intra-articular corticosteroid or a pharmaceutically acceptable salt thereof is released at a rate that does not cause adrenal insufficiency in the human subject.

[0016] In some embodiments, the liposomal composition comprises (a) a lipid

mixture comprising one or more phospholipids; and (b) the intra-articular corticosteroid or a pharmaceutically acceptable salt thereof. An exemplary intra-articular corticosteroid according to the present disclosure is dexamethasone sodium phosphate (DSP). An exemplary lipid mixture comprises dioleoylphosphatidylcholine (DOPC) and dioleoylphosphatidylglycerol (DOPG).

[0017] In some embodiments, the intra-articular corticosteroid is present in an amount ranging from about 6 mg to about 18 mg, or about 12 mg, in the liposomal composition. In other embodiments, the intra-articular corticosteroid is present in an amount ranging from about 6 mg to about 18 mg, or about 12 mg, per mL of the liposomal composition.

[0018] In some embodiments, the subject has knee osteoarthritis of Kellgren-Lawrence Grade 2 or Grade 3. The effective amount of IACS or a pharmaceutically acceptable salt thereof in a liposomal composition is about 12 mg per mL or about 12 mg. The liposomal composition comprises (a) the IACS or a pharmaceutical acceptable salt thereof and (b) a mixture of DOPC, DOPG and cholesterol at a ratio of 56.25-72.5 : 7.5-18.75 : 10-33 by mole percent based on the total moles of the mixture.

[0019] According to the change in pain from baseline, treatment of osteoarthritis in a human subject with the sustained release liposomal composition according to some embodiments of this disclosure by single or multiple injections demonstrates the safety and the robustness of the efficacy response to a long term treatment with IACS, particularly to Group B and Group C steroid by Coopman Classification in the liposomal composition according to the present disclosure.

[0020] Other objectives, advantages and novel features of the invention will become more apparent from the following detailed description when taken in conjunction with the accompanying drawings.

BRIEF DESCRIPTION OF THE DRAWINGS

[0021] Figure 1 is a graph illustrating the trial design of the Phase 3 study in the present disclosure.

[0022] Figure 2 is an overview of the number of patients completing Injection Periods 1 and 2.

[0023] Figure 3 is a graph illustrating the least square (LS) Mean change from baseline in WOMAC pain score for TLC599 treated group versus placebo group during Injection 1 Period.

[0024] Figure 4 is a graph illustrating the mean change from baseline in WOMAC Function score for all treatment groups during Injection 1 Period.

[0025] Figure 5 is a graph illustrating the cumulative weekly total rescue medication consumption for all treatment groups from week 1 through week 24.

[0026] Figure 6 is a graph illustrating the mean change from baseline in weekly mean of ADP during Injection 1 Period (from week 0 through week 24) by all treatment groups. The abbreviation “NRS” stands for numeric rating scale.

[0027] Figure 7 is a graph illustrating changes in patient’s ADP for all treatment groups from baseline through Week 52. The first injection was administered at baseline (week 0) and the second injection was administered at week 24.

DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENTS

[0028] As employed above and throughout the disclosure, the following terms, unless otherwise indicated, shall be understood to have the following meanings.

[0029] As used herein, the singular forms “a”, “an” and “the” include the plural reference unless the context clearly indicates otherwise.

[0030] All numbers herein may be understood as modified by “about,” which, when referring to a measurable value such as an amount, a temporal duration, and the like, is meant to encompass variations of $\pm 10\%$, preferably $\pm 5\%$, more preferably

$\pm 1\%$, and even more preferably $\pm 0.1\%$ from the specified value, as such variations are appropriate to obtain a desired amount of liposomal drug, unless other specified.

[0031] The term “treating,” “treated,” or “treatment” as used herein includes preventative (e.g. prophylactic), slowing, arresting or reversing progressive structural tissue damage causing joint pain. The terms “treatment” or “treatments” may also refer to compositions or medicaments. Throughout this disclosure, by treating is meant a method of reducing, alleviating, inhibiting or delaying one or more symptoms or signs of osteoarthritis or the amelioration of joint pain as detected by art-known techniques or reduction in use of pain control medications. These include, but are not limited to, clinical examination, imaging, or analysis of serum or joint aspirate (for example, rheumatoid factors, erythrocyte sedimentation rate).

[0032] Art recognized methods are available to evaluate pains and its symptoms. These include, but are not limited to, 6-point descriptive pain rating scale, 11-point NPRS, visual analog scale, average daily pain (ADP), Western Ontario and McMaster Universities Osteoarthritis Index (WOMAC) pain, Wisconsin Brief Pain Questionnaire, Brief Pain Inventory, The McGill Pain Questionnaire and the short-form, McGill Pain Questionnaire and other scoring methods including Patient Global Assessment (PGA) of the method of pain control. For a human subject, self-reporting, for example using a graded scale of (0) no pain to (10) maximum pain, could be used to identify level of pain. The ADP, for example, measures daily pain intensity on a 0 to 10 numeric rating scale (NRS). Alternatively, the WOMAC index is a self-administered questionnaire including 3 subscales, with scores ranging from 0 to 4 for each subscale: pain (5 items), stiffness (2 items), and physical function (17 items). Optionally, functional magnetic resonance imaging (fMRI) could be used in a subject to identify decreased pain following administration of a liposomal composition of the present disclosure. For example, a disclosed method is considered to be a treatment if

there is about or at least 1%, 5%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90% or 100% reduction of joint pain in a subject when compared to the subject prior to treatment or to control subjects. The treatment includes single articular injection or multiple articular injections within a desired interval.

[0033] The term “joint pain” refers to a joint disorder or condition that involves inflammation and/or pain of one or more joints. The term “joint pain,” as used herein, encompasses a variety of types and subtypes of arthritis of various etiologies and causes, either known or unknown, including, but not limited to, rheumatoid arthritis, osteoarthritis, infectious arthritis, psoriatic arthritis, gouty arthritis, and lupus-related arthritis or painful local tissues affected by bursitis, tenosynovitis, epicondylitis, synovitis and/or other disorders.

[0034] “Pharmaceutically acceptable salts” of the intra-articular corticosteroid (IACS) of the present disclosure are salts of an acidic IACS formed with bases, namely base addition salts such as alkali and alkaline earth metal salts, such as sodium, lithium, potassium, calcium, magnesium, as well as ammonium salts, such as ammonium, trimethyl-ammonium, diethylammonium, and tris-(hydroxymethyl)-methyl-ammonium salts. Similarly, acid addition salts, such as of mineral acids, organic carboxylic and organic sulfonic acids, e.g., hydrochloric acid, methanesulfonic acid, maleic acid, may also be provided to a basic IACS.

[0035] “Long term” treatment or dosing regimen as used herein generally refers to a treatment or a dosing regimen that lasts for a period of at least 52 weeks when a therapeutic agent is administered to a patient to provide its therapeutic effect. This includes administering the therapeutic agent either in a single dose, multiple doses or repeated doses to the patient over, or for, a period of at least 52 weeks. In many cases, the period of long term treatment or dosing regimen may even be extended for 53 weeks, 54 weeks, 55 weeks, 56 weeks, 57 weeks, 58 weeks, 59 weeks, 60 weeks, 61

weeks, 62 weeks, 63 weeks, 64 weeks, 65 weeks, 66 weeks, 67 weeks, 68 weeks, 69 weeks, 70 weeks, 71 weeks, 72 weeks, 73 weeks, 74 weeks, 75 weeks, 76 weeks, 77 weeks, 78 weeks, 79 weeks, 80 weeks, or more depending on patients' need to improve, heal or recover from their original diseased state.

[0036] The term “HPA axis” refers to the hypothalamic-pituitary-adrenal axis, an important endocrine system in the body that regulates the stress response and the release of cortisol. Cortisol is the main adrenal glucocorticoid and plays a central role in control of glucose metabolism and body's response to stress. Cortisol produced in the adrenal cortex will negatively feedback to inhibit both the hypothalamus and the pituitary gland. This reduces the secretion of corticotropin-releasing hormone (CRH) and vasopressin, and directly reduces the cleavage of proopiomelanocortin (POMC) into adrenocorticotrophic hormone (ACTH) and β -endorphins. Use of exogenous glucocorticoids is known to cause suppression of the HPA axis. Therefore, chronic use of glucocorticoids for their potent anti-inflammatory and pharmacological effects, may exacerbate to secondary adrenal insufficiency due to its long exposure (Younes A. (2017). *Translational Pediatrics*, 17; 6(4), 269–273).

[0037] The term “adrenal insufficiency” may be classified clinically into primary, secondary, and tertiary causes. “Primary adrenal insufficiency” occurs when there is a pathology affecting the adrenal gland itself. “Secondary adrenal insufficiency” results from a decreased level of adrenocorticotrophin hormone (ACTH) released from the pituitary gland, and “Tertiary adrenal insufficiency” results from a decreased level of corticotrophin-releasing hormone (CRH) released from the hypothalamus. It was known that glucocorticoid therapy may prolong suppression of the production of corticotropin-releasing hormone, cause suppression of the HPA axis through negative feedback, further lead to secondary adrenal insufficiency. Some of the clinical manifestations of secondary adrenal insufficiency include pale skin without marked

anemia, amenorrhea, decreased libido and potency, scanty axillary and pubic hair, small testicles, secondary hypothyroidism, prepubertal growth deficit, delayed puberty, headache, visual symptoms and diabetes insipidus (Oelkers W. (1996). *The New England journal of medicine*, 335(16), 1206–1212). The diagnosis of adrenal insufficiency is suggested by clinical features and confirmed by biochemical tests, e.g., insulin tolerance test (ITT), metyrapone stimulation test, or ACTH stimulation test. The characteristic features of adrenal insufficiency include fever, hypoglycemia, long-lasting fatigue, anorexia, diarrhea, weight loss, muscle weakness, abdominal pain, nausea, vomiting, low blood pressure that drops further when standing up and causing dizziness or fainting, irritability and depression, craving salty foods, irregular or no menstrual periods, loss of interest in sex, or other potential clinical signs and symptoms.

Lipid mixture and liposomal compositions containing the same

[0038] In some embodiments, the disclosure provides a sustained release liposomal composition comprising a lipid mixture and an effective amount of an intra-articular corticosteroid (IACS) or a pharmaceutically acceptable salt thereof, wherein the lipid mixture comprising one or more phospholipids and the amount of phospholipids in the sustained release liposomal composition is about 20 μmol to 150 μmol per 1 mL.

[0039] In some embodiments, the liposomal compositions described herein and administered via single or repeat dose(s) sustain the release of the IACS for up to 3 months, 4 months, 5 months, 6 months, 7 months, 8 months, 9 months, 10 months, 11 months, 12 months, 13 months, 14 months, 15 months, 16 months, 17 months, 18 months, 19 months, 19.5 months, 20 months, 2 weeks, 3 weeks, 4 weeks, 5 weeks, 6 weeks, 7 weeks, 8 weeks, 9 weeks, 10 weeks, 11 weeks, 12 weeks, 13 weeks, 14 weeks, 15 weeks, 16 weeks, 17 weeks, 18 weeks, 19 weeks, 20 weeks, 21 weeks, 22 weeks, 23 weeks, 24 weeks, 25 weeks, 26 weeks, 27 weeks, 28 weeks, 29 weeks, 30

weeks, 31 weeks, 32 weeks, 33 weeks, 34 weeks, 35 weeks, 36 weeks, 37 weeks, 38 weeks, 39 weeks, 40 weeks, 41 weeks, 42 weeks, 43 weeks, 44 weeks, 45 weeks, 46 weeks, 47 weeks, 48 weeks, 49 weeks, 50 weeks, 51 weeks, 52 weeks, 53 weeks, 54 weeks, 55 weeks, 56 weeks, 57 weeks, 58 weeks, 59 weeks, 60 weeks, 61 weeks, 62 weeks, 63 weeks, 64 weeks, 65 weeks, 66 weeks, 67 weeks, 68 weeks, 69 weeks, 70 weeks, 71 weeks, 72 weeks, 73 weeks, 74 weeks, 75 weeks, 76 weeks, 77 weeks, 78 weeks, 79 weeks, 80 weeks, half year, one year, or 1.5 year.

[0040] In some embodiments, the efficacy of the liposomal composition disclosed herein is increased, relative to the efficacy of a liposomal composition having more than 150 μmol of phospholipids per 1 mL of liposomal composition. In yet another embodiment, the liposomal composition disclosed herein sustains the therapeutic efficacy of the IACS and reduces the side effects associated with the IACS.

[0041] In some embodiments, the total amount of the phospholipids is about 50 μmol to less than about 140 μmol per 1 mL of the liposomal composition. In another embodiment, the total amount of the phospholipids is about 45 μmol to less than about 135 μmol per 1 mL of the liposomal composition. In another embodiment, the total amount of the phospholipids is about 70 μmol to less than about 150 μmol per 1 mL of the liposomal composition. In another embodiment, the total amount of the phospholipids is about 90 μmol to less than about 150 μmol per 1 mL of the liposomal composition. In yet another embodiment, the total amount of the phospholipids is about 50 μmol to less than about 120 μmol per 1 mL of the liposomal composition. In yet another embodiment, the total amount of the phospholipids is about 60 μmol to less than about 110 μmol per 1 mL of the liposomal composition.

[0042] In some embodiments, the liposomal composition further comprises at least one pharmaceutically acceptable excipient, diluent, vehicle, carrier, medium for the

active ingredient, a preservative, cryoprotectant, or a combination thereof.

[0043] In some embodiments, the liposomal composition of the present disclosure is prepared by mixing one or more phospholipids, with or without cholesterol, and one or more buffers to form liposomes, lyophilizing the liposomes with one or more bulking agents to form a lipid mixture in a form of cake and reconstituting the lipid mixture cake with a solution containing the IACS to form an aqueous suspension.

[0044] In some embodiments, the liposomal composition of the present disclosure is prepared by mixing one or more phospholipids, with or without cholesterol, in a solvent, then removing the solvent to form a lipid mixture in a form of powder or film, and reconstituting the lipid mixture powder or film with a solution containing the IACS to form an aqueous suspension. In another embodiment, the liposomal composition of the present disclosure is prepared by mixing one or more phospholipids, with or without cholesterol, in a solvent, followed by injection of the dissolved lipid solution into an aqueous solution to form liposomes. Liposomes are then sized down by filtering through track-etched polycarbonate membranes. Solvent is removed by diafiltration against buffer by means of a semi-automated tangential-flow filtration (TFF) system. The diafiltrated liposome solution is then lyophilized in a form of powder, and the lipid mixture powder or film is reconstituted with a solution containing the IACS to form an aqueous suspension.

[0045] In some embodiments, the liposomal composition of the disclosure comprises about 10% to about 50% of lipid-associated IACS or about 50% to about 90% of non-associated IACS. The term “non-associated form” refers to the IACS molecules separable via gel filtration from the phospholipid/cholesterol fraction of the liposomal composition and provides immediate release component. In other embodiments, the weight ratio of the combination of the phospholipid and cholesterol to the IACS is about 5-80 to 1. In yet another embodiment, the weight ratio of the

combination of the phospholipid and cholesterol to the IACS is about 5-40 to 1. For example, the weight ratio of the combination of the phospholipid and cholesterol to the IACS may be about 5, 10, 15, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75 or 80 to 1.

[0046] In some embodiments, the IACS of the liposomal composition of the present disclosure is at a concentration of at least or about 10 mM, 11 mM, 12 mM, 13 mM, 14 mM, 15 mM, 16 mM, 17 mM, 18 mM, 19 mM, 20 mM, 21 mM, 22 mM, 23 mM, 24 mM, 25 mM, 26 mM, 27 mM, 28 mM, 29 mM, 30 mM, 31 mM, 32 mM, 33 mM, 34 mM or 35 mM; or ranging from about 10 mM to about 40 mM, from about 15 mM to about 40 mM, from about 20 mM to about 40 mM, from about 15 mM to about 35 mM, from about 15 mM to about 30 mM, from about 15 mM to about 25 mM, or from about 20 mM to about 25 mM.

[0047] In some embodiments, the total amount of the liposomal composition for each administration ranges from about 0.5 mL to about 1.5 mL, and preferably about 1.0 mL.

[0048] The lipid mixture of the liposomal composition provided herein refers to a phospholipid or a mixture of phospholipids. The lipid mixture may be, but not limited to, in a form of film, cake, granules or powders before being added to the liposomal composition.

[0049] In some embodiments, the phospholipid or the mixture of phospholipids, with or without cholesterol, are pre-formed into liposomes before being further processed into a lipid mixture.

[0050] In some embodiments, the phospholipid or mixture of phospholipids, with or without cholesterol, are not pre-formed into liposomes before being further processed into a lipid mixture.

[0051] The liposomes may be nano-sized and comprise a lipid bilayer surrounding

an internal aqueous agent-carrying component. Non-limiting examples of liposomes include small unilamellar vesicles (SUV), large unilamellar vesicles (LUV), multivesicular liposome (MVL) and multi-lamellar vesicles (MLV).

[0052] The lipid mixture may be prepared from a variety of lipids capable of either forming or being incorporated into a unilayer or bilayer structure. The lipids used in the disclosure include one or more phospholipids, including but are not limited to, phosphatidylcholine (PC), phosphatidylglycerol (PG), phosphatidylethanolamine (PE), phosphatidylserine (PS), phosphatidic acid (PA), phosphatidylinositol (PI) or combinations thereof. In some embodiments, the lipid mixture comprises egg phosphatidylcholine (EPC), egg phosphatidylglycerol (EPG), egg phosphatidylethanolamine (EPE), egg phosphatidylserine (EPS), egg phosphatidic acid (EPA), egg phosphatidylinositol (EPI), soy phosphatidylcholine (SPC), soy phosphatidylglycerol (SPG), soy phosphatidylethanolamine (SPE), soy phosphatidylserine (SPS), soy phosphatidic acid (SPA), soy phosphatidylinositol (SPI) or combinations thereof. In another embodiments, the lipid mixture comprises dipalmitoylphosphatidylcholine (DPPC), 1,2-dioleoyl-sn-glycero-3-phosphatidylcholine (DOPC), dimyristoylphosphatidylcholine (DMPC), dipalmitoylphosphatidylglycerol (DPPG), dioleoylphosphatidylglycerol (DOPG), dimyristoylphosphatidylglycerol (DMPG), hexadecylphosphocholine (HEPC), hydrogenated soy phosphatidylcholine (HSPC), distearoylphosphatidylcholine (DSPC), distearoylphosphatidylglycerol (DSPG), dioleoylphosphatidylethanolamine (DOPE), palmitoylstearylphosphatidylcholine (PSPC), palmitoylstearylphosphatidylglycerol (PSPG), monooleoylphosphatidylethanolamine (MOPE), 1-palmitoyl-2-oleoyl-sn-glycero-3-phosphatidylcholine (POPC), polyethyleneglycol distearoylphosphatidylethanolamine (PEG-DSPE), dipalmitoylphosphatidylserine (DPPS), 1,2-dioleoyl-sn-glycero-3-phosphatidylserine

(DOPS), dimyristoylphosphatidylserine (DMPS), distearoylphosphatidylserine (DSPS), dipalmitoylphosphatidic acid (DPPA), 1,2-dioleoyl-sn-glycero-3-phosphatidic acid (DOPA), dimyristoylphosphatidic acid (DMPA), distearoylphosphatidic acid (DSPA), dipalmitoylphosphatidylinositol (DPPI), 1,2-dioleoyl-sn-glycero-3-phosphatidylinositol (DOPI), dimyristoylphosphatidylinositol (DMPI), distearoylphosphatidylinositol (DSPI), or combinations thereof.

[0053] In some embodiments, the lipid mixture comprises a first phospholipid and a second phospholipid. In some embodiments, the first phospholipid is selected from the group consisting of EPC, EPE, SPC, SPE, DPPC, DOPC, DMPC, HEPC, HSPC, DSPC, DOPE, PSPC, MOPE, POPC, and mixtures thereof; and the second phospholipid is selected from the group consisting of PG, PS, PA, PI and mixtures thereof. In some embodiments, the first phospholipid is EPC, EPE, SPC, SPE, DPPC, DOPC, DMPC, HEPC, HSPC, DSPC, DOPE, PSPC, MOPE, POPC, or mixtures thereof; and the second phospholipid is selected from the group consisting of EPG, EPS, EPA, EPI, SPG, SPE, SPS, SPA, SPI, DPPG, DOPG, DMPG, DSPG, PSPG, DPPS, DOPS, DMPS, DSPS, DPPA, DOPA, DMPA, DSPA, DPPI, DOPI, DMPI, DSPI, a hydrophilic polymer with a long chain of highly hydrated flexible neutral polymer attached to a phospholipid molecule, and mixtures thereof. Examples of the hydrophilic polymer include, but are not limited to, polyethylene glycol (PEG) with a molecular weight about 2,000 to about 5,000 daltons, methoxy PEG (mPEG), ganglioside GM₁, polysialic acid, polylactic acid (also termed polylactide), polyglycolic acid (also termed polyglycolide), polylacticpolyglycolic acid, polyvinyl alcohol, polyvinylpyrrolidone, polymethoxazoline, polyethyloxazoline, polyhydroxyethyloxazoline, polyhydroxypropyloxazoline, polyaspartamide, polyhydroxypropyl methacrylamide, polymethacrylamide, polydimethylacrylamide, polyvinylmethylether, polyhydroxyethyl acrylate, derivatized celluloses such as

hydroxymethylcellulose or hydroxyethylcellulose and synthetic polymers.

[0054] In some embodiments, the lipid mixture further comprises a sterol. Sterol used in the present disclosure is not particularly limited, but examples thereof include cholesterol, phytosterol (sitosterol, stigmasterol, fucosterol, spinasterol, brassicasterol, and the like), ergosterol, cholestanone, cholestenone, coprostenol, cholesteryl-2'-hydroxyethyl ether, and cholesteryl-4'-hydroxybutyl ether. The sterol component of the lipid mixture, when present, maybe any of those sterols conventionally used in the field of liposome, lipid vesicle or lipid particle preparation. In another embodiment, the lipid mixture comprises about 10% to about 33% of cholesterol, about 15% to less than about 30 mole % of cholesterol, about 18% to about 28 mole % of cholesterol or about 20% to about 25 mole % of cholesterol.

[0055] In some embodiments, the lipid mixture comprises the first phospholipid, the second phospholipid and the sterol at a mole percent of 29.5% to 87% : 3% to 37.5% : 10 % to 33%.

[0056] In some embodiments, the first phospholipid is DOPC, POPC, SPC, or EPC and the second phospholipid is PEG-DSPE or DOPG.

[0057] In some embodiments, the first phospholipid is DOPC and the second phospholipid is DOPG.

[0058] In some embodiments, the lipid mixture is free of fatty acid or cationic lipid (i.e., a lipid carrying a net positive charge at a physiological pH).

[0059] The liposomal compositions prepared in this disclosure may be generated by conventional techniques used to prepare vesicles. These techniques include the ether injection method (Deamer et al., *Acad. Sci.* (1978) 308: 250), the surfactant method (Brunner et al., *Biochim. Biophys. Acta.* (1976) 455: 322), the freeze-thaw method (Pick et al., *Arch. Biochim. Biophys.* (1981) 212: 186), the reverse-phase evaporation method (Szoka et al., *Biochim. Biophys. Acta.* (1980) 601: 559 71), the

ultrasonic treatment method (Huang et al., *Biochemistry* (1969) 8: 344), the ethanol injection method (Kremer et al., *Biochemistry* (1977) 16: 3932), the extrusion method (Hope et al., *Biochim. Biophys. Acta.* (1985) 812: 55-65), the French press method (Barenholz et al., *FEBS Lett.* (1979) 99: 210) and methods detailed in Szoka, F., Jr., et al., *Ann. Rev. Biophys. Bioeng.* 9: 467 (1980). All of the above processes are basic technologies for the formation of vesicles and these processes are incorporated by reference herein. After sterilization, the pre-formed liposomes are placed aseptically into a container and then lyophilized to form a powder or a cake. In the embodiment where the lipid mixture comprising pre-formed liposomes, said liposomes are obtained by solvent injection method and followed by lyophilization in the presence or absence of bulking agent(s) and/or buffering agent(s) to form the lipid mixture. In one embodiment, the lipid mixture comprises one or more bulking agents. In one embodiment, the lipid mixture further comprises one or more buffering agents.

[0060] The bulking agents include, but are not limited to, polyols or sugar alcohols such as mannitol, glycerol, sorbitol, dextrose, sucrose, and/or trehalose; and amino acids such as histidine, or glycine. One preferred bulking agent is mannitol.

[0061] The buffering agents include, but are not limited to, sodium phosphate monobasic dihydrate and sodium phosphate dibasic anhydrous.

[0062] In some embodiments where the lipid mixture comprises lipids that are not pre-formed into liposomes, the lipid mixture may be prepared by dissolving in a suitable organic solvent, including, but not limited to, ethanol, methanol, t-butyl alcohol, ether and chloroform, and drying by heating, vacuum evaporation, nitrogen evaporation, lyophilization, or other conventional means of solvent removal.

[0063] Specific examples of lipid mixture preparation in support of the disclosure will be described below.

Intra-articular corticosteroids

[0064] Intra-articular corticosteroid (IACS) is a current treatment recommendation for individuals with osteoarthritis by Osteoarthritis Research Society International (OARSI) guidelines for the non-surgical management of knee, hip and polyarticular osteoarthritis (Bannuru RR et al. *Osteoarthritis and Cartilage* (2019) 27: 1578-1589).

[0065] The IACS useful in the present disclosure includes any naturally occurring steroid hormones, synthetic steroids and their derivatives. Examples of the IACS, derivatives or a pharmaceutically acceptable salt thereof include, but are not limited to, Group B and Group C corticosteroid according to Coopman Classification (S. Coopman *et al.*, "Identification of cross-reaction patterns in allergic contact dermatitis from topical corticosteroids" *Br J Dermatol.* 1989 Jul; 121(1):27-34).

[0066] The pharmaceutically acceptable salts of the IACS include non-toxic salts formed from non-toxic inorganic or organic bases. For example, non-toxic salts may be formed with inorganic bases such as an alkali or alkaline earth metal hydroxide, e.g., potassium, sodium, lithium, calcium, or magnesium; and with organic bases such as an amine and the like.

[0067] The pharmaceutically acceptable salts of the IACS also include non-toxic salts formed from non-toxic inorganic or organic acids. Examples of organic and inorganic acids are, for example, hydrochloric acid, sulfuric acid, phosphoric acid, acetic acid, succinic acid, citric acid, lactic acid, maleic acid, fumaric acid, palmitic acid, cholic acid, pantoic acid, mucic acid, D-glutamic acid, glutaric acid, glycolic acid, phthalic acid, tartaric acid, lauric acid, stearic acid, salicylic acid, sorbic acid, benzoic acid and the like.

[0068] In some embodiments, the IACS includes, but is not limited to, hydrocortisone acetate, methylprednisolone acetate, dexamethasone sodium acetate, dexamethasone sodium phosphate, betamethasone acetate, prednisolone, triamcinolone acetonide, and triamcinolone hexacetonide, which may be administered

at a dose ranging from about 0.1 mg to about 300 mg, from about 0.1 mg to about 100 mg, from about 0.1 mg to about 20 mg, from about 0.1 mg to about 18 mg, from about 1 mg to about 300 mg, from about 1 mg to about 100 mg, from about 1 mg to about 20 mg, from about 1 mg to about 18 mg, from about 4 mg to about 300 mg, from about 4 mg to about 100 mg, from about 4 mg to about 20 mg, from about 4 mg to about 18 mg, from about 6 mg to about 18 mg, from about 6 mg to about 16 mg, from about 8 mg to about 16 mg, from about 6 mg to about 12 mg, per mL of the liposomal composition.

[0069] In one example, the IACS is dexamethasone sodium phosphate (DSP). The DSP is in a form of a solution and used as the solution containing the IACS as mentioned above to reconstitute the lipid mixture in a form of a cake to obtain the liposomal composition of the present disclosure, resulting in the IACS of the liposomal composition at a concentration of about 2 mg/mL to about 100 mg/mL, about 4 mg/mL to about 80 mg/mL, about 5 mg/mL to about 60 mg/mL, about 6 mg/mL to about 40 mg/mL, about 8 mg/mL to about 20 mg/mL, or about 10 mg/mL to about 16 mg/mL.

[0070] Effective dosages of the IACS in the disclosure in human may be higher than a recommended or standard dosage known in the art; for example, see Wernecke, C. et al. *Orthop J Sports Med.*, 3(5), 2325967115581163 (DOI: 10.1177/2325967115581163), which is incorporated by reference herein. For example, while the recommended effective and tolerable dosage of triamcinolone hexacetonide as the IACS is 20 mg, the dosage of the IACS in the present compositions and methods may be at least 20 mg or higher.

[0071] The dosage of the IACS administered will also depend on the severity of the condition being treated, the particular formulation, and other clinical factors such as weight and the general condition of the recipient and severity of the side effect.

[0072] In some embodiments, the liposomal composition may further comprise a target molecule including, but are not limited to, TNF- α and B cell surface antigen, such as CD20. Other antigens, such as CD19, HER-3, GD2, Gp75, CS1 protein, mesothelin, cMyc, CD22, CD4, CD44, CD45, CD28, CD3, CD123, CD138, CD52, CD56, CD74, CD30, Gp75, CD38, CD33, GD2, VEGF, or TGF may also be used. The target molecule could be in a form of a lipid-conjugate of an antibody or a peptide that acts as a targeting moiety to enable to specifically bind to a target cell bearing a target molecule to deliver the IACS to a desired microenvironment to achieve desired disease-modifying therapies.

Administration of the liposomal composition

[0073] The liposomal composition may be administered intra-articularly in a single dose regimen or in multiple dose regimen, over a period of time appropriate to the condition being treated. The liposomal composition may conveniently be administered at appropriate dosing frequency, for example, once over a period of a week, a fortnight, 6 weeks, a month, two months, at least 3 months, at least 6 months, at least 9 months, or until the symptoms and signs of the medical condition (i.e., joint pain) are alleviated, improved or cured.

[0074] In some embodiments, the liposomal composition may be administered either before or after a non-liposomal composition in a multiple dose regimen with a dosing interval selected from the group consisting of 2 weeks, 3 weeks, 4 weeks, 5 weeks, 6 weeks, 7 weeks, 8 weeks, 9 weeks, 10 weeks, 11 weeks, 12 weeks, 13 weeks, 14 weeks, 15 weeks, 16 weeks, 17 weeks, 18 weeks, 19 weeks, 20 weeks, 21 weeks, 22 weeks, 23 weeks, 24 weeks, 25 weeks, 26 weeks, 27 weeks, 28 weeks, 29 weeks, 30 weeks, 31 weeks, 32 weeks, 33 weeks, 34 weeks, 35 weeks, and 36 weeks.

[0075] In a group of embodiments, the liposomal composition may be administered according to a multiple dose regimen which includes but is not limited to at least two

articular injections with a dosing interval selected from the group consisting of 2 weeks, 3 weeks, 4 weeks, 5 weeks, 6 weeks, 7 weeks, 8 weeks, 9 weeks, 10 weeks, 11 weeks, 12 weeks, 13 weeks, 14 weeks, 15 weeks, 16 weeks, 17 weeks, 18 weeks, 19 weeks, 20 weeks, 21 weeks, 22 weeks, 23 weeks, 24 weeks, 25 weeks, 26 weeks, 27 weeks, 28 weeks, 29 weeks, 30 weeks, 31 weeks, 32 weeks, 33 weeks, 34 weeks, 35 weeks, and 36 weeks.

[0076] In some embodiments, the multiple dose regimen includes at least two articular injections administered with a dosing interval of at least 12 weeks, such as 13 weeks to six months. In another group of embodiments, the multiple dose regimen described above may further includes one, two, three, four or more additional articular injections at an appropriate dosing interval following the two articular injections as long as the subject does not experience any side effect after exposure of the liposomal composition.

[0077] In some embodiments, the multiple dose regimen includes at least two articular injections administered with a dosing interval of at least 24 weeks, such as 25 weeks to 36 weeks or longer. In another group of embodiments, the multiple dose regimen described above may be further administered by one, two, three, four or more additional articular injections following the two articular injections as long as the subject does not experience any side effect after exposure of the liposomal composition.

[0078] In some embodiments, the liposomal composition is administered at an amount ranging from about 0.5 mL to about 1.5 mL, about 0.6 mL to about 1.2 mL, about 0.8 mL to about 1.2 mL, or about 1.0 mL per articular injection.

[0079] In an exemplary embodiment, the liposomal composition is administered in a single dose treatment, wherein the effective amount of intra-articular corticosteroid ranges from about 6 mg to about 18 mg, from about 10 mg to about 18 mg, from

about 12 mg to about 18 mg, from about 10 mg to about 15 mg, from about 11 mg to about 13 mg, about 8 mg, about 9 mg, about 10 mg, about 11 mg, about 12 mg, about 13 mg, about 14 mg, or about 15 mg. The lipid mixture comprises the first phospholipid, the second phospholipid and the sterol at a mole percent ratio of 29.5% to 87% : 3% to 37.5% : 10 % to 33%; and the total amount of the phospholipids is about 50 μ mol to about 140 μ mol , about 45 μ mol to about 135 μ mol, about 50 μ mol to about 120 μ mol, or about 60 μ mol to about 110 μ mol.

[0080] In some embodiments , the liposomal composition is administered in a multiple doses treatment, wherein the effective amount of each dose of intra-articular corticosteroid ranges from about 6 mg to about 18 mg, from about 10 mg to about 18 mg, from about 12 mg to about 18 mg, from about 10 mg to about 15 mg, from about 11 mg to about 13 mg, about 8 mg, about 9 mg, about 10 mg, about 11 mg, about 12 mg, about 13 mg, about 14 mg, or about 15 mg. The lipid mixture comprises the first phospholipid, the second phospholipid and the sterol at a mole percent ratio of 29.5% to 87% : 3% to 37.5% : 10 % to 33%; and the total amount of the phospholipids is about 50 μ mol to about 140 μ mol , about 45 μ mol to about 135 μ mol, about 50 μ mol to about 120 μ mol, or about 60 μ mol to about 110 μ mol.

[0081] In some embodiments, the liposomal composition is administered as a separate dose from the non-liposomal composition in a multiple dose treatment, wherein the effective amount of intra-articular corticosteroid in the non-liposomal composition ranges from about 0.1 mg to about 300 mg.

The method of treating joint pain

[0082] One aspect of this disclosure is directed to a method of treating joint pain in a subject, comprising administering an effective amount of the liposomal composition as described herein to the subject in need thereof, whereby the side effects induced by the IACS are reduced compared to the side effects in a subject following the

administration of an immediate release or standard IACS formulation, and/or the efficacy and the release rate of the IACS of the liposomal composition is increased compared to the efficacy and the release rate of a liposomal composition with more than about 150 μmol of phospholipid per ml of the liposomal composition.

[0083] In some embodiments, the subject has arthritis such as osteoarthritis, rheumatoid arthritis, acute gouty arthritis, psoriatic arthritis, reactive arthritis, arthritis due to Ehlers-Danlos Syndrome, haemochromatosis, hepatitis, Lyme disease, Sjogren's disease, Hashimoto's thyroiditis, celiac disease, non-celiac gluten sensitivity, inflammatory bowel disease, Henoch-Schönlein purpura, Hyperimmunoglobulinemia D with recurrent fever, sarcoidosis, Whipple's disease, TNF receptor associated periodic syndrome, Granulomatosis with polyangiitis, familial Mediterranean fever, or systemic lupus erythematosus.

[0084] A plain radiograph of knee joint or magnetic resonance imaging (MRI) may be used for evaluation of patients of osteoarthritis of knee joint. MRI is preferred as a diagnostic tool for evaluating the changes in bones as well as soft tissues in osteoarthritis of knee. Kellgren Lawrence grade (K-L Grade) of osteoarthritis could be evaluated on the basis of radiography of the joint of knee.

[0085] Kellgren and Lawrence classification system has been vastly used for classification of severity of osteoarthritis. Below is the original description:

grade 0 (none): definite absence of x-ray changes of osteoarthritis;

grade 1 (doubtful): doubtful narrowing of joint space and possible osteophytic lipping;

grade 2 (minimal): definite osteophytes and possible narrowing of joint space on posteroanterior weight-bearing radiograph;

grade 3 (moderate): moderate multiple osteophytes, definite narrowing of joint space and some sclerosis and possible deformity of bone ends;

grade 4 (severe): large osteophytes, marked narrowing of joint space, severe sclerosis and definite deformity of bone ends.

[0086] Osteoarthritis is deemed present in patient classified as grade 2 based on the minimal severity as diagnosed. This classification was proposed by Kellgren & Lawrence in 1957 and later accepted by the World Health Organization (WHO) in 1961 as the radiological definition of OA for the purpose of epidemiological studies.

[0087] The efficacy refers to the ability of the IACS to induce a favorable clinical response in a disease. The efficacy also refers to the reduction of clinical signs, such as joint pain, tenderness, transient morning stiffness, and crepitus on joint motion that leads to instability and physical disability. In some embodiments, the efficacy of the IACS is determined by the WOMAC OA index, ADP score, VAS score or the like.

In some embodiments, the sustained, steady state release of the IACS from the liposomal composition described herein will not induce side effects including, but not limited to, articular cartilage damage or destruction, such as chondrocyte apoptosis, proteoglycan loss, cysts in articular cartilage, articular cartilage degradation, joint destruction, suppression of the serum cortisol level, suppression of the hypothalamic-pituitary-adrenal axis (HPA axis), or adrenal insufficiency. The reduction in side effects in a subject described herein may range from 1%, 5%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90% or 100% when compared with a subject injected with the IACS not formulated in the form of liposomal compositions described herein, e.g., without a lipid mixture.

[0088] The liposomal composition provided herein may be used in combination with any of a variety of additional chemical entities, including but not limited to, analgesics (e.g., bupivacaine, ropivacaine, or lidocaine) or hyaluronic acid preparations (e.g., Synvisc One). In some embodiments, the claimed liposomal composition and additional chemical entities are formulated into a single therapeutic

composition, and the claimed liposomal composition and the additional chemical entities are administered simultaneously. Alternatively, the claimed liposomal composition and the additional chemical entities are separate from each other, e.g., each is formulated into a separate therapeutic composition, and the claimed liposomal composition and the additional chemical entities are administered simultaneously, or at different times during a treatment regimen by the same route or different routes, as a single dose or multiple doses.

[0089] The disclosure will be further described with reference to the following specific, non-limiting examples.

EXAMPLES

[0090] The following examples illustrate the preparation and properties of certain embodiments of the present disclosure.

Example 1: Preparation of a lipid mixture

[0091] The lipids, including DOPC, DOPG and cholesterol, were combined at a mole percentage ratio of 56.25-72.5 : 7.5-18.75 : 10-33, for example 67.5 : 7.5 : 25, and dissolved in 99.9% ethanol at about 40°C in a flask to form a lipid solution. A tabletop ultrasonic bath was used for lipid dissolution.

[0092] The dissolved lipid solution was added to 1.0 mM sodium phosphate aqueous solution at 100mL/min by a peristaltic pump to form a pro-liposome suspension. The pro-liposome suspension was then passed 6 to 10 times through a polycarbonate membrane with a pore size of 0.2 µm. A liposome mixture was obtained and the liposomes had an average vesicle diameter of about 120-140 nm (measured by Malvern ZetaSizer Nano ZS-90, Malvern Instruments Ltd, Worcestershire, UK).

[0093] The liposome mixture was dialyzed and concentrated by a tangential flow filtration system with Millipore Pellicon 2 Mini Ultrafiltration Module Biomax-100C (0.1m²) (Millipore Corporation, Billerica, MA, USA) and then sterilized using a 0.2 µm sterile filter.

[0094] The lipid concentration of the filtered liposome mixture was quantified by phosphorous assay and the filtered liposome mixture was formulated with mannitol at a concentration of 2% mannitol and then sterilized again using a 0.2 µm sterile filter. The sterilized liposome mixture was then subject to lyophilization to obtain a lipid mixture in a form of cake.

Example 2: Preparation of a liposomal composition

[0095] A liposomal composition in accordance with the present disclosure was prepared by mixing the lipid mixture described in Example 1 with a dexamethasone sodium phosphate (DSP; C₂₂H₂₈FN₂O₈P; molecular weight: 516.41 g/L) aqueous solution, which comprises 13.2 mg/ml DSP and 4 mg/ml sodium citrate as DSP composition used hereafter, whereby each mL of the liposomal composition as formed (also known as liposomal DSP hereinafter) includes about 12.0 mg/mL of DSP and about 90 µmol to 100 µmol of phospholipid(s).

Example 3. A randomized, double-blinded controlled study of efficacy and safety of liposomal dexamethasone sodium phosphate (DSP) in patients with knee osteoarthritis

[0096] Liposomal DSP was prepared by methods described previously, such as Examples 1 and 2. In a Phase 3, randomized, double-blinded, placebo- and active-controlled clinical trial, a liposomal DSP (denoted as “TLC599” below) is intended for administration by local injection to provide sustained relief of knee OA pain. The

study was conducted to confirm that TLC599 provided pain relief in OA patients within 24 weeks in previous studies, while also to study the benefits of repeated injections of TLC599 over one year.

[0097] The current study was to evaluate the efficacy and safety of TLC599 in single or repeat doses, in patients with K-L Grade 2-3 OA of the knee. A total of 504 patients (506 patients were enrolled but 2 patients were withdrawn) were randomized in 3 groups by a 2:1:1 ratio to receive an injection of TLC599 12 mg (TLC599 treated group), DSP 4 mg (DSP treated group), and saline placebo (placebo group) respectively at Day 1 (baseline, or Week 0). At Week 24, a total of 203 eligible patients in TLC599 treated group who received an initial injection of TLC599 were given the second injection of TLC599. A total of eligible 89 patients in DSP treated group who received an initial injection of DSP were given the injection of TLC599. A total of eligible 94 patients in placebo group who received an initial injection of placebo were given the second injection of placebo. The study design described above is shown in Figure 1, and Figure 2 provides an overview of the number of patients completing the study in both Injection Periods 1 and 2. Efficacy and safety were assessed during Injection 1 Period (defined as period up to 24 weeks following the administration of Injection 1) and Injection 2 Period (defined as period up to 28 weeks following the administration of Injection 2 (or up to week 52 post baseline)).

[0098] Among efficacy parameters, Western Ontario and McMaster Universities Osteoarthritis Index (WOMAC) pain score, WOMAC function score, and average daily pain (ADP) in the index knee were collected and assessed using statistical methods such as Mixed Models for Repeated Measures (MMRM) and Analysis of Covariance (ANCOVA), respectively. Meanwhile, safety analysis was based on all safety information collected through the overall study period, including adverse events (AEs), clinical laboratory data, vital signs, electrocardiogram (ECG), and knee

radiographs.

[0099] Demographic and baseline characteristics of patients are summarized in Table 1.

Table 1. Demographic and baseline characteristics

	TLC599 (N=252)	DSP (N=126)	Placebo (N=126)
	n(%)	n(%)	n(%)
Gender			
Male	106 (42)	55 (44)	47 (37)
Female	146 (58)	71 (56)	79 (63)
Race			
Caucasian	195 (77)	94 (75)	95 (75)
African American	48 (19)	26 (21)	20 (16)
Asian	8 (3)	5 (4)	5 (4)
Other	1 (0.4)	1 (1)	6 (5)
Region			
United States	224 (89)	114 (90)	109 (87)
Australia	28 (11)	12 (10)	17 (13)
Kellgren-Lawrence (K-L) grade			
Grade 2	105 (42)	51 (41)	55 (44)
Grade 3	146 (58)	75 (59)	71 (56)
	Mean (SD)	Mean (SD)	Mean (SD)
Age (years)	61.5 (8.5)	59.9 (9.5)	61.3 (9.3)
BMI (kg/m ²)	31 (4.8)	32 (5.1)	31 (4.6)
Time since OA diagnosis (years)	8.9 (8.9)	7.5 (7.0)	8.8 (8.7)
Baseline Characteristics			
WOMAC Pain (0-4)	2.3 (0.5)	2.2 (0.4)	2.3 (0.4)
WOMAC Function (0-4)	2.3 (0.5)	2.2 (0.5)	2.3 (0.5)
ADP (0-10)	6.6 (1.0)	6.7 (1.0)	6.8 (1.0)

WOMAC = Western Ontario McMaster Universities Osteoarthritis Index.

ADP = Average Daily Pain.

[0100] For one of the efficacy endpoints as change from baseline WOMAC pain at Week 12 (ANCOVA), TLC599 treated group was statistically significantly better than placebo group with a least square mean difference of -0.171 and a P value of 0.0372 (Table 2). Moreover, TLC599 treated group was also numerically superior to placebo group at all time points through week 24, as shown in Figure 3.

Table 2. Change from Baseline in WOMAC Pain Score at Week 12

Endpoint	LS Mean		
	Difference (SE)	95% CI	P value
Change from Baseline in WOMAC Pain at Week 12 (TLC599 vs placebo)	-0.171 (0.0819)	(-0.331, -0.010)	0.0372

[0101] Table 3 summarizes the changes from baseline in WOMAC Function score for TLC599 versus placebo in Injection 1 Period. The treatment difference was statistically significant through Week 12 and at Weeks 16, Week 20, and Week 24 was numerically better in favor of TLC599 at every other visit until Week 24. The change from baseline in WOMAC Function score for all treatment groups during Injection 1 Period was displayed in Figure 4.

Table 3. Change from Baseline in WOMAC Function Score in Injection 1 Period for TLC599 Versus Placebo – ANCOVA

Visit	LS Mean (SE)	95% CI	P value
Week 1	-0.351 (0.0795)	(-0.507, -0.195)	<0.0001
Week 2	-0.279 (0.0811)	(-0.438, -0.120)	0.0006
Week 4	-0.214 (0.0799)	(-0.370, -0.057)	0.0075
Week 8	-0.206 (0.0823)	(-0.367, -0.045)	0.0124
Week 12	-0.168 (0.0826)	(-0.330, -0.006)	0.0416
Week 16	-0.178 (0.0851)	(-0.345, -0.011)	0.0363
Week 20	-0.177 (0.0860)	(-0.345, -0.008)	0.0397
Week 24	-0.190 (0.0868)	(-0.360, -0.020)	0.0286

[0102] Use of rescue medication (e.g., protocol-permitted acetaminophen, opioid or NSAIDs) was another efficacy assessment for the potential clinical benefits.

Acetaminophen in tablets of 500mg was provided as rescue medication in this study and was dispensed at Screening as needed. Between screening and end of study/early termination, patients recorded the previous 24-hour use of acetaminophen rescue medication daily into their diary. Rescue medication was reviewed at every study visit. The daily allowed acetaminophen dosage was up to 3 g/day. No acetaminophen was allowed within 24 hours before each study visit. As summarized in Table 4, the

analysis of cumulative rescue medication consumption through Week 12 and Week 24 by ANCOVA showed a statistically significant difference for TLC599 treated group versus placebo group, with less rescue medicine consumed in total by TLC599 treated group, which supports the clinical improvement significantly on average pain relief in TLC599 treated group. A graphical presentation of the cumulative weekly total rescue medication consumed by various treatment groups during Injection 1 Period is illustrated in Figure 5.

Table 4. Analysis of Total Rescue Acetaminophen Consumption for TLC599 Versus Placebo Through Weeks 12 and 24 – ANCOVA

Visit	Treatment	LS Mean (SE)	95% CI	P value
Injection 1	TLC599 (N=252)	15.568 (3.1902)	(9.316, 21.821)	0.0109
Weeks 1-12	Placebo (N=126)	29.803 (4.5893)	(20.808, 38.798)	
	Treatment difference: TLC599 vs placebo	-14.234 (5.5894)	(-25.190, -3.279)	
Injection 1	TLC599 (N=252)	42.990 (6.9093)	(29.448, 56.532)	0.0143
Weeks 1-24	Placebo (N=126)	72.663 (9.9617)	(53.137, 92.188)	
	Treatment difference: TLC599 vs placebo	-29.673 (12.1078)	(-53.404, -5.942)	

[0103] For other knee pain related endpoints including change from baseline weekly mean of average daily pain (ADP) at Week 12 and AUC of change from baseline in WOMAC Pain for the time interval of Baseline through Week 12, both showed that TLC599 treated group were statistically significantly better than placebo group for improvement of OA knee pain. Specifically, TLC599 treated group was numerically and statistically superior ($p < 0.05$) to placebo group at all time points during Injection 1 period as shown in Figure 6. As summarized in Table 5 for the change from Injection 1 in weekly mean of ADP for TLC599 versus placebo, statistically significant treatment differences were observed at Week 1, Week 12 and Week 24.

Table 5. Analysis of Change from Injection 1 in Weekly Mean of Average Daily**Pain in the Index Knee for TLC599 Versus Placebo at Weeks 1, 12, and 24 –****MMRM**

Visit	Treatment	LS Mean (SE)	95% CI	P value
Injection 1 Week 1	TLC599 (N=252)	-2.641 (0.1140)	(-2.865, -2.416)	<0.0001
	Placebo (N=126)	-1.560 (0.1614)	(-1.877, -1.242)	
	Treatment difference: TLC599 vs Placebo	-1.081 (0.1976)	(-1.470, -0.692)	
Injection 1 Week 12	TLC599 (N=252)	-3.209 (0.1388)	(-3.482, -2.936)	0.0154
	Placebo (N=126)	-2.620 (0.1978)	(-3.010, -2.231)	
	Treatment difference: TLC599 vs Placebo	-0.589 (0.2417)	(-1.065, -0.113)	
Injection 1 Week 24	TLC599 (N=252)	-2.969 (0.1389)	(-3.242, -2.696)	0.0186
	Placebo (N=126)	-2.398 (0.1977)	(-2.787, -2.009)	
	Treatment difference: TLC599 vs Placebo	-0.572 (0.2416)	(-1.047, -0.096)	

[0104] In addition, the efficacy of repeat doses of TLC599 in patients with OA of the knee was further evaluated. Eligible patients in the TLC599 treated group and placebo group received a second blinded injection of the same treatment at week 24, while patients in the DSP treated group received a blinded injection of TLC599 at the same time, refer to Figures 1 and 2. The change from baseline of weekly mean ADP scores recorded during Injection 1 Period and Injection 2 Period by various treatment groups are graphically displayed in Figure 7 for the eligible patients who received 2 injections (N=386). TLC599 treated groups demonstrated numerically better treatment effects than placebo group among patients receiving 2 injections from Week 1 through Week 52. Similar trend has been observed for patients receiving repeated injections of TLC599 and patients receiving DSP followed by TLC599 during the Injection 2 Period.

[0105] For the evaluation of safety, the results of clinical laboratory examination, vital signs, ECG, physical examination, and knee radiographs were unremarkable and did not raise safety concerns. There were no glucose and HbA1c results that were significantly outside the normal range that were observed throughout the study.

[0106] It is known that chronic use of corticosteroid may lead to suppression of the

HPA axis through negative feedback, leading to adrenal insufficiency. Normal cortisol levels are usually highest early in the morning (ranging from 10 to 20 µg/dL) and lowest about midnight. Therefore, the safety assessment, indicating potential adrenal insufficiency was performed by measuring morning cortisol level for various groups at the pre-determined time points/visits. Any patient with a morning serum cortisol <10 µg/dL who also had potential clinical signs and symptoms, e.g., fever, hypoglycemia, long-lasting fatigue, anorexia, diarrhea, weight loss, muscle weakness, abdominal pain, nausea, vomiting, low blood pressure that drops further when standing up, irritability and depression, craving salty foods, irregular or no menstrual periods, loss of interest in sex, suggestive of adrenal insufficiency during the study were subjected to an ACTH stimulation test to determine if he/she suffers from adrenal insufficiency. Specifically, the ACTH stimulation test was performed using the pre-dose serum cortisol sample collected before 9 AM as the baseline level, and the post-stimulation cortisol sample was collected approximately 1 hour after the injection of reconstituted 0.25 mg of cosyntropin for assessing the post-stimulation serum cortisol level. Patients who failed the ACTH stimulation test (post-stimulation cortisol level ≤18 µg/dL [or ≤497 nmol/L]) were referred to an endocrinologist for further evaluation and treatment.

[0107] In accordance with the study protocol, patients had their cortisol levels measured at Week 20 for eligibility to receive the second injection. There were 4 patients (3 patients in the TLC599 group and 1 patient in the Placebo group) with morning serum cortisol levels at Week 20 imputed as “0” due to specimen quality issues. All patients received the second injection after confirming their re-tested cortisol levels were above 10 µg/dL. At Week 20, 9 patients across the 3 treatment groups exhibited morning serum cortisol levels below 5 µg/dL. The incidence of patients with morning serum cortisol levels below 5 µg/dL was similar across the 3

groups: 4 out of 252 patients in the TLC599 group (1.6%); 3 out of 126 patients in the DSP group (2.4%), and 2 out of 126 patients in the Placebo groups (1.6%). The incidence of morning serum cortisol levels below 5 µg/dL in the TLC599 group was numerically lower than DSP group and comparable to the Placebo group, which suggested this represents the background incidence rate.

[0108] In addition, at Week 20, 113 patients displayed morning serum cortisol levels ranging from 5 to 10 µg/dL. These patients were less frequent in the TLC599 group (52 out of 252 patients, 20.6%) than the DSP group (32 out of 126 patients, 25.4%) and Placebo group (29 out of 126 patients, 23.0%). Notably, the incidences of morning serum cortisol levels ranging from 5 to 10 µg/dL in the TLC599 group (20.6%) were numerically lower than those in the DSP group (25.4%) and the Placebo group (23.0%), which showed comparable incidences in these 3 treatment groups. Importantly, none of the patients with cortisol levels below 5 µg/dL or between 5 and 10 µg/dL at Week 20 reported any signs and/or symptoms of adrenal insufficiency.

[0109] The proportion of patients with low cortisol (morning serum cortisol <5 µg/dL) by treatment group is presented for patients who received at least one injection (Table 6) and for patients who received 2 injections (Table 7). The results in Table 6 and Table 7 reveal an incidence in the Placebo group ranging from 1.0% to 7.7% of low cortisol values during Week 1 to Week 52 (end of study). These represent normal physiologic sporadic variation of low cortisol levels. In other words, there is an inherent background incidence of low cortisol levels which do not indicate a safety concern.

[0110] Regarding the changes in cortisol level during Injection Period 1, as shown in Table 6, there were more shifts at Week 1 from baseline to low cortisol values for the TLC599 group (23.6%), than for either the DSP 4 mg (0%) or the Placebo group (1.7%). However, at Week 2, the shift to low cortisol values for the TLC599 (4.7%)

and DSP group (3.5%) groups were comparable while the incidence was 1.7% for the Placebo group. At Week 4 the shifts to low cortisol values were comparable for the TLC599 (3.4%), DSP (3.5%), and Placebo groups (4.5%).

[0111] A similar pattern was seen for Injection Period 2, as shown in Table 7. One week after TLC599 injection at Week 24, 28.6% of patients who initially received TLC599, and 31.6% of patients who initially received DSP 4 mg had low cortisol values at Week 25, compared to 1.2% of patients who received placebo at both injections. At Week 26 (2 weeks following the second injection), 5.2% of patients in the TLC599 group and 6.6% of patients in the DSP 4 mg group had low cortisol values, compared to 1.3% of patients in the Placebo group. At Week 28, the incidence was comparable among the Placebo and DSP 4 mg groups, and lower (1.7%) in the TLC599 group.

[0112] Overall, no clinically significant serum cortisol values outside the normal range were observed throughout the study. The results indicate no accumulated effects on HPA axis after repeat injections of TLC599 and no patient with low cortisol values reported any treatment-emergent adverse event (TEAE) related to adrenal insufficiency throughout the whole period of the study. Specifically, there were no events recorded with preferred terms of adrenal insufficiency, adrenal androgen deficiency, adrenal suppression, adrenocortical insufficiency acute, glucocorticoid deficiency, Cushingoid, Cushing's syndrome, hyperadrenalism, or hyperadrenocorticism.

Table 6. Incidence of Low Cortisol Values by Time Period – Patients Who Received at Least 1 Injection

Visit	TLC599 12 mg (N=252)			DSP 4 mg (N=126)			Placebo (N=126)		
	N	n	%	N	n	%	N	n	%
Week 1	242	57	23.6%	122		0.0%	121	2	1.7%
Week 2	232	11	4.7%	113	4	3.5%	116	2	1.7%
Week 4	234	8	3.4%	115	4	3.5%	112	5	4.5%
Week 8	237	7	3.0%	114	3	2.6%	108	2	1.9%
Week 12	231	4	1.7%	99	3	3.0%	107	3	2.8%
Week 16	224	4	1.8%	102	5	4.9%	110	4	3.6%
Week 20	216	3 ^a	1.4%	101	3	3.0%	101	2	2.0%
Week 24	210	8	3.8%	94	2	2.1%	99	1	1.0%

^a One patient was not included in this count, due to the morning serum cortisol was collected on Day 151, which was out of window.

Table 7. Incidence of Low Cortisol Values by Time Period – Patients Who Received 2 Injections

Visit	TLC599 12 mg (N=203)			DSP 4 mg → TLC599 12 mg (N=89)			Placebo (N=94)		
	N	n	%	N	n	%	N	n	%
Week 25	168	48	28.6%	79	25	31.6%	85	1	1.2%
Week 26	172	9	5.2%	76	5	6.6%	79	1	1.3%
Week 28	180	3	1.7%	79	5	6.3%	78	6	7.7%
Week 32	179	3	1.7%	78	3	3.8%	85	3	3.5%
Week 36	173	2	1.2%	75	3	4.0%	78	2	2.6%
Week 40	171	2	1.2%	76	1	1.3%	81	1	1.2%
Week 44	171	4	2.3%	74	3	4.1%	82	2	2.4%
Week 48	171	5	2.9%	74	7	9.5%	81	1	1.2%
Week 52	161	8	5.0%	72	2	2.8%	74	4	5.4%

[0113] In summary, the liposomal DSP, i.e., TLC599, demonstrated a benefit over placebo in ADP and WOMAC pain in patients with OA of the knee after a single injection that was sustained to 24 weeks or beyond. A second injection of TLC599 at Week 24 extended the benefit to Week 52. Furthermore, the safety profile

demonstrated that during both injection periods the low incidence of AEs, the absence of treatment-related SAEs, and the lack of symptomatic adrenal insufficiency has reassured. These results suggest that TLC599 may provide prolonged efficacy in OA of the knee without adverse side effects and instill confidence in the favorable risk-benefit balance of TLC599 over multiple doses. The present disclosure demonstrates the safety and benefits of repeat injections of liposomal DSP in OA of the knee and offers an alternative treatment for the management of OA knee pain over an extended period (for example 52 weeks or beyond) as may be beneficial for the OA patients.

WHAT IS CLAIMED IS:

1. A method for treating joint pain in a human subject with osteoarthritis in long term, comprising intra-articularly administering to the human subject with repeat doses of liposomal composition, each dose comprising an effective amount of intra-articular corticosteroid or a pharmaceutically acceptable salt thereof and a lipid mixture, wherein administration of the repeat doses of liposomal composition does not trigger signs and/or symptoms of adrenal insufficiency in the human subject.
2. The method of claim 1, wherein the repeat doses of liposomal composition are administered with a dosing interval selected from the group consisting of 3 weeks, 4 weeks, 5 weeks, 6 weeks, 7 weeks, 8 weeks, 9 weeks, 10 weeks, 11 weeks, 12 weeks, 13 weeks, 14 weeks, 15 weeks, 16 weeks, 17 weeks, 18 weeks, 19 weeks, 20 weeks, 21 weeks, 22 weeks, 23 weeks, 24 weeks, 25 weeks, 26 weeks, 27 weeks, 28 weeks, 29 weeks, 30 weeks, 31 weeks, 32 weeks, 33 weeks, 34 weeks, 35 weeks, and 36 weeks.
3. The method of claim 1, wherein the intra-articular corticosteroid or the pharmaceutically acceptable salt thereof is released over a duration selected from the group consisting of 52 weeks, 53 weeks, 54 weeks, 55 weeks, 56 weeks, 57 weeks, 58 weeks, 59 weeks, 60 weeks, 61 weeks, 62 weeks, 63 weeks, 64 weeks, 65 weeks, 66 weeks, 67 weeks, 68 weeks, 69 weeks, 70 weeks, 71 weeks, 72 weeks, 73 weeks, 74 weeks, 75 weeks, 76 weeks, 77 weeks, 78 weeks, 79 weeks and 80 weeks.
4. The method of claim 1, wherein the intra-articular corticosteroid or the pharmaceutically acceptable salt thereof is released at a rate that does not adversely suppress the hypothalamic-pituitary-adrenal axis (HPA axis).
5. The method of claim 1, wherein the intra-articular corticosteroid or the

pharmaceutically acceptable salt thereof is released at a rate that does not adversely suppress cortisol levels in the human subject.

6. The method of claim 1, wherein the intra-articular corticosteroid or the pharmaceutically acceptable salt thereof is selected from the group consisting of dexamethasone sodium phosphate, dexamethasone, betamethasone, betamethasone sodium phosphate, betamethasone acetate, betamethasone dipropionate, betamethasone valerate, mometasone furonate, triamcinolone acetonide, triamcinolone hexacetonide, triamcinolone diacetate, methylprednisolone sodium succinate, methylprednisolone acetate, prednisolone tebutate, hydrocortisone acetate, alclometasone dipropionate, halcinonide, fluocortolone, fluocinolone acetonide, and combinations thereof.
7. The method of claim 1, wherein the intra-articular corticosteroid is dexamethasone sodium phosphate (DSP) with an effective amount ranging from about 6 mg to about 18 mg.
8. The method of claim 7, wherein the effective amount of DSP is about 12 mg.
9. The method of claim 1, wherein the lipid mixture comprises dioleoylphosphatidylcholine (DOPC) and dioleoylphosphatidylglycerol (DOPG).
10. The method of claim 9, wherein the lipid mixture further comprises cholesterol at a mole percent of about 10 to about 33 based on the total amount of the lipid mixture.
11. The method of claim 1, wherein the osteoarthritis is knee osteoarthritis.
12. A dosing regimen for joint pain treatment in long term, comprising:
administering intra-articularly in a joint of an osteoarthritic patient with repeat doses of liposomal composition, each dose comprising an effective amount of the intra-articular corticosteroid and a lipid mixture, wherein the dosing regimen does not trigger signs and/or symptoms of adrenal insufficiency in the osteoarthritic

patient.

13. The dosing regimen of claim 12, wherein the repeat doses of liposomal composition are administered with a dosing interval selected from the group consisting of 3 weeks, 4 weeks, 5 weeks, 6 weeks, 7 weeks, 8 weeks, 9 weeks, 10 weeks, 11 weeks, 12 weeks, 13 weeks, 14 weeks, 15 weeks, 16 weeks, 17 weeks, 18 weeks, 19 weeks, 20 weeks, 21 weeks, 22 weeks, 23 weeks, 24 weeks, 25 weeks, 26 weeks, 27 weeks, 28 weeks, 29 weeks, 30 weeks, 31 weeks, 32 weeks, 33 weeks, 34 weeks, 35 weeks, and 36 weeks.
14. The dosing regimen of claim 12, wherein the intra-articular corticosteroid is released over a duration selected from the group consisting of 52 weeks, 53 weeks, 54 weeks, 55 weeks, 56 weeks, 57 weeks, 58 weeks, 59 weeks, 60 weeks, 61 weeks, 62 weeks, 63 weeks, 64 weeks, 65 weeks, 66 weeks, 67 weeks, 68 weeks, 69 weeks, 70 weeks, 71 weeks, 72 weeks, 73 weeks, 74 weeks, 75 weeks, 76 weeks, 77 weeks, 78 weeks, 79 weeks and 80 weeks.
15. The dosing regimen of claim 12, wherein the intra-articular corticosteroid is released at a rate that does not adversely suppress the hypothalamic-pituitary-adrenal axis (HPA axis).
16. The dosing regimen of claim 12, wherein the intra-articular corticosteroid is released at a rate that does not adversely suppress cortisol levels in the human subject.
17. The dosing regimen of claim 12, wherein the intra-articular corticosteroid is selected from the group consisting of dexamethasone sodium phosphate, dexamethasone, betamethasone, betamethasone sodium phosphate, betamethasone acetate, betamethasone dipropionate, betamethasone valerate, mometasone furonate, triamcinolone acetonide, triamcinolone hexacetonide, triamcinolone diacetate, methylprednisolone sodium succinate, methylprednisolone acetate,

prednisolone tebutate, hydrocortisone acetate, alclometasone dipropionate, halcinonide, fluocortolone, fluocinolone acetonide, and combinations thereof.

18. The dosing regimen of claim 12, wherein the intra-articular corticosteroid is dexamethasone sodium phosphate (DSP) with an effective amount ranging from about 6 mg to about 18 mg.
19. The dose regimen of claim 18, wherein the effective amount of DSP is about 12 mg.
20. The dosing regimen of claim 12, wherein the lipid mixture comprises dioleoylphosphatidylcholine (DOPC) and dioleoylphosphatidylglycerol (DOPG).
21. The dosing regimen of claim 20, wherein the lipid mixture further comprises cholesterol at a mole percent of about 10 to about 33 based on the total amount of the one or more phospholipids.
22. The dosing regimen of claim 12, wherein the osteoarthritic patient is a patient with knee osteoarthritis.