Abstract:
The present invention relates to isolated ligands of anti-Mullerian hormone marked so as to be directly detectable by means of magnetic resonance imaging in the endometriotic lesions. In particular, such ligands can be used in a method for diagnosing in vivo endometriosis wherein said method comprises a passage of localizing and/or evaluating the entity of the endometriosic lesions in a patient.

FIGURE 1A

FIGURE 1B

Title: LABELLED LIGANDS OF ANTI-MULLERIAN HORMONE FOR THE DIAGNOSIS OF ENDOMETRIOSIS
LABELLED LIGANDS OF ANTI-MULLERIAN HORMONE FOR THE DIAGNOSIS OF ENDOMETRIOSIS

DESCRIPTION

The present invention relates to isolated ligands of anti-Mullerian hormone marked so as to be directly detectable by means of imaging techniques in the endometriosic lesions. In particular, such ligands can be used in a method for the in vivo diagnosis of endometriosis wherein said method comprises a passage of localizing and/or evaluating the entity of the endometriosic lesions in a patient.

STATE OF PRIOR ART

Endometriosis is defined as a recurrent and benign gynaecological disorder characterized by the presence of endometrial tissue (glands and stroma) outside the cavity of uterus. It is one of the most common diseases in the gynaecological field, affecting about 10% of the female population in reproductive age, whereas its frequency rises up to 20-50% in women with fertility problems (Baldi A. et al. Endometriosis: pathogenesis, diagnosis, therapy and association with cancer. Oncology Reports 2008;19:843-846).

The endometriosic neoformations mainly are localized on the pelvic peritoneum and ovaries, but they can be commonly found in the sub-peritoneal areas and, more rarely, in any anatomic district, such as for example pericardium, pleurae, pulmonary parenchyma and even brain (Giudice LC , and Kao LC: Endometriosis. The Lancet, 364: 1789-1799, 2004; Signorile PG et al.. Rectovaginal septum endometriosis: an immunohistochemical analysis of 62 cases. In Vivo 2009;23,459-464).


Recently the presence of endometriosic lesions in the female foetus has been described and this represents the first demonstration of a different pathogenetic theory based upon defects of embryogenesis (Signorile PG, Baldi A: Endometriosis: new concepts in the pathogenesis. Int J Biochem Cell Biol 2010; 42:778-780).
The anti-Mullerian hormone (AMH) is a glycoprotein belonging to the superfamily of the "Transforming Growth Factor-beta" (TGF-beta). The AMH is produced by the cells of the Sertoli in the male foetus and it is responsible for the regression of the Mullerian ducts (La Marca A et al.: Anti-Mullerian hormone (AMH): what do we still need to know? Hum Reprod, 24: 2264-2275, 2009). The AMH expression in the ovarian follicles starts in the female foetus, around the 32th week of gestation and keeps for the woman's whole fertile life. The AMH expression levels are considered good indicators of a woman's ovarian reserve; they decay with menopause (Lee MM et al.: Mullerian inhibiting substance in humans: normal levels from infancy to adulthood. J Clin Endocrinol Metab, 81: 571-576, 1996). Furthermore, an anti-cancer action for AMH has been proposed in the ovarian epithelial tumours and different experimental proofs seem supporting the cytotoxic effect on tumour cells (La Marca A., Volpe A: The anti-Mullerian Hormone and ovarian cancer. Hum Reprod., 13: 265-273, 2007). Recent studies have demonstrated that AMH, as well as a receptor thereof (MISRII), are expressed in the adult woman even at the level of endometrium, wherein probably they performs a function of paracrine type.

Up to now no marker has been described allowing localizing exactly in vivo the endometriosic lesions, both cystic and connective solid ones. In particular, several of the endometriosic neoformations can even have very reduced sizes (smaller than 1 cm), which makes practically impossible, with the currently available analysis methods, to highlight in vivo the localization both of cystic endometriosic lesions smaller than two millimetres and of the connective solid lesions smaller than one centimetre.

Still nowadays endometriosis is a disease therefor the one and only effective therapeutic strategy is the surgical removal of the endometriosic lesions: there is no resolving pharmacological therapy and the only pharmacological treatments used by the medical-scientific community are able only to act on symptoms, by relieving them. However, the success of the surgical procedure is substantially based upon the possibility of displaying in vivo the endometriosic lesion, which display is strictly connected even to the size of the lesion itself. It follows that, the effectiveness of the surgical treatment is limited by the fact that, as the disease is multicentric and often microscopic, the surgeon not always succeeds in eliminating all disease foci.

Therefore, in the state of art it is highly felt the need of detecting procedures allowing to obtain a precise picture regarding the localization and the sizes of the disease's different foci (endometriosic formations) in the patient, so as to be able to
diagnose and intervene in the most effective way in patients with endometriosis even in the states wherein the lesions have very reduced sizes.

The scope of the present invention is to overcome the problems associated to the detection of the endometriosis formations and, in particular, of the endometriosis neoformations, so as to develop alternative methods for diagnosing and/or treating endometriosis.

SUMMARY OF THE INVENTION

The present invention relates to isolated ligands of anti-Mullerian hormone marked so as to be directly detected in the endometriosis lesions by means of magnetic resonance imaging. In particular, such ligands can be used in an in vivo method for diagnosing endometriosis including a passage of localizing and/or evaluating the entity of the endometriosis lesions in a patient.

The invention subject of the present description is based upon the scientific observation, made by the inventors themselves, that the anti-Mullerian hormone (AMH) is over-expressed in the endometriosis lesions as shown in figure 1. From such observation derives the intuition of the inventors of being able to use AMH as target to detect foci (formations and/or neoformations) of the endometriosis disease.

In particular, as highlighted in the section "Examples", it was demonstrated that AMH can be used in an effective way as cellular target to allow to detect in vivo the exact localization of the endometriosis lesions. In fact, as shown in example 2 herebelow, a xenotransplant of human endometriosis tissue in nude mice can be subsequently displayed by means of using a marked ligand such as, for example, a marked anti-AMH antibody so as to be able to be detected by means of in vivo magnetic resonance imaging techniques (figures 2 and 3).

In particular, the use of a ligand able to link the AMH, marked so as to be able to be detected by means of magnetic resonance imaging in vivo techniques, demonstrated to be effective in detecting not only the endometriosis lesions with appreciable sizes but even anatomic localizations of endometriosis with small sizes, lower than 0.5 centimetres of diameter. These data suggest that the ligand of the invention can be used advantageously not only for localizing the lesions but even for evaluating, by means of the sizes of the lesions themselves, the entity/gravity of endometriosis.

It follows that the ligand of the invention can be used even for learning the real extension of the endometriosis disease since the intra-organ lesions, which are not detectable by surgery, can be effectively localized too before performing the operation.
From what said above, it appears clear that the use of the anti-AMH antibody of the invention can make the passages of diagnosing the endometriosis and/or surgical treatment of the pathology more selective and effective by defining the precise localization and extension of the endometriotic lesions.

Furthermore, as the here described approach is not invasive since it mainly consists in administrating the ligand able to link the AMH to the patient and displaying in vivo the sites wherein it accumulates as consequence of the link to the AMH deposits, the ligand of the invention can be advantageously used even to monitor in time the progress of the endometriotic pathology such as, for example but not only, in case the patient is subjected to schemes of pharmacological and/or surgical therapies.

Therefore the subject of the present application is:
- the isolated ligand of anti-Mullerian hormone suitable to be detected directly by means of magnetic resonance imaging for use in a in vivo method of diagnosing endometriosis comprising a passage of localization and/or evaluation of the entity of the endometriotic lesions in a patient;
- a formulation for use in a in vivo method of localizing and/or evaluating the entity of the endometriotic lesions in a patient comprising a ligand according to the invention and at least pharmaceutically acceptable carrier and/or excipient;
- kit for localizing and/or evaluating in vivo the entity of the endometriotic lesions in a patient comprising at least a ligand of the invention or a formulation of the invention and means useful to administer said ligand or said formulation to said patient.

Additional advantages, as well as the features and the use modes of the present invention will result evident from the following detailed description of some preferred embodiments, shown purely by way of example and not with limitative purpose.

**DETAILED DESCRIPTION OF THE FIGURES**

**Figure 1A e 1B:** Examples of the AMH hormone expression in the in vivo endometriotic structures, by means of immunohistochemistry method; the AMH expression is detected by the colouring of intense dark colour.

**Figure 2A and 2B:** total-body Magnetic Resonance Image of a small female mouse before (figure 2A) and after (figure 2B) the inoculation of gadolinium-conjugated antibody against AMH: the area corresponding to the sub-cutaneous
ectopic transplant of connective solid endometriosic tissue and the tail area wherein
the inoculation of the gadolinium-antibody compound for AMH took place are circled in
white.

**Figure 3A e 3B**: Magnetic Resonance Image in cross-section of a female
mouse before (figure 3A) and after (figure 3B) the inoculation of gadolinium-
conjugated antibody against AMH: the area corresponding to the ectopic transplant is
circled in white.

**Figure 4A-D**: histological and immunohistochemical analysis of the
transplanted tissue. Figures A and B show the histological structure of the transplant
with colouring by means of Hematoxylin-Van Gieson and Hematoxylin-Eosin; figures C
and D show the expression (immunohistochemical colouring of intense black colour),
respectively of CD10 and AMH in the transplanted tissue.

**DETAILED DESCRIPTION OF THE INVENTION**

The present invention, as already shown in the previous section, relates
to an isolated ligand of the marked anti-Mullerian hormone (AMH) able to be detected
by means of magnetic resonance imaging in the endometriosic lesions.

Under ligand of the anti-Mullerian hormone (Ab anti-AMH) in the present invention a
natural or synthetic molecule is meant, able to link, preferably with high affinity, at least
a specific epitope of AMH protein.

As it is known to the person skilled in the art the term epitope or antigenic determinant
relates to a site on the antigen, in this case the AMH protein, which is specifically
recognized and linked by an immunoglobulin. The epitopes can be formed by a
sequence of contiguous aminoacids or by juxtaposed aminoacids in the three-
dimensional shape of the protein. Preferably the ligand of the present invention is able
to link an AMH epitope not present in other proteins, so as to avoid the aspecific link
with proteins different from the anti-Mullerian hormone.

The anti-Mullerian hormone is a glycated protein of homodimeric 140 kDa
belonging to the superfamily of the "Transforming Growth Factor-beta" (TGF-beta).
The nucleotidic sequence and the coding aminoacidic sequence for the AMH of
different origins (human, murine, bovine) is described in the known state of art. In
particular, the aminoacid sequence of the monomeric AMH of human origin (sequence
of 535 aa) is described in the bank UniProtKB/Swiss-Prot, version 133, last
modification 16 May 2012; http://www.uniprot.org/) and identified with number P03971.

Preferably, the ligand of the invention is able to recognize and link an epitope of
the human anti-Mullerian hormone.
In an embodiment, the ligand of the invention can be an antibody or a receptor able to link in specific way at least an epitope of the AMH hormone. By way of example and not for limiting purpose, the isolated receptor to be used as ligand according to what described herein is the receptor of type II of the anti-Mullerian hormone (MISIIIR) (The Mullerian duct: recent insights into its development and regression Klattig J, Englert C. Sex Dev. 2007;1(5):271-8).

Under the term "antibody" in the present invention complete antibodies, antibodies with single chain, synthetic antibodies, chimeric antibodies, humanizing antibodies, not human antibodies, conjugates of antibodies and fragments or their derivatives are meant. In particular, under "complete antibodies" in the present invention proteins or glycoproteins are referred to, comprising at least two heavy chains and at least two light chains inter-connected by means of disulphide bridges.

Each heavy chain is composed of a variable region (V_H) and a constant region (C_H).

The constant region (C_H) comprises three domains C_H1, C_H2 and C_H3.

Each light chain is composed of a variable region (V_L) and a constant region (C_L).

The variable regions of the heavy chain (V_H) and of the light chain (V_L) can be further divided into iper-variable regions known as "Complementarity determining regions" (CDR). Such regions CDR are ipervariable with respect to the more preserved regions known as Framework region (FR). Each V_H and V_L is composed of 3 CDR and four FR, arranged from the terminal amino end to the terminal carboxy end in the following order: FR1, CDR1, FR2, CDR2, FR3, CDR3, FR4. The variable regions of the light and heavy chains contain the domain interacting with the antigen, by linking it. By way of pure example, such fragment can be: a Fab fragment consisting of the domains V_L, V_H, C_L and C_H1; a fragment consisting of the domains V_H and C_H1; a Fv fragment consisting of domains V_L and V_H; a fragment consisting of a single variable domain isolated by a CDR region; F(ab')2 fragment comprising two linked fragments Fab; Fv molecules with single chain wherein a domain V_L and V_H are linked by a connecting peptide promoting the association between the two domains so as to form a linkage site for the antigen. Examples of possible forms and structures of the antibodies are described in Holliger&Hudson (2006) Nature Biotechnology 23(9): 1126-1136; Carter (2006) Nature Reviews Immunology 6:343:357.

In case the ligand is an antibody, however alternative embodiments can be provided such as a human, humanized, murine, chimeric, rabbit, sheep antibody or however of any origin provided that it is able of recognizing and linking at least an epitope of the AMH hormone. Under the term "humanized" an antibody is meant comprising a human framework region (FR) and one or more regions determining the
complementarity (CDR) of not human origin, for example murine. In a preferred embodiment of the invention, the antibody is of human origin. By purely way of example an antibody able to recognize the human AMH is the one commercialized by ABCAM, # cat. ab103233, MIS Antibody(C-20): sc-6886 of Santa Cruz; antibody against AMH # cat (MM0475-7H26) of Novus Biologicals; hormone against AMH # cat AM05878SU-N, of Acris Antibodies.

Furthermore, the antibody, as well as the receptor able to link AMH, can be both a recombinant protein and a protein usually present in nature. Under recombinant protein a molecule is meant which is produced in organisms and host cells which do not produce naturally the interest protein, for example either an anti-AMH antibody or an AMH receptor. The antibody can be both a monoclonal and a polyclonal antibody or, as it is known to the person skilled in the art, an antibody with a single linking specificity or obtained from antibodies produced by different colonies of lymphocytes B.

The ligand of the AMH subject of the present invention is an isolated ligand marked so as to be directly detected by means of magnetic resonance imaging in the endometriosic lesions.

Under the term "isolated" in the present invention ligands in substantially free form are meant, for example in case of ligands present in the cells, free from any cellular material. In other words, in case the ligand, for example, is an AMH receptor, such receptor will be in a different form from that in which it is in nature, that is without interactions with cellular components, such as for example, the plasmatic membranes thereto it is usually associated. In case of an anti-AMH antibody, instead, it will be free from antibodies having different antigenic specificity.

In particular, the detection of the marked ligand by means of magnetic resonance imaging can be performed by using techniques such as for example, and without being limited thereto: ecography, radiography, computed tomography, nuclear magnetic resonance, tomography with emission of positrons, scintigraphy or however any other imaging method useful to detect the antibody of the invention. Such techniques are well known to the person skilled in the art and therefore do not request herein further examinations. A description of the magnetic resonance imaging techniques useful to the purpose of the present invention is however present in Sutton's Textbook of Radiology & Imaging 7th Edition, published by Churchill Livingstone.

To the detection purpose, the ligand can be marked by using any agent suitable to the detection by means of magnetic resonance imaging and, as it will be
understood, the type of agent used to mark the ligand mainly will depend upon the type of techniques which will be chosen for displaying the endometriosic lesions. Generally, the ligand can be marked with at least one of the agents chosen in the group comprising: paramagnetic contrast agents, iodized contrast agents, intravenous contrast agents, radioisotopes.

By purely way of example and not for limitative purposes, the paramagnetic contrast agents can be chosen among: gadolinium or manganese; the iodized contrast agents can be chosen among: ioexolo, ioversolo, iopromide, iopamidolo, iodixanolo; the intravenous contrast agents can be chosen among: sulphur hexafluoride; the radioisotopes can be chosen among: Tecnezio 99, Iodine 131, Thallium 201, Iodine 125, Fluorine 18, Carbon 14.

In particular, for the Nuclear Magnetic resonance, the ligand of the invention can be marked for example with: gadodiamide (Omniscan®), gadobenic acid (Multihance®), gadobutrol (Gadovist®), gadofosveset (Vasovist®), gadopentetic acid (Magnevist®), gadoteric acid (Dotaren®), gadoteridol (Prohance®) and gadoxetic acid (Primovist®).

For the detection by means of the computerized tomography, iodized agents can be used such as: monomers such as ioexolo (Omnipaque®), ioversolo (Optiray®), iopromide (Ultravist®), iopamidol (for example lopamiro®) or dimers such as iodixanol (Visipaque®). For the ecography, the ligand of the invention for example can be marked with intravenous contrast agents constituted by microbubbles of sulphur hexafluoride or other graphic contrast agents for ultrasounds.

In a preferred embodiment of the invention, the ligand is a polyclonal or monoclonal antibody able to recognize and link the AMH of human origin marked with gadolinium. The marking and conjugation of a protein with a detecting agent, such as those shown above, nowadays is performed by means of techniques well known to the person skilled in the art. By way of example, the methods which can be used for conjugating or marking the ligand of the invention are described in Kuriu Y et al. Monoclonal antibody conjugated to gadolinium as a contrast agent for magnetic resonance imaging of human rectal carcinoma. J Surg Oncol. 2006 Aug 1;94(2):144-148; and Molecular Imaging and Contrast Agent Database (MICAD) [Internet]. Bethesda (MD): National Center for Biotechnology Information (US); 2004-2013 (available in: http://www.ncbi.nlm.nih.gov/books/NBK23053/).

As shown previously, the herein described ligand demonstrated to be useful in particular to the purpose of localizing and/or evaluating the entity of the endometriosic lesions directly in vivo in a patient suffering from, or supposed suffering from, endometriosis. Under endometriosis lesion, analogously to what reported in literature,
the presence of an endometrial tissue, both glandular and stromal tissue, outside the
cavity of uterus is meant. In the specific case, the anti-AMH antibody of the invention
allows detecting in vivo both cystic and connective solid endometriosic lesions. The
evaluation of the entity of the lesions, in this case, substantially relates to the analysis
to the purposes of diagnosing or treating the size of the foci of the endometriosic
disease. The size of the endometriosis lesions can be very variable and in case of
neoformed lesions the sizes can be so reduced that they do not allow the localization
thereof by the physician. Advantageously, the ligand of the invention allows displaying
even endometriosic lesions with diameter smaller or larger than 1 centimetre and 0.5
centimetres. Under evaluation of the entity of the endometriosis lesions herein the
physician's determination of the sizes and/or the spreading level of the disease foci is
meant in order to understand the endometriosis progress stage and, in case, to define
the best therapeutic approach to be followed.

The subject of the present invention then is also a formulation to be used in an
in vivo method for localizing and/or evaluating the entity of the endometriosis lesions in
a patient comprising at least a ligand of the invention and at least a pharmaceutically
acceptable carrier and/or excipient.
In a preferred embodiment the formulation is administered to the patient wherein one
wants to localize and/or evaluate the entity of the endometriosis lesions, by injection or
infusion or even in case by means of oral administration. A pharmaceutically
acceptable carrier can be chosen, for example, among buffer aqueous solutions,
sterile water, balanced saline physiological solutions, ions, additives.
By pure way of example, the buffer aqueous solutions can be chosen among tris
(hydroxyethyl) amino methane and the salts, phosphate, citrate and bicarbonates;
the balanced ionic solutions, instead, can be selected among chlorides and
bicarbonates of cations chosen among Ca, Na, K, Mg and other halides, carbonates,
sulphates, phosphates and Na, K, Mg and Ca; the excipients can be chosen among
glycerol, polyethylene glycol, and dextran. In any case, the carriers and the excipients
which can be comprised in the formulation of the invention can be chosen among
those commonly known and considered useful by the person skilled in the art for the
present invention.

The subject of the present invention is also a kit for localizing and/or evaluating the
in vivo entity of the endometriosis lesions in a patient comprising at least a ligand
of the invention or a formulation as above described and means useful for the
administration of said ligand or said formulation to the patient. By purely way of
example, such means can comprise physiological solutions, needles, syringes, sterilizing solutions, etc.

Furthermore, herein also an *in vivo* method is described for localizing and/or evaluating the *in vivo* entity of the endometriosis throughout the patient comprising a passage of administering the ligand or the formulation of the invention to the patient itself. As already designated previously, under localization the possibility of detecting precisely the site wherein there is the endometriosis is meant, whereas under evaluation substantially the analysis of the sizes of the localized lesions is referred to. From this point of view, then, the *in vivo* method can even include an operating passage wherein the subject, thereto the ligand or the formulation of the invention was administrated, is subsequently subjected to a technique of magnetic resonance imaging. By pure way of example and not for limitative purposes, such techniques can be: ecography, radiography, computed tomography, nuclear magnetic resonance, tomography with emission of positrons.

The just described method can be performed, if the person skilled in the art can consider to be useful, even on *in vitro* tissue samples and in this case then the *in vitro* method for localizing and/or evaluating the entity of the endometriosis will include a passage of incubating a tissue sample, obtained from the patient under analysis, with a ligand or a formulation of the invention. In a way analogous to what described above, the sample can be subsequently subjected to a technique of magnetic resonance imaging with the purpose of allowing to display the site and the sizes of the endometriosis disease foci.

**EXAMPLES**

**Example 1. In vivo expression of AMH hormone in the endometriosis lesions by means of immunohistochemical methods.**

This experiment represents the first scientific demonstration of the fact that the AMH hormone is clearly and abundantly expressed in the endometriosis lesions, both in the glandular and in the stromal component. For this demonstration, collections of tissue were performed at the sub-peritoneal level from 10 patients affected by endometriosis; the tissues were fixed in 10%-buffered paraformaldehyde, included in paraffin and coloured with Hematoxylin and Eosin to highlight the glandular and stromal structures of endometriosis. On stained sections, immediately subsequent to the ones coloured with Hematoxylin and Eosin, immunohistochemical colourings were
performed, by using with proper dilutions an antibody specific for AMH (anti-AMH antibody of ABCAM, # cat. ab103233) with the dilution of 1 to 100, the ABC system and the colouring with Diaminobenzidine to detect the antigen-antibody complexes. Such experiment allowed demonstrating that the AMH hormone is constantly and abundantly expressed in the glandular and stromal component of the endometriosic lesions. Figure 1 shows two examples of such expression.

Example 2. Xenotransplant of human endometriosic tissue in nude mice

Fragments of human connective solid endometriosic tissue (max diameter about 3 mm) collected from two different patients during surgical removal operation by laparoscopic way were transplanted subcutaneously in the left side of two female nude mice. After implanting endometriosic tissue, performed in total anaesthesia, the small female mice were stabled for two weeks with food and water ad libitum and, limited to the first week, with antibiotic therapy (5% enrofloxacin in the beverage water). The imaging evaluation was performed by means of using a 0.2-Tesla magnetic resonance for veterinary use. The small female mice were soothed with tiletamine + zolazepam + xylazine in order to be able to perform the imaging studies. After being positioned in the apparatus, total-body and local studies were performed for the abdominal area with sections of 2 mm. Subsequently the small female mice were removed from the apparatus for a second administration of sedative and in order to be able to perform the intravenous inoculation (tail vein) of the antibody (10 μl of a 0.2 mg/ml concentrated solution of anti AMH antibody conjugated with gadolinium). The small female mice were repositioned and total-body and loco-regional studies were performed. Both in the total-body study (Figure 2) (wherein subcutaneous captation is found with residue of antibodies in the inoculation site in the caudal vein) and in some cross sections (Figure 3), antibody captation is highlighted in the site of transplanting the endometriosic tissue. In particular, in the cross section of an animal before the treatment, the subcutaneous mass not having captation signs is found. After the experiment, the animals were brought in animal house and sacrificed to explant the ectopic tissue. Such tissue was then analysed with histological and immunohistochemical examination. These examinations confirmed that the transplant histological aspect was that of a connective solid endometriosic tissue. At last, by means of immunohistochemical examination, performed by using the same method shown before, it was demonstrated that such transplanted tissue expressed CD10 (marker of endometriosic tissue) and the codifying protein for AMH (Figure 4).
CLAIMS

1. An isolated ligand of anti-Mullerian hormone suitable to be directly detected by means of imaging techniques for use in a *in vivo* method for the diagnosis of endometriosis comprising a passage of localizing and/or evaluating the entity of the endometriosic lesions in a patient.

2. The ligand according to claim, consisting in an anti-Mullerian anti-hormone antibody or the receptor of type II of anti-Mullerian hormone (MISIIR).

3. The ligand according to claim 1 or 2, wherein said antibody is human, humanized, murine or chimeric.

4. The ligand according to anyone of claims 1 to 3, wherein said antibody is a polyclonal or monoclonal antibody.

5. The ligand according to anyone of claims 1 to 4, wherein said ligand is detected by means of: ecography, radiography, computed tomography, nuclear magnetic resonance, tomography with emission of positrons, scintigraphy.

6. The ligand according to anyone of claims 1 to 5, wherein said ligand is marked with at least one of the agents chosen in the group comprising: paramagnetic contrast agents, iodized contrast agents, intravenous contrast agents, radioisotopes.

7. The ligand according to claim 6, wherein
   - said paramagnetic contrast agents are chosen among: gadolinium or manganese;
   - said iodized contrast agents are chosen among: ioexolo, ioversolo, iopromide, iopamidolo, iodixanolo;
   - said intravenous contrast agents are chosen among: sulphur hexafluoride;
   - said radioisotopes are chosen among: Tecnezio 99, Iodine 131, Thallium 201, Iodine 125, Fluorine 18, Carbon 14.

8. The ligand according to anyone of claims 1 to 7, wherein said lesions are endometriosic neoformations with diameter smaller or larger than 1 centimetre.
9. A formulation for use in an *in vivo* method for localizing and/or evaluating the entity of the endometriotic lesions in a patient comprising a ligand according to anyone of claims 1 to 8 and at least pharmaceutically acceptable carrier and/or excipient.

10. A kit for localizing and/or evaluating the *in vivo* entity of the endometriotic lesions in a patient comprising at least a ligand according to anyone of claims 1-8 or a formulation according to claim 9 and means useful to the administration of said ligand or said formulation to said patient.
INTERNATIONAL SEARCH REPORT

International application No
PCT/IB2014/063673

A. CLASSIFICATION OF SUBJECT MATTER

A61K51/08 A61K51/10
ADD. A61K38/22

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

B. FIELD SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
A61K A61P

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, BIOSIS, CHEM ABS Data, EMBASE

C. DOCUMENTS CONSIDERED TO BE RELEVANT

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<td>wo 95/16709 A2 (BIOGEN INC [US]; INSERM U 293 [FR]; CATE RICHARD L [US]; J0SS0 NATHALI) 22 June 1995 (1995-06-22) page 22, lines 5-23</td>
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Further documents are listed in the continuation of Box C. See patent family annex.

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Authorized officer
Vi l lard, Anne-Laure
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<td>NAMKUNG JE0NG ET AL: &quot;Mullerian inhibiting substance induces apoptosis of human endometrial stromal cells in endometriosis &quot;, THE JOURNAL OF CLINICAL ENDOCRINOLOGY AND METABOLISM SEP 2012, vol. 97, no. 9, September 2012 (2012-09), pages 3224-3230, XP002723721, ISSN: 1945-7197 abstract page 3229, right-hand column, paragraphs 2,3</td>
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