Title: APOL3 FOR USE IN THE TREATMENT OF TRYPANOSOMA INFECTIOUS DISEASES

Abstract: The present invention is related to a pharmaceutical composition comprising (human) ApoL-3-derived protein(s), especially for use in the treatment or the prevention of Trypanosoma Sp. Infection.
ApoL3 for use in the treatment of Trypanosoma infectious diseases

Field of the invention

[0001] The present invention is in the field of Molecular Biology and is related to Apolipoprotein L-3 derived proteins and/or peptides and their pharmaceutical (therapeutical or prophylactic) use, especially for a treatment and/or a prevention of diseases induced in mammals, especially in human, preferably infections induced by Trypanosoma, especially African Trypanosoma, more particularly Trypanosoma brucei gambiense.

Background of the invention and state of the art

[0002] Tytler et al. (1995 Molecular and Biochemical Parasitology, 69, 9-17) identified the presence of trypanolytic factors on a subspecies of human high-density lipoproteins. Among them are factors they called “ApoL-I” (for Trypanosoma Lytic Factor apolipoprotein) and “ApoL-III”. Both these factors are secreted and their apparent electrophoretic mobility is of about 80 and 40 kDa. However, no sequence was provided. None of the identified factors was individually cytotoxic.

[0003] Apolipoprotein L-I (ApoL-1) was later one identified as a minor component in a subclass of high-density lipoproteins (HDL) and further sequenced (Duchateau et al., J. Biol. Chem., 1997, vol 272, pages 25576-25582).
Several other Apolipoproteins L were subsequently identified, including Apolipoprotein L-III (Duchateau et al., J. Lipid Res., 2001, vol 42, pages 620-630).

The size of the "ApoL-I" as mentioned by Tytler (80 kDa) differs from the size of the ApoL-I as consistently reported since the publication of Duchateau (about 40 kDa).

The "ApoL-III" disclosed in Tytler is secreted, while the ApoL-III (to which the present application relates) was found to be intracellular.

Therefore, the functional nomenclature used in the publication of Tytler cannot correspond to the Apolipoproteins L mentioned in the databases (or in the present patent application).

Although it is postulated that these 30-40 kDa proteins are involved in the lipid transport and metabolism, the function of Apolipoproteins L is not fully characterized.

The inventors have previously discovered that endogenous human ApoL-1 selectively and naturally kills some Trypanosomal species, and they have reproduced this phenomenon in vitro using recombinant ApoL-1.

By refining their searches, the inventors identified variants of human ApoL-1 that are able to kill Trypanosomes that resisted to the lysis caused by wild-type ApoL-I, such as Trypanosoma brucei rhodesiense (ex. PCT/EP2009/060687 and PCT/EP2010/062065).

(Human) Apolipoprotein L-III (ApoL-3) is another member of this family. Contrary to ApoL-1, (human) ApoL-3 is found in the cytoplasm of cells (i.e. this protein is not secreted and is not naturally found on human HDLs). This protein is also thought to interfere with the lipid metabolism.
[0011] Trypanosoma brucei gambiense still remains a considerable health issue in Africa, as sleeping sickness potentially affects the live of millions of persons.

[0012] Being cytoplasmic, one cannot find or postulate any role for human ApoL-3 in controlling trypanosomal infections.

**Summary of the invention**

[0013] The present invention is related to a pharmaceutical composition comprising (human) Apolipoprotein L-III- (ApoL-3-) derived protein(s) or peptide(s) (or a nucleotide sequence encoding it) and adequate (suitable or compatible) pharmaceutical carrier (or diluent).

[0014] Preferably, this pharmaceutical composition is for use in the treatment (or in the prevention) of infections caused by Trypanosoma (preferably not by Trypanosoma cruzi) and, more preferably, caused by Trypanosoma brucei, (and/or by African Trypanosoma, more particularly Trypanosoma brucei brucei, Trypanosoma brucei rhodesiense, Trypanosoma brucei gambiense, Trypanosoma conglobense, trypanosome evansi and/or trypanosoma vivax) in a mammal subject (in particular in a cattle), preferably in a human patient.

[0015] Advantageously, this pharmaceutical composition is for use in the treatment or the prevention of infections caused by Trypanosoma (brucei) expressing TgsGP and/or the sequence SEQ.ID.NO.6.

[0016] Alternatively (or in addition) this pharmaceutical composition is for use in the treatment or the prevention of infections caused Trypanosoma brucei gambiense.

[0017] Preferably, these ApoL-3-derived protein(s) or peptide(s) and/or this pharmaceutical composition
comprise(s) the sequence SEQ.ID.NO.1 (i.e. IEKLRALANGIEEV), more preferably SEQ.ID.NO.2, still more preferably SEQ.ID.NO.4, SEQ.ID.NO.5 or SEQ.ID.NO.8.

[0018] Alternatively (or in addition), these ApoL-3-derived protein(s) and/or this pharmaceutical composition comprise(s) the sequence SEQ.ID.NO.3.

[0019] Possibly, these ApoL-3-derived protein(s) and/or this pharmaceutical composition comprise(s) a protein or a peptide selected from the group consisting of the sequence SEQ.ID.NO.1, SEQ.ID.NO.2, SEQ.ID.NO.3, SEQ.ID.NO.4, SEQ.ID.NO.5 and SEQ.ID.NO.8, wherein this protein or peptide is further submitted to one, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more mutation(s), and/or this mutated protein or peptide keeps at least 85, 90, 95, 96, 97, 98 or 99% of identity with the corresponding wild-type (non mutated) SEQ.ID.NO.1, SEQ.ID.NO.2, SEQ.ID.NO.3, SEQ.ID.NO.4, SEQ.ID.NO.5 and SEQ.ID.NO.8 peptide sequences.

[0020] Preferably, these mutations in ApoL-3-derived protein(s) or peptide(s) are conservative mutations.

[0021] Alternatively, or in addition, these mutations in these ApoL-3-derived protein(s) or peptide(s) keep (or even increase) the properties of the (corresponding) wild-type ApoL-3-derived protein(s) or peptide(s), especially lysis capacities (lysis of Trypanosoma, such as T. brucei gambiense).

[0022] By keeping its properties, it is meant that less than 150% (\(W_{\text{mutated}}:W_{\text{wild-type}}\)) (preferably less than 120% \(w:w\)) of mutated ApoL-3 (compared to the (corresponding) wild-type ApoL-3) protein or peptide is required to promote the lysis of Trypanosoma species, such as T. brucei gambiense in a delay of about 30 minutes to about 24 hours, preferably in a delay of about 6 hours to about 8 hours.
[0023] More preferably, this ApoL-3-derived protein or peptide is (and/or this pharmaceutical composition comprises) the sequence SEQ.ID.NO.4.

[0024] Alternatively, this ApoL-3-derived protein or peptide is (and/or this pharmaceutical composition comprises) the sequence SEQ.ID.NO.3.

[0025] Alternatively, this ApoL-3-derived protein or peptide is (and/or this pharmaceutical composition comprises) the sequence SEQ.ID.NO.5.

[0026] Alternatively, this ApoL-3-derived protein or peptide is (and/or this pharmaceutical composition comprises) the sequence SEQ.ID.NO.8.

[0027] Possibly and preferably, this ApoL-3-derived protein or peptide (wild-type or mutated) further comprises a signal peptide for an extracellular secretion of the protein or peptide.

[0028] A related aspect of the invention is a secretable ApoL-3-derived protein comprising a peptide selected from the group consisting of SEQ.ID.NO.1, SEQ.ID.NO.2, SEQ.ID.NO.3, SEQ.ID.NO.4 and SEQ.ID.NO.5 (and even SEQ.ID.NO.8) sequence.

[0029] Advantageously, this secretable ApoL-3-derived protein further comprises at its N-terminus a signal peptide and/or a (10-60 amino acids) peptidic sequence able to target this secretable ApoL-3-derived protein to the secretory pathway of a cell and/or preferably to the surrounding medium of this cell; this N-terminus signal peptide being possibly the sequence SEQ.ID.NO.7.

[0030] Preferably, this N-terminus signal peptide is a peptide signal cleavable (from the secretable ApoL-3-derived protein) in a cell secretory pathway.

[0031] Alternatively, or in addition, the (secretable) ApoL-3-derived protein comprises a peptide selected from the group consisting of the sequences
SEQ.ID.NO.1, SEQ.ID.NO.2, SEQ.ID.NO.3, SEQ.ID.NO.4, SEQ.ID.NO.5 and SEQ.ID.NO.8 and further comprises a proteic tag, preferably selected from the group consisting of HA, FLAG, His<sub>6</sub>, Myc and isopeptag, and being more preferably His<sub>6</sub>, possibly this proteic tag being located at the C-terminus of this (secretable) ApoL-3-derived protein, possibly after a glycine-rich linker, such as GGLE tetrapeptide.

[0032] The present invention is also related to the (recombinant) nucleotide sequence(s) encoding this (these) ApoL-3-derived protein(s) or peptide(s), possibly including the secretable and/or the tagged form(s).

[0033] Possibly, this nucleotide sequence is present upon a vector, preferably an extrachromosomial replicon, such a plasmid.

[0034] The present invention is also related to a (recombinant) (eukaryote) cell secreting (to the surrounding medium) ApoL-3-derived protein of the present invention and possibly transformed by this vector or comprising this extrachromosomal replicon.

[0035] This (recombinant) cell has preferably incorporated the vector or the (recombinant) nucleotide sequence encoding the (secretable) protein of the present invention and/or is able to express the proteic sequence(s) of the present invention.

[0036] Preferably, this (recombinant) cell is a eukaryotic cell, and more preferably is a mammal cell with the proviso that this cell is not a human embryonic cell.

[0037] Alternatively, this (recombinant) eukaryotic cell is a (ApoL-3-resistant) protozoan.

[0038] Another aspect of the present invention is related to one or more isolated high-density lipoprotein (HDL) particle(s) for use as a medicament, wherein this HDL
particle comprises one or more of these ApoL-3-derived protein(s) or peptide(s).

[0039] Preferably, these ApoL-3-derived protein(s) or peptide(s) (of this pharmaceutical composition in the form of HDL particle(s)) comprise one more of the genetic sequences selected from the group consisting of the sequence SEQ.ID.NO.1 and more preferably the sequence SEQ.ID.NO.2, still more preferably the sequence SEQ.ID.NO.4, SEQ.ID.NO.5 or SEQ.ID.NO.8.

[0040] Alternatively, or in addition, these ApoL-3-derived protein(s) (of this pharmaceutical composition in the form of HDL particle(s)) comprise the sequence SEQ.ID.NO.3.

[0041] Another aspect of the present invention is related to a transgenic animal (such as a protozoan, a rodent or a cow) expressing secretable (human) ApoL-3-derived protein(s) (in the blood of this transgenic animal).

[0042] Preferably, these ApoL-3-derived protein(s) or peptide(s) (expressed by this transgenic animal) comprise the sequence SEQ.ID.NO.1 and more preferably the sequence SEQ.ID.NO.2, still more preferably the sequence SEQ.ID.NO.4, SEQ.ID.NO.5 or SEQ.ID.NO.8.

[0043] Alternatively, or in addition these ApoL-3-derived protein(s) (expressed by this transgenic animal) comprise the sequence SEQ.ID.NO.3.

[0044] A related aspect of the invention is a transgenic rodent (such as a mouse) being deficient in (murine) ApoL-7 and/or in (murine) ApoL-11 (being the murine apolipoproteins corresponding to human ApoL-3).

[0045] Another aspect of the present invention is related to an inhibitor of TgsGP (SEQ.ID.NO.6) for use in the treatment of *Trypanosoma brucei gambiense* infection.
[0046] Possibly, this inhibitor of TgsGP (SEQ.ID.NO.6) is in the form of a siRNA sequence (ora sequence that possibly at least comprise an antisense sequence complementary to TgsGP mRNA, this sequence comprising at least 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 60, 65, 70, 75, 85 or more nucleotides and preferably having about 100% of complementary with TgsGP mRNA).

[0047] Another aspect of the present invention is related to a pharmaceutical composition comprising TgsGP (SEQ.ID.NO.6) and possibly a suitable pharmaceutical carrier.

[0048] Possibly, this pharmaceutical composition comprising an adequate pharmaceutical (adequate or compatible) carrier (or diluent) and TgsGP (SEQ.ID.NO.6), is for use in the treatment (or in the prevention) of renal disease(s).

[0049] Possibly, this TgsGP (SEQ.ID.NO.6) is in the form of a variant, being devoid of Glycosylphosphatidylinositol (GPI) anchor, possibly either because of a specific point mutation(s) or being the wild-type protein with no, or with a lost, GPI (post-translational) modification.

[0050] Another aspect of the present invention is a diagnostic method comprising the step of measuring the presence of TgsGP (SEQ.ID.NO.6) in the blood of a patient having, or suspected to have an infection by trypanosoma.

[0051] Another aspect of the present invention is a diagnostic kit comprising isolated (and purified) TgsGP (SEQ.ID.NO.6) protein or a fragment thereof or a nucleotide sequence encoding TgsGP (SEQ.ID.NO.6) protein or a fragment thereof, or the complementary nucleotide sequence, able to hybridize with this nucleotide sequence encoding TgsGP
(SEQ.ID.NO.6) protein or a fragment thereof and possibly others means for a (suitable and efficient) genetic detection.

[0052] The present invention concerns also a method for a screening of one or more (pharmaceutical active) compounds (preferably inhibitor(s)) able to interact (to bind, to block or to inactivate) the isolated (and purified) TgsGP (SEQ.ID.NO:6) or its encoding nucleotide sequence, and that could be used in the treatment or the prevention of diseases, especially diseases induced by Trypanosoma, especially Trypanosoma brucei gambiense; this method comprising the step of putting in contact compound(s) to be tested with this TgsGP (or with one fragment thereof) protein or its encoding nucleotide sequence and recovering the compounds bound to the TgsGP protein (or to one fragment thereof) or its corresponding nucleotide sequence.

**Brief description of the figures and tables**

[0053] Fig. 1 discloses a sequence alignment of fragments and isoforms of ApoL-3 (SEQ.ID.NO.s.2, 3, 4, 5 and 8).

[0054] Fig. 2 shows the sensitivity of several Trypanosomal species to recombinant ApoL-3.

[0055] Fig. 3 shows the sensitivity towards normal human serum of Trypanosoma brucei gambiense mutants having lost TgsGP (SEQ.ID.NO.6).

**Detailed description of the invention**

[0056] The inventors have expressed a recombinant secretable apolipoprotein L-III (ApoL-3) and purified the recombinant apolipoprotein L-III (ApoL-3) (SEQ.ID.NO.8) having lost its signal peptide (SEQ.ID.NO.7). This
recombinant ApoL-3 is >99% identical to human ApoL-3 (e.g. SEQ.ID.NO.4).

[0057] They have found in vitro that this recombinant apolipoprotein L-III (ApoL-3) rapidly kills several Trypanosomal species (see for instance Fig. 2).

[0058] Especially noticeable is the fact that Trypanosoma brucei gambiense is lysed by this ApoL-3, but is not lysed by ApoL-1.

[0059] The inventors further performed additional modifications and developments.

[0060] The inventors incorporated fragments of ApoL-3, such as the sequences SEQ.ID.NO.1 and SEQ.ID.NO.2 in a pharmaceutical composition.

[0061] The inventors also used other isoforms of ApoL-3 such as the sequences SEQ.ID.NO.3 and SEQ.ID.NO.5.

[0062] Furthermore, in order to promote the secretion of these ApoL-3 proteins or peptides produced in a recombinant cell, the inventors added (at the genetic sequence encoding ApoL-3, the genetic sequence encoding a signal sequence in order to produce ApoL-3 proteins or peptides having at the N-terminus) a signal peptide derived from the human ApoL-1 (being SEQ.ID.NO.7) that will be cleaved in the secretory pathway of this recombinant cell:

MEGAALLRVSVLCIWMSALFLGVGVRAEEAGARVQONVPSGTDTGDPQSKPLGDAAG

[0063] The inventors further produced high-density lipoproteins particles comprising ApoL-3 (protein(s) or peptide(s)), in order to increase the lytic properties of ApoL-3 (peptide(s)).

[0064] The inventors further generate recombinant animals expressing human ApoL-3 (protein(s) or peptide(s)).

[0065] The first animal is a cow expressing secretable human ApoL-3 (protein(s) or peptide(s)), in order to have these protein(s) or peptide(s) in the blood of the animal.
[0066] This transgenic cow is useful as a model for the (long-term) toxicity of human ApoL-3, when present in the blood, rather than to be confined in the cytoplasm.

[0067] Moreover, this transgenic cow is resistant to Trypanosomal (such as T. brucei) infections, meaning both increased potential in agriculture and a reduced reservoir for the parasite.

[0068] Finally, this transgenic cow directly produce the pharmaceutical compositions in the form of HDL particles (comprising human ApoL-3 peptide(s)) (that remain to be purified from the blood of this transgenic cow) or in the form of human ApoL-3 peptide(s) (that remain to be purified purified from the blood or another biological fluid of this transgenic cow).

[0069] Another transgenic animal is a protozoan expressing secretable human ApoL-3 protein (peptide(s)). The inventors have further infected cattle (a cow) with this transgenic protozoan and have observed HDL particle comprising the human ApoL-3 protein according to the present invention.

[0070] Another transgenic animal is a mouse expressing secretable human ApoL-3 (peptide(s)). Although mice express apolipoproteins that correspond to human ApoL-3 (murine ApoL-7 and murine ApoL-11), these two proteins are expressed in the cytoplasm and are not secreted in the blood. This transgenic animal, secreting ApoL-3, is therefore a suitable model for ApoL-3-induced (long-term) toxicity.

[0071] The inventors further generated transgenic mice having no ApoL-7 and/or no ApoL-11 protein production.

[0072] These transgenic mice having no ApoL-7 and/or ApoL-11 proteins are useful to study the physiological role (especially in immunology, including a role in auto-immune disease) of proteins corresponding to human ApoL-3.
Conversely, the inventors have found in vitro that *Trypanosoma brucei gambiense*, when submitted to a total suppression of TgsGP (SEQ.ID.NO.6) expression, are sensitive to normal human serum (comprising ApoL-1) (see fig. 2).

Therefore, inhibitors of this protein, including (specific) siRNA, are useful in the treatment of Trypanosomal diseases.

On the other hand, since this TgsGP protein (SEQ.ID.NO.6) interferes with ApoL-1-induced toxicity, the inventors used this TgsGP (SEQ.ID.NO.6) protein (possibly in a soluble form) as a specific medicament, especially to reduce this toxicity and/or in the case of focal segmental glomerulosclerosis (renal disease) caused by mutated ApoL-1 (especially with the double deletion in (human) ApoL-1 of N388 and N389, as previously observed by the inventors).

Since this TgsGP (SEQ.ID.NO.6) protein is associated with the resistance to normal human serum, the inventors further developed a diagnostic test based on the detection of this protein.

A related diagnostic method encompasses the detection in a blood sample of this TgsGP (SEQ.ID.NO.6) protein. Consequently, if this protein (or a fragment thereof) is present, there is a need to apply treatment(s) for trypanosomal infections and/or, preferably, the ApoL-3 peptide(s) herein disclosed.

**Examples**

**Material and method**

Unless stated otherwise, the experiments, including *Trypanosoma* culture and the tests of human sera for their lytic activities were carried-out in a manner similar to the ones already published (Lecordier L. et al.,
2009); C-terminal mutants of apolipoprotein L-I efficiently kill both *Trypanosoma brucei brucei* and *Trypanosoma brucei rhodesiense* (PLoS Pathog. 2009 Dec;5(12):e1000685).

**Example 1:**

[0079] The inventors used three different Trypanosomes: one *T. brucei* resistant to (WT human) ApoL-1 (ETat1.2R), one *T. brucei* sensitive to (WT human) ApoL-1 (ETat1.2S) and one *T. brucei gambiense* (LiTat1.3).

[0080] When the surrounding medium was supplemented with 40 µg/ml of recombinant ApoL-3 (SEQ.ID.NO.8), all these trypanosomes were rapidly lysed (Fig. 2.A.), and a significant vacuolization is visible within the parasite (Fig. 2.B.).

**Example 2:**

[0081] The inventors then searched for the factor conferring resistance of *T. brucei gambiense* towards ApoL-1. The inventors produced *T. brucei gambiense* having lost their TgsGP protein. In practice, the TgsGP gene was deleted by telomere truncation, or replaced by a gene encoding a selectable marker (resistance to phleomycin).

[0082] These mutant trypanosomes were found sensitive to normal human serum (comprising ApoL-1 HDL particles and/or ApoL-1 protein) (see Fig. 3.A).

[0083] This almost equates the sensitivity of ‘sensitive’ *T. brucei* variants towards ApoL-1 (see Fig. 3.B.).
CLAIMS

1. An apolipoprotein L-III-derived peptide for use in the treatment or the prevention of Trypanosoma sp. infection.

2. The peptide of claim 1 wherein the Trypanosoma is selected from the group consisting of Trypanosoma brucei, Trypanosoma congoense, trypanosome evansi and trypanosoma vivax.

3. The peptide of claim 1 or 2 comprising the sequence selected from the group consisting of the sequences SEQ.ID.NO.1, SEQ.ID.NO.2 and SEQ.ID.NO.3.

4. The peptide according to any of the preceding claims 1 to 3 comprising the sequence SEQ.ID.NO.4, SEQ.ID.NO.5 or SEQ.ID.NO.8.

5. The peptide according to any of the preceding claims 1 to 4 for use in the treatment or prevention of Trypanosoma sp. infection, wherein the said Trypanosoma expresses the sequence SEQ.ID.NO.6 and/or is Trypanosoma brucei gambiense.

6. A nucleotide sequence encoding the peptide according to any of the preceding claims 1 to 5 for use in the treatment of Trypanosoma sp. infection, being preferably Trypanosoma sp. expressing the sequence SEQ.ID.NO.6 and/or of Trypanosoma brucei gambiense.

7. Isolated High-density lipoprotein (HDL) particles for use as a medicament, wherein the said HDL particles comprise a peptide sequence selected from the group consisting of the sequence SEQ.ID.NO.1, SEQ.ID.NO.2 and SEQ.ID.NO.3.

8. The HDL particle of claim 7 comprising the sequence SEQ.ID.NO.4, SEQ.ID.NO.5 or SEQ.ID.NO.8.

9. A transgenic non-human animal expressing a secretable ApoL-3-derived protein, wherein the ApoL-3-derived protein comprises a peptide selected from the group
consisting of the sequence SEQ.ID.NO.1, SEQ.ID.NO.2,  
SEQ.ID.NO.3, SEQ.ID.NO.4, SEQ.ID.NO.5 and SEQ.ID.NO.8.

10. A secretable ApoL-3-derived protein  
comprising a peptide selected from the group consisting of
the sequences SEQ.ID.NO.1, SEQ.ID.NO.2, SEQ.ID.NO.3,  
SEQ.ID.NO.4, SEQ.ID.NO.5 and SEQ.ID.NO.8.

11. A nucleotide sequence encoding the  
secretable ApoL-3 protein of claim 10.

12. A cell comprising the nucleotide sequence  
of claim 11.

13. An inhibitor of TgsGP (SEQ.ID.NO.6) for  
use in the treatment or prevention of Trypanosoma brucei  
gambiense infection.

14. A protein comprising the sequence  
SEQ.ID.NO.6, for use as a medicament.

15. A protein comprising the sequence  
SEQ.ID.NO.6, for use in the treatment or prevention of  
renal disease.

16. A screening method of one or more  
compounds able to interact with the TgsGP (SEQ.ID.NO.6) or  
its encoding nucleotide sequence, which comprises the step  
of putting into contact the compounds to be tested with the  
said TgsGP or its encoding nucleotide sequence and  
recovering the compounds bound to the said TgsGP or its  
encoding nucleotide sequence.
Multiple sequence alignment of ApoL-3 proteins

Seq. 5  MGLGQWGWEASCFACLIRSSCCQVVNTTFPPGQGSLESNGYADARLEVGSTQLR  60

Seq. 5  RTAGSCSHFSKRSFL---------------------------------  75

Seq. 8  MSKKRFTEATKYYREVSVPVLQILTNNEAWKRFVTAAELPRDEADAYEALKLR  59
Seq. 3  -----------------------------------------------
Seq. 4  MSKKRFTEATKYYREVSVPVLQILTNNEAWKRFVTAAELPRDEADAYEALKLR  60
Seq. 2  EKKRFTTEATKYYREVSVPVLQILTNNEAWKRFVTAAELPRDEADAYEALKLR  57
Seq. 5  EKKRFTTEATKYYREVSVPVLQILTNNEAWKRFVTAAELPRDEADAYEALKLR  132

Seq. 8  TYAIEEYVQKDEQFREWFLKEFPQVERKIQESIEKLRALANGIEEHGCTISNVVS  119
Seq. 3  -----------------------------------------------
Seq. 4  TYAIEEYVQKDEQFREWFLKEFPQVERKIQESIEKLRALANGIEEHGCTISNVVS  120
Seq. 2  TYAIEEYVQKDEQFREWFLKEFPQVERKIQESIEKLRALANGIEEHGCTISNVVS  117
Seq. 5  TYAIEEYVQKDEQFREWFLKEFPQVERKIQESIEKLRALANGIEEHGCTISNVVS  192

Seq. 8  SSGAASGIMSLAGLVLAPFTAGTSIALTAAGVGIGAASAVGITTSSIVHSTSSAEAE  179
Seq. 3  -----------------------------------------------
Seq. 4  SSGAASGIMSLAGLVLAPFTAGTSIALTAAGVGIGAASAVGITTSSIVHSTSSAEAE  179
Seq. 2  SSGAASGIMSLAGLVLAPFTAGTSIALTAAGVGIGAASAVGITTSSIVHSTSSAEAE  179
Seq. 5  SSGAASGIMSLAGLVLAPFTAGTSIALTAAGVGIGAASAVGITTSSIVHSTSSAEAE  252

Seq. 8  ASRLTATSDRLKVFKEVRNGTIPNNLSSLNNYATQTIGSEIRARQAARARLRVT  239
Seq. 3  -----------------------------------------------
Seq. 4  ASRLTATSDRLKVFKEVRNGTIPNNLSSLNNYATQTIGSEIRARQAARARLRVT  239
Seq. 5  ASRLTATSDRLKVFKEVRNGTIPNNLSSLNNYATQTIGSEIRARQAARARLRVT  312

Seq. 8  WRISAGGGQAERTIAGTRAVSRGARILTSGTGFLALDVYNLYE5KHLHEGKAS  299
Seq. 3  -----------------------------------------------
Seq. 4  WRISAGGGQAERTIAGTRAVSRGARILTSGTGFLALDVYNLYE5KHLHEGKAS  299
Seq. 4  WRISAGGGQAERTIAGTRAVSRGARILTSGTGFLALDVYNLYE5KHLHEGKAS  300
Seq. 5  WRISAGGGQAERTIAGTRAVSRGARILTSGTGFLALDVYNLYE5KHLHEGKAS  371

Seq. 8  AEELRQAQLEENLMELTQYQLNCPCTGGLHHHHH------------------  340
Seq. 3  AEELRQAQLEENLMELTQYQLNCPCTGGLHHHHH------------------  340
Seq. 4  AEELRQAQLEENLMELTQYQLNCPCTGGLHHHHH------------------  331

Fig. 1.
Fig. 2.
Fig. 3.
INTERNATIONAL SEARCH REPORT

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.:

1-8, 10-12

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.
**A. CLASSIFICATION OF SUBJECT MATTER**

INV. C07K14/775 A61K38/17 A61P33/02
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)

C07K A61K

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

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

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

**C. DOCUMENTS CONSIDERED TO BE RELEVANT**

<table>
<thead>
<tr>
<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>TYTLER E M ET AL: &quot;Reconstitution of the trypanolytic factor from components of a subspecies of human high-density lipoproteins&quot;, MOLECULAR AND BIOCHEMICAL PARASITOLOGY, ELSEVIER SCIENCE PUBLISHERS, AMSTERDAM, NL, vol. 69, no. 1, 1 January 1995 (1995-01-01), pages 9-17, XP001155959, ISSN: 0166-6851, DOI: DOI:10.1016/0166-6851(94)00172-J</td>
<td>1-4,6-8, 10-12</td>
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<td>WO 2010/129267 A2 (UNIV GEORGIA [US]; HARRINGTON JOHN M [US]; HAJDUK STEPHEN L [US]) 11 November 2010 (2010-11-11) claims; figures</td>
<td>1-8, 10-12</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)
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**Date of the actual completion of the international search**

4 April 2012

**Date of mailing of the international search report**

03/07/2012

Name and mailing address of the ISA/

European Patent Office, P.B. 5818 Patentlaan 2
NL - 2280 HV Rijswijk
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Authorized officer

Langer, Astrid
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<td>WO 2007138023 A1</td>
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</table>
This International Searching Authority found multiple (groups of) inventions in this international application, as follows:

1. claims: 1-8, 10-12

   Pharmaceutical compositions/uses comprising apolipoprotein L-3 (ApoL-3), an active fragment thereof, a polynucleotide encoding ApoL-3, a cell transformed with the latter polynucleotide. Use of these compositions for the treatment or prevention of Trypanosoma infection, in particular of infection by T. b. gambiense.

2. claim: 9

   A non-human genetically modified animal, expressing a secretable ApoL-3-derived protein.

3. claims: 13, 16

   An inhibitor of TgsGP for the treatment of Trypanosoma brucei, screening methods for compounds interacting with TgsGP

4. claims: 14, 15

   TgsGP (SEQ ID NO: 6) for use as a medicament, in particular for the treatment or prevention of renal disease