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(54) Title: PEPTIDE IMAGING AGENT TARGETED TO THE EXTRACELLULAR PORTION OF CD115

(57) Abstract: The invention relates to imaging agents and peptides which bind to a human CD115 receptor as well as to the use of the imaging agents for identifying CD115 expression and patients at high risk of acute ischemic attack.



PEPTIDE IMAGING AGENT TARGETED TO THE EXTRACELLULAR PORTION OF CD115

Field of the Invention

- 5 The invention relates to an imaging agent which specifically targets Cluster of Differentiation 115 (CD115), also known as Colony Stimulating Factor 1 Receptor (CSFR1), and Macrophage Colony-Stimulating Factor Receptor (M-CSFR).

Background to the Invention

- 10 It has long been known that monocyte arterial infiltration is central to atherosclerosis development. Their progressive accumulation within atherosclerotic lesions not only promotes plaque growth but, more importantly, contributes to lesion vulnerability (erosion/rupture), in turn leading to acute cardiovascular thrombotic events including myocardial infarction and stroke.

15

- Technical advances in experimental imaging modalities applied to experimental animal models of atherosclerosis, which enable monocyte *in vivo* tracking, have promoted an understanding of monocyte biology during disease progression and it is now clear that monocyte accumulation within atherosclerotic plaques results from an imbalance between
- 20 their ingress to, and egress from, lesions. The pharmacological/genetic manipulation of key molecules that regulate these events has been shown to impact importantly on disease progression in animal models, and may provide potential novel anti-atherogenic strategies exploitable in human disease.

- 25 However, such technical advances in the pre-clinical setting have not been paralleled by similar development of diagnostic modalities that would be clinically applicable for the characterisation and monitoring of plaque composition in a longitudinal and non-invasive manner. As a consequence, the translatability of pre-clinical findings to the clinic as regards the anti-inflammatory/anti-atherogenic effect of conventional and novel emerging
- 30 pharmacological therapies has been severely limited.

One reported method for non-invasive imaging of monocytes/macrophages in humans is iron-oxide based imaging by magnetic resonance imaging (MRI), which relies on the phagocytic ability of monocytes and macrophages to take up iron oxide particles whose accumulation

within tissue can be then visualized as a signal void on T2-weighted images or quantified with T2* mapping sequences. However, this approach carries important limitations in terms of specificity and sensitivity for myeloid cells, since iron particles are taken up by cell types other than monocytes and macrophages such as endothelial cells, and artefacts at tissue
5 interfaces related to motion or calcification can mimic the signal void.

An alternative approach uses PET/CT (positron emission tomography-computed tomography) imaging. Specifically, ^{18}F -fluorodeoxyglucose (^{18}F FDG)-PET which is currently used clinically, especially for cancer imaging, has been proposed as an alternative approach to
10 detect arterial inflammation, based on pre-clinical evidence that macrophages take up ^{18}F FDG more avidly than other cell types, thus providing intense signal within inflamed tissues. However, it has been reported that ^{18}F FDG uptake can be altered in patients with metabolic disorders (obesity and diabetes), since it depends on cellular glucose transport, which is deranged in such conditions; and this potentially constitutes an important limiting factor to its
15 usefulness in patients at high cardiovascular risk. Moreover, image acquisition of the coronary arteries is compromised by background noise due to myocardial tracer uptake.

There is therefore a need to develop novel non-invasive imaging approaches that would be applicable in the clinic in a longitudinal and non-invasive manner, to provide reliable
20 information on the degree of plaque inflammation, thus improving the accuracy of cardiovascular risk stratification related to plaque vulnerability so that preventative strategies can be more effectively targeted (e.g. with more intensive anti-platelet or lipid-lowering therapies). An imaging tool, able to detect vascular inflammation in humans in a non-invasive manner, would also implement clinical and pharmaceutical research with a
25 diagnostic technology thus enabling a better understanding of human atherosclerosis progression and response to therapy. This would promote major advances in the study of inflammatory events in the context of atherosclerosis and would be informative of the anti-inflammatory efficacy of conventional, as well as emerging, drug therapies.

30 The inventors have developed a new imaging agent comprising a portion of the amino acid sequence which encodes for the human macrophage colony-stimulating factor (M-CSF), which is the autologous ligand of CD115 (Chitu *et al*, Curr Opin Immunol. 2006 Feb; 18(1): 39-48). Chen X, *et al*. (Chen X, *et al*. Proc Natl Acad Sci USA 2008; 105:18267-18272)

conducted an X-ray crystallographic analysis of the interface between murine M-CSF and its cognate ligand. The human M-CSF protein, in the form of a truncated isoform binding the human CD115, has been characterised by Panditi J *et al* (Panditi J, *et al.* Science 1992; 258(5086):1358-1362). The imaging agent of the present invention is targeted to the
5 extracellular portion of CD115, which is a universal marker of myeloid cell lineage, equally and highly expressed on both human and murine monocytes (Ingersoll MA, *et al.* Blood 2010; 115: e10-e19). Low levels of expression also are found on granulocytes. Also, other cell types belonging to the myeloid cell lineage express CD115 on their extracellular membrane, including Kupffer cells, microglia, and osteoclasts. Moreover, abnormal expression of
10 CD115 has been described in breast and ovarian cancer and myeloid leukemic blast cells. Increased expression of CD115 has also been reported in astrocytes and neurons following injury.

The imaging agent of the present invention specifically localises within, and enhances,
15 inflamed atherosclerotic vessels without any evidence of accumulation within disease-free regions of the arterial wall. The localisation of the imaging agent within atherosclerotic plaques is not attributable to non-specific accumulation of the peptide in the vascular wall, but rather to a targeted action. Indeed, this imaging agent has high specificity for myeloid cells given its ability to bind to the extracellular domain of CD115. Targeted MRI-imaging agents
20 have been developed and described in the literature by other groups, but they generally consist of iron-oxide/microcelle particles which, due to their molecular mass, show poor tissue permeability. In addition, they generally undergo cellular internalisation by targeted cells so that accumulation within tissues persists for weeks before removal via the reticuloendothelial system. On the contrary, the present imaging agent remains localised within the extracellular
25 space and has a low molecular mass. These features not only allow for excellent tissue penetration but may also account for the more favourable pharmacokinetic profile compared to nanoparticles since the imaging agent of the present invention undergoes rapid (within hours) renal clearance. Binding to CD115 also occurs without activation of the M-CSF receptor, and this makes the present imaging agent biologically inert. Compared to the
30 PET/CT techniques described above, the present approach does not necessitate the use of radiotracers, and this is an important and additional safety consideration. Moreover, the present imaging agent is suitable for MRI imaging which provides improved anatomical resolution. Other targeted MRI imaging agents have been developed (US2006/0239913), but

the specificity of the present imaging agent for CD115 makes it uniquely specific for the detection of myeloid cells.

5 Summary of the Invention

In a first aspect of the invention, there is provided an imaging agent comprising:

- a peptide having the amino acid sequence of SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 3 or SEQ ID NO: 4, or a sequence that differs from SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 3 or SEQ ID NO: 4 by 1, 2 or 3 amino acids, wherein the peptide binds to a human CD115 receptor; and
- a signal entity.

The inventors have surprisingly found that this imaging agent is specifically targeted to the extracellular portion of CD115. The specificity of the imaging agent for CD115 makes it uniquely specific for detection of monocytes/macrophages, as well as of other cell types belonging to the myeloid cell lineage and expressing CD115. The imaging agent of the present invention localises within atherosclerotic plaques and gives plaque enhancement without any evidence of accumulation within healthy vessels. Furthermore, the imaging agent of the present invention has a low molecular mass (~ 1 kDa) and does not bind to plasma proteins such as albumin, (molecules with molecular mass < 10 kDa are freely filtered by the glomeruli when unbound to plasma proteins) and this not only allows for excellent tissue penetration but also accounts for a favourable pharmacokinetic profile by undergoing rapid (within hours) renal clearance. Binding to CD115 by the imaging agent does not cause receptor activation. These are important safety considerations. Additionally, the imaging agent of the present invention is suitable for MRI imaging which provides optimal anatomical resolution in a non-invasive manner.

The peptide has the amino acid sequence of SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 3 or SEQ ID NO: 4, or a sequence that differs from SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 3 or SEQ ID NO: 4 by 1, 2 or 3 amino acids. This means that the peptide is selected from the amino acid sequence of SEQ ID NO: 1 with 1, 2 or 3 amino acid changes, the amino acid sequence of SEQ ID NO: 2 with 1, 2 or 3 amino acid changes, the amino acid sequence of

- SEQ ID NO: 3 with 1, 2 or 3 amino acid changes, or the amino acid sequence of SEQ ID NO: 4 with 1, 2 or 3 amino acid changes. In one embodiment, the peptide is selected from a peptide having the amino acid sequence of SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 3 or SEQ ID NO: 4, or a sequence that differs from SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 3 or SEQ ID NO: 4 by 1 or 2 amino acids. In another embodiment, the peptide is selected from a peptide having the amino acid sequence of SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 3 or SEQ ID NO: 4, or a sequence that differs from SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 3 or SEQ ID NO: 4 by one amino acid.
- 10 In a preferred embodiment, the peptide has the sequence of SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 3 or SEQ ID NO: 4. In a particularly preferred embodiment, the peptide has the sequence of SEQ ID NO: 1 or SEQ ID NO: 4.

- In one embodiment, the imaging agent may comprise more than one peptide as defined above.
- 15 For example, the imaging agent may comprise a single signal entity which is bound to a plurality of peptides, preferably two peptides. The plurality of peptides may, or may not be, identical.

- As herein described the term "peptide" refers to any peptide comprising amino acids joined to each other by peptide bonds or modified peptide bonds, i.e., peptide isosteres. The term "peptide" as used herein is generally between 5 and 15 amino acids in length. The peptide generally will contain naturally occurring amino acids but may include amino acid sequences modified either by natural processes, such as post-translational processing, or by chemical modification techniques which are well known in the art. Such modifications are well described in basic texts. Modifications can occur anywhere in a peptide, including the peptide backbone, the amino acid side-chains and the amino or carboxyl termini. It will be appreciated that the same type of modification may be present in the same or varying degrees at several sites in a given peptide. Also, a given peptide may contain many types of modifications. In addition to proteinogenic amino acids, the peptide may comprise amino acid isomers. Such isomers are well known to one skilled in the art and may include, for example, norleucine and norvaline.
- 20
- 25
- 30

As used herein a "deletion" is defined as a change in an amino acid sequence in which one or more amino acid residues, respectively, are absent. As used herein an "insertion" or "addition" is a change in an amino acid sequence which has resulted in the addition of one or more amino acid residues, respectively, as compared to the amino acid sequence of SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 3 or SEQ ID NO: 4. As used herein "substitution" is defined as the replacement of one or more amino acids by different amino acids, respectively.

The peptide may have deletions, insertions or substitutions of amino acid residues which produce a silent change and result in a functionally equivalent peptide, i.e., a peptide that binds to human CD115. For example, amino acid substitutions may be made on the basis of similarity in polarity, charge, solubility, hydrophobicity, hydrophilicity, and/or the amphipathic nature of the residues. For example, negatively charged amino acids include aspartic acid and glutamic acid; positively charged amino acids include lysine and arginine; and amino acids with uncharged polar head groups having similar hydrophilicity values include leucine, isoleucine, valine, glycine, alanine, asparagine, glutamine, serine, threonine, phenylalanine, and tyrosine.

The peptide has the amino acid sequence of SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 3 or SEQ ID NO: 4, or a sequence that differs from SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 3 or SEQ ID NO: 4 by 1, 2 or 3 amino acids. The amino acid differences include any substitution of, variation of, modification of, replacement of, deletion of or addition of an amino acid. As will be appreciated by those skilled in the art a number of different changes can be made, for example, one amino acid could be substituted and another amino acid added, provided the peptide only differs by 3 amino acids from SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 3 or SEQ ID NO: 4. One skilled in the art can determine which amino acids to change. For example, one skilled in the art would be able to determine which amino acids can be changed without affecting the peptide's tertiary structure.

As indicated above, the peptide binds to a human CD115 receptor. Methods for determining that the peptide binds to the human CD115 receptor are known in the art, and are described in detail elsewhere in this document.

In one embodiment of the invention, the signal entity is any signal entity that enables imaging.

In particular, the signal entity is suitable for medical imaging. Medical imaging includes magnetic resonance imaging (MRI), nuclear medicine, x-ray, ultra-sound, optical imaging and fluoroscopy. In a preferred embodiment, the medical imaging is MRI.

- 5 A wide variety of signal entities are known by those skilled in the art and may be used to enable imaging. For example, suitable signal entities include radionuclides, enzymes, fluorescent agents, chemiluminescent agents, chromogenic agents, magnetic particles, and the like.
- 10 In one embodiment of the invention the signal entity is a metal chelate. The chelate may be a linear or macrocyclic chelate. Chelates (chelators, chelating ligands) for magnetic resonance imaging are selected to form stable complexes with paramagnetic metal ions, such as Gd (III), Dy (III), Fe (III), Mn (III) and Mn (II), and include the residue of a polyaminopolycarboxylic acid, either linear or cyclic, in racemic or optically active form, such as
- 15 ethylenediaminetetracetic acid (EDTA), diethylenetriaminopentaacetic acid (DTPA), N-[2-[bis(carboxymethyl)amino]-3-(4-ethoxyphenyl)propyl]-N-[2-[bis(carboxymethyl)amino]ethyl]-L-glycine (EOB-DTPA), N,N-bis[2-[bis(carboxymethyl)amino]ethyl]-L-glutamic acid (DTPA-GLU), N,N-bis[2-[bis(carboxymethyl)amino]ethyl]-L-lysine (DTPA-LYS), the DTPA mono- or bis-amide
- 20 derivatives, such as N,N-bis[2-[carboxymethyl[(methylcarbamoyl)methyl]amino]ethyl]glycine (DTPA-BMA), 4-carboxy-5,8,11-tris(carboxymethyl)-1-phenyl-2-oxa-5,8,11-triazatridecan-13-oic acid (BOPTA), 1,4,7,10-tetraazacyclododecan-1,4,7,10-tetraacetic acid (DOTA), 1,4,7,10-tetraazacyclododecan-1,4,7-triacetic acid (DO3A), 10-(2-hydroxypropyl)-1,4,7,10-tetraazacyclododecan-1,4,7-triacetic acid (HPDO3A), 2-methyl-1,4,7,10-
- 25 tetraazacyclododecan-1,4,7,10-tetraacetic acid (MCTA), (alpha, alpha', alpha'', alpha''')-tetramethyl-1,4,7,10-tetraazacyclododecan-1,4,7,10-tetraacetic acid (DOTMA), 3,6,9,15-tetraazabicyclo[9.3.1]pentadeca-1(15),11,13-triene-3,6,9-triacetic acid (PCTA), or of a derivative thereof wherein one or more of the carboxylic groups are in the form of the corresponding salts, esters, or amides; or of a corresponding compound wherein one or more
- 30 of the carboxylic groups is replaced by a phosphonic and/or phosphinic group, such as for instance 4-carboxy-5,11-bis(carboxymethyl)-1-phenyl-12-[(phenylmethoxy)methyl]-8-(phosphonomethyl)-2-oxa-5,8,11-triazatridecan-13-oic acid, N,N'-[(phosphonomethylimino)di-2,1-ethanediyl]bis[N-(carboxymethyl)glycine], N,N'-

[(phosphonomethylimino)di-2,1-ethanediyl]bis[N-(phosphonomethyl)glycine], N,N'-[(phosphinomethylimino)di-2,1-ethanediyl]bis[N-(carboxymethyl)glycine], 1,4,7,10-tetraazacyclododecane-1,4,7,10-tetrakis[methylen(methylphosphonic)]acid, or 1,4,7,10-tetraazacyclododecane-1,4,7,10-tetrakis[methylen(methylphosphinic)]acid. Usable chelates
 5 may also be DOTA gadofluorins, DO3A, HPDO3A, TETA, TRITA, HETA, DOTA-NHS, M4DOTA, M4DO3A, PCTA and their derivatives, 2-benzyl-DOTA, alpha- (2-phenethyl) 1,4,7,10-tetraazacyclododecane-1-acetic-4,7,10-tris (methylacetic) acid, 2benzyl-cyclohexyldiethylenetriaminepentaacetic acid, 2-benzyl-6methyl-DTPA, and 6,6''-bis [N, N, N'', N'' tetra(carboxymethyl)aminomethyl]-4'-(3-amino-4-methoxyphenyl)-2,2':6',2''-terpyridine, N,N'-bis-(pyridoxal-5-phosphate) ethylenediamine-N,N'-diacetic acid (DPDP)
 10 and ethylenedinitrilotetrakis (methylphosphonic) acid (EDTP).

Appropriate chelates are not limited to this list; other chelates with good efficiency in imaging diagnostics may be used.

15

Preferred chelators are DTPA, DOTA, DTPA BMA, BOPTA, DO3A, HPDO3A, TETA, TRITA, HETA, M4DOTA, DOTMA, MCTA, PCTA and the derivatives thereof. In one embodiment the chelate is preferably DOTA.

20 When the signal entity is a chelate, the signal entity comprises a chelate complexed with a metal ion. Preferred metal ions are paramagnetic metal ions including ions of transition and lanthanide metals (i.e. metals having atomic number of 21 to 29, 42 to 44, or 58 to 70). In particular, ions of Mn, Fe, Co, Ni, Eu, Gd, Dy, Tm, and Yb are preferred, with those of Mn, Fe, Eu, Gd, and Dy being more preferred and Gd being the most preferred. In particular, Gd
 25 is preferred for use in clinical MRI.

In nuclear medicine diagnostics, the metal chelate is selected to form stable complexes with the metal ion chosen for the particular application. Chelators or bonding moieties for diagnostic radiopharmaceuticals are selected to form stable complexes with the radioisotopes
 30 that have imageable gamma ray or positron emissions such as ⁹⁹Tc, ¹¹⁷Sn, ¹¹¹In, ⁹⁷Ru, ⁶⁷Ga, ⁶⁸Ga, ⁸⁹Zr, ¹⁷⁷Lu, ⁴⁷Sc, ¹⁰⁵Rh, ¹⁸⁸Re, ⁶⁰Cu, ⁶²Cu, ⁶⁴Cu, ⁶⁷Cu, ⁹⁰Y, ¹⁵⁹Gd, ¹⁴⁹Pr, ¹⁶⁶Ho.

- Chelators for technetium are selected preferably from diaminedithiols, monoamine-monoamidedithiols, triamide-monothiol, monoamine-diamide-monothiol, diaminedioximes, and hydrazines. The chelators are generally tetradentate with donor atoms selected from nitrogen, oxygen and sulfur. Preferred reagents are comprised of chelators
- 5 having amine nitrogen and thiol sulfur donor atoms and hydrazine bonding units. The thiol sulfur atoms and the hydrazines may bear a protecting group which can be displaced either prior to using the reagent to synthesize a radiopharmaceutical or preferably in situ during the synthesis of the radiopharmaceutical.
- 10 Chelators for ^{111}In , ^{86}Y , copper and gallium isotopes are typically selected from cyclic and acyclic polyaminocarboxylates such as DTPA, DOTA, D03A, 2benzyl-DOTA, alpha- (2-phenethyl) 1,4,7,10-tetraazacyclododecane-1-acetic-4,7,10-tris(methylacetic)acid, 2-benzylcyclohexyldiethylenetriaminepentaacetic acid, 2-benzyl-6-methyl DTPA, and 6,6"-bis [N, N, N", N"-tetra(carboxymethyl)aminomethyl-4'-(3-amino-4-methoxyphenyl)-2,2':6',2"-
- 15 terpyridine.

According to another embodiment, the signal entity is a fluorescent agent for time-resolved fluorescence assays. The unique fluorescent properties of lanthanide metals, which are long-lived luminescence molecules, make them attractive for application in cellular imaging due to

20 the lack of overlapping spectra with autofluorescent molecules. Lanthanide chelates have been successfully applied to *in vivo* cellular imaging (microscopy and spectroscopy) as well as *in vitro* bioassays for protein detection. The fluorescent signal emitted by lanthanides upon excitation is generally detected by time-resolved fluorescence or used as a source of excitation light for near infrared fluorescent (NIRF) molecules. The excitation light is absorbed by the

25 NIRF molecule that then emits light (fluorescence) spectrally distinguishable (longer wavelength) from the excitation light.

Fluorescent agents are suited for studying biological phenomena, as has been done extensively in fluorescence microscopy. If fluorescent probes are to be used in living

30 systems, the choice is generally limited to the near infrared spectrum (600-1000 nm) to maximize tissue penetration by minimizing absorption by physiologically abundant absorbers such as hemoglobin (<550 nm) or water (>1200 nm). Typically the fluorochromes are designed to emit at 800+/-50 nm. A variety of NIRF molecules have been described and/or

are commercially available, including: Cy5.5 (Amersham, Arlington Heights, Ill.); NIR-1 (Dojindo, Kumamoto, Japan); 1RD382 (LI-COR, Lincoln, Nebr.); La Jolla Blue (Diatron, Miami, Fla.); ICG (Akorn, Lincolnshire, Ill.); and ICG derivatives (Serb Labs, Paris, France). Quantum dots derivatives (inorganic fluorophores comprising nanocrystal) may also be used.

- 5 Lanthanide chelates have also been used as energy donors to excite NIRF molecules, such a technique being applied to both *in vivo* imaging and *in vitro* bioassays.

In one embodiment of the invention the peptide is directly bound to the signal entity. In an alternative embodiment of the invention, the peptide is bound to the signal entity by a linker.

- 10 Preferably, the peptide is directly bound to the signal entity.

The signal entity may be bound to the peptide at any suitable position provided the peptide remains capable of binding to the human CD115 receptor. For example, the signal entity may be bound to the N-terminus or the C-terminus of the peptide. Alternatively, the signal entity
15 may be bound at some point between the N-terminus and the C-terminus of the peptide. In one embodiment the peptide is bound to the N-terminus. In another embodiment, the signal entity is bound to both the N-terminus and the C-terminus.

As indicated above, the signal entity may be bound to the peptide via a linker. Any suitable
20 linker may be used including peptide linkers and chemical linkers. Suitable linkers are well known to those skilled in the art.

A second aspect of the invention relates to the use of the imaging agent of the present invention for identifying the level of CD115 expression in an individual. Preferably an
25 altered level of CD115 expression is identified. By an altered level of CD115 expression it is meant an altered level compared to the level observed in a normal, healthy individual. Preferably, an increase in the level of CD115 expression is identified. In particular, an increased level of CD115 expression indicates the presence of tissue inflammation as indicated by the presence of CD115-expressing cells, predominately myeloid cells such as
30 monocytes and macrophages. Increased CD115 expression, or the presence of myeloid cells, can be identified in an individual or in a sample (e.g., tissue sample) obtained from an individual, in particular, a human. By identifying an increased level of CD115 expression, or

the presence of myeloid cells, it is possible to diagnose a disease associated with tissue inflammation or determine an individual's risk of developing an inflammation-related disease.

In the setting of cardiovascular disease, particular pathological conditions associated with
5 tissue infiltration by myeloid cells include atherosclerosis, arteritis (i.e Takayasu arteritis),
arterial aneurysms, myocardial infarction and myocarditis. Furthermore, by determining the
degree of atherosclerotic plaque inflammation in an individual it is possible to identify plaque
vulnerability and the associated risk of an acute ischemic event. In the context of
neurological diseases, stroke, Alzheimer's disease and autoimmune disorders (i.e. multiple
10 sclerosis) are known to be associated with myeloid cell infiltration of the central nervous
system (CNS) as well as with abnormal expression of CD115 by neurons and astroglia. As
regards oncological diseases, blast cells expressing CD115 characterise acute myeloid
leukemia; myeloid cell infiltration of solid tumours contributes to neoangiogenesis and cancer
vascularisation and growth, thus influencing the individual's prognosis; ovarian and breast
15 cancers can display increased level of CD115 on tumorigenic cells and this is considered a
negative prognostic factor; and liver cancer/metastasis replaces normal hepatic tissue with
absence of Kupffer cells, thus leading to a loss of hepatic CD115 expression. Finally,
increased colonisation of transplanted organs by myeloid cells predict the risk of allograft
rejection. The peptide and the signal entity are as defined above.

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In a particular embodiment, the invention provides a method of using the imaging agent of the
present invention for identifying the level of CD115 expression in an individual comprising
administering to an individual the imaging agent of the present invention and acquiring an
image of a site of concentration of said imaging agent in the individual by a diagnostic
25 imaging technique.

Preferably an altered level of CD115 expression is identified. By an altered level of CD115
expression it is meant an altered level compared to the level observed in a normal, healthy
individual. Preferably, an increase in the level of CD115 expression is identified. In
30 particular, an increased level of CD115 expression indicates the presence of tissue
inflammation as indicated by the presence of CD115-expressing cells, predominately myeloid
cells such as monocytes and macrophages.

The diagnostic imaging technique may include magnetic resonance imaging (MRI), nuclear medicine, x-ray, ultra-sound, optical imaging and fluoroscopy. Preferably the digital imaging technique is MRI. In an alternative embodiment, the invention provides a method of acquiring an image of a site of concentration of the imaging agent of the present invention, wherein the imaging agent has been previously administered to an individual. By being able to identify areas in which there is an increased level of CD115 expression, which indicates the presence of tissue inflammation as determined by the presence of CD115-expressing cells, predominately myeloid cells such as monocytes and macrophages, it is possible to monitor or diagnose a disease associated with the presence of monocytes and/or macrophages.

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In certain embodiments of the invention the imaging is for non-diagnostic purposes. In certain embodiments, the image is acquired without any physical contact with the individual's body, e.g., by scanning the body. As indicated above, in certain embodiments, the image is obtained on a tissue sample. Furthermore, in certain embodiments of the invention the image which is acquired will not lead to an immediate diagnosis but will contribute to a diagnosis.

15

A third aspect of the invention relates to the imaging agent of the present invention for use in diagnosing a disease or disease risk in which levels of CD115 expression are altered. Preferably the levels of CD115 expression are increased. Increase in the level of CD115 within organs indicates the presence of tissue inflammation as determined by the presence of CD115-expressing cells, predominately myeloid cells such as monocytes and macrophages. Particular cardiovascular diseases, neurological diseases, autoimmune disorders, cancers and organ transplantation where tissue infiltration by myeloid cells is clinically relevant are listed above. The peptide and the signal entity are as defined above.

20

A fourth aspect of the invention relates to the imaging agent of the present invention in the manufacture of an agent for diagnosing a disease or disease risk in which levels of CD115 expression are altered. Preferably the levels of CD115 expression are increased. Increase in the level of CD115 within organs his indicates the presence of tissue inflammation as determined by the presence of CD115-expressing cells, predominately myeloid cells such as monocytes and macrophages. Particular cardiovascular diseases, neurological diseases, autoimmune disorders, cancers and organ transplantation where tissue infiltration by myeloid

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cells is clinically relevant are listed above. The peptide and the signal entity are as defined above.

A fifth aspect of the invention relates to use of an imaging agent for diagnosing a disease or
5 disease risk in which levels of CD115 expression are altered. Preferably the levels of CD115
expression are increased. Increase in the level of CD115 within organs indicates the presence
of tissue inflammation as indicated by the presence of CD115-expressing cells, predominately
myeloid cells such as monocytes and macrophages. Particular cardiovascular diseases,
neurological diseases, autoimmune disorders, cancers and organ transplantation where tissue
10 infiltration by myeloid cells is clinically relevant are listed above. The peptide and the signal
entity are as defined above.

The term "diagnosing a disease or disease risk" as used herein refers to determining whether
an individual has a certain disease or has an increased risk of developing the disease
15 compared to the general population. In particular, high level of vascular tissue infiltration by
myeloid cells is an indicator of certain cardiovascular diseases, especially atherosclerosis, as
discussed above. Similarly, abnormal tissue infiltration by myeloid cells is indicative of a
inflammatory disorder.

20 A further aspect of the invention relates to a method of identifying a patient at high risk of an
acute ischemic attack by determining the degree of inflammation within atherosclerotic
plaques in the patient comprising administering to the patient the imaging agent of the present
invention and acquiring an image of a site of concentration of said imaging agent in the
patient by MRI. The peptide and the signal entity are as defined above.

25

The invention also provides a pharmaceutically acceptable composition comprising the
imaging agent of the present invention and one or more excipients.

The composition may comprise a plurality of signal entities, each signal entity having one or
30 more peptides bound to it. The peptides bound to each signal entity may, or may not be,
identical.

The pharmaceutical compositions may be for human or animal usage in human and veterinary medicine and will typically comprise one or more suitable excipients. Acceptable excipients for therapeutic use are well known in the pharmaceutical art, and are described, for example, in Remington's Pharmaceutical Sciences, Mack Publishing Co. (A. R. Gennaro edit. 1985).

5 The choice of pharmaceutical excipient can be selected with regard to the intended route of administration and standard pharmaceutical practice. The pharmaceutical compositions may comprise as, or in addition to, the excipient any suitable binder, lubricant, suspending agent, coating agent or solubilising agent.

10 Preservatives, stabilizers and dyes may be provided in the pharmaceutical composition. Examples of preservatives include sodium benzoate, sorbic acid and esters of p-hydroxybenzoic acid. Antioxidants and suspending agents may be also used.

There may be different composition/formulation requirements dependent on the different
15 delivery systems. By way of example, the pharmaceutical composition of the present invention may be formulated to be delivered parenterally in which the composition is formulated in an injectable form, for delivery, by, for example, an intravenous, intramuscular or subcutaneous route. For parenteral administration, the compositions may be best used in the form of a sterile aqueous solution which may contain other substances, for example
20 enough salts or monosaccharides to make the solution isotonic with blood.

Typically, a physician will determine the actual dosage which will be most suitable for an individual subject and it will vary with the disease, age, weight and response of the particular patient. The appropriate dosage can be determined by one skilled in the art.
25

The pharmaceutical compositions of the present invention may be administered by parental or mucosal routes. The composition may be formulated for parenteral (i.e. intramuscular, intravenous or subcutaneous) and mucosal administration.

30 Additionally, the invention provides a method of preparing an imaging agent comprising coupling the peptide to the signal entity. The peptide may be coupled to the signal entity directly or via a linker as described above. Techniques for doing so are well known to one skilled in the art. The peptide and the signal entity are as defined above.

A further aspect of the invention relates to a peptide having the amino acid sequence of SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 3 or SEQ ID NO: 4, or a sequence that differs from SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 3 or SEQ ID NO: 4 by 1, 2 or 3 amino acids, wherein the peptide binds to a human CD115 receptor. In one embodiment, the peptide has the amino acid sequence of SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 3 or SEQ ID NO: 4, or a sequence that differs from SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 3 or SEQ ID NO: 4 by 1 or 2 amino acids, wherein the peptide binds to a human CD115 receptor. In a further embodiment, the peptide has the amino acid sequence of SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 3 or SEQ ID NO: 4, or a sequence that differs from SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 3 or SEQ ID NO: 4 by one amino acid, wherein the peptide binds to a human CD115 receptor. In a preferred embodiment the peptide is SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 3 or SEQ ID NO: 4. In a more preferred embodiment, the peptide is SEQ ID NO: 1 or SEQ ID NO: 4.

15

The invention also provides for a kit comprising a peptide as defined above, a signal entity as defined above, and components for coupling the peptide to the signal entity. The components are generally reagents for enabling the coupling of the peptide to the signal entity, and may comprise a linker.

20

A skilled person will appreciate that all aspects of the invention, whether they relate to, for example, the imaging agent, its use, or a method of imaging, monitoring or diagnosing a disease in which monocytes and/or macrophages play a role, are equally applicable to all other aspects of the invention. In particular, aspects of the imaging agent for example, may have been described in greater detail than in other aspects of the invention, for example, the use of the imaging agent. However, the skilled person will appreciate where more detailed information has been given for a particular aspect of the invention, this information is generally equally applicable to other aspects of the invention.

30

Detailed Description of the Invention

The invention will now be described in detail by way of example only with reference to the figures in which:

5

Figure 1 shows the secondary structure of human and murine recombinant M-CSF (truncated isoforms). Areas of homology between the two species are highlighted in grey.

Figure 2 shows the results of a fluorescence antibody competition binding assay. The left
10 panel shows a dose-dependent decrease in the percentage of CD14⁺CD115⁺ monocytes as detected by whole blood flow cytometry following pre-treatment of human samples with Gd-MoCA at the different concentrations as indicated. (*p < 0.05 vs 0 mM (control).) Statistical analysis was by repeated measures ANOVA with Dunnett's post-test correction. The right
15 panel shows a time-course performed with Gd-MoCA (1.5 mM), M-CSF1 (100 ng/ml) and Magnevist (1.5 mM) as indicated. The dotted line indicates level of expression of CD115 in untreated blood at 37°C for 60 minutes (control), which was further reduced by M-CSF1 and Gd-MoCA, but not Magnevist treatment.

Figure 3 illustrates CyTOF-based characterisation of cellular distribution of Gd-MoCA in
20 human blood. Post-acquisition analysis was gated for all cell types as identified by DNA-Ir staining (i,ii,iii; upper box). Staining of whole blood with Gd-MoCA (on the left-hand side; lower box) identified two distinct cell populations: highly-expressing cells were mainly constituted of CD14⁺ cells (monocytes) whilst low-expressing cells mainly included CD15⁺ cells (neutrophils). Similar results were obtained in blood stained with a specific PE-
25 conjugated anti-human CD115 detected by a Gd-conjugated anti-PE secondary antibody (on the right-hand side), demonstrating that Gd-MoCA targets CD115-expressing cells and is able to distinguish between high- and low-expressing cell populations.

Figure 4 shows a DELFIA-based binding assay. In particular, Eu-MoCA binding to human
30 serum albumin (HSA) (A) and human recombinant CD115 (B). No binding was found for HSA. For the binding assay of Eu-MoCA to human recombinant CD115, the best fitting curve was One site - Specific Binding.

Figure 5 shows Gd-MoCA relaxivity in water at a 3T Achieva MR scanner (Philips Healthcare, Best, The Netherlands), demonstrating good paramagnetic properties on a clinical scanner ($r_1 = 8.224$ mmol/sec).

- 5 Figure 6 shows CD115 expression in total cell protein lysates from human PBMCs. Western Blotting of CD115 in human PBMCs incubated with the different compounds as indicated, for 1 hour at 37° C. The data confirm spontaneous reduction of CD115 expression in untreated blood incubated for 1 hour at 37° C as previously observed in the fluorescence competition binding assay. Exposure of PBMCs to M-CSF1 (100 ng/ml) induced CD115 activation with
10 consequent degradation of the receptor. Gd-MoCA (1.5 mM), similarly to the untargeted Magnevist (1.5 mM), did not affect CD115 expression in stimulated PBMCs (* $p < 0.05$ vs vehicle).

Figure 7 shows *in vivo* Magnevist-based DE-MRI in wild-type mice. Panel A shows an
15 abdominal scan of a wild-type mouse injected with Magnevist (0.2 mmol/kg of body weight). Acquisition was pre-injection (baseline) and at 30 and 60 minutes post-injection. Panel B reports SNR (signal-to-noise ratio) in the different organs and at the different time points as indicated. (* $p < 0.05$, ** $p < 0.01$, repeated measures ANOVA with Bonferroni's post test correction.)

20

Figure 8 shows *in vivo* Gd-MoCA-based DE-MRI in wild-type mice. Panel A shows an abdominal scan of wild-type mice injected with Gd-MoCA (0.2 mmol/kg of body weight). Acquisition was pre-injection (baseline) and at 30 and 60 minutes post-injection. Panel B reports SNR (signal-to-noise ratio) in the different organs and at the different time points as
25 indicated. (* $p < 0.05$, ** $p < 0.01$, repeated measures ANOVA with Bonferroni's post test correction.)

Figure 9 shows *in vivo* Gd-MoCA-based DE-MRI in ApoE^{-/-} mice. Panel A shows an abdominal scan of ApoE^{-/-} mice injected with Gd-MoCA (0.2 mmol/kg of body weight).
30 Acquisition was pre-injection (baseline) and at 30 and 60 minutes post-injection. Panel B reports SNR (signal-to-noise ratio) in the different organs and at the different time points as indicated. (* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, repeated measures ANOVA with Bonferroni's post test correction.)

Figure 10 shows *in vivo* T1 mapping. Images on the left-hand side and in the middle show the T1 and R1 maps respectively on the MRI abdominal scanning of ApoE^{-/-} mice injected with either Magnevist or Gd-MoCA (both at a dose of 0.2 mmol/ kg of body weight) as indicated, in comparison with the pre-injection scans. Corresponding DE-MRI on T1-weighted images are shown on the right-hand side. The graph displays the relaxivity (R1) in the different organs. (*p<0.05 vs pre-injection, repeated measures ANOVA with Bonferroni's post test correction.)

Figure 11 shows *in vivo* Gd-MoCA-based MRI of atherosclerotic plaques in ApoE^{-/-} mice. (A) 3D reconstruction and axial view time of flight (TOF) MR images of the aortic arch and associated vessels, showing the level of the axial scanning (green line). Abbreviations: LSC, left subclavian artery; LCC, left common carotid artery; BC, brachiocephalic artery; Ao, aorta. Delayed enhancement (DE) MRI image and composite DE and TOF images overlaid at 30 (top) and 60 minutes (bottom) post-Gd-MoCA injection. Arrow indicates the area of delayed enhancement corresponding to the posterior brachiocephalic wall. (B) Regions of interest (ROIs) were selected in the wall of the brachiocephalic, left common carotid and left subclavian arteries and an area outside of the scan defined as "noise", and the signal to noise ratios (SNRs) were calculated in three different slices of DE-MR images from each animal. (mean ± SEM, n = 3, *** p < 0.001, one way ANOVA with Bonferroni's post test correction.) (C) Modification of Gd-MoCA (1-DOTA) with a Gd-DOTA moiety also at the C-terminus via lysine addition to the amino acid sequence (2-DOTA) compromised the ability of the contrast agent to enhance atherosclerotic plaques.

Figure 12 shows a DELFIA-based binding assay, in particular, peptide binding to human recombinant CD115. Best fitting curve was One site - Specific Binding with Hill slope for both peptides.

Figure 13 shows a DELFIA-based binding assay (panel A), in particular, peptide SEQ ID NO: 4 binding to human recombinant CD115. Best fitting curve was One site - Specific Binding with Hill slope. Panel B shows *in vivo* MRI of atherosclerotic plaques in ApoE^{-/-} mice injected with SEQ ID NO: 4. 3D reconstruction and axial view time of flight (TOF) MR images of the aortic arch and associated vessels, showing the level of the axial scanning

(middle line). Abbreviations: LS, left subclavian artery; LCC, left common carotid artery; BCA, brachiocephalic artery. Delayed enhancement (DE) MRI image and composite DE and TOF images overlayed at 60 minutes post-contrast agent injection (0.2 mmol/Kg of body weight). Arrows indicate the area of delayed enhancement corresponding to the
5 brachiocephalic wall. Regions of interest (ROIs) were selected in the wall of the brachiocephalic, left common carotid and left subclavian arteries and an area outside of the scan defined as “noise”, and the signal to noise ratios (SNR) were calculated in three consecutive slices of DE-MR images. (mean $\pm$ SEM, $n = 3$, ** $p < 0.01$, one way ANOVA with Bonferroni’s post test correction.)

10

Figure 14 shows *in vivo* Gd-MoCA-based MRI of atherosclerotic plaques in ApoE^{-/-} mice at different time points of disease progression as indicated. On the left, 3D reconstruction (on the top) and axial view time of flight (TOF) MR images of the aortic arch and associated vessels, showing the level of the axial scanning (middle line). Abbreviations: LS, left
15 subclavian artery; LCC, left common carotid artery; BCA, brachiocephalic artery. Delayed enhancement (DE) MRI images (in the middle) and composite DE and TOF images overlayed (on the bottom) at 60 minutes post-Gd-MoCA injection (0.2 mmol/Kg of body weight). Arrows indicates the area of delayed enhancement corresponding to the posterior brachiocephalic wall. Regions of interest (ROIs) were selected in the wall of the
20 brachiocephalic artery and an area outside of the scan defined as “noise”, and the signal to noise ratios (SNRs) were calculated in three consecutive slices of DE-MR images from each animal. Data are reported in the graph on the right ($n = 4-5$ per group), ** $p = 0.03$, Mann Whitney test.)

25

Example 1

Design of Gd-MoCA (Gd³⁺-DOTA-SEQ ID NO: 1)

Gd-MoCA (Monocyte Imaging agent) is a 12-mer Gd³⁺-DOTA-conjugated peptide that was
30 designed to target the extracellular portion of CD115 (also known as c-fms or CSF-R1), which is a universal marker of myeloid cell lineage, equally expressed on both human and murine monocytes (Ingersoll MA, *et al.* Blood 2010; 115: e10-e19). The peptide consists of amino acid residues 55-66 (SEQ ID NO: 1) of the human macrophage colony-stimulating

factor (M-CSF, GeneBank: AAA59573.1), which is the autologous ligand of CD115. The 12 mer-peptide was selected among regions of homology between the murine and human species (Fig.1).

- 5 The compound was synthesised in the form of a DOTA-peptide at the N-terminus to allow for Gd^{3+} complexation, therefore providing paramagnetic properties to the molecule, thus making it suitable for an MRI application.

10 Example 2

MoCA complexation to lanthanides

MoCA was synthesised by Peptide Synthetics (Peptide Protein Research Limited, UK) in the form of a DOTA-peptide.

15

- For the *in vitro* experiments on human blood and the *in vivo* imaging described in the following paragraphs, the DOTA-peptide was complexed to the lanthanide metal gadolinium (III) (Gd^{3+}) according to a previously published protocol incorporated herein by reference (Kotek J *et al.* Synthesis and Characterization of Ligands and their Gadolinium(III) Complexes. In: The Chemistry of Imaging agents in Medical Magnetic Resonance Imaging, 2nd Edition, edited by Merbach A, Helm L and Toth E. London: John Wiley & Sons Ltd., 2013, p. 83-156.). Briefly, 10mg of MoCA (5.74×10^{-3} moles) was reconstituted in a total volume of 300 μ l of a solution containing dimethylsulphoxide (DMSO) (Sigma-Aldrich, UK) and dd.H₂O in a 1:3 ratio. Subsequently, $GdCl_3 \cdot 6H_2O$ (Sigma-Aldrich, UK) previously resuspended in dd.H₂O at a concentration of 169 mg/ml, was added to the DOTA-peptide at an equimolar concentration.
- 20
- 25

- For the purpose of determining the binding affinity of MoCA to its target CD115, the DOTA-peptide was complexed to the lanthanide metal Europium (Eu^{3+}) and tested by DELFIA time-resolved fluorescence assay. Briefly, 1 mg of MoCA was reconstituted in 1 ml ddH₂O (5.74×10^{-4} M), from which a stock solution of 2×10^{-5} M was prepared. An equimolar concentration of $EuCl_3 \cdot 6H_2O$ (Sigma-Aldrich, UK) previously resuspended in ddH₂O was added to the stock solution of MoCA.
- 30

In both cases, lanthanide complexation of MoCA occurred under conditions of constant stirring, at room temperature and at pH 7.0. The reaction was terminated when no free lanthanides could be detected in the solution as determined by the xylenol orange test (Barge
5 A, *et al.* Contrast Media & Molecular Imaging 1: 184-188, 2006).

Example 3

Study of MoCA specificity for myeloid cells and targeting of CD115

10

Fluorescence antibody competition binding assays and time of flight mass cytometry (CyTOF) were used to determine the specificity of Gd-MoCA for the myeloid cell lineage and its ability to specifically target CD115. Experiments were conducted *in vitro* on human whole blood obtained from healthy volunteers and collected in EDTA vacutainer tubes (Becton &
15 Dickinson, BD, UK). In addition, Eu-MoCA was tested by DELFIA time-resolved fluorescence assay against a human recombinant His-tagged CD115 protein (Life Technologies, UK) to assess its binding affinity to the target.

3.1. Fluorescence antibody competition binding assay

20 One-hundred microlitres of the whole human blood were incubated with increasing concentrations of Gd-MoCA (0 , 1×10^{-6} , 5×10^{-6} , 2×10^{-5} , 5×10^{-5} , 1×10^{-4} , 5×10^{-4} , 1×10^{-3} , 1.5×10^{-3} M) for 1 hour at 37°C . A time course (0 , 5 , 15 , 30 and 60 minute incubation) was also performed with Gd-MoCA at a final concentration of 1.5×10^{-3} M in blood. Samples were subsequently immunostained with a saturating concentration of phycoerythrin (PE)-
25 conjugated anti-human CD115 and fluorescein isothiocyanate (FITC)-conjugated anti-human CD14 (both from eBiosciences, UK). Antibody incubation was for 20 minutes at $+4^{\circ}\text{C}$ in the dark, followed by red cell lysing using Red Cell Lysing Solution (Becton & Dickinson, UK) for 10 minutes at room temperature (RT) in the dark. After washing twice in 1 ml filtered 0.2% bovine serum albumin/0.1% sodium azide in phosphate buffered saline
30 (0.2%BSA/0.1%SA/PBS), samples were fixed in 300 μl filtered 1% paraformaldehyde (1%PFA). The fluorescent signal emitted by the PE-conjugated CD115-specific antibody on CD14⁺ cells (monocytes) was detected by flow cytometry (FACSCalibur, BD, UK). A decay in the fluorescence signal for CD115 observed in Gd-MoCA pre-treated blood compared with

untreated blood (vehicle only) was taken as indicative of competitive binding of MoCA to CD115. Pre-incubation of the whole blood for 1 hour with the growth factor M-CSF (the endogenous ligand of CD115, 100 ng/ml; Cell Signalling, UK) prior to the dual staining for CD14 and CD115 was also conducted as positive control. Similar experiments were
5 performed by using Magnevist (un-targeted Gd-DTPA imaging agent, Bayer Healthcare, UK, 1.5×10^{-3} M) for comparison. At the flow cytometer, acquisition was performed using CellQuest Pro (BD) software. A total of 140,000 events were acquired. Post-acquisition analysis was performed using FlowJo Version X (Treestar Inc., OR, USA). Monocytes were identified on the basis of CD14 positivity and within this population, the expression of CD115
10 was evaluated and reported as percentage of double positive (CD14⁺CD115⁺) cells.

3.2. Time of flight mass spectrometry (CyTOF)

A 100 μ l aliquot of whole blood was incubated with either Gd-MoCA (1.5×10^{-3} M) or saturating concentrations of PE-conjugated anti-human CD115 antibody for 30 minutes at
15 +4°C. Erythrocytes were then lysed with 2 ml Red Cell Lysing Solution for 10 minutes at room temperature. Samples were washed as above in filtered 0.2% BSA/0.1% SA/PBS and then incubated for 30 minutes at room temperature with a cocktail of primary metal-conjugated antibodies (Er¹⁷⁰-CD3, Pr¹⁴³-CD14, Dy¹⁶⁴-CD15, Sm¹⁴⁷-CD20, and a secondary Gd-conjugated anti-PE antibody for detection of anti-human PE-CD115 antibody) (all from
20 DVS Sciences Ltd., UK). After washing in filtered 0.2% BSA/0.1% SA/PBS, samples were then fixed in 500 μ l 1% PFA overnight. The cell pellet was subsequently resuspended in 500 μ l 0.2% BSA/0.1% SA/PBS containing 10% saponin for permeabilisation, and incubated with 1 μ l of an Ir-DNA intercalating dye for 20 minutes at room temperature. Samples were washed as above and fixed in 500 μ l 1.6% PFA for at least 24 hours at +4°C before data
25 acquisition.

Acquisition was performed using a CyTOF mass cytometer (DVS Sciences Ltd., UK). A small aliquot of Ce¹⁴⁰-tagged beads were added to the fixed cell suspension prior to sample acquisition as an internal control. Post-acquisition analysis was performed using FlowJo
30 Version X. Intact cells were identified based on their positivity for the Ir-DNA intercalator. Monocytes, neutrophils, B-lymphocytes and T-lymphocytes were then distinguished accordingly with the expression of typical cluster differentiation antigens (CD14, CD15, CD20 and CD3 respectively). Unstained control preparations were used to define negative

signals. The expression pattern (i.e. high, low and negative signal) of Gd-MoCA and PE-conjugated anti-human CD115 antibody were then analysed among the different leukocytes.

3.3. Dissociation enhanced lanthanide fluoroimmunoassay (DELFLIA)

- 5 One mg of MoCA was reconstituted in 1 ml dd.H₂O, from which a stock solution of 2×10^{-5} M was prepared. EuCl₃.6H₂O was added at equimolar concentrations to the stock solution and complexation of the DOTA-peptide with the lanthanide occurred under conditions of constant stirring, at room temperature and at pH 7.0. The reaction was terminated when no free lanthanide could be detected in the solution as determined by the xylenol orange test.
- 10 The following concentrations of Eu-MoCA were tested: 1×10^{-5} , 5×10^{-6} , 1×10^{-6} , 5×10^{-7} , 1×10^{-7} and 5×10^{-8} M. These were added in triplicate to a 96-multiwell plate (50 µl/well) pre-coated overnight at 4 °C with human recombinant CD115 resuspended in PBS at a concentration of 10 µg/ml (100 µl/well). The same peptide concentrations were added to uncoated wells to determine the non-specific binding. Incubation was for 2 hours at room
- 15 temperature. Following 2 washes in PBS to remove unbound peptides, Eu-DOTA peptide binding to the immobilised CD115 was revealed by DELFIA solution (75 µl/well, Perkin Elmer, UK). Multiwell plates were acquired at a spectrofluorometer (TECAN, Infinite 200 PRO) with the following instrument settings : excitation 340 nm; emission 617 nm; delay 400 msec; window 400 msec; gain 100; Z-position 20000 mm. The intensity of fluorescence of
- 20 the different peptide concentrations were subtracted by the non-specific binding. Values were plotted against concentrations and analysis was by GraphPad Prism using non linear regression analysis (Specific Binding with or without Hill slope). Similar experiments were performed to assess the binding of MoCA to human serum albumin (HSA). For this purpose, the Eu-DOTA peptide was tested against immobilised HSA (Sigma, UK; 96-multiwell plate
- 25 coating with HSA was overnight at 4 °C using a 4.3% solution made in PBS).

3.4. Biological profile

- Western Blot analysis was used to determine whether MoCA caused CD115 receptor internalisation and degradation, as occurs in response to activation of the receptor by the
- 30 endogenous ligand M-CSF.

Approximately 50 ml of peripheral venous blood was obtained from three healthy volunteer donors by venipuncture into EDTA-coated blood collection tubes. Peripheral blood

mononuclear cells (PBMCs) were isolated by Lymphoprep® (Axis-Shield, Norway) gradient density centrifugation of the whole blood and were subsequently resuspended in 5 ml RPMI-1640 medium (Sigma-Aldrich, UK). One ml of the resuspended PBMC pellet was either processed immediately for cell protein extraction (as “fresh blood”) or incubated with either
5 50 µl 0.9% w/v saline solution, Magnevist® (1.5 mM), M-CSF (100 ng/ml) or Gd-MoCA (1.5 x 10⁻³ M) for 60 minutes at +37°C. Following incubation, PBMCs were washed in PBS and protein lysates were obtained by cell extraction with RIPA buffer (50 mM Tris, 150 mM NaCl, 0.1% SDS, 0.5% sodium deoxycholate and 1% Triton X, supplemented with protease and phosphates inhibitor cocktails, Sigma-Aldrich, UK). Protein concentration of the samples
10 was determined by bicinchoninic acid (BCA) assay. Forty micrograms of protein were then loaded and separated in a 10% SDS-PAGE gel (30% acrylamide, 1.5 M Tris, 10% SDS, 10% APS & 0.004% TEMED) and transferred to a polyvinylflouride (PVDF) membrane (Millipore, UK) by semi-dry transfer at 30 mA for 2 hours.

15 Membranes were subsequently incubated with rabbit anti-human CD115 antibody targeting the intracellular portion of the protein (Cell Signalling, UK) (1:250 in 5% milk-TBST, 2 hours at room temperature), followed by incubation with goat anti-rabbit horseradish peroxidase (HRP)-conjugated secondary antibody (1:5000 in 5% milk-TBST). The signal was revealed by ECL chemiluminescence substrate (Thermo Fisher Scientific, UK). Protein normalisation
20 was with rabbit anti-human β-actin (Cell Signalling Technologies, UK) (1:5000 in 5% milk-TBST).

Example 4

25 Gd-MoCA-based *in vivo* MRI

All experiments were carried out under the UK Animals (Scientific Procedures) Act 1986 (PPL 70/7097, PIL 70/20776). Two wild type C57Bl/6 male mice (Charles River Laboratories, Kent, UK) and five ApoE^{-/-} male mice (B6.129P2-apoEtm1Unc/J, Charles
30 River Laboratories) at the end of an 8-week on high fat diet (HFD) (21% fat from lard, 0.15% wt/wt cholesterol, Special Diets Services) were studied. MRI scans were performed in mice anaesthetised by isoflurane inhalation and were conducted using a 3T Philips Achieva MR

scanner (Philips Healthcare, Best, The Netherlands) equipped with a clinical gradient system (30mT m⁻¹, 200mT/m/ms) and a single-loop surface coil (diameter = 23mm).

4.1. Biodistribution study

5 A two-dimensional anatomic scan of the abdomen was performed (field of view (FOV) = 20×20×10mm, matrix = 160, slice thickness = 0.5 mm, repetition time (TR)/echo time (TE) = 37/7.7 ms, and flip angle = 60°) at baseline (pre-imaging agent injection), followed by high-resolution delayed enhancement (DE)-MRI and T1 mapping sequences. The high-resolution DE-MRI scan was preceded by a two-dimensional Look-Locker (LL) sequence to determine
10 the optimal inversion recovery time for blood signal nulling during the DE-MRI scan. Following the baseline (pre-injection) scanning, animals (two wild-type and one ApoE^{-/-} mice) were injected with a dose of 0.2 mmol/kg of either Magnevist® (preparation diluted 1:20 in PBS) or Gd-MoCA. The above imaging protocol was then repeated at 30 and 60 minutes post imaging agent injection.

15

Post acquisition image analysis was performed using OsiriX (OsiriX Foundation, CA, USA) for Mac. To understand the biodistribution of Gd-MoCA peptide, regions of interest (ROIs) were selected in the DE-MR images of the liver, kidneys and bladder on at least three consecutive image slices. The signal to noise ratio (SNR) was calculated by applying the
20 following formula: tissue signal/noise. Noise was determined as the standard deviation in air, lateral to the abdomen. T1 and relaxivity analyses were performed using an in-house developed programme (developed using MatLab R2012, Mathworks, Cambridge, UK). Similarly, ROIs were selected in the liver, kidneys and blood (abdominal aorta) and the T1 and relaxivity values were calculated and corrected for background noise. ROIs were selected
25 in at least three consecutive slices within each animal. T1 and relaxivity values of Gd-MoCA in vitro and in water were analysed with the same programme following acquisition at the 3 Tesla scanner of increasing concentration of the imaging agent (0-1.5 mM) using T1 mapping sequences.

30 4.2. Imaging of atherosclerotic plaques

In vivo MRI scanning of atherosclerotic plaques was performed in three ApoE^{-/-} mice on a high fat diet (HFD). Scanning was performed at the level of the aortic arch and brachiocephalic vessels. Animals were injected with a dose of 0.2 mmol/kg of Gd-MoCA.

For visualisation of the aortic arch and the brachiocephalic artery, a two-dimensional time of flight (TOF) scan was performed (FOV = 20×20×10 mm, matrix = 160, slice thickness = 0.5 mm, TR/TE = 37/7.7 ms, and flip angle = 60°). The subsequent high-resolution DE-MR images and T1 mapping sequences were then planned on a maximum intensity projection
5 (MIP) displaying the aortic arch and associated vessels.

Images were analysed as above, and the DE-MR SNR was calculated in the brachiocephalic (= diseased vessel), left common carotid and left subclavian arteries (= disease-free vessels). Areas of enhancement were only considered significant if they were clearly present in at least
10 three consecutive image slices.

For the purpose of MRI imaging of atherosclerotic vessels, Gd-MOCA was modified by the addition of a lysine residue at the C-terminus to allow binding to a second DOTA moiety at the C-terminus, so that the contrast agent could carry a double dose of Gd³⁺ per molecule
15 [Gd-DOTA-IDSQMETSCQIT (K)-DOTA-Gd]. This compound was injected at a dose of 0.2 mmol/kg in an ApoE^{-/-} mouse. MRI was performed as described above.

Example 5

20 Peptide Library Screening

Twenty-five peptides (between 6 and 12 amino acids) (Table 1) were designed to include truncated and overlapping sequences of MoCA. Additionally, MoCA was also modified with C-terminal amidation (IDSQMETSCQIT-NH₂ and IDSQMETSCQIT-NHMe).
25

All peptides were synthesised by Peptide Synthetics with conjugation of DOTA at the N-terminus and tested by DELFIA assay to assess their binding affinity to CD115, accordingly with the protocol described above.

ORIGINAL PEPTIDE:		IDSQMETSCQIT	
OVERLAPPING		TRUNCATION	
N-terminus overlapping		N-terminus truncation	
Criteria: 2 amino acid overlapping	RLIDSQMETSCQ	Criteria: 1 amino acid truncation	DSQMETSCQIT
Criteria: 4 amino acid overlapping	LQRLIDSQMETS		SQMETSCQIT
Criteria: 6 amino acid overlapping	QSLQRLIDSQME		QMETSCQIT
			METSCQIT
			ETSCQIT
			TSCQIT
			SCQIT
C-terminus overlapping		C-terminus truncation	
Criteria: 2 amino acid overlapping	SQMETSCQITFE	Criteria: 1 amino acid truncation	IDSQMETSCQI
Criteria: 4 amino acid overlapping	METSCQITFEFV		IDSQMETSCQ
Criteria: 6 amino acid overlapping	TSCQITFEFVDQ		IDSQMETSC
			IDSQMETS
			IDSQMET
			IDSQME
			IDSQM
		N-terminus/C-terminus truncation	
		Criteria: 1 amino acid truncation both sides	DSQMETSCQI
			SQMETSCQ
			QMETSC

Table 1 - List of peptides including truncated and overlapping sequences of MoCA.

5 Example 6

Gd-MoCA specifically targets CD115 expressed on the extracellular surface of human myeloid cells

- The fluorescence antibody competition binding assays showed that Gd-MoCA is capable of
- 10 displacing a specific anti-human CD115 antibody in a dose and time-dependent manner, thus demonstrating its specificity to the target when tested in human blood. Specifically, pre-treatment of the human blood with Gd-MoCA for 1 hour and at a dose of 1.5 mM prevented a PE-conjugated anti-human CD115 from binding to its target, leading to a statistically significant reduction of the percentage of CD115⁺ monocytes as detected by flow cytometry.
- 15 Reduction in the percentage of CD115⁺ monocytes was solely due to a competitive binding of Gd-MoCA to the CD115 site engaged by the anti-CD115 specific antibody. Indeed, as specified in Example 8, incubation of blood with Gd-MoCA did not decrease the expression of CD115 on the extracellular surface of myeloid cells in human blood.

Reduction in the percentage of CD115⁺ monocytes as obtained in blood pre-treated with the endogenous ligand of CD115 M-CSF (100 ng/ml), and absence of such an effect in samples pre-incubated with the untargeted Gd-DTPA imaging agent Magnevist (1.5 mM), validate the fluorescence competition binding assay (Figure 2).

5

Specificity of Gd-MoCA in targeting CD115-expressing cells in human blood was confirmed by CyTOF analysis. The distribution of Gd-MoCA on the extracellular surface of the different leucocytes in human blood was consistent with the CD115 expression on the different cell types as detected by the specific PE-conjugated anti-human CD115 (Figure 3).

10

Example 7

Gd-MoCA specifically targets the human CD115 extracellular receptor

15 The DELFIA-based binding assay showed a specific binding of Gd-MoCA to the target CD115 with $k_d = 9 \pm 1 \mu\text{M}$ and a percentage binding of 43%. The best-fitting curve was with one-site specific binding ($h=1.32$), suggesting that Gd-MoCA engages CD115 on a single contact site (Figure 4).

20

Example 8

Gd-MoCA in vitro relaxivity at 3 Tesla

The relaxivity of Gd-MoCA in water and at 3T Achieva MR scanner (Philips Healthcare, Best, The Netherlands) magnet was 8.224 mmol/sec, hence demonstrating good paramagnetic
25 properties of the contrast agent for MRI application by using a clinical scanner.

Example 9

Gd-MoCA binding to human CD115 does not activate the receptor in human cells

30

Western Blotting-based analysis of CD115 cellular content in PBMC following incubation with Gd-MoCA (1.5 mM) for 1 hour revealed no changes in protein expression compared to untreated cells (Figure 6). Reduction in the extracellular content of CD115 was observed only in PBMCs exposed to M-CSF1 (100 ng/ml), as a consequence of CD115 activation by the

growth factor that upon ligation of the extracellular receptor induces its internalization and degradation. The untargeted Gd-DTPA imaging agent Magnevist (1.5 mM) did not modify the expression of CD115 in PBMCs.

5

Example 10

In vivo Biodistribution of Gd-MoCA in murine animal models is consistent with CD115 tissue expression

- 10 Unlike the untargeted imaging agent Magnevist, Gd-MoCA was able to cause liver enhancement in both wild-type and ApoE^{-/-} mice at 30 and 60 minutes post-injection (0.2 mmol/kg of body weight). Increase in SNR observed in post-injection DE-MRI compared to pre-injection images, was further confirmed by the T1 mapping sequences that enabled *in vivo* analysis of tissue relaxivity, which is directly dependent upon Gd-tissue accumulation
- 15 (Figures 7 to 10). Indeed, Gd-MoCA but not Magnevist significantly reduced liver relaxivity.

Gd-MoCA displayed similar accumulation within the kidney and bladder at 30 and 60 minutes post-injection compared to Magnevist, consistent with renal excretion of Gd-based imaging agents.

20

Example 11

Gd-MoCA is able to enhance atherosclerotic, but not disease-free vessels, in ApoE^{-/-} mice

- 25 DE-MRI of the brachiocephalic artery in ApoE^{-/-} mice on HFD showed selective and discrete accumulation of Gd-MoCA in atherosclerotic lesions, but not in healthy vessels, at 30 minutes post-injection (0.2 mmol/kg of body weight) (Figure 11). Enhancement persisted at 60 minutes post-injection. Accumulation of the imaging agent within inflamed atherosclerotic lesions was not attributable to passive diffusion of the contrast agent from the bloodstream to
- 30 the arterial wall, but it was rather due to a targeted localisation. Indeed, modification of Gd-MoCA with addition of a lysine at the C-terminus complexed to a second DOTA moiety to enable dual dose of Gd³⁺ was tested *in vivo*. Although displaying ability to enhances the liver,

the modified Gd-MoCA completely lost its ability to give atherosclerotic plaque enhancement (even though carrying double the amount of Gd³⁺ per molecule) (Figure 11).

5 Example 12

Two additional Gd-DOTA-peptides (SEQ ID NO: 2 and SEQ ID NO: 3) were identified as capable of targeting human CD115

The peptide library screening identified 2 truncated sequences of Gd-MoCA that display
10 specific binding to the CD115, namely DOTA-METSCQIT and DOTA-IDSQMETSCQI. These two peptides correspond to SEQ ID NO: 2 and SEQ ID NO: 3. Without wishing to be bound by theory, the analysis of the truncated sequences suggest that the primary binding site of Gd-MoCA to CD115 lays within the -METSCQI- sequence. However, the lack of a consistent pattern in terms of ability to bind the target among the different C-terminus and/or
15 N-terminus truncated peptides that also contain this sequence, may point to a relevant role played by the other amino acid residues of Gd-MOCA (namely IDSQ) in determining a particular folded structure upon which it is able to bind to its target.

20 Example 13

Modified MoCA peptide (SEQ ID NO: 4) retains specificity of binding to human CD115 and ability to image atherosclerotic plaques *in vivo*

A modified isoform of MoCA was generated with replacement of the Methionine residue with
25 its synthetic analogue Norleucine (IDSQ(Nle)ETSCQIT). This peptide corresponds to SEQ ID NO: 4. The compound was synthesised by Peptide Synthetics with conjugation of DOTA at the N-terminus. It was tested by DELFIA assay to assess its binding affinity to human CD115 in comparison with the original sequence of MoCA (IDSQMETSCQIT) and by *in vivo* cardiovascular MRI in the ApoE^{-/-} animal model of atherosclerosis, accordingly with the
30 protocols described above.

The modified sequence of Gd-MoCA, namely DOTA- IDSQ(Nle)ETSCQIT, retained binding affinity to its target human CD115. The DELFIA-based binding assay showed a specific

binding of SEQ ID NO: 4 to the target CD115 with $k_d = 9.96 \pm 0.8 \mu\text{M}$. The best-fitting curve was with one-site specific binding ($h=1.34$), suggesting that SEQ ID NO: 4 engages CD115 on a single contact site (Figure 13).

- 5 DE-MRI of the brachiocephalic artery in ApoE^{-/-} mice on 8 weeks HFD showed selective and discrete accumulation of SEQ ID NO: 4 in atherosclerotic lesions, but not in healthy vessels, at 30 minutes post-injection (0.2 mmol/kg of body weight) with the arterial enhancement persisting at 60 minutes post-injection (Figure 13).
- 10 In conclusion, SEQ ID NO: 1 and SEQ ID NO: 4 display similar binding affinity to the human CD115 and similar efficacy in detecting atherosclerotic lesions *in vivo* by cardiovascular MRI.

15 Example 14

Gd-MoCA is able to detect atherosclerotic disease progression by cardiovascular MRI

Gd-MoCA was tested in the ApoE^{-/-} murine model of atherosclerosis at different stages of disease progression. For this purpose, eight week-old homozygous male ApoE^{-/-} mice
20 (B6.129P2-apoEtm1Unc/J) were switched from normal rodent chow to a Western high-fat diet (HFD) that contained 21% fat from lard and 0.15% (w/w) cholesterol (Special Diets Services, Witham, UK). Animals were scanned at 8 (n=5) and 12 weeks (n=4) post-commencement of HFD, since a significant and progressive increase in the content of myeloid cells within plaques has been previously demonstrated at these time points (Makowski MR, *et*
25 *al.* Circulation: Cardiovascular Imaging. 2011; 4: 295-30). Scanning was performed at the level of the aortic arch and brachiocephalic vessels, as described above.

A significant increase in Gd-MoCA accumulation within the brachiocephalic artery was observed with increase in duration of the HFD, as demonstrated by higher DE-MRI SNR in
30 the 12 week HFD group compared to the 8-week HFD animals (Figure 14).

All patent and literature references cited in the present specification are hereby incorporated by reference in their entirety.

Claims

1. An imaging agent comprising:
a peptide having the amino acid sequence of SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 3 or SEQ ID NO: 4, or a sequence that differs from SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 3 or SEQ ID NO: 4 by 1, 2 or 3 amino acids, wherein the peptide binds to a human CD115 receptor; and
a signal entity.
2. An imaging agent according to claim 1 wherein the peptide has the amino acid sequence of SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 3 or SEQ ID NO: 4, or a sequence that differs from SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 3 or SEQ ID NO: 4 by 1 or 2 amino acids.
3. An imaging agent according to claim 1 wherein the peptide has the amino acid sequence of SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 3 or SEQ ID NO: 4, or a sequence that differs from SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 3 or SEQ ID NO: 4 by one amino acid.
4. An imaging agent according to claim 1 wherein the peptide is SEQ ID NO: 1.
5. An imaging agent according to claim 1 wherein the peptide is SEQ ID NO: 2.
6. An imaging agent according to claim 1 wherein the peptide is SEQ ID NO: 3.
7. An imaging agent according to claim 1 wherein the peptide is SEQ ID NO: 4.
8. An imaging agent according to any preceding claim wherein the signal entity is a signal entity for medical imaging.
9. An imaging agent according to claim 8 wherein the medical imaging is magnetic resonance imaging.

10. An imaging agent according to claim 8 or claim 9 wherein the signal entity is a metal chelate.
11. The imaging agent according to claim 10, wherein the metal chelate comprises a
5 macrocyclic or linear chelate selected from DTPA, DOTA, DTPA BMA, BOPTA, DO3A, HPDO3A, TETA, TRITA, HETA, M4DOTA, DOTMA, MCTA, PCTA and the derivatives thereof.
12. An imaging agent according to claim 11 wherein the chelate is coupled to a metal
10 element chosen among an ion of a paramagnetic metal of atomic number 21-29, 42-44, or 58-70, or a radionuclide, typically ^{99}Tc , ^{117}Sn , ^{111}In , ^{97}Ru , ^{67}Ga , ^{68}Ga , ^{89}Zr , ^{177}Lu , ^{47}Sc , ^{105}Rh , ^{188}Re , ^{60}Cu , ^{62}Cu , ^{64}Cu , ^{67}Cu , ^{90}Y , ^{159}Gd , ^{149}Pr , ^{166}Ho .
13. An imaging agent according to claim 12 wherein the metal ion is Gd.
15
14. An imaging agent according to any preceding claim wherein the peptide is directly bound to the signal entity.
15. An imaging agent according to any of claims 1 to 13 wherein the peptide is bound to
20 the signal entity by a linker.
16. An imaging agent according to claim 1 wherein the peptide is SEQ ID NO: 1 or SEQ ID NO: 4 and the signal entity is DOTA coupled to a Gd metal ion.
- 25 17. Use of the imaging agent according to any one of claims 1 to 16 for identifying the level of CD115 expression in an individual.
18. The use of claim 17, wherein the level of CD115 expression is identified in a sample obtained from an individual.
30
19. The use of claim 17 or 18 wherein the level of CD115 expression is altered.
20. The use of claim 19 wherein the level of CD115 expression is increased.

21. The use of claim 20 wherein the increased level of CD115 expression within a tissue indicates the presence of myeloid cells.
22. A method of using the imaging agent according to any one of claims 1 to 16 for
5 identifying the level of CD115 expression in an individual comprising administering to an individual the imaging agent and acquiring an image of a site of concentration of said imaging agent in the individual.
23. A method of acquiring an image from an individual that has previously been
10 administered an imaging agent according to any one of claims 1 to 16 for identifying the level of CD115 expression in the individual.
24. The method of claim 22 or claim 23, wherein the image is obtained by performing
15 magnetic resonance imaging (MRI).
25. The method of any of claims 22 to 24 wherein the level of CD115 expression is altered.
26. The method of claim 25 wherein the level of CD115 expression is increased.
20
27. The imaging agent according to any one of claims 1 to 16 for use in diagnosing a disease or disease risk in which levels of CD115 expression are altered.
28. The imaging agent according to any one of claims 1 to 16 for use in the manufacture
25 of an agent for diagnosing a disease or disease risk in which levels of CD115 expression are altered.
29. The use of an imaging agent according to any one of claims 1 to 16 for diagnosing a disease or disease risk in which levels of CD115 expression are altered.
30
30. The imaging agent for use according to claims 27 and 28, and the use according to claim 29, wherein the disease in which levels of CD115 expression are altered is a cardiovascular disease, neurological disease, autoimmune disorder or cancer.

31. The imaging agent for use according to claim 30 wherein the disease in which levels of CD115 expression are altered is atherosclerosis.
- 5 32. A method of identifying a patient at high risk of an acute ischemic attack by determining the degree of atherosclerotic plaque inflammation in the patient comprising administering to the patient an imaging agent according to any one of claims 1 to 16 and acquiring an image of a site of concentration of said imaging agent in the patient by MRI.
- 10 33. A pharmaceutically acceptable composition comprising the imaging agent of any of claims 1 to 16 and an excipient.
34. A method of preparing the imaging agent of any of claims 1 to 16 comprising coupling
15 the peptide to the signal entity.
35. A peptide having the amino acid sequence of SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 3 or SEQ ID NO: 4, or a sequence that differs from SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 3 or SEQ ID NO: 4 by 1, 2 or 3 amino acids, wherein the peptide
20 binds to a human CD115 receptor.
36. A peptide according to claim 35 wherein the peptide has the amino acid sequence of SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 3 or SEQ ID NO: 4, or a sequence that differs from SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 3 or SEQ ID NO: 4 by 1 or
25 2 amino acids.
37. A peptide according to claim 35 wherein the peptide has the amino acid sequence of SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 3 or SEQ ID NO: 4, or a sequence that differs from SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 3 or SEQ ID NO: 4 by one
30 amino acid.
38. A peptide according to claim 35 wherein the peptide is SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 3 or SEQ ID NO: 4.

39. A kit comprising:
- i) a peptide according to any one of claims 35 to 38;
 - ii) a signal entity; and
 - 5 iii) components for coupling the peptide to the signal entity.

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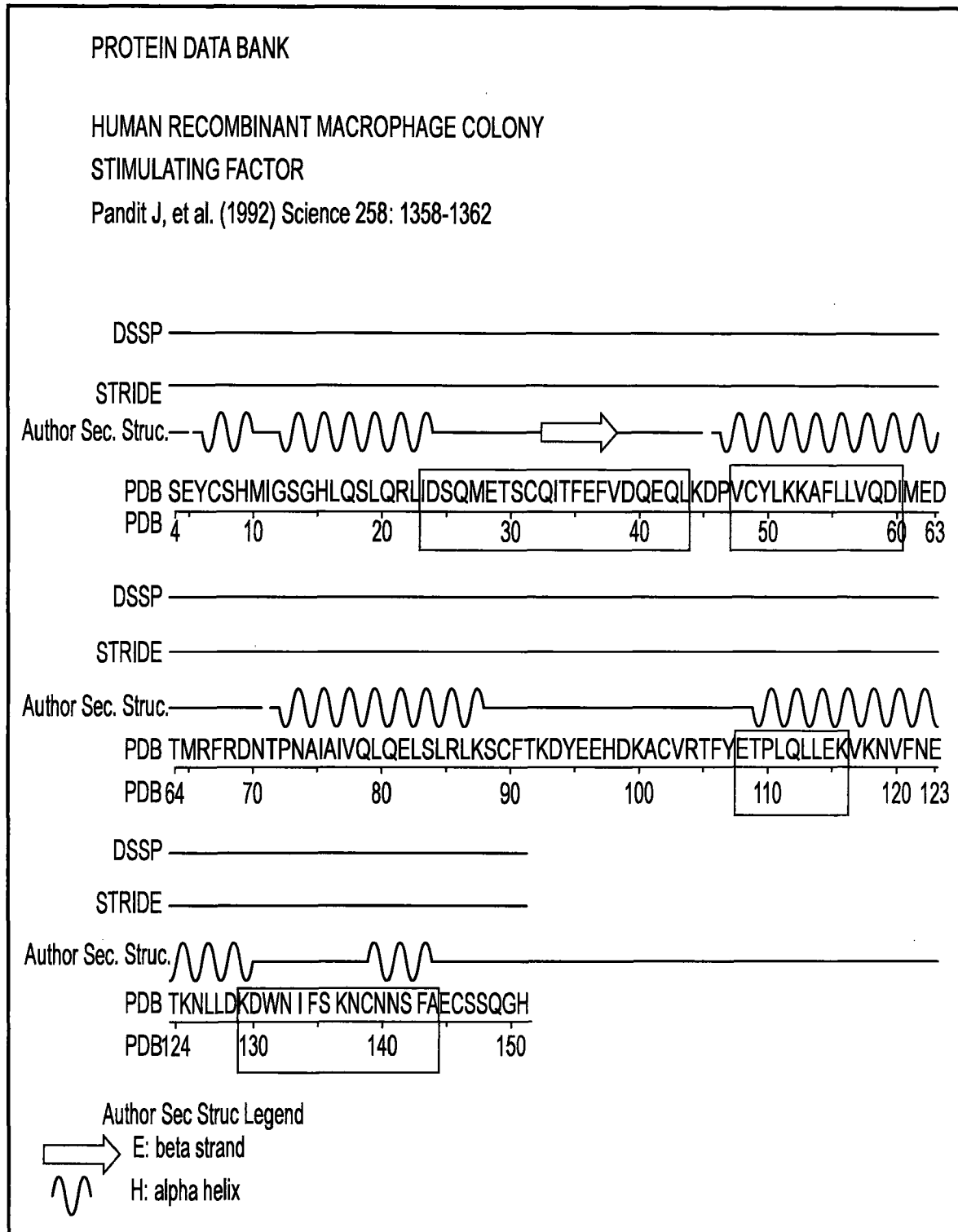


FIG. 1

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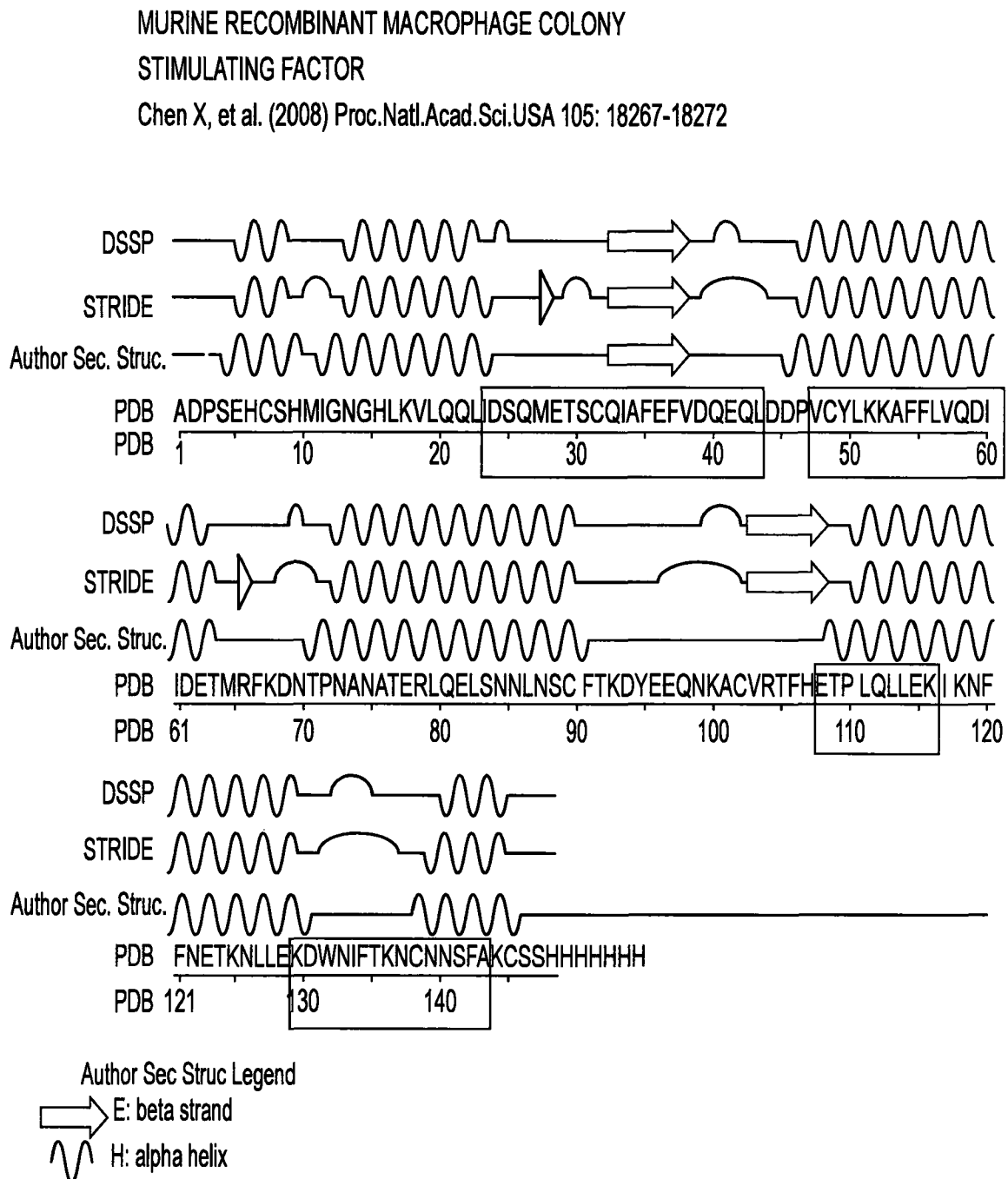


FIG. 1 (continued)

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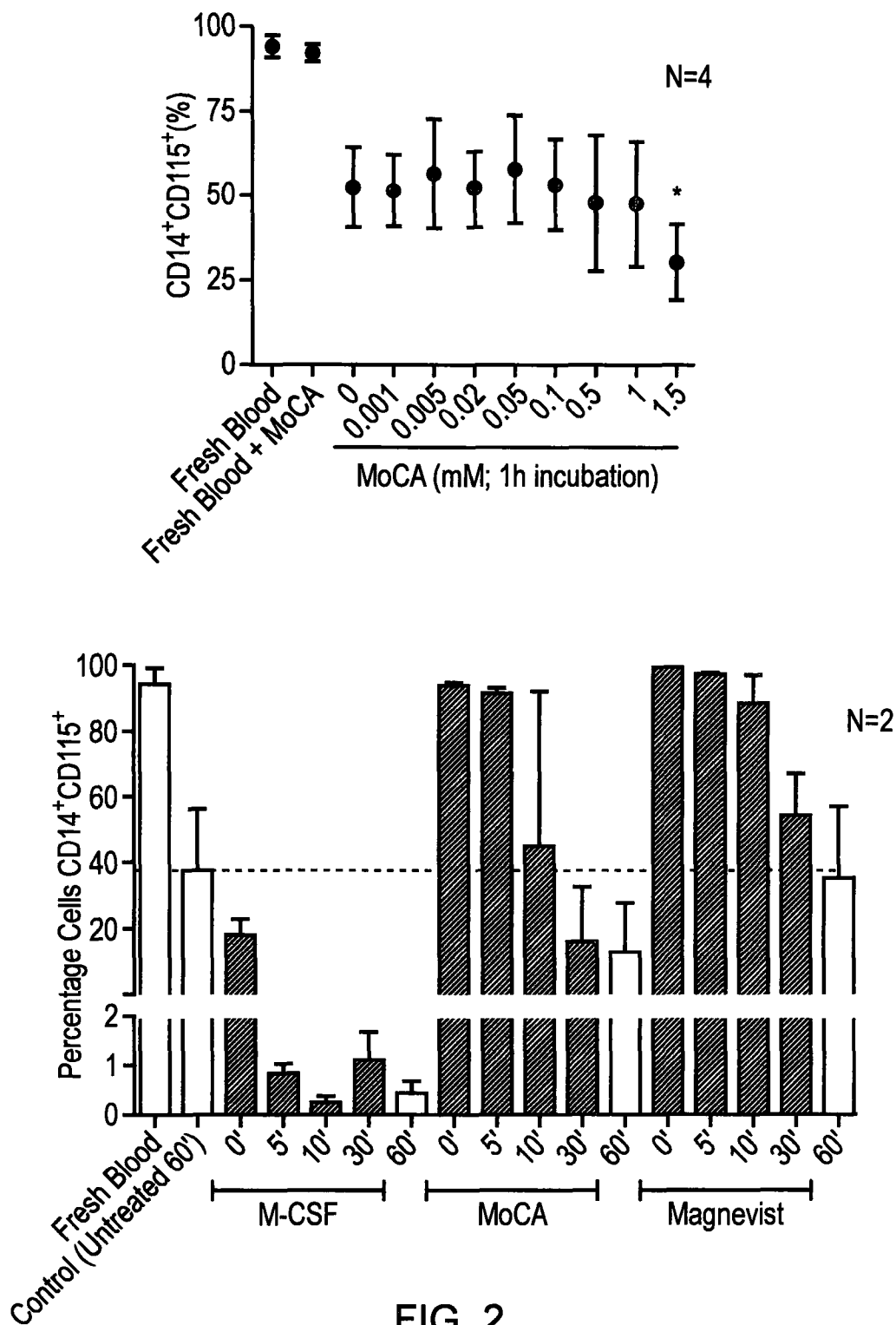


FIG. 2

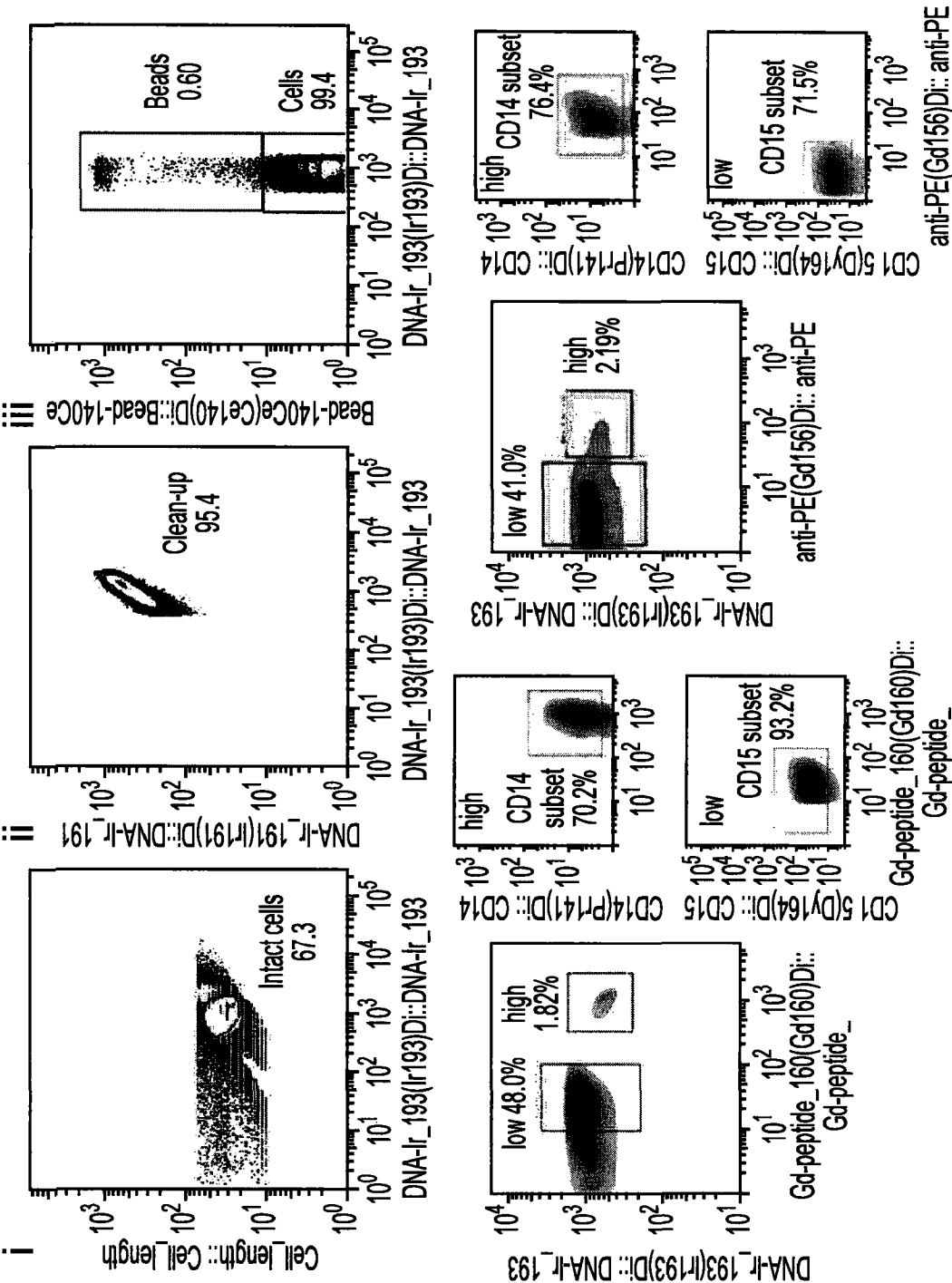


FIG. 3

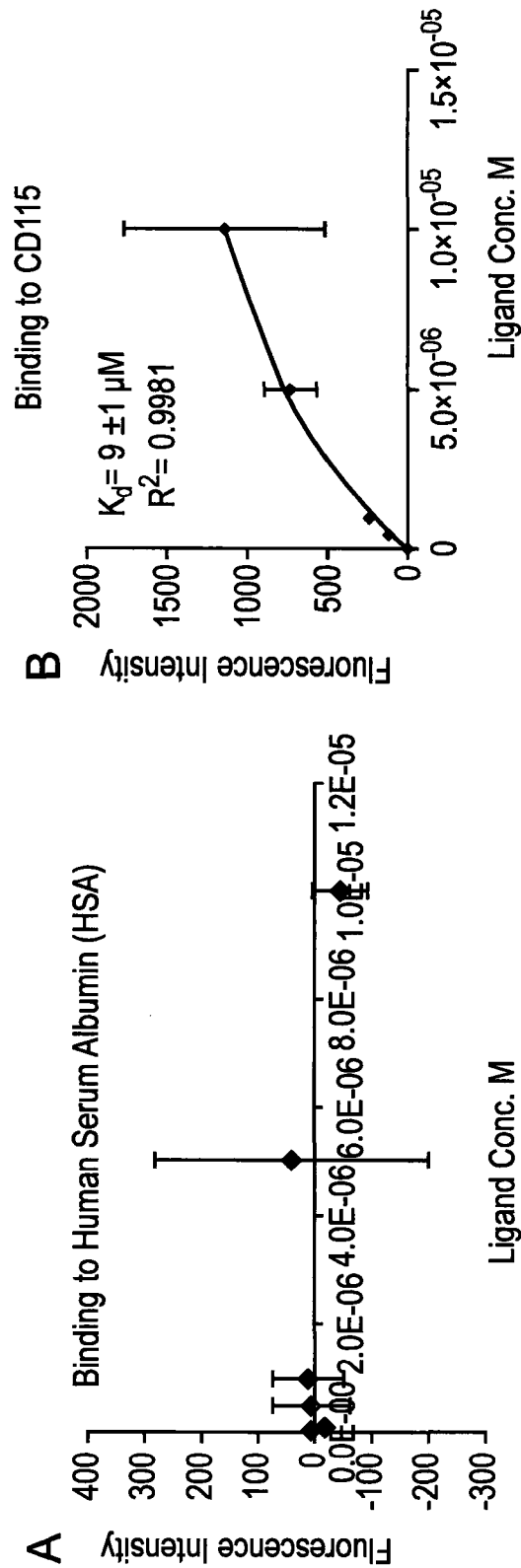


FIG. 4

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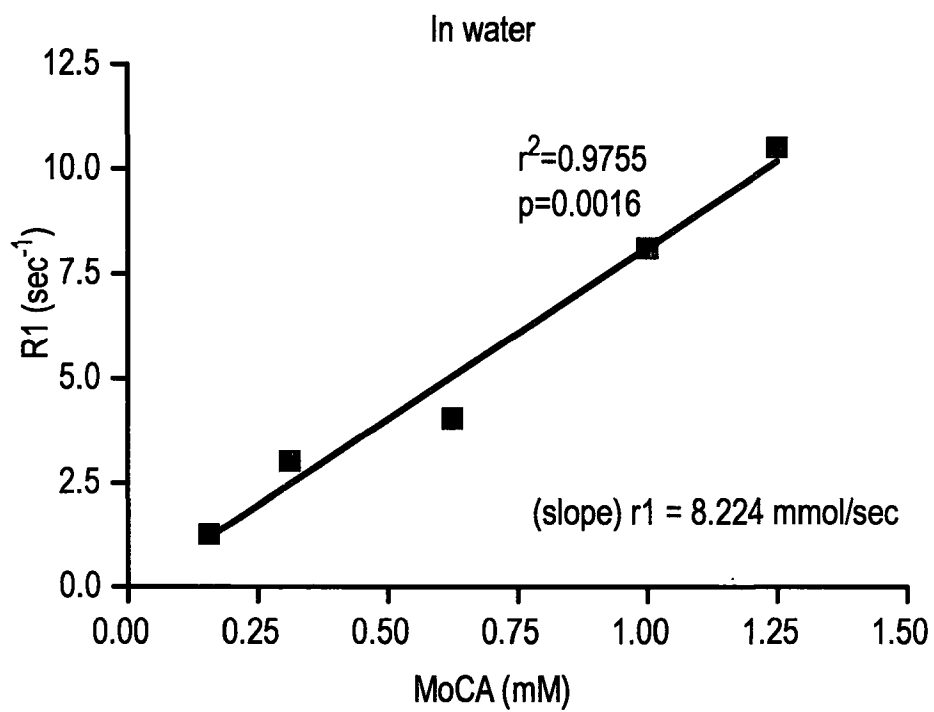


FIG. 5

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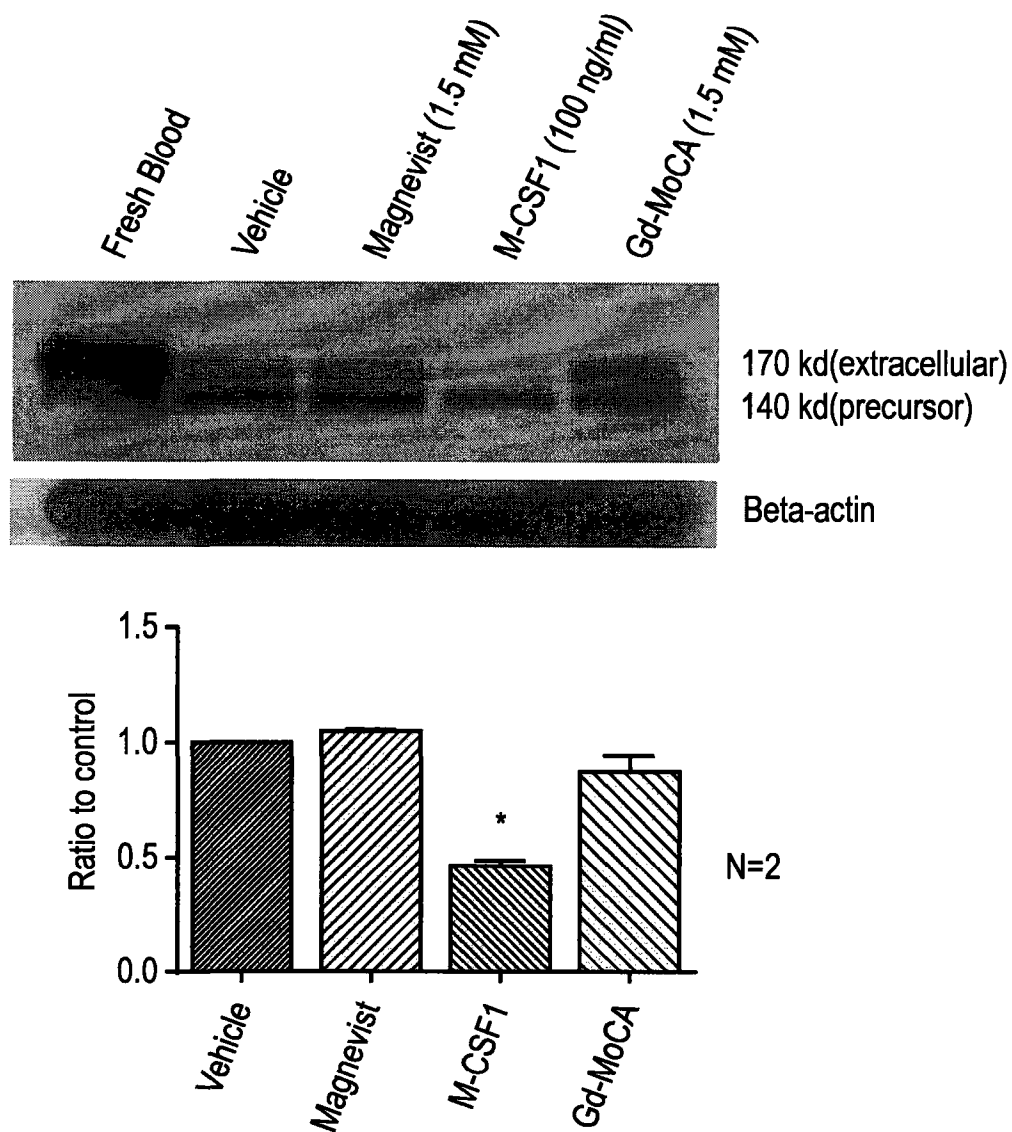


FIG. 6

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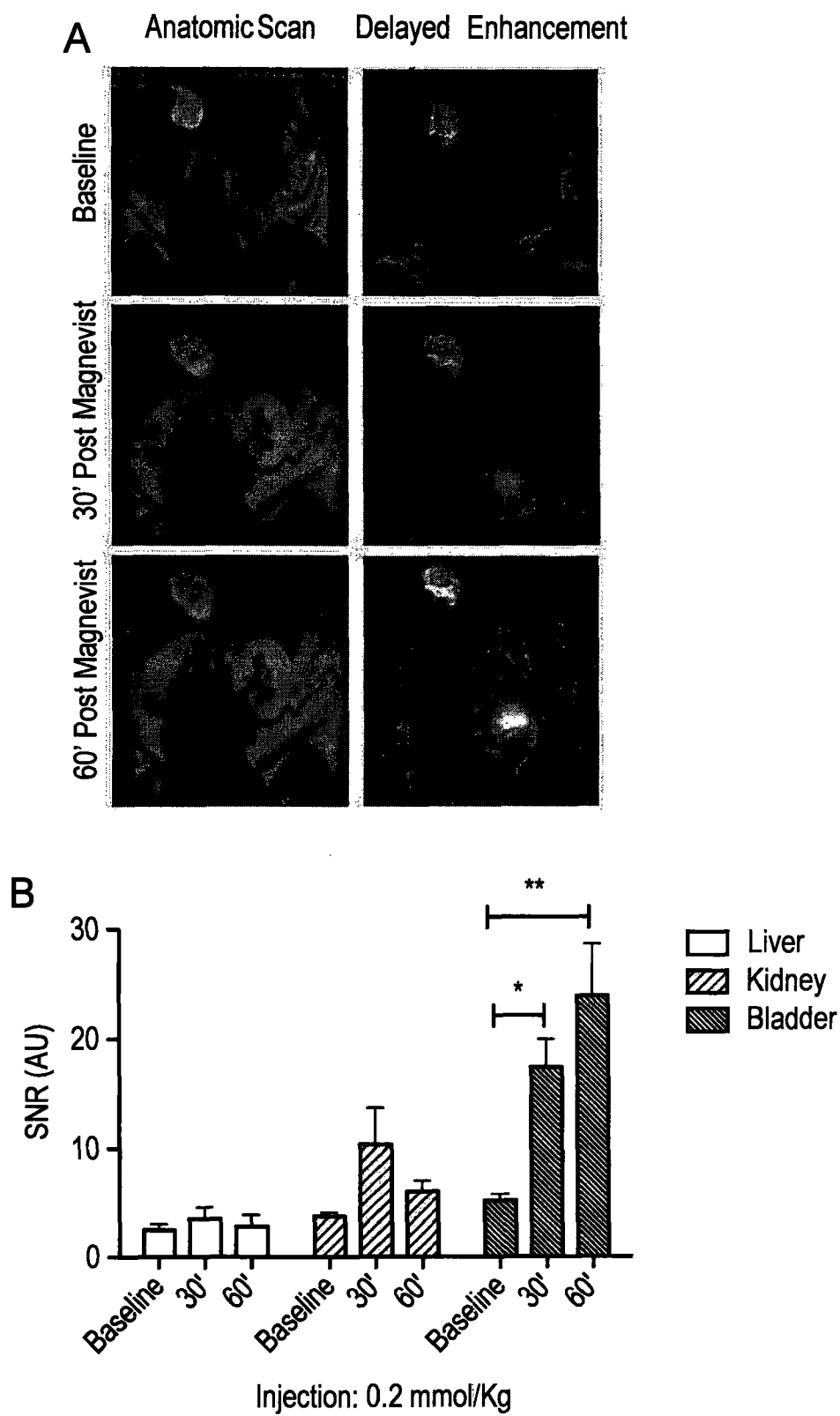


FIG. 7

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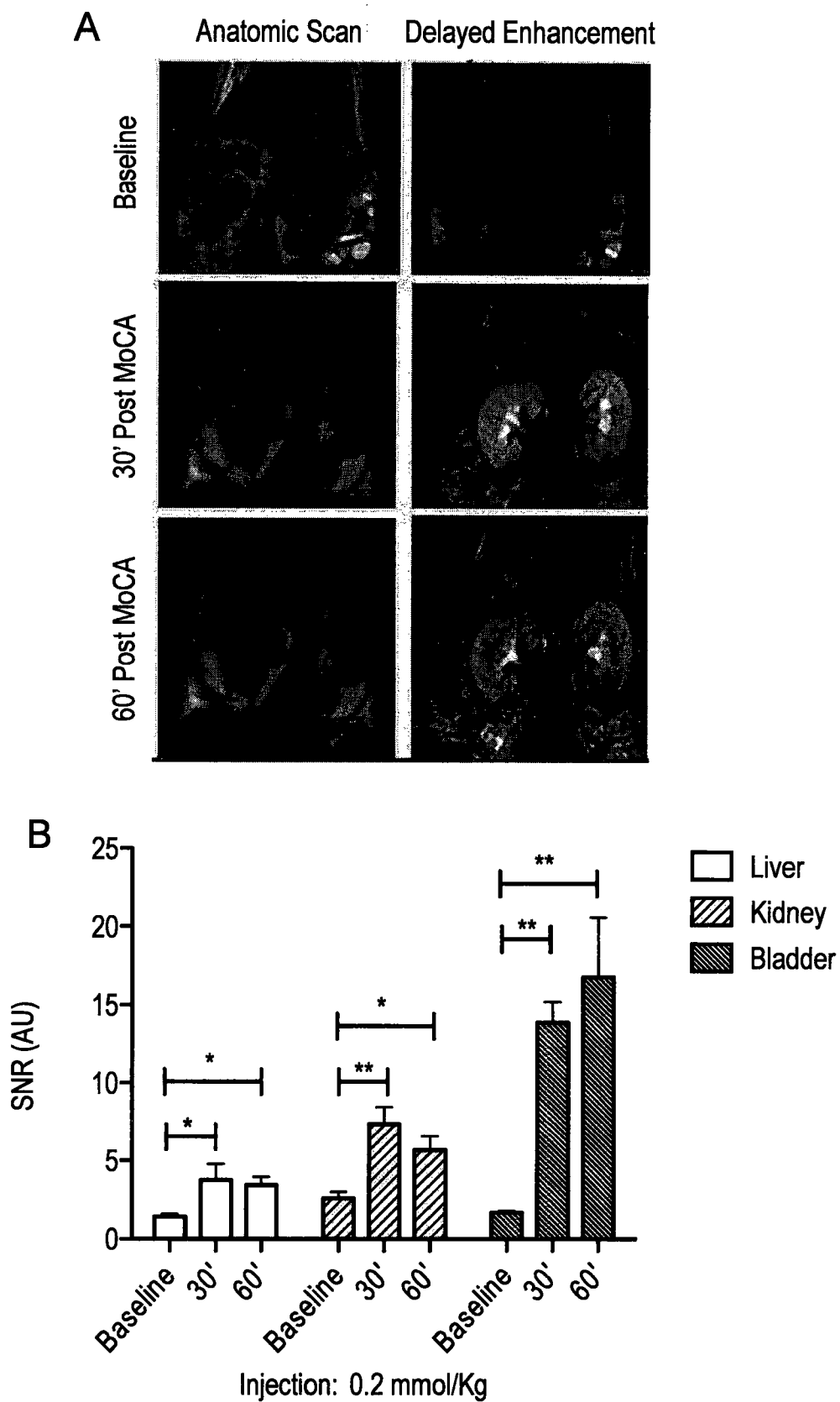


FIG. 8

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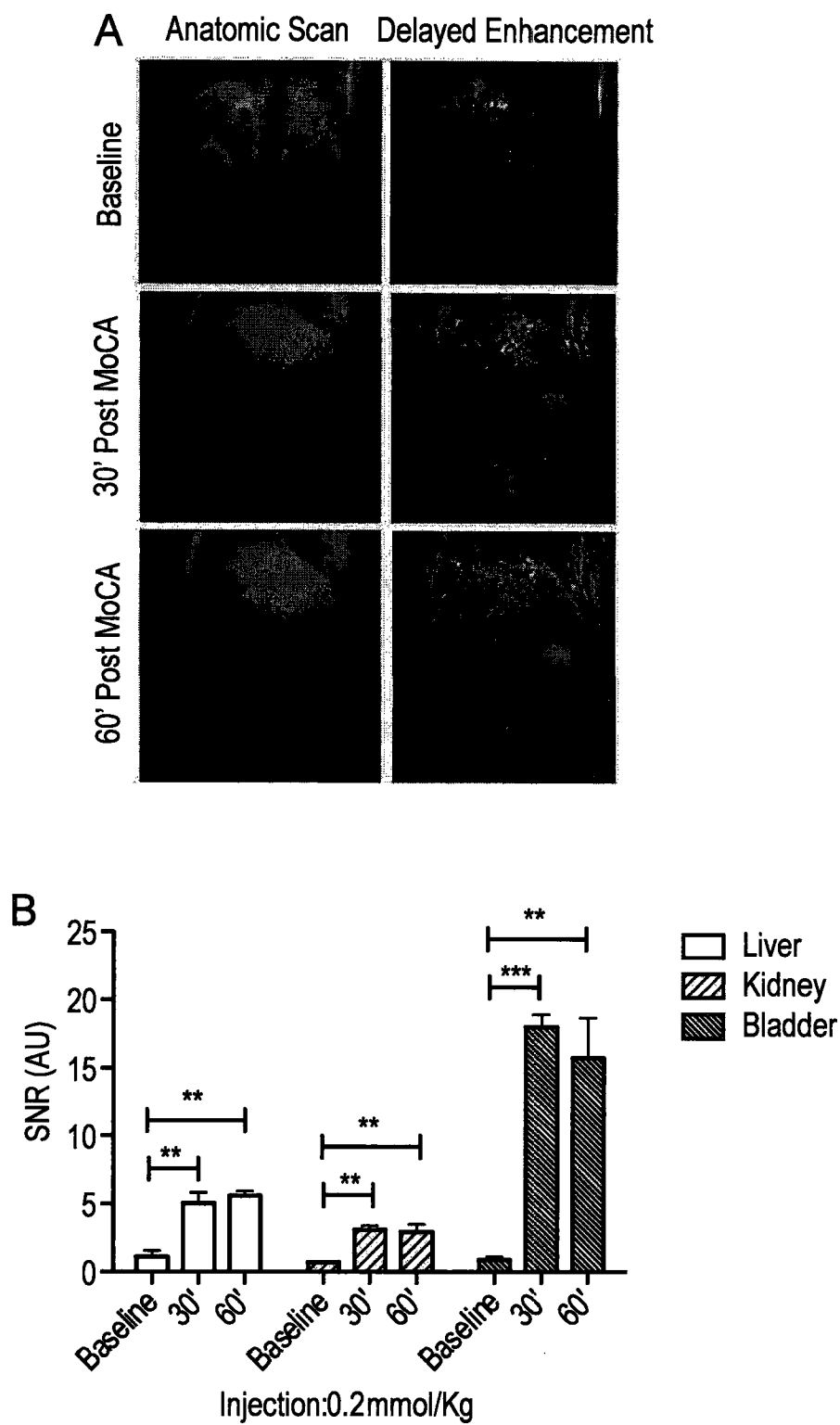


FIG. 9

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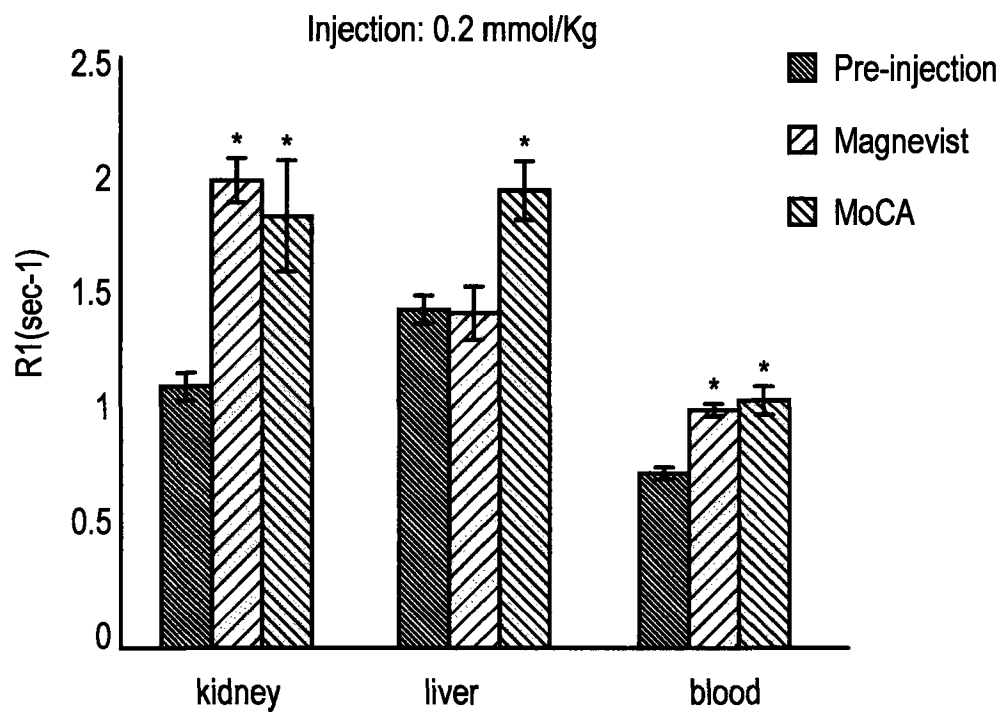
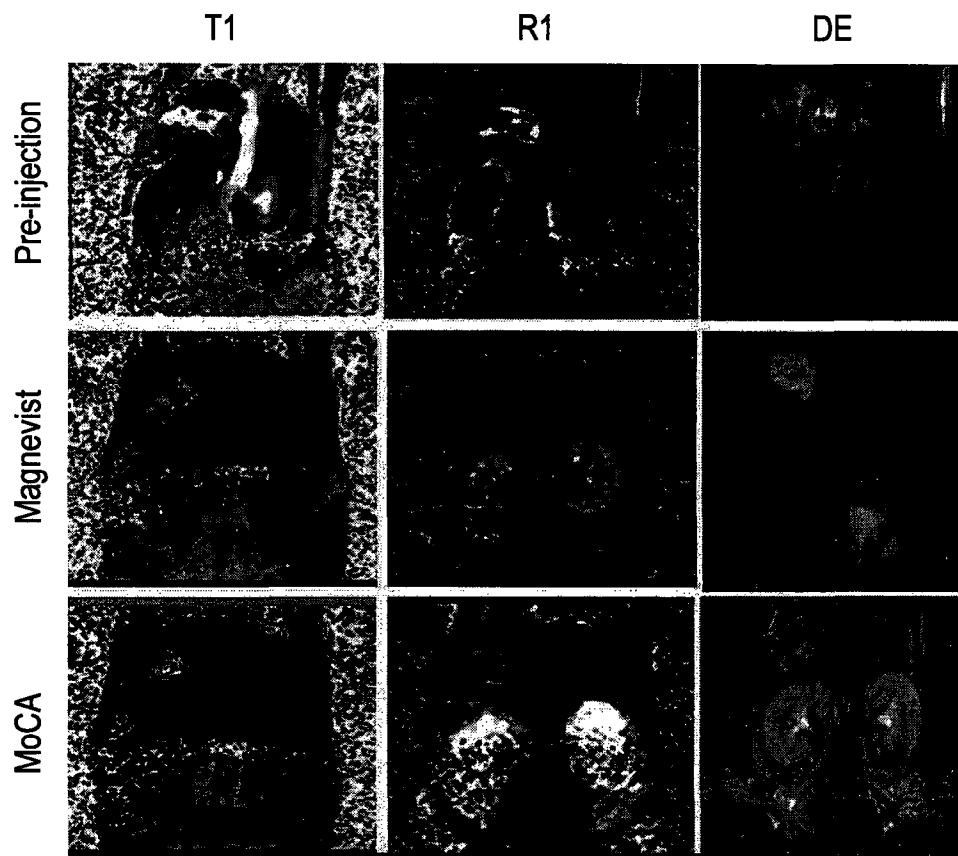


FIG. 10

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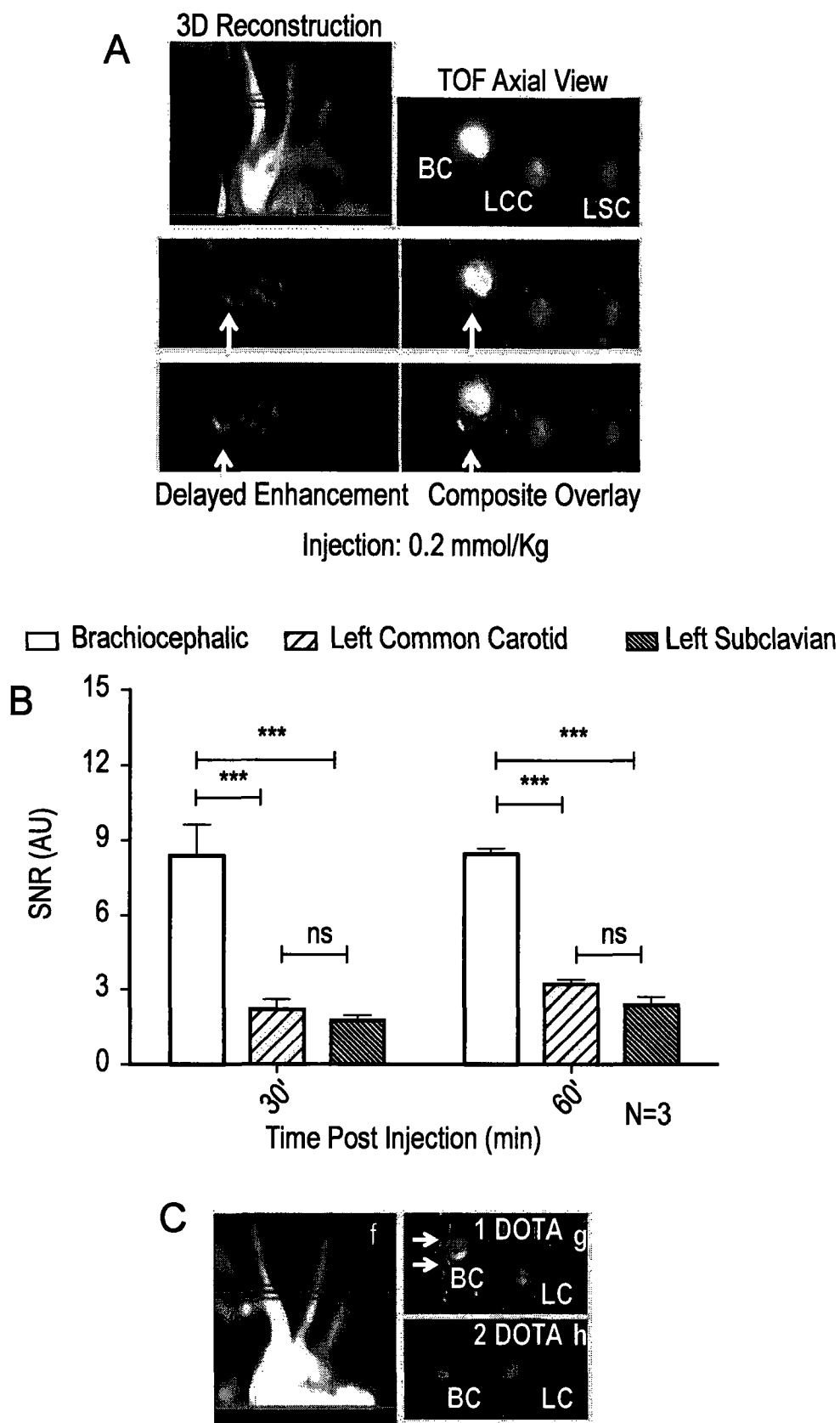


FIG. 11

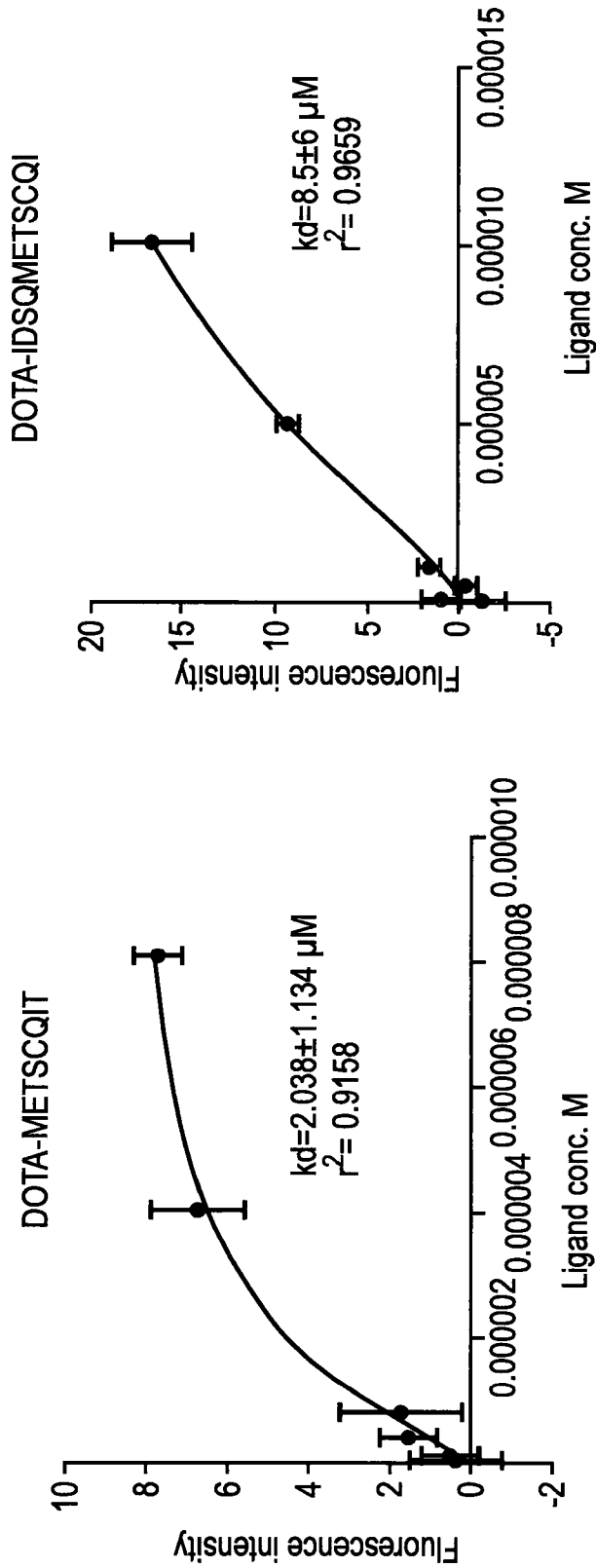


FIG. 12

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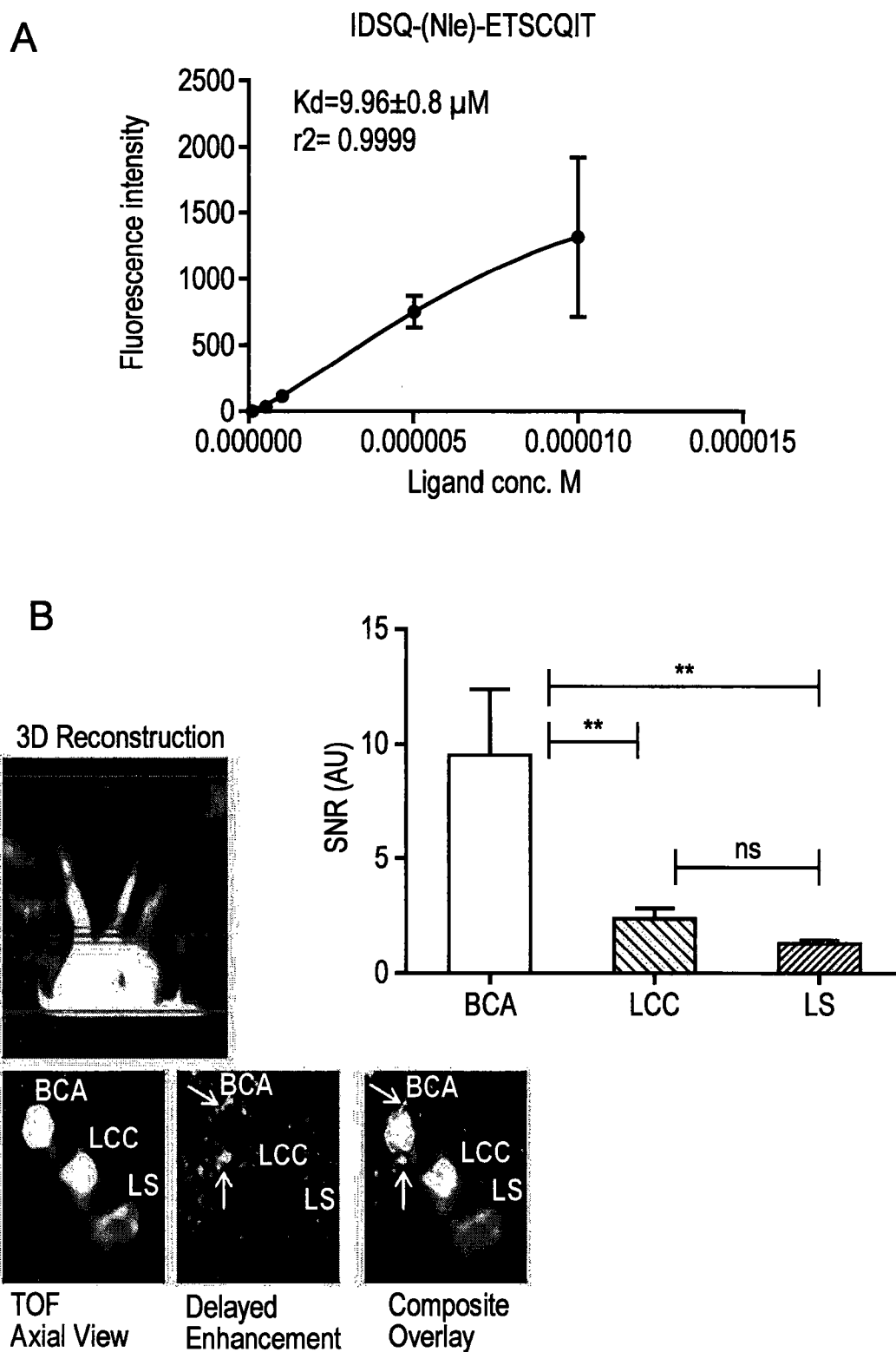


FIG. 13

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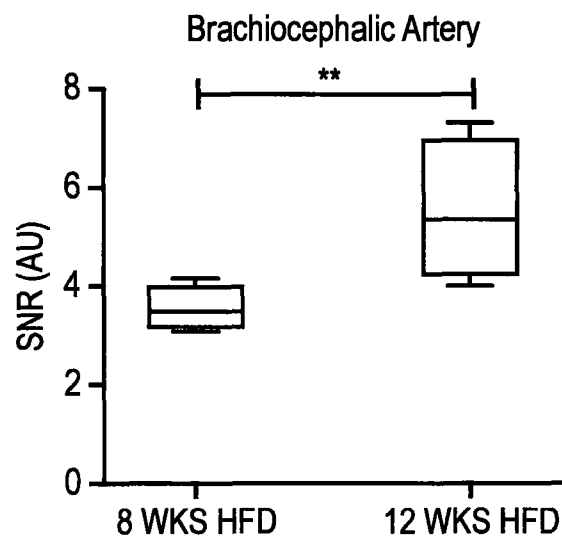
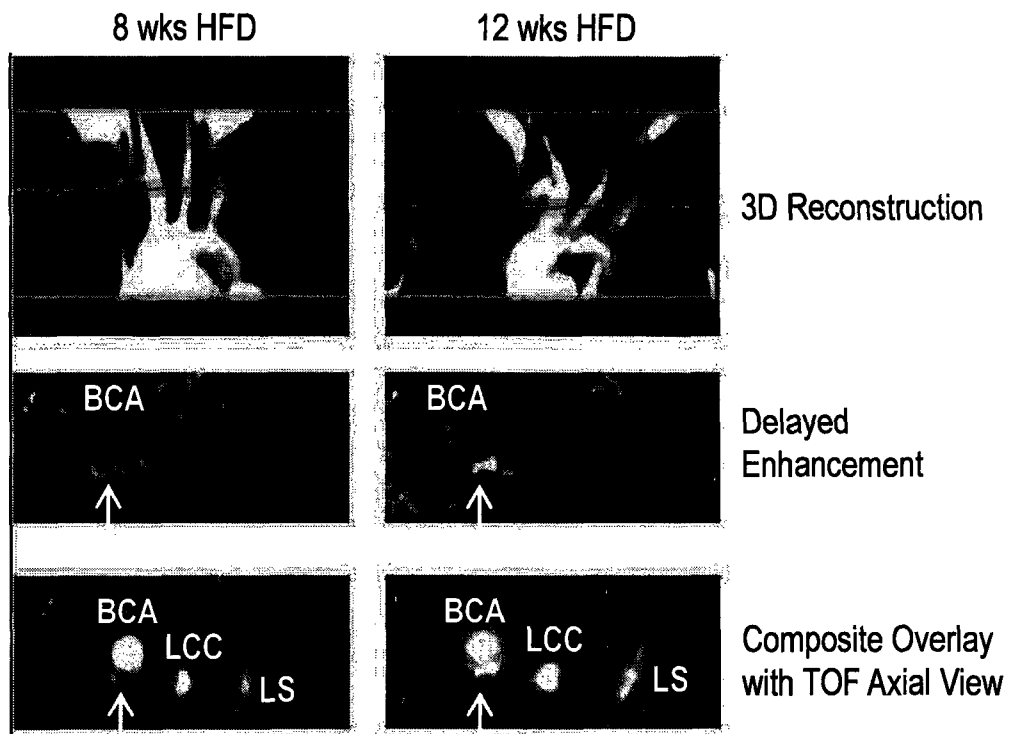


FIG. 14

INTERNATIONAL SEARCH REPORT

International application No
PCT/GB2015/051931

A. CLASSIFICATION OF SUBJECT MATTER		
INV.	A61K49/08 G01N33/58	A61K49/14 A61K51/08 C07K7/04 C07K7/08
ADD.		
According to International Patent Classification (IPC) or to both national classification and IPC		
B. FIELDS SEARCHED		
Minimum documentation searched (classification system followed by classification symbols) A61K C07K G01N		
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, WPI Data, EMBASE, BIOSIS		
C. DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	PANDIT J ET AL: "Three dimensional Structure of dimeric human recombinant Macrophage Colony stimulating factor", SCIENCE, AMERICAN ASSOCIATION FOR THE ADVANCEMENT OF SCIENCE, US, vol. 258, 20 November 1992 (1992-11-20), pages 1358-1362, XP002126391, ISSN: 0036-8075, DOI: 10.1126/SCIENCE.1455231 abstract ----- -/--	1-9,14, 15,28, 33,34
☒ Further documents are listed in the continuation of Box C. ☒ 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 18 September 2015		Date of mailing of the international search report 02/10/2015
Name and mailing address of the ISA/ European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Fax: (+31-70) 340-3016		Authorized officer Villard, Anne-Laure

INTERNATIONAL SEARCH REPORT

International application No

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C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT		
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A	Vardan Smbat Amirbekian Amirbekian ET AL: "Detecting and Assessing Macrophages in vivo to Evaluate Atherosclerosis Noninvasively Using Molecular MRI", Proceedings of the National Academy of Sciences of the United States of America, 16 January 2007 (2007-01-16), pages 961-966, XP055213945, United States DOI: 10.1073/pnas.0606281104 Retrieved from the Internet: URL:http://www.jstor.org/stable/25426216 abstract page 961, right-hand column, last paragraph page 962, right-hand column, paragraph 2 page 965, right-hand column, paragraph 1 -----	1-39
A	STEPHAN METZ ET AL: "Characterization of carotid artery plaques with USPIO-enhanced MRI: assessment of inflammation and vascularity as in vivo imaging biomarkers for plaque vulnerability", THE INTERNATIONAL JOURNAL OF CARDIAC IMAGING, KLUWER ACADEMIC PUBLISHERS, DO, vol. 27, no. 6, 24 October 2010 (2010-10-24), pages 901-912, XP019931099, ISSN: 1573-0743, DOI: 10.1007/S10554-010-9736-7 abstract -----	1-39
A	JEZOVNIK MATEJA KAJA ET AL: "Identification of inflamed atherosclerotic lesions in vivo using PET-CT.", INFLAMMATION APR 2014, vol. 37, no. 2, April 2014 (2014-04), pages 426-434, XP002744687, ISSN: 1573-2576 abstract -----	1-39
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A	US 2006/239913 A1 (PORT MARC [FR] ET AL) 26 October 2006 (2006-10-26) cited in the application paragraphs [0086] - [0088] page 8; compound B -----	1-39
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INTERNATIONAL SEARCH REPORT

International application No

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

Information on patent family members

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