Abstract:
The present invention relates to methods for diagnosing CATI-related diseases, wherein said methods comprises detecting CATI in a cell by a BLV.RBD ligand, or a variant or a fragment thereof. The present invention further relates to a BLV.RBD ligand, or a variant or a fragment thereof for use in the treatment of CATI-related diseases and/or BLV infections.
USE OF RECEPTOR-BINDING DOMAIN DERIVED FROM BOVINE LEUKEMIA VIRUS FOR THE DIAGNOSIS OR TREATMENT OF CATIONIC L-AMINO ACID TRANSPORTER-RELATED DISEASES

FIELD OF INVENTION

The present invention relates to the diagnosis or treatment of a cationic L-amino acid transporter-related disease, such as, for example, a CAT1-related disease. In particular, the present invention relates to methods comprising the detection of the binding of the cationic amino acid transporter CAT1/SLC7A1 with a receptor-binding domain (RBD) derived from the bovine leukemia virus (BLV) envelope glycoprotein.

BACKGROUND OF INVENTION

Arginine, lysine, histidine and ornithine are amino acids involved in diverse metabolic pathways. These pathways control the metabolism of fatty acids, glucose, amino acids and proteins but they are also involved in transport, processing and excretion of nitrogen, urea synthesis, and creatine and nitric oxide synthesis.

The dysregulation of one of these pathways is one of the hallmarks of diverse diseases such as cancer (including in particular carcinoma, sarcoma, and leukemia), diabetes, obesity, cardiovascular and inflammatory diseases that are characterized by an increased or decreased uptake of arginine, lysine, histidine and ornithine in cells, tissues or organs affected by such dysregulation. Therefore, there is growing interest in targeting pathways related to these amino acids. For instance, there have been many types of cancer characterized by a dysregulation of arginine-related pathways. Arginine deprivation has been one of the strategies to fight against these cancers. However, resistance to these treatments appeared besides undesirable side effects (Feun et al., 2015. Curr. Opin. Clin. Nutr. Metab. Care. 18(1):78-82; Qiu et al., 2015. Cancer Lett. 364(1): 1-7), therefore there is a need to develop new strategies and alternative therapies.
The state of the art teaches that the cationic L-amino acid transporter CAT1 is the main transporter for arginine (Closs et al., 2004. *J. Nutr.* **134**(10 Suppl):2752S-2759S) or for lysine, histidine and ornithine influx. So far, the Murine type C ecotropic retrovirus envelope glycoprotein is well known for its binding to mouse or rat CAT1 (Albritton et al., 1993. *J. Virol.* **67**(4):2091-2096). However, there is a lack of accurate ligand targeting other mammalian CAT1, in particular human, cattle and flock CAT1.

The inventors discovered that a ligand derived from the bovine leukemia virus (BLV) envelope glycoprotein bound specifically to CAT1 of different mammals including humans. Surprisingly, the inventors demonstrate that this ligand blocks arginine influx within cells, thereby preventing arginine accumulation within these cells.

The present invention thus relates to the use of a BLV.RBD for the diagnosis or treatment of CAT1-related diseases.

**SUMMARY**

The present invention thus relates to an *in vitro* method for detecting and/or quantifying the cationic amino acid transporter-1 (CAT1) in a cell, wherein said method comprises:

a. contacting said cell with at least one bovine leukemia virus (BLV).RBD ligand, a variant and/or a fragment thereof, and

b. determining and/or quantifying the binding of said at least one ligand variant and/or fragment thereof to CAT1.

In one embodiment, said method further comprises comparing the binding level determined and/or quantified at step b. with a reference value.

In one embodiment, said method is for diagnosing or monitoring a CAT1-related disease or a BLV infection in a subject.

In one embodiment, said at least one bovine leukemia virus (BLV).RBD ligand, variant and/or fragment thereof is selected from the group comprising SEQ ID NO: 21, 3, 4, 13, 15, 17, 19, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 47, 48 and 49, variants and fragments thereof.
The present invention further relates to a diagnostic composition comprising at least one BLV.RBD ligand, a variant and/or a fragment thereof coupled with at least one contrast agent, and a pharmaceutically acceptable excipient.

The present invention further relates to a BLV.RBD ligand, a variant and/or a fragment thereof, for use in the *in vivo* diagnosis of a CAT1-related disease, preferably by medical imaging.

The present invention further relates to a BLV.RBD ligand, a variant and/or a fragment thereof, for use in the treatment of a CAT1-related disease.

In one embodiment, said CAT1-related disease is selected from the group comprising arginine-related diseases, lysine-related diseases, histidine-related diseases, ornithine-related diseases, and inflammatory diseases.

Another object of the invention is a BLV.RBD ligand, a variant and/or a fragment thereof, for use in the treatment of a BLV infection.

Another object of the invention is a pharmaceutical composition comprising at least one BLV.RBD ligand, a variant and/or a fragment thereof for use as described hereinabove, and a pharmaceutically acceptable excipient.

The present invention further relates to a medicament comprising at least one BLV.RBD ligand, a variant and/or a fragment thereof for use as described hereinabove.

In one embodiment, the BLV.RBD ligand comprises SEQ ID NO: 21, 3, 4, 13, 15, 17, 19, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 47, 48 and/or 49, a variant and/or a fragment thereof.

**DEFINITIONS**

In the present invention, the following terms have the following meanings:

- The term "cell surface nutrient transporter" refers to the nutrient transporter CAT1. CAT1 may be anchored in the plasma membrane of a cell or within a cell.
"CAT1/SLC7A1" refers to a cationic L-amino acid transporter. CAT1 mediates sodium-independent and pH-insensitive transport of amino acids that include arginine, ornithine, lysine and histidine. CAT1 is ubiquitously expressed although it is thought to not be present on liver cells and lacrimal gland cells. CAT1 is herein identified as a specific receptor for BLV.RBD. In one embodiment, CAT1 is human CAT1 (accession number: AIC49738, SEQ ID NO: 1), encoded by SEQ ID NO: 2 (accession number: KJ892152). In one embodiment, CAT1 comprises or consists of an amino acid sequence presenting a sequence identity of at least 70% with SEQ ID NO: 1, preferably a sequence identity of at least 75, 80, 85, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99% or more with SEQ ID NO: 1. In one embodiment, CAT1 is encoded by a nucleotide sequence presenting a sequence identity of at least 70% with SEQ ID NO: 2, preferably a sequence identity of at least 75, 80, 85, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99% or more with SEQ ID NO: 2. In one embodiment, CAT1 comprises or consists of a fragment of SEQ ID NO: 1, preferably a fragment of at least about 100 amino acids, more preferably of at least about 150, 200, 250, 300, 350, 400, 450, 500, 550, 600 amino acids. In one embodiment, CAT1 is not mouse CAT1 (accession number: Q09143, SEQ ID NO: 23). In one embodiment, CAT is not rat CAT1 (accession number: P30823, SEQ ID NO: 24).

The term "diagnostic composition" refers to a composition to be administered in a subject in order to perform a diagnosis and in particular an in vivo diagnosis. In one embodiment, a diagnostic composition is for detecting cells wherein the function of a cationic L-amino acid transporter, preferably of CAT1, is dysregulated, preferably within the body of a subject. In another embodiment, the present invention relates to a diagnostic composition for detecting cells which are infected or not yet infected by BLV, preferably within the body of a subject.

The term "effective amount" refers to the level or amount of a ligand, preferably of the at least one BLV.RBD ligand that is aimed at binding to CAT1, without causing significant negative or adverse side effects to the subject wherein the function of a cationic L-amino acid transporter, preferably of CAT1, is dysregulated; or to the subject infected by BLV.
The term "therapeutically effective amount" means level or amount of agent that is aimed at, without causing significant negative or adverse side effects to the target, (1) delaying or preventing the onset of a CAT1-related disease or a BLV infection; (2) slowing down or stopping the progression, aggravation, or deterioration of one or more symptoms of a CAT1-related disease or a BLV infection; (3) bringing about ameliorations of the symptoms of a CAT1-related disease or a BLV infection; (4) reducing the severity or incidence of a CAT1-related disease or a BLV infection; or (5) curing a CAT1-related disease or a BLV infection. A therapeutically effective amount may be administered prior to the onset of a CAT1-related disease or a BLV infection, for a prophylactic or preventive action. Alternatively or additionally, the therapeutically effective amount may be administered after initiation of a CAT1-related disease or a BLV infection, for a therapeutic action.

The term "treatment" refers to both therapeutic treatment and prophylactic or preventative measures; wherein the object is to prevent or slow down (lessen) a CAT1-related disease or a BLV infection. Those in need of treatment include those already with a CAT1-related disease or a BLV infection as well as those prone to have a CAT1-related disease or a BLV infection or those in whom a CAT1-related disease or a BLV infection is to be prevented. A subject or mammal is successfully "treated" for a disease if, after receiving a therapeutic amount of a ligand according to the invention, the patient shows observable and/or measurable reduction in or absence of one or more of the following: reduction in the number of pathogenic cells; reduction in the percent of total cells that are pathogenic; and/or relief to some extent, of one or more of the symptoms associated with the specific disease or condition; reduced morbidity and mortality, and improvement in quality of life issues. The above parameters for assessing successful treatment and improvement in the disease are readily measurable by routine procedures familiar to a physician.

The term "pharmaceutically acceptable excipient" refers to an excipient that does not produce an adverse, allergic or other untoward reaction when administered to an animal, preferably a human. It includes any and all solvents, dispersion media, coatings, antibacterial and antifungal agents, isotonic and absorption delaying agents and the like. For human administration, preparations should meet sterility,
pyrogenicity, general safety and purity standards as required by regulatory offices, such as, for example, FDA Office or EMA.

- The term "polypeptide" refers to a linear polymer of amino acids (preferably at least 50 amino acids) linked together by peptide bonds.

- The term "protein" specifically refers to a functional entity formed of one or more polypeptides, and optionally of non-polypeptides cofactors.

- The term "identity", when used in a relationship between the sequences of two or more polypeptides or of two or more DNA sequences, refers to the degree of sequence relatedness between polypeptides or DNA sequences (respectively), as determined by the number of matches between strings of two or more amino acid residues or of two or more nucleotides, respectively. "Identity" measures the percent of identical matches between the smaller of two or more sequences with gap alignments (if any) addressed by a particular mathematical model or computer program (i.e., "algorithms"). Identity of related polypeptides or DNA sequences can be readily calculated by known methods. Such methods include, but are not limited to, those described in Arthur M. Lesk, *Computational Molecular Biology: Sources and Methods for Sequence Analysis* (New-York: Oxford University Press, 1988); Douglas W. Smith, *Biocomputing: Informatics and Genome Projects* (New-York: Academic Press, 1993); Hugh G. Griffin and Annette M. Griffin, *Computer Analysis of Sequence Data, Part I* (New Jersey: Humana Press, 1994); Gunnar von Heinje, *Sequence Analysis in Molecular Biology: Treasure Trove or Trivial Pursuit* (Academic Press, 1987); Michael Gribskov and John Devereux, *Sequence Analysis Primer* (New York: M. Stockton Press, 1991); and Carillo et al., 1988. *SIAM J. Appl. Math.* 48(5):1073-1082. Preferred methods for determining identity are designed to give the largest match between the sequences tested. Methods of determining identity are described in publicly available computer programs. Preferred computer program methods for determining identity between two sequences include the GCG program package, including GAP (Devereux et al., 1984, *Nucl. Acid. Res.* 12(1 Pt 1):387-395; Genetics Computer Group, University of Wisconsin Biotechnology Center, Madison, WI), BLASTP, BLASTN, TBLASTN and FASTA (Altschul et al., 1990, *J. Mol. Biol.* 215(3):403-410). The BLASTX program is
publicly available from the National Center for Biotechnology Information (NCBI) and other sources (BLAST Manual, Altschul et al. NCB/NLM/NIH Bethesda, Md. 20894; Altschul et al, 1990. J. Mol. Biol. 215(3):403-410). The well-known Smith Waterman algorithm may also be used to determine identity.

- The term "subject" refers to a mammal, preferably a human, cattle (e.g. cow, bull, calf, heifer), flock, ovine (e.g. sheep, lamb, ewe), caprine (goat, billy goat, kid goat), more preferably a human or a bovine. In one embodiment, a subject may be a "patient", i.e. a mammal, a warm-blooded animal, more preferably a human or a bovine, who/which is awaiting the receipt of, or is receiving medical care or was/is/will be the object of a medical procedure, or is monitored for the development of a disease.

- "About" preceding a figure means plus or less 10% of the value of said figure.

DETAILED DESCRIPTION

The present invention relates to an in vitro or in vivo method for detecting and/or quantifying the cell surface nutrient transporter CAT1 in a cell, wherein said method comprises:

a. contacting said cell with at least one ligand, preferably at least one bovine leukemia virus (BLV).RBD ligand, or a variant and/or a fragment thereof, and

b. determining and/or quantifying the binding of said at least one ligand, variant and/or fragment thereof to CAT1.

In one embodiment, the method of the invention comprises a step of comparing the binding determined and/or quantified at step b. with a reference binding value.

As used herein, the term "CAT1 present in a cell" may refer to CAT1 present at the surface of a cell or within the cell.
In one embodiment, the method of the invention is for assessing the expression level of CAT1 present on the cell surface. In another embodiment, the method of the invention is for assessing the expression level of CAT1 present within the cell.

In one embodiment, the method of the invention is for detecting and/or quantifying CAT1 in a sample.

In one embodiment, the sample was obtained from a subject prior to the implementation of the method of the invention. In one embodiment, the method of the invention does not comprise obtaining a sample from the subject, i.e., the method of the invention is non-invasive.

In one embodiment, the sample obtained from the subject to carry out the method of the invention is a biological sample.

In one embodiment, said biological sample is a body fluid sample. Examples of body fluids include, without being limited to, blood, plasma, serum, lymph, urine, cerebrospinal fluid or sweat.

In another embodiment, said biological sample is a cell sample. Examples of cell samples include, without being limited to, peripheral blood mononuclear cells (PBMC), peripheral white blood cells, cell samples obtained from tissue biopsies such as lymph nodes biopsies, intestinal or synovial biopsies, or cell sample obtained from broncho-alveolar lavage or cerebrospinal fluid.

In another embodiment, said biological sample is a tissue sample. Examples of tissue sample include, but are not limited to, biopsy samples.

In one embodiment, the sample obtained from the subject to carry out the method of the invention is a blood sample. In one embodiment, the sample obtained from the subject to carry out the method of the invention is a whole blood sample or a plasma sample.

In one embodiment, the method of the invention is not for detecting and/or quantifying CAT1 in a granulocyte. By "granulocyte" is meant a cell belonging to myelocyte or monocyte lines, such as neutrophils, eosinophils, basophils and mast cells.
*In vitro* methods for determining and/or quantifying a protein level in a sample are well-known in the art. Examples of such methods include, but are not limited to, immunohistochemistry, Multiplex methods (Luminex), western blot, enzyme-linked immunosorbent assay (ELISA), sandwich ELISA, fluorescent-linked immunosorbent assay (FLISA), enzyme immunoassay (EIA), radioimmunoassay (RIA), flow cytometry (FACS) and the like.

*In vivo* methods for determining and/or quantifying a protein level are well-known in the art. Examples of such methods include, but are not limited to, computed tomography (CT scan), endoscopic ultrasound (EUS), magnetic resonance imaging (MRI), positron-emission tomography (PET), single photon emission tomography (SPECT), magnetic resonance cholangiopancreatography, fluorimetry, fluorescence, and near-infrared (NIR) fluorescent imaging.

Methods for analyzing the presence of a protein on the cell surface or within a cell, sample, tissue or organ are well-known to the skilled artisan and include, without limitation, FACS analysis, immunohistochemistry, western blot associated with cell fractionation, enzyme-linked immunosorbent assay (ELISA), sandwich ELISA, fluorescent-linked immunosorbent assay (FLISA), enzyme immunoassay (EIA), radioimmunoassay (RIA), image analysis, for example high content analysis, computed tomography (CT scan), endoscopic ultrasound (EUS), magnetic resonance imaging (MRI), positron-emission tomography (PET), single photon emission tomography (SPECT), magnetic resonance cholangiopancreatography, fluorimetry, fluorescence, and near-infrared (NIR) fluorescent imaging and the like.

In one embodiment, in order to detect the protein on the cell surface, or within a cell, sample, tissue or organ, a step of fixation of said cell, sample, tissue or organ may be required before implementing one of the methods listed hereinabove. Technics for cell, sample, tissue or organ fixation are well known to the skilled artisan and include without limitation, perfusion, immersion with formaldehyde, or methanol/ethanol.

Methods for analyzing the presence of a protein on the cell surface are well-known to the skilled artisan and include, without limitation, FACS analysis, imaging flow cytometry
(e.g. AMNIS), immunohistochemistry, western blot associated with cell fractionation, enzyme-linked immunosorbent assay (ELISA), sandwich ELISA, fluorescent-linked immunosorbent assay (FLISA), enzyme immunoassay (EIA), radioimmunoassay (RIA), image analysis, for example high content analysis, computed tomography (CT scan), endoscopic ultrasound (EUS), magnetic resonance imaging (MRI), positron-emission tomography (PET), single photon emission tomography (SPECT), fluorimeter, fluorescence, and near-infrared (NIR) fluorescent imaging and the like.

The expression "determining and/or quantifying the binding" of the at least one ligand (preferably the at least one BLV.RBD ligand), variant and/or fragment thereof means that when the cell surface nutrient transporter CAT1 is present, a complex is formed between CAT1 and the ligand of the invention, variant and/or fragment thereof, that may be detected and optionally quantified.

In one embodiment, the complex can be detected if the ligand, variant and/or fragment thereof has been for example, but not limited to, covalently coupled with a detectable molecule such as an antibody constant fragment (Fc) or a fluorescent compound (e.g. Cyanine dye, Alexa dye, Quantum dye, etc.). The complex can also be detected if the ligand has been tagged with different means well known to the person skilled in the art. For example, but without limitation, a tag used in the invention can be a tag selected from the group comprising or consisting of Hemagglutinin tag, Poly Arginine tag, Poly Histidine tag, Myc tag, Strep tag, S-tag, HAT tag, 3x Flag tag, Calmodulin-Binding Peptide tag, SBP tag, Chitin Binding Domain tag, GST tag, Maltose-Binding Protein tag, Fluorescent Protein tag, T7 tag, V5 tag and X-press tag. These protein tags can be located N-terminally, C-terminally and/or internally of the BLV.RBD ligand of the invention.

The use of the ligand therefore allows on the one hand the identification and detection, and on the other hand the quantification of the complex formed. In one embodiment, detecting and/or quantifying binding is conducted by flow cytometry, immunofluorescence or image analysis, for example high content analysis.
In another embodiment, that complex can be detected if the ligand has been for example, but not limited to, covalently coupled with at least one contrast agent. In one embodiment, detecting and/or quantifying binding is conducted by medical imaging technics.

As used herein, the term "contrast agent" refers to agents used to improve the visibility of internal bodily structures in medical imaging technics, including, but not limited to, magnetic resonance imaging (MRI), X-ray-based imaging techniques such as computed tomography (CT), radiography, endoscopic ultrasound (EUS), positron-emission tomography (PET), single photon emission tomography (SPECT), fluoroscopy, fluorimetry, fluorescence, and near-infrared (NIR) fluorescent imaging.

In one embodiment, the ligand is coupled with at least one contrast agent, wherein said contrast agent may be a radiolabeled agent or a fluorescent agent.

In one embodiment, the radiolabeled agent of the invention is selected from the group comprising a non-metallic radioisotope, non-metallic or metallic dye, paramagnetic metal, or radioactive metal.

Examples of non-metallic radioisotopes comprise, but are not limited to, 1-125, 1-123, 1-131, C-11, F-18, Br-75, Br-76, Br-77, Br-80, and At-211. The non-metallic radioisotopes may be conjugated covalently to either terminus of the ligand, functional groups of amino acid side chains, be part of a linear stabilized peptide as an additional substituent, e.g. in an amino acid phenylalanine or tyrosine carrying fluorine, bromine or iodine, or as an additional substituent carboxy or methyl, or as a replacement of any regular carbon atom in the ligand. Preferably, the ligand is coupled with 1-125. These radioisotopes are useful in ligands as positron emission tomography (PET) probes or as single-photon emission computed tomography (SPECT) probes.

Examples of non-metallic or metallic dyes comprise, but are not limited to, organic molecules, e.g., commercial Alexa Fluor® dyes, fluorescein, rhodamine, or Cy® dyes (such as Cy3, Cy3.5, Cy5, Cy5.5, Cy7, and Cy7.5), complexes of transition metals, e.g. chelates of Eu³⁺, Tb³⁺, or nanoparticles (quantum dots) which adsorb and/or emit light in the visible range or in the near infrared. Organic dyes and chelating systems will be coupled to the ligands as described above for chelators. Conjugation of the ligands with
quantum dots is done by procedures known to those skilled in the art. These ligands carrying dyes are useful as optical imaging probes.

Examples of paramagnetic metals comprise, but are not limited to, Gd, Fe, Mn. The metals are attached to the ligands. These ligands are useful as magnetic resonance imaging (MRI) probes.

Examples of radioactive metals comprise, but are not limited to, Cu-64, Cu-67, Ga-67, Ga-68, Zr-89, Y-90, Te-99m, In-111, Tb-161, Lu-177, Re-186, Re-188, and Bi-213. The radioactive metals (and the paramagnetic metals mentioned above) are attached to the ligands of the invention through chelators as listed above, directly connected to the ligands or through a spacer.

Examples of fluorescent agents include, but are not limited to, GFP, mPlum®, mCherry®, tdTomato®, mStrawberry®, J-Red, DS-Red monomer®, mOrange®, mKO, mCitrine®, Venus, YPet®, EYFP, Emerald®, EGFP, CyPet, mCFP®, Cerulean®, T-Sapphire®, indocyanine green, ZW800-1, Cy5.5 and IRDye800CW.

In one embodiment, the contrast agent is 1-125.

Typical ligands include, but are not limited to, polypeptides and proteins.

In one embodiment, the ligand is an antibody specific to CATl, and the method of the invention comprises detecting and/or quantifying a complex formed between said antibody and CATl.

Consequently, in one embodiment, the present invention relates to an in vitro or in vivo method for detecting and/or quantifying the cell surface nutrient transporter CATl present in a cell, wherein said method comprises:

a. contacting said cell with at least one antibody specific to CATl, and

b. determining and/or quantifying the binding of said at least one antibody to CATl.

In one embodiment, the method of the invention comprises a step of comparing the binding determined and/or quantified at step b. with a reference binding value.
In one embodiment, the ligand is a receptor-binding domain (RBD) ligand and the method of the invention comprises detecting and/or quantifying a complex formed between said RBD ligand and CATI.

In one aspect of the invention, the ligand is a receptor-binding domain (RBD) ligand, preferably a BLV-RBD ligand, wherein said RBD ligand comprises a part or the totality of a receptor-binding domain (RBD) derived from the soluble part of the glycoprotein of an enveloped virus that interacts with CATI.

The expression "that interacts with CATI" means that the glycoprotein is liable to recognize the CATI receptor present in the cell. In one embodiment, a ligand that interacts with CATI will thus form a complex with CATI, which complex may be detected by a method as here above described.

The expression "derived from the soluble part of the glycoprotein of an enveloped virus" means that the ligand is a fragment or a part of a glycoprotein contained in the envelope of a virus and can be obtained, for example, by cloning.

The term "glycoprotein" is to be understood as meaning an envelope glycoprotein, a coat glycoprotein or a fusion glycoprotein, wherein the term "glycoprotein" refers to a protein containing oligosaccharide chains covalently attached to polypeptide side-chains.

RBDs are found, in particular, in glycoproteins of the envelope of viruses, therefore, the receptor-binding domain ligand contains the total RBD or a fragment or part of the RBD.

In one embodiment, the ligand of the invention comprises the cell surface (SU) domain of the glycoprotein envelope of a virus or a fragment of the SU domain, such as, for example, the BLV.RBD. In another embodiment, the ligand of the invention does not comprise the transmembrane (TM) domain of the glycoprotein envelope of a virus. Therefore, in one embodiment of the invention, the ligand of the invention is a soluble peptide, in particular a soluble BLV.RBD. As used herein, the term "soluble peptide" refers to a peptide which is not anchored within a membrane, such as, for example, by a transmembrane or a GPI anchor domain.
In one embodiment, the RBD ligand is not derived from the soluble part of the glycoprotein of Murine type C ecotropic retrovirus. In one embodiment, the RBD ligand is not derived from the soluble part of the glycoprotein of Moloney murine leukemia virus, isolate Shinnick (accession number: P03385, SEQ ID NO: 25). In one embodiment, the RBD ligand is not derived from the soluble part of the glycoprotein of Friend murine leukemia virus, strain 57 (accession number: P03390, SEQ ID NO: 26).

In one embodiment, the RBD ligand is derived from the soluble part of the glycoprotein of a deltaretrovirus. The deltaretrovirus genus includes viruses that infect humans, various simian species and cattle. Deltaretroviruses include, but are not limited to, Bovine Leukemia Virus (BLV), Human T-cell Leukaemia Viruses 1 to 4 (HTLV1-4), and Simian T-cell Leukaemia Viruses 1 to 4 (STLV1-4).

The deltaretroviruses encode an envelope of glycoprotein present in mature retrovirus viral particles. The envelope protein is synthesized in the form of a propeptide, which is cleaved in Golgi apparatus by furin peptidase, resulting in two polypeptides: the transmembrane (TM) and the cell surface (SU) components. The SU domain contains two major subdomains: a domain of interaction with the TM domain and the amino terminal RBD, the latest being liable to interact with host cell membrane receptors.

In one embodiment, the receptor-binding domain ligand is isolated from the envelope glycoprotein of Bovine Leukemia Virus, and is herein referred as BLV.RBD.

Consequently, in one embodiment, the ligand of the invention comprises the cell surface (SU) domain of the glycoprotein envelope of Bovine Leukemia Virus (BLV) or a fragment of the SU domain, such as, for example, the BLV.RBD. In another embodiment, the ligand of the invention does not comprise the transmembrane (TM) domain of the glycoprotein envelope of Bovine Leukemia Virus (BLV). Therefore, in one embodiment of the invention, the ligand of the invention is a soluble peptide, in particular a soluble BLV.RBD.
In one embodiment, the present invention thus relates to an *in vitro* or *in vivo* method for detecting and/or quantifying the cell surface nutrient transporter CAT1 present in a cell, wherein said method comprises:

a. contacting said cell with at least one bovine leukemia virus (BLV).RBD ligand, a variant and/or a fragment thereof, and

b. determining and/or quantifying the binding of said at least one BLV.RBD ligand, variant or fragment thereof to CAT1.

In one embodiment, the method of the invention comprises a step of comparing the binding determined and/or quantified at step b. with a reference binding value.

In one aspect of the invention, the ligand is a BLV.RBD ligand, wherein said BLV.RBD ligand comprises a part or the totality of a receptor-binding domain (RBD) derived from the soluble part of a glycoprotein of the BLV enveloped virus that interacts with CAT1.

In one embodiment, said BLV.RBD comprises or consists of the amino acid sequence SEQ ID NO: 21 (encoded by SEQ ID NO: 22 or by SEQ ID NO: 5), variants or fragments thereof.

In one embodiment, said fragment comprises or consists of amino acids 34 to 181 of SEQ ID NO: 21.

In one embodiment, said fragment comprises or consists of amino acids 1 to 149, 150, 151, 152, 153, 154, 155, 156, 157, 158, 159, 160, 161, 162, 163, 164, 165, 166, 167, 168, 169, 170, 171, 172, 173, 174, 175, 176, 177, 178, 179, or 180 of SEQ ID NO: 21.

In another embodiment, said fragment comprises or consists of amino acids 34 to 149, 150, 151, 152, 153, 154, 155, 156, 157, 158, 159, 160, 161, 162, 163, 164, 165, 166, 167, 168, 169, 170, 171, 172, 173, 174, 175, 176, 177, 178, 179, or 180 of SEQ ID NO: 21.

In one embodiment, said BLV.RBD comprises or consists of the amino acid sequence SEQ ID NO: 28, variants or fragments thereof.

In one embodiment, said fragment comprises or consists of amino acids 34 to 181 of SEQ ID NO: 28.
In one embodiment, said fragment comprises or consists of amino acids 1 to 149, 150, 151, 152, 153, 154, 155, 156, 157, 158, 159, 160, 161, 162, 163, 164, 165, 166, 167, 168, 169, 170, 171, 172, 173, 174, 175, 176, 177, 178, 179, or 180 of SEQ ID NO: 28.

In another embodiment, said fragment comprises or consists of amino acids 34 to 149, 150, 151, 152, 153, 154, 155, 156, 157, 158, 159, 160, 161, 162, 163, 164, 165, 166, 167, 168, 169, 170, 171, 172, 173, 174, 175, 176, 177, 178, 179, or 180 of SEQ ID NO: 28.

In one embodiment, said BLV.RBD comprises or consists of the amino acid sequence SEQ ID NO: 29, variants or fragments thereof.

In one embodiment, said fragment comprises or consists of amino acids 34 to 181 of SEQ ID NO: 29.

In one embodiment, said fragment comprises or consists of amino acids 1 to 149, 150, 151, 152, 153, 154, 155, 156, 157, 158, 159, 160, 161, 162, 163, 164, 165, 166, 167, 168, 169, 170, 171, 172, 173, 174, 175, 176, 177, 178, 179, or 180 of SEQ ID NO: 29.

In another embodiment, said fragment comprises or consists of amino acids 34 to 149, 150, 151, 152, 153, 154, 155, 156, 157, 158, 159, 160, 161, 162, 163, 164, 165, 166, 167, 168, 169, 170, 171, 172, 173, 174, 175, 176, 177, 178, 179, or 180 of SEQ ID NO: 29.

In one embodiment, said BLV.RBD comprises or consists of the amino acid sequence SEQ ID NO: 4, variants or fragments thereof.

In one embodiment, said fragment comprises or consists of amino acids 34 to 181 of SEQ ID NO: 4.

In one embodiment, said fragment comprises or consists of amino acids 1 to 149, 150, 151, 152, 153, 154, 155, 156, 157, 158, 159, 160, 161, 162, 163, 164, 165, 166, 167, 168, 169, 170, 171, 172, 173, 174, 175, 176, 177, 178, 179, or 180 of SEQ ID NO: 4.

In another embodiment, said fragment comprises or consists of amino acids 34 to 149, 150, 151, 152, 153, 154, 155, 156, 157, 158, 159, 160, 161, 162, 163, 164, 165, 166, 167, 168, 169, 170, 171, 172, 173, 174, 175, 176, 177, 178, 179, or 180 of SEQ ID NO: 4.
In one embodiment, said BLV.RBD comprises or consists of the amino acid sequence SEQ ID NO: 3, variants or fragments thereof.

In one embodiment, said fragment comprises or consists of amino acids 34 to 215 of SEQ ID NO: 3.

In one embodiment, said fragment comprises or consists of amino acids 1 to 182, 183, 184, 185, 186, 187, 188, 189, 190, 191, 192, 193, 194, 195, 196, 197, 198, 199, 200, 201, 202, 203, 204, 205, 206, 207, 208, 209, 210, 211, 212, 213 or 214 of SEQ ID NO: 3.

In another embodiment, said fragment comprises or consists of amino acids 34 to 182, 183, 184, 185, 186, 187, 188, 189, 190, 191, 192, 193, 194, 195, 196, 197, 198, 199, 200, 201, 202, 203, 204, 205, 206, 207, 208, 209, 210, 211, 212, 213 or 214 of SEQ ID NO: 3.

In one embodiment, said fragment comprises or consists of amino acids 1 to 182, 183, 184, 185, 186, 187, 188, 189, 190, 191, 192, 193, 194, 195, 196, 197, 198, 199, 200, 201, 202, 203, 204, 205, 206, 207, 208, 209, 210, 211, 212, 213 or 214 of SEQ ID NO: 30.

In one embodiment, said fragment comprises or consists of amino acids 34 to 215 of SEQ ID NO: 30.

In one embodiment, said fragment comprises or consists of amino acids 1 to 182, 183, 184, 185, 186, 187, 188, 189, 190, 191, 192, 193, 194, 195, 196, 197, 198, 199, 200, 201, 202, 203, 204, 205, 206, 207, 208, 209, 210, 211, 212, 213 or 214 of SEQ ID NO: 30.

In another embodiment, said fragment comprises or consists of amino acids 34 to 182, 183, 184, 185, 186, 187, 188, 189, 190, 191, 192, 193, 194, 195, 196, 197, 198, 199, 200, 201, 202, 203, 204, 205, 206, 207, 208, 209, 210, 211, 212, 213 or 214 of SEQ ID NO: 30.

In one embodiment, said BLV.RBD comprises or consists of the amino acid sequence SEQ ID NO: 31, variants or fragments thereof.

In one embodiment, said fragment comprises or consists of amino acids 34 to 215 of SEQ ID NO: 31.
In one embodiment, said fragment comprises or consists of amino acids 1 to 182, 183, 184, 185, 186, 187, 188, 189, 190, 191, 192, 193, 194, 195, 196, 197, 198, 199, 200, 201, 202, 203, 204, 205, 206, 207, 208, 209, 210, 211, 212, 213 or 214 of SEQ ID NO: 31.

In another embodiment, said fragment comprises or consists of amino acids 34 to 182, 183, 184, 185, 186, 187, 188, 189, 190, 191, 192, 193, 194, 195, 196, 197, 198, 199, 200, 201, 202, 203, 204, 205, 206, 207, 208, 209, 210, 211, 212, 213 or 214 of SEQ ID NO: 31.

In one embodiment, said BLV.RBD comprises or consists of the amino acid sequence SEQ ID NO: 32, variants or fragments thereof.

In one embodiment, said fragment comprises or consists of amino acids 34 to 215 of SEQ ID NO: 32.

In one embodiment, said fragment comprises or consists of amino acids 1 to 182, 183, 184, 185, 186, 187, 188, 189, 190, 191, 192, 193, 194, 195, 196, 197, 198, 199, 200, 201, 202, 203, 204, 205, 206, 207, 208, 209, 210, 211, 212, 213 or 214 of SEQ ID NO: 32.

In another embodiment, said fragment comprises or consists of amino acids 34 to 182, 183, 184, 185, 186, 187, 188, 189, 190, 191, 192, 193, 194, 195, 196, 197, 198, 199, 200, 201, 202, 203, 204, 205, 206, 207, 208, 209, 210, 211, 212, 213 or 214 of SEQ ID NO: 32.

In one embodiment, said BLV.RBD comprises or consists of the amino acid sequence SEQ ID NO: 13 (encoded by SEQ ID NO: 14), variants or fragments thereof.

In one embodiment, said BLV.RBD comprises or consists of the amino acid sequence SEQ ID NO: 15 (encoded by SEQ ID NO: 16), variants or fragments thereof.

In one embodiment, said BLV.RBD comprises or consists of the amino acid sequence SEQ ID NO: 17 (encoded by SEQ ID NO: 18), variants or fragments thereof.

In one embodiment, said BLV.RBD comprises or consists of the amino acid sequence SEQ ID NO: 19 (encoded by SEQ ID NO: 20), variants or fragments thereof.

In one embodiment, said BLV.RBD comprises or consists of the amino acid sequence SEQ ID NO: 33, variants or fragments thereof.
In one embodiment, said BLV.RBD comprises or consists of the amino acid sequence SEQ ID NO: 34, variants or fragments thereof.

In one embodiment, said BLV.RBD comprises or consists of the amino acid sequence SEQ ID NO: 35, variants or fragments thereof.

In one embodiment, said BLV.RBD comprises or consists of the amino acid sequence SEQ ID NO: 36, variants or fragments thereof.

In one embodiment, said BLV.RBD comprises or consists of the amino acid sequence SEQ ID NO: 37, variants or fragments thereof.

In one embodiment, said BLV.RBD comprises or consists of the amino acid sequence SEQ ID NO: 38, variants or fragments thereof.

In one embodiment, said BLV.RBD comprises or consists of the amino acid sequence SEQ ID NO: 39, variants or fragments thereof.

In one embodiment, said BLV.RBD comprises or consists of the amino acid sequence SEQ ID NO: 40, variants or fragments thereof.

In one embodiment, said BLV.RBD comprises or consists of the amino acid sequence SEQ ID NO: 41, variants or fragments thereof.

In one embodiment, said BLV.RBD comprises or consists of the amino acid sequence SEQ ID NO: 42, variants or fragments thereof.

In one embodiment, said BLV.RBD comprises or consists of the amino acid sequence SEQ ID NO: 43, variants or fragments thereof.

In one embodiment, said BLV.RBD comprises or consists of the amino acid sequence SEQ ID NO: 44, variants or fragments thereof.

In one embodiment, said BLV.RBD comprises or consists of the amino acid sequence SEQ ID NO: 45 (encoded by SEQ ID NO: 46), variants or fragments thereof.
In one embodiment, said BLV.RBD comprises or consists of the amino acid sequence SEQ ID NO: 47, variants or fragments thereof.

In one embodiment, said BLV.RBD comprises or consists of the amino acid sequence SEQ ID NO: 48, variants or fragments thereof.

In one embodiment, said BLV.RBD comprises or consists of the amino acid sequence SEQ ID NO: 49, variants or fragments thereof.

As used herein, "amino acids" are represented by their full name, their three letter code or their one letter code as well known in the art. Amino acid residues in peptides are abbreviated as follows: Phenylalanine is Phe or F; Leucine is Leu or L; Isoleucine is Ile or I; Methionine is Met or M; Valine is Val or V; Serine is Ser or S; Proline is Pro or P; Threonine is Thr or T; Alanine is Ala or A; Tyrosine is Tyr or Y; Histidine is His or H; Glutamine is Gin or Q; Asparagine is Asn or N; Lysine is Lys or K; Aspartic Acid is Asp or D; Glutamic Acid is Glu or E; Cysteine is Cys or C; Tryptophan is Trp or W; Arginine is Arg or R; and Glycine is Gly or G.

As used herein, the term "amino acids" includes both natural and synthetic amino acids, and both D and L amino acids. "Standard amino acid" or "naturally occurring amino acid" means any of the twenty standard L-amino acids commonly found in naturally occurring peptides. "Nonstandard amino acid residue" means any amino acid, other than the standard amino acids, regardless of whether it is prepared synthetically or derived from a natural source. For example, naphtylalanylamine can be substituted for tryptophan to facilitate synthesis. Other synthetic amino acids that can be substituted include, but are not limited to, L-hydroxypropyl, L-3,4-dihydroxyphenylalanyl, alpha-amino acids such as L-alpha-hydroxysyl and D-alpha-methylalanyl, L-alpha-methylalanyl, beta-amino acids, and isoquinolyl.

As used herein, "amino acid" also encompasses chemically modified amino acids, including but not limited to, salts, amino acid derivatives (such as amides), and substitutions. Amino acids contained within the polypeptides of the present invention, and particularly at the carboxy- or amino-terminus, can be modified by methylation, amidation, acetylation or substitution with other chemical groups which can change the
polypeptide's circulating half-life without adversely affecting their activity. Additionally, a disulfide linkage may be present or absent in the polypeptides of the invention.

The RBD ligands of the invention may comprise standard amino acids or non-standard amino acids. Polypeptide mimetics include polypeptides having the following modifications: i) polypeptides wherein one or more of the peptidyl -C(0)NR- linkages (bonds) have been replaced by a non-peptidyl linkage such as a -CH₂-carbamate linkage (-CH₂OC(0)NR-), a phosphonate linkage, a -CH₂-sulfonamide (-CH₂-S(0)₂NR-) linkage, a urea (-NHCO(NH-) linkage, a -CH₂-secondary amine linkage, or with an alkylated peptidyl linkage (-C(O)NR-) wherein R is C1-C4 alkyl; ii) polypeptides wherein the N-terminus is derivatized to a -NRR¹ group, to a -NRC(0)R group, to a -NRC(0)OR group, to a -NRS(0)₂R group, to a -NHCO(NH)- group where R and R¹ are hydrogen or C1-C4 alkyl with the proviso that R and R¹ are not both hydrogen; iii) polypeptides wherein the C terminus is derivatized to -C(0)R² where R² is selected from the group consisting of C1-C4 alkoxy, and -NR³R⁴ where R³ and R⁴ are independently selected from the group consisting of hydrogen and C1-C4 alkyl.

According to a preferred embodiment, the BLV.RBD ligands are selected from the group comprising the sequences SEQ ID NO: 21, 3, 4, 13, 15, 17, 19, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 47, 48 and 49, fragments and variants thereof, more preferably selected from the group comprising the sequences SEQ ID NO: 21 and 4, fragments and variants thereof, even more preferably selected from the group comprising the sequence SEQ ID NO: 21, fragments and variants thereof. According to another embodiment, receptor-binding domain ligands are encoded by a DNA sequence selected from the group comprising the sequence SEQ ID NO: 22, 5, 14, 16, 18, 20 and 46, variants and fragments thereof.

In one embodiment, the BLV.RBD ligand comprises or consists of a sequence presenting a sequence identity of at least 70% with one of the sequences SEQ ID NO: 21, 3, 4, 13, 15, 17, 19, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 47, 48 or 49, preferably a sequence identity of at least about 75, 80, 85, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99% or more with one of the sequences SEQ ID NO: 21, 3, 4, 13, 15, 17, 19, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 47, 48 or 49.
In another embodiment, the BLV.RBD ligand of the invention is encoded by a DNA sequence presenting a sequence identity of at least 70% with the sequence SEQ ID NO: 22, 5, 14, 16, 18, 20 or 46, preferably a sequence identity of at least about 75, 80, 85, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99% or more with the sequence SEQ ID NO: 22, 5, 14, 16, 18, 20 or 46.

In one embodiment, the BLV.RBD ligand of the invention is a variant of one of the polypeptide having the sequences SEQ ID NO: 21, 3, 4, 13, 15, 17, 19, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 47, 48 and 49.

A polypeptide "variant" as the term is used herein, is a polypeptide that typically differs from a polypeptide specifically disclosed herein in one or more substitutions, deletions, additions and/or insertions. Such variants may be naturally occurring or may be synthetically generated, for example, by modifying one or more of the above polypeptide sequences and evaluating one or more biological activities of the polypeptide as described herein and/or using any of a number of techniques well known in the art. Modifications may be made in the structure of polypeptides and still obtain a functional molecule that encodes a variant or derivative polypeptide with desirable characteristics.

When it is desired to alter the amino acid sequence of a polypeptide to create an equivalent, or even an improved, variant or portion of a ligand of the invention, one skilled in the art will typically change one or more of the codons of the encoding DNA sequence. For example, certain amino acids may be substituted by other amino acids in a protein structure without appreciable loss of its ability to bind cell surface receptor, preferably cell surface nutrient transporters. Since it is the binding capacity and nature of a protein that defines that protein's biological functional activity, certain amino acid sequence substitutions can be made in a protein sequence, and, of course, its underlying DNA coding sequence, and nevertheless obtain a protein with similar properties. It is thus contemplated that various changes may be made in the polypeptide sequences, or corresponding DNA sequences that encode said peptides without appreciable loss of their biological utility or activity. In many instances, a polypeptide variant will contain one or more conservative substitutions. A "conservative substitution" is one in which an amino acid is substituted by another amino acid that has similar properties, such that one skilled
in the art of peptide chemistry would expect the secondary structure and hydropathic nature of the polypeptide to be substantially unchanged. As outlined above, amino acid substitutions are generally therefore based on the relative similarity of the amino acid side-chain substituents, for example, their hydrophobicity, hydrophilicity, charge, size, and the like. Exemplary substitutions that take various of the foregoing characteristics into consideration are well known to those of skill in the art and include: arginine and lysine; glutamate and aspartate; serine and threonine; glutamine and asparagine; and valine, leucine and isoleucine. Amino acid substitutions may further 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 histidine, lysine and arginine; and amino acids with uncharged polar head groups having similar hydrophilicity values include leucine, isoleucine and valine; glycine and alanine; asparagine and glutamine; and serine, threonine, phenylalanine and tyrosine. Other groups of amino acids that may represent conservative changes include: (1) Ala, Pro, Gly, Glu, Asp, Gin, Asn, Ser, Thr; (2) Cys, Ser, Tyr, Thr; (3) Val, Ile, Leu, Met, Ala, Phe; (4) Lys, Arg, His; and (5) Phe, Tyr, Trp, His.

As used herein, the term ”conservative amino acid substitution” may further be defined as an amino acid exchange within one of the following five groups:

1. Small aliphatic, nonpolar or slightly polar residues: Ala, Ser, Thr, Pro, Gly,

2. Polar, negatively charged residues and their amides: Asp, Asn, Glu, Gin,

3. Polar, positively charged residues: His, Arg, Lys,

4. Large, aliphatic, nonpolar residues: Met, Leu, Ile, Val, Cys,

5. Large, aromatic residues: Phe, Tyr, Trp.

A variant may also, or alternatively, contain non-conservative changes. In a preferred embodiment, variant polypeptides differ from a native sequence by substitution, deletion or addition of 1, 2, 3, 4, 5, 6, 7, 8, 9 or 10 amino acids. Variants may also (or alternatively) be modified by, for example, the deletion or addition of amino acids that have minimal influence on the immunogenicity, secondary structure and hydropathic nature of the polypeptide.
In one embodiment, a variant of SEQ ID NO: 21, 3, 4, 13, 15, 17, 19, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 47, 48 or 49 is capable of binding to CAT1 with an affinity at least equivalent to the one of SEQ ID NO: 21, 3, 4, 13, 15, 17, 19, 28, 29, 30, 31, 32, respectively.

In one embodiment, a variant of SEQ ID NO: 21, 3, 4, 13, 15, 17, 19, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 47, 48 or 49 comprises conservative amino acid substitutions as compared to the sequence of SEQ ID NO: 21, 3, 4, 13, 15, 17, 19, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 47, 48 or 49, respectively, such as, for example, 1, 2, 3, 4, 5, 6, 7, 8, 9 or 10 conservative amino acid substitutions.

In another embodiment, a variant of SEQ ID NO: 21, 3, 4, 13, 15, 17, 19, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 47, 48 or 49 is a polypeptide wherein 1, 2, 3, 4, 5, 6, 7, 8, 9 or 10 amino acids from the sequence of SEQ ID NO: 21, 3, 4, 13, 15, 17, 19, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 47, 48 or 49, respectively, is/are absent, or substituted by any amino acid, or wherein 1, 2, 3, 4, 5, 6, 7, 8, 9 or 10 amino acids (either contiguous or not) is/are added.

In one embodiment of the invention, the RBD ligands as described here above are modified by means well-known in the art, for instance by the addition of one or more functional group such as a phosphate, acetate, lipid or carbohydrate group, and/or by the addition of one or more protecting group. For example, the RBD ligands can be modified by the addition of one or more functional groups such as phosphate, acetate, or various lipids and carbohydrates. The RBD ligands of the invention can also exist as polypeptide derivatives. The term "polypeptide derivative" refers to compound having an amino group (—NH—), and more particularly, a peptide bond. Polypeptides may be regarded as substituted amides. Like the amide group, the peptide bond shows a high degree of resonance stabilization. The C—N single bond in the peptide linkage has typically about 40 percent double-bond character and the C=O double bond about 40 percent single-bond character. "Protecting groups" are those groups that prevent undesirable reactions (such as proteolysis) involving unprotected functional groups. Specific examples of
amino protecting groups include formyl; trifluoroacetyl; benzyloxycarbonyl; substituted benzyloxycarbonyl such as (ortho- or para-) chlorobenzyloxycarbonyl and (ortho- or para-) bromobenzyloxycarbonyl; and aliphatic oxycarbonyl such as t-butoxycarbonyl and t-amiloxycarbonyl. The carboxyl groups of amino acids can be protected through conversion into ester groups. The ester groups include benzyl esters, substituted benzyl esters such as methoxybenzyl ester; alkyl esters such as cyclohexyl ester, cycloheptyl ester or t-butyl ester. The guanidino moiety may be protected by nitro; or arylsulfonyl such as tosyl, methoxybenzensulfonyl or mesitylenesulfonyl, even though it does not need a protecting group. The protecting groups of imidazole include tosyl, benzyl and dinitrophenyl. The indole group of tryptophan may be protected by formyl or may not be protected.

The modification of the RBD ligands aims in particular to improve their life time in vivo. One type of modification is the addition to the N- or C-termini of the RBD ligands of polyethylene glycol (PEG). PEG is known by the person skilled in the art to have many properties that make it an ideal carrier for polypeptides such as high water solubility, high mobility in solution and low immunogenicity. This modification also protects the polypeptides from exopeptidases and therefore increases their overall stability in vivo.

The other modifications used to prevent degradation of the polypeptides by endopeptidases or exopeptidases include N-terminal modifications such as acetylation or glycosylation, C-terminal modifications such as amidation and use of unnatural amino acids (β-amino and α-trifluoromethyl amino acids) at particular sites within the polypeptides. In one embodiment, the BLV.RBD ligand of the invention is glycosylated. In another embodiment, the BLV.RBD ligand of the invention is not glycosylated.

Another alternative to increase polypeptide molecular size is the genetic fusion of the polypeptides to the Fc domain of human immunoglobulin (including, for example, IgA, IgM and IgG) or the fusion of the polypeptides to albumin.

In one embodiment, the BLV.RBD ligand as described here above is a fusion protein comprising a part or the totality of a RBD fused to a detection tag, such as, for example, a Fc fragment or a GFP. Examples of Fc fragments include, but are not limited to, rabbit
Fc fragment (amino acid sequence SEQ ID NO: 8, encoded by SEQ ID NO: 9), mouse
Fc fragment (amino acid sequence SEQ ID NO: 10, encoded by SEQ ID NO: 11).

In one embodiment, the receptor-binding domain ligand is BLV.RBD fused to rabbit Fc fragment (that may be encoded, for example, by the DNA sequence SEQ ID NO: 12).

In one embodiment, the receptor-binding ligand of the invention is coupled with at least one contrast agent. Non-limiting examples of contrast agents are listed hereinabove. A preferred contrast agent is 1-125.

The RBD ligands of the invention described herein can be produced synthetically by chemical synthesis or enzymatic synthesis as it is well known in the art. Alternatively, nucleotide sequences encoding the polypeptides of the invention can be introduced into a protein expression vector and produced in a suitable host organism (e.g., bacteria, insect cells, etc.), then purified. In one embodiment, the receptor-binding domain ligand is obtained by a cloning method, such as, for example, using any production system known in the art, such as, for example, E. coli, yeast, baculovirus-insect cell, or mammalian cells such as HEK or CHO, expression system.

Another object of the invention is a BLV.RBD ligand as described hereinabove coupled with at least one contrast agent. In one embodiment, the at least one contrast agent is a radiolabeled agent or a fluorescent agent. In one embodiment, the at least one contrast agent is 1-125.

In one embodiment, the at least one BLV.RBD ligand coupled with at least one contrast agent may be used as a probe for medical imaging.

Methods for coupling at least one contrast agent to a RBD ligand are well known in the state of the art. For instance, the at least one contrast agent may be bound covalently or non-covalently.

For example, technics to couple polypeptides to 1-125 are well known in the state of the art. A non-limited example of such a method is the following: iodine present in a reduced form (Nal) reacts with the phenol group of a tyrosine or with the side chain of a histidine residue. These groups are pre-oxidized with an oxidizing agent (iodogen). The peptides
preparation (100 µg for lmci = 37 MBq) is then added to an iodogen solution and incubated for 10 minutes at 4°C. The reaction is stopped using a stop solution comprising for example 200 µL of PBS with sodium azide per marking. In parallel, a mouse serum is added onto a PD10 column. Then the reaction solution is added onto the PD10 column and the peptide coupled with the iodine is collected.

In one embodiment of the invention, the at least one BLV.RBD ligand, variant and/or fragment thereof coupled with at least one contrast agent of the invention is for use as a tracer. The term "tracer", as used herein, refers to a recognition agent providing insight into CAT1-related disease location, progression and/or structure for pre-, intra- and post-operative surgery.

The present invention thus further relates to an in vivo method for tracing cells infected or not yet infected by BLV or wherein the function of a cationic L-amino acid transporter, preferably the CAT1 function, is dysregulated, in a subject in need thereof, comprising:

a. administering an effective amount of at least one BLV.RBD ligand, a variant and/or a fragment thereof coupled with at least one contrast agent to the subject, and

b. detecting and/or quantifying said at the least one BLV.RBD ligand, variant and/or fragment thereof binding to said cells using medical imaging technics.

In one embodiment, said method is for use in pre-, intra-, or post-operative surgery. In another embodiment, said method is for use in fluorescence guided surgery.

Examples of specific medical imaging technics methods that may be used are well known to the skilled artisan and include, but are not limited to, for instance computer assisted tomography (CAT), magnetic resonance spectroscopy (MRS), magnetic resonance imaging (MRI), positron emission tomography (PET) or single-photon emission computed tomography (SPECT) and are described in Boonstra et al. (2015. Oncotarget. 6(16): 14260-73).

In one embodiment, the BLV.RBD ligand as described above is coupled with a radiolabeled agent or a fluorescent agent as described herein. Examples of radiolabeled agent or fluorescent agent that may be used for targeting include, but are not limited to,
I-125, 1-131, F-18 (i.e. 18-F-fluoro-2-deoxy-D-glucose, 18-F-fluoro-17-estradiol), H-11-C-acetate, Tc-99m, 0-15, N-13, Br-76, In-111, Cu-64, Ga-68, Zr-89, ZW800-1, Cy5.5, IRDye800CW.

The term "cationic L-amino acid transporter-related disease, preferably CAT1-related disease" as used herein refers to diseases wherein pathways involving arginine, lysine, ornithine or histidine homeostasis and/or metabolism are dysregulated.

Examples of cationic L-amino acid transporter-related diseases, preferably of CAT1-related diseases, include, but are not limited to, arginine-related diseases, lysine-related diseases, histidine-related diseases, ornithine-related diseases, nitric oxide-related diseases, and inflammatory diseases.

Examples of arginine-related diseases include, but are not limited to, arginine-related cancers, argininosuccinate lyase-related diseases, argininosuccinate synthetase-related diseases, cardiac fibrosis and muscle fibrosis.

Examples of arginine-related cancers include, but are not limited to, arginine-related tumors, arginine-related sarcomas, arginine-related leukemia, arginine-related carcinomas, arginine-related lymphoma, arginine-related metastasis, and arginine-related melanomas, argininosuccinate synthetase-related cancers, argininosuccinate lyase related cancers, arginine auxotroph tumors and acute myeloid leukemia.

Examples of argininosuccinate synthetase-related cancers include, but are not limited to, colorectal cancer, bladder cancer, pancreatic cancer, liver cancer, melanoma, nasopharyngeal carcinoma, hepatocellular carcinoma, myxofibrosarcoma, brain tumor, glioblastoma, uveal melanoma, lung carcinoma, small cell lung cancer, colon carcinoma, colorectal cancer, hepatic carcinoma, renal cell carcinoma, prostate cancer, breast cancer, non-Hodgkin's lymphoma, Hodgkin's lymphoma, pancreatic carcinoma, malignant melanoma, osteosarcoma, malignant pleural mesothelioma, esophageal adenocarcinoma, pulmonary metastasis, ovarian cancer, acute myelocystic leukemia, mesothelial cancer, urological cancer and Burkitt lymphoma.
Examples of argininosuccinate lyase-related diseases include, but are not limited to, liver cancer, liver fibrosis, colorectal cancer, argininosuccinate lyase deficiency, argininosuccinic aciduria, urea cycle disorders, hyperammonemia, developmental retardation, acute infection or stress to cognitive impairment, behavioral abnormalities, and/or learning disabilities, hepatomegaly, progressive encephalopathy, lethargy, seizures, attention deficit hyperactivity disorder, hepatitis, cirrhosis, systemic hypertension, hypokalemia and glioblastoma.

Examples of argininosuccinate synthetase-related diseases include, but are not limited to, citrullinemia I, hyperammonemia, urea cycle disorders, and argininosuccinate synthetase related cancers.

Examples of lysine-related diseases include, but are not limited to, hyperlysinemia, glutaric aciduria type I, L-2 hydroxyglutaric aciduria, D-2 hydroxyglutaric aciduria, and herpes simplex infection.

Examples of histidine-related diseases include, but are not limited to, histidinemia, and histidine ammonia-lyase deficiency.

Examples of ornithine-related diseases include, but are not limited to, ornithine transcarbamylase deficiency, cirrhosis, and carcinogenesis.

Examples of nitric oxide-related diseases include, but are not limited to, chronic renal failure, chronic heart failure, congestive heart failure, hypertension, atherosclerosis, stroke, and thrombosis.

Examples of metabolic diseases include, but are not limited to, obesity, diabetes, cardiovascular mortality, renal damage and ischemia.

Examples of inflammatory diseases include, but are not limited to, polyarthritis, rheumatoid arthritis, asthma, inflammatory bowel diseases, celiac diseases, autoimmune diseases and multiple sclerosis.

Bovine leukemia virus infects dairy and bovine or ovine cattle's blood cells and mammary tissue. The retrovirus is easily transmitted among cattle primarily through direct contact,
infected blood, milk and possibly by biting insects. In addition, recent studies demonstrated an increased risk for women exposed to BLV to develop breast cancer.

Examples of BLV infection include, but are not limited to, leukemia, persistent lymphocytosis, lymphoproliferation, lymphoid tumors, malignant B cell lymphoma, Enzootic bovine leucosis (in cows) and breast cancer.

In one embodiment, the method of the invention is for diagnosing a cationic L-amino acid transporter-related disease, preferably a CAT1-related disease, or a BLV infection. In one embodiment, the method of the invention is not for diagnosing an inflammatory state.

The present application thus relates to a method for the diagnosis of a cationic L-amino acid transporter-related disease, preferably a CAT1-related disease, or a BLV infection comprising the steps of:

a. contacting an effective amount of at least one BLV.RBD ligand, a variant and/or a fragment thereof to a cell, sample, tissue, and/or organ,

b. detecting and/or quantifying the binding of the at least one BLV.RBD ligand, variant and/or fragment thereof to CAT1 in said cell, sample, tissue, and/or organ.

In one embodiment, the method of the invention comprises a step of comparing the binding determined and/or quantified at step b. with a reference binding value.

As used herein, the term "reference" broadly encompasses any suitable reference binding level which may be used as a basis for comparison with respect to the determined binding. In one embodiment, the reference is constructed using algorithms and/or other methods of statistical and hierarchical classification. In another aspect, the reference binding level is stored in a database to provide a stored binding level and the stored binding level is used to determine the difference in the binding level. The database may, for example, be stored on a computer or a server.

In one embodiment, the reference binding level is an index value or is derived from one or more risk prediction algorithms or computed indices for the presence of cells wherein the function of a cationic L-amino acid transporter, preferably the CAT1 function,
dysregulated or cells infected or not yet infected by BLV. A reference binding level can be relative to a number or value derived from population studies, including without limitation, such populations of subjects having similar age range, subjects in the same or similar ethnic group.

The term "cells wherein a cationic L-amino acid transporter, preferably the CAT1 function is dysregulated" as used herein refers to cells wherein arginine or lysine or histidine or ornithine metabolism or influx is abnormally increased or decreased.

Arginine or lysine or histidine or ornithine metabolisms include their synthesis, catabolism but also dietary uptake. Arginine is synthetized by two key enzymes: argininosuccinate synthetase and argininosuccinate lyase, that exhibit deficient or abnormal activities in diverse pathologies which results in arginine accumulation in some organs, tissues, samples, or cells affected or not yet affected by these pathologies. Lysine and histidine are essential amino acids, and are therefore not synthesized in animals. Ornithine is involved in the production of urea via the action of enzyme arginase on L-arginine.

In one embodiment of the invention, the reference binding level is the binding level measured in a population of patients diagnosed with a cationic L-amino acid transporter-related disease, preferably a CAT1-related disease, or a BLV infection.

According to this embodiment, equivalence (i.e., an absence of difference) between the determined binding level and the reference binding level, or a determined binding level superior to the reference binding level may be indicative of the presence of a cationic L-amino acid transporter-related disease, preferably a CAT1-related disease, or an infection with BLV.

In one embodiment of the invention, the reference binding level is the binding level determined in a population of substantially healthy subjects, i.e., in a population of subjects not diagnosed with a cationic L-amino acid transporter-related disease, preferably a CAT1-related disease, or a BLV infection. According to this embodiment, a determined binding level superior to the reference binding level may be indicative of the
presence of a cationic L-amino acid transporter-related disease, preferably of a CAT 1-related disease, or a BLV infection.

In the present invention, two numeric values, in particular two binding levels, are considered as different if the first numeric value is higher (such as, for example, the first numeric value is about 20% higher than the second one, preferably is about 30, 40, 50, 60, 70, 80, 90% or more higher than the second one) or lower than the second one (such as, for example, the second numeric value is about 20% lower than the second one, preferably is about 30, 40, 50, 60, 70, 80, 90% or more lower than the second one).

In one embodiment, the reference value is a personalized reference, determined earlier in the same subject (such as, for example, before receiving a treatment for a cationic L-amino acid transporter-related disease, preferably a CAT 1-related disease, or a BLV infection).

In one embodiment, the at least one BLV.RBD ligand, variant and/or fragment thereof is a BLV.RBD ligand coupled with at least one contrast agent as described hereinabove.

In one embodiment, the diagnosis method of the invention is an \textit{in vivo} diagnosis method. Preferably, said diagnosis method is based on medical imaging.

In another embodiment, the diagnosis method of the invention is an \textit{in vitro} or \textit{ex vivo} method, \textit{i.e.}, the method of the invention is performed on a cell, sample, tissue and/or organ that was obtained from a patient prior to the implementation of the method of the invention. Consequently, in one embodiment, the method of the invention does not comprise obtaining a sample from the patient, \textit{i.e.}, the method of the invention is non-invasive.

In one embodiment, the method of the invention is for monitoring a cationic L-amino acid transporter-related disease, preferably a CAT 1-related disease, in a subject. In one embodiment, the method of the invention is for monitoring a BLV infection in a subject. The term "monitoring" as used herein refers to the determination of the amount of cells wherein arginine, lysine, histidine or ornithine metabolism is dysregulated in the body of a subject as a function of time, such as, for example, before, during and after a therapy.
against a cationic L-amino acid transporter-related disease, preferably against a CAT 1-related disease, or against a BLV infection.

The term "therapy against a cationic L-amino acid transporter-related disease, preferably against a CAT 1-related disease or against a BLV infection" as used herein may refer to arginine deprivation, chemotherapy, radiation, surgery, immunotherapy, and drugs known to the skilled artisan as drugs for treating a cationic L-amino acid transporter-disease (including a CAT 1-related disease) or a BLV infection.

In one embodiment, the method of monitoring of the invention comprises comparing two binding levels, such as, for example, a binding determined before treatment with a binding level determined after treatment.

In one embodiment, a decreased binding level of the at least one BLV.RBD ligand after treatment is indicative of the efficacy of the treatment.

In one embodiment, a binding level after treatment equivalent or superior to the one determined before treatment is indicative of the absence of efficacy of the treatment.

The present application also relates to a method for monitoring a cationic L-amino acid transporter-related disease, preferably a CAT 1-related disease, or a BLV infection in a subject comprising the steps of:

a. contacting an effective amount of at least one BLV.RBD ligand, a variant and/or a fragment thereof, preferably coupled with at least one contrast agent, to a cell, sample, tissue, and/or organ of said subject,

b. detecting and/or quantifying the binding of the at least one BLV.RBD ligand, variant and/or fragment thereof to CAT1 in said cell, sample, tissue, and/or organ, preferably by medical imaging,

c. treating the subject with a therapy against a cationic L-amino acid transporter-related disease, preferably against a CAT 1-related disease, or against a BLV infection,

d. contacting an effective amount of the at least one BLV.RBD ligand, variant and/or fragment thereof, preferably coupled with at least one contrast agent to a cell, sample, tissue, and/or organ of said subject, and
e. detecting and/or quantifying the binding of the at least one BLV.RBD ligand, variant and/or fragment thereof to CAT1 in said cell, sample, tissue, and/or organ.

In one embodiment, the method of the invention further comprises a step of comparing the binding determined in step e) with the binding determined in step b), thereby monitoring a cationic L-amino acid transporter-related disease, preferably a CAT1-related disease, or a BLV infection in the subject.

In one embodiment, the absence or the decrease of detection of CAT1 in a cell, sample, tissue, and/or organ after a therapy against a cationic L-amino acid transporter-related disease (including a CAT1-related disease), or against a BLV infection, is indicative of a remission. In particular, such disease may include for example nitric oxide-related diseases, preferably chronic renal failure and chronic heart failure.

In another embodiment, the presence or the increase of detection of CAT1 in a cell, sample, tissue, and/or organ after a therapy against a cationic L-amino acid transporter-related disease (including a CAT1-related disease) is indicative of a remission. In particular, such disease may include metabolic diseases such as for example, obesity, diabetes, cardiovascular mortality, renal damage or ischemia.

The present application further relates to a composition comprising, consisting or consisting essentially of at least one BLV.RBD ligand, a variant and/or a fragment thereof as described hereinabove.

The present application further relates to a pharmaceutical composition comprising, consisting or consisting essentially of at least one BLV.RBD ligand, a variant and/or a fragment thereof as described hereinabove and at least one pharmaceutically acceptable excipient.

The present application further relates to a medicament comprising, consisting or consisting essentially of at least one BLV.RBD ligand, a variant and/or a fragment thereof as described hereinabove.
As used herein, the term "consisting essentially of, with reference to a pharmaceutical composition or medicament, means that the at least one BLV.RBD ligand of the invention is the only one therapeutic agent or agent with a biologic activity within said pharmaceutical composition or medicament.

The present application also relates to a diagnostic composition comprising, consisting or consisting essentially of at least one BLV.RBD ligand, a variant and/or a fragment thereof coupled with at least one contrast agent as described hereinabove and at least one pharmaceutically acceptable excipient.

In one embodiment, the diagnostic composition of the invention is for diagnosing a cationic L-amino acid transporter-related disease, preferably a CAT1-related disease or for monitoring a cationic L-amino acid transporter-related disease, preferably a CAT1-related disease, according to the methods of the invention as described hereinabove. In another embodiment, the diagnostic composition of the invention is for diagnosing a BLV infection or for monitoring a BLV infection, according to the methods of the invention as described hereinabove. In one embodiment, the diagnostic composition of the invention is not for diagnosing an inflammatory state.

Pharmaceutically acceptable excipients include water, saline, Ringer's solution, dextrose solution, and solutions of ethanol, glucose, sucrose, dextran, mannose, mannitol, sorbitol, polyethylene glycol (PEG), phosphate, acetate, gelatin, collagen, Carbopol®, vegetable oils, and the like. One may additionally include suitable preservatives, stabilizers, antioxidants, antimicrobials, and buffering agents, such as, for example, BHA, BHT, citric acid, ascorbic acid, tetracycline, and the like.

Other examples of pharmaceutically acceptable excipients that may be used in the composition of the invention include, but are not limited to, ion exchangers, alumina, aluminum stearate, lecithin, serum proteins, such as human serum albumin, buffer substances such as phosphates, glycine, sorbic acid, potassium sorbate, partial glyceride mixtures of saturated vegetable fatty acids, water, salts or electrolytes, such as protamine sulfate, disodium hydrogen phosphate, potassium hydrogen phosphate, sodium chloride, zinc salts, colloidal silica, magnesium trisilicate, polyvinyl pyrrolidone, cellulose-based
substances, polyethylene glycol, sodium carboxymethylcellulose, polyacrylates, waxes, polyethylene- polyoxypropylene- block polymers, polyethylene glycol and wool fat.

In addition, pharmaceutically acceptable excipients may comprise some excipients, such as, for example, surfactants (e.g. hydroxypropylcellulose); suitable carriers, such as, for example, solvents and dispersion media containing, for example, water, ethanol, polyol (e.g. glycerol, propylene glycol, and liquid polyethylene glycol, and the like), suitable mixtures thereof, and vegetable oils, such as, for example, peanut oil and sesame oil; isotonic agents, such as, for example, sugars or sodium chloride; coating agents, such as, for example, lecithin; agents delaying absorption, such as, for example, aluminum monostearate and gelatin; preservatives, such as, for example, benzalkonium chloride, benzethonium chloride, chlorobutanol, thimerosal and the like; buffers, such as, for example, boric acid, sodium and potassium bicarbonate, sodium and potassium borates, sodium and potassium carbonate, sodium acetate, sodium biphosphate and the like; tonicity agents, such as, for example, dextrose, potassium chloride, propylene glycol, sodium chloride; antioxidants and stabilizers, such as, for example, sodium bisulfite, sodium metabisulfite, sodium thiosulfite, thiourea and the like; nonionic wetting or clarifying agents, such as, for example, polysorbate 80, polysorbate 20, poloxamer 282 and tyloxapol; viscosity modifying agents, such as, for example dextran 40, dextran 70, gelatin, glycerin, hydroxyethylcellulose, hydroxymethylpropylcellulose, lanolin, methylcellulose, petrolatum, polyethylene glycol, polyvinyl alcohol, polyvinylpyrrolidone, carboxymethylcellulose; and the like.

The present application also relates to at least one BLV.RBD ligand, composition, pharmaceutical composition or medicament as described hereinabove for treating or for use in treating a cationic L-amino acid transporter-related disease, preferably a CAT1-related disease, or a BLV infection.

In one embodiment, the at least one BLV.RBD ligand, composition, pharmaceutical composition or medicament as described hereinabove is not for treating or for use in treating an inflammatory state.
In one embodiment, the at least one BLV.RBD ligand, composition, pharmaceutical composition or medicament as described hereinabove is not a vaccine. Thus, in one embodiment, the at least one BLV.RBD ligand, composition, pharmaceutical composition or medicament as described hereinabove is not for generating or for use in generating antibodies, in particular, antibodies directed against BLV.

The present application also relates to a method for treating a cationic L-amino acid transporter-related disease, preferably a CAT1-related disease, or a BLV infection, wherein the method comprises administering to the subject a therapeutically effective amount of at least one BLV.RBD ligand as described here above.

The present application also relates to a method for targeting cells, samples, tissues, and/or organs infected or not yet infected by BLV or wherein the function of a cationic L-amino acid transporter, preferably the CAT1 function is dysregulated, wherein said method comprises administering at least one BLV.RBD ligand, a variant and/or a fragment thereof to a subject. Such method may be used, for example, for targeting therapeutic agents to cells, samples, tissues, and/or organs infected or not yet infected by BLV or wherein the function of a cationic L-amino acid transporter, preferably the CAT1 function is dysregulated. In one embodiment, the methods of the invention are for protecting a subject from other subjects already infected by BLV, or for preventing a BLV infection and in particular for preventing the propagation of a BLV infection.

In one embodiment, said at least one BLV.RBD ligand, preferably when coupled with at least one contrast agent, is encapsulated. The encapsulation of the at least one BLV.RBD ligand coupled with at least one contrast agent may avoid any degradation. The technics of encapsulation are well known in the state of the art.

Examples of capsule include, but are not limited to, phospholipids, polymers, liposomes and quantum dots.

In one embodiment, the at least one BLV.RBD ligand is encapsulated with a therapeutic agent to be specifically administered to cells, samples, tissues or organs infected or not yet infected by BLV or wherein the function of a cationic L-amino acid transporter, preferably of CAT1 function is dysregulated within the subject's body.
In one embodiment, the at least one BLV.RBD ligand, the at least one BLV.RBD ligand coupled with at least one contrast agent, the composition, the diagnostic composition, the pharmaceutical composition or the medicament of the invention is to be administered at a dose determined by the skilled artisan and personally adapted to each subject.

In one embodiment, the at least one BLV.RBD ligand, the at least one BLV.RBD ligand coupled with at least one contrast agent, the composition, the diagnostic composition, the pharmaceutical composition or the medicament of the invention is to be administered at an effective amount.

It will be understood that the usage of the at least one BLV.RBD ligand, the at least one BLV.RBD ligand coupled with at least one contrast agent, the composition, the diagnostic composition, the pharmaceutical composition or the medicament of the invention will be decided by the attending physician within the scope of sound medical judgment. The specific effective amount for any particular patient will depend upon a variety of factors including the specific composition employed, the age, body weight, general health, sex and diet of the subject; the time of administration, route of administration, and like factors well known in the medical arts.

In one embodiment, the at least one BLV.RBD ligand, the at least one BLV.RBD ligand coupled with at least one contrast agent, the composition, the diagnostic composition, the pharmaceutical composition or the medicament of the invention is to be administered by injection, orally, topically, nasally, buccally, rectally, vaginally, intratracheally, by endoscopy, transmucosally, or by percutaneous administration.

In one embodiment, the at least one BLV.RBD ligand, the at least one BLV.RBD ligand coupled with at least one contrast agent, the composition, the diagnostic composition, the pharmaceutical composition or the medicament of the invention is to be administered by injection, preferably is to be systemically injected. Examples of formulations adapted to systemic injections include, but are not limited to, liquid solutions or suspensions, solid forms suitable for solution in, or suspension in, liquid prior to injection. Examples of systemic injections include, but are not limited to, intravenous, subcutaneous, intramuscular, intradermal, intravitreal, and intraperitoneal injection, or perfusion. In
another embodiment, when injected, the composition, the diagnostic composition, the pharmaceutical composition or the medicament of the invention is sterile. Methods for obtaining a sterile composition, diagnostic composition, pharmaceutical composition or medicament include, but are not limited to, GMP synthesis (GMP stands for "Good manufacturing practice").

In one embodiment, the at least one BLV.RBD ligand, the at least one BLV.RBD ligand coupled with at least one contrast agent, the composition, the diagnostic composition, the pharmaceutical composition or the medicament of the invention is to be orally administered. Examples of formulations adapted to oral administration include, but are not limited to, solid forms, liquid forms and gels. Examples of solid forms adapted to oral administration include, but are not limited to, pill, tablet, capsule, soft gelatine capsule, hard gelatine capsule, caplet, compressed tablet, cachet, wafer, sugar-coated pill, sugar coated tablet, or dispersing/or disintegrating tablet, powder, solid forms suitable for solution in, or suspension in, liquid prior to oral administration and effervescent tablet.

Examples of liquid forms adapted to oral administration include, but are not limited to, solutions, suspensions, drinkable solutions, elixirs, sealed phial, potion, drench, syrup and liquor.

In another embodiment, the at least one BLV.RBD ligand, the at least one BLV.RBD ligand coupled with at least one contrast agent, the composition, the diagnostic composition, the pharmaceutical composition or the medicament of the invention is to be topically administered. Examples of formulations adapted to topical administration include, but are not limited to, sticks, waxes, creams, lotions, ointments, balms, gels, masks, leave-on washes and/or the like.

Depending on the cell(s), sample(s), tissue(s) and/or organ(s) targeted, the skilled artisan can determine the technology needed for the introduction of the at least one BLV.RBD ligand in the targeted cell(s), sample(s), tissue(s) and/or organ(s).

In one embodiment, the at least one BLV.RBD ligand, the at least one BLV.RBD ligand coupled with at least one contrast agent, the composition, the diagnostic composition, the pharmaceutical composition or the medicament of the invention is to be administered in
a sustained-release form. In another embodiment, the at least one BLV.RBD ligand, the at least one BLV.RBD ligand coupled with at least one contrast agent, the composition, the diagnostic composition, the pharmaceutical composition or the medicament of the invention comprises a delivery system that controls the release of the agent.

The "targeted cell(s), sample(s), tissue(s) and/or organ(s)" as used herein may refer to (a) cell(s), (a) sample(s), (a) tissue(s) and/or (an) organ(s) affected or suspected to be affected by a cationic L-amino acid transporter-related disease, preferably by a CAT1-related disease, or by a BLV infection.

In one embodiment, a therapeutically effective amount of the BLV.RBD ligand, the BLV.RBD ligand coupled with at least one contrast agent, the pharmaceutical composition or medicament of the invention is administered at least once a day, twice a day, or at least three times a day.

In another embodiment, a therapeutically effective amount of the BLV.RBD ligand, the BLV.RBD ligand coupled with at least one contrast agent, the pharmaceutical composition or medicament of the invention is administered every two, three, four, five, or six days.

In another embodiment, a therapeutically effective amount of the BLV.RBD ligand, the BLV.RBD ligand coupled with at least one contrast agent, the pharmaceutical composition or medicament of the invention is administered every week, twice a week, every two weeks, or once a month.

In another embodiment, a therapeutically effective amount of the at least one BLV.RBD ligand, the at least one BLV.RBD ligand coupled with at least one contrast agent, the pharmaceutical composition or the medicament of the invention is administered every month for a period at least 2; 3; 4; 5; or 6 months.

In another embodiment, a therapeutically effective amount of the at least one BLV.RBD ligand, the at least one BLV.RBD ligand coupled with at least one contrast agent, the pharmaceutical composition or the medicament of the invention ranges from about 1 µg to 5 g.
In another embodiment, a therapeutically effective amount of the at least one BLV.RBD ligand, the at least one BLV.RBD ligand coupled with at least one contrast agent, the pharmaceutical composition or the medicament of the invention is to be administered ranges from about 0.1 µg/kg to 1 g/kg.

In another embodiment, the at least one BLV.RBD ligand, the at least one BLV.RBD ligand coupled with at least one contrast agent, the composition, the pharmaceutical composition or the medicament of the invention as described here above is to be administered in combination with another treatment for a cationic L-amino acid transporter-related disease, preferably for a CAT1-related disease, or for a BLV infection.

Examples of agents for treating a cationic L-amino acid transporter-related diseases, preferably CAT1-related diseases, or BLV infections include, but are not limited to, arginine deprivation, chemotherapy, radiation, surgery, protein kinases inhibitors, microtubules inhibitors, anti-metabolite agents a tumor vaccine or an immunostimulatory antibody.

In one embodiment of the invention, the method for treating a cationic L-amino acid transporter-related disease, preferably a CAT1-related disease, or a BLV infection in a subject in need thereof, comprises administering to the subject the at least one BLV.RBD ligand, the at least one BLV.RBD ligand coupled with at least one contrast agent, the composition, the pharmaceutical composition or the medicament of the invention prior to, concurrent to and/or posterior to another treatment against a cationic L-amino acid transporter-related disease, preferably against a CAT1-related disease, or against a BLV infection.

In one embodiment, the subject is affected, preferably is diagnosed with a cationic L-amino acid transporter-related disease, preferably with a CAT1-related disease, or with a BLV infection. In another embodiment, the subject of the invention is at risk of developing a cationic L-amino acid transporter-related disease, preferably a CAT1-related disease, or a BLV infection. Examples of risk factor for developing a cationic L-amino acid transporter-related disease, preferably a CAT1-related disease, include, but are not limited to, genetic factors, smoking, obesity, diabetes, alcohol, and
environmental conditions. Examples of risk factor for developing a BLV infection, include, but are not limited to, environmental conditions such as, for example, exposure to other subjects infected by BLV.

In another embodiment, the subject of the invention is in a remission stage following a cationic L-amino acid transporter-related disease, preferably following a CAT1-related disease.

Another object of the present invention is a kit for implementing the method of the invention, wherein said kit comprises means for detecting and/or quantifying CAT1 in a cell, sample, tissue and/or organ, and in particular for determining the binding of the at least one BLV.RBD ligand to CAT1.

In one embodiment, the kit of the invention comprises at least one BLV.RBD ligand coupled with at least one contrast agent as described here above.

By "kit" is intended any manufacture (e.g., a package or a container) comprising at least one reagent (such as, for example, a BLV.RBD ligand coupled with at least one contrast agent) for specifically detecting and/or quantifying CAT1. The kit may be promoted, distributed, or sold as a unit for performing the methods of the present invention. Furthermore, any or all of the kit reagents may be provided within containers that protect them from the external environment, such as in sealed and sterile containers. The kits may also contain a package insert describing the kit and methods for its use.

Another object of the present invention is a screening method for detecting and/or quantifying compounds modulating CAT1 function in a cell comprising:

a. determining and/or quantifying the binding of a BLV.RBD ligand as described hereinabove to CAT1 expressed in a cell in the presence of said compound, and

b. comparing the binding measured at the previous step with a binding of a BLV.RBD ligand as described hereinabove to CAT1 expressed in said cell in the absence of said compound.
The terms "modulating" and "modulation" as used herein may thus refer to an increase or decrease of the presence of CAT1 in a cell.

The present application also relates to a method for the \textit{in vitro} or \textit{ex vivo} or \textit{in vivo} diagnosis of a cationic L-amino acid transporter-related disease, preferably of a CAT1-related disease, or a BLV infection comprising:

a. contacting at least one BLV.RBD ligand, a variant and/or a fragment thereof with a cell, a sample, a tissue or an organ, and

b. detecting and/or quantifying the at least one BLV.RBD ligand bound to CAT1 present in the cell, sample, tissue or organ within said subject.

The present application also relates to a method for the \textit{in vivo} diagnosis of a cationic L-amino acid transporter-related disease, preferably of a CAT1-related disease, or a BLV infection comprising:

a. administering to a subject in need thereof an effective amount of at least one BLV.RBD ligand, a variant and/or a fragment thereof, and

b. detecting and/or quantifying the at least one BLV.RBD ligand, variant and/or fragment thereof within said subject.

In one embodiment, of the invention, the BLV.RBD ligand is coupled with at least one contrast agent, and may be used for \textit{in vivo} diagnosis by medical imaging.

The present application also relates to a method for treating a cationic L-amino acid transporter-related disease, preferably a CAT1-related disease, or a BLV infection comprising:

a. diagnosing a cationic L-amino acid transporter-related disease, preferably a CAT1-related disease, or a BLV infection in a subject in need thereof according to the method of the invention, and

b. administering a therapeutically effective amount of a therapy against a cationic L-amino acid transporter-related disease, preferably a CAT1-related disease, or against a BLV infection to said subject diagnosed in step (a) with a cationic L-amino acid transporter-related disease, preferably with a CAT1-related disease, or with a BLV infection.
In one embodiment, the method for treating a cationic L-amino acid transporter-related disease, preferably a CAT1-related disease, or a BLV infection of the invention thus comprises:

a. determining the presence of cells wherein the function of a cationic L-amino acid transporter, preferably the CAT1 function, is dysregulated, or of cells infected by BLV (or not yet infected by BLV), in a subject in need thereof by:

i. administering an effective amount of at least one BLV.RBD ligand, a variant and/or a fragment thereof coupled with at least one contrast agent to a subject, and

ii. detecting and/or quantifying the at least one BLV.RBD ligand, variant and/or fragment thereof using medical imaging, and

b. administering a therapeutically effective amount of a therapy against a cationic L-amino acid transporter-related disease, preferably against a CAT1-related disease, or against a BLV infection, to a subject diagnosed in step a. with a cationic L-amino acid transporter-related disease, preferably with a CAT1-related disease, or with a BLV infection.

The present application also relates to a method for treating a cationic L-amino acid transporter-related disease, preferably a CAT1-related disease, or a BLV infection comprising administering a therapeutically effective amount of at least one BLV.RBD, a variant and/or a fragment thereof to a subject in need thereof.

The present application also relates to a method for treating a cationic L-amino acid transporter-related disease, preferably a CAT1-related disease, or a BLV infection comprising:

a. diagnosing a cationic L-amino acid transporter-related disease, preferably a CAT1-related disease, or a BLV infection in a subject in need thereof according to the method of the invention, and

b. administering a therapeutically effective amount of at least one BLV.RBD, a variant and/or a fragment thereof to said subject diagnosed in step a. with
a cationic L-amino acid transporter-related disease, preferably with a CAT1-related disease, or with a BLV infection.

The present application also relates to an in vivo method for detecting and/or quantifying CAT1 in a cell comprising:

a. administering at least one BLV.RBD ligand, a variant and/or a fragment thereof coupled with at least one contrast agent or the diagnostic composition of the invention to a body, an organ, a tissue or a cell,

b. detecting and/or quantifying the binding of the at least one BLV.RBD ligand, variant and/or fragment thereof binding to CAT1 in said body, organ, tissue or cell using medical imaging technics.

In one embodiment, the method of the invention further comprises comparing the binding determined in step b. with a reference binding.

The present application also relates to a method for inhibiting CAT1 activity in a subject in need thereof wherein a therapeutically effective amount of at least one BLV.RBD ligand is administered to said subject.

The term "inhibiting CAT1 activity" as used herein may refer to an inhibition of the flux of arginine, histidine, lysine and/or ornithine transport within a cell by CAT1.

**BRIEF DESCRIPTION OF THE DRAWINGS**

Figure 1 is a graph showing the BLV.RBD automated screen identifying CAT1 as the BLV.RBD cognate receptor. Screening for BLV.RBD binding on the 172 SLC members was performed with the Tecan robot and Cellomics microscope. Binding is expressed as total fluorescence intensity for each well.

Figure 2 is a set of graphs showing the specific BLV.RBD binding to CAT1/SLC7A1. HEK293T cells were transfected with either of siLUC, siCAT1, siCAT1 combined with CAT1 expression vector (rescue assay), empty vector (pcHix) or CAT1 expression vector only. CAT1 expression level was monitored using BLV.RBD ligand.
**Figure 3** is a set of 2 histograms showing the specific inhibition of arginine uptake by the receptor-binding domain of the bovine leukemia virus envelope glycoprotein (BLV.RBD). Uptake of radiolabeled arginine and glutamine by HEK293T cells transfected with either of: pCSImFc empty vector ("empty vector"), BLV.RBD fused to a rFc tag in pCSI expression vector ("BLV-RBD", SEQ ID NO: 12), or HTLV2.RBD fused to a rFc tag in pCSI expression vector ("HTLV2-RBD", SEQ ID NO: 7). N=3, bilateral Student test on unpaired data (**, p<0.01; all other differences are not statistically significant).

**Figure 4** is an histogram showing infectivity trials, using CHO cells. Infectiveness of BLV (left panel) and HTLV (right panel) were tested, in cells transfected with an empty pLXSN expression vector as negative control (LXSN), or a pLXSN vector expressing human CAT1 (Hu-CAT1), bovine CAT1 (B-CAT1) or human CAT2 (Hu-CAT2). Results are shown as the number of infectious particles/mL.

**EXAMPLES**

The present invention will now be further illustrated by, but is by no means limited to, the following examples.

**Example 1: Specific binding of BLV.RBD to CAT1**

**Materials and Methods**

**Cell culture and transfection**

All cell lines were maintained in DMEM culture medium (Dulbecco's Modified Eagle Medium) supplied by Life Technologies (ref: 11965-092) with 10% decomplemented fetal bovine serum (PAN Biotech); non-essential amino acids (11140-035, Life Technologies); glutamine (25030-081 Life Technologies) and antibiotics (penicillin and streptomycin). All were incubated at 37°C in a 5% CO2-95% air atmosphere.
20,000 QT6 cells were seeded in a 96-well plate. Cells were transfected with 100 ng of DNA using JetPrime transfection protocol. Conditions for binding with BLV.RBD were optimized for automated conditions.

*Staining for Tecan robot and Cellomics microscope*

Staining was performed 48 hours post-transfection, using a BLV.RBD-mFc ligand (SEQ ID NO: 27). For this purpose, RBD ligands were tested at a 1/10 (v:v) dilution and the anti-mouse IgG1 bound to Alexa Fluor® 488 was used (1 to 500 dilutions).

Incubation of the RBD ligand is performed at 37°C without shaking and the secondary anti-mouse IgG1-Alexa is incubated at room temperature (as opposed to 4°C in previous protocol). Cells were then fixed with 4% PFA and fluorescence intensity was measured using the Cellomics Array Scan XTI HCS reader (Thermo Scientific; available at the CNRS MRI platform).

The SLC candidate detected by the high-throughput screening was confirmed by both siRNA and overexpression in HEK293T cells (data not shown).

Because of the natural expression of the BLV receptor on 293T cells and the looser attachment of HEK293T cell monolayers to plastic, we choose to use quail QT6 cells, which had lowest natural binding of our BLV.RBD ligands and a monolayer attachment to plastic compatible with the automated screening of our SLC library. We chose to set up the Cellomics-based fluorescent automated assay that would allow the detection and quantification potentially isolated foci off the quail QT6 cell monolayer that would be highly fluorescent from expression of the BLV.RBD cognate receptor.

*Results*

A SLC library containing 172 of the nearly 400 described members, coming from the human ORFeome (MGC platform) completed with constructs that were derived in our laboratory was established. We proceeded to set up an automated protocol of transient overexpression in the quail QT6 cell line and binding with a BLV.RBD tagged in frame with the constant fragment of the mouse immunoglobulin (mFc), in order to detect and isolate the BLV receptor.
Accordingly, when HEK293T cells are transfected with siRNA specifically designed to target CAT1 mRNA, BLV.RBD binding has a 3.5-fold decrease as measured by flow cytometry \((n=3)\). In contrast, when HEK293T cells are transfected with CAT1/SLC7A1 expression vector, CAT1 overexpression in these cells is accompanied by a 3-time-increase of BLV.RBD binding when compared to HEK293T cells transfected with an empty vector control \((n=3)\).

Our screening method provides evidence for CAT1 (SLC7A1) as the BLV.RBD and BLV Env receptor (figure 1). From the results as seen in figure 1, we focused our attention on CAT1 (SLC7A1) as the putative receptor for BLV.RBD ligand. Accordingly, down modulation of CAT1 mRNA by specific siRNA (siCAT1 5'-UAAUUGCAUCAAAAGGCGTT-3' - SEQ ID NO: 6) resulted in a highly significant decrease of the binding with BLV.RBD on HEK293T cells \((135 \text{ vs } 49, \text{ figure 2})\). We performed a rescue assay (co-transfection of siCAT1 and CAT1 expression plasmid) and were able to restore CAT1 expression as monitored with BLV.RBD ligand \((49 \text{ vs } 218, \text{ figure 2})\). Moreover, BLV.RBD can be used to monitor specific increase expression of CAT1 when comparing cells either transfected with the pCHIX control empty vector or a human CAT1 expression vector \((92 \text{ vs } 335, \text{ figure 2})\).

**Example 2: BLV.RBD-induced inhibition of arginine uptake in cells**

**Materials and Methods**

**Uptake assays**

3.5x10⁵ HEK293T cells per well were seeded in a 6-well plate coated with poly-D lysine. The following day, the cells were transfected using calcium phosphate with the vectors: pCSI rabbit Fc (rFc), pCSI BLV.RBD rFc (SEQ ID NO: 12) et pCSI HTLV2.RBD rFc (SEQ ID NO: 7). 16 hours post-transfection, the cell medium was changed and 24 hours later, the cells were seeded in 24-well plates \((5x10^4 \text{ cells/well})\) for the uptake of L-arginine or L-glutamine radiolabeled or in 6-well plates \((3x10^5 \text{ cells/well})\) to lyse the cells and verify the expression of RBD by immunoblotting.

For uptake, cells were incubated in a volume of 250 µL for 30 minutes at 37°C with
5 µCi/mL of L-arginine monohydrochloride \([2,3,4-^3\text{H}]\) (NET1 123250UC, Perkin Elmer) or L-glutamine \([3,4-^3\text{H} (N)]\) (NET551001MC Perkin Elmer) diluted in DMEM classic. After two washes with cold PBS, cells were lysed with 500 µL Triton X-100 1% and mixed with 2 mL of liquid scintillation cocktail (ULTIMA GOLD, Perkin Elmer) before measuring the radioactivity.

The values of the assimilation correspond to the ratio between the captured quantity (pmol) of arginine or glutamine radiolabeled and the amount (mg) of proteins in each well. The results were calculated and are reported using GraphPad Prism 5. The bilateral statistical test for unpaired data Student was used.

**Results**

The ability of BLV.RBD to functionally alter its cognate receptor upon binding was tested. For this purpose, uptake assays were performed and it allowed us to monitor whether BLV.RBD tagged with mouse Fc (mFc) (data not shown) or rabbit Fc (rFc) can block the transporter function using radiolabeled arginine.

CAT1 is described to be ubiquitously expressed in all cell types except in liver and lacrimal gland and to mediate sodium-independent transport of cationic L-amino acids that include arginine, lysine, ornithine and histidine.

Using radiolabeled arginine monohydrochloride, L-[2,3,4-^3\text{H}] provides further evidence of BLV.RBD binding of CAT1/SLC7A1. Thus, arginine transport, which is mediated by CAT1, is inhibited upon introduction of BLV.RBD and data show that BLV.RBD can block CAT1 transport function (**Figure 3**).

These results demonstrate that BLV.RBD ligand can be used to monitor CAT1 cell-surface expression and can be applied in a large variety of biological samples. BLV.RBD ligand can therefore be used *in vitro* by flow cytometry, immunohistochemistry (not shown) and immunofluorescence or *in vivo* by SPECT-CT imaging.
Example 3: Test \textit{in vitro} of the effect of BLV.RBD on a BLV infection

Materials and Methods

Materials

Infection of A23 cell line stably expressing CAT1 from different mammal species (A23-LXSN: empty vector; A23-CAT1 human; A23-Cat1 cattle; A23-mouse Cat1 mouse and A23-hamster Cat1).

Culture medium: HEK293 cells are grown up in DMEM with 10% FBS, L-glutamine, nonessential amino acids and antibiotics (Penicillin streptomycin). The producing cells (HEK293) and the infected A23 cells will encode for GFP.

Method

At \textbf{Day 0 (DO)}: seed 4 Petri dishes (10 cm diameter) with HEK293 cells (ATCC) 3×10^7 cells/dish.

At \textbf{D1} (evening): transfection of HEK293 in order to produce viral pseudotypes coding for the following envelopes (HTLV2; BLV; Vesicular stomatitis Indiana virus (VSV) or without envelope). Co-transfection with the following vectors:

1. NCG (code for GFP): 10 tig,
2. BEB.GP57 (gag-pol): 5 µg,
3. Envelope glycoprotein: 5 ng.

Calcium phosphate transfection (500 µL of HeBS and 33 µE of CaC12).

At \textbf{D2} (morning): change the medium of the HEK293 cells.

At D3: collect and filter the supernatant of HEK293 cells (to keep for infection with the supernatant); detach and count the producing cells (for cell to cell infection).
Infection with the supernatant

Seed the A23 cells (LXSN; hCAT1; bovineCAT1; mouseCAT1 and hamsterCAT1): 5000 cells/well in a 96-well plate (make sure to have 5000 cells in 50 μL of medium). Add an equal volume (50 μL) of the viral supernatant. N = 4.

Cell to cell infection

Co-culture of the different indicator cells (A23) with the producing cells (HEK293). Seed 2500 cells/well of A23 with 2500 cells of pseudotype producing cells for each condition (e.g. A23-LXSN with HEK293 producing pseudotypes). N = 4.

At D5: (only for cell to cell infection). Add the selection agent (2 mg/ml of G418). In this way, we will eliminate the producer cells which are sensitive, while A23 are resistant.

D8: Image (10x) and fluorescence intensity acquiring with High Content Cellomics ArrayScan (available at Montpellier Rio Imaging Platform: MR!).

Example 4: Test in vitro of the retroviral infection by a BLV env glycoprotein harboring a BLV.RBD, via CAT1 binding

Viral infection

CHO cells stably transfected with the empty pLXSN expression vector or with a pLXSN construct expressing either human (Hu-) or bovine (B-) CAT1, or the human CAT2 isoform were plated in 6-well plates and infected the following day with serial dilutions of replication-defective pLKO-1-puro lentiviral vector pseudotyped with the BLV or HTLV Env glycoproteins. One day after viral challenge, cells were selected with 3 μg/ml. puromycin during 10 days and resistant cells were counted to determine viral titers due to entry using the BLV or HTLV Env.

Results

CHO cells susceptibility to HTLV Env-mediated infection is independent of CAT1 or CAT2 expression (Figure 4, right panel); in contrast, CHO cells, which are naturally resistant to BLV Env-mediated infection (Figure 4, left panel, black histogram), become
specifically susceptible to BLV Env-mediated infection when expressing either human (Figure 4, left panel, dark grey histogram) or bovine (Figure 4, left panel, grey histogram) CAT1, while remaining resistant upon expression of the human CAT2 isoform (Figure 4, left panel, light grey histogram).

These results confirm that the bovine leukemia virus (BLV) envelope glycoprotein binds specifically to CAT1 of different mammals, including cattle and humans, but not to CAT2.
CLAIMS

1. An in vitro method for detecting and/or quantifying the cationic amino acid transporter-1 (CAT1) in a cell, wherein said method comprises:
   a. contacting said cell with at least one bovine leukemia virus (BLV).RBD ligand, a variant and/or a fragment thereof, and
   b. determining and/or quantifying the binding of said at least one ligand variant and/or fragment thereof to CAT1.

2. The in vitro method according to claim 1, wherein said method further comprises comparing the binding level determined and/or quantified at step b. with a reference value.

3. The in vitro method according to claim 1 or 2, wherein said method is for diagnosing or monitoring a CAT1-related disease or a BLV infection in a subject.

4. The in vitro method according to any one of claims 1 to 3, wherein said at least one BLV.RBD ligand, variant and/or fragment thereof is selected from the group comprising SEQ ID NO: 21, 3, 4, 13, 15, 17, 19, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 47, 48 and 49, variants and fragments thereof.

5. A diagnostic composition comprising at least one BLV.RBD ligand, a variant and/or a fragment thereof coupled with at least one contrast agent, and a pharmaceutically acceptable excipient.

6. A BLV.RBD ligand, a variant and/or a fragment thereof, for use in the in vivo diagnosis of a CAT1-related disease, preferably by medical imaging.

7. A BLV.RBD ligand, a variant and/or a fragment thereof, for use in the treatment of a CAT1-related disease.

8. The BLV.RBD ligand, a variant and/or a fragment thereof, for use according to claim 6 or 7, wherein said CAT1-related disease is selected from the group comprising arginine-related diseases, lysine-related diseases, histidine-related
diseases, ornithine-related diseases, nitric oxide-related diseases, and inflammatory diseases.

9. A BLV.RBD ligand, a variant and/or a fragment thereof, for use in the treatment of a BLV infection.

10. A pharmaceutical composition comprising at least one BLV.RBD ligand, a variant and/or a fragment thereof for use according to any one of claims 7 to 9, and a pharmaceutically acceptable excipient.

11. A medicament comprising at least one BLV.RBD ligand, a variant and/or a fragment thereof for use according to any one of claims 7 to 9.

12. The BLV.RBD ligand for use according to any one of claims 6 to 9, the diagnostic composition according to claim 5, the pharmaceutical composition according to claim 10, the medicament according to claim 11, wherein said BLV.RBD ligand, variant or fragment thereof comprises SEQ ID NO: 21, 3, 4, 13, 15, 17, 19, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 47, 48 and/or 49, a variant and/or a fragment thereof.
L-Arginine uptake

pmol of Arg/mg of protein/30min

Empty vector  BLV-RBD  HTLV2-RBD

**

Glutamine uptake

pmol of Gln/mg of protein/30min

Empty vector  BLV-RBD  HTLV2-RBD

FIG. 3
FIG. 4
**INTERNATIONAL SEARCH REPORT**

**PCT/EP2016/078163**

**A. CLASSIFICATION OF SUBJECT MATTER**

**INV. G01N33/68**

**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)

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

**C. DOCUMENTS CONSIDERED TO BE RELEVANT**

<table>
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<th>Category*</th>
<th>Citation of document, with indication, where appropriate, of the relevant passages</th>
<th>Relevant to claim No.</th>
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<td>X</td>
<td>wo 2012/035166 AI (CENTRE NAT RECH SCI ENT [FR] ; TI ROUVANZIAM RABINDRA [US] ; LAVAL JULI E []) 22 March 2012 (2012-03-22) (p 13, last para) (p 5, para 5) (p 7, last para) (p 9, last para) (p 4, para 's 3-5; p 15, 1 24-25) (p 14, 1 6-7) (p 9, last para) (p 14, 1 8-11) (p 7, last para); claim 19</td>
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<tr>
<td>X</td>
<td>EP 0 284 492 AI (RHONE MERI EUX [FR]) 28 September 1988 (1988-09-28) claim 20; figure 6</td>
<td>6-12</td>
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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

- **A** document member of the same patent family

Date of the actual completion of the international search: 20 January 2017

Date of mailing of the international search report: 31/03/2017

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: Bi got-Maucher, Cora
This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. □ Claims Nos.:
   because they relate to subject matter not required to be searched by this Authority, namely:

2. □ Claims Nos.:
   because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. □ Claims Nos.:
   because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

This International Searching Authority found multiple inventions in this international application, as follows:

see additional sheet

1. □ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. □ As all searchable claims could be searched without effort justifying an additional fees, this Authority did not invite payment of additional fees.

3. □ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. □ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

   l-12 (partially)

Remark on Protest

- □ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.

- □ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.

- □ No protest accompanied the payment of additional search fees.
Method for detecting the catonic L-aminoc acid transporter CAT1 in a cell using BLV.RBD, a diagnostic composition comprising BLV.RBD, a BLV.RBD for use in vivo diagnosis, a BLV.RBD for use in treatment of CAT1-related disease, BLV.RBD for use in the treatment of BLV infection, a pharmaceutical composition comprising BLV.RBD, a medicament comprising BLV.RBD, where in the BLV.RBD has the SEQ ID No. 21

Method for detecting the catonic L-aminoc acid transporter CAT1 in a cell using BLV.RBD, a diagnostic composition comprising BLV.RBD, a BLV.RBD for use in vivo diagnosis, a BLV.RBD for use in treatment of CAT1-related disease, BLV.RBD for use in the treatment of BLV infection, a pharmaceutical composition comprising BLV.RBD, a medicament comprising BLV.RBD, where in the BLV.RBD has the SEQ ID No. 3

Method for detecting the catonic L-aminoc acid transporter CAT1 in a cell using BLV.RBD, a diagnostic composition comprising BLV.RBD, a BLV.RBD for use in vivo diagnosis, a BLV.RBD for use in treatment of CAT1-related disease, BLV.RBD for use in the treatment of BLV infection, a pharmaceutical composition comprising BLV.RBD, a medicament comprising BLV.RBD, where in the BLV.RBD has the SEQ ID No. 4

Method for detecting the catonic L-aminoc acid transporter CAT1 in a cell using BLV.RBD, a diagnostic composition comprising BLV.RBD, a BLV.RBD for use in vivo diagnosis, a BLV.RBD for use in treatment of CAT1-related disease, BLV.RBD for use in the treatment of BLV infection, a pharmaceutical composition comprising BLV.RBD, a medicament comprising BLV.RBD, where in the BLV.RBD has the SEQ ID No. 13

Method for detecting the catonic L-aminoc acid transporter CAT1 in a cell using BLV.RBD, a diagnostic composition comprising BLV.RBD, a BLV.RBD for use in vivo diagnosis, a BLV.RBD for use in treatment of CAT1-related disease, BLV.RBD for use in the treatment of BLV infection, a pharmaceutical composition comprising BLV.RBD, a medicament
6. **Claims: l-12 (partially)**

Method for detecting the catonic L-aminobutyric acid transporter CAT1 in a cell using BLV.RBD, a diagnostic composition comprising BLV.RBD, a BLV.RBD for use in vivo diagnosis, a BLV.RBD for use in treatment of CAT1 related disease, BLV.RBD for use in the treatment of BLV infection, a pharmaceutical composition comprising BLV.RBD, a medicament comprising BLV.RBD wherein the BLV.RBD has the SEQ ID No. 15

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7. **Claims: l-12 (partially)**

Method for detecting the catonic L-aminobutyric acid transporter CAT1 in a cell using BLV.RBD, a diagnostic composition comprising BLV.RBD, a BLV.RBD for use in vivo diagnosis, a BLV.RBD for use in treatment of CAT1 related disease, BLV.RBD for use in the treatment of BLV infection, a pharmaceutical composition comprising BLV.RBD, a medicament comprising BLV.RBD wherein the BLV.RBD has the SEQ ID No. 17

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8. **Claims: l-12 (partially)**

Method for detecting the catonic L-aminobutyric acid transporter CAT1 in a cell using BLV.RBD, a diagnostic composition comprising BLV.RBD, a BLV.RBD for use in vivo diagnosis, a BLV.RBD for use in treatment of CAT1 related disease, BLV.RBD for use in the treatment of BLV infection, a pharmaceutical composition comprising BLV.RBD, a medicament comprising BLV.RBD wherein the BLV.RBD has the SEQ ID No. 19

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9. **Claims: l-12 (partially)**

Method for detecting the catonic L-aminobutyric acid transporter CAT1 in a cell using BLV.RBD, a diagnostic composition comprising BLV.RBD, a BLV.RBD for use in vivo diagnosis, a BLV.RBD for use in treatment of CAT1 related disease, BLV.RBD for use in the treatment of BLV infection, a pharmaceutical composition comprising BLV.RBD, a medicament comprising BLV.RBD wherein the BLV.RBD has the SEQ ID No. 21

---

10. **Claims: l-12 (partially)**

Method for detecting the catonic L-aminobutyric acid transporter CAT1 in a cell using BLV.RBD, a diagnostic composition comprising BLV.RBD, a BLV.RBD for use in vivo diagnosis, a BLV.RBD for use in treatment of CAT1 related disease, BLV.RBD for use in the treatment of BLV infection, a pharmaceutical composition comprising BLV.RBD, a medicament
compri sing BLV.RBD wherei n the BLV.RBD has the SEQ ID No. 30

11. claims: l-12 (partially)

Method for detecting the cat onic L-ami no aci d transporter CAT1 in a cell usi ng BLV.RBD, a di a gnostic composition compri sing BLV.RBD, a BLV.RBD for use i n v i vo di a gnos i s, a BLV.RBD for use i n treatment of CAT1 rel ated di sease, BLV.RBD for use i n the treatment of BLV i nfect i on, a pharma ceuti cal composition compri sing BLV.RBD, a medi cament compri sing BLV.RBD wherei n the BLV.RBD has the SEQ ID No. 31

12. claims: l-12 (partially)

Method for detecting the cat onic L-ami no aci d transporter CAT1 in a cell usi ng BLV.RBD, a di a gnostic composition compri sing BLV.RBD, a BLV.RBD for use i n v i vo di a gnos i s, a BLV.RBD for use i n treatment of CAT1 rel ated di sease, BLV.RBD for use i n the treatment of BLV i nfect i on, a pharma ceuti cal composition compri sing BLV.RBD, a medi cament compri sing BLV.RBD wherei n the BLV.RBD has the SEQ ID No. 32

13. claims: l-12 (partially)

Method for detecting the cat onic L-ami no aci d transporter CAT1 in a cell usi ng BLV.RBD, a di a gnostic composition compri sing BLV.RBD, a BLV.RBD for use i n v i vo di a gnos i s, a BLV.RBD for use i n treatment of CAT1 rel ated di sease, BLV.RBD for use i n the treatment of BLV i nfect i on, a pharma ceuti cal composition compri sing BLV.RBD, a medi cament compri sing BLV.RBD wherei n the BLV.RBD has the SEQ ID No. 33

14. claims: l-12 (partially)

Method for detecting the cat onic L-ami no aci d transporter CAT1 in a cell usi ng BLV.RBD, a di a gnostic composition compri sing BLV.RBD, a BLV.RBD for use i n v i vo di a gnos i s, a BLV.RBD for use i n treatment of CAT1 rel ated di sease, BLV.RBD for use i n the treatment of BLV i nfect i on, a pharma ceuti cal composition compri sing BLV.RBD, a medi cament compri sing BLV.RBD wherei n the BLV.RBD has the SEQ ID No. 34

15. claims: l-12 (partially)

Method for detecting the cat onic L-ami no aci d transporter CAT1 in a cell usi ng BLV.RBD, a di a gnostic composition compri sing BLV.RBD, a BLV.RBD for use i n v i vo di a gnos i s, a BLV.RBD for use i n treatment of CAT1 rel ated di sease, BLV.RBD for use i n the treatment of BLV i nfect i on, a pharma ceuti cal composition compri sing BLV.RBD, a medi cament
compri sing BLV. RBD wherei n the BLV. RBD has the SEQ ID No. 35

16. claims: I-12 (partially)

Method for detecting the catonc L-ami no aci d transporter
CAT1 in a cell using BLV. RBD, a diagnosti c composition
compri sing BLV. RBD, a BLV. RBD for use in vivo diagnosis,
a BLV. RBD for use in treatment of CAT1 related disease,
BLV. RBD for use in the treatment of BLV infection, a
pharmaceutical composition compri sing BLV. RBD, a medicament
compri sing BLV. RBD wherei n the BLV. RBD has the SEQ ID No. 36

17. claims: I-12 (partially)

Method for detecting the catonc L-ami no aci d transporter
CAT1 in a cell using BLV. RBD, a diagnosti c composition
compri sing BLV. RBD, a BLV. RBD for use in vivo diagnosis,
a BLV. RBD for use in treatment of CAT1 related disease,
BLV. RBD for use in the treatment of BLV infection, a
pharmaceutical composition compri sing BLV. RBD, a medicament
compri sing BLV. RBD wherei n the BLV. RBD has the SEQ ID No. 37

18. claims: I-12 (partially)

Method for detecting the catonc L-ami no aci d transporter
CAT1 in a cell using BLV. RBD, a diagnosti c composition
compri sing BLV. RBD, a BLV. RBD for use in vivo diagnosis,
a BLV. RBD for use in treatment of CAT1 related disease,
BLV. RBD for use in the treatment of BLV infection, a
pharmaceutical composition compri sing BLV. RBD, a medicament
compri sing BLV. RBD wherei n the BLV. RBD has the SEQ ID No. 38

19. claims: I-12 (partially)

Method for detecting the catonc L-ami no aci d transporter
CAT1 in a cell using BLV. RBD, a diagnosti c composition
compri sing BLV. RBD, a BLV. RBD for use in vivo diagnosis,
a BLV. RBD for use in treatment of CAT1 related disease,
BLV. RBD for use in the treatment of BLV infection, a
pharmaceutical composition compri sing BLV. RBD, a medicament
compri sing BLV. RBD wherei n the BLV. RBD has the SEQ ID No. 39

20. claims: I-12 (partially)

Method for detecting the catonc L-ami no aci d transporter
CAT1 in a cell using BLV. RBD, a diagnosti c composition
compri sing BLV. RBD, a BLV. RBD for use in vivo diagnosis,
a BLV. RBD for use in treatment of CAT1 related disease,
BLV. RBD for use in the treatment of BLV infection, a
pharmaceutical composition compri sing BLV. RBD, a medicament
21. claims: l-12 (partly)

Method for detecting the catonic L-amino acid transporter CAT1 in a cell using BLV.RBD, a diagnostic composition comprising BLV.RBD, a BLV.RBD for use in vivo diagnosis, a BLV.RBD for use in treatment of CAT1 related disease, BLV.RBD for use in the treatment of BLV infection, a pharmaceutical composition comprising BLV.RBD, a medicament comprising BLV.RBD where n the BLV.RBD has the SEQ ID No. 40

22. claims: l-12 (partly)

Method for detecting the catonic L-amino acid transporter CAT1 in a cell using BLV.RBD, a diagnostic composition comprising BLV.RBD, a BLV.RBD for use in vivo diagnosis, a BLV.RBD for use in treatment of CAT1 related disease, BLV.RBD for use in the treatment of BLV infection, a pharmaceutical composition comprising BLV.RBD, a medicament comprising BLV.RBD where n the BLV.RBD has the SEQ ID No. 41

23. claims: l-12 (partly)

Method for detecting the catonic L-amino acid transporter CAT1 in a cell using BLV.RBD, a diagnostic composition comprising BLV.RBD, a BLV.RBD for use in vivo diagnosis, a BLV.RBD for use in treatment of CAT1 related disease, BLV.RBD for use in the treatment of BLV infection, a pharmaceutical composition comprising BLV.RBD, a medicament comprising BLV.RBD where n the BLV.RBD has the SEQ ID No. 42

24. claims: l-12 (partly)

Method for detecting the catonic L-amino acid transporter CAT1 in a cell using BLV.RBD, a diagnostic composition comprising BLV.RBD, a BLV.RBD for use in vivo diagnosis, a BLV.RBD for use in treatment of CAT1 related disease, BLV.RBD for use in the treatment of BLV infection, a pharmaceutical composition comprising BLV.RBD, a medicament comprising BLV.RBD where n the BLV.RBD has the SEQ ID No. 43

25. claims: l-12 (partly)

Method for detecting the catonic L-amino acid transporter CAT1 in a cell using BLV.RBD, a diagnostic composition comprising BLV.RBD, a BLV.RBD for use in vivo diagnosis, a BLV.RBD for use in treatment of CAT1 related disease, BLV.RBD for use in the treatment of BLV infection, a pharmaceutical composition comprising BLV.RBD, a medicament comprising...
compri sing BLV.RBD wherei n the BLV.RBD has the SEQ ID No. 45

26. claims: l-12 (partially)

Method for detecti ng the cati on compri sing BLV.RBD, a medicament componi ng BLV.RBD wherei n the BLV.RBD has the SEQ ID No. 47

27. claims: l-12 (partially)

Method for detecti ng the cati on compri sing BLV.RBD, a medicament componi ng BLV.RBD wherei n the BLV.RBD has the SEQ ID No. 48

28. claims: l-12 (partially)

Method for detecti ng the cati on compri sing BLV.RBD, a medicament componi ng BLV.RBD wherei n the BLV.RBD has the SEQ ID No. 49
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