United States Patent Application Publication	20250255901
Kind Code	A1
Publication Date	August 14, 2025
Inventor(s)	MEVORACH; Dror et al.

THERAPEUTIC APOPTOTIC CELLS FOR TREATMENT OF OSTEOARTHRITIS

Abstract

Methods of use for treating osteoarthritis or vanishing bone disease in a subject in need, including methods of direct administration of a composition of early apoptotic cells or an apoptotic supernatant into or adjacent to the affected joint or bone tissue. Methods of use may reduce pain, swelling, inflammation, bone loss, and or cartilage degeneration, and may increase freedom of movement at an affected joint.

Inventors: **MEVORACH; Dror (Jerusalem, IL), NOVIK; Shai (Ramat Hasharon, IL)**

Applicant: **ENLIVEX THERAPEUTICS R&D LTD** (Nes-Ziona, IL)

Family ID: **96661347**

Assignee: **ENLIVEX THERAPEUTICS R&D LTD (Nes-Ziona, IL)**

Appl. No.: **19/174936**

Filed: **April 10, 2025**

Related U.S. Application Data

parent US continuation-in-part 17637813 20220224 PENDING WO continuation-in-part PCT/IL2020/050919 20200823 child US 19174936
us-provisional-application US 62894982 20190903

Publication Classification

Int. Cl.: **A61K35/15** (20250101); **A61P19/02** (20060101)

U.S. Cl.:

CPC **A61K35/15** (20130101); **A61P19/02** (20180101);

Background/Summary

CROSS-REFERENCE TO RELATED APPLICATIONS [0001] This application is a Continuation-in-Part Application of U.S. application Ser. No. 17/637,813 filed Feb. 24, 2022, which filed as a National Phase Application of PCT International Application Number PCT/IL2020/050919, International filing date Aug. 23, 2020, which claims the benefit of U.S. Provisional Application Ser. No. 62/894,982 filed Sep. 3, 2019. All of these applications are hereby incorporated by reference in their entirety herein.

FIELD OF DISCLOSURE

[0002] Disclosed herein are compositions and methods thereof for treating osteoarthritis or vanishing bone disease in a subject. Methods disclosed herein comprise direct administration of an early apoptotic cell composition into or adjacent to an effected joint, or close to or adjacent to an affected bone tissue. Methods of treatment comprise reducing pain, swelling, cartilage degeneration, and bone erosion.

BACKGROUND

[0003] The in vitro and in vivo properties of apoptotic cells suggest their potential use in a broad range of inflammatory and autoimmunity and conditions associated with cytokine storm. Multiple mechanisms are used by apoptotic cells to create an immune homeostatic anti-inflammatory state in macrophages and dendritic cells (DCs). These include direct binding to phosphatidyl serine (PtdSer) and indirect binding to Tyro3, Axl, and Mer (TAM) receptors, as well as signaling via opsonins/bridging molecules that use additional integrins and Scavenger Receptors (ScRs) to inhibit Toll-like receptors (TLRs) as well as NF-κb, STAT1, and IFN signaling, and to activate Liver X Receptors (LXR), Suppression of Cytokine Signaling (SOCS 1/3), Peroxisome Proliferator-Activated Receptors (PPAR)-6, and hepatic growth factor (HGF). The sum of these events leads to downregulation of the inflammatory characteristics of macrophages and DCs, repair, and peripheral tolerance.

[0004] Indeed, autoimmune and autoinflammatory conditions, including type 1 diabetes in non-obese diabetic mice, experimental auto immune encephalomyelitis, arthritis, colitis, pulmonary fibro sis, fulminant hepatitis, contact hypersensitivity, acute- and chronic-graft rejection, hematopoietic cell engraftment, acute graft-versus-host disease (GvHD), and reduction of infarction size after acute myocardial infarction, were treated quite successfully by apoptotic cell infusion (Saas et al., (2016) “*Concise Review: Apoptotic Cell-Based Therapies—Rationale, Preclinical Results and Future Clinical Developments*.” Stem Cells Volume 34(6): 1464-1473). In the majority of the studies reviewed, administration of the apoptotic cells was systemic, wherein the study specifically examining treatment of arthritis administration was by intravenous or intraperitoneal injection (i.v. or i.p., respectively).

[0005] Among the autoimmune and autoinflammatory conditions, rheumatoid arthritis (RA) models were included in the review by Saas et al. (Saas et al., ibid). In the collagen induced arthritis (CIA) model, Gray et al. (M. Gray, K. Miles, D. Salter, D. Gray, and J. Savill. (2007) “*Apoptotic cells protect mice from autoimmune inflammation by the induction of regulatory B cells*.” Proc Natl Acad Sci USA. August 28; 104(35): 14080-14085.) identified the mechanism of action in RA. The data presented in Grey et al., indicated that, in two different model systems and

three different strains of mice, the administration of apoptotic cells was able to affect splenic B cells such that they secreted IL-10 and enhanced secretion of the immunosuppressive cytokine IL-10 from antigen-specific effector CD4+ T cells. In addition, they inhibited the severity of inflammatory arthritis and the generation of pathogenic autoantibody despite immunization in the presence of a powerful adjuvant, CFA. Thus, administration of apoptotic cells or the passive transfer of B cells from AC-treated mice protected the mice from CIA. Apoptotic cells themselves did not secrete immunosuppressive cytokines, such as IL-10 or TGF-β, and were unable to affect the progression of a passively induced arthritis. This demonstrates that apoptotic cells are able to directly affect B cell function and cytokine production inducing regulatory B cells. Furthermore, in the CIA model they found that injecting apoptotic cells by the i.v. or i.p. route was protective because both deliver apoptotic cells to the spleen. The authors clearly suggest that if the chosen route of administration does not deliver apoptotic cells to the spleen such that the apoptotic cells can interact with B cells, protection is unlikely to be elicited.

[0006] In additional studies using experimental streptococcal cell wall (SCW)-induced arthritis Perruche et al. (2009) (Sylvain Perruche, Philippe Saas, and Wanjun Chen. "*Apoptotic cell-mediated suppression of streptococcal cell wall-induced arthritis is associated with alteration of macrophage function and local regulatory T-cell increase: a potential cell-based therapy*?" Arthritis Res Ther. 11(4): R104.), demonstrated again that the apoptotic cell effect in RA model is via lymphoid organ and via i.p (equivalent to I.V.) route of administration. They showed that only apoptotic cells administered by i.p. injection profoundly suppressed joint swelling and destruction typically observed during the acute and chronic phases of SCW-induced arthritis. This was suggested to be a result of higher Foxp3+ Tregs in the lymphoid organs, especially in the draining lymph nodes.

[0007] Last, but not least, Notley et al. (Notley CA1, Brown M A, Wright G P, Ehrenstein M R. (2011) "*Natural IgM is required for suppression of inflammatory arthritis by apoptotic cells*." J Immunol. April 15; 186(8):4967-72. doi: 10.4049/jimmunol.1003021.) using Ag-induced model of inflammatory arthritis, showed that the enhanced production of IL-10 by T cells from draining lymph nodes and splenic marginal zone B cells, driven by the systemic infusion of apoptotic cells, was abrogated in the absence of natural IgM. Apoptotic cells were administered by i.v. injection and were present shortly after administration in the splenic marginal zone.

[0008] The clearance of dying cells is vital for re-establishing tolerance during inflammation and has potent immunoregulatory consequences. Because natural IgM plays a key role in the removal of apoptotic cells, Notely et al. (ibid) investigated whether the immune modulatory properties of apoptotic cells depended on its presence. Using an Ab-independent, Ag-induced model of inflammatory arthritis, they tested whether natural IgM is essential for the arthritis-suppressing properties of apoptotic cells. Whereas administration of apoptotic cells reduced joint inflammation and damage in normal mice accompanied by suppression of the Th17 response, no protection was afforded in secreted IgM-deficient (Sμ(−)) mice. The enhanced production of IL-10 by T cells from draining lymph nodes and splenic marginal zone B cells, driven by the infusion of apoptotic cells, was abrogated in the absence of natural IgM. Apoptotic cells were present shortly after administration in the splenic marginal zone, concluding that natural IgM is a critical factor in a chain of events triggered by the administration of apoptotic cells that promote splenic IL-10-secreting B and T cells and restrain the development of inflammation.

[0009] Taken together these results suggest that apoptotic cells modify RA via splenic effect on B and T cells and thus modify autoimmunity. A direct intra-joint effect on a non-autoimmune condition is not expected.

[0010] Osteoarthritis is the most prevalent musculoskeletal disorder and one for which there is no disease modifying therapy available at present. The current understanding of the disease mechanism of osteoarthritis is limited owing to a lacuna of knowledge about the development and maintenance of articular cartilage that is affected during osteoarthritis. During osteoarthritis,

articular cartilage expresses markers for transient cartilage differentiation. Moreover, blocking transient cartilage differentiation is sufficient for halting the progression of experimental osteoarthritis. A developmental biology inspired approach that combines restoration of tissue microenvironment, supplementation with engineered cartilage and built in mechanism to prevent transient cartilage differentiation could be an avenue for developing a disease modifying therapy for osteoarthritis. Non-steroidal anti-inflammatory drugs which might mitigates pain, do not arrest the progressive degeneration of articular cartilage. Therefore, non-steroidal anti-inflammatory drugs or corticosteroids are not considered of a therapeutic benefit in osteoarthritis, and in that regard apoptotic cells as an immune modulator were not really expected to modify osteoarthritis.

[0011] "Vanishing bone disease" is a clinical presentation mainly involving the hips and shoulders, but other joints as well, that could evolve mainly from rapidly destructive arthritis due to erosive osteoarthritis (Mavrogenis A F, Flevas D A, Panagopoulos G N, et al. (2015) "*Rapid destructive arthritis of the hip revisited*." Eur J Orthop Surg Traumatol. 25:1115-20) or spontaneous bone osteolysis due to proliferation of lymphangiomatous tissue, i.e, the Gorham-stout variant (Dellinger M T, Garg N, Olsen B R. (2014) "*Viewpoints on vessels and vanishing bones in Gorham-Stout disease*." Bone 63:47-52). No effective treatment has been identified for these conditions.

[0012] Thus, there remains an unmet need for compositions and methods of treatment of non-autoimmune conditions such as osteoarthritis and vanishing bone disease, including for the treatment for pain reduction, reduction of inflammation, reduction of swelling, inhibition or slowing the progressive degeneration of articular cartilage, inhibition or slowing of erosion of bone tissue, and treatment for increased movement including increased range of movements. Apoptotic cell infusion directly into a joint may present a novel and safe treatment for rebalancing the immune response in the bone and joint.

SUMMARY

[0013] In one aspect, this application discloses a method of treating osteoarthritis or vanishing bone disease in a subject in need, comprising the step of administering a composition comprising an early apoptotic cell population or an apoptotic supernatant, directly into a joint of said subject or at the site of the vanishing bone disease, wherein said administration treats osteoarthritis or vanishing bone disease, or a combination thereof in said subject.

[0014] In a related aspect, when treating osteoarthritis said treating comprises pain reduction, reduction of inflammation, reduction of swelling, inhibition of progressive degeneration of articular cartilage, reduction of progressive degeneration of articular cartilage, improving a quality of life, or any combination thereof. In a related aspect, when treating vanishing bone disease said treating comprises pain reduction, reduction of inflammation, reduction of swelling, inhibition of erosion of bone tissue, slowing of erosion of bone tissue, reduction in bone fractures, reduction of broken bones, inhibition of bone fractures, inhibition of broken bones, inhibiting loss of bone mass, inhibiting loss of bone density, reducing loss of bone mass, reducing loss of bone density, or improving a quality of life, or any combination thereof.

[0015] In another related aspect, the method of treating osteoarthritis increases movement in said joint, wherein increased movement comprises increased range of movement or increased movement with reduced pain, or a combination thereof.

[0016] In a related aspect, the joint comprises a synovial joint. In a further related aspect, the synovial joint comprises a knee joint, a hip joint, a shoulder joint, a joint between neck vertebrae, an elbow joint, an ankle joint, a wrist joint, a finger joint, a toe joint, or a thumb joint, a hand joint, a foot joint, or a combination thereof.

[0017] In a related aspect, the site of said vanishing bone disease comprises a shoulder, the skull, the pelvic girdle, the jaw, a rib or ribs, a collar bone, or the spine, or a combination thereof.

[0018] In a related aspect in methods of treatment disclosed herein, direct administration into the joint comprises infusion or injection of said early apoptotic cell population or said apoptotic supernatant.

[0019] In a related aspect in the methods of treatment disclosed herein, said early apoptotic cell population comprises an autologous early apoptotic cell population that is stable for greater than 24 hours; or an allogeneic early apoptotic cell population that is stable for greater than 24 hours. In a further related aspect, apoptotic supernatant is collected from cultured apoptotic cells, cultured early apoptotic cells, or cultured apoptotic cells with white blood cells. In yet another related aspect, the supernatants may be pooled. In still another related aspect, the early apoptotic cell population comprises a pooled population of early apoptotic cells. In another related aspect, pooled early apoptotic cell population comprises an irradiated, pooled population of early apoptotic cells. In a further related aspect, the pooled early apoptotic cell population comprises apoptotic cells prepared from single donor or from multiple donor mononuclear cells. In another related aspect, the early apoptotic cell population comprises an irradiated population of early apoptotic cells.
[0020] In a related aspect for the methods of treatment disclosed herein, the subject is a human subject.
[0021] In a related aspect for the methods of treatment disclosed herein, the administering comprises a single administration of said early apoptotic cell population or said apoptotic supernatant. In a further related aspect, the administering comprises multiple administrations of said apoptotic cell population or said apoptotic supernatant. In still a further related aspect, the multiple administration comprises daily or week administrations.
[0022] In a related aspect of the methods of treatment disclosed herein, the dose of each administration comprises between about 1×10.sup.6±20% to 1×10.sup.9 20% early apoptotic cells/kg subject. In a further related aspect, the dose of each administration comprises a supernatant collected from a culture comprising between about 1×10.sup.6±20% to 1×10.sup.9±20% apoptotic cells/kg subject.
[0023] In a related aspect of the methods of treatment disclosed herein, the administering reduces the concentration of at least one pro- or ant-inflammatory cytokine or chemokine in the synovial fluid present in the joint.

Description

BRIEF DESCRIPTION OF THE DRAWINGS
[0024] The subject matter regarded as the invention is particularly pointed out and distinctly claimed in the concluding portion of the specification. The invention, however, both as to organization and method of operation, together with objects, features, and advantages thereof, may best be understood by reference to the following detailed description when read with the accompanying drawings in which:
[0025] FIG. **1** presents a flow chart of the steps during some embodiments of a manufacturing process of early apoptotic cell populations, wherein anti-coagulants were included in the process (See Examples 1 and 2 for details of different embodiments). The mononuclear cells collected could be autologous or allogeneic, wherein non-matched mononuclear cells are used in some embodiments. Additional step includes irradiating the cells and pooling unmatched cells if multiple sources of cells are used.
[0026] FIG. **2** presents a series of bar graphs showing cytokine/chemokine levels in the right shoulder joint before and after treatment with early apoptotic cells. Cytokines/chemokines measurement was performed via Luminex MAGPIX system and analysis performed using Milliplex software (See Example 4 below).
[0027] FIG. **3** shows improvements in pain, function, and stiffness at 3 months and 6 months in a study of intra-articular Allocetra administration in moderate to severe knee osteoarthritis.
DETAILED DESCRIPTION
[0028] In the following detailed description, numerous specific details are set forth in order to

provide a thorough understanding of the early apoptotic cell population and use thereof for treating osteoarthritis. However, it will be understood by those skilled in the art that use of an early apoptotic cell population or compositions thereof may be practiced without these specific details. In other instances, well-known methods, procedures, and components have not been described in detail so as not to obscure the disclosure.

[0029] In some embodiments, disclosed herein is a method of treating osteoarthritis or vanishing bone disease, or a combination thereof, in a subject in need, comprising a step of administering a composition comprising an early apoptotic cell population directly into a joint of said subject or at the site of the vanishing bone disease, wherein said administration treats the osteoarthritis or vanishing bone disease in said subject. In other embodiments, disclosed herein is a method of treating osteoarthritis or vanishing bone disease, or a combination thereof, in a subject in need, comprising a step of administering a composition comprising an apoptotic cell supernatant directly into a joint of said subject or at the site of the vanishing bone disease, wherein said administration treats the osteoarthritis or vanishing bone disease in said subject.

[0030] Unlike rheumatoid arthritis, osteoarthritis and vanishing bone disease are not considered inflammatory autoimmune diseases or disorders. The methods described herein address multiple methods of treatment, not restricted to treating autoimmunity. In some embodiments, methods described herein by-pass the systemic immune system and the necessity of any response thereof as a required element of the effective therapy. In some embodiments, treatment is implemented by administering an early apoptotic cell population or a composition thereof, directly to the site of the osteoarthritis or vanishing bone disease. In some embodiments, treatment is implemented by administering an apoptotic cell supernatant or a composition thereof, directly to the site of the osteoarthritis or vanishing bone disease.

Apoptotic Cells

[0031] Production of early apoptotic cells (“ApoCells”) for use in compositions and methods as disclosed herein, has been described in WO 2014/087408, which is incorporated by reference herein in its entirety, and is described in brief in Example 1 below. In another embodiment, early apoptotic cells for use in compositions and methods as disclosed herein are produced in any way that is known in the art. In another embodiment, early apoptotic cells for use in compositions and methods disclosed herein are autologous with a subject undergoing therapy. In another embodiment, early apoptotic cells for use in compositions and methods disclosed herein are allogeneic with a subject undergoing therapy. In another embodiment, early apoptotic cells for use in compositions and methods disclosed herein are not match to a subject undergoing therapy, in other words they are “Off the shelf” (OTS). In another embodiment, a composition comprising early apoptotic cells comprises early apoptotic cells as disclosed herein or as is known in art.

[0032] A skilled artisan would appreciate that the term “autologous” may encompass a tissue, cell, nucleic acid molecule or polypeptide in which the donor and recipient is the same person.

[0033] A skilled artisan would appreciate that the term “allogeneic” may encompass a tissue, cell, nucleic acid molecule or polypeptide that is derived from separate individuals of the same species. In some embodiments, allogeneic donor cells are genetically distinct from the recipient.

[0034] In some embodiments, the source of cells for the early apoptotic population comprises mononuclear-enriched cells. In some embodiments, obtaining a mononuclear-enriched cell composition according to the production method disclosed herein is achieved by leukapheresis. A skilled artisan would appreciate that the term “leukapheresis” may encompass an apheresis procedure in which leukocytes are separated from the blood of a donor. In some embodiments, the blood of a donor undergoes leukapheresis and thus a mononuclear-enriched cell composition is obtained according to the production method disclosed herein. It is to be noted that the use of at least one anticoagulant during leukapheresis is required, as is known in the art, in order to prevent clotting of the collected cells.

[0035] In some embodiments, the leukapheresis procedure is configured to allow collection of

mononuclear-enriched cell composition according to the production method disclosed herein. In some embodiments, cell collections obtained by leukapheresis comprise at least 65% mononuclear cells. In other embodiments, at least 70%, or at least 80% mononuclear cells, as disclosed herein. In some embodiments, blood plasma from the cell-donor is collected in parallel to obtaining of the mononuclear-enriched cell composition. In the production method disclosed herein. In some embodiments, about 300-600 ml of blood plasma from the cell-donor are collected in parallel to obtaining the mononuclear-enriched cell composition according to the production method disclosed herein. In some embodiments, blood plasma is collected in parallel to obtaining the mononuclear-enriched cell composition according to the production method disclosed herein is used as part of the freezing and/or incubation medium. Additional detailed methods of obtaining an enriched mononuclear population of apoptotic cells for use in the compositions and methods as disclosed herein may be found in WO 2014/087408, which is incorporated herein by reference in its entirety.

[0036] In some embodiments, the early apoptotic cells for use in the methods disclosed herein comprise at least 85% mononuclear cells. In further embodiments, the early apoptotic cells for use in the methods disclosed herein contain at least 85% mononuclear cells, 90% mononuclear cells or alternatively over 90% mononuclear cells. In some embodiments, the early apoptotic cells for use in the methods disclosed herein comprise at least 90% mononuclear cells. In some embodiments, the early apoptotic cells for use in the methods disclosed herein comprise at least 95% mononuclear cells.

[0037] It is to be noted that, in some embodiments, while the mononuclear-enriched cell preparation at cell collection comprises at least 65%, preferably at least 70%, most preferably at least 80% mononuclear cells, the final pharmaceutical population, following the production method of the early apoptotic cells for use in the methods disclosed herein, comprises at least 85%, preferably at least 90%, most preferably at least 95% mononuclear cells.

[0038] In certain embodiments, the mononuclear-enriched cell preparation used for production of the composition of the early apoptotic cells for use in the methods disclosed herein comprises at least 50% mononuclear cells at cell collection. In certain embodiments, disclosed herein is a method for producing the pharmaceutical population wherein the method comprises obtaining a mononuclear-enriched cell preparation from the peripheral blood of a donor, the mononuclear-enriched cell preparation comprising at least 50% mononuclear cells. In certain embodiments, disclosed herein is a method for producing the pharmaceutical population wherein the method comprises freezing a mononuclear-enriched cell preparation comprising at least 50% mononuclear cells.

[0039] In some embodiments, the cell preparation comprises at least 85% mononuclear cells, wherein at least 40% of the cells in the preparation are in an early-apoptotic state, and wherein at least 85% of the cells in the preparation are viable cells. In some embodiments, the apoptotic cell preparation comprises no more than 15% CD15.sup.high expressing cells.

[0040] A skilled artisan would appreciate that the term “early-apoptotic state” may encompass cells that show early signs of apoptosis without late signs of apoptosis. Examples of early signs of apoptosis in cells include exposure of phosphatidylserine (PS) and the loss of mitochondrial membrane potential. Examples of late events include propidium iodide (PI) admission into the cell and the final DNA cutting. In order to document that cells are in an “early apoptotic” state, in some embodiments, PS exposure detection by Annexin-V and PI staining are used, and cells that are stained with Annexin V but not with PI or with only minimal PI staining are considered to be “early apoptotic cells” (An.sup.+ PI.sup.−). In some embodiments, minimal IP staining comprising less than or equal to (≤) 15% PI+ cells within the population of cells. In some embodiments, minimal IP staining comprising ≤10% PI+ cells within the population of cells. In some embodiments, minimal IP staining comprising ≤5% PI+ cells within the population of cells. In another embodiment, cells that are stained by both Annexin-V FITC and high PI are considered to be “late apoptotic cells”. In some embodiments, high IP staining comprises greater than (>) 15% PI+ cells within the

population of cells. In some embodiments, high IP staining comprises greater than or equal to (≥) 16% PI+ cells within the population of cells. In another embodiment, cells that do not stain for either Annexin-V or PI are considered non-apoptotic viable cells.

[0041] In some embodiments, at least 40% of the cells in a preparation are in an early apoptotic state. In some embodiments, at least 45% of the cells in a preparation are in an early apoptotic state. In some embodiments, at least 50% of the cells in a preparation are in an early apoptotic state. In some embodiments, at least 55% of the cells in a preparation are in an early apoptotic state. In some embodiments, at least 60% of the cells in a preparation are in an early apoptotic state. In some embodiments, at least 65% of the cells in a preparation are in an early apoptotic state. In some embodiments, at least 70% of the cells in a preparation are in an early apoptotic state. In some embodiments, at least 75% of the cells in a preparation are in an early apoptotic state. In some embodiments, at least 80% of the cells in a preparation are in an early apoptotic state. In some embodiments, at least 85% of the cells in a preparation are in an early apoptotic state. In some embodiments, at least 90% of the cells in a preparation are in an early apoptotic state. In some embodiments, at least 95% of the cells in a preparation are in an early apoptotic state.

[0042] In some embodiments, an early apoptotic cell preparation comprises less than or equal to (≤) 15% PI.sup.+ cells. In some embodiments, an early apoptotic cell preparation comprises ≤10% PI.sup.+ cells. In some embodiments, an early apoptotic cell preparation comprises ≤9% PI.sup.+ cells. In some embodiments, an early apoptotic cell preparation comprises ≤8% PI.sup.+ cells. In some embodiments, an early apoptotic cell preparation comprises ≤7% PI.sup.+ cells. In some embodiments, an early apoptotic cell preparation comprises ≤6% PI.sup.+ cells. In some embodiments, an early apoptotic cell preparation comprises ≤5% PI.sup.+ cells. In some embodiments, an early apoptotic cell preparation comprises ≤4% PI.sup.+ cells. In some embodiments, an early apoptotic cell preparation comprises ≤3% PI.sup.+ cells. In some embodiments, an early apoptotic cell preparation comprises ≤2% PI.sup.+ cells. In some embodiments, an early apoptotic cell preparation comprises ≤1% PI.sup.+ cells.

[0043] In some embodiments, at least 40% of the cells in a preparation are in an early apoptotic state (An.sup.+), wherein ≤15%, 10%, 9%, 8%, 7%, 6%, 5%, 4%, 3%, 2%, 1%, or 0% of the cells are PI.sup.+. In some embodiments, at least 45% of the cells in a preparation are in an early apoptotic state (An.sup.+), wherein ≤15%, 10%, 9%, 8%, 7%, 6%, 5%, 4%, 3%, 2%, 1%, or 0% of the cells are PI.sup.+. In some embodiments, at least 50% of the cells in a preparation are in an early apoptotic state (An.sup.+), wherein ≤15%, 10%, 9%, 8%, 7%, 6%, 5%, 4%, 3%, 2%, 1%, or 0% of the cells are PI.sup.+. In some embodiments, at least 55% of the cells in a preparation are in an early apoptotic state (An.sup.+), wherein ≤15%, 10%, 9%, 8%, 7%, 6%, 5%, 4%, 3%, 2%, 1%, or 0% of the cells are PI.sup.+. In some embodiments, at least 60% of the cells in a preparation are in an early apoptotic state (An.sup.+), wherein ≤15%, 10%, 9%, 8%, 7%, 6%, 5%, 4%, 3%, 2%, 1%, or 0% of the cells are PI.sup.+. In some embodiments, at least 65% of the cells in a preparation are in an early apoptotic state (An.sup.+), wherein ≤15%, 10%, 9%, 8%, 7%, 6%, 5%, 4%, 3%, 2%, 1%, or 0% of the cells are PI.sup.+. In some embodiments, at least 70% of the cells in a preparation are in an early apoptotic state (An.sup.+), wherein ≤15%, 10%, 9%, 8%, 7%, 6%, 5%, 4%, 3%, 2%, 1%, or 0% of the cells are PI.sup.+. In some embodiments, at least 75% of the cells in a preparation are in an early apoptotic state (An.sup.+), wherein ≤15%, 10%, 9%, 8%, 7%, 6%, 5%, 4%, 3%, 2%, 1%, or 0% of the cells are PI.sup.+. In some embodiments, at least 80% of the cells in a preparation are in an early apoptotic state (An.sup.+), wherein ≤15%, 10%, 9%, 8%, 7%, 6%, 5%, 4%, 3%, 2%, 1%, or 0% of the cells are PI.sup.+. In some embodiments, at least 85% of the cells in a preparation are in an early apoptotic state (An.sup.+), wherein ≤15% or ≤14%, 10%, 9%, 8%, 7%, 6%, 5%, 4%, 3%, 2%, 1%, or 0% of the cells are PI.sup.+. In some embodiments, at least 90% of the cells in a preparation are in an early apoptotic state (An.sup.+), wherein ≤10%, or ≤9%, 8%, 7%, 6%, 5%, 4%, 3%, 2%, 1%, or 0% of the cells are PI.sup.+. In some embodiments, at least 95%

of the cells in a preparation are in an early apoptotic state (An.sup.+), wherein ≤5%, ≤4%, 3%, 2%, 1%, or 0% of the cells are PI.sup.+.

[0044] A skilled artisan would appreciate that in some embodiments the terms “apoptotic cell”, “early apoptotic cell”, “Allocetra”, Autocetra”, “ALC”, and “ApoCell”, and grammatical variants thereof, may be used interchangeably to represent a population of “early apoptotic cells”, wherein said cell population is enriched for mononuclear cells and has unique characteristics (See for example, Example 1).

[0045] In some embodiments, Allocetra comprise a population of early apoptotic cells obtained from a single allogeneic donor. In some embodiments, Allocetra comprise a population of early apoptotic cells obtained from multiple allogeneic donors. In some embodiments, Allocetra comprise pooled populations of early apoptotic cells obtained from multiple allogeneic donors or from cells obtained from a blood bank. In some embodiments, Allocetra comprise pooled population of early apoptotic cells obtained from the same allogeneic donor. In some embodiments, Allocetra comprise an irradiated population of early apoptotic cells. In some embodiments, the term “Allocetra” may be used interchangeably with the term “Allocetra-OTS”. In some embodiments, the terms “Allocetra” and “Allocetra-OTS” encompass mononuclear early apoptotic cells, prepared as described in Example 1, independent of the source of said cells.

[0046] In some embodiments, Autocetra comprises a population of early apoptotic cells obtained as a single donation from an autologous donor. In some embodiments, Autocetra comprises a population of early apoptotic cells obtained as multiple donations from an autologous donor. In some embodiments, early apoptotic cells from an autologous donor, used in the methods for treating osteoarthritis or vanishing bone disease are irradiated. In some embodiments, Autocetra comprise an irradiated population of early apoptotic cells. In some embodiments, the term “Autocetra” may be used interchangeably with the term “Autocetra-OTS”.

[0047] The skilled artisan would appreciate that the compositions and methods described herein, in some embodiments comprise early apoptotic cells. In some embodiments, as described herein, early apoptotic cells are HLA matched to a recipient of a composition comprising the early apoptotic cells (a subject in need). In some embodiments, as described herein, early apoptotic cells are not matched to a recipient of a composition comprising the early apoptotic cells (a subject in need). In some embodiments, the early apoptotic cells not matched to a recipient of a composition comprising the early apoptotic cells (a subject in need) are irradiated as described herein in detail. In some embodiments, irradiated not matched cells are termed “Allocetra-OTS” or “ALC-OTS”.

[0048] In some embodiments, apoptotic cells comprise cells in an early apoptotic state. In another embodiment, apoptotic cells comprise cells wherein at least 90% of said cells are in an early apoptotic state. In another embodiment, apoptotic cells comprise cells wherein at least 80% of said cells are in an early apoptotic state. In another embodiment, apoptotic cells comprise cells wherein at least 70% of said cells are in an early apoptotic state. In another embodiment, apoptotic cells comprise cells wherein at least 60% of said cells are in an early apoptotic state. In another embodiment, apoptotic cells comprise cells wherein at least 50% of said cells are in an early apoptotic state.

[0049] In some embodiments, the composition comprising apoptotic cells further comprises an anti-coagulant.

[0050] In some embodiments, early apoptotic cells are stable. A skilled artisan would appreciate that in some embodiments, stability encompasses maintaining early apoptotic cell characteristics over time, for example, maintaining early apoptotic cell characteristics upon storage at about 2-8° C. In some embodiments, stability comprises maintaining early apoptotic cell characteristic upon storage at freezing temperatures, for example temperatures at or below 0° C.

[0051] In some embodiments, the mononuclear-enriched cell population obtained according to the production method of the early apoptotic cells for use in the methods disclosed herein undergoes freezing in a freezing medium. In some embodiments, the freezing is gradual. In some

embodiments, following collection the cells are maintained at room temperature until they are frozen. In some embodiments, the cell-preparation undergoes at least one washing step in washing medium following cell-collection and prior to freezing.

[0052] As used herein, the terms “obtaining cells” and “cell collection” may be used interchangeably. In some embodiments, the cells of the cell preparation are frozen within 3-6 hours of collection. In some embodiments, the cell preparation is frozen within up to 6 hours of cell collection. In some embodiments, the cells of the cell preparations are frozen within 1, 2, 3, 4, 5, 6, 7, 8 hours of collection. In other embodiments, the cells of the cell preparations are frozen up to 8, 12, 24, 48, 72 hours of collection. In other embodiments, following collection the cells are maintained at 2-8° C. until frozen.

[0053] In some embodiments, freezing according to the production of an early apoptotic cell population comprises freezing the cell preparation at about −18° C. to −25° C. followed by freezing the cell preparation at about −80° C. and finally freezing the cell preparation in liquid nitrogen until thawing. In some embodiments, the freezing according to the production of an early apoptotic cell population comprises: freezing the cell preparation at about −18° C. to −25° C. for at least 2 hours, freezing the cell preparation at about −80° C. for at least 2 hours and finally freezing the cell preparation in liquid nitrogen until thawing. In some embodiments, the cells are kept in liquid nitrogen for at least 8, 10 or 12 hours prior to thawing. In some embodiments, the cells of the cell preparation are kept in liquid nitrogen until thawing and incubation with apoptosis-inducing incubation medium. In some embodiments, the cells of the cell preparation are kept in liquid nitrogen until the day of hematopoietic stem cell transplantation. In non-limiting examples, the time from cell collection and freezing to preparation of the final population may be between 1-50 days, alternatively between 6-30 days. In alternative embodiments, the cell preparation may be kept in liquid nitrogen for longer time periods, such as at least several months.

[0054] In some embodiments, the freezing according to the production of an early apoptotic cell population comprises freezing the cell preparation at about −18° C. to −25° C. for at least 0.5, 1, 2, 4 hours. In some embodiments, the freezing according to the production of an early apoptotic cell population comprises freezing the cell preparation at about −18° C. to −25° C. for about 2 hours. In some embodiments, the freezing In the production of an early apoptotic cell population comprises freezing the cell preparation at about −80° C. for at least 0.5, 1, 2, 4, 12 hours.

[0055] In some embodiments, the mononuclear-enriched cell composition may remain frozen at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 20 months. In some embodiments, the mononuclear-enriched cell composition may remain frozen at least 0.5, 1, 2, 3, 4, 5 years. In certain embodiments, the mononuclear-enriched cell composition may remain frozen for at least 20 months.

[0056] In some embodiments, the mononuclear-enriched cell composition is frozen for at least 8, 10, 12, 18, 24 hours. In certain embodiments, freezing the mononuclear-enriched cell composition is for a period of at least 8 hours. In some embodiments, the mononuclear-enriched cell composition is frozen for at least about 10 hours. In some embodiments, the mononuclear-enriched cell composition is frozen for at least about 12 hours. In some embodiments, the mononuclear-enriched cell composition is frozen for about 12 hours. In some embodiments, the total freezing time of the mononuclear-enriched cell composition (at about −18° C. to −25° C., at about −80° C. and in liquid nitrogen) is at least 8, 10, 12, 18, 24 hours.

[0057] In some embodiments, the freezing at least partly induces the early-apoptotic state in the cells of the mononuclear-enriched cell composition. In some embodiments, the freezing medium comprises RPMI 1640 medium comprising L-glutamine, Hepes, Hes, dimethyl sulfoxide (DMSO) and plasma. In some embodiments, the plasma in the freezing medium is an autologous plasma of the donor which donated the mononuclear-enriched cells of the population. In some embodiments, the freezing medium comprises RPMI 1640 medium comprising 2 mM L-glutamine, 10 mM Hepes, 5% Hes, 10% dimethyl sulfoxide and 20% v/v plasma.

[0058] In some embodiments, the freezing medium comprises an anti-coagulant. In certain embodiments, at least some of the media used during the production of an early apoptotic cell population, including the freezing medium, the incubation medium and the washing media comprise an anti-coagulant. In certain embodiments, all media used during the production of an early apoptotic cell population which comprises an anti-coagulant comprising the same concentration of anti-coagulant. In some embodiments, anti-coagulant is not added to the final suspension medium of the cell population.
[0059] In some embodiments, addition of an anti-coagulant at least to the freezing medium improves the yield of the cell-preparation. In other embodiments, addition of an anti-coagulant to the freezing medium improves the yield of the cell-preparation in the presence of a high triglyceride level. As used herein, improvement in the yield of the cell-preparation relates to improvement in at least one of: the percentage of viable cells out of cells frozen, the percentage of early-state apoptotic cells out of viable cells and a combination thereof.
[0060] In some embodiments, early apoptotic cells are stable for at least 24 hours. In another embodiment, early apoptotic cells are stable for 24 hours. In another embodiment, early apoptotic cells are stable for more than 24 hours. In another embodiment, early apoptotic cells are stable for at least 36 hours. In another embodiment, early apoptotic cells are stable for 48 hours. In another embodiment, early apoptotic cells are stable for at least 36 hours. In another embodiment, early apoptotic cells are stable for more than 36 hours. In another embodiment, early apoptotic cells are stable for at least 48 hours. In another embodiment, early apoptotic cells are stable for 48 hours. In another embodiment, early apoptotic cells are stable for at least 48 hours. In another embodiment, early apoptotic cells are stable for more than 48 hours. In another embodiment, early apoptotic cells are stable for at least 72 hours. In another embodiment, early apoptotic cells are stable for 72 hours. In another embodiment, early apoptotic cells are stable for more than 72 hours.
[0061] A skilled artisan would appreciate that the term “stable” encompasses apoptotic cells that remain PS-positive (Phosphatidylserine-positive) with only a very small percent of PI-positive (Propidium iodide-positive). PI-positive cells provide an indication of membrane stability wherein a PI-positive cell permits admission into the cells, showing that the membrane is less stable. In some embodiments, stable early apoptotic cells remain in early apoptosis for at least 24 hours, for at least 36 hours, for at least 48 hours, or for at least 72 hours. In another embodiment, stable early apoptotic cells remain in early apoptosis for 24 hours, for 36 hours, for 48 hours, or for 72 hours. In another embodiment, stable early apoptotic cells remain in early apoptosis for more than 24 hours, for more than 36 hours, for more than 48 hours, or for more than 72 hours. In another embodiment, stable early apoptotic cells maintain their state for an extended time period.
[0062] In some embodiments, an apoptotic cell population is devoid of cell aggregates. In some embodiments, an apoptotic cell population is devoid of large cell aggregates. In some embodiments, an apoptotic cell population has a reduced number of cell aggregates compared to an apoptotic cell population prepared without adding an anticoagulant in a step other than cell collection (leukapheresis) from the donor. In some embodiments, an apoptotic cell population or a composition thereof, comprises an anticoagulant.
[0063] In some embodiments, apoptotic cells are devoid of cell aggregates, wherein said apoptotic cells were obtained from a subject with high blood triglycerides. In some embodiments, blood triglycerides levels of the subject are above 150 mg/dL. In some embodiments, an apoptotic cell population is devoid of cell aggregates, wherein said apoptotic cell population is prepared from cells obtained from a subject with normal blood triglycerides. In some embodiments, blood triglycerides levels of the subject are equal to or below 150 mg/dL. In some embodiments, cell aggregates produce cell loss during apoptotic cell production methods.
[0064] A skilled artisan would appreciate that the terms “aggregates” or “cell aggregates” may encompass the reversible clumping of blood cells under low shear forces or at stasis. Cell aggregates can be visually observed during the incubation steps of the production of the apoptotic

cells. Cell aggregation can be measured by any method known in the art, for example by visually imaging samples under a light microscope or using flow cytometry.
[0065] In some embodiments, the anti-coagulant is selected from the group comprising: heparin, acid citrate dextrose (ACD) Formula A and a combination thereof. In some embodiments, the anti-coagulant is selected from the group consisting of: heparin, acid citrate dextrose (ACD) Formula A and a combination thereof.
[0066] In some embodiments of methods of preparing an early apoptotic cell population and compositions thereof, an anticoagulant is added to at least one medium used during preparation of the population. In some embodiments, the at least one medium used during preparation of the population is selected from the group consisting of: the freezing medium, the washing medium, the apoptosis inducing incubation medium, and any combinations thereof.
[0067] In some embodiments, the anti-coagulant is selected from the group consisting of: Heparin, ACD Formula A and a combination thereof. It is to be noted that other anti-coagulants known in the art may be used, such as, but not limited to Fondaparinaux, Bivalirudin and Argatroban.
[0068] In some embodiments, at least one medium used during preparation of the population contains 5% of ACD formula A solution comprising 10 U/ml heparin. In some embodiments, anti-coagulant is not added to the final suspension medium of the cell population. As used herein, the terms “final suspension medium” and “administration medium” are used interchangeably having all the same qualities and meanings.
[0069] In some embodiments, at least one medium used during preparation of the population comprises heparin at a concentration of between 0.1-2.5 U/ml. In some embodiments, at least one medium used during preparation of the population comprises ACD Formula A at a concentration of between 1%-15% v/v. In some embodiments, the freezing medium comprises an anti-coagulant. In some embodiments, the incubation medium comprises an anti-coagulant. In some embodiments, both the freezing medium and incubation medium comprise an anti-coagulant. In some embodiments the anti-coagulant is selected from the group consisting of: heparin, ACD Formula A and a combination thereof.
[0070] In some embodiments, the heparin in the freezing medium is at a concentration of between 0.1-2.5 U/ml. In some embodiments, the ACD Formula A in the freezing medium is at a concentration of between 1%-15% v/v. In some embodiments, the heparin in the incubation medium is at a concentration of between 0.1-2.5 U/ml. In some embodiments, the ACD Formula A in the incubation medium is at a concentration of between 1%-15% v/v. In some embodiments, the anticoagulant is a solution of acid-citrate-dextrose (ACD) formula A. In some embodiments, the anticoagulant added to at least one medium used during preparation of the population is ACD Formula A containing heparin at a concentration of 10 U/ml.
[0071] In some embodiments, the apoptosis inducing incubation medium used in the production of an early apoptotic cell population comprises an anti-coagulant. In some embodiments, both the freezing medium and apoptosis inducing incubation medium used in the production of an early apoptotic cell population comprise an anti-coagulant. Without wishing to be bound by any theory or mechanism, in order to maintain a high and stable cell yield in different cell compositions, regardless of the cell collection protocol, in some embodiments addition of anti-coagulants comprising adding the anticoagulant to both the freezing medium and the apoptosis inducing incubation medium during production of the apoptotic cell population. In some embodiments, a high and stable cell yield within the composition comprises a cell yield of at least 30%, preferably at least 40%, typically at least 50% cells of the initial population of cells used for induction of apoptosis.
[0072] In some embodiments, both the freezing medium and the incubation medium comprise an anti-coagulant. In some embodiments, addition of an anti-coagulant both to the incubation medium and freezing medium results in a high and stable cell-yield between different preparations of the population regardless of cell-collection conditions, such as, but not limited to, the timing and/or

type of anti-coagulant added during cell collection. In some embodiments, addition of an anti-coagulant both to the incubation medium and freezing medium results in a high and stable yield of the cell-preparation regardless of the timing and/or type of anti-coagulant added during leukapheresis. In some embodiments, production of the cell-preparation in the presence of a high triglyceride level results in a low and/or unstable cell-yield between different preparations. In some embodiments, producing the cell-preparation from the blood of a donor having high triglyceride level results in a low and/or unstable cell-yield of the cell preparation. In some embodiments, the term “high triglyceride level” refers to a triglyceride level which is above the normal level of a healthy subject of the same sex and age. In some embodiments, the term “high triglyceride level” refers to a triglyceride level above about 1.7 mM/liter. As used herein, a high and stable yield refers to a cell yield in the population which is high enough to enable preparation of a dose which will demonstrate therapeutic efficiency when administered to a subject. In some embodiments, therapeutic efficiency refers to the ability to treat, prevent or ameliorate an immune disease, an autoimmune disease or an inflammatory disease in a subject. In some embodiments, a high and stable cell yield is a cell yield of at least 30%, possibly at least 40%, typically at least 50% of cells in the population out of cells initially frozen.

[0073] In some embodiments, in case the cell-preparation is obtained from a donor having a high triglyceride level, the donor will take at least one measure selected from the group consisting of: taking triglyceride-lowering medication prior to donation, such as, but not limited to: statins and/or bezafibrate, fasting for a period of at least 8, 10, 12 hours prior to donation, eating an appropriate diet to reduce blood triglyceride level at least 24, 48, 72 hours prior to donating and any combination thereof.

[0074] In some embodiments, cell yield in the population relates to cell number in the composition out of the initial number of cells subjected to apoptosis induction. As used herein, the terms “induction of early apoptotic state” and “induction of apoptosis” may be used interchangeably.

[0075] In some embodiments, the mononuclear-enriched cell composition is incubated in incubation medium following freezing and thawing. In some embodiments, there is at least one washing step between thawing and incubation. As used herein, the terms “incubation medium” and “apoptosis inducing incubation medium” are used interchangeably. In some embodiments, the incubation medium comprises RPMI 1640 medium supplemented with L-glutamine, Hepes methylprednisolone and plasma. In some embodiments, the washing medium comprises 2 mM L-glutamine, 10 mM Hepes and 10% v/v blood plasma. In some embodiments, the blood plasma in in the incubation medium is derived from the same donor from whom the cells of the cell preparations are derived. In some embodiments, the blood plasma is added to the incubation medium on the day of incubation. In some embodiments, incubation is performed at 37° C. and 5% CO.sub.2.

[0076] In some embodiments, the incubation medium comprises methylprednisolone. In some embodiments, the methylprednisolone within the incubation medium further induces the cells in the mononuclear-enriched cell composition to enter an early-apoptotic state. In some embodiments, the cells in the mononuclear-enriched cell composition are induced to enter an early-apoptotic state both by freezing and incubating in the presence of methylprednisolone. In some embodiments, the production of an early apoptotic cell population advantageously allows induction of an early-apoptosis state substantially without induction of necrosis, wherein the cells remain stable at said early-apoptotic state for about 24 hours following preparation.

[0077] In some embodiments, the incubation medium comprises methylprednisolone at a concentration of about 10-100 g/ml. In some embodiments, the incubation medium comprises methylprednisolone at a concentration of about 40-60 g/ml, alternatively about 45-55 g/ml. In some embodiments, the incubation medium comprises methylprednisolone at a concentration of 50 g/ml.

[0078] In some embodiments, the incubation is for about 2-12 hours, possibly 4-8 hours, typically for about 5-7 hours. In some embodiments, the incubation is for about 6 hours. In some embodiments, the incubation is for at least 6 hours. In a preferred embodiment, the incubation is for

6 hours.
[0079] In some embodiments, the incubation medium comprises an anti-coagulant. In some embodiments, addition of an anti-coagulant to the incubation medium improves the yield of the cell-preparation. In some embodiments, the anti-coagulant in the incubation medium is of the same concentration as within the freezing medium. In some embodiments, the incubation medium comprises an anti-coagulant selected from the group consisting of: heparin, ACD Formula A and a combination thereof. In some embodiments, the anti-coagulant used in the incubation medium is ACD Formula A containing heparin at a concentration of 10 U/ml.
[0080] In some embodiments, the incubation medium comprises heparin. In some embodiments, the heparin in the incubation medium is at a concentration of between 0.1-2.5 U/ml. In some embodiments, the heparin in the incubation medium is at a concentration of between 0.1-2.5 U/ml, possibly between 0.3-0.7 U/ml, typically about 0.5 U/ml. In certain embodiments, the heparin in the incubation medium is at a concentration of about 0.5 U/ml.
[0081] In some embodiments, the incubation medium comprises ACD Formula A. In some embodiments, the ACD Formula A in the incubation medium is at a concentration of between 1%-15% v/v. In some embodiments, the ACD Formula A in the incubation medium is at a concentration of between 1%-15% v/v, possibly between 4%-7% v/v, typically about 5% v/v. In some embodiments, the ACD Formula A in the incubation medium is at a concentration of about 5% v/v.
[0082] In some embodiments, improvement in the yield of the cell-preparation comprises improvement in the number of the early-apoptotic viable cells of the preparation out of the number of frozen cells from which the preparation was produced.
[0083] In some embodiments, addition of an anti-coagulant to the freezing medium contributes to a high and stable yield between different preparations of the pharmaceutical population. In preferable embodiments, addition of an anti-coagulant at least to the freezing medium and incubation medium results in a high and stable yield between different preparations of the pharmaceutical composition, regardless to the cell collection protocol used.
[0084] In some embodiments, the freezing medium comprises an anti-coagulant selected from the group consisting of heparin, ACD Formula A and a combination thereof. In some embodiments, the anti-coagulant used in the freezing medium is ACD Formula A containing heparin at a concentration of 10 U/ml. In some embodiments, the freezing medium comprises 5% v/v of ACD Formula A solution comprising heparin at a concentration of 10 U/ml.
[0085] In some embodiments, the freezing medium comprises heparin. In some embodiments, the heparin in the freezing medium is at a concentration of between 0.1-2.5 U/ml. In some embodiments, the heparin in the freezing medium is at a concentration of between 0.1-2.5 U/ml, possibly between 0.3-0.7 U/ml, typically about 0.5 U/ml. In certain embodiments, the heparin in the freezing medium is at a concentration of about 0.5 U/ml.
[0086] In some embodiments, the freezing medium comprises ACD Formula A. In some embodiments, the ACD Formula A in the freezing medium is at a concentration of between 1%-15% v/v. In some embodiments, the ACD Formula A in the freezing medium is at a concentration of between 1%-15% v/v, possibly between 4%-7% v/v, typically about 5% v/v. In some embodiments, the ACD Formula A in the freezing medium is at a concentration of about 5% v/v.
[0087] In some embodiments, addition of an anti-coagulant to the incubation medium and/or freezing medium results in a high and stable cell yield within the population regardless of the triglyceride level in the blood of the donor. In some embodiments, addition of an anti-coagulant to the incubation medium and/or freezing medium results in a high and stable cell yield within the composition of the invention when obtained from the blood of a donor having normal or high triglyceride level. In some embodiments, addition of an anti-coagulant at least to the incubation medium, results in a high and stable cell yield within the composition regardless of the triglyceride

level in the blood of the donor. In some embodiments, addition of an anti-coagulant to the freezing medium and incubation medium results in a high and stable cell yield within the composition regardless of the triglyceride level in the blood of the donor.
[0088] In some embodiments, the freezing medium and/or incubation medium and/or washing medium comprise heparin at a concentration of at least 0.1 U/ml, possibly at least 0.3 U/ml, typically at least 0.5 U/ml. In some embodiments, the freezing medium and/or incubation medium and/or washing medium comprise ACD Formula A at a concentration of at least 1% v/v, possibly at least 3% v/v, typically at least 5% v/v.
[0089] In some embodiments, the mononuclear-enriched cell composition undergoes at least one washing step following cell collection and prior to being re-suspended in the freezing medium and frozen. In some embodiments, the mononuclear-enriched cell composition undergoes at least one washing step following freezing and thawing. In some embodiments, washing steps comprise centrifugation of the mononuclear-enriched cell composition followed by supernatant extraction and re-suspension in washing medium.
[0090] In some embodiments, the mononuclear-enriched cell composition undergoes at least one washing step between each stage of the production of an early apoptotic cell population. In some embodiments, anti-coagulant is added to washing media during washing steps throughout the production of an early apoptotic cell population. In some embodiments, the mononuclear-enriched cell composition undergoes at least one washing step following incubation. In some embodiments, the mononuclear-enriched cell composition undergoes at least one washing step following incubation using PBS. In some embodiments, anti-coagulant is not added to the final washing step prior to re-suspension of the cell-preparation in the administration medium. In some embodiments, anti-coagulant is not added to the PBS used in the final washing step prior to re-suspension of the cell-preparation in the administration medium. In certain embodiments, anti-coagulant is not added to the administration medium.
[0091] In some embodiments, the cell concentration during incubating is about 5×106 cells/ml.
[0092] In some embodiments, the mononuclear-enriched cell composition is suspended in an administration medium following freezing, thawing and incubating, thereby resulting in the pharmaceutical population. In some embodiments, the administration medium comprises a suitable physiological buffer. Non-limiting examples of a suitable physiological buffer are saline solution, Phoshpate Buffered Saline (PBS), Hank's Balanced Salt Solution (HBSS), and the like. In some embodiments, the administration medium comprises PBS. In some embodiments, the administration medium comprises supplements conducive to maintaining the viability of the cells. In some embodiments, the mononuclear-enriched cell composition is filtered prior to administration. In some embodiments, the mononuclear-enriched cell composition is filtered prior to administration using a filter of at least 200p m.
[0093] In some embodiments, the mononuclear-enriched cell population is re-suspended in an administration medium such that the final volume of the resulting cell-preparation is between 100-1000 ml, possibly between 200-800 ml, typically between 300-600 ml.
[0094] In some embodiments, cell collection refers to obtaining a mononuclear-enriched cell composition. In some embodiments, washing steps performed during the production of an early apoptotic cell population are performed in a washing medium. In certain embodiments, washing steps performed up until the incubation step of the production of an early apoptotic cell population are performed in a washing medium. In some embodiments, the washing medium comprises RPMI 1640 medium supplemented with L-glutamine and Hepes. In some embodiments, the washing medium comprises RPMI 1640 medium supplemented with 2 mM L-glutamine and 10 mM Hepes.
[0095] In some embodiments, the washing medium comprises an anti-coagulant. In some embodiments, the washing medium comprises an anti-coagulant selected from the group consisting of heparin, ACD Formula A and a combination thereof. In some embodiments, the concentration of the anti-coagulant in the washing medium is the same concentration as in the freezing medium. In

some embodiments, the concentration of the anti-coagulant in the washing medium is the same concentration as in the incubation medium. In some embodiments, the anti-coagulant used in the washing medium is ACD Formula A containing heparin at a concentration of 10 U/ml.
[0096] In some embodiments, the washing medium comprises heparin. In some embodiments, the heparin in the washing medium is at a concentration of between 0.1-2.5 U/ml. In some embodiments, the heparin in the washing medium is at a concentration of between 0.1-2.5 U/ml, possibly between 0.3-0.7 U/ml, typically about 0.5 U/ml. In certain embodiments, the heparin in the washing medium is at a concentration of about 0.5 U/ml.
[0097] In some embodiments, the washing medium comprises ACD Formula A. In some embodiments, the ACD Formula A in the washing medium is at a concentration of between 1%-15% v/v. In some embodiments, the ACD Formula A in the washing medium is at a concentration of between 1%-15% v/v, possibly between 4%-7% v/v, typically about 5% v/v. In some embodiments, the ACD Formula A in the washing medium is at a concentration of about 5% v/v.
[0098] In some embodiments, the mononuclear-enriched cell composition is thawed several hours prior to the intended administration of the population to a subject. In some embodiments, the mononuclear-enriched cell composition is thawed at about 33° C.-39° C. In some embodiments, the mononuclear-enriched cell composition is thawed for about 30-240 seconds, preferably 40-180 seconds, most preferably 50-120 seconds.
[0099] In some embodiments, the mononuclear-enriched cell composition is thawed at least 10 hours prior to the intended administration of the population, alternatively at least 20, 30, 40 or 50 hours prior to the intended administration of the population. In some embodiments, the mononuclear-enriched cell composition is thawed at least 15-24 hours prior to the intended administration of the population. In some embodiments, the mononuclear-enriched cell composition is thawed at least about 24 hours prior to the intended administration of the population. In some embodiments, the mononuclear-enriched cell composition is thawed at least 20 hours prior to the intended administration of the population. In some embodiments, the mononuclear-enriched cell composition is thawed 30 hours prior to the intended administration of the population. In some embodiments, the mononuclear-enriched cell composition is thawed at least 24 hours prior to the intended administration of the population. In some embodiments, the mononuclear-enriched cell composition undergoes at least one step of washing in the washing medium before and/or after thawing.
[0100] In some embodiments, the composition further comprises methylprednisolone. At some embodiments, the concentration of methylprednisolone does not exceed 30 g/ml.
[0101] In some embodiments, the apoptotic cells are used at a high dose. In some embodiments, the apoptotic cells are used at a high concentration. In some embodiments, human apoptotic polymorphonuclear neutrophils (PMNs) are used. In some embodiments, a group of cells, of which 50% are apoptotic cells, are used. In some embodiments, apoptotic cells are verified by May-Giemsa-stained cytopreps. In some embodiments, viability of cells is assessed by trypan blue exclusion. In some embodiments, the apoptotic and necrotic status of the cells are confirmed by annexin V/propidium iodide staining with detection by FACS.
[0102] In some embodiments, apoptotic cells disclosed herein comprise no necrotic cells. In some embodiments, apoptotic cells disclosed herein comprise less than 1% necrotic cells. In some embodiments, apoptotic cells disclosed herein comprise less than 2% necrotic cells. In some embodiments, apoptotic cells disclosed herein comprise less than 3% necrotic cells. In some embodiments, apoptotic cells disclosed herein comprise less than 4% necrotic cells. In some embodiments, apoptotic cells disclosed herein comprise less than 5% necrotic cells.
[0103] In some embodiments, the apoptotic cells are prepared from cells obtained from a subject other than the subject that will receive said apoptotic cells. In some embodiments, the methods as disclosed herein comprise an additional step that is useful in overcoming rejection of allogeneic

donor cells, including one or more steps described in U.S. Patent Application Publication 20130156794, which is incorporated herein by reference in its entirety. In some embodiments, the methods comprise the step of full or partial lymphodepletion prior to administration of the apoptotic cells, which in some embodiments, are allogeneic apoptotic cells. In some embodiments, the lymphodepletion is adjusted so that it delays the host versus graft reaction for a period sufficient to allow the allogeneic apoptotic cells to control cytokine release. In some embodiments, the methods comprise the step of administering agents that delay egression of the allogeneic apoptotic T-cells from lymph nodes, such as 2-amino-2-[2-(4-octylphenyl)ethyl]propane-1,3-diol (FTY720), 5-[4-phenyl-5-(trifluoromethyl)thiophen-2-yl]-3-[3-(trifluoromethyl)pheny-1]1,2,4-oxadiazole (SEW2871), 3-(2-(-hexylphenylamino)-2-oxoethylamino)propanoic acid (W123), 2-ammonio-4-(2-chloro-4-(3-phenoxyphenylthio)phenyl)-2-(hydroxymethyl)but-yl hydrogen phosphate (KRP-203 phosphate) or other agents known in the art, may be used as part of the compositions and methods as disclosed herein to allow the use of allogeneic apoptotic cells having efficacy and lacking initiation of graft vs host disease. In another embodiment, MHC expression by the allogeneic apoptotic T-cells is silenced to reduce the rejection of the allogeneic cells.

[0104] In some embodiments, methods comprise producing a population of mononuclear apoptotic cell comprising a decreased percent of non-quiescent non-apoptotic viable cells; a suppressed cellular activation of any living non-apoptotic cells; or a reduced proliferation of any living non-apoptotic cells; or any combination thereof, said method comprising the following steps, obtaining a mononuclear-enriched cell population of peripheral blood; freezing said mononuclear-enriched cell population in a freezing medium comprising an anticoagulant; thawing said mononuclear-enriched cell population; incubating said mononuclear-enriched cell population in an apoptosis inducing incubation medium comprising methylprednisolone at a final concentration of about 10-100 g/mL and an anticoagulant; resuspending said apoptotic cell population in an administration medium; and inactivating said mononuclear-enriched population, wherein said inactivation occurs following apoptotic induction, wherein said method produces a population of mononuclear apoptotic cell comprising a decreased percent of non-quiescent non-apoptotic cells; a suppressed cellular activation of any living non-apoptotic cells; or a reduced proliferation of any living non-apoptotic cells; or any combination thereof.

[0105] In some embodiments, the methods comprise the step of irradiating a population of apoptotic cells derived from a subject prior to administration of the population of apoptotic cells to the same subject (autologous ApoCells; Autocetra). In some embodiments, the methods comprise the step of irradiating apoptotic cells derived from a subject prior to administration of the population of apoptotic cells to a recipient (allogeneic ApoCells; Allocetra).

[0106] In some embodiments, cells are irradiated in a way that will decrease proliferation and/or activation of residual viable cells within the apoptotic cell population. In some embodiments, cells are irradiated in a way that reduces the percent of viable non-apoptotic cells in a population. In some embodiments, the percent of viable non-apoptotic cells in an inactivated early apoptotic cell population is reduced to less than 50% of the population. In some embodiments, the percent of viable non-apoptotic cells in an inactivated early apoptotic cell population is reduced to less than 40% of the population. In some embodiments, the percent of viable non-apoptotic cells in an inactivated early apoptotic cell population is reduced to less than 30% of the population. In some embodiments, the percent of viable non-apoptotic cells in an inactivated early apoptotic cell population is reduced to less than 20% of the population. In some embodiments, the percent of viable non-apoptotic cells in an inactivated early apoptotic cell population is reduced to less than 10% of the population. In some embodiments, the percent of viable non-apoptotic cells in an inactivated early apoptotic cell population is reduced to 0% of the population.

[0107] In another embodiment, the irradiated apoptotic cells preserve all their early apoptotic-, immune modulation-, stability-properties. In another embodiment, the irradiation step uses UV radiation. In another embodiment, the radiation step uses gamma radiation. In another embodiment,

the apoptotic cells comprise a decreased percent of living non-apoptotic cells, comprise a preparation having a suppressed cellular activation of any living non-apoptotic cells present within the apoptotic cell preparation, or comprise a preparation having reduced proliferation of any living non-apoptotic cells present within the apoptotic cell preparation, or any combination thereof.

[0108] In some embodiments, irradiation of apoptotic cells does not increase the population of dead cells (PI+) compared with apoptotic cells not irradiated. In some embodiments, irradiation of apoptotic cells does not increase the population of dead cells (PI+) by more than about 1% compared with apoptotic cells not irradiated. In some embodiments, irradiation of apoptotic cells does not increase the population of dead cells (PI+) by more than about 2% compared with apoptotic cells not irradiated. In some embodiments, irradiation of apoptotic cells does not increase the population of dead cells (PI+) by more than about 3% compared with apoptotic cells not irradiated. In some embodiments, irradiation of apoptotic cells does not increase the population of dead cells (PI+) by more than about 4% compared with apoptotic cells not irradiated. In some embodiments, irradiation of apoptotic cells does not increase the population of dead cells (PI+) by more than about 5% compared with apoptotic cells not irradiated. In some embodiments, irradiation of apoptotic cells does not increase the population of dead cells (PI+) by more than about 6% compared with apoptotic cells not irradiated. In some embodiments, irradiation of apoptotic cells does not increase the population of dead cells (PI+) by more than about 7% compared with apoptotic cells not irradiated. In some embodiments, irradiation of apoptotic cells does not increase the population of dead cells (PI+) by more than about 8% compared with apoptotic cells not irradiated. In some embodiments, irradiation of apoptotic cells does not increase the population of dead cells (PI+) by more than about 9% compared with apoptotic cells not irradiated. In some embodiments, irradiation of apoptotic cells does not increase the population of dead cells (PI+) by more than about 10% compared with apoptotic cells not irradiated. In some embodiments, irradiation of apoptotic cells does not increase the population of dead cells (PI+) by more than about 15% compared with apoptotic cells not irradiated. In some embodiments, irradiation of apoptotic cells does not increase the population of dead cells (PI+) by more than about 20%, 25%, 30%, 35%, 40%, 45%, or 50% compared with apoptotic cells not irradiated.

[0109] In some embodiments, a cell population comprising a reduced or non-existent fraction of living non-apoptotic cells may in one embodiment provide a mononuclear early apoptotic cell population that does not have any living/viable cells. In some embodiments, a cell population comprising a reduced or non-existent fraction of living non-apoptotic cells may in one embodiment provide a mononuclear apoptotic cell population that does not elicit GVHD in a recipient.

[0110] In some embodiments, use of irradiated ApoCells removes the possible graft versus leukemia effect use of an apoptotic population (that includes a minor portion of viable cells) may cause, demonstrating that the effects shown here in the Examples (See Example 4) result from the apoptotic cells and not from a viable proliferating population of cells with cellular activity, present within the apoptotic cell population.

[0111] In another embodiment, the methods comprise the step of irradiating apoptotic cells derived from WBCs from a donor prior to administration to a recipient. In some embodiments, cells are irradiated in a way that will avoid proliferation and/or activation of residual viable cells within the apoptotic cell population. In another embodiment, the irradiated apoptotic cells preserve all their early apoptotic-, immune modulation-, stability-properties. In another embodiment, the irradiation step uses UV radiation. In another embodiment, the radiation step uses gamma radiation. In another embodiment, the apoptotic cells comprise a decreased percent of living non-apoptotic cells, comprise a preparation having a suppressed cellular activation of any living non-apoptotic cells present within the apoptotic cell preparation, or comprise a preparation having reduced proliferation of any living non-apoptotic cells present within the apoptotic cell preparation, or any combination thereof.

[0112] In some embodiments, apoptotic cells comprise a pooled mononuclear apoptotic cell

preparation. In some embodiments, a pooled mononuclear apoptotic cell preparation comprises mononuclear cells in an early apoptotic state, wherein said pooled mononuclear apoptotic cells comprise a decreased percent of living non-apoptotic cells, a preparation having a suppressed cellular activation of any living non-apoptotic cells, or a preparation having reduced proliferation of any living non-apoptotic cells, or any combination thereof. In another embodiment, the pooled mononuclear apoptotic cells have been irradiated. In another embodiment, disclosed herein is a pooled mononuclear apoptotic cell preparation that in some embodiments, originates from the white blood cell fraction (WBC) obtained from donated blood.

[0113] In some embodiments, the apoptotic cell preparation is irradiated. In another embodiment, said irradiation comprises gamma irradiation or UV irradiation. In yet another embodiment, the irradiated preparation has a reduced number of non-apoptotic cells compared with a non-irradiated apoptotic cell preparation. In another embodiment, the irradiated preparation has a reduced number of proliferating cells compared with a non-irradiated apoptotic cell preparation. In another embodiment, the irradiated preparation has a reduced number of potentially immunologically active cells compared with a non-irradiated apoptotic cell population.

[0114] In some embodiments, pooled blood comprises 3rd party blood not matched between donor and recipient (allogeneic).

[0115] A skilled artisan would appreciate that the term “pooled” may encompass blood collected from multiple donors, prepared and possibly stored for later use. This combined pool of blood may then be processed to produce a pooled mononuclear apoptotic cell preparation. In another embodiment, a pooled mononuclear apoptotic cell preparation ensures that a readily available supply of mononuclear apoptotic cells is available. In another embodiment, cells are pooled just prior to the incubation step wherein apoptosis is induced. In another embodiment, cells are pooled following the incubation step at the step of resuspension. In another embodiment, cells are pooled just prior to an irradiation step. In another embodiment, cells are pooled following an irradiation step. In another embodiment, cells are pooled at any step in the methods of preparation.

[0116] In some embodiments, a population of pooled mononuclear apoptotic cells comprises blood or apoptotic cells or cells from any step within the process of making early apoptotic cells that have been pooled. In some embodiments, cells are allogeneic. In some embodiments, cells are autologous.

[0117] In some embodiments, a pooled apoptotic cell preparation is derived from cells present in between about 2 and 25 units of blood. In another embodiment, said pooled apoptotic cell preparation is comprised of cells present in between about 2-5, 2-10, 2-15, 2-20, 5-10, 5-15, 5-20, 5-25, 10-15, 10-20, 10-25, 6-13, or 6-25 units of blood. In another embodiment, said pooled apoptotic cell preparation is comprised of cells present in about 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24 or 25 units of blood. The number of units of blood needed is also dependent upon the efficiency of WBC recovery from blood. For example, low efficiency WBC recovery would lead to the need for additional units, while high efficiency WBC recovery would lead to fewer units needed. In some embodiments, each unit is a bag of blood. In another embodiment, a pooled apoptotic cell preparation is comprised of cells present in at least 25 units of blood, at least 50 units of blood, or at least 100 units of blood.

[0118] In some embodiments, the units of blood comprise white blood cell (WBC) fractions from blood donations. In another embodiment, the donations may be from a blood center or blood bank. In another embodiment, the donations may be from donors in a hospital gathered at the time of preparation of the pooled apoptotic cell preparation. In another embodiment, units of blood comprising WBCs from multiple donors are saved and maintained in an independent blood bank created for the purpose of compositions and methods thereof as disclosed herein. In another embodiment, a blood bank developed for the purpose of compositions and methods thereof as disclosed herein, is able to supply units of blood comprising WBC from multiple donors and comprises a leukapheresis unit.

[0119] In some embodiments, the units of pooled WBCs are not restricted by HLA matching. Therefore, the resultant pooled apoptotic cell preparation comprises cell populations not restricted by HLA matching. Accordingly, in certain embodiments a pooled mononuclear apoptotic cell preparation comprises allogeneic cells.
[0120] An advantage of a pooled mononuclear apoptotic cell preparation that is derived from pooled WBCs not restricted by HLA matching, is a readily available source of WBCs and reduced costs of obtaining WBCs.
[0121] In some embodiments, pooled blood comprises blood from multiple donors independent of HLA matching. In another embodiment, pooled blood comprises blood from multiple donors wherein HLA matching with the recipient has been taken into consideration. For example, wherein 1 HLA allele, 2 HLA alleles, 3 HLA alleles, 4 HLA alleles, 5 HLA alleles, 6 HLA alleles, or 7 HLA alleles have been matched between donors and recipient. In another embodiment, multiple donors are partially matched, for example some of the donors have been HLA matched wherein 1 HLA allele, 2 HLA alleles, 3 HLA alleles, 4 HLA alleles, 5 HLA alleles, 6 HLA alleles, or 7 HLA alleles have been matched between some of the donors and recipient. Each possibility comprises an embodiment as disclosed herein.
[0122] In certain embodiments, some viable non-apoptotic cells (apoptosis resistant) may remain following the induction of apoptosis step described below (Example 1). The presence of these viable non-apoptotic cells is, in some embodiments, is observed prior to an irradiation step. These viable non-apoptotic cells may be able to proliferate or be activated. In some embodiments, the pooled mononuclear apoptotic cell preparation derived from multiple donors may be activated against the host, activated against one another, or both.
[0123] In some embodiments, an irradiated cell preparation as disclosed herein has suppressed cellular activation and reduced proliferation compared with a non-irradiated cell preparation. In another embodiment, the irradiation comprises gamma irradiation or UV irradiation. In another embodiment, an irradiated cell preparation has a reduced number of non-apoptotic cells compared with a non-irradiated cell preparation. In some embodiments, the irradiation comprises about 10 Gray units (Gy) to 60 Gy. In some embodiments, the irradiation comprises about 10 Gray units (Gy) to 6 Gy. In some embodiments, the irradiation comprises about 10 Gray units (Gy) to 50 Gy. In some embodiments, the irradiation comprises about 10 Gray units (Gy) to 40 Gy. In some embodiments, the irradiation comprises about 10 Gray units (Gy) to 30 Gy. In some embodiments, the irradiation comprises about 10 Gray units (Gy) to 20 Gy. In some embodiments, the irradiation comprises about 10 Gray units (Gy) to 15 Gy.
[0124] In another embodiment, the irradiation comprises about 10 Gray units (Gy). In another embodiment, the irradiation comprises about 15 Gray units (Gy). In another embodiment, the irradiation comprises about 20 Gray units (Gy). In another embodiment, the irradiation comprises about 25 Gray units (Gy). In another embodiment, the irradiation comprises about 30 Gray units (Gy). In another embodiment, the irradiation comprises about 35 Gray units (Gy). In another embodiment, the irradiation comprises about 40 Gray units (Gy). In another embodiment, the irradiation comprises about 45 Gray units (Gy). In another embodiment, the irradiation comprises about 50 Gray units (Gy). In another embodiment, the irradiation comprises about 55 Gray units (Gy). In another embodiment, the irradiation comprises about 60 Gray units (Gy). Gray. In another embodiment, an irradiated pooled apoptotic cell preparation maintains the same or a similar apoptotic profile, stability and efficacy as a non-irradiated pooled apoptotic cell preparation.
[0125] In some embodiments, a pooled mononuclear apoptotic cell preparation as disclosed herein is stable for up to 24 hours. In another embodiment, a pooled mononuclear apoptotic cell preparation is stable for at least 24 hours. In another embodiment, a pooled mononuclear apoptotic cell preparation is stable for more than 24 hours. In yet another embodiment, a pooled mononuclear apoptotic cell preparation as disclosed herein is stable for up to 36 hours. In still another embodiment, a pooled mononuclear apoptotic cell preparation is stable for at least 36 hours. In a

further embodiment, a pooled mononuclear apoptotic cell preparation is stable for more than 36 hours. In another embodiment, a pooled mononuclear apoptotic cell preparation as disclosed herein is stable for up to 48 hours. In another embodiment, a pooled mononuclear apoptotic cell preparation is stable for at least 48 hours. In another embodiment, a pooled mononuclear apoptotic cell preparation is stable for more than 48 hours.

[0126] In some embodiments, methods of producing the pooled cell preparation comprising an irradiation step preserves the early apoptotic, immune modulation, and stability properties observed in an apoptotic preparation derived from a single match donor wherein the cell preparation may not include an irradiation step. In another embodiment, a pooled mononuclear apoptotic cell preparation as disclosed herein does not elicit a graft versus host disease (GVHD) response.

[0127] Irradiation of the cell preparation is considered safe in the art. Irradiation procedures are currently performed on a routine basis to donated blood to prevent reactions to WBC.

[0128] In another embodiment, the percent of apoptotic cells in a pooled mononuclear apoptotic cell preparation as disclosed herein is close to 100%, thereby reducing the fraction of living non-apoptotic cells in the cell preparation. In some embodiments, the percent of apoptotic cells is at least 40%. In another embodiment, the percent of apoptotic cells is at least 50%. In yet another embodiment, the percent of apoptotic cells is at least 60%. In still another embodiment, the percent of apoptotic cells is at least 70%. In a further embodiment, the percent of apoptotic cells is at least 80%. In another embodiment, the percent of apoptotic cells is at least 90%. In yet another embodiment, the percent of apoptotic cells is at least 99%. Accordingly, a cell preparation comprising a reduced or non-existent fraction of living non-apoptotic cells may in one embodiment provide a pooled mononuclear apoptotic cell preparation that does not elicit GVHD in a recipient. Each possibility represents an embodiment as disclosed herein.

[0129] Alternatively, in another embodiment, the percentage of living non-apoptotic WBC is reduced by specifically removing the living cell population, for example by targeted precipitation. In another embodiment, the percent of living non-apoptotic cells may be reduced using magnetic beads that bind to phosphatidylserine. In another embodiment, the percent of living non-apoptotic cells may be reduced using magnetic beads that bind a marker on the cell surface of non-apoptotic cells but not apoptotic cells. In another embodiment, the apoptotic cells may be selected for further preparation using magnetic beads that bind to a marker on the cell surface of apoptotic cells but not non-apoptotic cells. In yet another embodiment, the percentage of living non-apoptotic WBC is reduced by the use of ultrasound.

[0130] In one embodiment the apoptotic cells are from pooled third-party donors.

[0131] In some embodiments, a pooled cell preparation comprises at least one cell type selected from the group consisting of: lymphocytes, monocytes and natural killer cells. In another embodiment, a pooled cell preparation comprises an enriched population of mononuclear cells. In some embodiments, a pooled mononuclear is a mononuclear enriched cell preparation comprises cell types selected from the group consisting of lymphocytes, monocytes and natural killer cells. In another embodiment, the mononuclear enriched cell preparation comprises no more than 15%, alternatively no more than 10%, typically no more than 5% polymorphonuclear leukocytes, also known as granulocytes (i.e., neutrophils, basophils and eosinophils). In another embodiment, a pooled mononuclear cell preparation is devoid of granulocytes.

[0132] In another embodiment, the pooled mononuclear enriched cell preparation comprises no more than 15%, alternatively no more than 10%, typically no more than 5% CD15high expressing cells. In some embodiments, a pooled apoptotic cell preparation comprises less than 15% CD15 high expressing cells.

[0133] In some embodiments, the pooled mononuclear enriched cell preparation disclosed herein comprises at least 80% mononuclear cells, at least 85% mononuclear cells, alternatively at least 90% mononuclear cells, or at least 95% mononuclear cells, wherein each possibility is a separate embodiment disclosed herein. According to some embodiments, the pooled mononuclear enriched

cell preparation disclosed herein comprises at least 85% mononuclear cells.
[0134] In another embodiment, any pooled cell preparation that has a final pooled percent of mononuclear cells of at least 80% is considered a pooled mononuclear enriched cell preparation as disclosed herein. Thus, pooling cell preparations having increased polymorphonuclear cells (PMN) with cell preparations having high mononuclear cells with a resultant “pool” of at least 80% mononuclear cells comprises a preparation as disclosed herein. According to some embodiments, mononuclear cells comprise lymphocytes and monocytes.
[0135] A skilled artisan would appreciate that the term “mononuclear cells” may encompass leukocytes having a one lobed nucleus. In another embodiment, a pooled apoptotic cell preparation as disclosed herein comprises less than 5% polymorphonuclear leukocytes.
[0136] In some embodiments, the apoptotic cells are T-cells. In another embodiment, the apoptotic cells are derived from the same pooled third-party donor T-cells as the CAR T-cells. In another embodiment, the apoptotic cells are derived from the CAR T-cell population.
Supernatants—Apoptotic Cell Supernatants & Apoptotic Cell-Phagocyte Supernatants
[0137] In some embodiments, apoptotic cell supernatants may be used in the methods and treatments as disclosed herein include an apoptotic cell supernatant and apoptotic-phagocyte supernatants, as disclosed herein. In some embodiments, the cells from which a supernatant is collected are autologous with a patient. In some embodiments, the cells from which a supernatant is collected are allogeneic from a donor. In some embodiments, the cells from which a supernatant is collected are allogeneic from a cells collected from a blood bank.
[0138] In some embodiments, the apoptotic cell supernatant is obtained by a method comprising the steps of a) providing apoptotic cells, b) culturing the apoptotic cells of step a), and c) separating the supernatant from the cells. In certain embodiments, the apoptotic cells used for obtaining a supernatant, are prepared by any of the methods disclosed herein. In certain embodiments, the apoptotic cells used for obtaining a supernatant, are prepared by any method known in the art.
[0139] In some embodiments, apoptotic cells for use making an apoptotic cell supernatant as disclosed herein are autologous with a subject undergoing therapy. In another embodiment, apoptotic cells for use in making an apoptotic cell supernatant disclosed herein are allogeneic with a subject undergoing therapy.
[0140] The “apoptotic cells” from which the apoptotic cell supernatant is obtained may be cells chosen from any cell type of a subject, or any commercially available cell line, subjected to a method of inducing apoptosis known to the person skilled in the art. The method of inducing apoptosis may be hypoxia, ozone, heat, radiation, chemicals, osmotic pressure, pH shift, X-ray irradiation, gamma-ray irradiation, UV irradiation, serum deprivation, corticoids or combinations thereof, or any other method described herein or known in the art. In another embodiment, the method of inducing apoptosis produces apoptotic cells in an early apoptotic state.
[0141] In some embodiments, the apoptotic cells are leukocytes.
[0142] In an embodiment, said apoptotic leukocytes are derived from peripheral blood mononuclear cells (PBMC). In another embodiment, said leukocytes are from pooled third-party donors. In another embodiment, said leukocytes are allogeneic.
[0143] According to some embodiments, the apoptotic cells are provided by selecting non-adherent leukocytes and submitting them to apoptosis induction, followed by a cell culture step in culture medium. “Leukocytes” used to make the apoptotic cell-phagocyte supernatant may be derived from any lineage, or sub-lineage, of nucleated cells of the immune system and/or hematopoietic system, including but not limited to dendritic cells, macrophages, mast-cells, basophils, hematopoietic stem cells, bone marrow cells, natural killer cells, and the like. The leukocytes may be derived or obtained in any of various suitable ways, from any of various suitable anatomical compartments, according to any of various commonly practiced methods, depending on the application and purpose, desired leukocyte lineage, etc. In some embodiments, the source leukocytes are primary leukocytes. In another embodiment, the source leukocytes are primary peripheral blood leukocytes.

[0144] Primary lymphocytes and monocytes may be conveniently derived from peripheral blood. Peripheral blood leukocytes include 70-95 percent lymphocytes, and 5-25 percent monocytes.
[0145] Methods for obtaining specific types of source leukocytes from blood are routinely practiced. Obtaining source lymphocytes and/or monocytes can be achieved, for example, by harvesting blood in the presence of an anticoagulant, such as heparin or citrate. The harvested blood is then centrifuged over a Ficoll cushion to isolate lymphocytes and monocytes at the gradient interface, and neutrophils and erythrocytes in the pellet.
[0146] Leukocytes may be separated from each other via standard immunomagnetic selection or immunofluorescent flow cytometry techniques according to their specific surface markers, or via centrifugal elutriation. For example, monocytes can be selected as the CD14.sup.+ fraction, T-lymphocytes can be selected as CD3.sup.+ fraction, B-lymphocytes can be selected as the CD19.sup.+ fraction, macrophages as the CD206.sup.+ fraction.
[0147] Lymphocytes and monocytes may be isolated from each other by subjecting these cells to substrate-adherent conditions, such as by static culture in a tissue culture-treated culturing recipient, which results in selective adherence of the monocytes, but not of the lymphocytes, to the cell-adherent substrate.
[0148] Leukocytes may also be obtained from peripheral blood mononuclear cells (PBMCs), which may be isolated as described herein.
[0149] One of ordinary skill in the art will possess the necessary expertise to suitably culture primary leukocytes so as to generate desired quantities of cultured source leukocytes as disclosed herein, and ample guidance for practicing such culturing methods is available in the literature of the art.
[0150] One of ordinary skill in the art will further possess the necessary expertise to establish, purchase, or otherwise obtain suitable established leukocyte cell lines from which to derive the apoptotic leukocytes. Suitable leukocyte cell lines may be obtained from commercial suppliers, such as the American Tissue Type Collection (ATCC). It will be evident to the person skilled in the art that source leukocytes should not be obtained via a technique which will significantly interfere with their capacity to produce the apoptotic leukocytes.
[0151] In another embodiment, the apoptotic cells may be apoptotic lymphocytes. Apoptosis of lymphocytes, such as primary lymphocytes, may be induced by treating the primary lymphocytes with serum deprivation, a corticosteroid, or irradiation. In another embodiment, inducing apoptosis of primary lymphocytes via treatment with a corticosteroid is affected by treating the primary lymphocytes with dexamethasone. In another embodiment, with dexamethasone at a concentration of about 1 micromolar. In another embodiment, inducing apoptosis of primary lymphocytes via irradiation is affected by treating the primary lymphocytes with gamma-irradiation. In another embodiment, with a dosage of about 66 rad. Such treatment results in the generation of apoptotic lymphocytes suitable for the co-culture step with phagocytes.
[0152] In a further embodiment, apoptotic cells may be apoptotic monocytes, such as primary monocytes. To generate apoptotic monocytes the monocytes are subjected to in vitro conditions of substrate/surface-adherence under conditions of serum deprivation. Such treatment results in the generation of non-pro-inflammatory apoptotic monocytes suitable for the co-culture step with phagocytes.
[0153] In other embodiments, the apoptotic cells may be any apoptotic cells described herein, including allogeneic apoptotic cells, third party apoptotic cells, and pools of apoptotic cells.
[0154] In other embodiments, the apoptotic cell supernatant may be obtained through the co-culture of apoptotic cells with other cells. In some embodiments, the apoptotic cell supernatant may be obtained through the co-culture of apoptotic cells with white blood cells. In some embodiments, the apoptotic cell supernatant may be obtained through the co-culture of apoptotic cells with dendritic cells. In some embodiments, the apoptotic cell supernatant may be obtained through the co-culture of apoptotic cells with macrophages. Macrophages may be produced in different ways including

M1 and M2 macrophages.
[0155] In some embodiments, the apoptotic cell supernatant may be obtained through the culture of white blood cells that have ingested apoptotic cells or fragments thereof. In some embodiments, the apoptotic cell supernatant may be obtained through the culture of dendritic cells that have ingested apoptotic cells or fragments thereof. In some embodiments, the apoptotic cell supernatant may be obtained through the culture of macrophages that have ingested apoptotic cells or fragments thereof.
[0156] In some embodiments, the apoptotic cell supernatant is an apoptotic cell supernatant obtained by a method comprising the steps of a) providing apoptotic cells, b) providing other cells, c) optionally washing the cells from step a) and b), d) co-culturing the cells of step a) and b), and optionally e) separating the supernatant from the cells.
[0157] A skilled artisan would appreciate that as used herein in certain embodiments, the term "supernatant" may be used interchangeably with "apoptotic cell supernatant", "apoptotic supernatant", "apoptotic-phagocyte supernatant", "apoptotic-white blood cell supernatant", "apoptotic-macrophage supernatant", "apoptotic-dendritic supernatant", or the like, having all the same meanings and qualities to affect cytokine release or treat osteoarthritis or vanishing bone disease or a combination thereof, though the source and method of preparation of the supernatant may differ.
[0158] In some embodiments, the other cells co-cultured with the apoptotic cells are white blood cells. In some embodiments, the white blood cells may be phagocytes, such as macrophages, monocytes, or dendritic cells. In some embodiments, the white blood cells may be B cells, T-cells, or natural killer (NK cells).
[0159] In certain embodiments, the apoptotic cell supernatant is an apoptotic cell-white blood cell supernatant obtained by a method comprising the steps of a) providing apoptotic cells, b) providing white blood cells, c) optionally washing the cells from step a) and b), d) co-culturing the cells of step a) and b), and optionally e) separating the supernatant from the cells. In certain embodiments, the apoptotic cell supernatant is an apoptotic cell-white blood cell supernatant obtained by a method comprising the steps of a) providing apoptotic cells, b) providing phagocytes, c) optionally washing the cells from step a) and b), d) co-culturing the cells of step a) and b), and optionally e) separating the supernatant from the cells. In certain embodiments, the apoptotic cell supernatant is an apoptotic cell-white blood cell supernatant obtained by a method comprising the steps of a) providing apoptotic cells, b) providing macrophages, c) optionally washing the cells from step a) and b), d) co-culturing the cells of step a) and b), and optionally e) separating the supernatant from the cells. In certain embodiments, the apoptotic cell supernatant is an apoptotic cell-white blood cell supernatant obtained by a method comprising the steps of a) providing apoptotic cells, b) providing dendritic cells, c) optionally washing the cells from step a) and b), d) co-culturing the cells of step a) and b), and optionally e) separating the supernatant from the cells. In certain embodiments, the apoptotic cell supernatant is an apoptotic cell-white blood cell supernatant obtained by a method comprising the steps of a) providing apoptotic cells, b) providing monocytes, c) optionally washing the cells from step a) and b), d) co-culturing the cells of step a) and b), and optionally e) separating the supernatant from the cells. In certain embodiments, the apoptotic cell supernatant is an apoptotic cell-white blood cell supernatant obtained by a method comprising the steps of a) providing apoptotic cells, b) providing B-cells, c) optionally washing the cells from step a) and b), d) co-culturing the cells of step a) and b), and optionally e) separating the supernatant from the cells. In certain embodiments, the apoptotic cell supernatant is an apoptotic cell-white blood cell supernatant obtained by a method comprising the steps of a) providing apoptotic cells, b) providing T-cells, c) optionally washing the cells from step a) and b), d) co-culturing the cells of step a) and b), and optionally e) separating the supernatant from the cells. In certain embodiments, the apoptotic cell supernatant is an apoptotic cell-white blood cell supernatant obtained by a method comprising the steps of a) providing apoptotic cells, b) providing Natural Killed (NK)

cells, c) optionally washing the cells from step a) and b), d) co-culturing the cells of step a) and b), and optionally e) separating the supernatant from the cells.
[0160] In some embodiments of methods of obtaining a cell supernatant, apoptotic cells are first cultured with white blood cells, in order that the white blood cells will ingest the apoptotic cells or portions thereof.
[0161] Thus, in some embodiments, the methods and treatments disclosed herein use apoptotic cell-phagocyte supernatants, or compositions thereof, as described in WO 2014/106666, which is incorporated by reference herein in its entirety. In another embodiment, apoptotic cell-phagocyte supernatants or compositions thereof, for use in the methods disclosed herein are produced in any way that is known in the art.
[0162] In some embodiments, the apoptotic cell-phagocyte supernatant is obtained from a co-culture of phagocytes with apoptotic cells,
[0163] In some embodiments, the apoptotic cell-phagocyte supernatant is obtained by a method comprising the steps of a) providing phagocytes, b) providing apoptotic cells, c) optionally washing the cells from step a) and b), d) co-culturing the cells of step a) and b), and optionally e) separating the supernatant from the cells.
[0164] The term “phagocytes” denotes cells that protect the body by ingesting (phagocytosing) harmful foreign particles, bacteria, and dead or dying cells. Phagocytes include for example cells called neutrophils, monocytes, macrophages, dendritic cells, and mast T-cells, preferentially dendritic cells and monocytes/macrophages. The phagocytes may be dendritic cells (CD4+ HLA-DR+ Lineage− BDCA1/BDCA3+), macrophages (CD14+ CD206+ HLA-DR+), or derived from monocytes (CD14+). Techniques to distinguish these different phagocytes are known to the person skilled in the art.
[0165] In an embodiment, monocytes are obtained by a plastic adherence step. Said monocytes can be distinguished from B and T-cells with the marker CD14+, whereas unwanted B cells express CD19+ and T-cells CD3+. After Macrophage Colony Stimulating Factor (M-CSF) induced maturation the obtained macrophages are in some embodiments, positive for the markers CD14+, CD206+, HLA-DR+.
[0166] In an embodiment, said phagocytes are derived from peripheral blood mononuclear cells (PBMC).
[0167] Phagocytes may be provided by any method known in the art for obtaining phagocytes. In some embodiments, phagocytes such as macrophages or dendritic cells can be directly isolated from a subject or be derived from precursor cells by a maturation step.
[0168] In some embodiments, macrophages may be directly isolated from the peritoneum cavity of a subject and cultured in complete RRPMI medium. Macrophages can also be isolated from the spleen.
[0169] Phagocytes are also obtainable from peripheral blood monocytes. In said example, monocytes when cultured differentiate into monocyte-derived macrophages upon addition of, without limitation to, macrophage colony stimulating factor (M-CSF) to the cell culture media.
[0170] For example, phagocytes may be derived from peripheral blood mononuclear cells (PBMC). For example, PBMC may be isolated from cytapheresis bag from an individual through Ficoll gradient centrifugation, plated in a cell-adherence step for 90 min in complete RPMI culture medium (10% FBS, 1% Penicillin/Streptomycin). Non-adherent T-cells are removed by a plastic adherence step, and adherent T-cells cultured in complete RPMI milieu supplemented with recombinant human M-CSF. After the culture period, monocyte-derived macrophages are obtained.
[0171] Phagocytes can be selected by a cell-adherence step. Said “cell adherence step” means that phagocytes or cells which can mature into phagocytes are selected via culturing conditions allowing the adhesion of the cultured cells to a surface, a cell adherent surface (e.g. a tissue culture dish, a matrix, a sac or bag with the appropriate type of nylon or plastic). A skilled artisan would appreciate that the term “Cell adherent surfaces” may encompass hydrophilic and negatively

charged, and may be obtained in any of various ways known in the art, In another embodiment by modifying a polystyrene surface using, for example, corona discharge, or gas-plasma. These processes generate highly energetic oxygen ions which graft onto the surface polystyrene chains so that the surface becomes hydrophilic and negatively charged. Culture recipients designed for facilitating cell-adherence thereto are available from various commercial suppliers (e.g. Coming, Perkin-Elmer, Fisher Scientific, Evergreen Scientific, Nunc, etc.).

[0172] B cells, T-cells and NK cells may be provided by any method known in the art for obtaining such cells. In some embodiments, B cells, T-cells or NK cells can be directly isolated from a subject or be derived from precursor cells by a maturation step. In another embodiment, the B, T or NK cells can be from a B, T or NK cell line. One of ordinary skill in the art will possess the necessary expertise to establish, purchase, or otherwise obtain suitable established B cells, T-cells and NK cell lines. Suitable cell lines may be obtained from commercial suppliers, such as the American Tissue Type Collection (ATCC).

[0173] In an embodiment, said apoptotic cells and said white blood cells, such as the phagocytes, B, T or NK cells, are cultured individually prior to the co-culture step d).

[0174] The cell maturation of phagocytes takes place during cell culture, for example due to addition of maturation factors to the media. In one embodiment said maturation factor is M-CSF, which may be used for example to obtain monocyte-derived macrophages.

[0175] The culture step used for maturation or selection of phagocytes might take several hours to several days. In another embodiment said pre-mature phagocytes are cultured for 2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28, 30, 32, 34, 36, 38, 40, 42, 44, 46, 48, 50, 52, 54, 56, 58 hours in an appropriate culture medium.

[0176] The culture medium for phagocytes is known to the person skilled in the art and can be for example, without limitation, RPMI, DMEM, X-vivo and Ultraculture milieus.

[0177] In an embodiment, co-culture of apoptotic cells and phagocytes takes place in a physiological solution.

[0178] Prior to this “co-culture”, the cells may be submitted to a washing step. In some embodiments, the white blood cells (e.g. the phagocytes) and the apoptotic cells are washed before the co-culture step. In another embodiment, the cells are washed with PBS.

[0179] During said co-culture the white blood cells (e.g. the phagocytes such as macrophages, monocytes, or phagocytes, or the B, T or NK cells) and the apoptotic cells may be mixed in a ratio of 10:1, 9:1; 8:1, 7:1, 6:1, 5:1, 4:1, 3:1, 2:1, or 1:1, or in a ratio of (white blood cells:apoptotic cells) 1:2, 1:3, 1:4, 1:5, 1:6, 1:7, 1:8, 1:9, or 1:10. In one example, the ratio of white blood cells to apoptotic cells is 1:5.

[0180] The co-culture of the cells might be for several hours to several days. In some embodiments, said apoptotic cells are cultured for 2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28, 30, 32, 34, 36, 38, 40, 42, 44, 46, 48, 50, 52 hours. A person skilled in the art can evaluate the optimal time for co-culture by measuring the presence of anti-inflammatory compounds, the viable amount of white blood cells and the amount of apoptotic cells which have not been eliminated so far. elimination of apoptotic cells by phagocytes is observable with light microscopy due to the disappearance of apoptotic cells.

[0181] In some embodiments, the culture of apoptotic cells, such as the co-culture with culture with white blood cells (e.g. phagocytes such as macrophages, monocytes, or phagocytes, or the B, T or NK cells), takes place in culture medium and/or in a physiological solution compatible with administration e.g. injection to a subject.

[0182] A skilled artisan would appreciate that a “physiological solution” may encompass a solution which does not lead to the death of white blood cells within the culture time. In some embodiments, the physiological solution does not lead to death over 2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28, 30, 32, 34, 36, 38, 40, 42, 44, 46, 48, 50, 52 hours. In other embodiment, 48 hours, or 30 hours.

[0183] In some embodiments, the white blood cells (e.g. phagocytes such as macrophages, monocytes, or phagocytes, or the B, T or NK cells) and the apoptotic cells are incubated in the physiological solution for at least 30 min. This time of culture allows phagocytosis initiation and secretion of cytokines and other beneficial substances.
[0184] In an embodiment, such a physiological solution does not inhibit apoptotic leukocyte elimination by leukocyte-derived macrophages.
[0185] At the end of the culture or the co-culture step, the supernatant is optionally separated from the cultured apoptotic cells or the co-cultured cells. Techniques to separate the supernatant from the cells are known in the art. For example, the supernatant can be collected and/or filtered and/or centrifuged to eliminate cells and debris. For example, said supernatant may be centrifuged at 3000 rpm for 15 minutes at room temperature to separate it from the cells.
[0186] The supernatant may be “inactivated” prior to use, for example by irradiation. Therefore, the method for preparing the apoptotic cell supernatant may comprise an optional additional irradiation step f). Said “irradiation” step can be considered as a disinfection method that uses X-ray irradiation (25-45 Gy) at sufficiently rate to kill microorganisms, as routinely performed to inactivate blood products.
[0187] Irradiation of the supernatant is considered safe in the art. Irradiation procedures are currently performed on a routine basis to donated blood to prevent reactions to WBC.
[0188] In an embodiment, the apoptotic cell supernatant is formulated into a pharmaceutical composition suitable for administration to a subject, as described in detail herein.
[0189] In some embodiments, the final product is stored at +4° C. In another embodiment, the final product is for use in the next 48 hours.
[0190] In some embodiments, the apoptotic cell supernatant, such as an apoptotic cell-phagocyte supernatant, or pharmaceutical composition comprising the supernatant, may be lyophilized, for example for storage at −80° C.
[0191] In one specific embodiment, as described in Example 1 of WO 2014/106666, an apoptotic cell-phagocyte supernatant may be made using thymic cells as apoptotic cells. After isolation, thymic cells are irradiated (e.g. with a 35 X-Gray irradiation) and cultured in complete DMEM culture medium for, for example, 6 hours to allow apoptosis to occur. In parallel, macrophages are isolated from the peritoneum cavity, washed and cultured in complete RPMI (10% FBS, Peni-Strepto, EAA, Hepes, NaP and 2-MercaptoEthanol). Macrophages and apoptotic cells are then washed and co-cultured for another 48 hour period in phenol-free X-vivo medium at a 1/5 macrophage/apoptotic cell ratio. Then, supernatant is collected, centrifuged to eliminate debris and may be frozen or lyophilized for conservation. Macrophage enrichment may be confirmed using positive staining for F4/80 by FACS. Apoptosis may be confirmed by FACS using positive staining for Annexin-V and 7AAD exclusion.
[0192] In an embodiment, the apoptotic cell supernatant is enriched in TGF-β levels both in active and latent forms of TGF-β, compared to supernatants obtained from either macrophages or apoptotic cells cultured separately. In an embodiment, IL-10 levels are also increased compared to macrophages cultured alone and dramatically increased compared to apoptotic cells cultured alone. In another embodiment, inflammatory cytokines such as IL-6 are not detectable and IL-1 and TNF are undetectable or at very low levels.
[0193] In an embodiment, the apoptotic cell supernatant, when compared to supernatants from macrophages cultured alone or from apoptotic cells cultured alone, has increased levels of IL-1ra, TIMP-1, CXCL1/KC and CCL2/JE/MCP1, which might be implicated in a tolerogenic role of the supernatant to control inflammation, in addition to TGF-β and IL-10.
[0194] In another specific embodiment, as described in Example 3 of WO 2014/106666, human apoptotic cell-phagocyte supernatant may be made from the co-culture of macrophages derived from peripheral blood mononuclear cells (PBMC) cultured with apoptotic PBMC. Thus, PBMC are isolated from cytapheresis bag from a healthy volunteer through, for example, Ficoll gradient

centrifugation. Then PBMC are plated for 90 min in complete RPMI culture medium (10% FBS, 1% Penicillin/Streptomycin). Then, non-adherenT-cells are removed and rendered apoptotic using, for example, a 35 Gy dose of X-ray irradiation and cultured in complete RPMI milieu for 4 days (including cell wash after the first 48 hrs of culture), in order to allow apoptosis to occur. In parallel, adherent T-cells are cultured in complete RPMI milieu supplemented with 50 g/mL of recombinant human M-CSF for 4 days including cell wash after the first 48 hrs. At the end of the 4-day culture period, monocyte-derived macrophages and apoptotic cells are washed and cultured together in X-vivo medium for again 48 hours at a one macrophage to 5 apoptotic cell ratio. Then supernatant from the latter culture is collected, centrifuged to eliminate cells and debris, and may be frozen or lyophilized for conservation and subsequent use.

[0195] In an embodiment, as described in WO 2014/106666, human apoptotic cell-phagocyte supernatant may be obtained in 6 days from peripheral blood mononuclear cells (PBMC). Four days to obtain PBMC-derived macrophages using M-CSF addition in the culture, and 2 more days for the co-culture of PBMC-derived macrophages with apoptotic cells, corresponding to the non-adherent PBMC isolated at day 0.

[0196] In an embodiment, as described in WO 2014/106666, a standardized human apoptotic cell-phagocyte supernatant may be obtained independently of the donor or the source of PBMC (cytapheresis or buffy coat). The plastic-adherence step is sufficient to obtain a significant starting population of enriched monocytes (20 to 93% of CD14+ cells after adherence on plastic culture dish). In addition, such adherent T-cells demonstrate a very low presence of B and T-cells (1.0% of CD19+ B cells and 12.8% of CD3+ T-cells). After 4 days of culture of adherent T-cells in the presence of M-CSF, the proportion of monocytes derived-macrophages is significantly increased from 0.1% to 77.7% of CD14+CD206+HLA-DR+ macrophages. At that time, monocyte-derived macrophages may be co-cultured with apoptotic non-adherent PBMC (47.6% apoptotic as shown by annexin V staining and 7AAD exclusion) to produce the apoptotic cell-phagocyte supernatant during 48 hours.

[0197] In an embodiment, the collected apoptotic cell-phagocyte supernatant, contains significantly more latent TGF than in the culture supernatant of monocyte-derived macrophages alone or monocyte-derived macrophages treated in inflammatory conditions (+LPS), and only contains trace or low level of inflammatory cytokines such as IL-10 or TNF.

[0198] In another embodiment, as described in Example 3 below, an apoptotic supernatant is prepared with early apoptotic cells and monocytes.

[0199] In some embodiments, the composition comprising the apoptotic cell supernatant further comprises an anti-coagulant. In some embodiments, the anti-coagulant is selected from the group consisting of: heparin, acid citrate dextrose (ACD) Formula A and a combination thereof.

[0200] In another embodiment, an anti-coagulant is added during the process of manufacturing the apoptotic cells used. In another embodiment, the anti-coagulant added is selected from the group comprising ACD and heparin, or any combination thereof. In another embodiment, ACD is at a concentration of 1%. In another embodiment, ACD is at a concentration of 2%. In another embodiment, ACD is at a concentration of 3%. In another embodiment, ACD is at a concentration of 4%. In another embodiment, ACD is at a concentration of 5%. In another embodiment, ACD is at a concentration of 6%. In another embodiment, ACD is at a concentration of 7%. In another embodiment, ACD is at a concentration of 8%. In another embodiment, ACD is at a concentration of 9%. In another embodiment, ACD is at a concentration of 10%. In another embodiment, ACD is at a concentration of between about 1-10%. In another embodiment, ACD is at a concentration of between about 2-8%. In another embodiment, ACD is at a concentration of between about 3-7%. In another embodiment, ACD is at a concentration of between about 1-5%. In another embodiment, ACD is at a concentration of between about 5-10%. In another embodiment, heparin is at a final concentration of 0.5 U/ml. In another embodiment, heparin is at a final concentration of about 0.1 U/ml-1.0 U/ml. In another embodiment, heparin is at a final concentration of about 0.2 U/ml-0.9

U/ml. In another embodiment, heparin is at a final concentration of about 0.3 U/ml-0.7 U/ml. In another embodiment, heparin is at a final concentration of about 0.1 U/ml-0.5 U/ml. In another embodiment, heparin is at a final concentration of about 0.5 U/ml-1.0 U/ml. In another embodiment, heparin is at a final concentration of about 0.01 U/ml-1.0 U/ml. In another embodiment, heparin is at a final concentration of 0.1 U/ml. In another embodiment, heparin is at a final concentration of 0.2 U/ml. In another embodiment, heparin is at a final concentration of 0.3 U/ml. In another embodiment, heparin is at a final concentration of 0.4 U/ml. In another embodiment, heparin is at a final concentration of 0.5 U/ml. In another embodiment, heparin is at a final concentration of 0.6 U/ml. In another embodiment, heparin is at a final concentration of 0.7 U/ml. In another embodiment, heparin is at a final concentration of 0.8 U/ml. In another embodiment, heparin is at a final concentration of 0.9 U/ml. In another embodiment, heparin is at a final concentration of 1.0 U/ml. In another embodiment, ACD is at a concentration of 5% and heparin is at a final concentration of 0.5 U/ml.

[0201] In some embodiments, the composition comprising the apoptotic cell supernatant further comprises methylprednisolone. At some embodiments, the concentration of methylprednisolone does not exceed 30 g/ml.

Apoptotic Cell Administration

[0202] Surprisingly, the apoptotic cells reduce production of pro- and anti-inflammatory cytokines/chemokines. In some embodiments, administration of apoptotic cells reduces production of pro-inflammatory cytokines/chemokines including but not limited to IL-1, IL-6, IL-8, IL-10, IL-1β, IL-2, IL-15, IL-22, MIP-1β, MCP-1, MDC, IP-10, fractalkine, IL-9, or TNFα, alone or in combination. In one embodiment, the apoptotic cells affect cytokine expression levels in synovial fluid. In one embodiment, the apoptotic cells affect cytokine expression levels in joint fluid.

[0203] In some embodiments, administration of apoptotic cells inhibits one or more pro- and anti-inflammatory cytokines. In some embodiments, administration of apoptotic cells inhibits one or more pro-inflammatory cytokine. In some embodiments, administration of apoptotic cells inhibits one or more anti-inflammatory cytokines. In some embodiments, the pro- and anti-inflammatory cytokine comprises IL-1, IL-6, IL-8, IL-10, IL-1β, IL-2, IL-15, IL-22, MIP-1β, MCP-1, MDC, IP-10, fractalkine, IL-9, or TNFα, or any combination thereof. In another embodiment, administration of apoptotic cells promotes the secretion of one or more anti-inflammatory cytokines. In some embodiments, at least one cytokine or chemokine having abnormal expression or content is downregulated by administration of apoptotic cells. In some embodiments, each cytokine or chemokine having abnormal expression or content is downregulated by administration of apoptotic cells.

[0204] In some embodiments, at least one anti-inflammatory cytokine or chemokine having abnormal expression or content is downregulated by administration of apoptotic cells. In some embodiments, at least one pro-inflammatory cytokine or chemokine having abnormal expression or content is downregulated by administration of apoptotic cells. In some embodiments, a combination of pro- and anti-inflammatory cytokines or chemokines having abnormal expression or content are downregulated by administration of apoptotic cells. In some embodiments, any of IL-1, IL-6, IL-8, IL-10, IL-1β, IL-2, IL-15, IL-22, MIP-1β, MCP-1, MDC, IP-10, fractalkine, IL-9, or TNFα, or any combination thereof, having abnormal expression or content, for example in synovial fluid or joint fluid in the area of a joint are downregulated by administration of apoptotic cells. In some embodiments, any of IL-1, IL-6, IL-8, IL-10, IL-1β, IL-2, IL-15, IL-22, MIP-1β, MCP-1, MDC, IP-10, fractalkine, IL-9, or TNFα, or any combination thereof, having abnormal expression or content, for example in the area of vanishing bone disease, are downregulated by administration of apoptotic cells. (See Example 4)

[0205] In some embodiments, a dose of about 100×10.sup.6-210×10.sup.6 apoptotic cells is administered. In some embodiments, a dose of about 140×10.sup.6-210×10.sup.6 apoptotic cells is administered. In some embodiments, a dose of about 100×10.sup.6-140×10.sup.6 apoptotic cells is

administered. In some embodiments, a dose of about 10-100×10.sup.6 apoptotic cells is administered.
[0206] In some embodiments, a dose of about 1×10.sup.6-×10.sup.9 apoptotic cells is administered. In some embodiments, a dose of about 10×10.sup.6-×10.sup.9 apoptotic cells is administered. In some embodiments, a dose of about 1×10.sup.6-×108 apoptotic cells is administered. In some embodiments, a dose of about 1×10.sup.7-×108 apoptotic cells is administered. In some embodiments, a dose of about 1×10.sup.6-×10.sup.7 apoptotic cells is administered. In some embodiments, a dose of about 1×10.sup.7-×10.sup.9 apoptotic cells is administered. In some embodiments, a dose of about 1×10.sup.8-×10.sup.9 apoptotic cells is administered.
[0207] In some embodiments, a dose of about 20×10.sup.6 apoptotic cells is administered. In some embodiments, a dose of about 30×10.sup.6 apoptotic cells is administered. In some embodiments, a dose of about 40×10.sup.6 apoptotic cells is administered. In some embodiments, a dose of about 50×10.sup.6 apoptotic cells is administered. In some embodiments, 60×10.sup.6 apoptotic cells is administered. In some embodiments, a dose of about 60×10.sup.6 apoptotic cells is administered. In some embodiments, a dose of about 70×10.sup.6 apoptotic cells is administered. In some embodiments, a dose of about 80×10.sup.6 apoptotic cells is administered. In some embodiments, a dose of about 90×10.sup.6 apoptotic cells is administered. In some embodiments, a dose of about 1-15×10.sup.7 apoptotic cells is administered. In some embodiments, a dose of about 10×10.sup.7 apoptotic cells is administered. In some embodiments, a dose of about 11×10.sup.7 apoptotic cells is administered. In some embodiments, a dose of about 12×10.sup.7 apoptotic cells is administered. In some embodiments, a dose of about 13×10.sup.7 apoptotic cells is administered. In some embodiments, a dose of about 14×10.sup.7 apoptotic cells is administered. In some embodiments, a dose of about 15×10.sup.7 apoptotic cells is administered.
[0208] In some embodiments, a dose of 10×10.sup.6 apoptotic cells is administered. In another embodiment, a dose of 10×10.sup.7 apoptotic cells is administered. In another embodiment, a dose of 10×10.sup.8 apoptotic cells is administered. In another embodiment, a dose of 10×10.sup.9 apoptotic cells is administered. In another embodiment, a dose of 10×10.sup.10 apoptotic cells is administered. In another embodiment, a dose of 10×10.sup.11 apoptotic cells is administered. In another embodiment, a dose of 10×10.sup.12 apoptotic cells is administered. In another embodiment, a dose of 10×10.sup.5 apoptotic cells is administered. In another embodiment, a dose of 10×10.sup.4 apoptotic cells is administered. In another embodiment, a dose of 10×10.sup.3 apoptotic cells is administered. In another embodiment, a dose of 10×10.sup.2 apoptotic cells is administered.
[0209] In some embodiments, a high dose of apoptotic cells is administered. In some embodiments, a dose of 35×10.sup.6 apoptotic cells is administered. In another embodiment, a dose of 210×10.sup.6 apoptotic cells is administered. In another embodiment, a dose of 70×10.sup.6 apoptotic cells is administered. In another embodiment, a dose of 140×10.sup.6 apoptotic cells is administered. In another embodiment, a dose of 35-210×10.sup.6 apoptotic cells is administered.
[0210] In some embodiments, a single dose of apoptotic cells is administered. In some embodiments, multiple doses of apoptotic cells are administered. In some embodiments, 2 doses of apoptotic cells are administered. In some embodiments, 3 doses of apoptotic cells are administered. In some embodiments, 4 doses of apoptotic cells are administered. In some embodiments, 5 doses of apoptotic cells are administered. In some embodiments, 6 doses of apoptotic cells are administered. In some embodiments, 7 doses of apoptotic cells are administered. In some embodiments, 8 doses of apoptotic cells are administered. In some embodiments, 9 doses of apoptotic cells are administered. In some embodiments, more than 9 doses of apoptotic cells are administered. In some embodiments, multiple doses of apoptotic cells are administered. In some embodiments, multiple doses of apoptotic cells are administered during the treatment of a subject in need.

[0211] Each of the doses described herein may in some embodiments be ±about 5%, 10%, 15%, 20%, or 25% of the dose of apoptotic cells. In some embodiments, the dose of apoptotic cells is ±about 5% of the dose of apoptotic cells. In some embodiments, the dose of apoptotic cells is ±about 10% of the dose of apoptotic cells. In some embodiments, the dose of apoptotic cells is ±about 15% of the dose of apoptotic cells. In some embodiments, the dose of apoptotic cells is ±about 20% of the dose of apoptotic cells. In some embodiments, the dose of apoptotic cells is ±about 25% of the dose of apoptotic cells. In some embodiments, the dose of apoptotic cells is between ±about 5%-25% of the dose of apoptotic cells. For example, but not limited to a dose of about 100×10.sup.6-210×10.sup.6±20%, or about 25×10.sup.6±about 20%, or about 50×10.sup.6±about 20%, or about 100×10.sup.6±about 20%, or a dose of 120×10.sup.6±about 20%, or a dose of 140×10.sup.6±about 20%, or a dose of 160×10.sup.6±about 20%, or a dose of 180×10.sup.6±about 20%, or a dose of 200×10.sup.6±about 20%, etc. Similar embodiments include doses ±5, 10, 15, 20, or 25%.

[0212] In some embodiments, a dose of apoptotic cells is administered daily. In some embodiments, a dose of apoptotic cells is administered weekly. In some embodiments, a dose of apoptotic cells is administered semi-weekly (twice a week). In some embodiments, a dose of apoptotic cells is administered bi-weekly (every two weeks). In some embodiments, a dose of apoptotic cells is administered monthly. In some embodiments, a dose of apoptotic cells is administered in a non-regular regime, for example daily for a given time period followed by semi-weekly, or weekly, or bi-weekly, or monthly administration, or a combination thereof.

[0213] In some embodiments, the apoptotic cells may be administered by any method known in the art that would apply cells directly to an area of need, including, but not limited to injection and infusion. In some embodiments, administration comprises injection and/or infusion directly into a joint. In some embodiments, administration comprises injection and/or infusion adjacent to a joint. In some embodiments, administration comprises injection and/or infusion directly at the site of vanishing bone disease. In some embodiments, administration comprises injection and/or infusion adjacent to the site of vanishing bone disease. Apoptotic cells for injection may be in the form of a pharmaceutical composition formulated as a sterile injectable solution.

[0214] In some embodiments, the apoptotic cells may be administered by topical administration, wherein cells or a pharmaceutical composition comprising the apoptotic cells are applied directly to an area of need. In some embodiments, administration comprises topical application directly at the site of a joint. In some embodiments, administration comprises topical application adjacent to a joint. In some embodiments, administration comprises topical application directly at the site of vanishing bone disease. In some embodiments, administration comprises topical application adjacent to the site of vanishing bone disease. Apoptotic cells for topical application may be in the form of a pharmaceutical composition formulated as a topical ointment, a cream, an oil, a patch, or a dermal patch. In some embodiments, the apoptotic cells may be administered by infiltrating cartilage in the area of need, wherein cells or a pharmaceutical composition comprising the apoptotic cells are targeted directly to an area of need. In some embodiments, administration comprises infiltrating a joint with apoptotic cells or a composition thereof. In some embodiments, administration comprises infiltrating a tissue adjacent to a joint with apoptotic cells or a composition thereof. In some embodiments, administration comprises infiltrating the area at the site of vanishing bone disease with apoptotic cells or a composition thereof. In some embodiments, administration comprises infiltrating a tissue adjacent to a site of vanishing bone disease with apoptotic cells or a composition thereof.

[0215] In some embodiments, application of apoptotic cells is for local use.

Apoptotic Cell Supernatant Administration

[0216] In some embodiments, an apoptotic cell supernatant reduces production of pro- and/or anti-inflammatory cytokines/chemokines. In some embodiments, administration of an apoptotic cell supernatant reduces production of pro-inflammatory cytokines/chemokines including but not

limited to IL-1, IL-6, IL-8, IL-10, IL-1β, IL-2, IL-15, IL-22, MIP-1β, MCP-1, MDC, IP-10, fractalkine, IL-9, or TNFα, alone or in combination. In one embodiment, the apoptotic cell supernatant affects cytokine expression levels in synovial fluid. In one embodiment, the apoptotic cell supernatant affects cytokine expression levels in joint fluid.

[0217] In some embodiments, administration of an apoptotic cell supernatant inhibits one or more pro- and anti-inflammatory cytokines. In some embodiments, administration of an apoptotic cell supernatant inhibits one or more pro-inflammatory cytokine. In some embodiments, administration of an apoptotic cell supernatant inhibits one or more anti-inflammatory cytokines. In some embodiments, the pro- and anti-inflammatory cytokine comprises IL-1, IL-6, IL-8, IL-10, IL-1β, IL-2, IL-15, IL-22, MIP-1β, MCP-1, MDC, IP-10, fractalkine, IL-9, or TNFα, or any combination thereof. In another embodiment, administration of an apoptotic cell supernatant promotes the secretion of one or more anti-inflammatory cytokines. In some embodiments, at least one cytokine or chemokine having abnormal expression or content is downregulated by administration of an apoptotic cell supernatant. In some embodiments, each cytokine or chemokine having abnormal expression or content is downregulated by administration of an apoptotic cell supernatant.

[0218] In some embodiments, at least one anti-inflammatory cytokine or chemokine having abnormal expression or content is downregulated by administration of an apoptotic cell supernatant. In some embodiments, at least one pro-inflammatory cytokine or chemokine having abnormal expression or content is downregulated by administration of an apoptotic cell supernatant. In some embodiments, a combination of pro- and anti-inflammatory cytokines or chemokines having abnormal expression or content are downregulated by administration of an apoptotic cell supernatant. In some embodiments, any of IL-1, IL-6, IL-8, IL-10, IL-1β, IL-2, IL-15, IL-22, MIP-1β, MCP-1, MDC, IP-10, fractalkine, IL-9, or TNFα, or any combination thereof, having abnormal expression or content, for example in synovial fluid or joint fluid in the area of a joint are downregulated by administration of an apoptotic cell supernatant. In some embodiments, any of IL-1, IL-6, IL-8, IL-10, IL-1β, IL-2, IL-15, IL-22, MIP-1β, MCP-1, MDC, IP-10, fractalkine, IL-9, or TNFα, or any combination thereof, having abnormal expression or content, for example in the area of vanishing bone disease, are downregulated by administration of an apoptotic cell supernatant.

[0219] In some embodiments, the apoptotic supernatant or a composition comprising a supernatant may be used at a total dose or aliquot of apoptotic cell supernatant derived from the co-culture of about 14×10.sup.9 of CD45+ cells obtained by cytapheresis equivalent to about 200 million of cells per kilogram of body weight (for a 70 kg subject). In some embodiments, such a total dose is administered as unit doses of supernatant derived from about 100 million cells per kilogram body weight, and/or is administered as unit doses at weekly intervals. Suitable total doses according to certain embodiments, include total doses of supernatant derived from about 10 million to about 4 billion cells per kilogram body weight. In another embodiment, the supernatant is derived from about 40 million to about 1 billion cells per kilogram body weight. In yet another embodiment the supernatant is derived from about 80 million to about 500 million cells per kilogram body weight. In still another embodiment, the supernatant is derived from about 160 million to about 250 million cells per kilogram body weight. Suitable unit doses according to this embodiment include unit doses of supernatant derived from about 4 million to about 400 million cells per kilogram body weight. In another embodiment, the supernatant is derived from about 8 million to about 200 million cells per kilogram body weight. In another embodiment, the supernatant is derived from about 16 million to about 100 million cells per kilogram body weight. In yet another embodiment, the supernatant is derived from about 32 million to about 50 million cells per kilogram body weight.

[0220] In another embodiment, a dose of apoptotic cell supernatant derived from the co-culture of about 10×10.sup.6 apoptotic cells is administered. In another embodiment, a dose derived from 10×10.sup.7 apoptotic cells is administered. In another embodiment, a dose derived from

10×10.sup.8 apoptotic cells is administered. In another embodiment, a dose derived from 10×10.sup.9 apoptotic cells is administered. In another embodiment, a dose derived from 10×10.sup.10 apoptotic cells is administered. In another embodiment, a dose derived from 10×10.sup.11 apoptotic cells is administered. In another embodiment, a dose derived from 10×10.sup.12 apoptotic cells is administered. In another embodiment, a dose derived from 10×10.sup.5 apoptotic cells is administered. In another embodiment, a dose derived from 10×10.sup.4 apoptotic cells is administered. In another embodiment, a dose derived from 10×10.sup.3 apoptotic cells is administered. In another embodiment, a dose derived from 10×10.sup.2 apoptotic cells is administered.

[0221] In some embodiments, a dose of apoptotic cell supernatant derived from 35×10.sup.6 apoptotic cells is administered. In another embodiment, a dose derived from 210×10.sup.6 apoptotic cells is administered. In another embodiment, a dose derived from 70×10.sup.6 apoptotic cells is administered. In another embodiment, a dose derived from 140×10.sup.6 apoptotic cells is administered. In another embodiment, a dose derived from 35-210×10.sup.6 apoptotic cells is administered.

[0222] In some embodiments, the apoptotic cell supernatant, or composition comprising said apoptotic cell supernatant, may be administered

[0223] In some embodiments, the apoptotic cell supernatants, such as apoptotic cell-phagocyte supernatants, reduces production of cytokines associated with the cytokine storm such as IL-6 (See Example 3 below).

[0224] In some embodiments, the apoptotic cell supernatants, such as apoptotic cell-phagocyte supernatants, affect cytokine expression levels in macrophages and DCs, but do not affect cytokine expression levels in the T-cells themselves.

[0225] In another embodiment, the apoptotic cell supernatants trigger death of T-cells, but not via changes in cytokine expression levels.

[0226] In another embodiment, apoptotic cell supernatants, such as apoptotic cell-phagocyte supernatants antagonize the priming of macrophages and dendritic cells to secrete cytokines. In another embodiment, apoptotic cell supernatants increase Tregs which suppress the inflammatory response and/or prevent excess release of cytokines.

[0227] In some embodiments, administration of apoptotic cell supernatants, such as apoptotic cell-phagocyte supernatants, inhibits one or more pro-inflammatory cytokines. In some embodiments, the pro-inflammatory cytokine comprises IL-1beta, IL-6, TNF-alpha, or IFN-gamma, or any combination thereof. In another embodiment, administration of apoptotic cell supernatants promotes the secretion of one or more anti-inflammatory cytokines. In some embodiments, the anti-inflammatory cytokine comprises TGF-beta, IL10, or PGE2, or any combination thereof.

[0228] In another embodiment, administration of apoptotic cell supernatants creates potentially tolerogenic dendritic cells, which in some embodiments, are capable of migration, and in some embodiments, the migration is due to CCR7. In another embodiment, administration of apoptotic cell supernatants elicits various signaling events which in one embodiment is TAM receptor signaling (Tyro3, Axl and Mer) which in some embodiments, inhibits inflammation in antigen-presenting cells. In some embodiments, Tyro-3, Ax1, and Mer constitute the TAM family of receptor tyrosine kinases (RTKs) characterized by a conserved sequence within the kinase domain and adhesion molecule-like extracellular domains. In another embodiment, administration of apoptotic cell supernatants activates signaling through MerTK. In another embodiment, administration of apoptotic cell supernatants activates the phosphatidylinositol 3-kinase (PI3K)/AKT pathway, which in some embodiments, negatively regulates NF-κB. In another embodiment, administration of apoptotic cell supernatants negatively regulates the inflammasome which in one embodiment leads to inhibition of pro-inflammatory cytokine secretion, DC maturation, or a combination thereof. In another embodiment, administration of apoptotic cell supernatants upregulates expression of anti-inflammatory genes such as Nr4a, Thbs1, or a

combination thereof. In another embodiment, administration of apoptotic cell supernatants induces a high level of AMP which in some embodiments, is accumulated in a Pannexin1-dependent manner. In another embodiment, administration of apoptotic cell supernatants suppresses inflammation.

[0229] In some embodiments, a single dose of an apoptotic cell supernatant is administered. In some embodiments, multiple doses of an apoptotic cell supernatant are administered. In some embodiments, 2 doses of an apoptotic cell supernatant are administered. In some embodiments, 3 doses of an apoptotic cell supernatant are administered. In some embodiments, 4 doses of an apoptotic cell supernatant are administered. In some embodiments, 5 doses of an apoptotic cell supernatant are administered. In some embodiments, 6 doses of an apoptotic cell supernatant are administered. In some embodiments, 7 doses of an apoptotic cell supernatant are administered. In some embodiments, 8 doses of an apoptotic cell supernatant are administered. In some embodiments, 9 doses of an apoptotic cell supernatant are administered. In some embodiments, more than 9 doses of an apoptotic cell supernatant are administered. In some embodiments, multiple doses of an apoptotic cell supernatant are administered. In some embodiments, multiple doses of an apoptotic cell supernatant are administered during the treatment of a subject in need.

[0230] Each of the doses described herein may in some embodiments be ±about 5%, 10%, 15%, 20%, or 25% of the dose of an apoptotic cell supernatant. In some embodiments, the dose of an apoptotic cell supernatant is ±about 5% of the dose of an apoptotic cell supernatant. In some embodiments, the dose of an apoptotic cell supernatant is ±about 10% of the dose of an apoptotic cell supernatant. In some embodiments, the dose of an apoptotic cell supernatant is ±about 15% of the dose of an apoptotic cell supernatant. In some embodiments, the dose of an apoptotic cell supernatant is ±about 20% of the dose of an apoptotic cell supernatant. In some embodiments, the dose of an apoptotic cell supernatant is ±about 25% of the dose of an apoptotic cell supernatant. In some embodiments, the dose of an apoptotic cell supernatant is between ±about 5%-25% of the dose of an apoptotic cell supernatant. For example, but not limited to a dose of about 100×10.sup.6-210×10.sup.6±20%, or about 100×10.sup.6±about 20%, or a dose of 120×10.sup.6±about 20%, or a dose of 140×10.sup.6±about 20%, or a dose of 160×10.sup.6±about 20%, or a dose of 180×10.sup.6±about 20%, or a dose of 200×10.sup.6±about 20%, etc. Similar embodiments include doses ±5, 10, 15, 20, or 25%.

[0231] In some embodiments, a dose of an apoptotic cell supernatant is administered daily. In some embodiments, a dose of an apoptotic cell supernatant is administered weekly. In some embodiments, a dose of an apoptotic cell supernatant is administered semi-weekly (twice a week). In some embodiments, a dose of an apoptotic cell supernatant is administered bi-weekly (every two weeks). In some embodiments, a dose of an apoptotic cell supernatant is administered monthly. In some embodiments, a dose of an apoptotic cell supernatant is administered in a non-regular regime, for example daily for a given time period followed by semi-weekly, or weekly, or bi-weekly, or monthly administration, or a combination thereof.

[0232] In some embodiments, an apoptotic cell supernatant may be administered by any method known in the art that would apply the cell supernatant directly to an area of need, including, but not limited to injection and infusion.

[0233] In some embodiments, administration comprises injection and/or infusion directly into a joint. In some embodiments, administration comprises injection and/or infusion adjacent to a joint. In some embodiments, administration comprises injection and/or infusion directly at the site of vanishing bone disease. In some embodiments, administration comprises injection and/or infusion adjacent to the site of vanishing bone disease. An apoptotic cell supernatant for injection may be in the form of a pharmaceutical composition formulated as a sterile injectable solution.

[0234] In some embodiments, an apoptotic cell supernatant may be administered by topical administration, wherein cells or a pharmaceutical composition comprising an apoptotic cell supernatant are applied directly to an area of need. In some embodiments, administration comprises

topical application directly at the site of a joint. In some embodiments, administration comprises topical application adjacent to a joint. In some embodiments, administration comprises topical application directly at the site of vanishing bone disease. In some embodiments, administration comprises topical application adjacent to the site of vanishing bone disease. An apoptotic cell supernatant for topical application may be in the form of a pharmaceutical composition formulated as a topical ointment, a cream, an oil, a patch, or a dermal patch. In some embodiments, the apoptotic cell supernatant may be administered by infiltrating cartilage in the area of need, wherein cells or a pharmaceutical composition comprising the an apoptotic cell supernatant are targeted directly to an area of need. In some embodiments, administration comprises infiltrating a joint with an apoptotic cell supernatant or a composition thereof. In some embodiments, administration comprises infiltrating a tissue adjacent to a joint with an apoptotic cell supernatant or a composition thereof. In some embodiments, administration comprises infiltrating the area at the site of vanishing bone disease with an apoptotic cell supernatant or a composition thereof. In some embodiments, administration comprises infiltrating a tissue adjacent to a site of vanishing bone disease with an apoptotic cell supernatant or a composition thereof.

[0235] In some embodiments, application of an apoptotic cell supernatant is for local use.

Compositions

[0236] As used herein, the terms “composition” and pharmaceutical composition” may in some embodiments, be used interchangeably having all the same qualities and meanings. In some embodiments, disclosed herein is a pharmaceutical composition for the treatment of a condition or disease as described herein. In some embodiments, disclosed herein is a pharmaceutical composition for the treatment of osteoarthritis. In some embodiments, disclosed herein is a pharmaceutical composition for the treatment of vanishing bone disease.

[0237] In some embodiments, a pharmaceutical composition comprises an early apoptotic cell population as described in detail above. In other embodiments, a pharmaceutical composition comprises a supernatant from an early apoptotic cell population as described in detail above. In some embodiments, a pharmaceutical composition comprises an apoptotic supernatant as described in detail above. In some embodiments, a pharmaceutical composition comprises an apoptotic-phagocytic supernatant as described in detail above.

[0238] In still another embodiment, a pharmaceutical composition for the treatment of osteoarthritis and vanishing bone disease, as described herein, comprises an effective amount of an early apoptotic cell population and a pharmaceutically acceptable excipient. In some embodiments, a composition comprising apoptotic cells is used in methods disclosed herein for example for treatment of pain caused by osteoarthritis. In some embodiments, a composition comprising apoptotic cells is used in methods to reduce the pain of osteoarthritis. In some embodiments, a composition comprising apoptotic cells is used in methods to reduce the inflammation in a joint caused by osteoarthritis. In some embodiments, a composition comprising apoptotic cells is used in methods to reduce the swelling in or around a joint caused by osteoarthritis. In some embodiments, a composition comprising apoptotic cells is used in methods to inhibit or slow the progressive degeneration of articular cartilage in a joint. In some embodiments, a composition comprising apoptotic cells is used in methods to inhibit or slow the progressive erosion of bone tissue caused by vanishing bone disease. In some embodiments, a composition comprising apoptotic cells is used in methods to increase movement in a joint affected by osteoarthritis. In some embodiments, a composition comprising apoptotic cells is used in methods to increase range of movement in a joint affected by osteoarthritis.

[0239] Dosages of early apoptotic cells are described in detail above. An effective amount of an early apoptotic cell population includes those dosages described above, for example but not limited to, dosages of early apoptotic cells in the range of about 10×10.sup.6 cells±20% through 1×10.sup.9 cells 20%. In some embodiments, a composition comprises an effective amount of an early apoptotic cell population and a pharmaceutically acceptable excipient.

[0240] In some embodiments, apoptotic cells comprised in a composition comprise apoptotic cells in an early apoptotic state. In some embodiments, apoptotic cells comprised in a composition are pooled third party donor cells. In some embodiments, apoptotic cells comprised in a composition are allogenic donor cells. In some embodiments, apoptotic cells comprised in a composition are autologous donor cells. In some embodiments, apoptotic cells comprised in a composition are pooled autologous donor cells. In some embodiments, apoptotic cells comprised in a composition are irradiated.

[0241] In still another embodiment, a pharmaceutical composition for the treatment of osteoarthritis and vanishing bone disease, as described herein, comprises an effective amount of an early apoptotic cell supernatant or an apoptotic supernatant or an apoptotic-phagocytic supernatant, and a pharmaceutically acceptable excipient. In some embodiments, a composition comprising an early apoptotic cell supernatant or an apoptotic supernatant or an apoptotic-phagocytic supernatant is used in methods disclosed herein for example for treatment of pain caused by osteoarthritis. In some embodiments, a composition comprising an early apoptotic cell supernatant or an apoptotic supernatant or an apoptotic-phagocytic supernatant is used in methods to reduce the pain of osteoarthritis. In some embodiments, a composition comprising an early apoptotic cell supernatant or an apoptotic supernatant or an apoptotic-phagocytic supernatant is used in methods to reduce the inflammation in a joint caused by osteoarthritis. In some embodiments, a composition comprising an early apoptotic cell supernatant or an apoptotic supernatant or an apoptotic-phagocytic supernatant is used in methods to reduce the swelling in or around a joint caused by osteoarthritis. In some embodiments, a composition comprising an early apoptotic cell supernatant or an apoptotic supernatant or an apoptotic-phagocytic supernatant is used in methods to inhibit or slow the progressive degeneration of articular cartilage in a joint. In some embodiments, a composition comprising an early apoptotic cell supernatant or an apoptotic supernatant or an apoptotic-phagocytic supernatant is used in methods to inhibit or slow the progressive erosion of bone tissue caused by vanishing bone disease. In some embodiments, a composition comprising an early apoptotic cell supernatant or an apoptotic supernatant or an apoptotic-phagocytic supernatant is used in methods to increase movement in a joint affected by osteoarthritis. In some embodiments, a composition comprising an early apoptotic cell supernatant or an apoptotic supernatant or an apoptotic-phagocytic supernatant is used in methods to increase range of movement in a joint affected by osteoarthritis.

[0242] Dosages of an early apoptotic cell supernatant or an apoptotic supernatant or an apoptotic-phagocytic supernatant are described in detail above. An effective amount of an early apoptotic cell supernatant or an apoptotic supernatant or an apoptotic-phagocytic supernatant includes those dosages described above, for example but not limited to, dosages of collected from apoptotic cell cultures or apoptotic-phagocytic cultures, wherein said apoptotic cells are in the range of about 1×10.sup.6 cells±20% through 1×10.sup.9 cells 20%. In some embodiments, a composition comprises an effective amount of an early apoptotic cell supernatant or an apoptotic supernatant or an apoptotic-phagocytic supernatant and a pharmaceutically acceptable excipient.

[0243] In some embodiments, an early apoptotic cell supernatant or an apoptotic supernatant or an apoptotic-phagocytic supernatant comprised in a composition comprise medium collected from apoptotic cells in an early apoptotic state. In some embodiments, an early apoptotic cell supernatant or an apoptotic supernatant or an apoptotic-phagocytic supernatant comprised in a composition comprises supernatants collected from pooled third-party donor cells. In some embodiments, an early apoptotic cell supernatant or an apoptotic supernatant or an apoptotic-phagocytic supernatant comprised in a composition are comprised of supernatants collected from allogenic donor cells. In some embodiments, an early apoptotic cell supernatant or an apoptotic supernatant or an apoptotic-phagocytic supernatant comprised in a composition comprise supernatants collected from autologous donor cells. In some embodiments, an early apoptotic cell supernatant or an apoptotic supernatant or an apoptotic-phagocytic supernatant comprised in a composition comprise

supernatant collected from pooled autologous donor cells. In some embodiments, an early apoptotic cell supernatant or an apoptotic supernatant or an apoptotic-phagocytic supernatant comprised in a composition comprise supernatants collected from cells that have been irradiated.
[0244] A skilled artisan would appreciate that a “pharmaceutical composition” may encompass a preparation of one or more of the active ingredients described herein with other chemical components such as physiologically suitable carriers and excipients. The purpose of a pharmaceutical composition is to facilitate administration of a compound to an organism.
[0245] A skilled artisan would appreciate that the phrases “physiologically acceptable carrier”, “pharmaceutically acceptable carrier”, “physiologically acceptable excipient”, and “pharmaceutically acceptable excipient”, may be used interchangeably may encompass a carrier, excipient, or a diluent that does not cause significant irritation to an organism and does not abrogate the biological activity and properties of the administered active ingredient.
[0246] A skilled artisan would appreciate that an “excipient” may encompass an inert substance added to a pharmaceutical composition to further facilitate administration of an active ingredient. In some embodiments, excipients include calcium carbonate, calcium phosphate, various sugars and types of starch, cellulose derivatives, gelatin, vegetable oils and polyethylene glycols.
[0247] Techniques for formulation and administration of drugs are found in “Remington's Pharmaceutical Sciences,” Mack Publishing Co., Easton, PA, latest edition, which is incorporated herein by reference.
[0248] In some embodiments, the composition as disclosed herein comprises a therapeutic composition. In some embodiments, the composition as disclosed herein comprises a therapeutic efficacy.
[0249] In some embodiments, a composition as disclosed herein is administered once. In another embodiment, the composition is administered twice. In another embodiment, the composition is administered three times. In another embodiment, the composition is administered four times. In another embodiment, the composition is administered at least four times. In another embodiment, the composition is administered more than four times. In another embodiment, the composition is administered multiple times.
[0250] In some embodiments, a composition as disclosed herein is administered daily, weekly, monthly, or in intervals that fits the underlying disease in a specific person. In some embodiments, a composition as disclosed herein is administered daily. In some embodiments, a composition as disclosed herein is administered semi-weekly. In some embodiments, a composition as disclosed herein is administered weekly. In some embodiments, a composition as disclosed herein is administered bi-weekly. In some embodiments, a composition as disclosed herein is administered monthly. In some embodiments, a composition as disclosed herein is administered using different regimes for example but not limited to a first administration daily that after a time period is changed to semi-weekly, or weekly, or bi-weekly administration. In some embodiments, a composition as disclosed herein is administered in intervals that fits the underlying disease, for example but not limited to osteroarthritis in a specific person.
[0251] In some embodiments, a composition is injected at the site of need, for example a joint. In some embodiments, a composition is injected near the site of need, for example adjacent to a joint. In some embodiments, a composition is injected at the site of need, for example a site of articular cartilage degeneration. In some embodiments, a composition is injected near the site of need, for example adjacent to a site of articular cartilage degeneration. In some embodiments, a composition is injected at the site of need, for example a site of bone erosion. In some embodiments, a composition is injected near the site of need, for example adjacent to a site of bone erosion.
[0252] In some embodiments, a composition is infused at the site of need, for example a joint. In some embodiments, a composition is infused near the site of need, for example adjacent to a joint. In some embodiments, a composition is infused at the site of need, for example a site of articular cartilage degeneration. In some embodiments, a composition is infused near the site of need, for

example adjacent to a site of articular cartilage degeneration. In some embodiments, a composition is infused at the site of need, for example a site of bone erosion. In some embodiments, a composition is infused near the site of need, for example adjacent to a site of bone erosion.
[0253] In some embodiments, a composition is infiltrated at the site of need, for example a joint. In some embodiments, the cartilage in a joint is infiltrated with apoptotic cells or a composition thereof, at the site of need. In some embodiments, a composition is infiltrated near the site of need, for example adjacent to a joint. In some embodiments, a composition is infiltrated at the site of need, for example a site of articular cartilage degeneration. In some embodiments, a composition is infiltrated near the site of need, for example adjacent to a site of articular cartilage degeneration. In some embodiments, a composition is infiltrated at the site of need, for example a site of bone erosion. In some embodiments, a composition infiltrated near the site of need, for example adjacent to a site of bone erosion.
[0254] In some embodiments, administration of compositions described herein reduces pro- and anti-inflammatory cytokine or chemokine release from synovium, or cartilage or from any joint component, or a combination thereof. In some embodiments, administration of compositions described herein reduces pro-inflammatory cytokine or chemokine release from synovium, or cartilage or from any joint component, or a combination thereof. In some embodiments, administration of compositions described herein reduces anti-inflammatory cytokine or chemokine release from synovium, or cartilage or from any joint component, or a combination thereof. In some embodiments, administration of compositions described herein rebalances the immune response in a joint. In some embodiments, administration of compositions described herein rebalances the immune response in synovial fluid present in a joint. In some embodiments, administration of compositions described herein rebalances the immune response in joint fluid. In some embodiments, administration of compositions described herein rebalances the immune response in cartilage within a joint. In some embodiments, administration of compositions described herein rebalances the immune response in at a site of vanishing bone disease.
Formulations
[0255] Pharmaceutical compositions disclosed herein comprising early apoptotic cell populations or comprising early apoptotic cell supernatants or apoptotic supernatants or apoptotic-phagocytic supernatants can be conveniently provided as sterile liquid preparations, e.g., isotonic aqueous solutions, suspensions, emulsions, dispersions, or viscous compositions, which may be buffered to a selected pH, Liquid preparations are normally easier to prepare than gels, other viscous compositions, and solid compositions. Additionally, liquid compositions are somewhat more convenient to administer, especially by injection. Viscous compositions, on the other hand, can be formulated within the appropriate viscosity range to provide longer contact periods with specific tissues. Liquid or viscous compositions can comprise carriers, which can be a solvent or dispersing medium containing, for example, water, saline, phosphate buffered saline, polyol (for example, glycerol, propylene glycol, liquid polyethylene glycol, and the like) and suitable mixtures thereof.
[0256] In some embodiments, a composition comprising early apoptotic cells or comprising early apoptotic cell supernatants or apoptotic supernatants, or apoptotic-phagocytic supernatants is comprised in a Ringer's lactate solution. In some embodiments, a composition of early apoptotic cells or comprising early apoptotic cell supernatants or apoptotic supernatants or apoptotic-phagocytic supernatants comprises known buffer solutions, for example but not limited to normal saline or PBS.
[0257] Sterile injectable solutions can be prepared by incorporating the early apoptotic cell population described herein or the early apoptotic cell supernatants or apoptotic supernatants or apoptotic-phagocytic supernatants described herein, and utilized in practicing the methods disclosed herein, in the required amount of the appropriate solvent with various amounts of the other ingredients, as desired. Such compositions may be in admixture with a suitable carrier, diluent, or excipient such as sterile water, physiological saline, glucose, dextrose, or the like. The

compositions can contain auxiliary substances such as wetting, dispersing, or emulsifying agents (e.g., methylcellulose), pH buffering agents, gelling or viscosity enhancing additives, preservatives, flavoring agents, colors, and the like, depending upon the route of administration and the preparation desired. Standard texts, such as “REMINGTON'S PHARMACEUTICAL SCIENCE”, 17th edition, 1985, incorporated herein by reference, may be consulted to prepare suitable preparations, without undue experimentation.

[0258] Various additives which enhance the stability and sterility of the compositions, including antimicrobial preservatives, antioxidants, chelating agents, and buffers, can be added. Prevention of the action of microorganisms can be ensured by various antibacterial and antifungal agents, for example, parabens, chlorobutanol, phenol, sorbic acid, and the like. Prolonged absorption of the injectable pharmaceutical form can be brought about by the use of agents delaying absorption, for example, aluminum monostearate and gelatin. According to the disclosure herein, however, any vehicle, diluent, or additive used would have to be compatible with the genetically modified immunoresponsive cells or their progenitors.

[0259] The compositions or formulations described herein can be isotonic, i.e., they can have the same osmotic pressure as blood and lacrimal fluid. The desired isotonicity of the compositions as disclosed herein may be accomplished using sodium chloride, or other pharmaceutically acceptable agents such as dextrose, boric acid, sodium tartrate, propylene glycol or other inorganic or organic solutes. Sodium chloride may be preferred particularly for buffers containing sodium ions.

[0260] Viscosity of the compositions, if desired, can be maintained at the selected level using a pharmaceutically acceptable thickening agent. Methylcellulose may be preferred because it is readily and economically available and is easy to work with.

[0261] Other suitable thickening agents include, for example, xanthan gum, carboxymethyl cellulose, hydroxypropyl cellulose, carbomer, and the like. The preferred concentration of the thickener will depend upon the agent selected. The important point is to use an amount that will achieve the selected viscosity. Obviously, the choice of suitable carriers and other additives will depend on the exact route of administration and the nature of the particular dosage form, e.g., liquid dosage form (e.g., whether the composition is to be formulated into a solution, a suspension, gel or another liquid form, such as a time release form or liquid-filled form).

[0262] Those skilled in the art will recognize that the components of the compositions or formulations should be selected to be chemically inert and will not affect the viability or efficacy of the early apoptotic cell populations as described herein, for use in the methods disclosed herein. This will present no problem to those skilled in chemical and pharmaceutical principles, or problems can be readily avoided by reference to standard texts or by simple experiments (not involving undue experimentation), from this disclosure and the documents cited herein.

[0263] One consideration concerning the therapeutic use of early apoptotic cells disclosed herein is the quantity of cells necessary to achieve an optimal effect. Similarly, a consideration concerning the therapeutic use of supernatants disclosed herein is the quantity of cells from which the supernatant is collected, necessary to achieve an optimal effect. The quantity of cells to be administered or cells from which a supernatant is collected may in some embodiments, vary for the subject being treated. In addition, for local administration to a joint, the size of the joint may in some embodiments, be taken into consideration.

[0264] In some embodiments, between 10.sup.5 to 10.sup.10, between 10.sup.6 to 10.sup.9, or between 10.sup.6 and 10.sup.8 early apoptotic cells are administered to a human subject. In some embodiments, a supernatant collected from between 10.sup.5 to 10.sup.10, between 10.sup.6 to 10.sup.9, or between 10.sup.6 and 10.sup.8 early apoptotic cells or apoptotic cells or apoptotic-phagocytic cells is administered to a human subject. The number of apoptotic cells for administration or for collection of a supernatant for administration may in some embodiments, comprise a range.

[0265] In some embodiments, between 1×10.sup.6-1×10.sup.9±20%. In some embodiments,

between 1×10.sup.6-1×10.sup.8±20%. In some embodiments, between 1×10.sup.6-1×10.sup.7±20%. In some embodiments, between 1×10.sup.7-1×10.sup.9±20%. In some embodiments, between 1×10.sup.8-1×10.sup.9±20%. In some embodiments, between 10×10.sup.6-500×10.sup.6±20%. In some embodiments, between 10×10.sup.6-210×10.sup.6±20%, or about 10×10.sup.6±about 20%, or a dose of 12×10.sup.6±about 20% or a dose collected from 12×10.sup.6±about 20% cells, or a dose of 14×10.sup.6±about 20% or a dose collected from 12×10.sup.6±about 20% cells, or a dose of 16×10.sup.6±about 20% or a dose collected from 16×10.sup.6±about 20% cells, or a dose of 18×10.sup.6±about 20% or a dose collected from 18×10.sup.6±about 20% cells, or a dose of 20×10.sup.6±about 20% or a dose collected from 20×10.sup.6±about 20% cells, or 25×10.sup.6±about 20% cells, or a dose of 50×10.sup.6±about 20% cells, or a dose collected from 50×10.sup.6±about 20% cells, or a dose of 100×10.sup.6±about 20% cells or a dose collected from 100×10.sup.6±about 20% cells. In some embodiments, between 100×10.sup.6-210×10.sup.6±20%, or about 100×10.sup.6±about 20%, or a dose of 120×10.sup.6±about 20% or a dose collected from 120×10.sup.6±about 20% cells, or a dose of 140×10.sup.6±about 20% or a dose collected from 140×10.sup.6±about 20% cells, or a dose of 160×10.sup.6±about 20% or a dose collected from 160×10.sup.6±about 20% cells, or a dose of 180×10.sup.6±about 20% or a dose collected from 180×10.sup.6±about 20% cells, or a dose of 200×10.sup.6±about 20% or a dose collected from 200×10.sup.6±about 20% cells, or a dose of 1×10.sup.6±about 20% or a dose collected from 1×10.sup.6±about 20% cells, or a dose of 1×10.sup.7±about 20% or a dose collected from 1×10.sup.7±about 20% cells, or a dose of 1×10.sup.8±about 20% or a dose collected from 1×10.sup.8±about 20% cells, or a dose of 1×10.sup.9±about 20% or a dose collected from 1×10.sup.9±about 20% cells.

[0266] In some embodiments, between 1×10.sup.6-1×10.sup.9±5%. In some embodiments, between 1×10.sup.6-1×10.sup.8±5%. In some embodiments, between 1×10.sup.6-1×10.sup.7±5%. In some embodiments, between 1×10.sup.7-1×10.sup.9±5%. In some embodiments, between 1×10.sup.8-1×10.sup.9±5%. In some embodiments, between 10×10.sup.6-500×10.sup.6±5%. In some embodiments, between 10×10.sup.6-210×10.sup.6±5%, or about 10×10.sup.6±about 5%, or a dose of 12×10.sup.6±about 5% or a dose collected from 12×10.sup.6±about 5% cells, or a dose of 14×10.sup.6±about 5% or a dose collected from 12×10.sup.6±about 5% cells, or a dose of 16×10.sup.6±about 5% or a dose collected from 16×10.sup.6±about 5% cells, or a dose of 18×10.sup.6±about 5% or a dose collected from 18×10.sup.6±about 5% cells, or a dose of 20×10.sup.6±about 5% or a dose collected from 20×10.sup.6±about 5% cells, or a dose of 25×10.sup.6±about 5% cells, or a dose collected from 25×10.sup.6±about 5% cells, or a dose of 50×10.sup.6±about 5% cells, or a dose collected from 50×10.sup.6±about 5% cells, or a dose of 100×10.sup.6±about 5% or a dose collected from 100×10.sup.6±about 5% cells. In some embodiments, between 100×10.sup.6-210×10.sup.6±5%, or about 100×10.sup.6±about 5%, or a dose of 120×10.sup.6±about 5% or a dose collected from 120×10.sup.6±about 5% cells, or a dose of 140×10.sup.6±about 5% or a dose collected from 140×10.sup.6±about 5% cells, or a dose of 160×10.sup.6±about 5% or a dose collected from 160×10.sup.6±about 5% cells, or a dose of 180×10.sup.6±about 5% or a dose collected from 180×10.sup.6±about 5% cells, or a dose of 200×10.sup.6±about 5% or a dose collected from 200×10.sup.6±about 5% cells, or a dose of 1×10.sup.6±about 5% or a dose collected from 1×10.sup.6±about 5% cells, or a dose of 1×10.sup.7±about 5% or a dose collected from 1×10.sup.7±about 5% cells, or a dose of 1×10.sup.8±about 5% or a dose collected from 1×10.sup.8±about 5% cells, or a dose of 1×10.sup.9±about 5% or a dose collected from 1×10.sup.9±about 5% cells.

[0267] In some embodiments, between 1×10.sup.6-1×10.sup.9±10%. In some embodiments, between 1×10.sup.6-1×10.sup.8±10%. In some embodiments, between 1×10.sup.6-1×10.sup.7±10%. In some embodiments, between 1×10.sup.7-1×10.sup.9±10%. In some embodiments, between 1×10.sup.8-1×10.sup.9±10%. In some embodiments, between 10×10.sup.6-500×10.sup.6±10%. In some embodiments, between 10×10.sup.6-210×10.sup.6±10%, or about

10×10.sup.6±about 10%, or a dose of 12×10.sup.6±about 10% or a dose collected from 12×10.sup.6±about 10% cells, or a dose of 14×10.sup.6±about 10% or a dose collected from 12×10.sup.6±about 10% cells, or a dose of 16×10.sup.6±about 10% or a dose collected from 16×10.sup.6±about 10% cells, or a dose of 18×10.sup.6±about 10% or a dose collected from 18×10.sup.6±about 10% cells, or a dose of 20×10.sup.6±about 10% or a dose collected from 20×10.sup.6±about 10% cells, or a dose of 25×10.sup.6±about 10% cells or a dose collected from 25×10.sup.6±about 10% cells, or a dose of 50×10.sup.6±about 10% cells or a dose collected from 50×10.sup.6±about 10% cells, or a dose of 100×10.sup.6±about 10% or a dose collected from 100×10.sup.6±about 10% cells. In some embodiments, between 100×10.sup.6-210×10.sup.6±10%, or about 100×10.sup.6±about 10%, or a dose of 120×10.sup.6±about 10% or a dose collected from 120×10.sup.6±about 10% cells, or a dose of 140×10.sup.6±about 10% or a dose collected from 140×10.sup.6±about 10% cells, or a dose of 160×10.sup.6±about 10% or a dose collected from 160×10.sup.6±about 10% cells, or a dose of 180×10.sup.6±about 10% or a dose collected from 180×10.sup.6±about 10% cells, or a dose of 200×10.sup.6±about 10% or a dose collected from 200×10.sup.6±about 10% cells, or a dose of 1×10.sup.6±about 10% or a dose collected from 1×10.sup.6±about 10% cells, or a dose of 1×10.sup.7±about 10% or a dose collected from 1×10.sup.7±about 10% cells, or a dose of 1×10.sup.8±about 10% or a dose collected from 1×10.sup.8±about 10% cells, or a dose of 1×10.sup.9±about 10% or a dose collected from 1×10.sup.9±about 10% cells.

[0268] In some embodiments, between 1×10.sup.6-1×10.sup.9±15%. In some embodiments, between 1×10.sup.6-1×10.sup.8±15%. In some embodiments, between 1×10.sup.6-1×10.sup.7±15%. In some embodiments, between 1×10.sup.7-1×10.sup.9±15%. In some embodiments, between 1×10.sup.8-1×10.sup.9±15%. In some embodiments, between 10×10.sup.6-500×10.sup.6±15%. In some embodiments, between 10×10.sup.6-210×10.sup.6±15%, or about 10×10.sup.6±about 15%, or a dose of 12×10.sup.6±about 15% or a dose collected from 12×10.sup.6±about 15% cells, or a dose of 14×10.sup.6±about 15% or a dose collected from 12×10.sup.6±about 15% cells, or a dose of 16×10.sup.6±about 15% or a dose collected from 16×10.sup.6±about 15% cells, or a dose of 18×10.sup.6±about 15% or a dose collected from 18×10.sup.6±about 15% cells, or a dose of 20×10.sup.6±about 15% or a dose collected from 20×10.sup.6±about 15% cells, or a dose of 25×10.sup.6±about 15% or a dose collected from 25×10.sup.6±about 15% cells, or a dose of 50×10.sup.6±about 15% or a dose collected from 50×10.sup.6±about 15% cells, or a dose of 100×10.sup.6±about 15% or a dose collected from 100×10.sup.6±about 15% cells. In some embodiments, between 100×10.sup.6-210×10.sup.6±15%, or about 100×10.sup.6±about 15%, or a dose of 120×10.sup.6±about 15% or a dose collected from 120×10.sup.6±about 15% cells, or a dose of 140×10.sup.6±about 15% or a dose collected from 140×10.sup.6±about 15% cells, or a dose of 160×10.sup.6±about 15% or a dose collected from 160×10.sup.6±about 15% cells, or a dose of 180×10.sup.6±about 15% or a dose collected from 180×10.sup.6±about 15% cells, or a dose of 200×10.sup.6±about 15% or a dose collected from 200×10.sup.6±about 15% cells, or a dose of 1×10.sup.6±about 15% or a dose collected from 1×10.sup.6±about 15% cells, or a dose of 1×10.sup.7±about 15% or a dose collected from 1×10.sup.7±about 15% cells, or a dose of 1×10.sup.8±about 15% or a dose collected from 1×10.sup.8±about 15% cells, or a dose of 1×10.sup.9±about 15% or a dose collected from 1×10.sup.9±about 15% cells. More effective cells or cell supernatants may be administered in even smaller numbers or collected from few cells, respectively. The precise determination of what would be considered an effective dose may be based on factors individual to each subject, including their size, age, sex, weight, condition of the particular subject, and site of application, for example the size of a synovial joint or area of bone being treated. Dosages can be readily ascertained by those skilled in the art from this disclosure and the knowledge in the art.

[0269] The skilled artisan can readily determine the number of cells or amount of a supernatant, and optional additives, vehicles, and/or carrier in compositions to be administered in methods

disclosed herein. Typically, any additives (in addition to the active cell(s) and/or agent(s)) are present in an amount of 0.001 to 50% (weight) solution in phosphate buffered saline, and the active ingredient is present in the order of micrograms to milligrams, such as about 0.0001 to about 5 wt %. In another embodiment about 0.0001 to about 1 wt %. In still another embodiment, about 0.0001 to about 0.05 wt % or about 0.001 to about 20 wt %. In a further embodiment, about 0.01 to about 10 wt %. In another embodiment, about 0.05 to about 5 wt %. Of course, for any composition to be administered to an animal or human, and for any particular method of administration, it is preferred to determine therefore: toxicity, such as by determining the lethal dose (LD) and LD50 in a suitable animal model e.g., rodent such as mouse; and, the dosage of the composition(s), concentration of components therein and timing of administering the composition(s), which elicit a suitable response. Such determinations do not require undue experimentation from the knowledge of the skilled artisan, this disclosure and the documents cited herein. And, the time for sequential administrations can be ascertained without undue experimentation.

Methods of Use

[0270] In some embodiments, disclosed herein is a method of treating a joint disease or disorder in a subject in need, comprising the step of administering a composition comprising an early apoptotic cell population directly into a joint of said subject, wherein said administration treats said joint disease or disorder in said subject.

[0271] A skilled artisan would appreciate that the term “joint disease or disorder” encompasses diseases in which the normal functioning or use of a joint is impaired. Impairment or loss of function may lead to discomfort or pain in or around the joint. Joint diseases and disorders may affect bone, joint capsule, cartilage, tendons, ligaments, tendon sheath, sac, synovial fluid, in or around the affected. Common diseases of the joints including, osteoarthritis, rheumatoid arthritis, gout, lupus, tendonitis, bursitis, carpal tunnel syndrome, sprains, and other. In some embodiments, the joint disease or disorder comprises osteoarthritis.

[0272] In some embodiments, methods of treatment disclosed herein for treating osteoarthritis treat inflammatory osteoarthritis. In some embodiments, methods of treatment disclosed herein for treating osteoarthritis treat erosive osteoarthritis (EOA). In some embodiments, methods of treatment disclosed herein for treating osteoarthritis treat Gorham disease with inflammatory osteoarthritis. In some embodiments, osteoarthritis comprises inflammatory arthritis, erosive osteoarthritis, and vanishing bone. In some embodiments, osteoarthritis comprises any combination of inflammatory arthritis, erosive osteoarthritis, and vanishing bone. In some embodiments, osteoarthritis comprises inflammatory arthritis, or erosive osteoarthritis, or vanishing bone, or a combination thereof.

[0273] Osteoarthritis is by definition a chronic disease. In some embodiments, methods of treatment disclosed herein for treating osteoarthritis treat moderate to severe osteoarthritis. In some embodiments, methods of treatment disclosed herein for treating osteoarthritis treat severe osteoarthritis. In some embodiments, methods of treatment disclosed herein for treating osteoarthritis treat advanced osteoarthritis. In some embodiments, methods of treatment disclosed herein for treating osteoarthritis treat knee osteoarthritis or end stage knee osteoarthritis. In some embodiments, methods of treatment disclosed herein for treating osteoarthritis treat a finger joint osteoarthritis such as a thumb joint osteoarthritis or a basal thumb joint osteoarthritis.

[0274] Methods of treatment comprising direct administration of early apoptotic cells are in some embodiments, also helpful for treating vanishing bone diseases. In some embodiments, disclosed herein is a method of treating osteoarthritis or vanishing bone disease, or a combination thereof in a subject in need, comprising the step of administering a composition comprising an early apoptotic cell population directly into a joint of said subject or at the site of the vanishing bone disease, wherein said administration treats osteoarthritis or vanishing bone disease, respectively, in said subject. In some embodiments, disclosed herein is a method of treating osteoarthritis or vanishing bone disease, or a combination thereof in a subject in need, comprising the step of administering a

composition comprising an early apoptotic cell supernatant, an apoptotic cell supernatant, or an apoptotic cell-phagocytic cell supernatant, directly into a joint of said subject or at the site of the vanishing bone disease, wherein said administration treats osteoarthritis or vanishing bone disease, respectively, or a combination thereof in said subject.

[0275] In some embodiments, treating of osteoarthritis or vanishing bone disease, or a combination thereof, comprises pain reduction, reduction of inflammation, reduction of swelling, inhibition of progressive degeneration of articular cartilage, reduction of progressive degeneration of articular cartilage, inhibition of erosion of bone tissue, or slowing of erosion of bone tissue, reduction in bone fractures, reduction of broken bones, inhibition of bone fractures, inhibition of broken bones. improving a quality of life, or any combination thereof in said subject suffering from the osteoarthritis or vanishing bone disease. In some embodiments, treating osteoarthritis or vanishing bone disease, or a combination thereof using the methods described herein rebalances the immune response within a joint or a bone tissue, or in the adjacent tissue thereof, or any combination thereof.

[0276] In some embodiments, methods of treatment disclosed herein provide a long-term treatment. In some embodiments, the effects of treatment last at least one-month post administration. In some embodiments, the effects of treatment last at least two months post administration. In some embodiments, the effects of treatment last at least 3, 4, 5, 6, 7, 8, 9, 10, or 11 months post administration. In some embodiments, the effects of treatment last between at least one-six months post administration. In some embodiments, the effects of treatment last between at least one-month and one-year post administration. In some embodiments, the effects of treatment last at least one-year post administration. In some embodiments, the effects of treatment last more than one-year post administration.

[0277] In some embodiments, disclosed herein is a method of treating osteoarthritis in a subject in need, comprising the step of administering a composition comprising an early apoptotic cell population directly into a joint of said subject, wherein said administration treats osteoarthritis in said subject. In some embodiments, disclosed herein is a method of treating osteoarthritis in a subject in need, comprising the step of administering a composition comprising an early apoptotic cell supernatant, an apoptotic cell supernatant, or an apoptotic cell-phagocytic cell supernatant, population directly into a joint of said subject, wherein said administration treats osteoarthritis in said subject. In some embodiments, osteoarthritis comprises degenerative osteoarthritis. In some embodiments, osteoarthritis comprises inflammatory osteoarthritis. In some embodiments, osteoarthritis comprises erosive osteoarthritis (EOA). In some embodiments, osteoarthritis comprises Gorham vanishing bone disease with inflammatory osteoarthritis

[0278] Osteoarthritis often affects synovial joints, such as at the knees, hips, shoulders, elbows, ankles, wrists, fingers, thumbs, neck, toes, thumb, hand, foot, and spine. Synovial joints consist of two bone ends covered by articular cartilage. Osteoarthritis may be caused by meniscal or ligament injury, pyogenic infection, ligamentous instability, joint fracture, obesity, or natural degenerative causes. Osteoarthritis often includes a progressive degeneration of articular cartilage at or in these joints. Joints affected by osteoarthritis may be painful, inflamed, swollen, and have a decreased range of movement.

[0279] Examples of the effects of osteoarthritis include but are not limited to the following: osteoarthritis in the hips can cause pain, stiffness, and severe disability. Patients may feel the pain in their hips, groin, inner thigh, buttocks, or knees. Osteoarthritis in the fingers may cause the fingers to become enlarged and gnarled. The disease may cause small, bony knobs to appear on the end joints of the fingers. Affected fingers may ache or be stiff and numb. More women than men suffer from osteoarthritis in the fingers, and they develop it especially after menopause. The base of the thumb joint (the 1.sup.st carpometacarpal joint) may also be similarly affected by osteoarthritis. Osteoarthritis in the neck and spine may cause stiffness and pain in the neck or in the lower back. It may also cause weakness or numbness of the arms or legs. Osteoarthritis in the neck and spine is

often debilitating and may result in the patient being bed-ridden.
[0280] One of the most common locations for osteoarthritis is in the knees. Osteoarthritis in the knee joint may cause the knee to be stiff, swollen, and painful-thus making it hard to walk, climb, and get in and out of chairs and bathtubs. If not treated, osteoarthritis in the knees can lead to permanent disability, and reduced quality of life.
[0281] In some embodiments, osteroarthritis is moderate to severe. In some embodiments, osteoarthritic is moderate. In some embodiments, osteoarthritis is severe. In some embodiments, osteroarthritis is at an end stage where the only remedy is joint replacement.
[0282] In some embodiments, a method of treating osteoarthritis in a subject in need, comprises treating osteoarthritis in a synovial joint. In some embodiments, a method of treating osteoarthritis in a subject in need, comprises treating osteoarthritis in a synovial joint selected from a knee joint, a hip joint, a shoulder joint, a joint between neck vertebrae, an elbow joint, an ankle joint, a wrist joint, a finger joint, a toe joint, a hand joint, a foot joint, or a thumb joint, or a combination thereof. In some embodiments, a method of treating osteoarthritis in a subject in need, comprises treating osteoarthritis in a knee joint. In some embodiments, a method of treating osteoarthritis in a subject in need, comprises treating osteoarthritis in a hip joint. In some embodiments, a method of treating osteoarthritis in a subject in need, comprises treating osteoarthritis in a shoulder joint. In some embodiments, a method of treating osteoarthritis in a subject in need, comprises treating osteoarthritis in a joint between neck vertebrae. In some embodiments, a method of treating osteoarthritis in a subject in need, comprises treating osteoarthritis in an elbow joint. In some embodiments, a method of treating osteoarthritis in a subject in need, comprises treating osteoarthritis in an ankle joint. In some embodiments, a method of treating osteoarthritis in a subject in need, comprises treating osteoarthritis in a wrist joint. In some embodiments, a method of treating osteoarthritis in a subject in need, comprises treating osteoarthritis in a finger joint. In some embodiments, a method of treating osteoarthritis in a subject in need, comprises treating osteoarthritis in a toe joint. In some embodiments, a method of treating osteoarthritis in a subject in need, comprises treating osteoarthritis in a hand joint. In some embodiments, a method of treating osteoarthritis in a subject in need, comprises treating osteoarthritis in a foot joint. In some embodiments, a method of treating osteoarthritis in a subject in need, comprises treating osteoarthritis in a thumb joint. In some embodiments, a method of treating osteoarthritis in a subject in need, comprises treating osteoarthritis in a 1.sup.st carpometacarpal joint of the thumb. In some embodiments, a method of treating osteoarthritis in a subject in need, comprises treating osteoarthritis in a combination of synovial joints.
[0283] In some embodiments, a method of treating osteoarthritis in a subject in need, comprises treating chronic osteoarthritis. In some embodiments, a method of treating osteoarthritis in a subject in need, comprises treating chronic osteoarthritis in a synovial joint selected from a knee joint, a hip joint, a shoulder joint, a joint between neck vertebrae, an elbow joint, an ankle joint, a wrist joint, a finger joint, a toe joint, a hand joint, a foot joint, or a thumb joint, or a combination thereof. In some embodiments, a method of treating osteoarthritis in a subject in need, comprises treating chronic and or moderate, and/or advanced osteoarthritis. In some embodiments, the term “chronic osteoarthritis” may be used interchangeably with “moderate to severe osteoarthritis”. In some embodiments, a method of treating osteoarthritis in a subject in need, comprises treating chronic and/or advanced osteoarthritis in a synovial joint selected from a knee joint, a hip joint, a shoulder joint, a joint between neck vertebrae, an elbow joint, an ankle joint, a wrist joint, a finger joint, a toe joint, a hand joint, a foot joint, or a thumb joint, or a combination thereof. In some embodiments, a method of treating osteoarthritis in a subject in need, comprises treating moderate to severe osteoarthritis in a synovial joint selected from a knee joint, a hip joint, a shoulder joint, a joint between neck vertebrae, an elbow joint, an ankle joint, a wrist joint, a finger joint, a toe joint, a hand joint, a foot joint, or a thumb joint, or a combination thereof. In some embodiments, a method of treating osteoarthritis in a subject in need, comprises treating end stage osteoarthritis. In

some embodiments, a method of treating osteoarthritis in a subject in need, comprises treating moderate osteoarthritis in a synovial joint selected from a knee joint, a hip joint, a shoulder joint, a joint between neck vertebrae, an elbow joint, an ankle joint, a wrist joint, a finger joint, a toe joint, a hand joint, a foot joint, or a thumb joint, or a combination thereof. In some embodiments, a method of treating osteoarthritis in a subject in need, comprises treating severe osteoarthritis in a synovial joint selected from a knee joint, a hip joint, a shoulder joint, a joint between neck vertebrae, an elbow joint, an ankle joint, a wrist joint, a finger joint, a toe joint, a hand joint, a foot joint, or a thumb joint, or a combination thereof. In some embodiments, a method of treating osteoarthritis in a subject in need, comprises treating end stage osteoarthritis in a synovial joint selected from a knee joint, a hip joint, a shoulder joint, a joint between neck vertebrae, an elbow joint, an ankle joint, a wrist joint, a finger joint, a toe joint, a hand joint, a foot joint, or a thumb joint, or a combination thereof.

[0284] In some embodiments, a method of treating osteoarthritis in a subject in need, comprises treating chronic osteoarthritis in a knee joint. In some embodiments, a method of treating osteoarthritis in a subject in need, comprises treating moderate and/or severe osteoarthritis in a knee joint. In some embodiments, a method of treating osteoarthritis in a subject in need, comprises treating advanced osteoarthritis in a knee joint. In some embodiments, a method of treating osteoarthritis in a subject in need, comprises treating end stage osteoarthritis in a knee joint.

[0285] A skilled artisan would appreciate that the term “treating”, and grammatical variations thereof, may encompass both therapeutic treatment and prophylactic or preventative measures, wherein the object is to prevent or lessen the targeted pathologic condition or disorder of osteoarthritis or vanishing bone disease, or combinations thereof. In some embodiments, treating may include directly affecting or curing, suppressing, inhibiting, preventing, reducing the severity of, delaying the onset of, reducing symptoms associated with osteoarthritis or vanishing bone disease, or a combination thereof. In some embodiments, “treating” may encompass delaying progression, speeding recovery, increasing efficacy of or decreasing resistance to alternative therapeutics, or a combination thereof. In some embodiments, “preventing” comprises delaying the onset of symptoms, preventing relapse to osteoarthritis or vanishing bone disease or a combination thereof, decreasing the number or frequency of relapse episodes, increasing latency between symptomatic episodes, or a combination thereof. In some embodiments, “suppressing” or “inhibiting”, comprises reducing the severity of symptoms, reducing the severity of an acute episode, reducing the number of symptoms, reducing the incidence of disease-related symptoms, reducing the latency of symptoms, ameliorating symptoms, reducing secondary symptoms, reducing secondary infections, prolonging patient survival, or a combination thereof.

[0286] In some embodiments, treating osteoarthritis comprises pain reduction, reduction of inflammation, reduction of swelling, inhibition of progressive degeneration of articular cartilage, reduction of progressive degeneration of articular cartilage, or any combination thereof in and or around the joint being treated. Joint function can be measured by evaluating the parameters such as the presence of discomfort or pain during the range of motion and movement, and by evaluating the range of motion itself.

[0287] In some embodiments, treating osteoarthritis comprises pain reduction in and or around the treated joint. In some embodiments, treatment alleviates pain localized around the joint. Alleviating or reducing pain may in some embodiments, be associated with a reduction or alleviation of pain during movement of a joint. Alleviating or reducing pain may in some embodiments, be associated with a reduction or alleviation of pain in a joint under static conditions. In some embodiments, treating osteoarthritis comprises reduction of stiffness in and or around the joint being treated.

[0288] In some embodiments, reduction or alleviation of pain allows for increased range of movement of the joint. In some embodiments, treating osteoarthritis using a method of directly administering early apoptotic cells or an apoptotic supernatant disclosed herein, increases movement in the treated joint. In some embodiments, the increased movement comprises increased

range of movement or increased movement with reduced pain, or a combination thereof. In some embodiments, the increased movement comprises increased range of movement. In some embodiments, the increased movement is accompanied by reduced pain.

[0289] In some embodiments, treating osteoarthritis comprises reduction of inflammation in and or around the treated joint. In some embodiments, treatment leading to reduced inflammation may be the result of reduction in the secretion of proinflammatory cytokines and chemokines. For example, but not limited to reduction of IL-1, IL-6, IL-8, IL-10, IL-1β, IL-2, IL-15, IL-22, MIP-1β, MCP-1, MDC, IP-10, fractalkine, IL-9, or TNFα, or any combination thereof. In some embodiments, treatment of osteoarthritis by direct administration of early apoptotic cells reduces pro- and anti-inflammatory cytokines and chemokines in the synovial fluid associated with the joint being treated. In some embodiments, the pro-inflammatory cytokines and chemokines reduced following treatment with early apoptotic cells or with an apoptotic supernatant, comprises any proinflammatory cytokine or chemokine.

[0290] A skilled artisan would recognize that a measure of treatment effectiveness may be performed by measuring the level of cytokines and or chemokines in the synovial fluid associated with the joint being treated. Methods of measuring the presence and concentration of cytokines and or chemokines is well known in the art, and any known method could be used. In some embodiments, synovial fluid will be collected in order to measure different cytokines and or chemokines in the joint fluid. In some embodiments, synovial fluid will be collected in order to measure different metalloproteinases in the joint fluid. In some embodiments, synovial fluid will be collected in order to measure different pro-inflammatory cytokines in the joint fluid. In some embodiments, synovial fluid will be collected in order to measure different anti-inflammatory cytokines in the joint fluid. In some embodiments, synovial fluid will be collected in order to measure different pro- and anti-inflammatory cytokines in the joint fluid. In some embodiments, synovial fluid will be collected in order to measure any of IL-1, IL-6, IL-8, IL-10, IL-1β, IL-2, IL-15, IL-22, MIP-13, MCP-1, MDC, IP-10, fractalkine, IL-9, or TNFα, or any combination thereof, in the joint fluid.

[0291] In some embodiments, synovial fluid is collected and assayed prior to treatment. In some embodiments, synovial fluid is collected and assayed prior to treatment and during treatment. In some embodiments, synovial fluid is collected and assayed prior to treatment, during treatment, and as a follow-up once treatment has been completed.

[0292] In some embodiments, synovial biopsies will be taken prior to treatment. In some embodiments, synovial biopsies will be taken prior to and during treatment. In some embodiments, synovial biopsies will be taken prior to and during treatment, and as a follow-up once treatment has been completed.

[0293] In some embodiments, methods of treating osteoarthritis by direct administration of a composition comprising early apoptotic cells or an apoptotic supernatant reduces the level of pro and anti-inflammatory cytokines or chemokines in the synovial fluid associated with the joint being treated. In some embodiments, the pro-inflammatory cytokines and chemokines reduced following treatment with early apoptotic cells or with an apoptotic cell supernatant, comprises any of IL-1, IL-6, IL-8, IL-10, IL-1β, IL-2, IL-15, IL-22, MIP-1β, MCP-1, MDC, IP-10, fractalkine, IL-9, or TNFα, or any combination thereof. In some embodiments, methods of treating osteoarthritis by direct administration of a composition comprising early apoptotic cells or comprising an apoptotic cell supernatant, modifies the level of pro and anti-inflammatory cytokines or chemokines in the synovial fluid associated with the joint being treated. In some embodiments, the pro-inflammatory cytokines and chemokines having their concentration altered following treatment with early apoptotic cells comprises any of IL-1, IL-6, IL-8, IL-10, IL-1β, IL-2, IL-15, IL-22, MIP-1β, MCP-1, MDC, IP-10, fractalkine, IL-9, or TNFα, or any combination thereof.

[0294] In some embodiments, methods of treating osteoarthritis by direct administration of a composition comprising early apoptotic cells or comprising an apoptotic cell supernatant, reduces

the level of IL-1 in the synovial fluid associated with the joint being treated. In some embodiments, methods of treating osteoarthritis by direct administration of a composition comprising early apoptotic cells or comprising an apoptotic cell supernatant, reduces the level of IL-6 in the synovial fluid associated with the joint being treated. In some embodiments, methods of treating osteoarthritis by direct administration of a composition comprising early apoptotic cells or comprising an apoptotic cell supernatant, reduces the level of IL-8 in the synovial fluid associated with the joint being treated. In some embodiments, methods of treating osteoarthritis by direct administration of a composition comprising early apoptotic cells or comprising an apoptotic cell supernatant, reduces the level of IL-10 in the synovial fluid associated with the joint being treated. In some embodiments, methods of treating osteoarthritis by direct administration of a composition comprising early apoptotic cells or comprising an apoptotic cell supernatant, reduces the level of IL-10 in the synovial fluid associated with the joint being treated. In some embodiments, methods of treating osteoarthritis by direct administration of a composition comprising early apoptotic cells or comprising an apoptotic cell supernatant, reduces the level of IL-2 in the synovial fluid associated with the joint being treated. In some embodiments, methods of treating osteoarthritis by direct administration of a composition comprising early apoptotic cells or comprising an apoptotic cell supernatant, reduces the level of IL-15 in the synovial fluid associated with the joint being treated. In some embodiments, methods of treating osteoarthritis by direct administration of a composition comprising early apoptotic cells or comprising an apoptotic cell supernatant, reduces the level of IL-22 in the synovial fluid associated with the joint being treated. In some embodiments, methods of treating osteoarthritis by direct administration of a composition comprising early apoptotic cells or comprising an apoptotic cell supernatant, reduces the level of IL-9 in the synovial fluid associated with the joint being treated. In some embodiments, methods of treating osteoarthritis by direct administration of a composition comprising early apoptotic cells or comprising an apoptotic cell supernatant, reduces the level of MIP-10 in the synovial fluid associated with the joint being treated. In some embodiments, methods of treating osteoarthritis by direct administration of a composition comprising early apoptotic cells or comprising an apoptotic cell supernatant, reduces the level of MCP-1 in the synovial fluid associated with the joint being treated. In some embodiments, methods of treating osteoarthritis by direct administration of a composition comprising early apoptotic cells or comprising an apoptotic cell supernatant, reduces the level of MDC in the synovial fluid associated with the joint being treated. In some embodiments, methods of treating osteoarthritis by direct administration of a composition comprising early apoptotic cells or comprising an apoptotic cell supernatant, reduces the level of IP-10 in the synovial fluid associated with the joint being treated. In some embodiments, methods of treating osteoarthritis by direct administration of a composition comprising early apoptotic cells or comprising an apoptotic cell supernatant, reduces the level of fractalkine in the synovial fluid associated with the joint being treated. In some embodiments, methods of treating osteoarthritis by direct administration of a composition comprising early apoptotic cells or comprising an apoptotic cell supernatant, reduces the level of TNFα in the synovial fluid associated with the joint being treated.

[0295] In some embodiments, methods of treating osteoarthritis by direct administration of a composition comprising early apoptotic cells or comprising an apoptotic cell supernatant, modifies the level of IL-1 in the synovial fluid associated with the joint being treated. In some embodiments, methods of treating osteoarthritis by direct administration of a composition comprising early apoptotic cells or comprising an apoptotic cell supernatant, modifies the level of IL-6 in the synovial fluid associated with the joint being treated. In some embodiments, methods of treating osteoarthritis by direct administration of a composition comprising early apoptotic cells or comprising an apoptotic cell supernatant, modifies the level of IL-8 in the synovial fluid associated with the joint being treated. In some embodiments, methods of treating osteoarthritis by direct administration of a composition comprising early apoptotic cells or comprising an apoptotic cell

supernatant, modifies the level of IL-10 in the synovial fluid associated with the joint being treated. In some embodiments, methods of treating osteoarthritis by direct administration of a composition comprising early apoptotic cells or comprising an apoptotic cell supernatant, modifies the level of IL-10 in the synovial fluid associated with the joint being treated. In some embodiments, methods of treating osteoarthritis by direct administration of a composition comprising early apoptotic cells or comprising an apoptotic cell supernatant, modifies the level of IL-2 in the synovial fluid associated with the joint being treated. In some embodiments, methods of treating osteoarthritis by direct administration of a composition comprising early apoptotic cells or comprising an apoptotic cell supernatant, modifies the level of IL-15 in the synovial fluid associated with the joint being treated. In some embodiments, methods of treating osteoarthritis by direct administration of a composition comprising early apoptotic cells or comprising an apoptotic cell supernatant, modifies the level of IL-22 in the synovial fluid associated with the joint being treated. In some embodiments, methods of treating osteoarthritis by direct administration of a composition comprising early apoptotic cells or comprising an apoptotic cell supernatant, modifies the level of MIP-10 in the synovial fluid associated with the joint being treated. In some embodiments, methods of treating osteoarthritis by direct administration of a composition comprising early apoptotic cells or comprising an apoptotic cell supernatant, modifies the level of MCP-1 in the synovial fluid associated with the joint being treated. In some embodiments, methods of treating osteoarthritis by direct administration of a composition comprising early apoptotic cells or comprising an apoptotic cell supernatant, modifies the level of MDC in the synovial fluid associated with the joint being treated. In some embodiments, methods of treating osteoarthritis by direct administration of a composition comprising early apoptotic cells or comprising an apoptotic cell supernatant, modifies the level of IP-10 in the synovial fluid associated with the joint being treated. In some embodiments, methods of treating osteoarthritis by direct administration of a composition comprising early apoptotic cells or comprising an apoptotic cell supernatant, modifies the level of fractalkine in the synovial fluid associated with the joint being treated. In some embodiments, methods of treating osteoarthritis by direct administration of a composition comprising early apoptotic cells or comprising an apoptotic cell supernatant, modifies the level of TNFα in the synovial fluid associated with the joint being treated.

[0296] In some embodiments, reduction of cytokines or chemokines is by about 10%-90%. In some embodiments, reduction of cytokines or chemokines is by about 20%-80%. In some embodiments, reduction of cytokines or chemokines is by about 30%-70%. In some embodiments, reduction of cytokines or chemokines is by about 40%-60%. In some embodiments, reduction of cytokines or chemokines is by about 80%-99%. In some embodiments, reduction of cytokines or chemokines is by about 10%. In some embodiments, reduction of cytokines or chemokines is by about 20%. In some embodiments, reduction of cytokines or chemokines is by about 30%. In some embodiments, reduction of cytokines or chemokines is by about 40%. In some embodiments, reduction of cytokines or chemokines is by about 50%. In some embodiments, reduction of cytokines or chemokines is by about 60%. In some embodiments, reduction of cytokines or chemokines is by about 70%. In some embodiments, reduction of cytokines or chemokines is by about 80%. In some embodiments, reduction of cytokines or chemokines is by about 90%. In some embodiments, reduction of cytokines or chemokines is by about 95%. In some embodiments, reduction of cytokines or chemokines is by about 99%.

[0297] A skilled artisan would appreciate that each cytokine or chemokine may be reduced by a different percentage, wherein the reduction of each cytokine or chemokine is by about 10%-90%. In some embodiments, reduction of cytokines or chemokines is by about 20%-80%. In some embodiments, reduction of each cytokine or chemokine is by about 30%-70%. In some embodiments, reduction of each cytokine or chemokine is by about 40%-60%. In some embodiments, reduction of each cytokine or chemokine is by about 80%-99%. In some embodiments, reduction of each cytokine or chemokine is by about 10%. In some embodiments,

reduction of each cytokine or chemokine is by about 20%. In some embodiments, reduction of each cytokine or chemokine is by about 30%. In some embodiments, reduction of each cytokine or chemokine is by about 40%. In some embodiments, reduction of each cytokine or chemokine is by about 50%. In some embodiments, reduction of each cytokine or chemokine is by about 60%. In some embodiments, reduction of each cytokine or chemokine is by about 70%. In some embodiments, reduction of each cytokine or chemokine is by about 80%. In some embodiments, reduction of each cytokine or chemokine is by about 90%. In some embodiments, reduction of each cytokine or chemokine is by about 95%. In some embodiments, reduction of each cytokine or chemokine is by about 99%.

[0298] In some embodiments, treating osteoarthritis comprises reduction of swelling in and or around the joint being treated. In some embodiments, treating osteoarthritis comprises inhibition of progressive degeneration of articular cartilage in the joint being treated. In some embodiments, treating osteoarthritis comprises reduction of progressive degeneration of articular cartilage in the joint being treated.

[0299] A skilled artisan would appreciate that inhibition of progressive degeneration may be measured using ultrasound or MRI or X ray analysis, or a combination thereof, wherein the size of the cartilage is measured. Further, pain scales used in the art for the amount of pain a subject is feeling may be used to gauge the benefit of treatment and reduction of pain. In addition, fluid analysis may be performed analyzing different components of test samples comprising for example but not limited to synovial fluid or joint fluid, and measuring cytokine levels, or chemokine levels, or levels of metalloproteases, or any combination thereof.

[0300] In some embodiments, a method of treating osteoarthritis in a subject in need, comprises the step of administering a composition comprising an early apoptotic cell population or comprising an apoptotic cell supernatant, directly into a joint of said subject, wherein said administration treats osteoarthritis. In some embodiments, said joint comprises a synovial joint. In some embodiments, a synovial joint comprises a comprises a knee joint, a hip joint, a shoulder joint, a joint between neck vertebrae, an elbow joint, an ankle joint, a wrist joint, a finger joint, a toe joint, or a thumb joint, a hand joint, a foot joint, or a combination thereof. In some embodiments, a subject in need suffers at a single joint. In some embodiments, a subject in need suffers at multiple joint.

[0301] In some embodiments, administration of a composition comprising an early apoptotic cell population or comprising an apoptotic cell supernatant, comprises direct administration into a synovial joint. In some embodiments, administration of a composition comprising an early apoptotic cell population or comprising an apoptotic cell supernatant, comprises direct administration into a knee joint. In some embodiments, administration of a composition comprising an early apoptotic cell population or comprising an apoptotic cell supernatant, comprises direct administration into a hip joint. In some embodiments, administration of a composition comprising an early apoptotic cell population or comprising an apoptotic cell supernatant, comprises direct administration into a shoulder joint. In some embodiments, administration of a composition comprising an early apoptotic cell population or comprising an apoptotic cell supernatant, comprises direct administration into a joint between neck vertebrae. In some embodiments, administration of a composition comprising an early apoptotic cell population or comprising an apoptotic cell supernatant, comprises direct administration into an elbow joint. In some embodiments, administration of a composition comprising an early apoptotic cell population or comprising an apoptotic cell supernatant, comprises direct administration into an ankle joint. In some embodiments, administration of a composition comprising an early apoptotic cell population or comprising an apoptotic cell supernatant, comprises direct administration into a wrist joint. In some embodiments, administration of a composition comprising an early apoptotic cell population or comprising an apoptotic cell supernatant, comprises direct administration into a finger joint. In some embodiments, administration of a composition comprising an early apoptotic cell population or comprising an apoptotic cell supernatant, comprises direct administration into a toe joint. In

some embodiments, administration of a composition comprising an early apoptotic cell population or comprising an apoptotic cell supernatant, comprises direct administration into a thumb joint. In some embodiments, administration of a composition comprising an early apoptotic cell population or comprising an apoptotic cell supernatant, comprises direct administration into a hand joint. In some embodiments, administration of a composition comprising an early apoptotic cell population or comprising an apoptotic cell supernatant, comprises direct administration into a foot joint. In some embodiments, administration of a composition comprising an early apoptotic cell population or comprising an apoptotic cell supernatant, comprises direct administration into a combination of synovial joints selected from comprises a knee joint, a hip joint, a shoulder joint, a joint between neck vertebrae, an elbow joint, an ankle joint, a wrist joint, a finger joint, a toe joint, a hand joint, a foot joint, or a thumb joint, or a combination thereof

[0302] A skilled artisan would appreciate that the term “direct” encompasses administration of the early apoptotic cells or apoptotic cell supernatant, or a composition thereof, respectively, within the joint being treated or immediately adjacent to the joint being treated. A direct treatment at a joint is in contrast to systemic administration for example but not limited to intravenous injection or intraperitoneal infusion. In some embodiments, administration of a composition comprising early apoptotic cell population or comprising an apoptotic cell supernatant, for treatment of osteoarthritis does not comprise a systemic administration.

[0303] In some embodiments, in methods disclosed herein direct administration comprises injection of a composition of early apoptotic cells or comprising an apoptotic cell supernatant, into a joint. In some embodiments, in methods disclosed herein direct administration comprises injection a composition of early apoptotic cells or comprising an apoptotic cell supernatant, into the tissue adjacent to a joint. In some embodiments, in methods disclosed herein direct administration comprises infusion of a composition of early apoptotic cells or comprising an apoptotic cell supernatant, into a joint. In some embodiments, in methods disclosed herein direct administration comprises infusion a composition of early apoptotic cells or comprising an apoptotic cell supernatant, into the tissue adjacent to a joint.

[0304] In some embodiments, in methods disclosed herein direct administration comprises infiltration of a composition of early apoptotic cells or comprising an apoptotic cell supernatant, into a cartilage tissue at a joint. In some embodiments, in methods disclosed herein direct administration comprises infiltration of a composition of early apoptotic cells or comprising an apoptotic cell supernatant, into the cartilage tissue adjacent to a joint.

[0305] Vanishing bone disease, also known as Gorham-Stout syndrome, disappearing bone disease, or massive osteolysis, is a rare disease of unknown etiology, characterized by destruction of osseous matrix and proliferation of vascular structures, is characterized by progressive bone loss. Bone tissue of subjects suffering from vanishing bone disease erode over time.

[0306] In some embodiments, a method of treatment disclosed herein comprises pain reduction, reduction of inflammation, reduction of swelling, inhibition of progressive degeneration of articular cartilage, reduction of progressive degeneration of articular cartilage, inhibition of erosion of bone tissue, or slowing of erosion of bone tissue, or any combination thereof. Affected individuals experience progressive destruction and resorption of bone. Multiple bones may become involved. Areas commonly affected include the ribs, spine, pelvis, skull, collarbone (clavicle), shoulder, and jaw. Pain and swelling in the affected area may occur. Bones affected are prone to reduced bone mass (osteopenia) and fracture. The severity can vary from one person to another and the disorder can potentially cause disfigurement and functional disability of affected areas.

[0307] In some embodiments, a method of treating vanishing bone disease in a subject in need, comprises treating vanishing bone disease present in any of a shoulder, the skull, the pelvic girdle or a portion thereof, the jaw, a rib or ribs, the collar bone or a portion thereof, or the spine or a combination thereof. In some embodiments, a method of treating vanishing bone disease in a subject in need, comprises treating a shoulder bone. In some embodiments, a method of treating

vanishing bone disease in a subject in need, comprises treating the skull. In some embodiments, a method of treating vanishing bone disease in a subject in need, comprises treating the pelvic girdle or a portion thereof. In some embodiments, a method of treating vanishing bone disease in a subject in need, comprises treating the jaw. In some embodiments, a method of treating vanishing bone disease in a subject in need, comprises treating a rib or ribs. In some embodiments, a method of treating vanishing bone disease in a subject in need, comprises treating the collar bone or a portion thereof. In some embodiments, a method of treating vanishing bone disease in a subject in need, comprises treating the spine. In some embodiments, a method of treating vanishing bone disease in a subject in need, comprises treating a combination of bone comprising a shoulder, the skull, the pelvic girdle or a portion thereof, the jaw, a rib or ribs, the collar bone or a portion thereof, or the spine.

[0308] In some embodiments, treating vanishing bone disease comprises pain reduction, reduction of inflammation, reduction of swelling, inhibition of erosion of bone tissue, reduction of erosion of bone tissue, inhibition of bone fractures at the site of the disease, reduction in bone fractures at the site of the disease, inhibition of bone breaks at the site of the disease, reduction in bone breaks at the site of the disease, inhibition of bone resorption at the site of the disease, reduction in bone resorption at the site of the disease, inhibition of reduction of bone mass at the site of the disease, slowing of reduction in bone mass at the site of the disease, inhibition of loss of bone density at the site of the disease, reduction in loss of bone density at the site of the disease, or any combination thereof. Measurements associated with bone mass and bone density may include any methods known in the art.

[0309] In some embodiments, treating vanishing bone disease comprises pain reduction in and or around the treated bone. In some embodiments, treatment alleviates pain localized around the bone. Alleviating or reducing pain may in some embodiments, be associated with a reduction or alleviation of pain during movement of the bone or in the area of the bone. Alleviating or reducing pain may in some embodiments, be associated with a reduction or alleviation of pain in a bone under static conditions. In some embodiments, treating osteoarthritis comprises reduction of stiffness in and or around the bone being treated.

[0310] In some embodiments, reduction or alleviation of pain allows for increased range of movement of the bone or area adjacent to the bone. In some embodiments, treating vanishing bone disease using a method of directly administering early apoptotic cells disclosed herein, increases movement in the treated bone or the region adjacent to the treated bond. In some embodiments, the increased movement comprises increased range of movement or increased movement with reduced pain, or a combination thereof. In some embodiments, the increased movement comprises increased range of movement. In some embodiments, the increased movement is accompanied by reduced pain.

[0311] In some embodiments, treating vanishing bone disease comprises reduction of inflammation in and or around the treated bone. In some embodiments, treatment leading to reduced inflammation may be the result of reduction in the secretion of anti-inflammatory or proinflammatory cytokines and chemokines, or a combination thereof. For example, but not limited to reduction of IL-1, IL-6, IL-8, IL-10, IL-1β, IL-2, IL-15, IL-22, MIP-1β, MCP-1, MDC, IP-10, fractalkine, IL-9, or TNFα, or any combination thereof. In some embodiments, treatment of vanishing bone disease by direct administration of early apoptotic cells or an apoptotic cell supernatant, reduces proinflammatory cytokines and chemokines in the tissue adjacent to the bone being treated. In some embodiments, the pro-inflammatory cytokines and chemokines reduced following treatment with early apoptotic cells or an apoptotic cell supernatant, comprises any proinflammatory cytokine or chemokine. In some embodiments, the pro-inflammatory cytokines and chemokines reduced following treatment with early apoptotic cells or an apoptotic cell supernatant, comprises any of IL-1, IL-6, IL-8, IL-10, IL-1β, IL-2, IL-15, IL-22, MIP-1β, MCP-1, MDC, IP-10, fractalkine, IL-9, or TNFα, or any combination thereof.

[0312] In some embodiments, reduction of cytokines or chemokines is by about 10%-90%. In some embodiments, reduction of cytokines or chemokines is by about 20%-80%. In some embodiments, reduction of cytokines or chemokines is by about 30%-70%. In some embodiments, reduction of cytokines or chemokines is by about 40%-60%. In some embodiments, reduction of cytokines or chemokines is by about 80%-99%. In some embodiments, reduction of cytokines or chemokines is by about 10%. In some embodiments, reduction of cytokines or chemokines is by about 20%. In some embodiments, reduction of cytokines or chemokines is by about 30%. In some embodiments, reduction of cytokines or chemokines is by about 40%. In some embodiments, reduction of cytokines or chemokines is by about 50%. In some embodiments, reduction of cytokines or chemokines is by about 60%. In some embodiments, reduction of cytokines or chemokines is by about 70%. In some embodiments, reduction of cytokines or chemokines is by about 80%. In some embodiments, reduction of cytokines or chemokines is by about 90%. In some embodiments, reduction of cytokines or chemokines is by about 95%. In some embodiments, reduction of cytokines or chemokines is by about 99%.

[0313] A skilled artisan would appreciate that each cytokine or chemokine may be reduced by a different percentage, wherein the reduction of each cytokine or chemokine is by about 10%-90%. In some embodiments, reduction of cytokines or chemokines is by about 20%-80%. In some embodiments, reduction of each cytokine or chemokine is by about 30%-70%. In some embodiments, reduction of each cytokine or chemokine is by about 40%-60%. In some embodiments, reduction of each cytokine or chemokine is by about 80%-99%. In some embodiments, reduction of each cytokine or chemokine is by about 10%. In some embodiments, reduction of each cytokine or chemokine is by about 20%. In some embodiments, reduction of each cytokine or chemokine is by about 30%. In some embodiments, reduction of each cytokine or chemokine is by about 40%. In some embodiments, reduction of each cytokine or chemokine is by about 50%. In some embodiments, reduction of each cytokine or chemokine is by about 60%. In some embodiments, reduction of each cytokine or chemokine is by about 70%. In some embodiments, reduction of each cytokine or chemokine is by about 80%. In some embodiments, reduction of each cytokine or chemokine is by about 90%. In some embodiments, reduction of each cytokine or chemokine is by about 95%. In some embodiments, reduction of each cytokine or chemokine is by about 99%.

[0314] In some embodiments, treating vanishing bone disease comprises reduction of inflammation in and or around the treated bone. In some embodiments, treatment leading to reduced inflammation may be the result of modifying the secretion of proinflammatory cytokines and chemokines. For example, but not limited to modifying secretion of IL-1, IL-6, IL-8, IL-10, IL-1β, IL-2, IL-15, IL-22, MIP-1β, MCP-1, MDC, IP-10, fractalkine, IL-9, or TNFα, or any combination thereof. In some embodiments, treatment of vanishing bone disease by direct administration of early apoptotic cells or an apoptotic cell supernatant, modifies proinflammatory cytokines and chemokines in the tissue adjacent to the bone being treated. In some embodiments, the pro-inflammatory cytokines and chemokines modified following treatment with early apoptotic cells or an apoptotic cell supernatant, comprises any proinflammatory cytokine or chemokine. In some embodiments, the pro-inflammatory cytokines and chemokines modified following treatment with early apoptotic cells or an apoptotic cell supernatant, comprises any of IL-1, IL-6, IL-8, IL-10, IL-1β, IL-2, IL-15, IL-22, MIP-1β, MCP-1, MDC, IP-10, fractalkine, IL-9, or TNFα, or any combination thereof.

[0315] In some embodiments, methods of administering a composition of early apoptotic cells or an apoptotic cell supernatant, reduces the concentration of at least one pro-inflammatory cytokine or chemokine in the synovial fluid present in the joint. In some embodiments, methods of administering a composition of early apoptotic cells or an apoptotic cell supernatant, reduces the concentration of at least one pro-inflammatory cytokine or chemokine secreted from cells adjacent to an affected bone. In some embodiments, methods of administering a composition of early

apoptotic cells or an apoptotic cell supernatant, reduces the concentration of at least one anti-inflammatory cytokine or chemokine in the synovial fluid present in the joint. In some embodiments, methods of administering a composition of early apoptotic cells or an apoptotic cell supernatant, reduces the concentration of at least one anti-inflammatory cytokine or chemokine secreted from cells adjacent to an affected bone. In some embodiments, methods of administering a composition of early apoptotic cells or an apoptotic cell supernatant, reduces the concentration of at least one pro-inflammatory cytokine or chemokine and one anti-inflammatory cytokine or chemokine in the synovial fluid present in the joint. In some embodiments, methods of administering a composition of early apoptotic cells or an apoptotic cell supernatant, reduces the concentration of at least one pro-inflammatory cytokine or chemokine and one anti-inflammatory cytokine or chemokines secreted from cells adjacent to an affected bone.

[0316] In some embodiments, treating vanishing bone disease comprises reduction of swelling in and or around the bone being treated.

[0317] In some embodiments, treating vanishing bone disease comprises inhibition of progressive erosion of the bone being treated. Erosion may in some embodiments, result in loss of bone mass, loss of bone density, and an increased occurrence of fracture or breaks in the affected bone. In some embodiments, treating vanishing bone disease comprises reduction of progressive erosion of the bone being treated. In some embodiments, treating vanishing bone disease comprises reduction of erosion of bone tissue. In some embodiments, treating vanishing bone disease comprises inhibition of bone fractures at the site of the disease. In some embodiments, treating vanishing bone disease comprises reduction in bone fractures at the site of the disease. In some embodiments, treating vanishing bone disease comprises inhibition of bone breaks at the site of the disease. In some embodiments, treating vanishing bone disease comprises reduction in bone breaks at the site of the disease. In some embodiments, treating vanishing bone disease comprises inhibition of bone resorption at the site of the disease. In some embodiments, treating vanishing bone disease comprises reduction in bone resorption at the site of the disease. In some embodiments, treating vanishing bone disease comprises inhibition of reduction of bone mass at the site of the disease. In some embodiments, treating vanishing bone disease comprises slowing of reduction in bone mass at the site of the disease. In some embodiments, treating vanishing bone disease comprises inhibition of loss of bone density at the site of the disease. In some embodiments, treating vanishing bone disease comprises reduction in loss of bone density at the site of the disease. In some embodiments, treating vanishing bone disease comprises a combination of pain reduction, reduction of inflammation, reduction of swelling, inhibition of erosion of bone tissue, reduction of erosion of bone tissue, inhibition of bone fractures at the site of the disease, reduction in bone fractures at the site of the disease, inhibition of bone breaks at the site of the disease, reduction in bone breaks at the site of the disease, inhibition of bone resorption at the site of the disease, reduction in bone resorption at the site of the disease, inhibition of reduction of bone mass at the site of the disease, slowing of reduction in bone mass at the site of the disease, inhibition of loss of bone density at the site of the disease, reduction in loss of bone density at the site of the disease.

[0318] In some embodiments, a method of treating vanishing bone disease in a subject in need, comprises the step of administering a composition comprising an early apoptotic cell population or comprising an apoptotic cell supernatant, directly at the site of the affected bone in said subject, wherein said administration treats vanishing bone disease. In some embodiments, said bone comprises of a shoulder, the skull, the pelvic girdle or a portion thereof, the jaw, a rib or ribs, the collar bone or a portion thereof, or the spine or a combination thereof.

[0319] In some embodiments, administration of a composition comprising an early apoptotic cell population or comprising an apoptotic cell supernatant, comprises direct administration at the site of a shoulder bone, the skull, the pelvic girdle or a portion thereof, the jaw, a rib or ribs, the collar bone or a portion thereof, or the spine, or a combination thereof. In some embodiments, administration of a composition comprising an early apoptotic cell population or comprising an

apoptotic cell supernatant, comprises direct administration at the site of a shoulder bone. In some embodiments, administration of a composition comprising an early apoptotic cell population or comprising an apoptotic cell supernatant, comprises direct administration at the site of the skull. In some embodiments, administration of a composition comprising an early apoptotic cell population or comprising an apoptotic cell supernatant, comprises direct administration at the site of the pelvic girdle or a portion thereof. In some embodiments, administration of a composition comprising an early apoptotic cell population or comprising an apoptotic cell supernatant, comprises direct administration at the site of the jaw or a portion thereof. In some embodiments, administration of a composition comprising an early apoptotic cell population or comprising an apoptotic cell supernatant, comprises direct administration at the site of a rib or ribs. In some embodiments, administration of a composition comprising an early apoptotic cell population or comprising an apoptotic cell supernatant, comprises direct administration at the site of the collar bone or a portion thereof. In some embodiments, administration of a composition comprising an early apoptotic cell population or comprising an apoptotic cell supernatant, comprises direct administration at the site of or the spine or a portion thereof. In some embodiments, administration of a composition comprising an early apoptotic cell population or comprising an apoptotic cell supernatant, comprises direct administration at multiple sites of vanishing bone disease.

[0320] A skilled artisan would appreciate that the term “direct” encompasses administration of the early apoptotic cells or an apoptotic cell supernatant, a composition thereof respectively, at or adjacent to the bone being treated. A direct treatment at a bone is in contrast to systemic administration for example but not limited to intravenous injection or intraperitoneal infusion. In some embodiments, administration of a composition comprising early apoptotic cell population or comprising an apoptotic cell supernatant, for treatment of vanishing bone disease does not comprise a systemic administration.

[0321] In some embodiments, in methods disclosed herein direct administration comprises injection of a composition of early apoptotic cells or an apoptotic cell supernatant, at the site of an affected bone. In some embodiments, in methods disclosed herein direct administration comprises injection a composition of early apoptotic cells or an apoptotic cell supernatant, adjacent to the site of an affected bone. In some embodiments, in methods disclosed herein direct administration comprises infusion of a composition of early apoptotic cells or an apoptotic cell supernatant, at the site of an affected bone. In some embodiments, in methods disclosed herein direct administration comprises infusion a composition of early apoptotic cells or an apoptotic cell supernatant, at the site adjacent to an affected bone.

[0322] In some embodiments, methods disclosed herein treating osteoarthritis or vanishing bone disease comprise direct administration into the joint or at the site of the affecting bone, said administration comprising infusion or injection of said early apoptotic cell population or an apoptotic cell supernatant.

[0323] Early apoptotic cell populations have been described in detail above including but not limited to methods of preparing early apoptotic cells and compositions thereof, and therapeutically effective doses thereof. Methods disclosed herein may in some embodiments utilize any of the early apoptotic cell populations or compositions thereof described above, including for example but not limited to ApoCells, Allocetra, Allocetra-OTS, Autocetra, and Autocetra-OTS. In some embodiments, early apoptotic cells comprise allogeneic or autologous cells that have been irradiated. Similarly, supernatants collected from early apoptotic cell populations or apoptotic cell populations or apoptotic-phagocytic cultures have been described in detail above including but not limited to methods of preparing these supernatant and compositions thereof, and therapeutically effective doses thereof. Methods disclosed herein may in some embodiments utilize any of the supernatants described or compositions thereof described above, including for example but not limited to supernatants collected from ApoCells, Allocetra, Allocetra-OTS, Autocetra, Autocetra-OTS, apoptotic cells, pooled apoptotic cell populations, and apoptotic-phagocytic cultures. In some

embodiments, the apoptotic cells used in culture to produce the supernatant have been irradiated.
[0324] In some embodiments, in the methods described herein, the early apoptotic cell population comprises an autologous early apoptotic cell population or an allogeneic early apoptotic cell population. The early apoptotic cells populations used in the methods of treatment described herein, may in some embodiments be stable at early apoptotic stage for an extended time period of greater than 24 hours. In some embodiments, in the methods described herein, the early apoptotic cell population comprises an autologous early apoptotic cell population that is stable for greater than 24 hours; or an allogeneic early apoptotic cell population that is stable for greater than 24 hours. In some embodiments, in the methods described herein, the supernatants used are collected from an autologous early apoptotic cell population or an allogeneic early apoptotic cell population. In some embodiments, in the methods described herein, the supernatants used are collected from an apoptotic cell population or pooled apoptotic cell populations. In some embodiments, in the methods described herein, the supernatants used are collected from an apoptotic cell-phagocytic culture.
[0325] Methods of treating osteoarthritis or vanishing bone disease with a composition comprising an early apoptotic cell population comprises use of single source or multiple source mononuclear enriched cells. In some embodiments, the early apoptotic cells comprise a pooled population of early apoptotic cells. In some embodiments, the early apoptotic cells comprise a pooled population of early apoptotic cells, wherein the starting population of mononuclear enriched cells were obtained from a single source. In some embodiments, the early apoptotic cells comprise a pooled population of early apoptotic cells, wherein the starting population of mononuclear enriched cells were obtained from multiple sources. Cells may be pooled prior to or after induction of apoptosis. In some embodiments, the single source of cells comprises cells from an unmatched donor.
[0326] In some embodiments, methods of treating osteoarthritis of vanishing bone disease comprise administration of a pooled early apoptotic cell population comprising an irradiated, pooled population of early apoptotic cells. In some embodiments, methods of treating osteoarthritis of vanishing bone disease comprise administration of a supernatant collected from pooled early apoptotic cell population comprising an irradiated, pooled population of early apoptotic cells, or from a pooled apoptotic cell population, which has been irradiated.
[0327] In some embodiments, methods of treating osteoarthritis or vanishing bone disease comprise use of irradiated early apoptotic cells, wherein the cells are irradiated in a way that will decrease proliferation and/or activation of residual viable cells within the apoptotic cell population. In some embodiments, in methods disclosed herein the cells are irradiated in a way that reduces the percent of viable non-apoptotic cells in a population. In some embodiments, in methods disclosed herein the percent of viable non-apoptotic cells in an inactivated early apoptotic cell population is reduced to less than 50% of the population. In some embodiments, in methods disclosed herein the percent of viable non-apoptotic cells in an inactivated early apoptotic cell population is reduced to less than 40% of the population. In some embodiments, in methods disclosed herein the percent of viable non-apoptotic cells in an inactivated early apoptotic cell population is reduced to less than 30% of the population. In some embodiments, in methods disclosed herein the percent of viable non-apoptotic cells in an inactivated early apoptotic cell population is reduced to less than 20% of the population. In some embodiments, in methods disclosed herein the percent of viable non-apoptotic cells in an inactivated early apoptotic cell population is reduced to less than 10% of the population. In some embodiments, in methods disclosed herein the percent of viable non-apoptotic cells in an inactivated early apoptotic cell population is reduced to 0% of the population.
[0328] In another embodiment, in methods disclosed herein the irradiated apoptotic cells preserve all their early apoptotic-, immune modulation-, stability-properties. In another embodiment, in methods disclosed herein the irradiation step uses UV radiation. In another embodiment, the radiation step uses gamma radiation. In another embodiment, in methods disclosed herein the apoptotic cells comprise a decreased percent of living non-apoptotic cells, comprise a preparation

having a suppressed cellular activation of any living non-apoptotic cells present within the apoptotic cell preparation, or comprise a preparation having reduced proliferation of any living non-apoptotic cells present within the apoptotic cell preparation, or any combination thereof.
[0329] In some embodiments, in methods disclosed herein a cell population comprising a reduced or non-existent fraction of living non-apoptotic cells may in one embodiment provide a mononuclear early apoptotic cell population that does not have any living/viable cells.
[0330] In some embodiments, use of irradiated early apoptotic cells, as shown here in the Examples) result from the apoptotic cells and not from a viable proliferating population of cells with cellular activity, present within the apoptotic cell population.
[0331] In some embodiments, apoptotic cells used in the methods described herein comprise a pooled mononuclear apoptotic cell preparation. In some embodiments, a pooled mononuclear apoptotic cell preparation comprises mononuclear cells in an early apoptotic state, wherein said pooled mononuclear apoptotic cells comprise a decreased percent of living non-apoptotic cells, a preparation having a suppressed cellular activation of any living non-apoptotic cells, or a preparation having reduced proliferation of any living non-apoptotic cells, or any combination thereof. In another embodiment, the pooled mononuclear apoptotic cells have been irradiated. In another embodiment, disclosed herein is a pooled mononuclear apoptotic cell preparation that in some embodiments, originates from the white blood cell fraction (WBC) obtained from donated blood.
[0332] In some embodiments, the apoptotic cell preparation used in methods described herein is irradiated. In another embodiment, said irradiation comprises gamma irradiation or UV irradiation. In yet another embodiment, the irradiated preparation has a reduced number of non-apoptotic cells compared with a non-irradiated apoptotic cell preparation. In another embodiment, the irradiated preparation has a reduced number of proliferating cells compared with a non-irradiated apoptotic cell preparation. In another embodiment, the irradiated preparation has a reduced number of potentially immunologically active cells compared with a non-irradiated apoptotic cell population. In some embodiments, methods of treating osteoarthritis or vanishing bone disease comprise direct administration of a composition comprising an early apoptotic cell population that comprises an irradiated population of early apoptotic cells.
[0333] In some embodiments, early apoptotic cells are prepared from pooled blood collected from a single autologous donor or from multiple donors, prepared and possibly stored for later use. This combined pool of blood may then be processed to produce a pooled mononuclear apoptotic cell preparation. In another embodiment, a pooled mononuclear apoptotic cell preparation ensures that a readily available supply of mononuclear apoptotic cells is available for methods of use treating osteoarthritis or vanishing bone disease.
[0334] In some embodiments, a method of treating osteoarthritis or vanishing bone disease comprises direct administration of a composition comprising a pooled early apoptotic cell population that comprises apoptotic cells prepared from single donor. In some embodiments, a method of treating osteoarthritis or vanishing bone disease comprises direct administration of a composition comprising a pooled early apoptotic cell population that comprises apoptotic cells prepared from multiple donor mononuclear cells. In some embodiments, a method of treating osteoarthritis or vanishing bone disease comprises direct administration of a composition comprising a pooled early apoptotic cell population that comprises apoptotic cells prepared from a single source donor or multiple donor mononuclear cells.
[0335] In some embodiments, in the method of treating osteoarthritis or vanishing bond disease, or a combination thereof, any apoptotic supernatant disclosed herein may be used, for example, but not limited to supernatants collected from apoptotic cells, early apoptotic cells, pooled apoptotic cells, or an apoptotic cell-phagocyte culture.
[0336] In some embodiments, in the method of treating osteoarthritis or vanishing bond disease, the subject is a mammalian subject. In some embodiments, in the method of treating osteoarthritis

or vanishing bond disease, the subject is a human subject. In some embodiments, the human subject is a child. In some embodiments, the human subject is an adult.
[0337] Dosages of early apoptotic cells or of supernatants have been described in detail above. The dosages described therein may in some embodiments, be used in the methods described herein for treating osteroarthritis or vanishing bone disease. In some embodiments, in methods of treating osteroarthritis or vanishing bone disease the direct administering of a composition of early apoptotic cells or of a supernatant comprises a single administration of the early apoptotic cell population cells or of said supernatant. In some embodiments, in methods of treating osteroarthritis or vanishing bone disease the direct administering of a composition of early apoptotic cells or of a supernatant comprises multiple administrations of said apoptotic cell population or of said supernatant. In some embodiments of methods described herein, the single direct administration is into an affected joint. In some embodiments of methods described herein, the single direct administration is into tissue as close as possible to an affected bone. In some embodiments of methods described herein, the single direct administration is adjacent to an affected joint. In some embodiments of methods described herein, the single direct administration is adjacent to an affected bone. In some embodiments of methods described herein, the multiple direct administrations are into an affected joint. In some embodiments of methods described herein, the multiple direct administrations are into tissue as close as possible to an affected bone. In some embodiments of methods described herein, the multiple direct administrations are adjacent to an affected joint. In some embodiments of methods described herein, the multiple direct administrations are adjacent to an affected bone. In some embodiments, direct multiple administrations comprise into a joint and adjacent to the joint. In some embodiments, direct multiple administrations comprise into tissue as close as possible to an affected bone and adjacent to the affected bone.
[0338] Dosage regimes have been described in detail above. Methods of use for treating osteoarthritis or vanishing bond disease may in some embodiments, use a dosage regime as described above with the early apoptotic cells or supernatants.
[0339] In some embodiments, in methods of treating osteroarthritis or vanishing bone disease comprising the direct administering of a composition of early apoptotic cells or of a supernatant, comprises daily or week administrations. In some embodiments, methods a dose of apoptotic cells or of a supernatant, is administered daily. In some embodiments, in methods of treating osteroarthritis or vanishing bone disease comprising the direct administering of a composition of early apoptotic cells or of a supernatant, comprises, a dose of apoptotic cells or of a supernatant, that is administered weekly. In some embodiments, a dose of apoptotic cells or of a supernatant, is administered semi-weekly (twice a week). In some embodiments, a dose of apoptotic cells or of a supernatant, is administered bi-weekly (every two weeks). In some embodiments, a dose of apoptotic cells or of a supernatant, is administered monthly. In some embodiments, a dose of apoptotic cells or of a supernatant, is administered in a non-regular regime, for example daily for a given time period followed by semi-weekly, or weekly, or bi-weekly, or monthly administration, or a combination thereof.
[0340] The dosage of early apoptotic cells or of a supernatant, has been described in detail above. Methods of use for treating osteoarthritis or vanishing bond disease may in some embodiments, use a dosage of early apoptotic cells or of a supernatant, as previously described.
[0341] In some embodiments, in methods of treating osteroarthritis or vanishing bone disease comprising the direct administering of a composition of comprising a supernatant collected from about 100×10.sup.6-210×10.sup.6 apoptotic cells. In some embodiments, in methods of treating osteroarthritis or vanishing bone disease comprising the direct administering of a composition of comprising a supernatant collected from about 1×10.sup.6-1×10.sup.9 apoptotic cells. In some embodiments, a dose of about 1×10.sup.6-1×10.sup.8 apoptotic cells is administered. In some embodiments, a dose of about 1×10.sup.6-1×10.sup.7 apoptotic cells is administered. In some

embodiments, a dose of about 1×10.sup.7-1×10.sup.9 apoptotic cells is administered. In some embodiments, a dose of about 1×10.sup.7-1×10.sup.8 apoptotic cells is administered. In some embodiments, a dose of about 1×10.sup.8-1×10.sup.9 apoptotic cells is administered. In some embodiments, a dose of about 1×10.sup.6 apoptotic cells is administered. In some embodiments, a dose of about 1×10.sup.7 apoptotic cells is administered. In some embodiments, a dose of about 1×10.sup.8 apoptotic cells is administered. In some embodiments, a dose of about 1×10.sup.9 apoptotic cells is administered.

[0342] In some embodiments, a dose of about 140×10.sup.6-210×10.sup.6 apoptotic cells is administered. In some embodiments, a dose of about 100×10.sup.6-140×10.sup.6 apoptotic cells is administered. In some embodiments, a dose of about 10-100×10.sup.6 apoptotic cells is administered. In some embodiments, a dose of about 20×10.sup.6 apoptotic cells is administered. In some embodiments, 25×10.sup.6 apoptotic cells is administered. In some embodiments, about 25×10.sup.6 apoptotic cells is administered. In some embodiments, a dose of about 30×10.sup.6 apoptotic cells is administered. In some embodiments, a dose of about 40×10.sup.6 apoptotic cells is administered. In some embodiments, a dose of about 50×10.sup.6 apoptotic cells is administered. In some embodiments, 50×10.sup.6 apoptotic cells is administered. In some embodiments, about 50×10.sup.6 apoptotic cells is administered. In some embodiments, 60×10.sup.6 apoptotic cells is administered. In some embodiments, a dose of about 60×10.sup.6 apoptotic cells is administered. In some embodiments, a dose of about 70×10.sup.6 apoptotic cells is administered. In some embodiments, a dose of about 80×10.sup.6 apoptotic cells is administered. In some embodiments, a dose of about 90×10.sup.6 apoptotic cells is administered. In some embodiments, a dose of about 1-15×10.sup.7 apoptotic cells is administered. In some embodiments, a dose of about 10×10.sup.7 apoptotic cells is administered. In some embodiments, a dose of about 11×10.sup.7 apoptotic cells is administered. In some embodiments, a dose of about 12×10.sup.7 apoptotic cells is administered. In some embodiments, a dose of about 13×10.sup.7 apoptotic cells is administered. In some embodiments, a dose of about 14×10.sup.7 apoptotic cells is administered. In some embodiments, a dose of about 15×10.sup.7 apoptotic cells is administered. In some embodiments, a dose of about 20×10.sup.7 apoptotic cells is administered. In some embodiments, a dose of 1×10.sup.6 apoptotic cells is administered. In some embodiments, a dose of 10×10.sup.6 apoptotic cells is administered. In another embodiment, a dose of 10×10.sup.7 apoptotic cells is administered. In another embodiment, a dose of 10×10.sup.8 apoptotic cells is administered. In another embodiment, a dose of 10×10.sup.9 apoptotic cells is administered. In another embodiment, a dose of 10×10.sup.10 apoptotic cells is administered. In another embodiment, a dose of 10×10.sup.11 apoptotic cells is administered. In another embodiment, a dose of 10×10.sup.12 apoptotic cells is administered. In another embodiment, a dose of 10×10.sup.5 apoptotic cells is administered. In another embodiment, a dose of 10×104 apoptotic cells is administered. In another embodiment, a dose of 10×10.sup.3 apoptotic cells is administered. In another embodiment, a dose of 10×10.sup.2 apoptotic cells is administered.

[0343] In some embodiments, methods of treating osteoarthritis or vanishing bone disease, or a combination thereof administer a high dose of apoptotic cells. In some embodiments, methods of treating osteoarthritis or vanishing bone disease, or a combination thereof administer a lower dose of apoptotic cells, dependent on the size of the joint. In some embodiments, a dose of 10×10.sup.6 apoptotic cells is administered. In some embodiments, a dose of 15×10.sup.6 apoptotic cells is administered. In some embodiments, a dose of 20×10.sup.6 apoptotic cells is administered. In some embodiments, a dose of 25×10.sup.6 apoptotic cells is administered. In some embodiments, a dose of 30×10.sup.6 apoptotic cells is administered. In some embodiments, a dose of 35×10.sup.6 apoptotic cells is administered. In some embodiments, a dose of 50×10.sup.6 apoptotic cells is administered. In some embodiments, a dose of 100×10.sup.6 apoptotic cells is administered. In some embodiments, a dose of 150×10.sup.6 apoptotic cells is administered. In some embodiments, a dose of 200×10.sup.6 apoptotic cells is administered. In another embodiment, a dose of

210×10.sup.6 apoptotic cells is administered. In another embodiment, a dose of 70×10.sup.6 apoptotic cells is administered. In another embodiment, a dose of 140×10.sup.6 apoptotic cells is administered. In another embodiment, a dose of 35-210×10.sup.6 apoptotic cells is administered.
[0344] In some embodiments, in methods of treating osteroarthritis or vanishing bone disease or a combination thereof, said method comprises the direct administering of a composition comprising a supernatant disclosed herein comprising a supernatant collected from about 100×10.sup.6-210×10.sup.6 apoptotic cells. In some embodiments, methods of treating osteroarthritis or vanishing bone disease comprises the direct administering of a composition comprising a supernatant collected from about 1×10.sup.6-1×10.sup.9 apoptotic cells. In some embodiments, a supernatant collected from about 1×10.sup.6-1×10.sup.8 apoptotic cells is administered. In some embodiments, a supernatant collected from about 1×10.sup.6-1×10.sup.7 apoptotic cells is administered. In some embodiments, a supernatant collected from about 1×10.sup.7-1×10.sup.9 apoptotic cells is administered. In some embodiments, a supernatant collected from about 1×10.sup.7-1×10.sup.8 apoptotic cells is administered. In some embodiments, a supernatant collected from about 1×10.sup.8-1×10.sup.9 apoptotic cells is administered. In some embodiments, a supernatant collected from about 1×10.sup.6 apoptotic cells is administered. In some embodiments, a supernatant collected from about 1×10.sup.7 apoptotic cells is administered. In some embodiments, a supernatant collected from about 1×10.sup.8 apoptotic cells is administered. In some embodiments, a supernatant collected from about 1×10.sup.9 apoptotic cells is administered.
[0345] In some embodiments, a supernatant collected from about 140×10.sup.6-210×10.sup.6 apoptotic cells is administered. In some embodiments, a supernatant collected from about 100×10.sup.6-140×10.sup.6 apoptotic cells is administered. In some embodiments, a supernatant collected from about 10-100×10.sup.6 apoptotic cells is administered. In some embodiments, a supernatant collected from about 20×10.sup.6 apoptotic cells is administered. In some embodiments, a supernatant collected from about 30×10.sup.6 apoptotic cells is administered. In some embodiments, a supernatant collected from about 40×10.sup.6 apoptotic cells is administered. In some embodiments, a supernatant collected from about 50×10.sup.6 apoptotic cells is administered. In some embodiments, a supernatant collected from about 60×10.sup.6 apoptotic cells is administered. In some embodiments, a supernatant collected from about 60×10.sup.6 apoptotic cells is administered. In some embodiments, a supernatant collected from about 70×10.sup.6 apoptotic cells is administered. In some embodiments, a supernatant collected from about 80×10.sup.6 apoptotic cells is administered. In some embodiments, a supernatant collected from about 90×10.sup.6 apoptotic cells is administered. In some embodiments, a supernatant collected from about 1-15×10.sup.7 apoptotic cells is administered. In some embodiments, a supernatant collected from about 10×10.sup.7 apoptotic cells is administered. In some embodiments, a supernatant collected from about 11×10.sup.7 apoptotic cells is administered. In some embodiments, a supernatant collected from about 12×10.sup.7 apoptotic cells is administered. In some embodiments, a supernatant collected from about 13×10.sup.7 apoptotic cells is administered. In some embodiments, a supernatant collected from about 14×10.sup.7 apoptotic cells is administered. In some embodiments, a supernatant collected from about 15×10.sup.7 apoptotic cells is administered. In some embodiments, a supernatant collected from 1×10.sup.6 apoptotic cells is administered. In some embodiments, a supernatant collected from 10×10.sup.6 apoptotic cells is administered. In another embodiment, a supernatant collected from 10×10.sup.7 apoptotic cells is administered. In another embodiment, a supernatant collected from 10×10.sup.8 apoptotic cells is administered. In another embodiment, a supernatant collected from 10×10.sup.9 apoptotic cells is administered. In another embodiment, a supernatant collected from 100×10.sup.10 apoptotic cells is administered. In another embodiment, a supernatant collected from 10×10.sub.11 apoptotic cells is administered. In another embodiment, a supernatant collected from 10×10.sup.12 apoptotic cells is administered. In another embodiment, a supernatant collected

from 10×10.sup.5 apoptotic cells is administered. In another embodiment, a supernatant collected from 10×104 apoptotic cells is administered. In another embodiment, a supernatant collected from 10×10.sup.3 apoptotic cells is administered. In another embodiment, a supernatant collected from 10×10.sup.2 apoptotic cells is administered.

[0346] In some embodiments, methods of treating osteoarthritis or vanishing bone disease, or a combination thereof administer a high dose of a supernatant collected from apoptotic cells or an apoptotic-phagocytic culture. In some embodiments, methods of treating osteoarthritis or vanishing bone disease, or a combination thereof administer a lower dose of a supernatant collected from apoptotic cells or an apoptotic-phagocytic cell culture, dependent on the size of the joint. In some embodiments, a supernatant collected from 10×10.sup.6 apoptotic cells is administered. In some embodiments, a supernatant collected from 15×10.sup.6 apoptotic cells is administered. In some embodiments, a supernatant collected from 20×10.sup.6 apoptotic cells is administered. In some embodiments, a supernatant collected from 25×10.sup.6 apoptotic cells is administered. In some embodiments, a supernatant collected from 30×10.sup.6 apoptotic cells is administered. In some embodiments, a supernatant collected from 35×10.sup.6 apoptotic cells is administered. In some embodiments, a supernatant collected from 50×10.sup.6 apoptotic cells is administered. In some embodiments, a supernatant collected from 100×10.sup.6 apoptotic cells is administered. In some embodiments, a supernatant collected from 150×10.sup.6 apoptotic cells is administered. In some embodiments, a supernatant collected from 200×10.sup.6 apoptotic cells is administered. In another embodiment, a supernatant collected from 210×10.sup.6 apoptotic cells is administered. In another embodiment, a supernatant collected from 70×10.sup.6 apoptotic cells is administered. In another embodiment, a supernatant collected from 140×10.sup.6 apoptotic cells is administered. In another embodiment, a supernatant collected from 35-210×10.sup.6 apoptotic cells is administered.

[0347] In some embodiments of methods described herein, the number of early apoptotic cells is ±5%, 10%, 15%, or 20% of the number of cells. In some embodiments of methods described herein, the number of apoptotic cells is ±5%, 10%, 15%, or 20% of the number of cells. For example, but not limited to the dose of each administration comprises between about 50×10.sup.6±5% to 200×10.sup.6±5% early apoptotic cells/kg subject, or 50×106±10% to 200×106±10% early apoptotic cells/kg subject, or 50×10.sup.6±15% to 200×10.sup.6±15% early apoptotic cells/kg subject, or 50×10.sup.6 20% to 200×10.sup.6 20% early apoptotic cells/kg subject. Similarly, supernatants may be collected from a culture of a range of apoptotic cells, being ±5%, 10%, 15%, or 20% of the number of cells.

EXAMPLES

Example 1: Apoptotic Cell Production

[0348] Objective: To produce early-apoptotic cells, including irradiated early apoptotic cells.

[0349] Methods: Methods of making populations of early-apoptotic cells have been well documented in International Publication No. WO 2014/087408 and United States Application Publication No. US2015/0275175-A1, see for example, the Methods section preceding the Examples at “Early apoptotic cell population Preparation” and “Generation of apoptotic cells” (paragraphs [0223] through [0288]), and Examples 11, 12, 13, and 14, which are incorporated herein in their entirety).

[0350] The flow chart presented in FIG. **1** provides an overview of one embodiment of the steps used during the process of producing a population of early apoptotic cells, wherein anticoagulants were included in the thawing and induction of apoptosis steps. As is described in detailed in Example 14 of International Publication No. WO 2014/087408 and United States Application Publication No. US US-2015-0275175-A1, early apoptotic cell populations were prepared wherein anti-coagulants were added at the time of freezing, or at the time of incubation, or at the time of freezing and at the time of incubation. The anticoagulant used was acid-citrate dextrose, NIH Formula A (ACD formula A) was supplemented with 10 U/ml heparin to a final concentration of 5% ACD of the total volume and 0.5 U/ml heparin.

[0351] The population of cells used as a starting population may vary in certain embodiments, yet methods producing the early apoptotic cell described herein result in the final products having the same qualities and characteristics. In some embodiments, cells used to make early apoptotic cells may be autologous, allogeneic from a donor, or allogeneic from a blood bank.
[0352] Briefly: The cells were collected and then frozen with addition of 5% anticoagulant citrate dextrose formula A and 10 U/ml heparin (ACDhep) to the freezing media. Thawing, incubation in an apoptosis induction media containing 5% ACDhep, and final product preparation were performed in a closed system.
[0353] Apoptosis and viability analysis, potency assay, and cell population characterization were performed in each experiment. In order to establish consistence in production of the early apoptotic cell product, the final product (FP) of initial batches of apoptotic cells were stored at 2-8° C. and examined at t0, t24 h, t48 h and t72 h. At each point apoptosis analysis, short potency assay (Applicants CD14+ frozen cells), trypan blue measurement and cell population characterization were performed. The FP was tested for cell count to assess average cell loss during storage and apoptosis and viability analysis.
[0354] The methods sections cited above and Example 11 of International Publication No. WO 2014/087408 and United States Application Publication No. US US-2015-0275175-A1 provide details of preparing other embodiment of apoptotic cell populations in the absence of anti-coagulants and are incorporated herein in full.
[0355] In certain instances, the early apoptotic cells were irradiated after they were prepared (after induction of apoptosis), in other words following the last step shown in FIG. **1**. In other embodiments, irradiation could occur at an earlier step of the procedure to produce irradiated early apoptotic cells.
[0356] Methods of preparing irradiated apoptotic cells: Similar methods were used to prepare an inactivated apoptotic cell population, wherein a mononuclear early apoptotic cell population, single source or from multiple sources, comprises a decreased percent of non-quiescent non-apoptotic cells, or a population of cells having a suppressed cellular activation of any living non-apoptotic cells, or a population of cells having a reduced proliferation of any living non-apoptotic cells, or any combination thereof.
[0357] Briefly, an enriched mononuclear cell fraction was collected via leukapheresis procedure from healthy, eligible donors. Following apheresis completion, cells were washed and resuspended with freezing media comprising 5% Anticoagulant Citrate Dextrose Solution-Formula A (ACD-A) and 0.5 U†ml heparin. Cell were then gradually frozen and transferred to liquid nitrogen for long term storage. In some embodiments, multiple fractions from different donors or the same donor were pooled.
[0358] For preparation of irradiated ApoCells (early apoptotic cells), cryopreserved cells were thawed, washed and resuspended with apoptosis induction media comprising 5% ACD-A, 0.5 U†ml heparin sodium and 50 g/ml methylprednisolone. Cells were then incubated for 6 hours at 37° C. in 5% CO.sub.2. At the end of incubation, cells were collected, washed and resuspended in Hartmann's solution using a cell processing system (Fresenius Kabi, Germany). In some embodiments, collected apoptotic cell fractions were pooled to create a pooled, apoptotic cell fraction. Following manufacturing completion (induction of apoptosis), ApoCells were irradiated at 4000 cGy using g-camera at the radiotherapy unit, Hadassah Ein Kerem. Apoptosis and viability of ApoCell determined using AnnexinV and PI (MBL, MA, USA) staining (≥40% and ≤15%, respectively) via Flow cytometer. Results analyzed using FCS express software. In some embodiments, following irradiation, single source irradiated, apoptotic cells were pooled, wherein the source was the same or different donors. In other embodiments, multiple single source or multiple different source apoptotic cells were pooled prior to irradiation.
[0359] This irradiated ApoCell population is considered to include early apoptotic cells, wherein any viable cells present have suppressed cellular activity and reduced or no proliferation

capabilities. In certain cases, the ApoCell population has no viable non-apoptotic cells.
Results:
[0360] The stability of the FP produced with inclusion of anticoagulant at freezing and incubation (apoptotic induction) and then stored at 2-8° C. are shown below in Table 1.
TABLE-US-00001 TABLE 1 Cell count*- performed using a MICROS 60 hematology analyzer. FP Time Cell concentration % of point (×l0.sup.6cells\ml) cell loss t0 20.8 NA t24 h 20.0 −3.85 t48 h 20.0 −3.85 t72 h 19.7 −5.3 *Results Representative of 6 (six) experiments.
[0361] When manufacturing the cells without including an anticoagulant in the induction medium, cells were stable for 24 hours and less stable thereafter. Use of anticoagulants unexpectedly extended the stability of the apoptotic cell population for at least 72 hours, as shown in Table 1.
TABLE-US-00002 TABLE 2 Trypan blue measurement trypan blue FP Time positive cells point (%) t0 3.0 t24 h 5.9 t48 h 5.2 t72 h 6.5
[0362] The results of Table 2 show viability of the FP remained high for at least 72 hours.
TABLE-US-00003 TABLE 3 Apoptosis analysis- (AnPI staining) performed using Flow Cytometry 1.5 mM Ca FP Time An−PI− An+PI− An+PI+ point (%) (%) (%) t0 44.3 50.9 4.8 t24 h 39.0 55.9 5.1 t48 h 34.8 60.1 5.1 t72 h 33.4 60.5 6.1
[0363] The results of Table 3 show that the percent apoptotic cells versus necrotic cells was maintained over at extended time period of at least 72 hours post preparation of the cells, as was the percentage of early apoptotic cells.
[0364] The data in Table 3 confirms that the majority of cells in the population produced are in early apoptosis, wherein the percent of cells in the population in early apoptosis (An+PI−) was greater than 50% and in some instances greater than 60%. The cell population produced comprises a minimal percent of cells in late apoptosis or dead cells (less than or equal to 6%). See also Table 5 below.
[0365] Inclusion of anticoagulants both at the time of freezing and during induction of apoptosis resulted in the most consistently high yield of stable early-apoptotic cells (average yield of early apoptotic cells 61.3±2.6% % (An+PI−) versus 48.4±5.0%, wherein 100% yield is based on the number of cells at freezing). This high yield was maintained even after 72 hours storage at 2-8° C.
[0366] Next a comparison was made between the inclusion of the anticoagulant at freezing or thawing or both, wherein percent (%) recovery was measured as well as stability. Anticoagulant was included in the apoptotic incubation mix for all populations. Table 4 presents the results of these studies.
TABLE-US-00004 TABLE 4 Yield and stability comparison of final products (FP) manufactured from cells collected, with (“+”) or without (“−”) addition of anticoagulant during freezing (“F”) and thawing (“Tha”) # of Collected Cells % Cell Recovery in Final Product of Collected Cells Donor (×10.sup.9, FP t0 FP t24 h* ID 100%) F−/Tha− F−/Tha+ F+/Tha+ F+/Tha− F−/Tha− F−/Tha+ F+/Tha+ F+/Tha− 1 13.3 52.1 53.4 62.5 62 52.1 48.9 62.5 62 2 13.6 50.5 36.7 53.5 63.5 47.6 36.7 53.1 59.7 3 15.0 42.7 42 53.6 58.4 42.7 41.7 53.6 57.8 Avg 14.0 48.4 ± 5.0 44.0 ± 8.5 56.5 ± 5.2 61.3 ± 2.6 47.5 ± 4.7 42.4 ± 6.1 56.4 ± 5.3 59.8 ± 2.1
[0367] Additional population analysis comparisons of early apoptotic cell populations (batches of cells) prepared with and without anti-coagulant added, show the consistency of these results.
TABLE-US-00005 TABLE 5 Cell population analysis comparison between batches prepared with and without anticoagulant ApoCell At ApoCell Time 24 h Thawing Time 0 h Storage Test Specification w\o ACDhep +ACDhep w\o ACDhep +ACDhep w\o ACDhep +ACDhep Change in Total >35.0% 85.5 82.8 49.9 66.7 49.0 66.7 Cell Count (79.5-92.5) (67.7-96.4) (46.6-52.3) (62.5-71.2) 46.6-50.3) (62.5-71.2) Percent change (min-max) Changes in 90.0 ± 10.0% 100 100 98.2 100 ApoCell (96.2-100) Percent change Range (min- max) Cell viability PI >85.0% 98.0 96.0 98.5 94.6 97.7 94.5 exclusion (97.4-98.4) (91.9-98.1) (97.9-99.2) (93.5-95.5) (96.4-98.6) (93.4-95.1) Percent viable Range (min- max) Identity/ CD3 (T 75.7 66.5 73.3 62.8 71.6 64.2 Purity cells): (71.6-81.4) (60.1-70.1) (70.3-78.3) (61.1-65.3) (61.5-79.1) (61.6-68.1) Analysis of

cell 71.9 phenotype (50.0-85.0) Average (%) ApoCell (maximal CD3: calculated 71.6 range) (50.0-85.0) CD19 (B 7.5 9.8 9.0 9.9 9.5 9.7 cells): (4.0-11.1) (8.6-12.0) (7.6-10.2) (9.3-10.2) (8.6-10.3) (9.2-10.4) 9.3 (3.0-15.0) ApoCell CD19: 9.5 (4-15) CD14 9.8 14.0 11.6 15.4 9.3 16.1 (monocytes): (6.4-13.0) (8.8-22.1) (10.2-13.3) (8.2-19.3) (4.8-17.2) (9.0-20.4) 10.1 (2.5-22.0) ApoCell CD14: 10.6 (2.5-22.0) CD15.sup.high 0.2 0.46 0.2 0.083 0.1 0.09 (granulocytes): (0-0.3) (0.18-0.69) (0.1-0.4) (0.08-0.09) (0.1-0.2) (0.07-0.1) 0.4 (0-6.0) ApoCell CD15.sup.high: 0.2 (0-2.0) CD 56 (NK): 7.4 10.1 4.7 11.2 4.9 10.0 7.2 (2.4-11.0) (6.6-14.2) (2.7-8.0) (7.2-14.2) (2.2-9.2) (6.4-13.0) (1.5-22.0) ApoCell CD56: 5.2 (1.5-15.0)

[0368] Percentage of final product cells (yield) in the presence or absence of anticoagulants. Similar to the results presented above at Table 1, the data presented in Table 4 demonstrates that early apoptotic cells manufactured from cells frozen in the presence of anticoagulant had a beneficial effect on average yield of fresh final product (FP t0) as compared to cells frozen without anticoagulant. The beneficial effect was seen when anticoagulant was used while freezing only (61.3±2.6% versus 48.4±5.0%), or both freezing and thawing (56.5±5.2% versus 48.4±5.0%). The beneficial effect was less significant when anticoagulant was used upon thawing only (44.0±8.5% versus 48.4±5.0%). These were non-high triglyceride samples.

[0369] Effect of anticoagulants on aggregation. No cell aggregations were seen in these 3 non-high triglyceride samples, or in 21 additional samples (data not shown). However, in 41 other non-high triglyceride samples manufactured without anticoagulants (data not shown), mild aggregates were seen in 10 (24.4%) and severe aggregates in 5 (12.2%); thus, anticoagulants avoid completely cell aggregates.

[0370] Effect of anticoagulants on stability. Fresh FPs manufactured with- or without anticoagulants were stored at 2-8° C. for 24 hours to determine whether addition of ACDhep to the manufacturing procedure impairs the stability of the FP. Cells were sampled following 24 hours of storage and yield was calculated. Similar to the results shown in Table 1 for extended time periods (up to 72 hours), Table 4 shows that the beneficial effect was kept and observed when anticoagulant was used while freezing only (59.8±2.1% versus 47.5±4.7%), or both freezing and thawing (56.4±5.3% versus 47.5±4.7%). The beneficial effect was less significant when anticoagulant was added only upon thawing (42.4±6.1% versus 47.5±4.7%). These were all non-high triglyceride samples. These results show minimal cell loss following 24 hours of FP storage in all treatments with significant advantage to cells treated with anticoagulant during both freezing and thawing. Average loss of cells treated with anticoagulant during freezing only was 2.3±3.2% compared to 1.9±3.3% without anticoagulants, upon thawing only was 3.0±4.7 compared to 1.9±3.3% without anticoagulants, and 0.2±0.4% compared to 1.9±3.3% without anticoagulants when cells were both frozen and thawed with ACDhep. In summary, the beneficial effect of anticoagulants on yield was kept for at least 24 hours.

[0371] The characteristics of a representative cell population of the FP are shown below in Table 6.

TABLE-US-00006 TABLE 6 Characterization of the cell population of fresh (t0) FP manufactured from cells collected with (“+”) or without (“−”) addition of anticoagulant during freezing (“F”) and thawing (“Tha”) procedures* FP t0 F−/Tha− F−/Tha+ F+/Tha+ Donor CD3+ CD19+ CD56+ CD14+ CD15+ CD3+ CD19+ CD56+ CD14+ CD15+ CD3+ ID (%) (%) (%) (%) (%) (%) (%) (%) (%) (%) (%) (%) 1-3 62.2 ± 6.1 5.6 ± o.7 9.8 ± 0.9 13.5 ± 1.1 0 ± 0 61 ± 6.1 8.6 ± 0.4 8.6 ± 0.9 14.1 ± 1.1 0 ± 0 63.9 ± 5.8 FP t0 F+/Tha+ F+/Tha− Donor CD19+ CD56+ CD14+ CD15+ CD3+ CD19+ CD56+ CD14+ CD15+ ID (%) (%) (%) (%) (%) (%) (%) (%) (%) 1-3 7.4 ± 0.6 9.4 ± 0.8 13.3 ± 1.9 0 ± 0 61.9 ± 6.0 11.5 ± 1.1 10.1 ± 1.0 14.3 ± 1.3 0 ± 0 *Induction of apoptosis was performed using a medium containing anticoagulant for all batches.

[0372] The results of Table 6 show the cell characteristics of the final products (FP) manufactured with or without anticoagulant at freezing and thawing. Batches were sampled, stained for mononuclear markers, and analyzed via flow cytometry to determine the cell distribution in each sample and to examine whether the addition of anticoagulant affected the cell population. As

presented in Table 5, there were no significant differences detected in cell populations manufactured with or without anticoagulants at freezing or thawing. The average T cell population (CD3+ cells) in fresh FP was 62.3±1.2% between treatments compared to 62.9±1.1% before freezing; the average B cell population (CD19+ cells) was 8.3±2.5% between treatments compared to 3.1±0.8% before freezing; the average natural killer cell population (CD56+ cells) was 9.5±0.7% between treatments compared to 12.9±0.5% before freezing; the average monocyte cell population (CD14+ cells) was 13.8±0.5% between treatments compared to 17.5±0.3% before freezing; and the average granulocyte population (CD15+ cells) was 0.0% in the fresh FP compared to 0.35±0.2% at freezing.

[0373] The potency of the early apoptotic population was also examined.

TABLE-US-00007 TABLE 7 Potency analysis of fresh (t0) FP manufactured from cells with ("+") or without ("−") addition of anticoagulant during freezing ("F") and thawing ("Tha") procedures.

FP t0 Donor ID # Treatment	F−/Tha−	F−/Tha+	F+/Tha+	F+/Tha−
Median DR CD86 DR CD86 DR CD86 DR CD86 fluorescence DCs 1:2 Early apoptotic cell population + LPS	3% 28%	4% 24%	5% 24%	9% 15%
from LPS DCs 1:4 Early apoptotic cell population + LPS	4% 38%	6% 35%	6% 34%	6% 24%
DCs 1:8 Early apoptotic cell population + LPS	13% Not done	10% 45%	15% 54%	8% 48%

[0374] The results presented in Table 7 are from a potency assay performed to determine the ability of each final product to enhance a tolerogenic state in immature dendritic cells (iDCs) following stimulation with (LPS). The tolerogenic effect was determined by assessing downregulation of co-stimulatory molecule HLA-DR and CD86 expression on iDCs following interaction with the early apoptotic cell populations and different treatments leading to LPS upregulation. The analysis was performed on DCsign+ cells. Results represent the percent delay in maturation following interaction with early apoptotic cell population and following addition of LPS versus LPS-induced maturation. The experiment tested the potency of fresh FP (t0) manufactured with- or without anticoagulant. Results presented in Table 7 show that apoptotic cells manufactured with or without anticoagulant enhance the tolerance effect of both co-stimulatory markers in a dose-dependent manner.

[0375] The early apoptotic cells produced herein were from non-high triglyceride samples. This consistent high yield of stable early apoptotic cells was produced even in the cases when the donor plasma is high in triglycerides (See for example, Examples 12 and 13 of International Publication No. WO 2014/087408 and United States Application Publication No. US US-2015-0275175-A1). Note that anti-coagulants were not added to the PBS media used for formulation of the final early apoptotic cell dose for infusion.

SUMMARY

[0376] The objective of this study was to produce a stable, high yield early apoptotic cell population. The rational for use of anticoagulants was that aggregates were seen first in patients with high-triglycerides, but later in a significant portion of other patients. A concern here was the disclosure in U.S. Pat. No. 6,489,311 that the use of anticoagulants prevented cell apoptosis.

[0377] In short, with minimal impact on the composition, viability, stability, and the apoptotic nature of the cells, there was a significant improvement of at least 10-20% in the number of collected cells in the final product (Yield) when anticoagulant was added. In this study an up to 13% increase in yield was shown, which represents 26.8% augmentation in yield in controlled conditions but in real GMP conditions it went up to 33% and more augmentations in cell number then can be produced in a single collection. This effect is crucial, since it may avoid the need for a second apheresis from a donor.

[0378] This effect was surprising because the anticipated impact was expected to be dissolution of mild aggregates. It had been hypothesize that thawing cells with anticoagulant reduced the amount of aggregates. When formed, these aggregates eventually lead to massive cell loss. Cells collected and frozen without anticoagulant demonstrated aggregate formation at thawing, immediately after

wash. Furthermore, a high level of aggregates was also detected in cells that were frozen without anticoagulant and resuspended with media containing anticoagulant. No aggregates were seen in cells that were both frozen and resuspended with media containing anticoagulant. Taken together, it was conclude that the addition of anticoagulants during freezing and apoptosis induction is of high importance, and did not appear to negatively impact the induction of early apoptosis on the cell population.

[0379] Recovery of early apoptotic cells was further tested, for example, following 24 hours of storage at 2-8° C., for stability purposes, during which an average cell loss of 3-4.7% was measured, regardless of manufacturing conditions, with favorable results for cells that were both frozen and thawed with media containing anticoagulant (0.2±0.4% cell loss following 24 hours of FP storage), suggesting that addition of anticoagulant is critical during freezing and thawing, but once finally formulated, the early apoptotic cell population is stable. Extended time point studies showed this stability to at least 72 hours.

[0380] Apoptosis and viability, as well as cell composition of the FP product were not significantly affected by the addition of anticoagulant at the freezing and/or thawing stage. Values measured from a wide variety of characteristics were similar, indicating the ACDhep did not change the early apoptotic cell characteristics and the final product met the acceptance criteria of >40% apoptotic cells.

[0381] The assay used to test apoptotic cells potency was based on immature dendritic cells (iDCs), DCs that are characterized by functions such as phagocytosis, antigen presentation, and cytokine production.

[0382] The HLA-DR (MHC class II) membrane molecule and co-stimulatory molecule CD86 were selected as markers to detect the tolerogenic effects of antigen-presenting cells (APCs). Using flow cytometry, changes in expression of HLA-DR and CD86 on iDCs were measured following stimulation with LPS, as well as in the presence of the early apoptotic cell population manufactured with- or without anticoagulant and stimulated with LPS. Early apoptotic cell populations were offered to DCs in ascending ratios of 1:2, 1:4, and 1:8 iDCs: early apoptotic cell population. As presented in Table 6, it was shown that early apoptotic cell population enhanced the tolerogenic effect over stimulated DCs in a dose-dependent manner, with slightly better results for early apoptotic cell population manufactured with anticoagulant both at freezing and apoptosis induction.

[0383] Taken together, it was concluded that addition of anticoagulant to both freezing and apoptosis media is of high importance to increase cell recovery and avoid massive cell loss due to aggregates, and to avoid in many cases a second round of apheresis from a donor. It was shown that all cells met acceptance criteria for the validated FP, indicating that the addition of anticoagulant does not impair the FP.

Example 2: Preparation and Use of Pooled Apoptotic Cell

[0384] Objective: Produce an irradiated multiple donor single apoptotic cell infusion (a pooled mononuclear irradiated apoptotic cell preparation).

[0385] Methods: Apoptotic cells were prepared as per Example 1 above, except that in the current experiments, preparation was done simultaneously from multiple (4) donors. Following preparation from 4 donors, the cell preparations were combined at the last step (prior to irradiation), irradiated immediately after, and were ready for use. Irradiation was at 25 Gy.

Results and Summary:

[0386] Analysis in a GvHD mouse model showed that the single infusion of multiple-donor irradiated apoptotic cells successfully and significantly improved life expectancy in a mouse model of GvHD. (Data not shown)

Example 3: Preparation of Apoptotic Cell Supernatant

[0387] Objective: To obtain a supernatant from early-apoptotic cells and monocytes/macrophages/dendritic cells.

[0388] Methods: Preparation from apoptotic cells: CD14+ monocytes and other mononuclear

enriched white blood cells were cultured and triggered to undergo apoptosis. The number of apoptotic cells was between 1 to 100 million cells per well in a 12-well plate. In some instances 8 million cells were cultured per well. After incubation for 24, 36, and 48 hours, the cells were centrifuge (290 g, 4 degrees Celsius, 10 minutes). The supernatant was collected and frozen in aliquots at −80 degrees Celsius until use.

[0389] Methods: Preparation from monocytes/macrophages/dendritic cells and apoptotic cells: CD14+ monocytes were cultured with apoptotic cells as prepared above at a ratio of 1:16, for 24 h or 36 h or 48 h. The number of monocytes was: 0.5 million cells per well in a 12-well plate and the number of apoptotic cells was: 8 million cells per well in a 12-well plate. After incubation for 24 hours the cells were centrifuge (290 g, 4 degrees Celsius, 10 minutes). The supernatant was collected and frozen in aliquots at −80 degrees Celcius until use. Similar procedures could be performed at different ratios of monocytes:apoptotic cells and/or using other sources of cells derived from monocytes, such as different types of macrophages including M1/M2 and dendritic cells.

[0390] Results: The apoptotic supernatant prepared herein either from apoptotic mononuclear or from co-culturing monocytes/macrophages/dendritic cells with apoptotic mononuclear, was capable of down regulating pro-inflammatory cytokines under conditions of a cytokine storm (data not shown). These apoptotic supernatants, could in certain embodiments, be effectively used in methods disclosed herein for treating osteoarthritis.

Example 4: Use of Apoptic Cells to Treat Osteoarthritis

[0391] Objective: Treat osteoarthritis in a subject who was non-responsive to other therapies.

[0392] Methods: Early apoptotic cells prepared as described above in Example 1

[0393] Subject treated was a 70-year-old female with hypothyroidism. She presented with a 4-year inflammatory and erosive process of her right shoulder. In 2015, she experienced right upper extremity swelling and limited range-of-motion of the right shoulder, without known injury. Complete destruction of the humeral head on X-ray, significant inflammatory reaction on MRI, and significantly elevated erythrocyte sedimentation rate (ESR) and C-reactive protein (CRP) were seen. No serum autoantibodies or bacterial growth on needle aspiration of the joint were detected. ESR and CRP decreased with systemic steroids, without significant clinical improvement. After an uneventful total reverse shoulder arthroplasty, swelling, ESR and CRP elevations gradually subsided. Two years later the patient returned with shoulder swelling and significant CRP elevation. Repeated aspirations, debridements, and finally removal of the prosthesis, were performed, followed by long-term antibiotic treatment for the possibility of infection. Swelling and CRP levels were ameliorated only via continuous shoulder drain. During the last year she was hospitalized >9 months. Compassionate treatment using her own irradiated apoptotic cells was authorized by the Ethical Committee.

[0394] Irradiated, autologous early apoptotic cells were prepared as in Example 1 above (termed “Allocetra-OTS” in FIG. **2**). The patient underwent leukapheresis to get the starting material and irradiated, early apoptotic cells were prepared from this starting material. The irradiated, early apoptotic cells (100×10.sup.6) were administrated by intra-joint injection in the right shoulder joint for 5 consecutive weeks. Specifically, each dose of cells administered contained 100×10.sup.6±20% irradiated, early apoptotic cells in 15 ml Ringer's Lactate Solution. Administration was by intra-joint infusion.

[0395] Measurement of cytokines and chemokines present in synovial flued was performed via a Luminex MAGPIX system (Luminex USA) using Milliplex software (Merck).

Results:

[0396] After weekly intra-joint apoptotic cell infusions for 5 consecutive weeks, the patient improved dramatically, with significant reduction in shoulder swelling, redness, and tightness over 6 weeks. Fluid drainage from the shoulder declined from 150-250 ml/day to <60 ml/day. CRP declined from 7.34, pretreatment, to 0.49 (normal range ≤0.5). Synovial fluid samples were

collected before treatment and weekly during/after treatment. Measurement of pro-inflammatory cytokines/chemokines related to monocyte, macrophage, dendritic cell, osteoclast, neutrophil, and T cell activation showed down regulation of IL-6, IL-8, IL-1β, IL-2, IL-15, IL-22, MIP-1β, IL-9 and TNFα, following 2 injections of irradiated early apoptotic cells (FIG. **2**). FIG. **2** shows that most notably IL-22 (dysregulation of wound healing of synovial tissue), IL-8 (neutrophilic chemotactic factor), IL-6 (innate immunity), IL-9 (apoptosis prevention) and MIP-1-3 (chronic inflammation) were downregulated.

[0397] Blood biochemistry and CBC were not significantly changed. An episode of drain-induced infection 3 weeks after the end of treatment was treated with antibiotics, debridement, and drain exchange. The drain was removed 3 months after treatment due to low fluid drainage and hospitalization was no longer required. At 6-month follow-up CRP remained low.

[0398] The effect observed in the first five weeks of treating the patient was maintained for nine months, where she remained at home and stable following the intervention. Surprisingly and unexpectedly, whereas Il-6 and perhaps IL-8 are cytokines that have been described in vanishing bone disease and could have been predicted to be elevated, this is the first description of high IL-22 which is related to synovial healing dysregulation. Neither its elevation nor the effect of treatment with early apoptotic cells were expected. The same can be said for IL-9 that can avoid apoptosis of inflammatory cells and therefore maintain chronic inflammation, whereas IL-9 down regulation may provoke activation induced cell death and terminate immune response. In addition, it was surprising that TNF was not elevated at all. In that regard anti-cytokine like anti-TNF and even anti IL-6 would not have been a good choices and only apoptotic cells had this global effect.

Summary

[0399] HLA-matched apoptotic cell infusion was previously reported to be safe and beneficial in prevention of graft-versus-host disease (Mevorach D, Zuckerman T, Reiner I, et al. (2014) “Single infusion of donor mononuclear early apoptotic cells as prophylaxis for graft-versus-host disease in myeloablative HLA-matched allogeneic bone marrow transplantation: a phase IIIa clinical trial.” Biol Blood Marrow Transplant., 20:58-65), but this is the first time autologous early apoptotic cells, specifically prepared as described herein, have been used as an intra-joint infusion.

[0400] The early apoptotic cell preparation prepared for treatment of this patient was autologous, yet one skilled in the art would appreciate that it would also have been possible to administer a non-autologous preparation of these early apoptotic cells to achieve the same results. Early apoptotic cells could have been obtained from either a third-party subject or could be a third-party pooled cell apoptotic preparation (allogeneic), followed by an irradiation step prior to administration and successfully treatments of osteoarthritis.

[0401] The mechanism of action of this treatment is suggested to be related to monocyte-, macrophage-, dendritic cell-, and osteoclast cell signaling (Trahtemberg U, Mevorach D. (2017) “Apoptotic cells induced signaling for immune homeostasis in macrophages and dendritic cells.” Front Immunol., 8:1356).

[0402] Considering the conclusions of Grey et al., (ibid) that if the chosen route of administration does not deliver apoptotic cells to the spleen such that the apoptotic cells can interact with B cells, protection is unlikely to be elicited, it was highly unexpected that intra joint infusion to a non-autoimmune condition such as erosive osteoarthritis would be successful, as the apoptotic cells administered by intra joint infusion did not have any access to the spleen or to immune organ. Therefore, it was surprising and unexpected that the intra joint infusion terminated increased pro- and anti-inflammatory cytokine/chemokine release in the synovial fluid. Specially, the down regulation of high IL-22 which is related to synovial healing dysregulation and IL-9 that can avoid apoptosis of inflammatory cells and therefore maintain chronic inflammation whereas its down regulation may provoke termination of chronic inflammation.

Example 5: Manufacturing of Allocetra

[0403] Allocetra is composed of mononuclear cells induced to and stabilized in an apoptotic state

(See Example 1). The starting material for Allocetra production was a fresh apheresate containing a mononuclear-enriched cell fraction collected from non-HLA matched healthy pre-screened adult donors via leukapheresis. Donor eligibility was determined using a process that includes screening of the donor for relevant communicable disease agents or diseases, predefined inclusion/exclusion criteria, and testing of the donor's blood sample for human pathogens. Donor eligibility criteria were defined to comply with local and international regulations, as applicable in regions in which clinical studies are performed.

[0404] As provided in Example 1, the apheresate was cryopreserved in liquid nitrogen for long-term storage. Following thawing and removal of freezing medium, cells were resuspended in a medium that induces apoptosis and transiently stabilizes the cells in an apoptotic stage. Induction medium was then removed, and Allocetra was resuspended in the final formulation solution. As part of the process, the cells were irradiated to prevent further T-cell proliferation and therefore to avoid possible development of an unwanted immune response by the Allocetra cells.

[0405] The biological activity and potency of the Allocetra product was demonstrated by its ability to induce immune modulation by reduction of the TNF-α secretion in macrophages in the presence of LPS.

[0406] Allocetra was provided as a frozen cell suspension, in two cell concentrations 25×10.sup.6 cells/ml and 50×10.sup.6 cells/ml, suspended in Plasma-Lyte and diluted 50% with CryoStor5©, to a final DMSO concentration of 2.5% (named Allocetra FDP25 and FDP50, respectively). The product was frozen in different package volumes, ready to be used post thawing in appropriate doses for the clinical studies.

Example 6: Use of Intra-Articular Allocetra in Knee Osteoarthritis

[0407] Objective: This example describes a double blind, randomized, multi-center study to evaluate the safety and efficacy of intra-articular administration of Allocetra compared to placebo in patients with symptomatic knee osteoarthritis (OA), ongoing in Israel, Moldova, and Denmark.

[0408] Methods: Up to 160 adult patients with symptomatic knee osteoarthritis who have failed to respond adequately to conventional therapy for osteoarthritis were and are being enrolled, in two phases: (1) open label, multiple dose safety run-in, to characterize the safety and tolerability of Allocetra injections at 3 different doses (50×10.sup.6, 100×10.sup.6, 200×10.sup.6 cells, 15 patients), and determine the dose and dose regimen for the randomized phase; (2) double-blinded controlled phase, in which patients are being randomized 1:1 to receive three injections of either Allocetra (200×10.sup.6 cells) or placebo. Patients had chronic (moderate to severe) knee OA with radiographic classification of Kelgren-Lawrence grade 2-3. The main study eligibility criteria are provided below. Patients were followed for safety assessment following injections, and responses to treatment were assessed by self reporting of pain (numerical rating scale (NRS) 0-10) and Western Ontario and McMaster Universities Arthritis Index (WOMAC) questionnaires. Responses to treatment were assessed in accordance with the OMERACT-OARSI responder criteria. Study treatment (Allocetra was prepared in Examples 1 and 5) was and is being injected to the index knee three times with a 2-week interval between injections. Patients were and are being followed-up for safety and efficacy for up to 12 months.

[0409] Results: Up to Feb. 10, 2025, 142 patients were treated under this study. Fifteen patients were enrolled and treated with Allocetra on the safety run-in stage, which was completed, and all patients have been followed to at least 6 months following the last injection. These patients demonstrated a favorable safety profile involving related AEs which were mostly short-term mild injection site reactions or joint related signs and symptoms including arthralgia and/or knee swelling. Events were distributed across the different dose cohorts, with no clear relation of severity or frequency to a specific dose cohort. Following data safety monitoring board (DSMB) review of the safety data and confirmation, the highest Allocetra dose was selected for the randomization phase.

[0410] Results obtained from the safety run-in phase in this study also showed evidence of efficacy.

Fifteen patients were treated with Allocetra in the Phase I stage of the study. All patients had symptomatic disease, with knee pain NRS at baseline average of 6.3 (95% CI: 5.6-7.0). All patients received three intra-articular (IA) Allocetra injections at intervals of 2-4 weeks between doses, apart from one patient who received 2 injections. Patients were assessed weekly for pain NRS, and at 3 and 6 months for WOMAC pain, stiffness and function. To date, all safety run-in patients completed at least 3 months of assessment following the last injection and twelve patients have completed follow-up to at least 6 months following the last injection. Reduction of pain, compared to the pain level at baseline was reported at 3 and 6 months, indicating a potentially durable effect. The reported reduction in pain was consistent with the results of the WOMAC questionnaire at 3 and 6 months following the last treatment, demonstrating also improvement in functionality. Pain reduction: patients reported a statistically significant average pain reduction of 47-50% (NRS) and 51-52% (WOMAC) from baseline at 3- and 6-months post-injections (one patient received a steroid injection to the target knee 2 months prior to 6 months assessment). Functionality: 42-46% statistically significant improvement in functionality at 3- and 6-months post-injections. All results were statistically significant as assessed by T-Test; pain (assessed by NRS or WOMAC) $P<0.0001$, function $P<0.001$, stiffness $P<0.05$ (see Figure). In total, 75% of the patients qualified as responders per the OMERACT-OARSI responder criteria at 3 months following the last treatment, with an additional patient reporting pain reduction >40%, and 83% at 6 months.

[0411] Up to Feb. 10, 2025, 127 patients were treated in the randomized phase of this study with enrollment ongoing. No Serious AEs were reported. As in the safety run-in stage, related AEs were mostly transient injection site reactions or joint related signs and symptoms including arthralgia and/or knee swelling.

Inclusion Criteria:

[0412] 1. Age 45-80 years. [0413] 2. Chronic (moderate to severe) osteoarthritis of index knee with knee-related joint pain. [0414] 3. Radiographic evidence of knee osteoarthritis of K-L Grade 2 or 3 in the index knee. [0415] 4. Knee pain assessed daily over a period of 7 days during the screening period and following wash-out of pain medications. [0416] 5. Patients with chronic (moderate to severe) knee pain for at least 3 months who have failed to respond adequately to conventional therapy. [0417] 6. Willing to abstain from other intra-articular treatments and adhere to the protocol restrictions for concomitant medications and therapies during the study. [0418] 7. Women of childbearing potential and all men must agree to use 2 methods of an adequate contraception prior to study entry and for the duration of study participation through 4 weeks following IP administration.

Exclusion Criteria:

[0419] 1. Wheelchair bound. [0420] 2. Immunosuppressive therapy. [0421] 3. Any known current or prior tumor of the index knee. [0422] 4. Any known history or current intra-articular or osseous infection of the index knee. [0423] 5. Any evidence of clinically significant active infection. [0424] 6. Any known history of inflammatory arthropathy or crystal-deposition arthropathy. [0425] 7. Any known severe systemic cartilage and/or bone disorder, such as, but not limited to, chondrodysplasia, osteogenesis imperfecta. [0426] 8. Body Mass Index (BMI) >40. [0427] 9. Any major surgical cartilage treatment within 6 months. [0428] 10. Any ligamentous repair or malalignment correction in the index knee within 6 months. [0429] 11. Major injury to the index knee, such as torn ligament or severe sprain within 6 months. [0430] 12. Clinically relevant knee instability of the index knee. [0431] 13. Severe hip osteoarthritis ipsilateral to the index knee. [0432] 14. Clinically significant widespread pain syndromes, e.g., fibromyalgia, long COVID syndrome. [0433] 15. Known coagulopathy, or use of anticoagulation medication or antiaggregant medication. [0434] 16. Certain tumors, severe cardiac, respiratory or hematologic diseases as defined in the protocol.

Example 7: Use of Allocetra in End Stage Knee Osteoarthritis

[0435] Objective: This example describes a pilot study designed to assess the safety and tolerability

of Allocetra therapy in subjects suffering from end stage knee osteoarthritis and indicated for total knee replacement.

[0436] Methods: This study is ongoing in a single site in Israel. Eighteen adult patients were enrolled and treated with Allocetra (as prepared in Examples 1 and 5) at a dose of 200×10.sup.6 cells injected to the index knee, with follow-up for up to 2 years. A second dose of Allocetra was offered following 6-12 months. Patients were adults with pain and functional disability due to advanced knee OA which was indicated for knee replacement surgery. The main study eligibility criteria are provided below. Patients were followed for safety reactions following injection. Responses to treatment were assessed by patient self-reporting of pain (scale 0-10) and Western Ontario and McMaster Universities Arthritis Index (WOMAC) questionnaires.

[0437] Results: Up to Feb. 10, 2025, all 18 patients were treated under this study, and of these 10 patients were treated twice. Patients reported transient events of discomfort/pain or swelling in the knee following injection, which were conservatively managed. These are expected with intra-articular injections may be associated with transient AEs. Two SAEs were reported in a single patient, one of them a Serious Adverse Reaction of moderate rash.

[0438] Results obtained in this study show evidence of responses to treatment. Patients had advanced OA, with a radiographic classification of Kellgren-Lawrence grade 4 (13 patients) or 3-4 (5 patients), and an average pain of 7 at baseline. Patients were treated with an IA injection of Allocetra to the target knee on Day 0, and went on to report pain and WOMAC pain, stiffness and function at defined timepoints up to 2 years. To date, all patients completed at least 3 months of follow-up, demonstrating an average 29.5% reduction of reported pain, compared to baseline. This improvement was maintained at 6 months, where 42.9% of patients still reported a reduction of at least 30% in their knee pain, compared to baseline. These results were also supported by the WOMAC scores reported at 3 months, reflecting a consistent improvement in both pain and function. Moreover, to date, only 2/18 patients proceeded to undergo knee replacement surgery.

Inclusion Criteria:

[0439] 1. Age 18 or older. [0440] 2. Diagnosed with end-stage knee osteoarthritis and are scheduled or offered surgery. [0441] 3. X-ray positive for knee osteoarthritis. [0442] 4. Pain and functional disability from osteoarthritis. [0443] 5. Acceptable blood workup results (CBC, electrolytes, kidney and liver function) from up to three months before treatment. [0444] 6. Mentally and physically able to fully comply with the study protocol. [0445] 7. Signed Informed Consent form.

Exclusion Criteria:

[0446] 1. Evidence of active local infection in the vicinity of the knee joint. [0447] 2. Previous surgery of total or partial knee replacement in the injected knee. [0448] 3. Patients unable to provide informed consent due to language barrier or mental status. [0449] 4. Patients with a major medical condition that would affect quality of life and influence the results of the study. [0450] 5. Patients unwilling to be followed for the duration of the study. [0451] 6. Acute infection requiring intravenous antibiotics at the time of screening. [0452] 7. Other limb pain of unknown etiology. [0453] 8. Pain in the limb clinically assessed to arise from an origin which is not the affected knee joint. [0454] 9. Known neurological disease or rheumatic condition other than osteoarthritis. [0455] 10. Bleeding disorders. [0456] 11. Known cognitive disorder. [0457] 12. Concurrent participation in any other clinical study. Participation in an interventional investigational study within 30 days prior to enrollment. [0458] 13. Physician objection. [0459] 14. Positive pregnancy test.

Example 8: Use of Intra-Articular Allocetra in Osteoarthritis of the 1.SUP.st .Carpo-Metacarpal Joint

[0460] Objective: This example describes a randomized, placebo-controlled Phase I/II trial designed to evaluate the safety of Allocetra therapy in subjects suffering from basal thumb joint osteoarthritis (osteoarthritis of the 1.sup.st carpometacarpal joint).

[0461] Methods: A total of up to 56 subjects were and are planned to be enrolled and treated, in two stages: (1) a safety run-in stage in which 16 patients were enrolled and treated in two Allocetra

dose cohorts, each receiving a single injection of Allocetra (as prepared in Examples 1 and 5) into the basal thumb joint (8 patients/cohort; 25×10.sup.6-50×10.sup.6 cells), following which Allocetra dose for the randomization stage was to be determined; (2) a double-blinded randomized stage of the study, in which up to 40 patients are to be randomized in a 1:1 ratio for treatment with Allocetra at the selected dose or placebo. The main study eligibility criteria are provided below.

[0462] Results: The safety run-in stage was completed and the highest Allocetra dose was selected for the randomization phase. A total of 16 patients were enrolled and treated in the safety run-in stage. Patients had symptomatic basal thumb joint OA with radiographic classification of Kelgren-Lawrence grade 2-4, with pain at baseline average 6.3. Patients were treated with an intrarticular (IA) injection of Allocetra to the basal thumb joint on Day 0, and went on to report pain and functional questionnaires at the follow-up. The FIHOA (Functional Index of Hand Osteoarthritis) is a 10-item questionnaire to assess the functional capacity of hands in OA-affected patients. The Quick Disabilities of the Arm, Shoulder, and Hand (DASH) questionnaire is an 11-item questionnaire that measures upper-extremity specific symptoms and disability.

[0463] Patients reported transient events of discomfort/pain or swelling in the thumb joint following injection, which were conservatively managed and with no observed dose relationship of injection reactions, allowing the study to proceed to the randomized stage of the study with the highest Allocetra dose tested.

[0464] Interim results of efficacy are also available. To date, patients completed at least 3 months of follow-up, with 9/15 responders demonstrating an average improvement of 58.2% in the reported pain (range 33.3-100% improvement in responding patients), compared to baseline. Overall, patients demonstrated an average 36% improvement in the reported pain, compared to baseline reported by NRS.

[0465] These results were also supported by the FIHOA and QuickDASH questionnaire scores reported at 3 months, reflecting improvement in both pain and functional capacity. The second stage of the study is a double-blind, randomized, placebo-controlled stage, which was initiated following the selection of the safe and tolerable dose from the safety run-in stage. Up to Feb. 10, 2025, 4 patients were treated in the randomized phase of this study at the time of the submission of this meeting request, with enrollment ongoing. No SAEs were reported in this study.

Inclusion Criteria:

[0466] Subjects eligible for this clinical study must fulfill all of the following: [0467] 1. Age 40 years or older. [0468] 2. Patients with OA of the first CMC joint of the target thumb (basal thumb joint) who have failed conventional therapies, with pain assessed when not taking analgesic medications. [0469] 3. Score of 6 or higher on the Functional Index for Hand Osteoarthritis (FIHOA). [0470] 4. X-ray confirming OA of the first CMC joint of the target thumb with a Grade of 2 or 3 according to Eaton classification. [0471] 5. Blood tests from up to three months before treatment within protocol-defined limits.

Exclusion Criteria:

[0472] Subjects not eligible for this study include those that have any of the following: [0473] 1. Any significant injury, fracture, surgery, active local infection, deformity, severe Carpal Tunnel Syndrome (CTS), DeQuervain's tenosynovitis, trigger finger, or a ganglion cyst of the target hand. [0474] 2. History of chondrocalcinosis in the target joint, concomitant rheumatic disease. [0475] 3. Previous intra-articular injection of steroid, hyaluronate, or other agent, into the target joint within 3 months prior to screening visit. [0476] 4. Other limb pain of unknown etiology, or clinically significant widespread pain syndrome, e.g., fibromyalgia. [0477] 5. Pain in the limb clinically assessed to arise from an origin which is not the affected thumb joint (wrist pain, shoulder pain, etc.). [0478] 6. Secondary OA such as gout, hemochromatosis, rheumatoid/psoriatic arthritis. [0479] 7. Bleeding disorders, cognitive disorder, neurologic disease or other major medical condition which may interfere with study participation, treatment, assessments, or results. [0480] 8. For women of childbearing potential, a positive pregnancy test.

[0481] As used herein, the singular form “a”, “an” and “the” include plural references unless the context clearly dictates otherwise.
[0482] Throughout this application, various embodiments of this invention may be presented in a range format. It should be understood that the description in range format is merely for convenience and brevity and should not be construed as an inflexible limitation on the scope of the invention. Accordingly, the description of a range should be considered to have specifically disclosed all the possible sub ranges as well as individual numerical values within that range. For example, description of a range such as from 1 to 6 should be considered to have specifically disclosed sub ranges such as from 1 to 3, from 1 to 4, from 1 to 5, from 2 to 4, from 2 to 6, from 3 to 6 etc., as well as individual numbers within that range, for example, 1, 2, 3, 4, 5, and 6. This applies regardless of the breadth of the range.
[0483] Whenever a numerical range is indicated herein, it is meant to include any cited numeral (fractional or integral) within the indicated range. The phrases “ranging/ranges between” a first indicate number and a second indicate number and “ranging/ranges from” a first indicate number “to” a second indicate number are used herein interchangeably and are meant to include the first and second indicated numbers and all the fractional and integral numerals there between.
[0484] While certain features have been illustrated and described herein, many modifications, substitutions, changes, and equivalents will now occur to those of ordinary skill in the art. It is, therefore, to be understood that the appended claims are intended to cover all such modifications and changes as fall within the true spirit of the methods of use presented herein.

Claims

1. A method of treating knee osteoarthritis in a subject in need thereof, comprising the step of administering a composition comprising an early apoptotic cell population directly into a knee joint of said subject, thereby treating knee osteoarthritis in said subject.
2. The method of claim 1, wherein the osteoarthritis comprises moderate to severe osteoarthritis or end stage knee osteoarthritis.
3. The method of claim 1, wherein said method results in pain reduction, reduction of inflammation, reduction of swelling, inhibition of progressive degeneration of articular cartilage, reduction of progressive degeneration of articular cartilage, improving quality of life, or any combination thereof.
4. The method of claim 1, wherein said method results in increased range of movement of said knee joint, or increased movement of said joint with reduced pain, or a combination thereof.
5. The method of claim 1, wherein said early apoptotic cell population comprises an autologous early apoptotic cell population or an allogeneic early apoptotic cell population.
6. The method of claim 1, wherein said early apoptotic cell population comprises an irradiated population of early apoptotic cells.
7. The method of claim 1, wherein said early apoptotic cell population comprises a pooled population of early apoptotic cells.
8. The method of claim 7, wherein said pooled early apoptotic cell population comprises an irradiated, pooled population of early apoptotic cells.
9. The method of claim 7, wherein said pooled early apoptotic cell population comprises apoptotic cells prepared from mononuclear cells derived from a single donor or from multiple donors.
10. The method of claim 1, wherein said administering comprises a single administration or multiple administrations of said early apoptotic cell population.
11. A method of treating a finger joint osteoarthritis in a subject in need thereof, comprising the step of administering a composition comprising an early apoptotic cell population directly into a finger joint of said subject, thereby treating a finger joint osteoarthritis in said subject.
12. The method of claim 11, wherein the finger joint osteoarthritis comprises a thumb joint

osteoarthritis or a basal thumb joint osteoarthritis.

13. The method of claim 11, wherein said method results in pain reduction, reduction of inflammation, reduction of swelling, inhibition of progressive degeneration of articular cartilage, reduction of progressive degeneration of articular cartilage, improving quality of life, or any combination thereof.

14. The method of claim 11, wherein said method results in increased range of movement of said finger joint, or increased movement of said finger joint with reduced pain, or a combination thereof.

15. The method of claim 11, wherein said early apoptotic cell population comprises an autologous early apoptotic cell population or an allogeneic early apoptotic cell population.

16. The method of claim 11, wherein said early apoptotic cell population comprises an irradiated population of early apoptotic cells.

17. The method of claim 11, wherein said early apoptotic cell population comprises a pooled population of early apoptotic cells.

18. The method of claim 17, wherein said pooled early apoptotic cell population comprises an irradiated, pooled population of early apoptotic cells.

19. The method of claim 17, wherein said pooled early apoptotic cell population comprises apoptotic cells prepared from mononuclear cells derived from a single donor or from multiple donors.

20. The method of claim 11, wherein said administering comprises a single administration or multiple administrations of said early apoptotic cell population.