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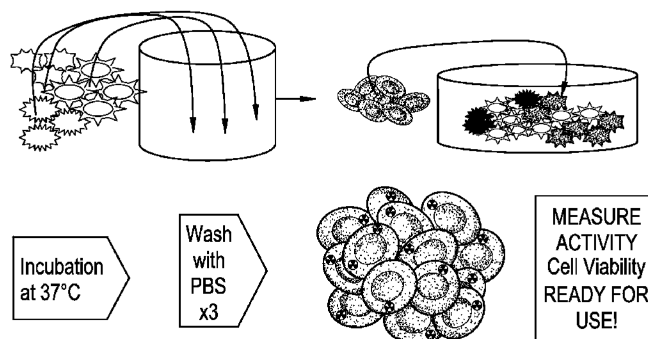
[Continued on next page]

(54) Title: ZIRCONIUM-89 COMPLEXES, METHODS OF LABELING CELLS, LABELED CELLS, KITS, AND METHODS OF USE THEREOF

FIG. 1



(57) Abstract: Described herein are ^{89}Zr PET imaging complexes, methods of labeling biological cells with ^{89}Zr, and cells labeled with ^{89}Zr. Also described are kits for labeling of cells with ^{89}Zr. The labeled cells can be administered to a subject and then imaged using PET imaging.

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ZIRCONIUM-89 COMPLEXES, METHODS OF LABELING CELLS, LABELED CELLS, KITS, AND METHODS OF USE THEREOF

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] The present application claims the benefit of U.S. Provisional Application Serial No. 61/611,964, which is incorporated by reference in its entirety.

STATEMENT OF GOVERNMENT SUPPORT

[0002] This invention was made in part with government support from the National Institutes of Health. The government has certain rights in this invention.

FIELD OF THE DISCLOSURE

[0003] This disclosure relates to zirconium-89 complexes, methods of labeling biological cells with the complexes, zirconium-89-labeled biological cells, and kits for the labeling of biological cells.

BACKGROUND

[0004] The advent of molecular imaging approaches such as Positron Emission Tomography (PET) has enabled the measurement of molecular and cellular mechanisms throughout the body in preclinical and clinical settings. Such measurements have widespread diagnostic utility, and their use for evaluation of treatment responses and to assist in drug development, is expanding rapidly. Cell based therapies have become an important method of treating inflammatory, autoimmune and neoplastic disease. These therapies include stem cell transplants, injecting stimulated natural killer or T-lymphocytes, chimeric antigen receptor (CAR)-engineered lymphocytes for cancer therapy, and highly engineered dendritic cells to induce immune mediated responses. Numerous questions remain regarding the viability and biology; including survival, trafficking and homing to bone marrow or tumor, of such injected cells in the living subject. A problem common to all cell-based strategies involving either administration of autologous or allogeneic cells is to identify and monitor the cells in the host to better understand the mode of action of the therapy and, ultimately, to improve upon it.

[0005] In one method, called indium leukocyte imaging, white blood cells are removed from a patient, labeled with indium-111, injected into the patient, and the labeled cells are imaged by single-photon emission computed tomography (SPECT). The labeled

leukocytes can be used to localize areas of new infection or to differentiate conditions such as osteomyelitis. A disadvantage of indium-111 SPECT analysis is that indium is a single photon emitter, which is an inefficient process, thus large amounts of radioactivity, often higher than 1 mCi, are required. Moreover, SPECT imaging has inherently low spatial resolution and the SPECT cameras are poorly adapted to the energies of the gamma rays emitted by indium-111 decay.

[0006] Unlike SPECT imaging, PET imaging detects a pair of gamma rays emitted by a positron-emitting radionuclide after the positron interacts with an electron. Because two gamma rays moving in opposite directions are detected, less radioactivity may be required than, for example, in a SPECT experiment in which single photon emission is detected. In certain cases amounts of radioactivity that are 10-fold or less than a in a SPECT experiment can be used in PET imaging. Moreover, resolution is improved compared to SPECT. While ^{18}F-imaging has proved useful for PET imaging, this isotope is limited because of its relatively short half-life of 110 minutes, which limits long-term monitoring of cells which typically require days to track. Thus, improved cell labeling is needed for in vivo and in vitro imaging of cell-based therapies.

SUMMARY

[0007] In an aspect, a PET imaging complex comprises a first component that is ^{89}Zr; a second component that is a polycation; and an optional third component that is an anionic polysaccharide.

[0008] In another aspect, a kit for labeling biological cells for PET imaging comprises a first component that is ^{89}Zr; a second component that is a polycation; and an optional third component that is an anionic polysaccharide.

[0009] In a third aspect, included is a biological cell labeled with ^{89}Zr, wherein the ^{89}Zr is not linked to an antibody or a drug molecule.

[0010] In another aspect, a method of labeling a biological cell with the PET imaging complex described above comprises contacting a biological cell in culture medium with the first, second and optionally the third components, wherein the PET imaging complexes are formed in the culture medium, or contacting a biological cell in culture medium with pre-formed PET imaging complexes, wherein contacting is done under conditions to label the biological cell with complexes.

[0011] In yet another aspect, a method of detecting a biological cell in a subject, comprises administering to the subject a labeled biological cell comprising PET imaging

complexes, and examining at least a portion of the subject by PET imaging, thereby detecting the labeled biological cell in the subject.

[0012] In a further aspect, a method of transplanting a biological cell into a subject comprises administering to the subject a labeled biological cell comprising the PET imaging complexes, examining at least a portion of the subject by PET imaging, detecting the migration pattern and/or cellular distribution pattern of the labeled biological cell in the subject, and optionally administering additional biological cells over a period of hours to days.

[0013] In a still further aspect, a method of treating a subject with a disease or injury comprises administering to the subject with the disease or injury a labeled biological cell comprising the PET imaging complexes described herein, examining at least a portion of the subject by PET imaging, detecting the migration pattern and/or cellular distribution pattern of the labeled biological cell in the subject, and optionally administering additional biological cells.

DESCRIPTION OF THE DRAWING

[0014] Figure 1 is a schematic of ^{89}Zr labeling of biological cells as described herein.

[0015] Figure 2 shows an assessment of Annexin V, TRAIL and NKG2D antibody binding by flow cytometry analysis (FACS) analysis on radiolabeled (^{89}Zr-H-PS) and non-radiolabeled (HPS treated) NK cells. A) Apoptosis in NK cells was determined through Annexin V- FITC staining by flow cytometry at 2 hours post labeling. The percentage of apoptotic (Annexin V positive) and live cells is shown. B) The left-most histogram in each panel represents cells stained by the corresponding isotype control antibody, and the right-most histograms in each panel represent non-radiolabeled (H-P treated) and blue indicates ^{89}Zr-H-PS treated NK cells.

[0016] Figure 3 shows results from cytotoxic function of radiolabeled (^{89}Zr-H-PS) or nonradiolabeled (H-P treated) NK cells on K562 cells in vitro. A) The percentage of apoptotic (Annexin V positive) and live K562 cells is shown at 24, 48, 72 hrs. B) At a 1:1 NK (effector) to K562 (target) cells ratio, the percentage of lysis of K562 cells by radiolabeled or non-radiolabeled NK cells is shown at 24, 48, 72 hrs post labeling.

[0017] Figure 4 is a PET scan of a whole pig injected with ^{89}Zr complexes by bolus intravenous injection.

[0018] Figure 5 is a PET scan of a whole pig injected with pig autologous granulocytes labeled with ^{89}Zr PET imaging complexes.

[0019] Figure 6 is a PET scan of a whole pig treated with a bolus intra-bone infusion of the ^{89}Zr labeled human CD34 positive cells. Panel A is a whole body image and panel B shows injection site images.

[0020] Figure 7 is a PET scan of a whole pig treated with a slow intra-bone infusion of the ^{89}Zr labeled human CD34 positive cells. Panel A is a whole body image and panel B shows injection site images.

[0021] Figure 8 shows the signal intensity loss on T2*WI sequences for USPIO (ultrasmall particle of iron oxide such as FeraHeme®) -PS-H treated cells when compared to the USPIO-PS treated cells in dual labeling method.

[0022] Figure 9 shows MR and PET Imaging of USPIO/^{89}Zr-labeled cells.

[0023] The above-described and other features will be appreciated and understood by those skilled in the art from the following detailed description, drawings, and appended claims.

DETAILED DESCRIPTION

[0024] Described herein are ^{89}Zr PET-imaging complexes, methods of labeling biological cells with the complexes, ^{89}Zr-labeled biological cells, and kits for ^{89}Zr-labeling of biological cells. The ^{89}Zr PET-imaging complexes comprise ^{89}Zr, a polycation, and an optional anionic polysaccharide. Cells are labeled by contacting them either with pre-formed complexes, or by contacting the cells with the components of the complexes, which self-assemble and allow the ^{89}Zr to be taken up into the cell. In an embodiment, the cells are further contacted with an MR imaging agent such as a superparamagnetic nanoparticle to allow for PET-MR imaging.

[0025] While ^{89}Zr-labeling has been used previously to image cells, the ^{89}Zr in the prior art methods was conjugated either to an antibody or a drug. This type of labeling is limited by the requirement for the antibody or drug to bind to a receptor, typically on the surface of the cell. Advantageously, in the present methods, the ^{89}Zr is not complexed with an antibody or drug molecule, either covalently or noncovalently. Instead of requiring direct binding, the ^{89}Zr-labeled complexes are taken up nonspecifically into the cells, likely into endosomes, leading to a higher density of ^{89}Zr in the cells than can be achieved with labeled antibodies. Thus, fewer cells are needed to produce an image and the technique is not limited to the available antibodies.

[0026] The specific results shown in the examples herein reveal that white blood cells (WBC) and natural killer (NK) cells can be labeled in vitro with ^{89}Zr. Labeling was

optimized when the cells were incubated for a minimum of 30 minutes with the ^{89}Zr -H-PS mixture at 37°C in the absence of plasma and under pH conditions of 6.5-7.2, however, other conditions can also be employed. The elution of ^{89}Zr as measured by free ^{89}Zr was increased when cells were exposed to an acidic solution causing cell death or in the presence of plasma within the cell media. Without being held to theory, the retention of ^{89}Zr-HPS complex was 6-8 times higher than ^{89}Zr alone indicating a likely role of endocytosis.

[0027] As used herein, PET imaging also includes PET/CT imaging, positron emission tomography/computed tomography. There is increasing interest in using new radiotracers (especially long-lived PET radiotracers) to monitor the in vivo behavior of transplanted cells. The choice of the positron emitter is an important factor for a successful cell labeling procedure with PET. Only two long-lived positron emitters seem well suited for imaging cells, namely ^{89}Zr (t1/2=78.4 h) and ^{124}I (t1/2=100.3 h). ^{89}Zr can be obtained in high yield and with high radionuclide purity, which makes it an attractive target material because of its 100% natural abundance. As a result, production costs are relatively low. Moreover, ^{89}Zr has no incidental emissions of gamma rays that can hamper image quality and accurate quantification. Recently, a large-scale production of radionuclidically pure ^{89}Zr (specific activity ≥0.15 GBq nmol^{-1}) with a small cyclotron (Y^{89}-target; E_p 14 MeV) was achieved. Thus, ^{89}Zr is suitable and is a very attractive radiotracer for PET imaging of cells.

[0028] Advantages of ^{89}Zr as a label for cells include the use of a desirable PET imaging agent with a suitably long half-life for extended cell tracking; the ability to take advantage of the inherent advantages of PET emitters in terms of sensitivity and spatial resolution; the techniques require low doses of radiation to be effective; a wide variety of cells can be labeled, including cells of lymphocytic lineage including T cells, natural killer T-cells, macrophages, dendritic cells, and stem cells with minimal damage to the cells; and the PET imaging agent can be readily combined with an MR imaging agent for combined PET/MR imaging of cells.

[0029] The ^{89}Zr PET-imaging complexes described herein comprise ^{89}Zr and a polycation. ^{89}Zr is produced, for example, via an ^{89}Y(p,n)^{89}Zr or ^{89}Y(d,2n)^{89}Zr reaction. In a ^{89}Y(p,n)^{89}Zr reaction, a proton bean with about 14 MeV energy is used to bombard a target such as a yttrium foil mounted onto an aluminum/copper disk. In a ^{89}Y(d,2n)^{89}Zr reaction, about a 14 MeV deuteron beam is used to irradiate a yttrium pellet. The ^{89}Zr is liberated from the target, typically in an acidic solution such as an oxalic acid solution. Thus, in an embodiment, the ^{89}Zr is provided as an ^{89}Zr-oxalic acid solution.

[0030] The ^{89}Zr PET-imaging complexes comprise a polycation. Without being held to theory, it is believed that the polycation acts as a transfection agent to facilitate uptake of the complexes into cells. The polycation is an organic polycation having a molecular weight of about 300 to about 200,000 and a valence of about 3 to about 1000 at pH 7.0. The polycation includes natural or synthetic amino acids, peptides, proteins, or polyamines. Nonlimiting examples of polycations include polyarginine, polyornithine, protamines, polylysine, histone, lipofectamine, spermine, and spermidine. In an embodiment, the polycation is a polycationic polypeptide having an amino acid composition in which arginine residues comprise at least 30% of the amino acid residues of the polypeptide. In an embodiment, the polycation is a protamine, specifically protamine sulfate.

[0031] The ^{89}Zr PET-imaging complexes optionally further comprise an anionic polysaccharide, that is, a polysaccharide containing a carboxyl, sulfuryl, phosphoryl, or other group containing an oxygen capable of ionization to a negatively-charged oxygen. The acid form or salt form of the anionic polysaccharide can be used. An example of an anionic polysaccharide is an anionic glycosoaminoglycan. Exemplary glycosoaminoglycans include heparin, heparan sulfate, chondroitin sulfate, dermatan sulfate, keratan sulfate, hyaluronic acid (hyaluronan), and combinations thereof. Glycosaminoglycans are linear unbranched polysaccharide chains comprising a repeating disaccharide unit that typically comprise hexosamine and a hexose or a hexuronic acid linked together by a glycosidic bond. The glycosaminoglycan may be naturally occurring or synthetically produced. Glycosaminoglycans form a major component of the extracellular matrix and of glycoproteins typically found on the surface of cells, and are the most abundant heteropolysaccharides in the body. Often they are attached to proteins to form proteoglycans. Their diverse functions in the cell include, for example, providing structural rigidity that is needed for cell migration, contributing to the viscosity of the fluid of which they are a part, and ligand-receptor recognition. In an embodiment, the anionic polysaccharide is heparin.

[0032] The ^{89}Zr PET-imaging complexes are formed by contacting ^{89}Zr, a polycation and an anionic polysaccharide under conditions suitable to form a complex. For example, 10 μCi to 1 mCi or more of ^{89}Zr (e.g., 50 to 100 μCi of ^{89}Zr) is combined with 20 to 100 μg/mL of polycation such as protamine sulfate (e.g., 40 μg/mL) and optionally 0.5 to 5 U/mL of anionic polysaccharide such as heparin (e.g., 2 U/mL).

[0033] In a specific embodiment, the ratio of the ^{89}Zr to the polycation to the anionic polysaccharide is 50/40/2 μCi/μg/U/ml:100/40/2 μCi/μg/U/ml:50/40 μCi/μg/ml

[0034] While protamine and heparin have been used previously to form complexes with iron oxide particles such as ferumoxytol, there was no expectation that such complexes could be formed with ^{89}Zr and that the complexes would readily be taken up by cells. Ferumoxytol is a superparamagnetic magnetite (Fe_3O_4) nanoparticle associated with a low molecular weight semi-synthetic carbohydrate, polyglucose sorbitol carboxymethyl ether. ^{89}Zr, in contrast, is a radioactive metal in solution, not an oxide nanoparticle in solution and has no magnetic or paramagnetic properties. Thus, the prior work with ferumoxytol provides no expectation of the excellent results obtained with ^{89}Zr.

[0035] In addition, given that labeling of antibodies with ^{89}Zr generally proceeds in the presence of a chelator such as deferoxamine mesylate, one might expect the ^{89}Zr-polycation complexes and subsequent labeling of cells to proceed better in the presence of a chelator for the ^{89}Zr. However, it was found that the use of a chelator such as deferoxamine mesylate actually inhibits the uptake of the ^{89}Zr-complexes into the cells. Advantageously, it has been found that washing cells with a chelator after ^{89}Zr-labeling can be used to remove any ^{89}Zr that was not taken up by the cells, thus reducing the exposure of the cells and the patient to the radioactivity.

[0036] In an embodiment, the ^{89}Zr PET-imaging complexes are used to label biological cells, particularly mammalian cells. Figure 1 shows a schematic of labeling cells with ^{89}Zr PET-imaging complexes. Also included herein is a biological cell labeled with ^{89}Zr. The term "biological cell' as used herein, refers to a cellular structure having biological functionality including, but not limited to, endocytosis and/or extracellular ligand binding sites. A biological cell may be naturally occurring or synthetic and is preferably viable. In an embodiment, a biological cell is a leukocyte or white blood cell. Such biological cells may be a stem cell including, but not limited to, bone marrow-derived stem cells such as bone marrow stromal cells, embryonic stem cells, adult stem cells, hemopoeitic stem cells, mesenchymal stem cells, epidermal stem cells, endothelial stem cells, endothelial progenitor cells, resident cardiac stem cells, induced pluripotent stem cells, adipose-derived stem cells, amniotic fluid stem cells, uterine stem cells, neural stem cells, neural progenitor cells, cancer stem cells (i.e., for example, a leukemic hemopoeitic stem cell, solid tumor stem cells), umbilical cord blood stem cells, or skeletal myoblasts. Alternatively, biological cells include, but are not limited to, brain cells, liver cells, muscle cells, nerve cells, chondrocytes, lymphocytes, intestinal cells, pancreatic cells, liver cells, heart cells, lung cells, colon cells, bladder cells, uterine cells, prostate cells, urethra cells, testicular cells, immune cells (T-cells, B-cells, monocytes, dendritic cells, tissue macrophages, and the like), and/or epithelial cells.

Further, a biological cell may be a cancerous cell, for example, a breast cancer cell. In an embodiment, the biological cell is genetically engineered in order to stay immortalized or is a genetically altered vector-producing cell, expressing particular therapeutic genes that activate the inactive prodrugs to toxic drugs or that deliver growth factors or antibodies to the targeted locations.

[0037] In an embodiment, the biological cell is in cell culture, that is, the cells are *ex-vivo*. When in cell culture, the biological cell is typically in a vessel such as a culture dish that contains a nutrient broth called a culture medium.

[0038] A specific type of cell to be labeled with ^{89}Zr is a leukocyte or a white blood cell. In comparison, antibody labeling methods require the presence of a specific receptor, a high affinity, specific antibody and uptake of the ^{89}Zr depends on the number of such receptors. Since only a minority of receptors can be occupied by the antibody and not all antibody-receptor interactions lead to internalization of the antibody, this is a relatively inefficient process compared to direct endocytosis of the ^{89}Zr. In addition, previous methods of ^{89}Zr- labeling using antibodies are disadvantageous because most antibodies are non-human or chimeric proteins that can induce allergic responses or induce host immune reactivity. In an embodiment, the white blood cells of a patient are labeled with ^{89}Zr, then after a time, for example 6-24 hours, the patient is scanned using PET imaging and the labeled cells are detected. The labeled cells travel to areas of tumor inflammation or infection thus allowing the identification of these areas. Such techniques are particular useful in the detection of osteomyelitis, an infection of the bone or bone marrow.

[0039] In another embodiment, a cord-blood sample including cord-blood cells is ^{89}Zr-labeled as described herein. Hematopoietic stem cells are parent cells that give rise to all of the cellular elements in the blood. They also have the ability to self-renew. In adult life, they usually reside only in the bone marrow and can be made to enter into the blood stream by administration of mobilizing drugs. They are also present in cord blood at the time of birth. Hematopoietic stem cell transplantation is a life-saving therapy for many malignant and non-malignant diseases of the blood and bone marrow. At present, sources of hematopoietic stem cells used in transplantation may be obtained from bone marrow, mobilized into peripheral blood or from cord blood. Traditionally these graft sources are administered to a recipient, after the appropriate conditioning, via infusion into a peripheral vein. The stem cells then circulate and make their way to the bone marrow (trafficking and homing) where the environment exists for them to survive and replicate and produce all the cellular elements of the blood. Many patients in need of a stem cell transplantation are

unable to obtain an HLA (human leukocyte antigen) matched related or unrelated donor. The advantage of cord blood as an alternative hematopoietic stem cell source is that a recipient can tolerate a greater degree of HLA mismatch than with bone marrow or peripheral blood stem cells and thus there is a greater probability of finding a suitable cord blood graft than a matched unrelated donor. The disadvantage with cord blood is that there are 100 times less stem cells in the product than that which can be obtained from peripheral blood stem cells. This creates problems such as a higher risk of graft failure (the graft not taking) and a longer time to engraftment (hematopoietic reconstitution) placing the recipient at risk of life threatening infection.

[0040] Cord blood is used as a hematopoietic stem cell source for allogeneic stem cell transplantation in individuals who do not have an HLA matched sibling or unrelated donor available. One of the problems with cord blood stem cell transplantation is delayed or failed engraftment relative to peripheral blood hematopoietic stem cell (HSC) transplantation, due to the lower mononuclear cell dose available in the cord blood unit. Graft versus host disease occurs when the stem cell graft produces immune competent white blood cells that recognize the recipient tissues as foreign and attacks them. This finding has important applicability for transplantation of all sources of stem cells since graft versus host disease is the major contributor to mortality and morbidity following stem cell transplantation. The immediate fate of stem cells injected into the bone marrow is not known. The hypothesis is that they are retained within the marrow overcoming attrition of stem cells in other organs such as the lungs, liver, and spleen where they are lost after administration via a peripheral vein. However, there is compelling circumstantial evidence to suggest that they will eventually enter the circulation just as in intravenous administration. The trafficking of stem cells after intra bone injection needs to be studied, the procedure needs to be optimized and the safety established so that there can be wider adoption in human stem cell transplantation.

[0041] Animal experimental models of human HSC xenotransplantation in immunodeficient mice suggest that transplantation via intra-bone delivery of cord blood improves engraftment compared with intravenous cord blood transplantation alone. In human clinical trials, intra bone transplantation of cord blood demonstrates improved platelet engraftment compared with standard intravenous cord blood infusion and may reduce the risk of severe acute graft versus host disease. Abrogation of graft versus host disease is also shown in a murine allogeneic transplantation model in favor of intra bone compared with intravenous donor lymphocyte injections. The optimal method of intra bone injection for the purpose of HSC transplantation is not known. When tracking was evaluated in the single

human trial, single bilateral injections were utilized. In the ongoing trial of intra bone cord blood transplantation in Italy, 4 aliquots of approximately 5 mL of cord blood were injected each into 4 different pelvic bone access points. No preclinical studies were reported by this group to optimize the retention in the marrow cavity of intra bone delivered cord blood cells.

[0042] The intraosseus (or intra bone) route is an established route for vascular access in trauma and hypovolemic shock situations when peripheral or central venous access cannot be obtained. Infusion of crystalloid or red cells via the intra bone route achieves systemic distribution and can restore hemodynamic parameters as effectively as intravenous infusion alone. A single human experiment showed that Tc-99m labeled bone marrow cells infused via the intravenous and intra bone routes resulted in cardiac enhancement at the same time period. The bone marrow thus behaves as a vascular bed. Although these studies did not determine the fate of nucleated hematopoietic cells, they raise the concern that cells injected directly into the bone marrow space may not necessarily be retained in the bone marrow. Furthermore, complications can be associated with intra bone injections including pain and fat embolization to the lung. Nonetheless, the clinical outcome in the single human trial ongoing in Italy investigating intra bone marrow delivery of cord blood hematopoietic stem cells has shown favorable differences compared to traditional IV cord blood transplantation including an improvement in platelet engraftment and a reduction in severe acute graft versus host disease without any reported procedural complications. To date, there have been no large animal experiments reported studying this phenomenon.

[0043] The compositions and methods described herein are particularly useful for the ^{89}Zr-labeling of cord blood cells. Once they are labeled, the cord-blood cells are injected into the bone marrow of a patient, such as a patient with acute lymphoblastic leukemia in remission. In an embodiment, the injection is an intrabone injection. An advantage of labeling the cells and PET imaging as the cells are injected is that trafficking and homing of hematopoeitic progenitor cells can be studied during alternate, i.e., intrabone route of delivery and monitoring for escape of cells from the bone marrow into the muscles surrounding the bone.

[0044] In an embodiment, biological cells in culture are labeled by contacting the biological cells with a solution/suspension containing ^{89}Zr PET-imaging complexes in an amount and for a time sufficient to label the cells with ^{89}Zr. In another embodiment, the biological cells in culture are contacted with a solution/suspension(s) including the individual components of the nanoparticles in an amount and for a time sufficient to label the biological cells with ^{89}Zr. Culture medium is defined as a liquid that covers biological cells in a culture

dish and that contains nutrients to nourish and support the cells. Culture medium may include growth factors and other additives to produce desired changes in the cells. In an embodiment, the culture medium is a serum-free medium, that is, a medium that contains animal-free recombinant ingredients. Serum-free media include, for example, water, sugars, salts, albumin, insulin, transferrin, growth factors, and other optional additives that improve cell growth. Advantages of serum-free media include simplified purification of cells, batch-to-batch consistency of media, and the reduction of the risk of introducing unwanted agents. An exemplary serum-free medium is RPMI medium, which contains a bicarbonate buffering system and varying amounts of amino acids and vitamins depending upon the type of cultured cell.

[0045] In an embodiment, a method of labeling a biological cell with ^{89}Zr PET-imaging complexes comprises contacting a biological cell in culture medium with ^{89}Zr PET-imaging complexes, wherein the ^{89}Zr PET-imaging complexes are formed in the culture medium that is either mixed with growing cells or the ^{89}Zr PET-imaging complex components can be premixed, lyophilized and then placed on cells expanding in culture immediately or at later time.

[0046] In another embodiment, a method of labeling a biological cell with ^{89}Zr PET-imaging complexes comprises contacting a biological cell in culture medium with ^{89}Zr, a polycation and optionally an anionic polysaccharide, wherein the ^{89}Zr PET-imaging complexes are formed in the culture medium. As used herein, labeling of a cell with ^{89}Zr PET-imaging complexes means that the complex is internalized into the cell or associated with the cell such that the complex and thus the cell can be detected such as by PET imaging.

[0047] In another embodiment, ^{89}Zr PET-imaging complexes are pre-assembled before adding them to the cells. In specific embodiments, the ^{89}Zr PET-imaging complexes are formed by premixing the three components (e.g., heparin, protamine sulfate, and ^{89}Zr) together in sterile cell media or PBS and 1) lyophilizing the complexes or 2) incubating complexes at room temperature allowing complexes to form and letting them settle, which is followed by filtration. The ^{89}Zr PET-imaging complexes can be dried and then reconstituted in sterile serum free culture media for later labeling of the cells at the time of need.

[0048] In an embodiment, labeling of a biological cell in culture with ^{89}Zr PET-imaging complexes comprises internalization of the ^{89}Zr PET-imaging complexes into endosomes. Endocytosis of the ^{89}Zr PET-imaging complexes into endosomes allows for subsequent imaging of the endosomes by PET imaging, for example. In an embodiment, cultured biological cells labeled with ^{89}Zr PET-imaging complexes endocytose and

encapsulate the ^{89}Zr PET-imaging complexes into endosomes that are visible by PET imaging.

[0049] In an embodiment, after labeling with ^{89}Zr, the cells are washed with a solution containing a chelator such as deferoxamine mesylate to remove any free ^{89}Zr. Additional chelators include Deferasirox, a newer iron chelating medication that comes in a tablet form, and hydroxyethyl starch deferoxamine (HES-deferoxamine). The chemical attachment of deferoxamine to a hydroxyethyl starch polymer creates a high-molecular-weight chelator with affinity for iron identical to, but a vascular half-life 10 to 30 times longer than, that of standard deferoxamine.

[0050] In an embodiment, the ^{89}Zr PET-imaging complexes further comprise a binding moiety that specifically binds to a cell-surface receptor or antigen. Such a binding moiety will allow the ^{89}Zr PET-imaging complex to bind the surface of the cell where it is stably bound or subsequently internalized into the cell.

[0051] In an embodiment, included herein is a kit for the labeling of biological cells. The kit comprises ^{89}Zr, a polycation, and optionally an anionic polysaccharide. In an embodiment, a kit includes a first container comprising ^{89}Zr, and a second container comprising a polycation. The container containing the polycation optionally also includes an anionic polysaccharide. Alternatively, the kit optionally includes a third container comprising the anionic polysaccharide. In an embodiment, a kit further comprises a superparamagnetic nanoparticle.

[0052] In an embodiment, the ^{89}Zr-labeled cells are administered to a subject and then the labeled cells are imaged by PET. For use in a therapeutic regimen, methods of administration/delivery of cells include injections and use of special devices to implant cells in various organs. The present disclosure is not limited to any particular delivery method. For example, labeled cells can be imaged following either a focal implantation directly into tissues, subcutaneously, subdermally, or by intravenous injection. Exemplary injection techniques include intravenous, intra-arterial, intraperitoneal and/or direct tissue injection including dermal and subdermal. Cells may be inserted into a delivery device that facilitates introduction by injection or implantation into the subjects. Such delivery devices include tubes, e.g., catheters, for injecting cells and fluids into the body of a recipient subject. In an embodiment, the tubes additionally have a needle, e.g., a syringe, through which the cells can be introduced into the subject at a desired location. The cells may be prepared for delivery in a variety of different forms. For example, the cells may be suspended in a solution or gel or embedded in a support matrix when contained in such a delivery device. Cells may be mixed

with a pharmaceutically acceptable carrier or diluent in which the cells remain viable. Pharmaceutically acceptable carriers and diluents include saline, aqueous buffer solutions, solvents, and/or dispersion media. The use of such carriers and diluents is well known in the art. The solution is preferably sterile and fluid. In specific embodiments, the solution is stable under the conditions of manufacture and storage and preserved against the contaminating action of microorganisms such as bacteria and fungi by use of preservatives. Solutions may be prepared by incorporating cells as described herein in a pharmaceutically acceptable carrier or diluent and, as required, other ingredients enumerated above, followed by filtered sterilization.

[0053] In another embodiment, a ^{89}Zr-labeled cell further comprises an MR imaging agent such as a superparamagentic agent to allow for PET-MRI. An exemplary superparamagnetic agent is a superparamagnetic nanoparticle optionally associated (e.g., coated) with a polymer. Superparamagnetism means a form of magnetism, which appears in small ferromagnetic or ferrimagnetic nanoparticles. Like the paramagnetic materials, the superparamagnetic materials do not maintain their magnetism in the absence of an externally applied magnetic field. Superparamagnetic nanoparticles are particles having at least one dimension of 1 nm to 100 nm and that exhibit superparamagnetic properties. Superparamagnetic nanoparticles include iron oxide, dysprosium oxide, gadolinium oxide, manganese oxide, gold oxide, silver oxide and combinations thereof. Iron oxides include, for example, Fe_3O_4, γ-Fe_2O_3, FeOOH, and α-Fe_2O_3. The nanoparticle may be any shape including sphere, rod, or platelet. An exemplary superparamagnetic agent is FeraHeme®.

[0054] In an embodiment, the superparamagnetic nanoparticle is optionally associated with a polymer. In an embodiment, the polymer substantially coats at least a portion of the nanoparticle. Without being held to theory, it is believed that the polymer can facilitate *in vivo* transport of the nanoparticle throughout a subject, and facilitates uptake and retention of the nanoparticles by tissues and cells. The polymer can be a natural or a synthetic polymer. Exemplary synthetic polymers include poly(acrylic acid) and its derivates, poly(methacrylic acid), poly(ethylmethacrylic acid), poly(butylmethacrylic acid), poly(laurylmethacrylic acid), poly(hydroxyethylmethacrylic acid), poly(hydroxypropylmethacrylic acid), poly(acrylamide), poly(isocyanate), poly(styrene), poly(ethylene imine), poly(siloxane), poly(glutamic acid), poly(aspartic acid), poly(lysine), polypropylene glycol, poly(vinyl alcohol), poly(vinyl pyrrolidone), polyethylene oxide (PEG), derivatives thereof, and combinations thereof. Exemplary natural or semi-synthetic polymers include chitosan, dextran (e.g., carboxymethyl dextran), cellulose, hyaluronic acid, alginate, their carboxymethyl or other derivatives, and

combinations thereof. The polymers can be modified to include functional groups such as carboxymethyl or reduced carboxymethyl groups. The polymers can also be crosslinked or grafted to other polymers. In an embodiment, the polymer is a dextran such as a carboxymethylated dextran. In another embodiment, the polymer is a carboxymethylated dextran polymer. In a specific embodiment, the polymer associated with the superparamagnetic nanoparticle is polyglucose sorbitol carboxymethyl ether.

[0055] In a specific embodiment, the superparamagnetic nanoparticle is ferumoxytol (FeraHeme®), a superparamagnetic magnetite (Fe_3O_4) nanoparticle associated with a low molecular weight semi-synthetic carbohydrate, polyglucose sorbitol carboxymethyl ether, with potential anti-anemic and imaging properties. Ferumoxytol is commercially available as an aqueous colloidal drug. The overall colloidal particle size in the product commercially available from AMAG Pharmaceuticals is 17-31 nm in diameter. The chemical formula of FeraHeme® is $Fe_{5874}O_{8752}$-$C_{11719}H_{18682}O_{9933}Na_{414}$ with an apparent molecular weight of 750 kDa.

[0056] In another embodiment, the superparamagnetic nanoparticle is ferumoxide (Feridex IV®), a non-stoichiometric magnetite associated with dextran.

[0057] In a further embodiment, the superparamagnetic nanoparticle is complexed with a polycation and/or an anionic polysaccharide, such as those described herein. Without being held to theory, it is believed that the anionic polysaccharide and the polycation form a complex with the superparamagnetic nanoparticles that facilitates uptake of the nanoparticles into cells and allows for labeling of cells for MRI. The self-assembling complexes described herein are nontoxic to cells and do not substantially affect cell viability, apoptosis, proliferation, metabolic, functional or differentiation capacity when compared to unlabeled cells.

[0058] In an embodiment, for use as MRI contrast agents, it is preferred that the superparamagnetic complexes have a neutral or positive zeta potential in water and a negative zeta potential in balanced isotonic salt solutions. In an embodiment, the superparamagnetic complexes, such as HPF complexes, have a positive zeta potential of 5 to 25, more specifically 10 to 20 mV in water and/or 0 to -15 mV in isotonic salt solutions.

[0059] The self-assembled superparamagnetic complexes can also be characterized by their physical properties such as size and polydispersion index. In specific embodiments, the HPF complexes have an average size 100 to 500 nm, specifically 125 to 250 nm in sterile water, and a polydispersion index (PDI) in sterile water of 0.01 to 0.3, specifically about <0.1 immediately after complex formation. In specific embodiments, the HPF complexes

aggregate up to an average size of 1 μm in sterile water with a PDI of >0.5 and flocculate out of water, allowing the collection or lyophilization of the pre-formed complexes for future use. In specific embodiments, the HPF complexes have an average size of 100 to 650 nm, specifically 125 to 200 nm in isotonic salt solutions and a PDI of 0.01 to 0.4, specifically about 0.05 to 0.3 and remain suspended in the solutions.

[0060] In a specific embodiment, the ratio of the first to second to third component is H 5 - 14 μg/ml: P 20 μg/ml:F 50 μg/ml to H 75 – 210 μg/ml:P 300 μg/ml:F1000μg/ml in isotonic salt solutions at pH 7.0-7.4. Ordinarily, 1 mg of commercially available heparin of Na salt or Ba salt preparation contains approximately 70-180 IU of heparin. Since heparin, a biological-based molecule is purified from different sources, the molecular weight of heparin is largely dependent on the preparation of the specimens, for example, source, purity, preparation methods, lot number and the company. International Units (IU) of heparins is commonly used in clinic to standardize the drug action and it indicates the required amount of solution to prolong the clotting of 1 ml of whole blood for three minutes. The molecular weight of the heparin is not critical. Low molecular weight heparin, for example, can have an average molecular weight of less than about 8000 Daltons. High molecular weight heparin has a molecular weight of greater than 8000 Daltons, and can be about 15000 Daltons or higher. Similarly, the molecular weight of protamine is not critical. Typically, protamine has a molecular weight of about 5000 Daltons, however, chain shortened versions of protamine are also available and can be used in the complexes described herein. In a an embodiment, the ratio of the heparin to protamine to ferumoxytol (H:P:F) in the complexes is 1-3 IU/ml: 20-90 μg /ml: 50-200 μg/ml (e.g., 2 IU/ml:60 μg/ml:50 μg /ml) or multiples of the ratios up to 10x each product per ml. In a specific embodiment, the ratio is H 1 IU/ml : P 20μg/ml: F of 50-100 μg/ml.

[0061] In an aspect, heparin and protamine when mixed in specific ratios of about 1 molar heparin to about 2 molar protamine self-assemble and form a complex that incorporates the ferumoxytol in solution to form the complex (HPF complex) that has a positive zeta potential or surface charge in sterile water.

[0062] The term “detect” includes imaging to ascertain the presence or absence of a labeled molecule or cell, particularly by a PET technique. In an embodiment, PET, PET/CT, and/or PET-MRI allows the determination of the extent of migration of the ^{89}Zr-labeled cells, whether more cells are needed for repair or replacement of damaged tissue, and/or whether genetically altered vector producing therapeutic cells have entered into their target prior to

initiating treatment. The imaging information obtained will also allow the clinicians to associate the clinical findings and therapeutic index as it relates to the presence of the cells or the mechanisms behind their workings in order to optimize the therapeutic regimes.

[0063] Cells labeled with the complexes described herein will also allow for tracking the migration of ^{89}Zr-labeled stem cells and other hematopoeitic cells in the body by a non-invasive PET and/or PET-MRI techniques. Cell therapies administered by intravenous, intra-arterial, and/or direct tissue injection are limited by the lack of clinically available imaging methods to detect or track the *in vivo* fate of the administered cells. A robust imaging technology for serial, non-invasive, *in vivo* assessment of stem cell or hematopoeitic cell fate after delivery in humans is a powerful tool, both for pharmaceutical companies conducting trials of cell therapies, as well as for clinicians who would ultimately implement the imaging technology to guide clinical decision-making. Furthermore, such an imaging technology would be expected to be useful in the research arena, giving new physiologic insights into stem cell trafficking and enabling the monitoring of engineered cells to improve cellular delivery to target tissues.

[0064] The ^{89}Zr-complexes disclosed herein will allow the direct transplantation of ^{89}Zr-labeled stem cells, leukocytes including T-cells, B-cells and natural killer cells, neutrophils, dendritic cells, T reg cells, and the like, into tissue for purposes of repair (e.g., replace damaged organ) or for revascularization (e.g., increase blood flow to area) or therapeutic approaches (e.g., a cell containing an expression cassette that directs the expression of a polynucleotide coding sequence) to track the migration pattern and/or cellular distribution of the labeled cells noninvasively and repeatedly as necessary.

[0065] The ^{89}Zr-complexes disclosed herein will allow the direct transplantation of ^{89}Zr-labeled stem cells into tissue for purposes of repair (e.g., replace a damaged organ). The labeled stem cells have the advantage that their migration pattern and cellular distribution can be tracked noninvasively. Stem cell therapy has the potential to improve organ regeneration in a large spectrum of diseases, e.g., after ischemic, metabolic or toxic organ injury. Furthermore, it has been shown, for example, that mesenchymal stem cells (MSCs) have an immunomodulatory effect that can be used for avoiding graft rejections after organ transplantations, graft versus host disease after stem cell transplantations and autoimmune diseases. MSCs are also interesting for a cell-based regenerative medicine, as they can be stimulated to differentiate towards lineages of the mesenchymal tissue, including bone, cartilage, fat, muscle, tendon, and marrow stroma. MSCs have been employed in preclinical studies to regenerate bone in massive bone defects that the body cannot naturally repair.

[0066] A method of repairing damaged tissue in a human subject in need of such repair comprises contacting an effective amount of ^{89}Zr-labeled stem cells with the damaged tissue of the subject. The cells may be introduced into the body of the subject by localized injection, or by systemic injection. The cells may be introduced into the body of the subject in conjunction with a suitable matrix implant. The matrix implant may provide additional genetic material, cytokines, growth factors, or other factors to promote growth and differentiation of the cells.

[0067] In another embodiment, the ^{89}Zr-labeled cells disclosed herein are used for revascularization (e.g., increase blood flow to area). The ^{89}Zr-labeled stem cells have the advantage that their migration pattern and cellular distribution can be tracked noninvasively. Revascularization restores function and heals injured tissue. Revascularization occurs by at least three distinct processes. Vasculogenesis involves the development of blood vessels. Vasculogenesis occurs in blood islands of the embryonic yolk sac, and was originally thought to occur primarily in the embryo. Angiogenesis involves the sprouting of new vessels from previously existing vessels. Arteriogenesis involves the maturation of pre-existing collateral vessels that enlarge after blockage or damage to a primary vessel. Inducing vascularization from stem cells is especially effective in increasing cardiac or peripheral (i.e. limb) vascularization. Therefore, the method is especially effective in treating cardiac and peripheral ischemia. Patients suffering from other conditions also require vascularization. Such conditions include patients having undergone bone marrow transplant, patients suffering from sickle cell anemia and thalassemia.

[0068] In another embodiment, immune cells, dendritic cells, B-cells, T-cells, natural killer cells or other genetically altered cells are labeled with the ^{89}Zr-complexes disclosed herein to non-invasively monitor their trafficking into tissues or lesions in autoimmune or inflammatory diseases, ischemic diseases of the heart and central nervous system, genetically deficient disease states, and into malignancy as part of a therapeutic approach. Local administration of ^{89}Zr-labeled complexes can be used conjunction with systemic administration of immunotherapy, that is, immunotherapy or vaccine therapy administered to the whole body.

[0069] The ^{89}Zr-complexes disclosed herein can be employed in therapeutic approaches (e.g., genetically altered vector producing cells) to track the migration pattern and/or cellular distribution of the labeled cells noninvasively and repeatedly as necessary.

[0070] In an embodiment, the ^{89}Zr-labeled biological cell is a genetically altered vector-producing cell, that is, a cell containing an expression cassette that directs the

expression of a polynucleotide coding sequence. Candidate polynucleotides for gene therapyinclude, for example, genes encoding interferon, sodium symporter, HSVTk, Apolipoprotein E (which has been correlated with risk for Alzheimer's disease and cardiovascular disease), MTHFR (variants of which have been associated with increased homocysteine levels and risk of stroke), Factor V (which has been correlated with risk of thrombosis), ACE (variants of which have been correlated with risk of heart disease), CKR-5 (which has been associated with resistance to HIV), HPRT (hypoxanthine-guanine phosphoribosyl transferase, the absence of which results in Lesch-Nyhan disease), PNP (purine nucleoside phosphorylase, the absence of which results in severe immunodeficiency disease), ADA (adenosine deaminase, the absence of which results in severe combined immunodeficiency disease), p21 (which has been proposed as a candidate gene for treatment for ataxia telangiectasia), p47 (the absence of which is correlated with lack of oxidase activity in neutrophils of patients with chronic granulomatous disease), Rb (the retinoblastoma susceptibility gene associated with tumor formation), KVLQT1 (a potassium channel protein, with aberrant forms associated with cardiac arrhythmias), the dystrophin gene (associated with Duchenne muscular dystrophy), CFTR (the transmembrane conductance regulator associated with cystic fibrosis), phosphatidylinositol 3-kinase (associated with ataxia telangiectasia), and VHL (loss or mutation of the protein is associated with Von-Hippel Lindau disease). Other diseases which can be treated effectively using these genetically altered, nanoparticle-labeled cells include, Factor IX deficiency, adenosine deaminase deficiency (associated with severe combined immunodeficiency disease, or SCIDS), and diabetes, and deficiencies in glucocerebrosidase, α-iduronidase. In addition, for cancer, gene therapy may include a therapeutic gene that can activate a nontoxic drug to a chemotherapeutic drug, such as TK/Ganciclovir, CD/5-FC,CE/CPT-11, GUS/DOX-GA3, and the like. Additional candidates for gene therapy include nerve growth factor, brain-derived neurotropic factor gene delivery for Alzheimer's disease, and the like.

[0071] As used herein, a "polynucleotide coding sequence" or a sequence that "encodes" a selected polypeptide, is a nucleic acid molecule that is transcribed (in the case of DNA) and translated (in the case of mRNA) into a polypeptide *in vivo* when placed under the control of appropriate regulatory sequences (or "control elements"). The boundaries of the coding sequence are determined by a start codon at the 5′ (amino) terminus and a translation stop codon at the 3′ (carboxy) terminus. An "expression cassette" refers to an assembly that is capable of directing the expression of a sequence or gene of interest. The expression cassette includes a promoter that is operably linked to the sequences or gene(s) of interest.

Other control elements may be present as well. Expression cassettes described herein may be contained within a plasmid construct. In addition to the components of the expression cassette, the plasmid construct may also include a bacterial origin of replication, one or more selectable markers, a signal which allows the plasmid construct to exist as single-stranded DNA (e.g., a M13 origin of replication), a multiple cloning site, and a "mammalian" origin of replication (e.g., a SV40 or adenovirus origin of replication).

[0072] The polynucleotides can be operatively linked to an inducible promoter so that levels of protein, e.g., enzyme can be regulated. Exemplary inducible promoter systems include a mutated ligand-binding domain of the human estrogen receptor (ER) attached to the protein to be produced. This would require that the individual ingest tamoxifen to allow expression of the protein. Alternatives are tetracycline on or off systems, RU486, and a rapamycin inducible system. An additional method to obtain relative selective expression is to use tissue specific promoters. For instance in the brain, one can introduce a transgene driven by the neuron-specific enolase promoter (Ad-NSE) or the glial fibrillary acidic protein promoter (GFAP) promoter, which will allow almost exclusive expression in brain tissue. Likewise, endothelial expression only may be obtained by using the Tec promoter or the VE-cadherin promoter.

[0073] Cells isolated can be genetically modified by introducing DNA or RNA into the cell by a variety of methods known to those of skill in the art. These methods are generally grouped into four major categories: (1) viral transfer, including the use of DNA or RNA viral vectors, such as retroviruses (including lentiviruses), Simian virus 40 (SV40), adenovirus, Sindbis virus, and bovine papillomavirus for example; (2) chemical transfer, including calcium phosphate transfection and DEAE dextran transfection methods; (3) membrane fusion transfer, using DNA-loaded membranous vesicles such as liposomes, red blood cell ghosts, and protoplasts, for example; and (4) physical transfer techniques, such as microinjection, electroporation, or direct "naked" DNA transfer. Biological cells can be genetically altered by insertion of pre-selected isolated DNA, by substitution of a segment of the cellular genome with pre-selected isolated DNA, or by deletion of or inactivation of at least a portion of the cellular genome of the cell. Deletion or inactivation of at least a portion of the cellular genome can be accomplished by a variety of means, including but not limited to genetic recombination, by antisense technology (which can include the use of peptide nucleic acids, or PNAs), or by ribozyme technology, for example. Insertion of one or more pre-selected DNA sequences can be accomplished by homologous recombination or by viral integration into the host cell genome. The desired gene sequence can also be incorporated

into the cell, particularly into its nucleus, using a plasmid expression vector and a nuclear localization sequence. Methods for directing polynucleotides to the nucleus have been described in the art. The genetic material can be introduced using promoters that will allow for the gene of interest to be positively or negatively induced using certain chemicals/drugs, to be eliminated following administration of a given drug/chemical, or can be tagged to allow induction by chemicals (including but not limited to the tamoxifen responsive mutated estrogen receptor) expression in specific cell compartments (including but not limited to the cell membrane).

[0074] As described herein, when the ^{89}Zr-labeled cells also comprise an MR imaging agent, nuclear magnetic resonance techniques may be used to detect populations of MR imaging agent-labeled cells. The term “detect” when referring to MRI includes more sophisticated measurements, including quantitative measurements and two- or three-dimensional image generation. For example, MRI may be used to generate images of such cells. In many instances, the labeled cells are administered to a living subject. Following administration of the cells, some portion of the subject, or the entire subject, is examined by MRI to generate an MRI data set. A “data set” means raw data gathered during magnetic resonance probing of the subject material, as well as information processed, transformed, or extracted from the raw data. Examples of processed information include two-dimensional or three-dimensional pictorial representations of the subject material.

[0075] MRI examination may be conducted according to a suitable methodology known in the art. Many different types of MRI pulse sequences, or the set of instructions used by the MRI apparatus to orchestrate data collection, and signal processing techniques (e.g., Fourier transform and projection reconstruction) have been developed over the years for collecting and processing image data. The reagents and methods described herein are not tied to any particular imaging pulse sequence or processing method of the raw NMR signals. For example, MRI methods include spin-echo, stimulated-echo, gradient-echo, free-induction decay based imaging, and any combination thereof. The development of new and improved pulse sequence and signal processing methods is a continuously evolving field, and persons skilled in the art can devise multiple ways to image the labeled cells in their anatomical context.

[0076] Also included herein are methods of lymphatic system imaging using the ^{89}Zr-complexes disclosed herein, optionally in combination with a superparamagnetic nanoparticle such as FeraHeme®. Lymphatic system imaging is particularly useful to track macrophage uptake *in vivo*. Without being held to theory, it is believed that intradermal administration of

^{89}Zr-complexes leads to uptake by the lymphatic system, wherein the particles localize to the lymph nodes. Thus, in one embodiment, method of imaging the lymphatic system of an individual, comprising intradermally administering the PET imaging complexes described herein and detecting the migration pattern and/or cellular distribution pattern of the PET imaging complexes in the subject.

[0077] Further included herein are methods for treating various diseases and/or injuries using ^{89}Zr-labeled biological cells. In an embodiment, the progress of such treatment may be monitored by labeling the biological cells before administration to the patient. The fate of the biological cells may be determined, with precision, as to their location and status. For example, one therapeutic biological cell comprises a stem cell. Alternatively, a therapeutic biological cell comprises a non-stem cell. In an embodiment, the non-stem cell comprises a cancer cell, wherein the growth and/or metastasis of a tumor are detected and/or monitored using PET imaging. Another non-stem cell is an immune cell such as T-cells, B-cells, natural killer cells, or dendritic cells that may be used to treat cancer or autoimmune diseases or degenerative diseases.

[0078] Diseases treatable with ^{89}Zr-labeled stem cells include cardiovascular diseases, treatment of injured cardiovascular vessels, treatment of diabetes, imaging of cancer cells, and imaging of neurodegenerative disorders.

[0079] In an embodiment, a cardiovascular disease is treated with a ^{89}Zr-labeled stem cell. Stem cells can be used to treat heart failure, heart diseases resulting from cardiomyocyte death, to repair or regenerate tissue damaged by myocardial infarction, and treatment of injured cardiovascular vessels.

[0080] Adult bone marrow-derived mesenchymal stem cells (MSCs) may be useful to treat heart diseases resulting from cardiomyocyte death, for example, as treatment strategies to repair the heart, including delivery of reparative cells to injured tissue. Cell-based therapies, using for example bone marrow-derived cells, may be used to repair or regenerate tissue damaged by myocardial infarction. Bone-marrow derived cells have been suggested as potential candidates for this purpose as reported in clinical trials of intracoronary infusion of autologous unfractionated bone marrow. In an embodiment, ^{89}Zr-labeled stem cells are administered locally after myocardial infarction.

[0081] In an embodiment, ^{89}Zr-labeled endothelial stem cells and/or endothelial progenitor cells are locally administered to promote vessel wall healing and to track the progress of the healing by PET imaging that is capable of imaging cell attachment and growth. Injury to cardiovascular vessels can occur by a variety of insults, such as certain

medical procedures. For example, medical procedures including, but not limited to, angioplasty or stenting may result in the denuding of endothelial cells from the cardiovascular vessel wall. Alternatively, some surgical procedures may also leave injury to cardiovascular vessels that undergo a post-surgical healing process. Sometimes the healing process may comprise stenosis or restenosis, characterized by a narrowing of the vessel.

[0082] In another embodiment, ^{89}Zr-labeled pancreatic stem cells or pancreatic islets are used to treat diabetes. The transplanted stem cells can be used to promote organ regeneration and to track the progress of the organ regeneration by PET imaging.

[0083] In another embodiment, cancer cells are contacted with ^{89}Zr-complexes, such that the complexes are internalized into the cancer cells to track the progress of cancer therapy and to detect non-palpable metastasis by PET imaging. Cancer progression can be imaged in almost any organ or tissue, such as the lung, breast, colon, liver, prostate, breast, skin, bones, brain, nerve tissue, cervical, lymph, white blood cells, ovary, testes, thyroid, or uterus.

[0084] In yet another embodiment, nerve cells are contacted with ^{89}Zr-complexes, such that the complexes are internalized within the nerve cells to track the progress of neuronal regeneration and/or neurodegeneration by PET imaging. Neurodegenerative disorders that can be treated include Alzheimer’s disease, Parkinson’s disease, and dementia. Because of the central role of the presence of αβ plaques in Alzheimer’s disease and death of dopaminergic neurons in Parkinson’s disease and dementia, there has been a wide interest in developing radiolabeled ligands that bind to and allow imaging of such abnormalities. Superparamagnetic nanoparticles can contain a radiolabeled ligand allowing for PET-MRI to be performed.

[0085] In yet another embodiment, the ^{89}Zr-complexes are electrostatically bound to DNA. Without being held to theory, it is believed hat protamine, which contains large numbers of the amino acid arginine, can also be used as a transfection agent to incorporate DNA into cells. Thus, the same self-assembling complexes can also be directed to be bound to specific receptors or antigens on the surface of cells, allowing for the monitoring of DNA delivery into specific cells and tissues.

[0086] Also provided are pharmaceutical compositions (e.g., an ^{89}Zr-labeled biological cell). The pharmaceutical compositions may be administered in a number of ways depending upon whether local or systemic treatment is desired and upon the area to be treated. Administration may be topical (including ophthalmic and to mucous membranes including vaginal and rectal delivery), pulmonary (e.g., by inhalation or insufflation of

powders or aerosols, including by nebulizer); intratracheal, intranasal, epidermal and transdermal, intradermal, oral, or parenteral. Parenteral administration includes intravenous, intraarterial, subcutaneous, intraperitoneal or intramuscular injection or infusion; or intracranial, e.g., intrathecal or intraventricular administration.

[0087] Compositions and formulations for parenteral, intrathecal or intraventricular administration may include sterile aqueous solutions that may also contain buffers, diluents and other suitable additives such as, but not limited to, penetration enhancers, carrier compounds and other pharmaceutically acceptable carriers or excipients.

[0088] Pharmaceutical compositions include, but are not limited to, solutions, emulsions, and liposome-containing formulations. These compositions may be generated from a variety of components that include, but are not limited to, preformed liquids, self-emulsifying solids and self-emulsifying semisolids.

[0089] The pharmaceutical formulations, which may conveniently be presented in unit dosage form, may be prepared according to conventional techniques well known in the pharmaceutical industry. Such techniques include the step of bringing into association the active ingredients with the pharmaceutical carrier(s) or excipient(s). In general the formulations are prepared by uniformly and intimately bringing into association the active ingredients with liquid carriers or finely divided solid carriers or both, and then, if necessary, shaping the product.

[0090] The compositions may additionally contain other adjunct components conventionally found in pharmaceutical compositions. Thus, for example, the compositions may contain additional, compatible, pharmaceutically-active materials such as, for example, antipruritics, astringents, local anesthetics or anti-inflammatory agents, or may contain additional materials useful in physically formulating various dosage forms of the compositions of the present invention, such as dyes, flavoring agents, preservatives, antioxidants, opacifiers, thickening agents and stabilizers. However, such materials, when added, should not unduly interfere with the biological activities of the components of the compositions of the present invention. The formulations can be sterilized and, if desired, mixed with auxiliary agents, e.g., lubricants, preservatives, stabilizers, wetting agents, emulsifiers, salts for influencing osmotic pressure, buffers, colorings, flavorings and/or aromatic substances and the like which do not deleteriously interact with the nucleic acid(s) of the formulation.

[0091] The invention is further illustrated by the following non-limiting examples.

EXAMPLES

Methods

[0092] Isolation of human or swine WBCs from Whole Blood: Fifty milliliters of fresh human venous blood were collected in acid citrate dextrose (ACD) anticoagulant. Thirty five-milliliters diluted of this blood were carefully layered on 15 ml of Ficoll-Paque™ in 50 ml conical plastic tube. The tubes were centrifuged at 400xg for 20-30 min at room temperature. Two distinct bands were obtained from this operation: Linear band at the plasma-medium interface was composed primarily of mononuclear leukocytes and was collected. This band was carefully transferred to another plastic tube and washed once with PBS solution. The upper plasma layer was also saved in a different tube and centrifuged at 450xg for 10 min to obtain platelet poor plasma. The pelleted cells were reconstituted with PBS and were subjected to further labeling experiments with ^{89}Zr-based methods. The viability and counting of these cells immediately performed.

[0093] Preparation of Human Bone Marrow-Derived Stem Cells: Peripheral blood stem cell donors were treated by subcutaneous injection with recombinant human G-CSF (Amgen Inc, Thousand Oaks, CA) at a dose of 16 μg/kg for 5 days. Leukapheresis was performed using a Cobe® Spectra continuous flow blood cell separator (Cobe Laboratories, Lakewood, CO) on 2 consecutive days beginning on day 4 of G-CSF administration. Aliquots of the GPBMC product were collected after written informed consent using forms approved by the Institutional Review Board for collection of samples for research purposes. Cells were washed twice in Hanks' balanced salt solution (Gibco- BRL, Rockville, MD), and erythrocytes were removed by hypotonic lysis. Cells that could not be processed immediately were cryopreserved. Magnetic bead enrichment of CD34 cells was accomplished with a CD34 isolation kit (Miltenyi Biotec, Auburn, CA) and the sensitive mode of positive selection on an AutoMacs® magnetic separation apparatus (Miltenyi Biotec). CD34 cells from both sources were isolated to >95% purity by monoclonal antibody staining and flow cytometry (fluorescence-activated cell sorting [FACS]). Briefly, pre-enriched cells were labeled with HPCA-2-phycoerythrin (Becton Dickinson Immunocytometry Systems, San Jose, CA) and selected on a Becton Dickinson Vantage II with gates for exclusion of nonviable cells (propidium iodide positive) and cells with high side scatter. All antibodies were from Becton Dickinson.

[0094] ^{89}Zr Only Labeling of the Cells: After simple mixing of the ^{89}Zr (50 to 100 μCi)–protamine sulfate (PS) (40 μg/mL) -heparin (H) (2U/mL) or ^{89}Zr (50 to 100 μCi)–PS (40 μg/mL) complex, the indicated number of cells were added into these complexes into

cell-appropriate serum free cell media and then the cells were incubated for at least 30 min at 37°C. The cells were then washed three times and the tubes changed once. Alternatively, the cells could be washed with deferoxamine mesylate USP (desferal) in order to remove the unbound-free ^{89}Zr in the last washing step. The amount of radioactivity associated with the cells before and after the labeling procedure was routinely determined by using gamma counter/dose calibrator. Importantly, the cell viability was assessed immediately after the labeling procedure. The labeled cells were then used for *in vitro* or injection into animals or humans. For human use, all of these procedures could be performed under Good Manufacturing Practices (GMP) as provided by the FDA.

[0095] To determine the suitable labeling condition, the WBC cells were labeled with ^{89}Zr alone (increasing radioactive dose from 0- 100 microCi), ^{89}Zr with protamine sulfate (PS) (increasing PS dose of 0-2-40 microgram/mL or ^{89}Zr with PS (increasing PS dose from 0 to 40 microgram/mL and heparin (H) (increasing H dose from 0 to 2.2 U/mL) mixture.

[0096] Dual Labeling (^{89}Zr and Feromyxtol) of the Cells: After mixing and shaking of the feromyxtol (Fe) (100 μg/mL) –protamine sulfate(PS) (40 μg/mL) -heparin (H) (2U/mL) or Fe (100 μg/mL) –PS (40 μg/mL) complex for 10 minutes, the indicated number of cells were added into these complexes and were incubated for at least 2 hours at 37°C in the cell appropriate serum free media. The cells were then washed twice with PBS and resuspended with serum free media. Later, the ^{89}Zr was added into the already labeled cells either with Fe-PS-H or Fe-PS complex and incubated for 30 min 37°C. The cells were then washed three times and changed the tubes once. Alternatively, the cells could be washed with deferoxamine mesylate USP (desferal) in order to remove the unbound-free ^{89}Zr in the last washing step. The amount of radioactivity associated with the cells before and after the labeling procedure was routinely determined by using gamma counter/dose calibrator. Importantly, the cell viability was assessed immediately after the labeling procedure. The labeled cells were then used for *in vitro* studies or injection into animals or humans.

[0097] Recovery of Labeled Cells from Whole blood *in Vitro*: After labeling of autologous WBC with ^{89}Zr, the pelleted cells were reconstituted with 200 μL PBS and mixed with citrated whole blood and incubated 30 minutes at 37°C with occasional gentle mixing. As a controls, unlabeled cells and free ^{89}Zr were also added in different citrated whole blood containing tubes and incubated at the same time. After incubation, the whole blood samples were layered on top of a linear Ficoll® gradient and then centrifuged at 400xg for 20-30 min at room temperature. The tubes were immediately imaged using clinical PET/CT scan. The fraction of plasma, mononuclear layers and red blood cell were carefully collected in the 1.5

ml eppendorf tubes. These tubes then imaged by using clinical PET/CT scan. After the imaging, all the samples were counted in the gamma counter.

[0098] Evaluation of the stability of radiolabeled NK cells: The retention of the radionuclides within the expended NK cells was assessed by incubating radiolabelled NK cells with X-VIVO™ 20 media at 37° C for 0, 1, 2 and 3 hrs. Cell samples were withdrawn and centrifuged for 5 min at 2000x rpm at 4°C. The stability of the radiolabel in the serial samples was expressed as percent radioactivity in the cell pellet relative to total radioactivity in both the cell pellet and the corresponding supernatant.

[0099] Indirect (relative) labeling of erythrocytes and leukocytes in vitro: To determine the indirect labeling of erythrocytes or leukocytes in vitro, radiolabeled ^{89}Zr-PS-H mixture or non-radiolabeled, control PS-H mixture was added in autologous ACD anticoagulated whole blood and incubated 30 min at 37°C with gentle mixing. Then, the whole blood samples were layered on top of a linear Ficoll® gradient and then centrifuged at 400xg for 20-30 min at 20°C. The tubes were immediately imaged. The fraction of plasma, mononuclear layers and red blood cell were carefully collected in the 1.5 ml eppendorf tubes and imaged. After imaging, all the samples were counted in a gamma counter to determine radioactivity.

[0100] Determination of cell bound ^{89}Zr uptake- Evaluation of the incorporation and Release of the ^{89}Zr: Two sets of experiments were performed to determine the elution of the label from cells. WBC were labeled with the standard ^{89}Zr labeling protocol as described above and washed 3 times. Gamma counter was used to measure the radioactivity of the WBC pellets after labeling. Freeze thawing procedure was applied to destroy the pellet and 1 mL of PBS was subsequently added. These samples were centrifuged at 12000 x rpm for 10 min and supernatants and pellet were separated and the radioactivity of each fraction (pellet-cell bound ^{89}Zr and supernatant-free ^{89}Zr was counted in a gamma counter. To determine the stability of the label in tumor cells, after labeling of MKN45 cells with the ^{89}Zr-PSH mixture, cells were incubated 2 hr at 37°C in 3 different incubation buffers; PBS or 50% sodium citrate buffer or 100% sodium citrate buffer (pH=4.0). After incubation, the cell viability was assessed immediately and the cells and supernatant were separated. The radioactivity obtained from the cells and supernatant was expressed as a percentage of the activity of the whole suspension.

[0101] Trypan Blue Viability assay: The labeled cells were suspended in 1× PBS at the concentration of 1×10^6/ml and mixed with 0.4% of trypan blue dye at the 1:1 ratio. Ten μl of this mixture was loaded into hemocytometer, after which cells were counted. Cells with

an intact membrane excluded the dye and were considered as live cells. The percentage of live and dead cells was determined.

[0102] In vitro PET imaging of ^{89}Zr labeled cells: PET scans were performed using either a home built microPET (ATLAS) or clinical PET/CT scanner (Gemini TF, Philips Medical Systems). For the ATLAS scanner, the samples were placed in the center of scanner field of view and 5 min emission scans (two bed positions) were acquired with a 100–700 keV energy window. Further details regarding image acquisition, processing, and analysis are provided in the supplemental information. For the clinical PET/CT, the samples were imaged in 3-dimensional (3D) time-of-flight mode on a Gemini TF (Philips Medical Systems) camera with a spatial resolution of 4.8 mm at the center of the field of view. The images were reconstructed using the default row-action maximum-likelihood algorithm iterative reconstruction, with standard corrections for randoms, scatter, attenuation, and normalization. For each sample, ROIs were manually drawn over the samples. The maximum counts per pixel within the tubes were obtained from multiple ROIs.

[0103] Flow cytometry analysis of labeled expanded NK cells: To determine the effect of ^{89}Zr on cell viability and function, either expanded NK cells were mixed with ^{89}Zr-H-PS or H-PS alone or unmanipulated NK cells then were assessed by flow cytometry (FACS Calibur™ BD Biosciences, San Jose, CA) with the following anti-human monoclonal antibodies (BD Biosciences Pharmingen San Diego, CA): anti-CD56-APC, anti-CD16-PECy7, anti- tumor necrosis factor–related apoptosis-inducing ligand (TRAIL)-PE, anti-NKG2D-APC. Cell viability was determined by staining with Annexin V-FITC. Cells were also stained with their corresponding isotype-matched control monoclonal antibodies (IgG1-APC, IgG1-PE and IgG1 PE-Cy 7).

[0104] Flow cytometry killing assay of NK cells. NK cell cytotoxicity was assessed using flow cytometry. NK cells expanded in tissue culture flanks for 20 days were washed twice in X-VIVO™ 20 medium without serum. NK cells were then labeled with either ^{89}Zr-H-PS or a HPS control mixture. NK cells were plated into duplicate wells ($1x10^6$ NK cells/well) in 96-well tissue culture plates with or without $1x10^6$ K562 target cells in 200 μL of X-VIVO™ 20 media (no IL-2). After 2 hours of incubation at 37°C, cells were washed and stained with CD56 PE, Annexin V APC and IgG1 PE. The remaining NK cells were recultured with X-VIVO™ 20 medium with IL-2 and evaluated for K562 cytotoxicity 24 hr and 48 hrs after labeling. The percentage of Annexin V+ cells (dying cells) in CD56 negative K562 cells was assessed by flow cytometry and the effect of exposure to ^{89}Zr-H-PS and H-PS on NK cytotoxicity against K562 cells was determined.

[0105] Statistics. All experiments were performed in duplicate unless otherwise indicated and each experiment was repeated at least twice. Data are expressed as mean ± SD. The significance of differences in means was assessed using the Student t-test. Differences were considered to be significant when $P < 0.05$.

[0106] Swine Protocol: Female or male domestic pigs weighing 40–60 kg were used in the animal studies. Animal handling and care followed the principles stated in the federal law on animal experiments and the national animal research committee approved the protocol. The animals were anesthetized with isoflurane and an IV catheter was placed in an ear vein for venous access, and the animals were intubated with an appropriately sized endotracheal tube. At that time, placement of Foley urinary catheter was performed under anesthesia. For planning purposes, the surgical site was the right or left hip area. Hair was removed from all sides (dorsal, ventral and lateral) of the abdomen from the last rib to the perineum. Hair was also removed from all sides of the right rear leg down to the level of the hock. The animal was placed in left lateral recumbency and secured on the operating table. The right or left hind leg was included in the sterile prep and draped so as to facilitate movement cranially or caudally during the procedure. The surgical site was scrubbed with alternating Betadine and alcohol (repeated 3 times), followed by an application of Betadine solution. The animal was then surgically draped, using aseptic technique. The skin incision started cranially over the cranial dorsal iliac spine and continues caudally parallel to the midline to near the hip joint. Subcutaneous tissues and gluteal fascia and fat were incised on the same line to expose the cranial and caudal dorsal iliac spines. An incision was then made in the periosteal origin of the middle gluteal muscle on the edge of the ilium near the cranial dorsal iliac spine and ending beyond the caudal dorsal spine. The middle gluteal muscle was elevated subperiosteally using periosteal elevators. The elevation continues caudally to the caudal dorsal iliac spine. Using the specially design Intraosseous infusion system, a catheter is placed into the bone marrow on the dorsal spine of the ilium. The Samba Preclin 420/transducer was then inserted through this catheter to measure pressure within the bone marrow during the injection of stem cells. Using a proprietary bone marrow infusion system, a hole was then drilled on the cranial aspect of the ilium for delivering labeled stem cells. Fluoroscopy /CT was used to validate the correct placement of both catheters. The superficial fascia of the middle gluteal muscle was then be sutured to the wing of the ilium around the two catheters. This was followed by a layer closure of the gluteal fascia, gluteal fat, subcutaneous fascia and skin (subcuticular) around them so they for easy access during the stem cell delivery. The surgical site was then covered to protect the catheters. The pigs

were then transported to the PET facilities using approved transportation route. LAMS technicians were accompanied the anesthetized pigs and remained with the animal throughout the PET procedure (injection of the cells). This was a non-survival procedure. Therefore, after the animals scanned in the PET, they were then humanely euthanized with an intravenous bolus of KCl (2 mEq/kg) while under anesthesia.

[0107] *In vitro* PET Imaging of ^{89}Zr labeled Cells: PET scans were performed using the NIH Advanced Technology Laboratory Animal Scanner (ATLAS). The ATLAS detector modules possess two layers of scintillator crystals, with different scintillation time constants, 40 ns (LSO) and 60 ns (GSO) that enable a two–level depth–of-interaction discrimination. The central point source resolution is 1.8 mm with filtered back projection and a ramp filter with cut–off at the Nyquist frequency. The scanner has a transverse field–of–view (FOV) of 6.8 cm and an axial FOV of 2 cm. The samples were placed in the center of scanner FOV, and 5 min emission scans (two bed positions) were acquired with a 100–700 keV energy window. The images were reconstructed by a 2–dimensional ordered-subsets expectation maximization (2D–OSEM) algorithm, without correction applied for attenuation or scatter. For each samples, ROIs were manually drawn over the samples. The maximum counts per pixel within the tubes were obtained from multiple ROIs (counts).

[0108] *In vivo* PET Imaging of Pigs: A 3-dimensional (3D) time-of-flight mode on a Gemini TF (Philips Medical Systems) camera with a spatial resolution of 4.8 mm at the center of the field of view. The images were reconstructed using the default row-action maximum-likelihood algorithm iterative reconstruction (ordered-subset expectation maximization for the LS Discovery scanner), with standard corrections for randoms, scatter, attenuation, and normalization. After a low-dose transmission CT scan of the chest, abdomen and pelvis, each pig received an intravenous bolus/slow or bolus intra-bone injection of ^{89}Zr labeled cells (range of dose, 2–20 μCi), immediately followed by alternating 10-min static images were acquired per bed position with total of 6 bed position for whole body imaging of pig. In case of imaging of the whole blood and fractioned blood samples, we used the clinical Gemini TF PET scanner as well.

[0109] *In vitro* MRI imaging of Dual Labeling (^{89}Zr and Feromyxtol) of the Cells: MR images were acquired on a Philips Achieva 3.0 T clinical MR scanner with a custom-built 22 mm-diameter × 22 mm-long small-animal receiver coil. After a tri-planar survey scan of the 200 μL of suspended samples within the MRI tubes which placed in the water containing plastic mold, T2*-WI using a gradient echo (GRE) sequence with TR=272 ms, and TE= 4,10,16, 22 & 28 ms was acquired for each tube.

Example 1: ^{89}Zr labeling of cells

[0110] ^{89}Zr-H-PS, ^{89}Zr –PS and ^{89}Zr alone were compared for cell uptake in WBC and NK cells. An excellent labeling efficiency was achieved after incubating WBC with either with 50 or 100 μCi dose of ^{89}Zr for 30 min in the presence of the PS (40 μg/mL)- H (2U/mL) mixture resulting increased gamma counting and pixel intensity on the images of the samples.

Table 1: Labeling of isolated human WBCs with ^{89}Zr in Different Experimental Conditions

Heparin, U/mL	2.2	1.1	0.5	0.1	2.2	1.1	-	-	-	-
Protamine sulfate μg/mL	40	20	10	2	-	-	40	2	-	-
^{89}Zr μCi	<1	<1	<1	<1	<1	<1	<1	<1	<1	<1
CPM in cells, thousands	60	54	27	20	7	4.4	34	15	5.8	5.4

[0111] Based on the gamma counting and in vitro micro PET imaging, there was significantly increased counts and imaging signal seen in the ^{89}Zr -H-PS treated samples when compared to other test conditions.

Table 2: Labeling capacity of isolated human WBCs with ^{89}Zr

Heparin, U/mL	2	2	2	2	2	2	2	2	2	2
Protamine sulfate μg/mL	40	40	40	40	40	40	40	40	40	40
^{89}Zr μCi	0	1	10	50	100	100	0	100	100	0
Human Serum 10%									Yes	Yes
CPM in cells, Millions	0	0.02	0.35	1.58	1.64	1.49	0	1.28	0.04	0

[0112] Importantly, inhibition of ^{89}Zr was observed by adding either with serum or deferoxamine mesylate USP (desferal) into mixture (^{89}Zr -H-PS), confirming presence of high binding affinity of ^{89}Zr to serum and to the desferal as a chelator for ^{89}Zr (data not shown).

[0113] The retention of ^{89}Zr in WBC and NK cells following labeling with different conditions is summarized in Table 3. The presence of PS in the labeling mixture significantly increased the retention of ^{89}Zr in the cells. No significant labeling of the cells was observed

with ^{89}Zr alone. ^{89}Zr cell uptake was also evaluated with in vitro micro PET imaging in WBC and NK cells and revealed that exposure of cells to ^{89}Zr for 30 min either in the presence of PS (40 μg/mL) or in the presence of the mixture of PS (40 μg/mL)-H (2U/mL) resulted in higher activity when compared to exposure to ^{89}Zr alone or the PBS control.

Table 3: Retention of ^{89}Zr in WBC and NK cells following labeling

	PBS	^{89}Zr-H-PS	^{89}Zr-P	^{89}Zr	^{89}Zr
WBC (mil)	0.0071	1.69	0.717	0.203	0.193
NK (thous)	0.45	268.2	129.9	34.89	43.51

[0114] In order to determine the optimal incubation time, the suspended WBC were incubated with ^{89}Zr for 10, 30, 60 min at 37°C. The maximum cell uptake of ^{89}Zr-H (2U/mL)-PS (40 μg/mL) peaked at 30 min and slightly decreased at 60 min (Table 4). These timed uptake experiments suggest that incubation with the ^{89}Zr-HPS mixture at 37°C for 30 minutes is optimal compared to either the 10 or 60 min incubation time.

Table 4: Determination of optimal incubation time

	^{89}Zr-H2-PS40 10 min	^{89}Zr-H2-PS40 30 min	^{89}Zr-H2-PS40 60 min	^{89}Zr-H2-PS120 30 min	Control
Radioactivity CPM (mil)	0.287	0.919	0.838	0.773	0.00082

[0115] Based on gamma counting and in vitro micro-PET imaging, there was significantly increased uptake with ^{89}Zr -H-PS treated samples when compared to other test conditions. Importantly, complete inhibition of ^{89}Zr uptake was observed by adding either serum or deferoxamine mesylate USP (desferal) into the mixture (^{89}Zr -H-PS), suggesting that the strong binding of ^{89}Zr to serum proteins and ^{89}Zr chelation with desferal competes with weaker binding of ^{89}Zr to the PS-H nanocomplex (data not shown).

Example 2: Viability of WBC and NK cells after labeling

[0116] The viability of WBC by the trypan blue method for ^{89}Zr-H-PS, ^{89}Z- PS or ^{89}Zr alone-treated cells and control cells was not significantly different (estimated to be 86%, 88%, 89% and 92% respectively). Similarly, the viability of NK cells was 86% for ^{89}Zr-H-PS labeled, 81% for ^{89}Z- PS labeled, 90% for ^{89}Zr alone-treated and 87% for control cells.

No statistically significant difference in cell death was observed with ^{89}Zr-treated cells vs. control cells (P>0.05). Light microscopy revealed WBC to have a normal appearance being spherical and intact. Expended NK cells were also preserved their shape and integrity without significant difference being observed between ^{89}Zr- labeled and control cells.

Example 3: Determination of cell bound ^{89}Zr uptake- Evaluation of the incorporation and washout of the ^{89}Zr

[0117] To confirm cell-associated ^{89}Zr, labeled cells underwent a freeze-thaw cycle and lysed cells were centrifuged. The supernatant and pellet radioactivity were then gamma counted to determine the percentage of radioactivity that was trapped in the pellet. From this experiment, we found that about 80-85% of the radioactivity was recovered in the pellet, providing evidence for cell associated ^{89}Zr labeling (data not shown). The effect of cell media on stability of ^{89}Zr cell labeling was studied with labeled MKN45 cells suspended in PBS, PBS or 50% sodium citrate buffer or 100% sodium citrate buffer (pH=4.0); PBS media resulted 100% of the activity in the pellet and 0% in the supernatant, in 50% sodium citrate buffer pellet retention was 46% and supernatant activity was 56%, and in 100% sodium citrate buffer (pH=4.0) pellet retention was 35% and supernatant was 75%. In this experiment, the viability of the labeled cells were 95% in PBS, 30% in 50% sodium citrate buffer; 8% in 100% sodium citrate buffer. The elution was significantly greater in the 100% sodium citrate buffer and was associated with significant cell death causing leakage of ^{89}Zr into the supernatant. These results indicate that the endocytosed ^{89}Zr remained stable in the intracellular milieu.

Example 4: Recovery of labeled cells from whole blood *in vitro*

[0118] Twenty milliliters of leukocyte rich blood obtained by the buffy coat method were labeled with the ^{89}Zr-H-PS mixture or unlabeled H-PS. Once radioactivity of labeled WBC and viability were determined, 4.7x10^{6} radiolabeled (0.024 MBq or non-radiolabeled WBC were mixed in 4 mL of autologous ACD anticoagulated whole blood. For the control sample, about 0.085 MBq of ^{89}Zr was added into in 4 mL of autologous ACD anticoagulated whole blood as well. The whole blood samples were incubated and then layered on the top of a linear Ficoll® gradient and centrifuged. In order to determine the amount of the radioactivity confined to each of the compartments (mononuclear leukocytes, erythrocytes or plasma), the samples were layered on top of a linear Ficoll®-Hypaque density gradient centrifugation. After imaging of the tubes, the mononuclear leukocytes, erythrocytes and

plasma were recovered with a Pasteur pipet, collected in eppendorf tubes and imaged (data not shown). When free ^{89}Zr was used to label whole blood, the majority (96%) of the total radioactivity was in the plasma layer and only 4% of the total radioactivity was recovered in cells. No radioactivity was measured in the mononuclear leukocytes indicating no labeling of WBC. When labeled WBC were mixed with whole blood, the mononuclear leukocyte layer contained 57% of the total radioactivity, the granulocytes layer contained 35 % of the radioactivity with only small amounts of activity retained in the erythrocytes (3.9%) and plasma (5.2%). These results were consistent with those derived from imaging of the each of the whole blood samples after Ficoll®-Hypaque density gradients.

Example 5: Relative labeling of erythrocytes and leukocytes *in vitro*

[0119] After simple mixing of the ^{89}Zr (1.85 MBq)–PS (40 μg/mL) – H (2U/mL) or ^{89}Zr (1.85 MBq)—PS (40 μg/mL) complex or ^{89}Zr (1.85 MBq)– heparin (H) (2U/mL) or ^{89}Zr (1.85 MBq) alone or non-radiolabeled control, the complexes were added to 4 mL of ACD anticoagulated whole blood and then incubated at 37°C for 30 min. The samples were layered on top of a linear Ficoll-Hypaque density gradient-centrifugation. After performing PET/CT imaging of the tubes, the cells and plasma fractions were removed and collected in eppendorf tubes. Later, fractioned WBC, erythrocytes, and plasma samples were imaged (data not shown) and a gamma camera was used to measure radioactivity. Most of the radioactivity (greater than 97%) was seen in the plasma fractions of ^{89}Zr containing mixtures but not in the control sample. There was small amount of radioactivity in erythrocytes (1.7-3 %) and no radioactivity was observed in mononuclear leukocytes.

Example 6: Labeling efficiencies

[0120] The mean labeling efficiency of WBC with ^{89}Zr-H-PS mixture was 2-4% using 1.0×10^{6} cells and 8-10% using 4.6×107 WBC, with comparable mean total activity before labeling (range 1.85–3.7 MBq); the mean cell-associated radioactivity was about 0.037 MBq and 0.37 MBq, respectively.

Example 7: *In vitro* stability of the ^{89}Zr-labeled NK cells

[0121] After the radiolabeling with ^{89}ZrH-PS, the percentage of ^{89}Zr eluted from the labeled NK cells after 3 washings at 1, 2, and 3 hr was found to be 10%±2% indicating a high degree of stability of intracellular ^{89}Zr with little extracellular leakage.

Example 8: Phenotyping of NK cells after radiolabeling

[0122] To evaluate the phenotypic changes associated with ^{89}Zr labeling on expanded NK cells in vitro, expanded NK cells were incubated in a mixture of ^{89}Zr-H-PS or H-P alone, i.e. subjected to the same labeling procedures in the absence of ^{89}Zr. NK cells were analyzed by flow cytometry at 20 days following in vitro expansion using CD56-APC, CD16-PE-Cy7, TRAIL-PE, NKG2D-APC antibodies. There was no change in NK cell surface expression of CD56, TRIAL and NKG2D between ^{89}Zr-H-PS labeled and H-P treated NK cells (Fig. 2). Cell viability was determined by staining with Annexin V-FITC. ^{89}Zr -labeling did not affect cell viability; apoptosis in ^{89}Zr-H-PS labeled and H-P treated NK cells were 14% and 16%, respectively.

Example 9: Cytotoxic function of ^{89}Zr-H-PS labeled vs. non-labeled (H-P) NK cells

[0123] The effect of labeling on the functional activity of radiolabeled or unlabeled NK cells exposed to H-P was assessed by testing the lysis of K562 target cells in vitro. At a 1:1 effector to target ratio, lysis of K562 cells was not different in radiolabeled or non-radiolabeled NK cells ($P>0.05$) (Fig. 3); radiolabeled NK cells lysed 28.6 % at day 1, 28.5% at day 2 and 78% at day 3 while non-radiolabeled NK cells lysed 28.2% at day 1, 31.3% at day 2 and 78% at day 3. These results suggest the lytic activity of NK cells is maintained regardless of the presence of ^{89}Zr and therefore, ^{89}Zr had no effect on the cytotoxic function for up to 3 days post labeling.

Example 10: In vivo Experiment- Infusion of ^{89}Zr Labeled cells via intravenous or intra-bone infusion in a swine model

[0124] First, the biodistribution of free ^{89}Zr was performed in a swine model. For that, about 1.7 mCi of ^{89}Zr in was injected intravenously as a bolus injection. Static whole body PET/CT imaging of the pig was performed up to 4 hours post injection of free ^{89}Zr under general anesthesia. The PET images from this study revealed that there was persistent blood pool activity up to 4 hr. In addition, there was a constant bone activity at the epiphysis seen after 2 hours post injection of free ^{89}Zr, suggesting specific accumulation of free ^{89}Zr in the non-soft tissue, mineralized constituents of bone (Fig. 4). In another experiment, we have injected deferoxamine mesylate USP (desferal) intravenously which resulted in near clearance of free ^{89}Zr via kidneys (data not shown).

[0125] Human CD34 positive cells and pig autologous granulocytes were selected using immune-magnetic beads, resuspended in serum-free appropriate cell medium and the

cell number was determined. The cells were labeled with ^{89}Zr as described above. Briefly, after mixing of ^{89}Zr-H-PS mixture, the cells were added into this mixture and incubated for 30 minutes at 37°C. Preliminary experiments showed that the viability of these cells was not adversely affected by this labeling procedure (data not shown); the level of radioisotope was sufficient to produce high quality images taken with a clinical PET scanner. These labeled cells were then used for the intravenous for only human CD34 positive and pig autologous granulocytes and intrabone infusion for the human CD34 positive cells.

[0126] After intravenous infusion of the autologous granulocytes, human CD34 cells, increased activity associated with these cells was detected primarily in the lungs. (Fig. 5) Up to 3 hours of imaging, there was no other uptake observed in the remainder of the body other than lungs, suggesting persistent accumulation of labeled cells in the lungs. Without being held to theory, it is believed that this phenomenon is the result of several factors. Granulocytes spend more time in contact with the pulmonary endothelium than they do with the systemic vascular endothelium. This is caused in part by the fact that the mean driving pressure across the pulmonary circulation is lower than that in the systemic circulation. Cell size is another factor. To pass through the pulmonary capillaries, which have an average diameter of 5.5 μm, granulocytes, which have an average diameter of 8 μm, must undergo cytoskeletal deformation. During the labeling procedure, granulocytes are activated and stiffen and are less easily deformed. Their transit through the pulmonary vessels consequently is slowed. Activated granulocytes adhere to the pulmonary capillaries for a longer period than do nonactivated granulocytes, further impeding their passage through the lungs. There are data to suggest that the in vitro labeling procedure itself causes prolonged pulmonary transit of granulocytes, presumably because of cell trauma during the labeling process. Pulmonary activity on WBC studies, therefore, is best assessed on images acquired more than 4 hours after injection of labeled leukocytes. Similarly, human CD34 were also trapped within the pulmonary capillaries, which is consistent with other previously reported studies. The primary factors attributed to the lodging of these cells within lungs are probably cellular diameter and cellular attachment potential.

[0127] For the intra-bone infusion, we performed a slow or bolus infusion of the ^{89}Zr labeled human CD34 positive cells. Peak IM pressures during bolus hand IB injection were high, substantially exceeding systemic systolic arterial pressures. In contrast, IM pressures during slow IB infusion were significantly lower, remaining well below diastolic arterial pressures. During manual sequential hand IB injection of 5 ml aliquots of contrast at two different sites in the ipsilateral iliac crest, dynamic CT images revealed leakage from the

initial access site after the first injection as well as immediate drainage into the ipsilateral iliac vein. Following manual hand injection of ^{89}Zr labeled human CD34+ cells (^{89}Zr-hCD34+) given IV in swine via the external jugular vein, there was persistent PET activity noted in the lungs for up to 3 hrs. Bolus hand IB injection of ^{89}Zr labeled swine BM MNCs or ^{89}Zr-hCD34+ cells revealed PET activity in the iliac bone as well as activity in the lungs. (Fig. 6) Furthermore, PET activity following bolus hand IB injection was also noted in surrounding tissues outside the bone when more than a single ipsilateral injection site was used. In contrast, slow infusion of ^{89}Zr labeled swine BMMNCs or ^{89}Zr-hCD34+cells resulted in PET activity that was limited to the iliac bone, indicating retention of cells within the marrow space with no leakage of cells to the lungs. (Fig. 7) There were foci of uptake seen in the active marrow spaces in the long bones after 2 hours post injection of the labeled cells, suggesting of redistribution of the these cells.

Example 11: Dual Labeling of the Cells and *In Vitro* MRI and PET Imaging

[0128] *In vitro* MRI of labeled WBC cells revealed that both USPIO PS and USPIO-PS-H mixture labeling methods resulted in a significant but slightly better signal intensity (SI) loss on T2*WI sequences on the USPIO-PS-H treated cells when compared to the USPIO-PS treated cells (63% and 54%, respectively). (Fig. 8) For the *in vitro* PET imaging, there was significant increased activity in the seen in the USPIO-PS-H-^{89}Zr treated cells and USPIO-PS-^{89}Zr cells but slightly more activity in the USPIO-PS-H-^{89}Zr sample when compared to the USPIO-PS-^{89}Zr. The ^{89}Zr only treated cells revealed virtually no activity in the tube. (Fig. 9)

[0129] The compositions and methods described herein have general utility in improving the understanding of and optimization of cell based therapies. This includes bone marrow and stem cell transplants, activated and genetically engineered cell labeled immunotherapy and in cell based vaccine therapy. Cell based therapies are becoming an important new form of therapy against a variety of diseases. In particular, the fate of adult stem cells is important to understanding their optimization. Using this labeling technique, the labeled stem cells/progenitor cells should contribute to our understanding of cell migration processes in the context of numerous diseases, such as neurologic and muscular diseases, myocardial infarction, and cancer. The ability to directly monitor and assess cell-based therapies in patients will be invaluable as it will allow us to investigate these therapies directly in the living subject.

[0130] The ^{89}Zr-complexes described herein provide distinct advantages over prior art labeling with indium and fluorine, for example. Unlike Indium 11-labeled cells, ^{89}Zr labeling provides PET scans with at least 10 times the sensitivity and better resolution. Unlike conventional PET radioisotopes such as Fluorine 18, ^{89}Zr has an extended half-life of 78.4 hours in contrast to ^{18}F with a half-life of 110 minutes. The time for data acquisition can be limited to 6 hours or less. This is relevant for the prolonged monitoring of cells. Using the ^{89}Zr-labeled cells disclosed herein, the migration of intravenously injected cells can be followed with PET imaging for 6 days or more. The processes described herein include methods for labeling cells *ex vivo* with agents that can be detected by PET/CT or PET/MRI techniques. The labeled cells can be administrated to patients/animals and the trafficking of the labeled cells can be tracked *in vivo*.

[0131] Using the combination of ^{89}Zr -H-PS, successful *ex vivo* labeling was achieved in WBC and in vitro expended human NK cells. Cell labeling was stable with nearly 90% of the activity retained in the cells by three hours. ^{89}Zr labeling did not effect the viability, proliferation, and cytotoxic function of NK cells. Specifically, ^{89}Zr labeling at 1.85 to 3.7 MBq, preserved viability and function in NK cells while effectively labeling the cells to permit imaging and quantitation.

[0132] Based on this data we have developed a protocol for cell labeling with ^{89}Zr, which results in optimal label retention, visualization on clinical PET/CT and insignificant cytotoxicity. The optimal ^{89}Zr labeling utilizing the H-PS complex was achieved within 30 minutes in serum free media. Because both H and PS are FDA approved and widely accepted in clinical use, and the development of ^{89}Zr based labeling procedure is compliant with good manufacturing practices, it will be feasible to translate ^{89}Zr-H-PS labeling of human cells into clinical trials. The use of a radionuclide with a long half-life could be justified by the low amount of radioactivity that is needed to obtain satisfactory imaging results.

[0133] As used herein, the term nanoparticle means a particle that has at least one dimension that is 1 to 100 nm. As used herein, the term complex means a noncovalently (e.g., electrostatic) bonded complex containing one or more nanoparticles that has at least one dimension that is 1 to 500 nm. Particle sizes can be measured, for example, by TEM or by dynamic light scattering. As used herein, the term self-assembled means formed by a process in which a disordered system of pre-existing components forms an organized structure or pattern as a consequence of specific, local interactions among the components themselves,

without external direction. A self-assembled complex is typically held together by electrostatic and/or van der Waals forces, not covalent bonds.

[0134] The use of the terms "a," "an," "the," and similar referents in the context of describing the invention (especially in the context of the following claims) are to be construed to cover both the singular and the plural, unless otherwise indicated herein or clearly contradicted by context. The terms "comprising," "having," "including," and "containing" are to be construed as open-ended terms (i.e., meaning "including, but not limited to,") unless otherwise noted. "Or" means "and/or." Recitation of ranges of values are merely intended to serve as a shorthand method of referring individually to each separate value falling within the range, unless otherwise indicated herein, and each separate value is incorporated into the specification as if it were individually recited herein. Ranges are inclusive of endpoints. The term "wt%" refers to percent by weight. All methods described herein can be performed in any suitable order unless otherwise indicated herein or otherwise clearly contradicted by context. The use of any and all examples, or exemplary language (e.g., "such as"), is intended merely to better illuminate the invention and is not meant as a limitation on the scope of the invention unless otherwise claimed. No language in the specification should be construed as indicating any non-claimed element as essential to the practice of the invention. The term "combination thereof" is inclusive of a combination that includes one or more of the recited elements, optionally together one or more like elements not recited.

[0135] All cited references are incorporated herein by reference in their entirety.

[0136] Preferred embodiments are described herein, including the best mode known to the inventors for carrying out the invention. Variations of those preferred embodiments may become apparent to those of ordinary skill in the art upon reading the foregoing description. The inventors expect skilled artisans to employ such variations as appropriate, and the inventors intend for the invention to be practiced otherwise than as specifically described herein. Accordingly, this invention includes all modifications and equivalents of the subject matter recited in the claims appended hereto as permitted by applicable law. Moreover, any combination of the above-described elements in all possible variations thereof is encompassed by the invention unless otherwise indicated herein or otherwise clearly contradicted by context.

What is claimed is:

1. A PET imaging complex, comprising
a first component that is ^{89}Zr;
a second component that is a polycation; and
an optional third component that is an anionic polysaccharide.

2. The PET imaging complex of claim 1, wherein the polycation is polyarginine, polyornithine, protamine, polylysine, histone, lipofectamine, spermine, spermidine, or a combination thereof.

3. The PET imaging complex of claim 1, wherein the polycation is a protamine sulfate.

4. The PET imaging complex of claim 1, comprising the anionic polysaccharide.

5. The PET imaging complex of claim 4, wherein the anionic polysaccharide is an anionic glycosoaminoglycan.

6. The PET imaging complex of claim 5, wherein the anionic glycosoaminoglycan is a heparin, heparan sulfate, chondroitin sulfate, dermatan sulfate, keratan sulfate, hyaluronic acid, or a combination thereof.

7. The PET imaging complex of claim 1, further comprising a superparamagnetic nanoparticle.

8. A kit for labeling biological cells for PET-imaging, comprising
a first component that is ^{89}Zr;
a second component that is a polycation; and
an optional third component that is an anionic polysaccharide.

9. The kit of claim 8, comprising the anionic polysaccharide.

10. The kit of claim 8, wherein the ^{89}Zr is in a first container and the polycation and optionally the anionic polysaccharide are in a second container.

11. The kit of claim 8, further comprising a superparamagnetic nanoparticle.

12. A biological cell labeled with ^{89}Zr, wherein the ^{89}Zr is not linked to an antibody or drug molecule.

13. The biological cell of claim 12, further comprising a superparamagnetic nanoparticle composition.

14. A method of labeling a biological cell with the PET imaging complex of claim 1, comprising

contacting a biological cell in culture medium with the first, second and optionally the third components, wherein the PET-imaging complexes of claim 1 are formed in the culture medium, or

contacting a biological cell in culture medium with pre-formed PET imaging complexes of claim 1,

wherein contacting is done under conditions to label the biological cell with complexes.

15. The method of claim 14, wherein the PET imaging complexes comprise the anionic polysaccharide.

16. The method of claim 14, wherein labeling with PET imaging complexes comprises internalization of the PET-imaging complexes into endosomes.

17. The method of claim 14, wherein the biological cell is a white blood cell, a stem cell, an immune cell, or a cancer cell.

18. The method of claim 14, further comprising contacting the biological cell in culture with superparamagnetic nanoparticles.

19. A method of detecting a biological cell in a subject, comprising,

administering to the subject a labeled biological cell comprising PET imaging complexes of claim 1, and

examining at least a portion of the subject by PET imaging, thereby detecting the labeled biological cell in the subject.

20. The method of claim 19, wherein the PET imaging complexes comprise the anionic polysaccharide.

21. The method of claim 19, wherein administering the labeled biological cell comprises intravenous, intra-arterial, and/or direct tissue injection.

22. The method of claim 19, wherein examining at least a portion of the subject by PET imaging comprises tracking the migration of the labeled biological cell in the subject.

23. The method of claim 19, wherein the biological cell further comprises superparamagnetic nanoparticles, and examining at least a portion of the subject by PET imaging comprises PET-MR imaging.

24. A method of transplanting a biological cell into a subject, comprising

administering to the subject a labeled biological cell comprising the PET imaging complexes of claim 1,

examining at least a portion of the subject by PET imaging,

detecting the migration pattern and/or cellular distribution pattern of the labeled biological cell in the subject, and

optionally administering additional biological cells.

25. The method of claim 24, wherein the PET imaging complexes comprise the anionic polysaccharide.

26. The method of claim 24, wherein the labeled biological cells are stem cells and administering the labeled biological cells is for organ repair or revascularization.

27. The method of claim 26, wherein administering to the subject comprises administering the labeled biological cells to a damaged tissue in the subject by localized or systemic injection.

28. The method of claim 24, wherein the labeled biological cells are administered in a composition comprising a matrix implant.

29. The method of claim 24, wherein the labeled biological cells are injected intravenously or intra-arterially, and the labeled biological cells are detected in two or more organs of the subject.

30. The method of claim 24, wherein the labeled biological cells are immune cells, dendritic cells, B-cells, T-cells, or natural killer cells.

31. The method of claim 30, wherein the immune cells, dendritic cells, B-cells, T-cells, or natural killer cells are administered with systemic immunotherapy.

32. The method of claim 24, wherein the labeled biological cells comprise an expression cassette that directs the expression of a polynucleotide coding sequence.

33. The method of claim 24, wherein the labeled biological cells comprise white blood cells.

34. The method of claim 24, wherein the labeled biological cells are in a cord blood sample, and administration is intra-bone delivery.

35. The method of claim 24, wherein the biological cell further comprises superparamagnetic nanoparticles, and examining at least a portion of the subject by PET imaging comprises PET-MR imaging.

36. A method of treating a subject with a disease or injury, comprising

administering to the subject with a disease or injury a labeled biological cell comprising the PET imaging complexes of claim 1,

examining at least a portion of the subject by PET imaging,

detecting the migration pattern and/or cellular distribution pattern of the labeled biological cell in the subject, and

optionally administering additional biological cells.

37. The method of claim 36, wherein the PET imaging complexes comprise the anionic polysaccharide.

38. The method of claim 36, wherein the biological cell is a stem cell.

39. The method of claim 38, wherein the disease is a cardiovascular disease, diabetes, a neurodegenerative disorder, or cancer.

40. The method of claim 36, wherein the biological cell further comprises superparamagnetic nanoparticles, and examining at least a portion of the subject by PET imaging comprises PET-MR imaging.

41. A method of imaging the lymphatic system of an individual, comprising intradermally administering the PET imaging complexes of claim 1 and detecting the migration pattern and/or cellular distribution pattern of the PET imaging complexes in the subject.

42. The method of claim 41, further comprising intradermally administering superparamagnetic nanoparticles.

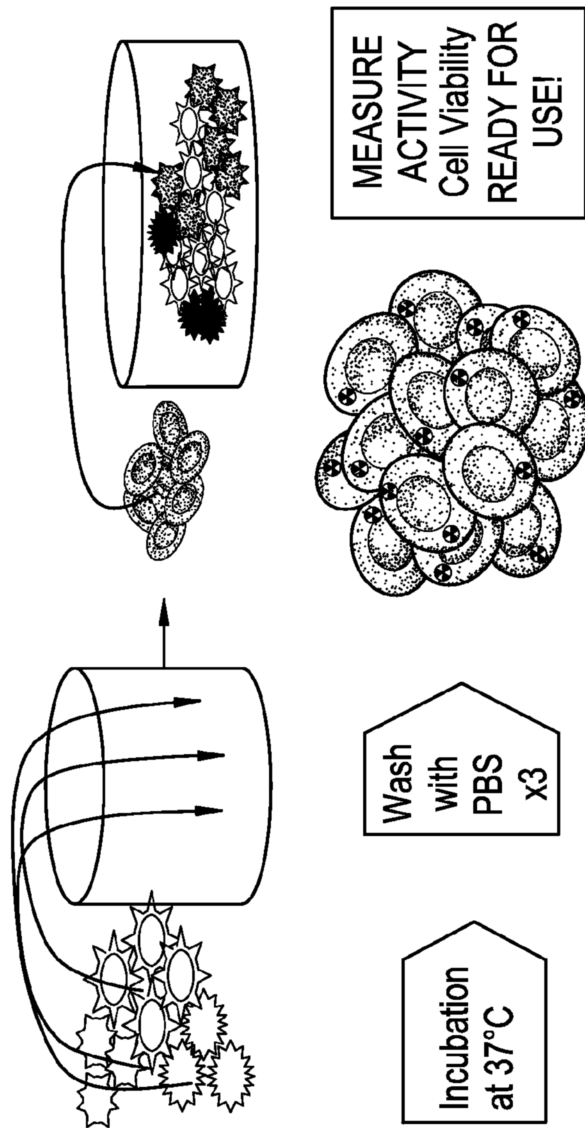


FIG. 1

FIG. 2A

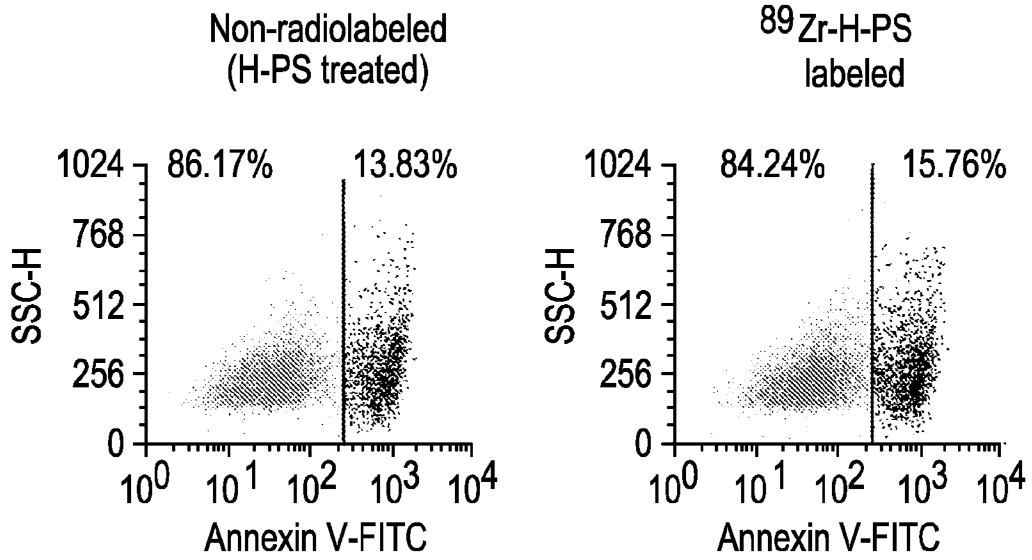
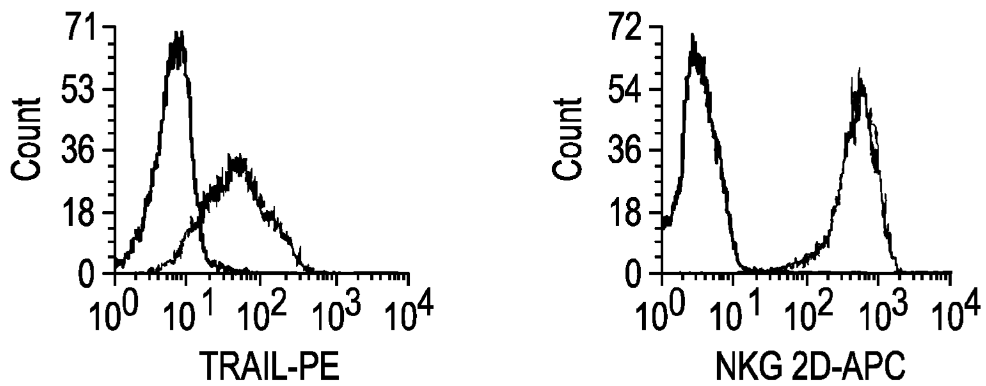


FIG. 2B



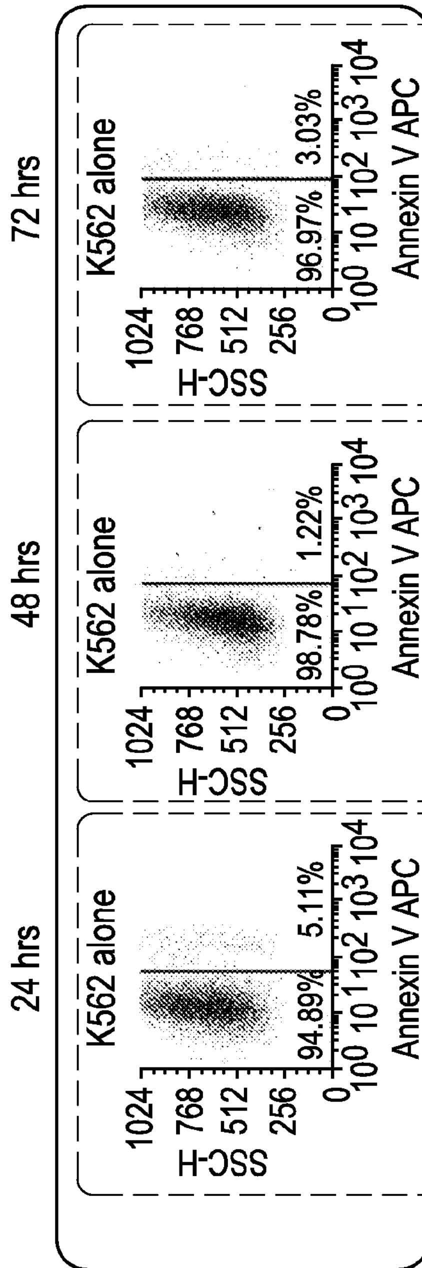


FIG. 3A
24 hrs
48 hrs
72 hrs
K562 alone
94.89%
5.11%
98.78%
1.22%
96.97%
3.03%
SSC-H
1024 768 512 256 0
Annexin V APC
10^0 10^1 10^2 10^3 10^4
FIG. 3B
Non-radiolabeled (H-PS treated)
89 Zr-H-PS labeled
FSC-H
0 256 512 768 102
Co-culture with NK cells
71.84%
28.16%
68.75%
31.25%
22.28%
77.72%
71.40%
28.60%
71.55%
28.45%
22.00%
78.00%

FIG. 4

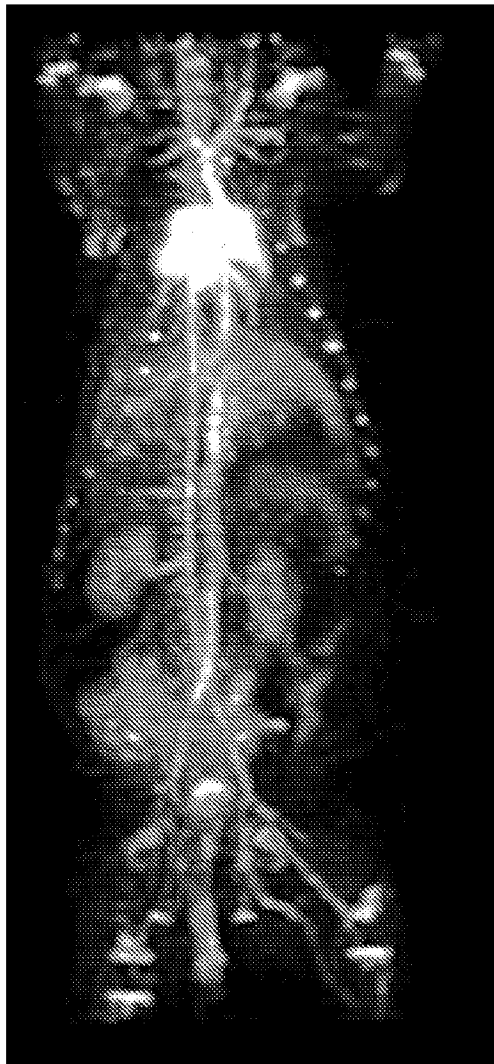


FIG. 5

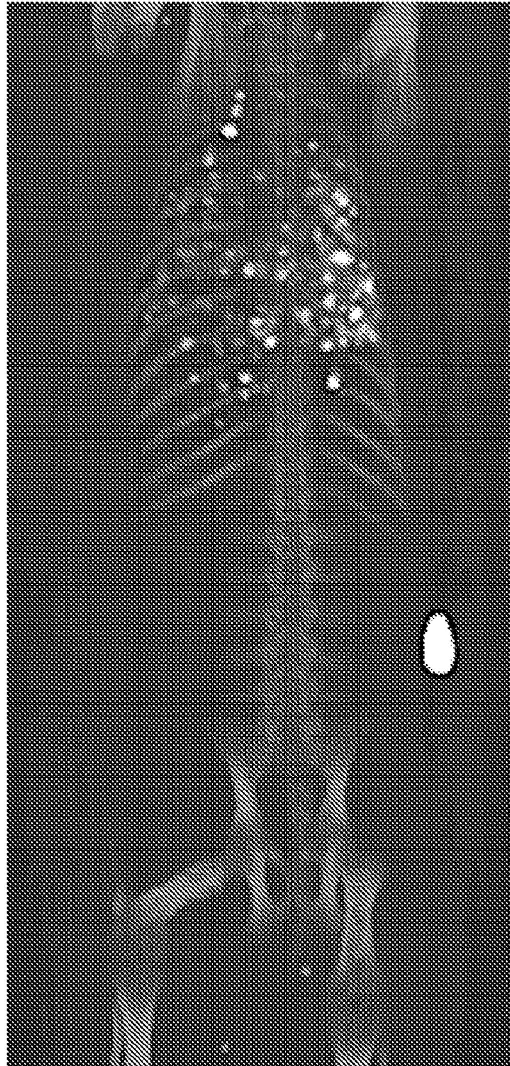


FIG. 6A

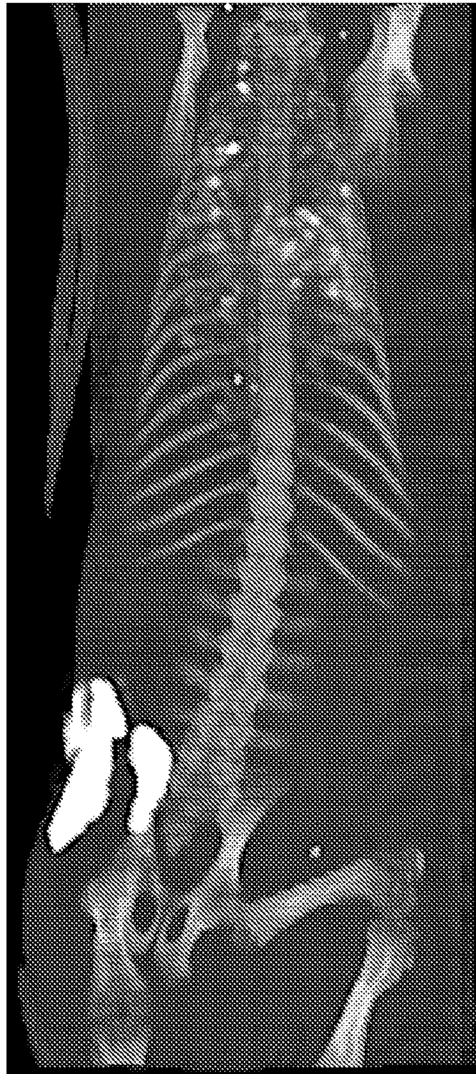


FIG. 6B

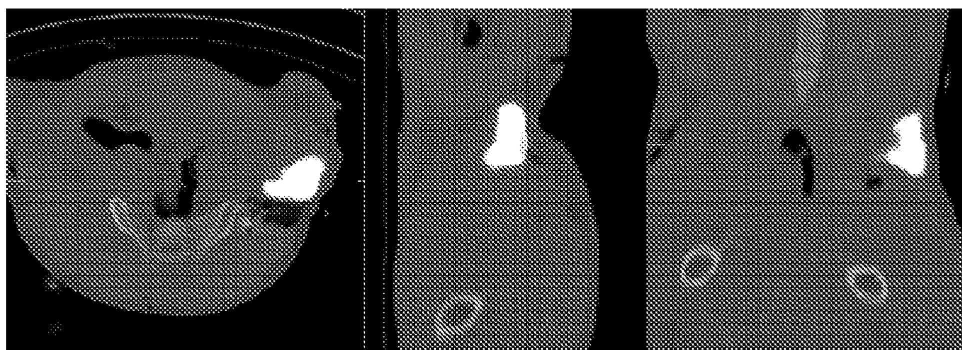


FIG. 7

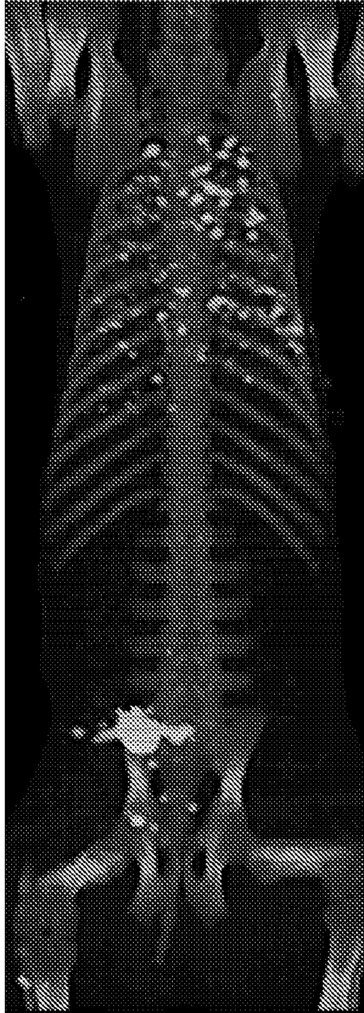


FIG. 8

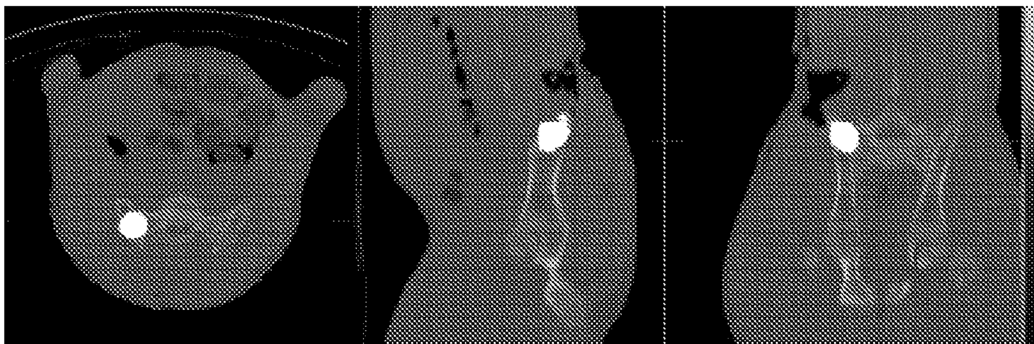
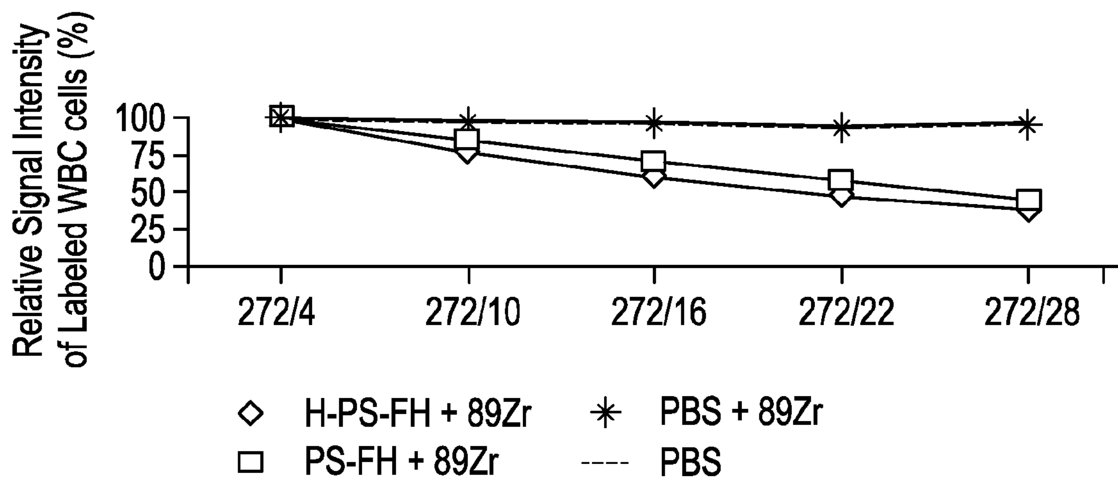
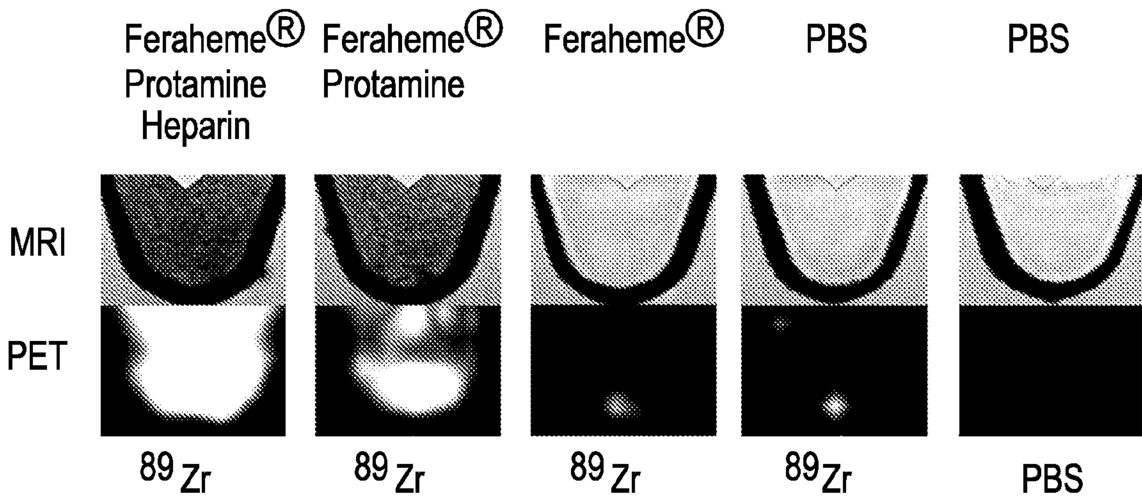


FIG. 9





INTERNATIONAL SEARCH REPORT

International application No
PCT/US2013/031944

A. CLASSIFICATION OF SUBJECT MATTER
INV. A61K51/04 A61K51/08 A61K51/06 A61K51/12
ADD. A61K103/00

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

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
A61K

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

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

EPO-Internal, BIOSIS, WPI Data, EMBASE, INSPEC, COMPENDEX

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 90/13256 A1 (WISCONSIN ALUMNI RES FOUND [US]) 15 November 1990 (1990-11-15) page 5, lines 8-18 page 8, lines 15-18,30-31 page 9, lines 1-4 page 16, line 14 page 17, lines 23-31 claims -----	1-3,7,8, 10-14, 16-18

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[X] Further documents are listed in the continuation of Box C. [X] See patent family annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
24 April 2013	14/05/2013

Name and mailing address of the ISA/	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Fax: (+31-70) 340-3016	Villard, Anne-Laure

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 2009/129578 A1 (UNIV AUSTRALIAN [AU]; STEPHENS ROSS WENTWORTH [AU]; SENDEN TIMOTHY JOH) 29 October 2009 (2009-10-29) page 1, lines 10-12 page 8, line 15 page 15, lines 4-10 page 16, line 32 - page 17, line 1 page 19, line 1 page 22, lines 29-32 page 25, lines 21-25 claims 1,10,11,14 -----	1-3,8, 10,12, 14,16,41
X	US 2007/122346 A1 (UZGIRIS EGIDIJUS E [US] ET AL UZGIRIS EGIDIJUS EDWARD [US] ET AL) 31 May 2007 (2007-05-31) paragraphs [0054], [0058], [0063], [0070] -----	1,2,8,10
X	KELIHER EDMUND J ET AL: "89Zr-labeled dextran nanoparticles allow in vivo macrophage imaging.", BIOCONJUGATE CHEMISTRY 21 DEC 2011, vol. 22, no. 12, 21 December 2011 (2011-12-21), pages 2383-2389, XP002695935, ISSN: 1520-4812	1,4, 7-23,41, 42
Y	page 2383, right-hand column figure 1A page 2384, left-hand column, last paragraph - right-hand column, paragraph 1 page 2385, left-hand column figure 2 pages 2385-2387, section "In vivo imaging" figure 4 page 2387, right-hand column, paragraph 2 -& KELIHER EDMUND J ET AL: "Supporting Information: 89Zr-labeled dextran nanoparticles allow in vivo macrophage imaging.", BIOCONJUGATE CHEMISTRY, vol. 22, no. 12, 21 December 2011 (2011-12-21), XP002696426, the whole document ----- -/--	19-40

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	TERROVITIS JOHN V ET AL: "Assessment and Optimization of Cell Engraftment After Transplantation Into the Heart", CIRCULATION RESEARCH, vol. 106, no. 3, February 2010 (2010-02), pages 479-494, XP002695936, ISSN: 0009-7330 pages 485-486, section "Direct Radiolabeling for Single Photon Emission Tomography or Positron Emission Tomography" -----	19-40
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INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No
PCT/US2013/031944

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