



(43) International Publication Date
13 September 2012 (13.09.2012)

WIPO | PCT

(10) International Publication Number
WO 2012/120129 A1

(51) International Patent Classification:

A61K 38/17 (2006.01) *C12Q 1/68* (2006.01)
G01N 33/569 (2006.01) *A61P 31/00* (2006.01)

(21) International Application Number:

PCT/EP2012/054152

(22) International Filing Date:

9 March 2012 (09.03.2012)

(25) Filing Language:

English

(26) Publication Language:

English

(30) Priority Data:

11305259.1 10 March 2011 (10.03.2011) EP

(71) Applicant (for all designated States except US): **INSERM (INSTITUT NATIONAL DE LA SANTÉ ET DE LA RECHERCHE MÉDICALE)** [FR/FR]; 101 rue de Tolbiac, F-75013 Paris (FR).

(72) Inventors; and

(75) Inventors/Applicants (for US only): **ESTAQUIER, Jérôme** [FR/FR]; Unité Inserm 955, Hôpital Henri Mondor, 51 avenue du mal de Lattre de Tassigny, F-94010 Creteil (FR). **LAFORGE, Mireille** [FR/FR]; Unité Inserm 955, Hôpital Henri Mondor, 51 avenue du Mal de Lattre de Tassigny, F-94010 Creteil (FR).

(74) Agent: **HIRSCH, Denise**; 7 rue Watt, F-75013 Paris (FR).

(81) Designated States (unless otherwise indicated, for every kind of national protection available): AE, AG, AL, AM, AO, AT, AU, AZ, BA, BB, BG, BH, BR, BW, BY, BZ, CA, CH, CL, CN, CO, CR, CU, CZ, DE, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IS, JP, KE, KG, KM, KN, KP, KR, KZ, LA, LC, LK, LR, LS, LT, LU, LY, MA, MD, ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PE, PG, PH, PL, PT, QA, RO, RS, RU, RW, SC, SD, SE, SG, SK, SL, SM, ST, SV, SY, TH, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW.

(84) Designated States (unless otherwise indicated, for every kind of regional protection available): ARIPO (BW, GH, GM, KE, LR, LS, MW, MZ, NA, RW, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European (AL, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV, MC, MK, MT, NL, NO, PL, PT, RO, RS, SE, SI, SK, SM, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

Published:

- with international search report (Art. 21(3))
- with sequence listing part of description (Rule 5.2(a))

(54) Title: METHODS AND PHARMACEUTICAL COMPOSITION FOR THE TREATMENT OF INFECTIOUS DISEASES

(57) Abstract: The present invention relates to DRAM polypeptide for use in the treatment of infectious diseases. The invention also relates to a pharmaceutical composition for use in the treatment of infectious diseases comprising a therapeutically effective amount of DRAM polypeptide according to the invention, or a nucleic acid according to the invention, or a plasmid according to the invention, or an expression vector according to the invention, or a fusion protein according to the invention along with at least one pharmaceutically acceptable excipient. The invention also relates to an activator of DRAM gene expression for use in the treatment of infectious diseases. Finally, the invention relates to a pharmaceutical composition for use in the treatment of infectious diseases comprising a therapeutically effective amount of an activator of DRAM gene expression.



WO 2012/120129 A1

METHODS AND PHARMACEUTICAL COMPOSITION FOR THE TREATMENT OF INFECTIOUS DISEASES

FIELD OF THE INVENTION:

The present invention relates to DRAM polypeptide or activator of DRAM gene expression for use in the treatment of infectious diseases.

BACKGROUND OF THE INVENTION:

Several proteolytic machineries are involved in programmed cell death and, over the last 10 years, lysosomal membrane permeabilization (LMP) and mitochondrial outer membrane permeabilization (MOMP) have been identified as major events triggering programmed cell death. Thus, in a number of cell death models, lysosomal destabilization and the ensuing efflux of cathepsins play early and important roles in cell destruction. This process is partly mediated by the activation of a caspase-dependent pathway causing the proteolytic activation of Bid and Bax, resulting in cytochrome c release and MOMP. LMP may also be a lethal event in itself, because the ectopic production of lysosomal proteases in the cytosol leads to the digestion of vital proteins and results in cell death, independently of caspase activation [Bidere N. et al., 2003]. By analogy to apoptosis, this process may be named Lysoptosis. It has been suggested that the tumor suppressor p53 can also trigger a primary lysosomal destabilization that contributes to cell death.

Activation of p53 and induction of its proapoptotic target genes in virally infected cells have been considered as an altruistic suicide mechanism that limits viral infection. Thus, many viruses, including Simian Virus 40 (SV40), Human papilloma virus (HPV) and Adenoviruses (Ad), have evolved mechanisms designated to abrogate p53 responses [O'Shea CC et al., 2005] whereas active p53 was detected with several other types of virus, such as Vesicular stomatitis virus (VSV), Newcastle disease virus (NDV) [Takaoka A. et al., 2003], and Human immunodeficiency virus (HIV) [Corbeil J. et al., 2001]. We have demonstrated that, in CD4+ T cells productively infected with HIV-1, destined to die through a caspase-independent death pathway [Moutouh L; et al., 1998 and Petit F. et al., 2002], lysosomes undergo limited membrane permeabilization and lysosomal efflux of cathepsins to the cytosol

13. In this system, LMP is induced by both the X4 and R5 laboratory strains and by HIV-1 isolates from infected patients [Laforge M. et al., 2007]. Until now, anything has been known about the link between p53 and lysosomal destabilization in virally infected cells.

The importance of lysosomes is also highlighted by their ability to regulate the terminal steps of autophagy [Shintani T. et al., 2004; Levine B. et al., 2005 and Levine B. et al., 2007]. Interestingly, autophagy participates in the elimination of certain intracellular bacteria, such as invading group A streptococci, *Mycobacterium tuberculosis*, and *Shigella flexneri* [Nakagawa I. et al., 2004; Gutierrez MG et al., 2004; Ogawa M. et al., 2005 and Singh SB et al., 2006]. It has been suggested that autophagy is involved in the death of uninfected CD4⁺ T cells following the interaction of the HIV envelope glycoprotein and its co-receptor CXCR4 [Espert L. et al., 2006]. Given the importance of autophagy in host defense against intracellular pathogens, microbial virulence may be linked to the subversion of autophagy [Orvedahl A. et al., 2007]. The Damage-regulated autophagy modulator (DRAM), a lysosomal protein, has recently been reported to link p53 to autophagy [Crichton D. et al., 2006]. However, the question of the potential role of DRAM in the context of host cell/microbe interactions and cell death has never before been addressed.

Now, the inventors highlight the major role played by DRAM in the regulation of LMP and autophagy in HIV-infected CD4⁺ T cells downstream from p53 activation. The use of a specific siRNA to block DRAM protein expression inhibited LMP and resulted in the dramatic rescue of HIV-infected CD4⁺ T cells. The data may be consistent with the notion that DRAM is involved in the elimination of microbe-infected cells through ~~lyseptosis~~ lysosomal membrane destabilization and constitutes a critical aspect of antiviral immunity.

SUMMARY OF THE INVENTION:

The invention relates to the fact that DRAM or an activator of the gene expression of DRAM may be used for the treatment of infectious diseases.

Thus, the present invention relates to DRAM polypeptide for use in the treatment of infectious diseases.

The invention also relates to a pharmaceutical composition for use in the treatment of infectious diseases comprising a therapeutically effective amount of DRAM polypeptide according to the invention, or a nucleic acid according to the invention, or a plasmid according to the invention, or an expression vector according to the invention, or a fusion

protein according to the invention along with at least one pharmaceutically acceptable excipient.

The invention also relates to an activator of DRAM gene expression for use in the treatment of infectious diseases.

5 The invention also relates to a pharmaceutical composition for use in the treatment of infectious diseases comprising a therapeutically effective amount of an activator of DRAM gene expression.

10 Finally, the invention relates to an ex vivo method of diagnosing or predicting a faster progression of HIV infection in a subject, which method comprises detecting a polymorphism in the DRAM gene in a sample obtained from said subject, wherein the presence of said polymorphism is indicative of a faster progression of HIV infection.

DETAILED DESCRIPTION OF THE INVENTION:

15

Protein and uses thereof:

A first object of the invention relates to DRAM polypeptide for use in the treatment of infectious diseases.

20

As used herein, the term “DRAM” has its general meaning in the art and refers to the Damage-Regulated Autophagy Modulator that is a lysosomal protein. The amino acid sequence of DRAM is described as the amino acid sequence SEQ ID NO 1 (number sequence in UniProtKB/Swiss-Prot: Q8N682).

25

In a preferred embodiment, the DRAM polypeptide may be DRAM itself or an active fragment thereof.

30 As used herein, the term “an active fragment” denotes a fragment of a protein that retains the activity of the complete protein. For example, an active fragment of DRAM denotes a fragment of the protein, which conserves the capacity to have lysosomal activity.

In a preferred embodiment, said active fragment of DRAM comprises at least 75% identity over said DRAM, even more preferably at least 80%, at least 85%, at least 90%, at least 95%, at least 97%, at least 99%.

In a particular embodiment, DRAM polypeptides according to the invention may be fused to another polypeptide.

For example, it is often advantageous to include one or more additional amino acid sequences which may contain secretory or leader sequences, pro-sequences, sequences which aid in purification, for example an histidine tag, or sequences that confer higher protein stability, for example during recombinant production.

Alternatively or additionally, the DRAM polypeptide may be fused with another polypeptide which comprises or consists of a sequence which allows transduction in the cell cytoplasm preferably without toxicity, like a protein transduction domain (PTD), a cell penetrating peptide, or a cell targeting peptide (in particular a breast, ovarian, bladder, colorectal or gastric cancer cell targeting peptide). Examples of protein transduction domains or cell penetrating peptides are the HIV TAT transduction domain, the Antennapedia homeodomain (Antp) protein from *Drosophila* and in particular the penetratin peptide, the VP22 protein from herpes simplex virus, transportan (Pooga et al. (2001) FASEB J. 15: 1451-1453.), FGF-4, MPG (Rothe et al. (2008) Curr Protoc Protein Sci 18: 18.11), a polyarginine peptide (Matsui et al. (2003) Nippon Yakurigaku Zasshi. 121: 435-439) (Michiue et al. (2005) J Biol Chem 280(9):8285-9) and a polyhistidine peptide (Ranki et al. (2007) Gene Ther. 14(1): 58-67), and a polyarginine peptide.

More preferably, the protein transduction domain (PDT), the cell penetrating peptide or the cell targeting peptide is the HIV TAT protein transduction domain, penetratin, a V1 or DV3 peptide derived from the viral chemokine vMIP-II and a polyarginine peptide.

The fusion of the two polypeptides can be realized at the carboxy-terminal or the amino-terminal end of each of them.

As intended herein the "amino-terminal end" of a polypeptide refers to the start of polypeptide terminated by an amino acid with a free amine group (-NH₂). The carboxy-terminal end of a polypeptide refers to the end of the amino acid chain terminated by a free carboxyl group (-COOH).

So, in a preferred embodiment, the DRAM polypeptide according to the invention is linked to a polypeptide consisting of a protein transduction domain, a cell penetrating peptide or a cell targeting peptide.

DRAM polypeptide may be produced by any technique known per se in the art, such as, without limitation, any chemical, biological, genetic or enzymatic technique, either alone or in combination(s).

Knowing the amino acid sequence of the desired sequence, one skilled in the art can readily produce a relevant part of the DRAM polypeptide, by standard techniques for production of proteins. For instance, they can be synthesized using well-known solid phase method, preferably using a commercially available protein synthesis apparatus (such as that made by Applied Biosystems, Foster City, California) and following the manufacturer's instructions.

Alternatively, DRAM polypeptide can be synthesized by recombinant DNA techniques as is now well known in the art. For example, these fragments can be obtained as DNA expression products after incorporation of DNA sequences encoding the desired polypeptide into expression vectors and introduction of such vectors into suitable eukaryotic or prokaryotic hosts that will express the desired protein or fragment of the protein, from which they can be later using well-known techniques.

DRAM polypeptide can be used in a vector, such as a membrane or lipid vesicle (e.g. a liposome).

In specific embodiments, it is contemplated that DRAM polypeptide used in the therapeutic methods of the present invention may be modified in order to improve their therapeutic efficacy. Such modification of therapeutic compounds may be used to decrease toxicity, increase circulatory time, or modify biodistribution. For example, the toxicity of potentially important therapeutic compounds can be decreased significantly by combination with a variety of drug carrier vehicles that modify biodistribution. In example adding dipeptides can improve the penetration of a circulating agent in the eye through the blood retinal barrier by using endogenous transporters.

A strategy for improving drug viability is the utilization of water-soluble polymers. Various water-soluble polymers have been shown to modify biodistribution, improve the mode of cellular uptake, change the permeability through physiological barriers; and modify the rate of clearance from the body. To achieve either a targeting or sustained-release effect, water-soluble polymers have been synthesized that contain drug moieties as terminal groups, as part of the backbone, or as pendent groups on the polymer chain.

Polyethylene glycol (PEG) has been widely used as a drug carrier, given its high degree of biocompatibility and ease of modification. Attachment to various drugs, proteins,

and liposomes has been shown to improve residence time and decrease toxicity. PEG can be coupled to active agents through the hydroxyl groups at the ends of the chain and via other chemical methods; however, PEG itself is limited to at most two active agents per molecule. In a different approach, copolymers of PEG and amino acids were explored as novel biomaterials which would retain the biocompatibility properties of PEG, but which would have the added advantage of numerous attachment points per molecule (providing greater drug loading), and which could be synthetically designed to suit a variety of applications.

Those of skill in the art are aware of PEGylation techniques for the effective modification of drugs. For example, drug delivery polymers that consist of alternating polymers of PEG and tri-functional monomers such as lysine, have been used by VectraMed (Plainsboro, N.J.). The PEG chains (typically 2000 daltons or less) are linked to the α - and ϵ -amino groups of lysine through stable urethane linkages. Such copolymers retain the desirable properties of PEG, while providing reactive pendent groups (the carboxylic acid groups of lysine) at strictly controlled and predetermined intervals along the polymer chain. The reactive pendent groups can be used for derivatization, cross-linking, or conjugation with other molecules. These polymers are useful in producing stable, long-circulating pro-drugs by varying the molecular weight of the polymer, the molecular weight of the PEG segments, and the cleavable linkage between the drug and the polymer. The molecular weight of the PEG segments affects the spacing of the drug/linking group complex and the amount of drug per molecular weight of conjugate (smaller PEG segments provides greater drug loading). In general, increasing the overall molecular weight of the block co-polymer conjugate will increase the circulatory half-life of the conjugate. Nevertheless, the conjugate must either be readily degradable or have a molecular weight below the threshold-limiting glomerular filtration (e.g., less than 45 kDa).

In addition, to the polymer backbone being important in maintaining circulatory half-life, and biodistribution, linkers may be used to maintain the therapeutic agent in a pro-drug form until released from the backbone polymer by a specific trigger, typically enzyme activity in the targeted tissue. For example, this type of tissue activated drug delivery is particularly useful where delivery to a specific site of biodistribution is required and the therapeutic agent is released at or near the site of pathology. Linking group libraries for use in activated drug delivery are known to those of skill in the art and may be based on enzyme kinetics, prevalence of active enzyme, and cleavage specificity of the selected disease-specific enzymes. Such linkers may be used in modifying the protein or fragment of the protein described herein for therapeutic delivery.

In a preferred embodiment, infectious diseases are selected from infections caused by a virus, infections caused by a bacterium, infection cause by a parasite, or infections caused by a fungus.

5 In a preferred embodiment, infections caused by a virus are selected from HIV, HCV, HBV, HPV, VSV, NDV, Influenza A.

In a preferred embodiment, infections caused by a bacterium are selected from Sphingomonas paucimobilis, Neisseria Burkholderia, Pseudomallei Brucella, Rhodococcus equi, Francisella tularensis, Anaplasma phagocytophilum, Mycobacterium lepraemurium, 10 Mycobacterium tuberculosis, Mycobacterium marinum, Yersinia pestisCoxiella burnetti, Salmonella enterica, Legionella pneumophila, Listeria monocytogenes, Leishmania spp. Leishmania amazonensis. Trypanosoma cruzi Toxoplasma gondii, Candida albicans

In a preferred embodiment, infections caused by a fungus are selected from Cryptococcus neoformans, Candida, Aspergillus, Cryptococcus, Histoplasma, Pneumocystis, 15 Stachybotrys.

As used herein, the terms "treating" or "treatment", denotes reversing, alleviating, inhibiting the progress of, or preventing the disorder or condition to which such term applies, or one or more symptoms of such a disorder or condition.

20 According to the invention, the term "patient" or "individual" to be treated is intended for a human or non-human mammal (such as a rodent (mouse, rat), a feline, a canine, or a primate) affected or likely to be affected with vision defects. Preferably, the subject is a human.

25

Nucleic acids, vectors, recombinant host cells and uses thereof

A second aspect of the invention relates to a nucleic acid molecule encoding DRAM polypeptide for use in the treatment of infectious diseases.

30

A "coding sequence" or a sequence "encoding" an expression product, such as a RNA, polypeptide, protein, or enzyme, is a nucleotide sequence that, when expressed, results in the production of that RNA, polypeptide, protein, or enzyme, i.e., the nucleotide sequence

encodes an amino acid sequence for that polypeptide, protein or enzyme. A coding sequence for a protein may include a start codon (usually ATG) and a stop codon.

These nucleic acid molecules may be obtained by conventional methods well known to those skilled in the art, in particular by site-directed mutagenesis of the gene encoding the native protein. Typically, said nucleic acid is a DNA or RNA molecule, which may be included in a suitable vector, such as a plasmid, cosmid, episome, artificial chromosome, phage or viral vector.

So, a further object of the present invention relates to a vector and an expression cassette in which a nucleic acid molecule of the invention is associated with suitable elements for controlling transcription (in particular promoter, enhancer and, optionally, terminator) and, optionally translation, and also the recombinant vectors into which a nucleic acid molecule in accordance with the invention is inserted. These recombinant vectors may, for example, be cloning vectors, or expression vectors.

The terms "vector", "cloning vector" and "expression vector" mean the vehicle by which a DNA or RNA sequence (e.g. a foreign gene) may be introduced into a host cell, so as to transform the host and promote expression (e.g. transcription and translation) of the introduced sequence.

Any expression vector for animal cell may be used, as long as a gene encoding a polypeptide or chimeric derivative of the invention can be inserted and expressed. Examples of suitable vectors include pAGE107, pAGE103, pHSG274, pKCR, pSG1 beta d2-4) and the like.

Other examples of plasmids include replicating plasmids comprising an origin of replication, or integrative plasmids, such as for instance pUC, pcDNA, pBR, and the like.

Other examples of viral vector include adenoviral, retroviral, herpes virus and AAV vectors. Such recombinant viruses may be produced by techniques known in the art, such as by transfecting packaging cells or by transient transfection with helper plasmids or viruses. Typical examples of virus packaging cells include PA317 cells, PsiCRIP cells, GPenv+ cells, 293 cells, etc. Detailed protocols for producing such replication-defective recombinant viruses may be found for instance in WO 95/14785, WO 96/22378, US 5,882,877, US 6,013,516, US 4,861,719, US 5,278,056 and WO 94/19478.

Examples of promoters and enhancers used in the expression vector for animal cell include early promoter and enhancer of SV40, LTR promoter and enhancer of Moloney mouse leukemia virus, promoter and enhancer of immunoglobulin H chain and the like.

The invention also includes gene delivery systems comprising a nucleic acid molecule of the invention, which can be used in gene therapy in vivo or ex vivo. This includes for instance viral transfer vectors such as those derived from retrovirus, adenovirus, adeno associated virus, lentivirus, which are conventionally used in gene therapy. This also includes
5 gene delivery systems comprising a nucleic acid molecule of the invention and a non-viral gene delivery vehicle. Examples of non viral gene delivery vehicles include liposomes and polymers such as polyethylenimines, cyclodextrins, histidine/lysine (HK) polymers, etc.

Another object of the invention is also a prokaryotic or eukaryotic host cell genetically transformed with at least one nucleic acid molecule according to the invention.

10 The term "transformation" means the introduction of a "foreign" (i.e. extrinsic or extracellular) gene, DNA or RNA sequence to a host cell, so that the host cell will express the introduced gene or sequence to produce a desired substance, typically a protein or enzyme coded by the introduced gene or sequence. A host cell that receives and expresses introduced DNA or RNA has been "transformed".

15 Preferably, for expressing and producing the proteins, and in particular DRAM polypeptide, eukaryotic cells, in particular mammalian cells, and more particularly human cells, will be chosen.

Typically, cell lines such as CHO, BHK-21, COS-7, C127, PER.C6 or HEK293
20 derivatives.

The construction of expression vectors in accordance with the invention, the transformation of the host cells can be carried out using conventional molecular biology techniques. The V-ATPase c-subunit derivatives of the invention, can, for example, be obtained by culturing genetically transformed cells in accordance with the invention and
25 recovering the derivative expressed by said cell, from the culture. They may then, if necessary, be purified by conventional procedures, known in themselves to those skilled in the art, for example by fractionated precipitation, in particular ammonium sulphate precipitation, electrophoresis, gel filtration, affinity chromatography, etc.

In particular, conventional methods for preparing and purifying recombinant proteins
30 may be used for producing the proteins in accordance with the invention.

In another embodiment, the invention relates to a plasmid comprising a nucleic acid sequence according to the invention for use in the treatment of infectious diseases.

In still another embodiment, the invention relates to an expression vector containing a nucleic acid sequence according to the invention for use in the treatment of infectious diseases.

5

Activator of DRAM gene expression

A third object of the invention relates to an activator of DRAM gene expression for use in the treatment of infectious diseases.

10

In one embodiment, said activator of DRAM gene expression may be a low molecular weight agonist, e. g. a small organic molecule (natural or not).

15

The term "small organic molecule" refers to a molecule (natural or not) of a size comparable to those organic molecules generally used in pharmaceuticals. The term excludes biological macromolecules (e. g., proteins, nucleic acids, etc.). Preferred small organic molecules range in size up to about 10000 Da, more preferably up to 5000 Da, more preferably up to 2000 Da and most preferably up to about 1000 Da.

20

Pharmaceutical compositions

25

A fourth object of this invention is a pharmaceutical composition, which includes a therapeutically effective amount of at least DRAM polypeptide according to the invention, along with at least one pharmaceutically acceptable excipient. Alternatively, the pharmaceutical composition of the invention may contain a therapeutically effective amount of a nucleic acid according to the invention or a plasmid or a vector that contains at least one nucleic acid sequence that codes for DRAM polypeptide according to the invention, along with at least one adjuvant and/or a pharmaceutically acceptable excipient. Said vector may be used in gene therapy.

30

Alternatively, the pharmaceutical composition of the invention may contain a therapeutically effective amount of a fusion protein according to the invention, along with at least one pharmaceutically acceptable excipient.

Alternatively, the pharmaceutical composition of the invention may contain a therapeutically effective amount of an activator of DRAM gene expression, along with at least one pharmaceutically acceptable excipient.

By a "therapeutically effective amount" is meant a sufficient amount of the chimeric derivative of the invention to treat a disease associated with retinal degenerative disorder at a reasonable benefit/risk ratio applicable to any medical treatment.

5

It will be understood that the total daily dosage of the compounds and compositions of the present invention will be decided by the attending physician within the scope of sound medical judgment. The specific therapeutically effective dose level for any particular patient will depend upon a variety of factors including the disorder being treated and the severity of the disorder; activity of the specific compound employed; the specific composition employed, the age, body weight, general health, sex and diet of the patient; the time of administration, route of administration, and rate of excretion of the specific compound employed; the duration of the treatment; drugs used in combination or coincidental with the specific polypeptide employed; and like factors well known in the medical arts. For example, it is well within the skill of the art to start doses of the compound at levels lower than those required to achieve the desired therapeutic effect and to gradually increase the dosage until the desired effect is achieved. However, the daily dosage of the products may be varied over a wide range from 0.01 to 1,000 mg per adult per day. Preferably, the compositions contain 0.01, 0.05, 0.1, 0.5, 1.0, 2.5, 5.0, 10.0, 15.0, 25.0, 50.0, 100, 250 and 500 mg of the active ingredient for the symptomatic adjustment of the dosage to the patient to be treated. A medicament typically contains from about 0.01 mg to about 500 mg of the active ingredient, preferably from 1 mg to about 100 mg of the active ingredient. An effective amount of the drug is ordinarily supplied at a dosage level from 0.0002 mg/kg to about 20 mg/kg of body weight per day, especially from about 0.001 mg/kg to 7 mg/kg of body weight per day.

25 The active products of the invention (proteins or vectors) may be administered for the treatment of infectious diseases.

The therapeutically effective amount of the active product of the invention [proteins or vectors (constructions)] that should be administered, as well as the dosage for the treatment of a pathological condition with the proteins and/or pharmaceutical compositions of the invention, will depend on numerous factors, including the age and condition of the patient, the severity of the disturbance or disorder, the method and frequency of administration and the particular peptide to be used.

30 The presentation of the pharmaceutical compositions that contain the proteins or vectors (constructions) of the invention may be in any form that is suitable for administration,

e.g., solid, liquid or semi-solid, such as creams, ointments, gels or solutions, and these compositions may be administered by any suitable means, for example, orally, parenterally, inhalation or topically, so they will include the pharmaceutically acceptable excipients necessary to make up the desired form of administration. A review of the different pharmaceutical forms for administering medicines and of the excipients necessary for obtaining same may be found, for example, in the "Tratado de Farmacia Galénica" (Treatise on Galenic Pharmacy), C. Fauli Trillo, 1993, Luzán 5, S.A. Ediciones, Madrid.

In the pharmaceutical compositions of the present invention for oral, sublingual, subcutaneous, intramuscular, intravenous, transdermal, local, pulmonary, eye drop, intraocular or rectal administration, the active principle, alone or in combination with another active principle, can be administered in a unit administration form, as a mixture with conventional pharmaceutical supports, to animals and human beings. Suitable unit administration forms comprise oral route forms such as tablets, gel capsules, powders, granules and oral suspensions or solutions, sublingual and buccal administration forms, aerosols, implants, subcutaneous, transdermal, topical, intraperitoneal, intramuscular, intravenous, subdermal, transdermal, intrathecal and intranasal administration forms, intraocular and rectal administration forms.

Preferably, the pharmaceutical compositions contain vehicles, which are pharmaceutically acceptable for a formulation capable of being injected. These may be in particular isotonic, sterile, saline solutions (monosodium or disodium phosphate, sodium, potassium, calcium or magnesium chloride and the like or mixtures of such salts), or dry, especially freeze-dried compositions which upon addition, depending on the case, of sterilized water or physiological saline, permit the constitution of injectable solutions.

The pharmaceutical forms suitable for injectable use include sterile aqueous solutions or dispersions; formulations including sesame oil, peanut oil or aqueous propylene glycol; and sterile powders for the extemporaneous preparation of sterile injectable solutions or dispersions. In all cases, the form must be sterile and must be fluid to the extent that easy syringability exists. It must be stable under the conditions of manufacture and storage and must be preserved against the contaminating action of microorganisms, such as bacteria and fungi.

Solutions comprising compounds of the invention as free base or pharmacologically acceptable salts can be prepared in water suitably mixed with a surfactant, such as hydroxypropylcellulose. Dispersions can also be prepared in glycerol, liquid polyethylene

glycols, and mixtures thereof and in oils. Under ordinary conditions of storage and use, these preparations contain a preservative to prevent the growth of microorganisms.

The DRAM polypeptide or the fusion protein according to the invention can be formulated into a composition in a neutral or salt form. Pharmaceutically acceptable salts include the acid addition salts (formed with the free amino groups of the protein) and which are formed with inorganic acids such as, for example, hydrochloric or phosphoric acids, or such organic acids as acetic, oxalic, tartaric, mandelic, and the like. Salts formed with the free carboxyl groups can also be derived from inorganic bases such as, for example, sodium, potassium, ammonium, calcium, or ferric hydroxides, and such organic bases as isopropylamine, trimethylamine, histidine, procaine and the like.

The carrier can also be a solvent or dispersion medium containing, for example, water, ethanol, polyol (for example, glycerol, propylene glycol, and liquid polyethylene glycol, and the like), suitable mixtures thereof, and vegetable oils. The proper fluidity can be maintained, for example, by the use of a coating, such as lecithin, by the maintenance of the required particle size in the case of dispersion and by the use of surfactants. The prevention of the action of microorganisms can be brought about by various antibacterial and antifungal agents, for example, parabens, chlorobutanol, phenol, sorbic acid, thimerosal, and the like. In many cases, it will be preferable to include isotonic agents, for example, sugars or sodium chloride. Prolonged absorption of the injectable compositions can be brought about by the use in the compositions of agents delaying absorption, for example, aluminium monostearate and gelatin.

Sterile injectable solutions are prepared by incorporating the active polypeptides in the required amount in the appropriate solvent with several of the other ingredients enumerated above, as required, followed by filtered sterilization. Generally, dispersions are prepared by incorporating the various sterilized active ingredients into a sterile vehicle which contains the basic dispersion medium and the required other ingredients from those enumerated above. In the case of sterile powders for the preparation of sterile injectable solutions, the preferred methods of preparation are vacuum-drying and freeze-drying techniques which yield a powder of the active ingredient plus any additional desired ingredient from a previously sterile-filtered solution thereof.

Upon formulation, solutions will be administered in a manner compatible with the dosage formulation and in such amount as is therapeutically effective. The formulations are easily administered in a variety of dosage forms, such as the type of injectable solutions described above, but drug release capsules and the like can also be employed.

For parenteral administration in an aqueous solution, for example, the solution should be suitably buffered if necessary and the liquid diluent first rendered isotonic with sufficient saline or glucose. These particular aqueous solutions are especially suitable for intravenous, intramuscular, subcutaneous and intraperitoneal administration. In this connection, sterile aqueous media, which can be employed, will be known to those of skill in the art in light of the present disclosure. For example, one dosage could be dissolved in 1 ml of isotonic NaCl solution and either added to 1000 ml of hypodermoclysis fluid or injected at the proposed site of infusion. Some variation in dosage will necessarily occur depending on the condition of the subject being treated. The person responsible for administration will, in any event, determine the appropriate dose for the individual subject.

The DRAM polypeptide or the fusion protein of the invention may be formulated as a therapeutic mixture to comprise about 0.0001 to 1.0 milligrams, or about 0.001 to 0.1 milligrams, or about 0.1 to 1.0 or even about 10 milligrams per dose or so. Multiple doses can also be administered.

In addition to the compounds of the invention formulated for parenteral administration, such as intravenous or intramuscular injection, other pharmaceutically acceptable forms include, e.g. tablets or other solids for oral administration; liposomal formulations; time-release capsules; and any other form currently used.

An additional object of this invention relates to the DRAM or the active fragment thereof or of vectors that contain at least one sequence that codes for DRAM or the active fragment thereof for the treatment of infectious diseases including but not limited to infections caused by a virus, infections caused by a bacterium, infection cause by a parasite, or infections caused by a fungus.

In addition, the invention provides a method for the treatment of infectious diseases in a patient which consists of administering to said mammal suffering from said pathological disease a therapeutically effective amount of at least the DRAM polypeptide, or of a vector containing at least one DNA sequence that codes for DRAM polypeptide, preferably in the form of a pharmaceutical composition that contains it.

Method of diagnosing and predicting

A fifth object of the invention is an ex vivo method of diagnosing or predicting a faster progression of HIV infection in a subject, which method comprises detecting a polymorphism in the DRAM gene in a sample obtained from said subject, wherein the presence of said polymorphism is indicative of a faster progression of HIV infection.

5

In a preferred embodiment the method of diagnosing or predicting a faster progression of HIV infection in a subject, which method comprises detecting the SNP rs17032033 (SEQ ID NO 6) or the SNP rs4764839 (SEQ ID NO 7) or the SNP rs12299074 (SEQ ID NO 8) in the DRAM gene in a sample obtained from said subject, wherein the presence of allele (T) in the SNP rs17032033 or in the SNP rs4764839 or in the SNP rs12299074 is indicative of a faster progression of HIV infection.

As used herein, the term "polymorphism" denotes a mutation in the normal sequence of a gene, which can be found in the exons, introns, or the coding region of the gene, or in the sequences that control expression of the gene. Complete gene sequencing often identifies numerous allelic variants (sometimes hundreds) for a given gene.

As used herein, the term "Allele" denotes an alternative form of a gene (one member of a pair) that is located at a specific position on a specific chromosome which, when translated result in functional or dysfunctional (including non-existent) gene products.

As used herein, the term "SNP" or "Single Nucleotide Polymorphism" denotes to a single nucleotide variation in a genetic sequence that occurs at appreciable frequency in the population. The single nucleotide variation can be a substitution but also an addition or a deletion. There are millions of SNPs in the human genome. Most commonly, these variations are found in the DNA between genes. When SNPs occur within a gene or in a regulatory region near a gene, they may play a more direct role in disease by affecting the gene function.

Preferably, the identification of the SNP of a patient is determined on a nucleic acid sample obtained from a biological sample from said patient.

The nucleic acid sample may be obtained from any cell source or tissue biopsy. Non-limiting examples of cell sources available include without limitation blood cells, buccal cells, epithelial cells, fibroblasts, or any cells present in a tissue obtained by biopsy. Cells may also

be obtained from body fluids, such as blood or lymph, etc. DNA may be extracted using any methods known in the art, such as described in Sambrook J. et al., 1989.

The SNP may be detected in the nucleic acid sample, preferably after amplification. For instance, the isolated DNA may be subjected to amplification by polymerase chain reaction (PCR), using oligonucleotide primers that are specific for one defined genotype or that enable amplification of a region containing the SNP of interest. According to a first alternative, conditions for primer annealing may be chosen to ensure specific reverse transcription (where appropriate) and amplification; so that the appearance of an amplification product be a diagnostic of the presence of a particular genotype. Otherwise, DNA may be amplified, after which the genotype is determined in the amplified sequence by hybridization with a suitable probe or by direct sequencing, or any other appropriate method known in the art.

As used herein, the terms “primer” (or “amplification primer”) and “probe” denote to oligonucleotides, which have different functions. A primer is typically extended by polymerase or ligation following hybridization to the target but a probe typically is not. A hybridized oligonucleotide may function as a probe if it is used to capture or detect a target sequence, and the same oligonucleotide may function as a primer when it is employed as a target binding sequence in an amplification primer.

Actually numerous strategies for SNP identification are available. For example, RFLP (restriction fragment length polymorphism) may be used. Briefly, the nucleic acid molecule may be tested for the presence or absence of a restriction site. When a base substitution creates or abolishes the recognition site of a restriction enzyme, this allows a simple direct PCR test for the SNP. Further strategies include, but are not limited to, direct sequencing, hybridization with allele-specific oligonucleotides (ASO) that are short synthetic probes which hybridize only to a perfectly matched sequence under suitably stringent hybridization conditions; allele-specific PCR; PCR using mutagenic primers; ligase-PCR, real-time quantitative PCR, or oligonucleotide ligation assay (OLA). OLA may be used for revealing SNPs. According to this method, two oligonucleotides are constructed that hybridize to adjacent sequences in the target nucleic acid, with the join sited at the position of the mutation. DNA ligase will covalently join the two oligonucleotides only if they are perfectly hybridized. Direct sequencing may be accomplished by any method, including without limitation enzymatic sequencing, using the Sanger method; mass spectrometry sequencing;

sequencing using a chip-based technology. Alternatively, scanning methods can be employed followed by one approach allowing the exact identification of base modification, as for example HOT cleavage; denaturing gradient gel electrophoresis (DGGE), temperature-denaturing gradient gel electrophoresis (TGGE), single-stranded conformational polymorphism (SSCP) and denaturing high performance liquid chromatography, high resolution melting (HRM).

Preferably, DNA from the patient is first subjected to amplification by polymerase chain reaction (PCR) using specific amplification primers. However several other methods are available, allowing DNA to be studied independently of PCR, such as the rolling circle amplification (RCA), the Invader™ assay. Moreover other amplification strategies allowing DNA amplification and PCR-free may also be suitable such as 3SR (Fahy E et al, 1991).

Using DNA chip technologies as those described in documents WO 2004106546 and WO 2006001627 may identify the SNPs of the invention.

Nucleic acids having at least 10 nucleotides and exhibiting sequence complementarity or homology to the sequence of interest herein finds utility as hybridization probes or amplification primers. It is understood that such nucleic acids need not be identical, but are typically at least about 80% identical to the homologous region of comparable size, more preferably 85% identical and even more preferably 90-95% identical. In certain embodiments, it will be advantageous to use nucleic acids in combination with appropriate means, such as a detectable label, for detecting hybridization. A wide variety of appropriate indicators are known in the art including, fluorescent, radioactive, and enzymatic or other ligands (e. g. avidin/biotin). Probes typically comprise single-stranded nucleic acids of between 10 to 1000 nucleotides in length, for instance of between 10 and 800, more preferably of between 15 and 700, typically of between 20 and 500.

Primers typically are shorter single-stranded nucleic acids, of between 10 to 25 nucleotides in length, designed to perfectly or almost perfectly match a nucleic acid of interest, to be amplified. The probes and primers are "specific" to the nucleic acids they hybridize to, i.e. they preferably hybridize under high stringency hybridization conditions (corresponding to the highest melting temperature T_m , e.g., 50 % formamide, 5x or 6x SCC. SCC is a 0.15 M NaCl, 0.015 M Na-citrate).

According to another aspect of the invention, the SNP of interest are detected by contacting the nucleic sample of the patient with a nucleic acid probe, which is optionally labelled. Primers may also be useful to amplify or sequence the portion of genes of the invention, e.g. DRAM, containing the SNP of interest.

The invention will be further illustrated by the following examples. However, these examples should not be interpreted in any way as limiting the scope of the present invention.

5

Table 1: Genetic association study.

The inventors indicated the name of the gene, the chromosome location (Chr), the number of genotyped SNPs, the SNPs significantly associated with the progression ($p < 0.05$), the genotype, the frequency in each population (LTNP: long-term non-progressor, CTR: control, RP: rapid progressor), the p values and the potential correlation with the Genevar and Dixon mRNA expression databases when significant.

Gene	Chr	Nb of d SNPs	SNP associated to progression	Genotype	LTNP	CTR	RP	p value LTNP vs CTR	p value RP vs CTR	Genevar	Dixon
DRAM	12	15	rs17032033	CC	1,87%	1,01%	7,14%	-	1,96E-3	-	-
			rs4764839	CC	2,22%	0,86%	5,95%	-	1,03E-2	-	-
			rs12299074	CC	6,30%	4,03%	11,90%	-	1,08E-2	-	$p = 6.8 \times 10^{-7}$ (decreased DRAM expression)

15

EXAMPLE:

Material & Methods

Isolation of lymphocytes and viral infection.

PBMC were isolated from the peripheral blood of healthy volunteers (Etablissement Français du Sang). CD4⁺ T cells were obtained by negative selection with a CD4 T-cell Isolation kit (Miltenyi Biotec). The CD4⁺ T-cell preparation was at least 98% pure. Monocytes (5%) were added to the purified cells, to ensure full T-cell activation. The cells were incubated with HIV-1LAI for 12 h at a multiplicity of infection (MOI) of 0.01, and activated with 1 µg/ml ConA (Sigma-Aldrich) and 100 units/ml recombinant human IL-2 (Roussel-Uclaf, France), as previously described [Laforge M, et al., 2007]. Absolute numbers of live cells were also counted under a light microscope, based on apoptotic cell morphology and/or trypan blue uptake, as previously described [Laforge M, et al., 2007].

Immunofluorescence analyses.

The reagents used for immunofluorescence studies were: rabbit polyclonal antibodies recognizing anti-MAP LC3 (H-50) purchased from Santa Cruz, anti phospho-p53 (Ser 15) antibodies purchased from Cell Signaling Technology, anti-DRAM antibodies purchased from φProSci, anti-cathepsin D antibodies from Zymed Laboratories, anti-p53 mAb (DO-1) purchased from Santa Cruz, anti-Lamp-2 mAb from Calbiochem, and a sheep anti-cytochrome c antiserum from Sigma. Intracellular p24 antigen was assessed by flow cytometry after fixation and permeabilization of the cells (Intraprep permeabilization reagent, Coulter), and which were then stained with FITC- or RD1-labeled mAb against p24 antigen (KC-57, Beckman coulter). Otherwise, the cells were fixed by incubation with 1% paraformaldehyde, spun on glass slides, washed with PBS, and permeabilized by incubation with 0.05 % Triton X-100. The cells were washed and incubated with the antibodies indicated in PBS supplemented with 0.5% BSA and 2% FCS. The cells were stained with an Alexa-conjugated secondary antibody (Molecular Probes). Nuclei were counterstained for 5 minutes with 5 µM DAPI (Molecular Probes). The cells were examined by conventional or confocal fluorescence microscopy (Zeiss Microsystems).

Immunoblotting.

Pellets of 1x10⁶ of CD4⁺ T-cells were either directly resuspended in Laemmli buffer containing 2% SDS and 10% 2-ME and boiled for 5 minutes, or lysed in Nonidet P-40 buffer (1% NP-40, 50 mM Tris-HCl (pH 7.4), 150 mM NaCl) supplemented with protease inhibitors. Cytosolic and nuclear fractions were obtained by extraction with the NE-PER kit (Nuclear and Cytoplasmic Extraction Reagents from PIERCE). Lysates were then subjected to electrophoresis in NUPAGE 4-20% polyacrylamide gels (Invitrogene). The proteins were

transferred onto polyvinylidene difluoride membranes (Amersham Bioscience) and then incubated with primary antibodies and with horseradish peroxidase-coupled secondary reagents (Amersham Biosciences). The primary antibodies used for western blotting were: rabbit antisera against Beclin 1 (H-300, Santa Cruz), phospho-p53 (Ser 15) (Cell Signaling Technology), DRAM (Stressgen), Atg5 (Novus) and tubulin (Santa-Cruz); mouse mAbs against p53 (DO-1, Santa Cruz), phospho-ATM (Ser 1981) and phospho-ATR (Ser 428) (Cell Signaling Technology) and lamin B (Ab-1, Oncogene Research Products Calbiochem). Rabbit antisera against MAP LC3 was purchased from MBL. The blots were then developed by enhanced chemiluminescence methods (ECL+ from GE Healthcare) and photographed with a CCD camera (GBOX, SYNGENE).

Transfection experiments.

Predesigned small interfering RNA (siRNA) molecules targeting p53, DRAM, BECLIN 1 and ATG5 were synthesized by Dharmacon. Scrambled controls were also used. Gene expression was silenced by the small interfering RNA (siRNA) technique 13, using duplexes of 21-nucleotide siRNAs with two 3'-overhanging TT residues (Proligo). The sense strand of the siRNA used to silence the BECLIN 1 gene was CAGTTTGGCACAATCAATATT (SEQ ID NO 2), that of ATG5 gene seq 1 was GCAACTCTGGATGGGATTGTT (SEQ ID NO 3) and that of seq 2 was CATCTGAGCTACCCGGATATT ((SEQ ID NO 4), whereas that of the DRAM gene was CCACAGAAATCAATGGTGATT (SEQ ID NO 5). For p53 we used a smart pool. Purified resting CD4+ T cells were transfected, by electroporation with siRNAs (0.75 μ M / 4 x 10⁶ cells), mediated by the Nucleofection® system (Amaxa). Cells were allowed to rest for 16 hours, exposed to HIV-1 cultured for an additional 12 h and then stimulated for four to five days with Con A and IL-2. We also used the autophagy inhibitor 3-methyladenine in some experiments, but the results obtained were unconvincing, due to a strong toxicity of this drug in primary cultures of human cells.

Genetic association study.

The GRIV (Genomics of Resistance to Immunodeficiency Virus) cohort was established in France in 1995 to generate a large collection of DNAs for genetic studies to identify host genes associated with rapid and long-term non-progression to AIDS. The long-term non-progressors (LTNP, n=275) and the rapid progressors (RP, n=86) were French seroprevalent subjects defined as follows: an asymptomatic HIV-1 infection for more than 8

years, no antiretroviral treatment and a CD4 T-cell count consistently above 500/mm³ for LTNP; and a drop of CD4 T-cell count below 300/mm³ less than 3 years after the last seronegative test for RP. The control group used for comparison with GRIV subjects comprised 697 French HIV-1 seronegative individuals from the D.E.S.I.R. program 24.

5 Genotyping data were obtained for the GRIV cohort and the control group using the Illumina Infinium II HumanHap300 BeadChips 24. Quality control filters were applied to ensure reliable genotyping data. Potential population stratification was also considered using the Eigenstrat software 25 and the top ten most significant principal components were included as covariates in the regression models described below.

10 Bioinformatics analysis. For each SNP, we performed a standard case-control analysis using a logistic regression and a recessive model, including as covariates the 10 principal components identified by Eigenstrat. For each significant association ($p < 0.05$), we eliminated a potential association with HIV-1 infection by checking that the frequency is similar between the second GRIV case group and the control group 24.

15 Electron microscopy.

Pellets of uninfected or infected CD4⁺ T cells were fixed by incubation for 1 h in phosphate buffer pH 7.2 supplemented with 1.6% glutaraldehyde and were then postfixed by incubation for 2 h in 0.1M phosphate buffer supplemented with 1% osmium tetroxide. Pieces
20 of cell pellet were washed for five minutes in water and then dehydrated in a series of increasing concentrations of ethanol before embedding in Epon 812 26. Ultrathin sections were cut and stained with 4% uranyl acetate and lead citrate. They were then examined under a ZEISS 902 electron microscope, at 80 KV, or under a FEI Technai 12 microscope at 80 KV.

25 Statistical analysis.

Data are reported as means $\pm$ SEM. The significance of differences was assessed by Student's t test (Prism software) with $p < 0.05$ considered significant.

Results

30 a) HIV-1 infection increases DRAM expression downstream to p53 activation.

As previously shown [Laforge M. et al., 2007 and Petit F. et al., 2002], the number of infected cells (p24⁺) peaked five days after infection, and a concomitant increase in lysosomal destabilization, as demonstrated by the release of cathepsins D (B and L, not

shown), was observed. We next investigated the phosphorylation status of p53 in HIV-1-infected CD4⁺ T cells using a specific antibody recognizing p53 phosphorylated on serine 15. We fractionated HIV-infected and non-infected CD4⁺ T cells into cytosolic and nuclear fractions. An analysis of the kinetics of p53 antigen expression indicated that the phosphorylation of this protein began four days after HIV infection. By confocal immunofluorescence microscopy, the phosphorylation of p53 occurred in CD4⁺ T cells with productive HIV-1 infection (p24⁺), but not in non-infected cells (p24⁻), and was selectively detected in the nuclei of the cells. Thus, HIV infection induces the phosphorylation of the p53 protein in virally infected CD4⁺ T cells. Because DRAM is linked to p53 activation [Crighton D. et al., 2006], we then analyzed the expression of DRAM on western blots. We detected at day 5 more DRAM protein in HIV-infected CD4⁺ T cells than in uninfected cells. Cells were also fractionated by separating detergent soluble membrane and detergent resistant membrane (DRM) using 1% NP-40 and 0.5% SDS, respectively. This process made it possible to isolate specific proteins in the DRM fraction such as lipid raft. We observed an increase in the amount of DRAM in the DRM fraction of virally-infected cells. Interestingly, we found that the molecular weight of DRAM is slightly higher in HIV-infected CD4⁺ T cells than in control cells suggesting possible post-translational modifications associated with greater segregation of DRAM within this DRM fraction. We then investigated the function of DRAM, using specific siRNAs targeting DRAM and p53 mRNA. As expected, the inhibition of DRAM protein production had no effect on p53 expression, whereas the siRNA targeting p53 resulted in lower levels of both the p53 and DRAM proteins in HIV-infected CD4⁺ T cells.

b) HIV-1 infection induces autophagic machinery in CD4⁺ T cells.

DRAM is a protein located at the lysosome membrane that has been reported to induce autophagy [Crighton D. et al., 2006]. Macroautophagy is a process wherein bulk cytosolic proteins and damaged organelles are sequestered and degraded via the lysosome. Electron microscopy analysis of HIV-infected CD4⁺ T cells with budding viruses showed that these cells contained large numbers of vacuoles with double-membrane structures. No such vacuoles were observed in non-infected cells. The presence of cytoplasmic material within a double-membrane structure identified autophagosomes, the first autophagic-related structures to be produced, on ultrastructural analysis of HIV-infected CD4⁺ T cells. We also observed lysosomes located close to the autophagosomes. The fusion of lysosomes with autophagosomes, which generates autophagolysosomes, associated with degradation of the

sequestered content, was also observed in HIV-infected CD4⁺ T cells. However, by contrast to what has been reported for macrophages infected with HIV [Kyei GB et al., 2009], we detected no viral particles in autophagosome structures. Thus, HIV infection is associated with the conversion of autophagosomes into degrading autolysosomes, which is characteristic of the autophagy pathway.

Autophagy, like apoptosis, is a dynamic process. Thus, the identification of several genes encoding proteins responsible for the execution of autophagy has facilitated detection and manipulation of the autophagy pathway. We investigated LC3 status, to identify the machinery involved in autophagy at the biochemical level. LC3 is initially synthesized in an unprocessed form, proLC3, which is proteolytically processed to generate LC3-I, which is then modified to give rise to a phosphatidylethanolamine (PE)-conjugated form, LC3-II. The binding of LC3-II to phosphatidylethanolamine (PE) results in aggregates of LC3-II, which have a greater electrophoretic mobility than LC3-I, as shown by western blot analysis [Klionsky et al., 2008]. LC3-II is the only protein marker reliably associated with autophagosomes [Klionsky et al., 2008].

On day 5, more LC3-II was found to have accumulated in HIV-infected CD4⁺ T-cell extracts isolated in the presence of SDS than in extracts derived from uninfected CD4⁺ T cells, suggesting that LC3 associates with the autophagosomal membrane in a stable manner. We also stained LC3 proteins (in red) and analyzed the formation of LC3-II aggregates by confocal microscopy. Consistent with immunoblotting results, larger numbers of CD4⁺ T cells with punctate LC3 staining were found in HIV-infected cultures than in uninfected cultures. We compared LC3 staining in uninfected (p24⁻) and HIV-1 infected CD4⁺ T cells (p24⁺, green) five days after infection and found that the formation of LC3 aggregates was largely confined to p24⁺ cells.

The initiation of autophagy involves a complex of Beclin 1 and PIK3C3, whereas Atg5 (30 kDa) is required for autophagosome-precursor synthesis. Atg5 forms a complex with Atg12 (the Atg5-Atg12 complex, which has a molecular weight of 64 kDa). We further investigated the early events associated with the autophagy pathway, by carrying out western blots to analyze the production of these proteins during LC3-II production, in cell extracts isolated in the presence of 1% NP-40 detergent, making it possible to detect both LC3-I and LC3-II. On day 5, Beclin 1 and Atg5 (30 kDa) levels, and LC3-II accumulation were higher in HIV-infected CD4⁺ T cells than in uninfected CD4⁺ T cells and the infected cells also displayed a band at 64 kDa band corresponding to the Atg5/Atg12 complex.

c) DRAM polymorphism is associated with faster progression to Aids

Given the importance of autophagy in host defense against intracellular pathogens, microbial virulence may be linked to the subversion of autophagy 22. The GRIV cohort, comprising subjects exhibiting extreme profile of AIDS progression (LTNP versus RP), constitutes a powerful contrasting tool to unravel new genetic factors associated with AIDS progression 24. We performed a genetic case-control association study on the ATG5, ATG12, BECN1 (Beclin-1), and DRAM genes in the GRIV cohort in order to assess the impact of genetic variants on disease progression. Our results obtained for each gene and the potential correlation with the Genevar 32 and the Dixon 33 mRNA expression databases are shown in Table 1. Interestingly, we found that rs12299074-CC allele from DRAM exhibited an association with faster progression to AIDS ($p=1.08 \times 10^{-2}$). This allele is associated with a lower expression of the DRAM gene according to the Dixon mRNA database ($p=6.8 \times 10^{-7}$). These observations support the hypothesis that defect in DRAM expression could participate in faster disease progression.

d) DRAM triggers autophagy and lysosomal destabilization in HIV-1-infected CD4 + T cells

Because defective activation or lower expression of DRAM may facilitate viral dissemination and increase susceptibility to Aids, we then investigated whether the impact to knocking down DRAM and p53 affected autophagy. Immunoblotting showed that the inhibition of DRAM and p53 protein production decreased the formation of LC3-II. Moreover, whereas staining for LC3-II remained punctate in cells treated with control siRNA (mock), cells treated with siRNA targeting DRAM or p53 displayed lower levels of punctate staining for LC3-II in p24+ CD4+ T cells than in uninfected cells. Thus, DRAM triggers autophagy in HIV-infected CD4+ T cells.

We also found that the knockdown of p53 and DRAM greatly decreased LMP, as demonstrated by levels of cathepsin D release. These results demonstrated for the first time that DRAM plays a key role in inducing LMP. As we previously reported that cathepsin D release from lysosomes is an early event resulting in mitochondrial destabilization in HIV-1-infected CD4+ T cells [Laforge M. et al., 2007], we investigated whether the knockdown of DRAM and p53 affected MOMP and cell death. Our data clearly show that siRNAs specific for DRAM and p53 inhibit MOMP, as assessed by cytochrome c release, and cell death. The siRNAs targeting p53 and DRAM clearly resulted in the presence of larger numbers of HIV-

infected CD4⁺ T cells than we observed with the control siRNA (mock). Thus, the knockdown of DRAM and p53 protein levels by specific siRNAs resulted in numbers of infected cells three to four times larger than for the control. This benefit effect is better than that observed after Beclin 1 and ATG5 knockdown.

5 These results suggest that the DRAM-dependent death in virally infected cells contributes to limiting virus replication. Thus, DRAM acts upstream from LMP and autophagy, and LMP is the major event involved in the death of HIV-infected CD4⁺ T cells. Altogether our data are consistent with a major role for DRAM in regulating host cell/pathogen interactions.

10 **Discussion**

Many different viruses have been described a long time ago to induce lysosomal damage and to kill the cells in which they replicate. Thus, our data provide new clues in virus-cell interaction showing a clear relationship between p53 activation, DRAM and LMP with
15 regards to cell death induction in HIV-infected CD4⁺ T cells. The data presented here demonstrated that DRAM controls LMP in addition to autophagy; the depletion of p53 and DRAM prevented LMP and cell death. These results reenforced the concept that productive HIV infection induces a caspase-independent cell death pathway associated with early lysosomal destabilization. The discovery of the role of DRAM during HIV infection identifies
20 this molecule as a new regulator of host cell-pathogen interactions contributing in the control of viral infection.

Lysosomes are permeabilized in CD4⁺ T lymphocytes productively infected with HIV-1, resulting in the early release of cathepsins into the cytosol. The released cathepsin D acts upstream from the conformational change in Bax and MOMP [Laforge M. et al., 2007].
25 Accumulating data now show that lysosomes function as death signal integrators in response to a wide variety of death stimuli [Jaattela M. et al., 2004]. Herein, we demonstrated that the inhibition of DRAM by specific siRNA prevents cathepsin D release, demonstrating for the first time that DRAM is critical for LMP. It was initially shown that the overexpression of DRAM was not sufficient in itself for inducing autophagy and cell death [Crichton D. et al.,
30 2006]. This initial observation suggests that additional partners and/or translational modifications are probably required for DRAM-mediated LMP. Herein, we found higher amount of DRAM within the DRM fraction in virally infected cells than in uninfected cell. Moreover, consistent with a shift in the molecular weight of DRAM, we found, by computer-based analyses, putative phosphorylation and sumoylation sites in the sequence of the DRAM

protein. It is therefore tempting to speculate that the stability of DRAM may reflect the intensity to permeabilize lysosomes like for Bax/Bak-mediated MOMP. Further analyses are currently underway to determine the role of these posttranslational modifications of DRAM in the context of microbial infections.

5 Our data demonstrated that inhibition of p53 by specific siRNA prevents DRAM expression and autophagy. The infection of T-cell lines and primary CD4⁺ T cells with HIV was initially reported to be associated with stronger expression of pro-apoptotic genes, such as those encoding Bax, p21 and MDM2 [Genini D. et al., 2001 and Imbeault M. et al., 2009]. CD4⁺ T cells productively infected with HIV displayed phosphorylation of p53 on serine 15
10 leading to the accumulation of p53 within the nucleus in agreement with other observation [Crichton D. et al., 2006]. We and others have shown that resting CD4⁺ T cells infected by HIV and stimulated with mitogens accumulate predominantly in the G0/G1 phase of the cell cycle at day 5, and no syncytia were observed in the culture [Petit F. et al., 2002; Laforge M. et al., 2007 and Dabrowska A. et al., 2008]. Other candidates encoded by HIV such as Vpr
15 [Roshal M. et al., 2003], and the gp120 envelope glycoprotein [Castedo M. et al., 2001] have been proposed to induce p53 phosphorylation, however, these proteins are dispensable for HIV infection-mediated cell death [Dabrowska A. et al., 2008 and Lenardo MJ et al., 2002]. Although virus lacking Nef are also cytopathic [Dabrowska A. et al., 2008 and Lenardo MJ et al., 2002], Nef enhances virus replication in vivo and in vitro in primary CD4⁺ T cells and
20 clearly associated with disease outcome [Kestler HW et al., 1991; Spina CA et al., 1994 and Viollet L. et al., 2006]. Moreover, it is conceivable that activation of p53 – the guardian of the genome – could be viewed as a sensor detecting pathogen replication in order to eliminate infected cells [Takaoka A. et al., 2003]. Thus, prompt induction of apoptosis of virally-infected cells via p53/DRAM activation will be beneficial for the host as an altruistic suicide
25 in limiting virus dissemination.

We show here that DRAM is required for autophagy in virally-infected CD4⁺ T cells downstream to active p53, consistent with a previous report [Crichton D. et al., 2006]. Autophagy is an evolutionarily conserved process first defined genetically in yeast [Kuma A. et al., 2004 and Klionsky S. et al., 2000] and autophagy may be activated by pro-death
30 stimuli, to induce caspase-independent cell death [Shimizu S. et al., 2004]. Within HIV-infected CD4⁺ T cells, we have observed (i) ultrastructural autophagy-associated structures, such as autophagosomes and autophagolysosomes; (ii) higher levels of Beclin 1, which is essential for the induction of autophagy; (iii) Atg5-Atg12 complexes, which are considered essential for membrane elongation, and (iv) LC3-II aggregates in p24⁺ cells, involved in the

formation of the autophagosome membrane. However, siRNAs blocking the production of the Atg5 and Beclin 1 proteins had no effect on LMP and cathepsin D release, consistent with the transient beneficial effect observed supporting the view that several autophagy-associated genes can be important for viral replication [Brass AL et al., 2008]. Our data revealed that autophagy is observed only in virally-infected CD4⁺ T cells. Thus, autophagy-mediated cell death due to interaction between the envelope protein and CXCR4 expressed on uninfected CD4⁺ T cells, as previously described Espert L. et al., 2006], therefore does not occur in our model. Altogether, our results demonstrated that DRAM acts upstream from LMP and autophagy in HIV-infected CD4⁺ T cells, and that blocking LMP, we prevent death of these virally-infected cells. Interestingly, our genetic association study showed that an allele of the DRAM gene (rs12299074-CC) is associated with a down-regulation of DRAM expression in rapid progressors that merits to be confirmed in other independent AIDS cohorts. As viral replication is thought to play a direct role in inducing CD4⁺ T-cell death during the acute phase of HIV infection, particularly in the intestine [Monceaux V. et al., 2003; Mattapallil JJ et al., 2005; Li G. et al., 2005 and Hurtel B. et al., 2005], altogether these result suggest the hypothesis that an absence of DRAM could be favourable for viral dissemination and persistence of virally-infected CD4⁺ T-cells in HIV individuals. Pharmacological intervention to modulate DRAM in HIV-infected CD4⁺ T cells may thus help eliminate viral reservoirs and delay development of clinical AIDS.

Our results demonstrate for the first time that the destabilization of lysosomes is induced by DRAM in CD4⁺ T cells productively infected with HIV and is an early central event in the commitment to cell death contributing in the control of viral infection.

REFERENCES:

Throughout this application, various references describe the state of the art to which this invention pertains. The disclosures of these references are hereby incorporated by reference into the present disclosure.

Bidere N, Lorenzo HK, Carmona S, et al. Cathepsin D triggers Bax activation, resulting in selective apoptosis-inducing factor (AIF) relocation in T lymphocytes entering the early commitment phase to apoptosis. J Biol Chem. 2003;278:31401-31411.

Brass AL, Dykxhoorn DM, Benita Y, et al. Identification of host proteins required for HIV infection through a functional genomic screen. *Science*. 2008;319:921-926.

Castedo M, Ferri KF, Blanco J, et al. Human immunodeficiency virus 1 envelope glycoprotein complex-induced apoptosis involves mammalian target of rapamycin/FKBP12-rapamycin-associated protein-mediated p53 phosphorylation. *J Exp Med*. 2001;194:1097-1110.

Corbeil J, Sheeter D, Genini D, et al. Temporal gene regulation during HIV-1 infection of human CD4+ T cells. *Genome Res*. 2001;11:1198-1204.

Crichton D, Wilkinson S, O'Prey J, et al. DRAM, a p53-induced modulator of autophagy, is critical for apoptosis. *Cell*. 2006;126:121-134.

Dabrowska A, Kim N, Aldovini A. Tat-induced FOXO3a is a key mediator of apoptosis in HIV-1-infected human CD4+ T lymphocytes. *J Immunol*. 2008;181:8460-8477.

Daniel R, Kao G, Taganov K, et al. Evidence that the retroviral DNA integration process triggers an ATR-dependent DNA damage response. *Proc Natl Acad Sci U S A*. 2003;100:4778-4783.

Esper L, Denizot M, Grimaldi M, et al. Autophagy is involved in T cell death after binding of HIV-1 envelope proteins to CXCR4. *J Clin Invest*. 2006;116:2161-2172.

Foghsgaard L, Wissing D, Mauch D, et al. Cathepsin B acts as a dominant execution protease in tumor cell apoptosis induced by tumor necrosis factor. *J Cell Biol*. 2001;153:999-1010.

Genini D, Sheeter D, Rought S, et al. HIV induces lymphocyte apoptosis by a p53-initiated, mitochondrial-mediated mechanism. *Faseb J*. 2001;15:5-6.

Gutierrez MG, Master SS, Singh SB, Taylor GA, Colombo MI, Deretic V. Autophagy is a defense mechanism inhibiting BCG and Mycobacterium tuberculosis survival in infected macrophages. *Cell*. 2004;119:753-766.

Hurtrel B, Petit F, Arnoult D, Muller-Trutwin M, Silvestri G, Estaquier J. Apoptosis in SIV infection. *Cell Death Differ*. 2005;12 Suppl 1:979-990.

Imbeault M, Ouellet M, Tremblay MJ. Microarray study reveals that HIV-1 induces rapid type-I interferon-dependent p53 mRNA up-regulation in human primary CD4+ T cells. *Retrovirology*. 2009;6:5.

Jaattela M. Multiple cell death pathways as regulators of tumour initiation and progression. *Oncogene*. 2004;23:2746-2756.

Kestler HW, 3rd, Ringler DJ, Mori K, et al. Importance of the nef gene for maintenance of high virus loads and for development of AIDS. *Cell*. 1991;65:651-662.

Klionsky DJ, Emr SD. Autophagy as a regulated pathway of cellular degradation. *Science*. 2000;290:1717-1721.

Klionsky DJ, Abeliovich H, Agostinis P, et al. Guidelines for the use and interpretation of assays for monitoring autophagy in higher eukaryotes. *Autophagy*. 2008;4:151-175.

Kuma A, Hatano M, Matsui M, et al. The role of autophagy during the early neonatal starvation period. *Nature*. 2004;432:1032-1036.

Kyei GB, Dinkins C, Davis AS, et al. Autophagy pathway intersects with HIV-1 biosynthesis and regulates viral yields in macrophages. *J Cell Biol*. 2009;186:255-268.

Laforge M, Petit F, Estaquier J, Senik A. Commitment to apoptosis in CD4(+) T lymphocytes productively infected with human immunodeficiency virus type 1 is initiated by lysosomal membrane permeabilization, itself induced by the isolated expression of the viral protein Nef. *J Virol*. 2007;81:11426-11440.

Lenardo MJ, Angleman SB, Bounkeua V, et al. Cytopathic killing of peripheral blood CD4(+) T lymphocytes by human immunodeficiency virus type 1 appears necrotic rather than apoptotic and does not require env. *J Virol*. 2002;76:5082-5093.

Levine B. Eating oneself and uninvited guests: autophagy-related pathways in cellular defense. *Cell*. 2005;120:159-162.

Levine B, Deretic V. Unveiling the roles of autophagy in innate and adaptive immunity. *Nat Rev Immunol*. 2007;7:767-777.

Li N, Zheng Y, Chen W, et al. Adaptor protein LAPF recruits phosphorylated p53 to lysosomes and triggers lysosomal destabilization in apoptosis. *Cancer Res*. 2007;67:11176-11185.

Li Q, Duan L, Estes JD, et al. Peak SIV replication in resting memory CD4+ T cells depletes gut lamina propria CD4+ T cells. *Nature*. 2005;434:1148-1152.

Mattapallil JJ, Douek DC, Hill B, Nishimura Y, Martin M, Roederer M. Massive infection and loss of memory CD4+ T cells in multiple tissues during acute SIV infection. *Nature*. 2005;434:1093-1097.

Monceaux V, Estaquier J, Fevrier M, et al. Extensive apoptosis in lymphoid organs during primary SIV infection predicts rapid progression towards AIDS. *Aids*. 2003;17:1585-1596.

Moutouh L, Estaquier J, Richman DD, Corbeil J. Molecular and cellular analysis of human immunodeficiency virus-induced apoptosis in lymphoblastoid T-cell-line-expressing wild-type and mutated CD4 receptors. *J Virol*. 1998;72:8061-8072.

Nakagawa I, Amano A, Mizushima N, et al. Autophagy defends cells against invading group A *Streptococcus*. *Science*. 2004;306:1037-1040.

Ogawa M, Yoshimori T, Suzuki T, Sagara H, Mizushima N, Sasakawa C. Escape of intracellular *Shigella* from autophagy. *Science*. 2005;307:727-731.

5 Orvedahl A, Alexander D, Talloczy Z, et al. HSV-1 ICP34.5 confers neurovirulence by targeting the Beclin 1 autophagy protein. *Cell Host Microbe*. 2007;1:23-35.

O'Shea CC, Fried M. Modulation of the ARF-p53 pathway by the small DNA tumor viruses. *Cell Cycle*. 2005;4:449-452.

10 Petit F, Arnoult D, Lelievre JD, et al. Productive HIV-1 infection of primary CD4+ T cells induces mitochondrial membrane permeabilization leading to a caspase-independent cell death. *J Biol Chem*. 2002;277:1477-1487. Roshal M, Kim B, Zhu Y, Nghiem P, Planelles V. Activation of the ATR-mediated DNA damage response by the HIV-1 viral protein R. *J Biol Chem*. 2003;278:25879-25886.

15 Shimizu S, Kanaseki T, Mizushima N, et al. Role of Bcl-2 family proteins in a non-apoptotic programmed cell death dependent on autophagy genes. *Nat Cell Biol*. 2004;6:1221-1228.

Shintani T, Klionsky DJ. Autophagy in health and disease: a double-edged sword. *Science*. 2004;306:990-995.

20 Singh SB, Davis AS, Taylor GA, Deretic V. Human IRGM induces autophagy to eliminate intracellular mycobacteria. *Science*. 2006;313:1438-1441.

Spina CA, Kwok TJ, Chowder MY, Guatelli JC, Richman DD. The importance of nef in the induction of human immunodeficiency virus type 1 replication from primary quiescent CD4 lymphocytes. *J Exp Med*. 1994;179:115-123.

25 Stoka V, Turk B, Schendel SL, et al. Lysosomal protease pathways to apoptosis. Cleavage of bid, not pro-caspases, is the most likely route. *J Biol Chem*. 2001;276:3149-3157.

Takaoka A, Hayakawa S, Yanai H, et al. Integration of interferon-alpha/beta signalling to p53 responses in tumour suppression and antiviral defence. *Nature*. 2003;424:516-523.

Toledo F, Wahl GM. Regulating the p53 pathway: in vitro hypotheses, in vivo veritas. *Nat Rev Cancer*. 2006;6:909-923.

30 Viollet L, Monceaux V, Petit F, et al. Death of CD4+ T cells from lymph nodes during primary SIVmac251 infection predicts the rate of AIDS progression. *J Immunol*. 2006;177:6685-6694.

Vousden KH, Lane DP. p53 in health and disease. *Nat Rev Mol Cell Biol*. 2007;8:275-283.

Yuan XM, Li W, Dalen H, et al. Lysosomal destabilization in p53-induced apoptosis. Proc Natl Acad Sci U S A. 2002;99:6286-6291.

CLAIMS:

1. DRAM polypeptide for use in the treatment of infectious diseases.
2. The DRAM polypeptide for use according to claim 1 wherein infectious diseases are
5 selected from infections caused by a virus, infections caused by a bacterium, infection
cause by a parasite, or infections caused by a fungus.
3. The DRAM polypeptide for use according to the claim 2 wherein infections caused by
a virus are selected from HIV, HCV, HBV, HPV, VSV, NDV, Influenza A.
4. An isolated nucleic acid sequence coding for a polypeptide according the claims from
10 1 to 2 for use in the treatment of infectious diseases.
5. A plasmid comprising a nucleic acid sequence according to claim 4 for use in the
treatment of infectious diseases.
6. An expression vector containing a nucleic acid sequence according to claim 4 for use
in the treatment of infectious diseases.
7. A fusion protein containing the amino acids sequences of DRAM or active fragment
15 thereof for use in the treatment of infectious diseases.
8. A fusion protein for use according to claim 6 wherein DRAM is linked to a
polypeptide consisting of a protein transduction domain, a cell penetrating peptide or a
cell targeting peptide.
9. A pharmaceutical composition for use in the treatment of infectious diseases
20 comprising a therapeutically effective amount of DRAM polypeptide according to
claims 1 or 2, or an nucleic acid according to claim 3, or a plasmid according to claim
4, or an expression vector according to claim 5, or a fusion protein according to claim
7 along with at least one pharmaceutically acceptable excipient.
10. An activator of DRAM gene expression for use in the treatment of infectious diseases.
25

11. A pharmaceutical composition for use in the treatment of infectious diseases comprising a therapeutically effective amount of an activator of DRAM gene expression.

12. An ex vivo method of diagnosing or predicting a faster progression of HIV infection in a subject, which method comprises detecting a polymorphism in the DRAM gene in a sample obtained from said subject, wherein the presence of said polymorphism is indicative of a faster progression of HIV infection.

13. An ex vivo method according to claim 12 wherein the method comprises detecting the SNP rs17032033 (SEQ ID NO 6) or the SNP rs4764839 (SEQ ID NO 7) or the SNP rs12299074 (SEQ ID NO 8) in the DRAM gene in a sample obtained from said subject, wherein the presence of allele (T) in the SNP rs17032033 or in the SNP rs4764839 or in the SNP rs12299074 is indicative of a faster progression of HIV infection.

INTERNATIONAL SEARCH REPORT

International application No.

PCT/EP2012/054152

Box No. I Nucleotide and/or amino acid sequence(s) (Continuation of item 1.c of the first sheet)

1. With regard to any nucleotide and/or amino acid sequence disclosed in the international application and necessary to the claimed invention, the international search was carried out on the basis of:
 - a. (means)

☐

 on paper
 - ☒

 in electronic form
 - b. (time)

☒

 in the international application as filed
 - ☐

 together with the international application in electronic form
 - ☐

 subsequently to this Authority for the purpose of search
2.

☐

 In addition, in the case that more than one version or copy of a sequence listing and/or table relating thereto has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that in the application as filed or does not go beyond the application as filed, as appropriate, were furnished.
3. Additional comments:

INTERNATIONAL SEARCH REPORT

International application No
PCT/EP2012/054152

A. CLASSIFICATION OF SUBJECT MATTER

INV. A61K38/17 G01N33/569 C12Q1/68 A61P31/00
ADD.

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

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

A61K

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 practical, search terms used)

EPO-Internal, BIOSIS, CHEM ABS Data, COMPENDEX, EMBASE, WPI Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	PARK SUN-MI ET AL: "Reduced expression of DRAM2/TMEM77 in tumor cells interferes with cell death", BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS, vol. 390, no. 4, December 2009 (2009-12), pages 1340-1344, XP002640039, page 1341 ----- -/--	1-13



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 document but published on or after the international filing date

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

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

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

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

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

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

"&" document member of the same patent family

Date of the actual completion of the international search

4 April 2012

Date of mailing of the international search report

16/04/2012

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

Bochelen, Damien

INTERNATIONAL SEARCH REPORT

International application No
PCT/EP2012/054152

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	KERLEY-HAMILTON ET AL: "The direct p53 target gene, FLJ11259/DRAM, is a member of a novel family of transmembrane proteins", BIOCHIMICA ET BIOPHYSICA ACTA . GENE STRUCTURE AND EXPRESSION, ELSEVIER, AMSTERDAM, NL, vol. 1769, no. 4, 1 April 2007 (2007-04-01), pages 209-219, XP022069343, ISSN: 0167-4781, DOI: DOI:10.1016/J.BBAEXP.2007.02.002 page 215 -----	1-13
A	CRIGHTON DIANE ET AL: "DRAM, a p53-induced modulator of autophagy, is critical for apoptosis", CELL, vol. 126, no. 1, July 2006 (2006-07), pages 121-134, XP002640040, ISSN: 0092-8674 pages 123-124 -----	1-13
A	WO 2010/063977 A1 (SILAB SA [FR]; PAUFIQUE JEAN [FR]) 10 June 2010 (2010-06-10) the whole document -----	1-13
A	ESPERT LUCILE ET AL: "Involvement of autophagy in viral infections: antiviral function and subversion by viruses", JOURNAL OF MOLECULAR MEDICINE (BERLIN), vol. 85, no. 8, August 2007 (2007-08), pages 811-823, XP019529781, the whole document -----	1-11

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No

PCT/EP2012/054152

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 2010063977 A1	10-06-2010	EP 2364136 A1	14-09-2011
		FR 2939316 A1	11-06-2010
		US 2011243983 A1	06-10-2011
		WO 2010063977 A1	10-06-2010
