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(54) Title: METHODS FOR SELECTING AN HIV TREATMENT REGIMEN

(57) Abstract: The invention features methods for selecting an HIV treatment regimen for HIV positive subjects. Such regimens include the administration of CD59 inhibitors including ÏLY domain 4. CD59 activity is associated with a decreased sensitivity to endogenous and exogenous anti-HIV antibodies. Desirably, administration of ILY domain 4 polypeptides is sufficient to inhibit CD59 activity while avoiding the general toxicity associated with full length ILY.

METHODS FOR SELECTING AN HIV TREATMENT REGIMEN

Field of the invention

This invention relates to the treatment of HIV.

The complement regulatory protein CD59 is expressed on the surface of mammalian cells to protect host cells from the bystander effects of complement activation. CD59 activity inhibits formation of the membrane attack complex of complement (MAC) by binding to complement proteins C8 and C9 and preventing C9 incorporation and polymerization. During maturation by budding, a number of enveloped viruses, such as human cytomegalovirus, HCMV, human T-cell leukemia virus type 1 (HTLV-1), HIV-1, simian immunodeficiency virus, Ebola virus, influenza virus, and vaccinia virus, capture CD59 and use it to evade the complement system. Other virsuses, (e.g., Herpesvirus saimiri) express a CD59-like molecule that aids the virus in avoiding the complement attack. Additionally, microbial parasites have been identified which also express a CD59-like molecule (e.g., Naegleria fowleri and Schistosoma manosni. These parasites, many of which are intracellular, are protected from human complement mediated lysis by CD59 and also use CD59 for infectivity.

Streptococcus intermedius intermedilysin (ILY) is a cholesterol-dependent cytolysin secreted by Streptococcus intermedius (SI), long suspected to play an important role in the pathogenesis of infectious disease. SI, a gram-positive bacterium, can cause purulent infections in the mouth and internal organs, specifically in the brain and liver. Infections with SI in the brain and liver can lead to abscesses. The protein ILY was assigned to the cholesterol-dependent cytolysin family as the pneumolysin secreted by *Streptococcus pneumoniae* and shows the specific hemolytic activity towards only human erythrocytes, but not towards other animal erythrocytes.

Summary of the Invention

In one aspect, the invention features a method of selecting an ILY domain 4 treatment regimen by detecting or providing CD59 expression levels on T-cells obtained from a subject. Based on this detection or provision, the treatment regimen includes a high dosage of a CD59 antagonist (e.g., ILY domain 4) therapy if elevated levels of CD59 expression are detected or provided, a low dosage of a CD59 antagonist (e.g., ILY domain 4) therapy if low levels of CD59 expression are detected or provided; and the treatment regimen does not include a CD59 antagonist if no CD59 is detected or provided (e.g., the amount of CD59 antagonist included in the HIV treatment regimen is commensurate with CD59 expression levels).

In the above method, detecting CD59 expression levels can include contacting a sample including the subject T-cells with a CD59 binding agent, and measuring the binding of the CD59 binding agent to the T-cells (e.g., by using FACS analysis). An elevated binding of the CD59 binding agent to the T-cells indicates an elevated level of CD59 expression. The CD59 binding agent can be a protein conjugated to a fluorescent or radioactive marker.

In another aspect, the invention features a method of selecting an ILY domain 4 treatment regimen by detecting or providing the anti-HIV antibody level in the plasma of a subject. Based on this detection or provision, the treatment regimen includes a high dosage of a CD59 antagonist (e.g., ILY domain 4) therapy if a low level of anti-HIV antibody is detected or provided and a low dosage of a CD59 antagonist (e.g., ILY domain 4) therapy if an elevated level of anti-HIV antibody is detected or provided.

In another aspect, the invention features a method of selecting an ILY domain 4 treatment regimen by detecting or providing the anti-HIV antibody level in the serum of subject. In this method, the treatment regimen includes anti-HIV therapeutic antibodies if an insufficient level of HIV antibody is detected or provided. The measuring the levels of anti-HIV antibody can include contacting heat-inactivated serum from the HIV positive subject with a source of complement (e.g., serum derived from a non-HIV positive subject) in the absence and presence of saturating concentrations of a CD59 binding agent,

and measuring the amount of viral p24 protein released from the resulting HIV virolysis. Elevated virolysis in the presence of saturating concentrations of CD59 binding agent compared to virolysis in the absence of CD59 binding agent indicates the presence of sufficient anti-HIV antibodies.

In a related aspect, the invention features a method of selecting an ILY domain 4 treatment regimen in an HIV positive subject based on measuring or providing both the expression of CD59 on T cells and the level of anti-HIV antibodies as set forth above.

In any of the foregoing aspects, the invention may further include communicating to an individual (e.g., a doctor or subject) the details of the treatment regimen, or the actual administration to the subject of the indicated treatment regimen.

In any of the forgoing aspects, the CD59 binding agent can be a protein including an anti-CD59 antibody, ILY domain 4 (e.g., a protein with 80%, 90%, 95%, or 100% identity with ILY domain 4 protein), vaginolysin, and fragments thereof.

By "patient" or "subject" is meant any mammal that can be infected with HIV, e.g., a human.

By "intermedilysin" or "ILY" is meant a polypeptide having the activity of a *Streptococcus intermedius* intermedilysin polypeptide. ILY can be purified from *Streptococcus intermedius*, or can be produced recombinantly. An exemplary Genbank Accession number corresponding to the nucleic acid sequence of ILY is AB029317, and an exemplary Genbank Accession number corresponding to the polypeptide sequence of ILY is BAE16324. By ILY is also meant a polypeptide with at least 50%, 60%, 70%, 80%, 90%, 95%, or 99% percent sequence identity to the ILY polypeptide. Additionally and alternatively, ILY is defined as a polypeptide encoded by a nucleic acid that hybridizes under high stringency conditions to a nucleic acid of ILY and has ILY activity. ILY can be isolated from any *Streptococcus intermedius* strain (e.g., strains 1208-1, UNS35, UNS46, and ATCC27335).

By "domain 4 of ILY polypeptide," "ILY domain 4 protein," or "rILYd4" is meant a protein including a fragment of ILY having the activity of

the ILY domain 4 polypeptide. Specifically excluded from this definition is the full length ILY protein having the Genbank Accession number BAE16324. This term is meant to include a protein containing a peptide sequence GALTLNHDGAFVARFYVYWEELGHDADGYETIRSRSWSGNGYNRGA HYSTTLRFKGNVRNIRVKVLGATGLAWEPWRLIYSKNDLPLVPQRNIS TWGTTLHPQFEDKVVKDNTD (SEQ ID NO:1) or RNIRVKVLGATGLAWEPWRLIYSKNDLPLVPQRNISTWGTTLHPQFED KVVKDNTD (SEQ ID NO:2), or a fragment thereof having ILY domain 4 activity. By ILY domain 4 polypeptide is also meant a polypeptide with at least 50%, 60%, 70%, 80%, 90%, 95%, or 99% percent sequence identity to SEQ ID NO:1 or 2. Additionally, ILY domain 4 polypeptide is defined as a polypeptide encoded by a nucleic acid that hybridizes under high stringency conditions to a nucleic acid of the ILY domain 4 polypeptide and has ILY domain 4 activity. The terms are also meant to include any conservative substitutions of amino-acid residues in an ILY domain 4 polypeptide. The term "conservative substitution" refers to replacement of an amino acid residue by a chemically similar residue, e.g., a hydrophobic residue for a separate hydrophobic residue, a charged residue for a separate charged residue, etc. Examples of conserved substitutions for non-polar R groups are alanine, valine, leucine, isoleucine, proline, methionine, phenylalanine, and tryptophan. Examples of substitutions for polar, but uncharged R groups are glycine, serine, threonine, cysteine, asparagine, or glutamine. Examples of substitutions for negatively charged R groups are aspartic acid or glutamic acid. Examples of substitutions for positively charged R groups are lysine, arginine, or histidine. Furthermore, the term ILY domain 4 polypeptide includes conservative substitutions with non-natural amino-acids. This term explicitly excludes full length ILY.

By "fragment" is meant a portion of a polypeptide that contains, preferably, at least 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 95%, or more of the entire length of the reference polypeptide. A fragment may contain at least 10, 20, 30, 40, 50, 60, 70, 80, 90, 100, 110, or 114 amino acids or more.

By "ILY domain 4 activity" is meant the activity of a peptide that antagonizes human CD59 but does not directly cause substantial lysis of human red blood cells (RBCs) in the lysis assay (described in International Application No. PCT/US2008/004191, which is hereby incorporated by reference in its entirety) (e.g., less than 50%, 40%, 30%, 20%, 10%, or 5% lysis when administered at a concentration of 6.4×10^{-7} M).

By "ILY domain 4 therapy" is meant the administration of ILY domain 4 protein to a subject in order to sensitize cells to an antibody mediated immune response. By "high dosage ILY domain 4 therapy" is meant a dosage corresponding to a significant fraction of the maximum tolerated dosage of ILY domain 4 therapy (e.g., 30%, 40%, 50%, 60%, 70%, 80%, 85%, 90%, 95%, 99%, or 100% of the maximum tolerated dosage of ILY domain 4 therapy). By "low dosage of ILY domain 4 therapy" is meant a dosage less than the maximum tolerated dosage of ILY domain 4 therapy.

By "maximum tolerated dosage" is meant a dosage above which, at least some adverse effects would be expected.

By "antagonizing human CD59" is meant decreasing the human CD59 binding to complement proteins C8 and C9, resulting in increased formation of the membrane attack complex of complement (MAC).

By "CD59-like molecule" is meant a molecule expressed by a pathogen that binds domain 4 of the ILY polypeptide. Cells expressing CD59-like molecules are resistant to the lytic effect of complement by inhibiting complete formation of the membrane attack complex of complement.

By "normal serum" is meant serum obtained from a subject that is not HIV positive, and preferably has never been HIV positive.

The term "CD59 binding agent" as used herein refers to one or more compounds that can bind to CD59 (e.g., human CD59). Such binding agents include, among others, ILY domain 4 proteins, or functional fragments thereof, antibodies or antibody-like molecules such as monoclonal antibodies, polyclonal antibodies, antibody fragments, single antibody domains and related molecules, such as scFv, diabodies, engineered lipocalins, camelbodies,

nanobodies and related structures. Also included are soluble mediators, synthetic molecules, or other structures that are known to bind CD59.

By "viral p24 protein" is meant HIV p24 antigen, a major structure core component of HIV-1 (Saifuddin, et al AIDS Res Hum Retroviruses, 1994, 10 (7): 829-37).

By "protein" or "polypeptide" or "peptide" means any chain of more than two natural or unnatural amino acids, regardless of post-translational modification (e.g., glycosylation or phosphorylation), constituting all or part of a naturally-occurring or non-naturally occurring polypeptide or peptide, as is described herein.

As used herein, a natural amino acid is a natural α-amino acid having the L-configuration, such as those normally occurring in natural proteins. Unnatural amino acid refers to an amino acid, which normally does not occur in proteins, e.g., an amino acid having the unnatural D-configuration; or a (D,L)-isomeric mixture thereof; or a homologue of such an amino acid, for example, a β-amino acid, an α,α-disubstituted amino acid, or an α-amino acid wherein the amino acid side chain has been shortened by one or two methylene groups or lengthened to up to 10 carbon atoms, such as an α-amino alkanoic acid with 5 up to and including 10 carbon atoms in a linear chain, an unsubstituted or substituted aromatic (α-aryl or α-aryl lower alkyl), for example, a substituted phenylalanine or phenylglycine.

As used herein, a "peptide of the invention" refers to a linear compound comprising the amino acid sequences of an ILY domain 4 polypeptide and containing only natural amino acids which are linked by peptide bonds and which are in an unprotected form.

The present invention also provides derivatives of the peptides of the invention. Such derivatives may be linear or circular, and include peptides having unnatural amino acids. Derivatives of the invention also include molecules wherein a peptide of the invention is non-covalently or preferably covalently modified by substitution, chemical, enzymatic or other appropriate means with another atom or moiety including another peptide or protein. The

moiety may be "foreign" to a peptide of the invention as defined above in that it is an unnatural amino acid, or in that one or more natural amino acids are replaced with another natural or unnatural amino acid. Conjugates comprising a peptide or derivative of the invention covalently attached to another peptide or protein are also encompassed herein. Attachment of another moiety may involve a linker or spacer, e.g., an amino acid or peptidic linker. Derivatives of the invention also included peptides wherein one, some, or all potentially reactive groups, e.g., amino, carboxy, sulfhydryl, or hydroxyl groups are in a protected form.

The atom or moiety derivatizing a peptide of the invention may serve analytical purposes, e.g., facilitate detection of the peptide of the invention, favor preparation or purification of the peptide, or improve a property of the peptide that is relevant for the purposes of the present invention. Such properties include binding to an human CD59 or suitability for *in vivo* administration, particularly solubility or stability against enzymatic degradation. Derivatives of the invention include a covalent or aggregative conjugate of a peptide of the invention with another chemical moiety, the derivative displaying essentially the same activity as the underivatized peptide of the invention, and a "peptidomimetic small molecule" which is modeled to resemble the three-dimensional structure of any of the amino acids of the invention. Examples of such mimetics are retro-inverso peptides (Chorev et al., Acc. Chem. Res. 26: 266-273, 1993). The designing of mimetics to a known pharmaceutically active compound is a known approach to the design of drugs based on a "lead" compound. This may be desirable, e.g., where the "original" active compound is difficult or expensive to synthesize, or where it is unsuitable for a particular mode of administration, e.g., peptides are considered unsuitable active agents for oral compositions as they tend to be quickly degraded by proteases in the alimentary canal.

Additional examples of derivatives within the above general definitions include the following:

(I) Cyclic peptides or derivatives including compounds with a disulfide bridge, a thioether bridge, or a lactam. Typically, cyclic derivatives containing

a disulfide bond will contain two cysteines, which may be L-cysteine or D-cysteine. Advantageously, the N-terminal amino acid and the C-terminal amino acids are both cysteines. In such derivatives, as an alternative to cysteine, penicillamine (β,β-dimethyl-cysteine) can be used. Peptides containing thioether bridges are obtainable, e.g., from starting compounds having a free cysteine residue at one end and a bromo-containing building block at the other end (e.g., bromo-acetic acid). Cyclization can be carried out on solid phase by a selective deprotection of the side chain of cysteine. A cyclic lactam may be formed, e.g., between the γ-carboxy group of glutamic acid and the ε-amino group of lysine. As an alternative to glutamic acid, it is possible to use aspartic acid. As an alternative to lysine, ornithine or diaminobutyric acid may be employed. Also, it is possible to make a lactam between the side chain of aspartic acid or glutamic acid at the C-terminus and the α-amino group of the N-terminal amino acid. This approach is extendable to β-amino acids (e.g., β-alanine). Alternatively, glutamine residues at the N-terminus or C-terminus can be tethered with an alkenedyl chain between the side chain nitrogen atoms (Phelan et al., J. Am. Chem. Soc. 119:455-460, 1997).

(II) Peptides of the invention, which are modified by substitution. In one example, one or more, preferably one or two, amino acids are replaced with another natural or unnatural amino acid, e.g., with the respective D-analog, or a mimetic. For example, in a peptide containing Phe or Tyr, Phe or Tyr may be replaced with another building block, e.g., another proteinogenic amino acid, or a structurally related analogue. Particular modifications are such that the conformation in the peptide is maintained. For example, an amino acid may be replaced by a α,α-disubstituted amino acid residue (e.g., α-aminoisobutyric acid, 1-amino-cyclopropane-1-carboxylic acid, 1-amino-cyclopentane-1-carboxylic acid, 1 -amino-cyclohexane-1 -carboxylic acid, 4-amino piperidine-4-carboxylic acid, and 1-amino-cycloheptane-1-carboxylic acid).

(III) Peptides of the invention detectably labeled with an enzyme, a fluorescent marker, a chemiluminescent marker, a metal chelate, paramagnetic

particles, biotin, or the like. In such derivatives, the peptide of the invention is bound to the conjugation partner directly or by way of a spacer or linker group, e.g., a (peptidic) hydrophilic spacer. Advantageously, the peptide is attached at the N- or C-terminal amino acid. For example, biotin may be attached to the N-terminus of a peptide of the invention via a serine residue or the tetramer Ser-Gly-Ser-Gly.

(IV) Peptides of the invention carrying one or more protecting groups at a potentially reactive side group, such as amino-protecting group, e.g., acetyl, or a carboxy-protecting group. For example, the C-terminal carboxy group of a compound of the invention may be present in form of a carboxamide function. Suitable protecting groups are commonly known in the art. Such groups may be introduced, for example, to enhance the stability of the compound against proteolytic degradation.

By a "derivative" of a peptide of the invention is also meant a compound that contains modifications of the peptides or additional chemical moieties not normally a part of the peptide. Modifications may be introduced into the molecule by reacting targeted amino acid residues of the peptide with an organic derivatizing agent that is capable of reacting with selected side chains or terminal residues. Methods of derivatizing are described below.

Cysteinyl residues most commonly are reacted with α-haloacetates (and corresponding amines), such as chloroacetic acid or chloroacetamide, to give carboxymethyl or carboxyamidomethyl derivatives. Cysteinyl residues also are derivatized by reaction with bromotrifluoroacetone, α-bromo-β-(5- imidazolyl) propionic acid, chloroacetyl phosphate, N-alkylmaleimides, 3-nitro-2-pyridyl disulfide, methyl 2-pyridyl disulfide, p-chloromercuribenzoate, 2-chloromercuri-4-nitrophenol, or chloro-7-nitrobenzo-2-oxa-1,3-diazole.

Histidyl residues are generally derivatized by reaction with diethylprocarbonate at pH 5.5-7.0 because this agent is relatively specific for the histidyl side chain. Para-bromophenacyl bromide also is useful; the reaction is preferably performed in 0.1 M sodium cacodylate at pH 6.0.

Lysinyl and amino terminal residues are reacted with succinic or other carboxylic acid anhydrides. Derivatization with these agents has the effect of

reversing the charge of the lysinyl residues. Other suitable reagents for derivatizing α-amino-containing residues include imidoesters such as methyl picolinimidate; pyridoxal phosphate; pyridoxal; chloroborohydride; trinitrobenzenesulfonic acid; O-methylisourea; 2,4-pentanedione; and transaminase-catalyzed reaction with glyoxylate.

Arginyl residues are modified by reaction with one or several conventional reagents, among them phenylglyoxal, 2,3-butanedione, 1,2-cyclohexanedione, and ninhydrin. Derivatization of arginine residues requires that the reaction be performed in alkaline conditions because of the high pK_a of the guanidine functional group.

Carboxyl side groups (aspartyl or glutamyl) are selectively modified by reaction with carbodiimides (R'--N--C--N--R') such as 1-cyclohexyl-3-(2-morpholinyl-(4-ethyl) carbodiimide or 1-ethyl-3 (4 azonia 4,4-dimethylpentyl) carbodiimide. Aspartyl and glutamyl residues can also be converted to asparaginyl and glutaminyl residues by reaction with ammonium ions.

Glutaminyl and asparaginyl residues are frequently deamidated to the corresponding glutamyl and aspartyl residues. Alternatively, these residues are deamidated under mildly acidic conditions. Either form of these residues falls within the scope of this invention.

Polypeptides or derivatives thereof may be fused or attached to another protein or peptide, e.g., as a glutathione-S-transferase (GST) fusion polypeptide. Other commonly employed fusion polypeptides include, but are not limited to, maltose-binding protein, *Staphylococcus aureus* protein A, polyhistidine, and cellulose-binding protein.

By a "peptidomimetic small molecule" of a peptide is meant a small molecule that exhibits substantially the same ILY domain 4 activity as the peptide itself.

By "substantially pure polypeptide" is meant a polypeptide or peptide that has been separated from the components that naturally accompany it. Typically, the polypeptide is substantially pure when it is at least 60%, by weight, free from the proteins and naturally-occurring organic molecules with which it is naturally associated. Preferably the polypeptide is an ILY domain 4

polypeptide that is at least 75%, more preferably at least 90%, and most preferably at least 99%, by weight, pure. A substantially pure ILY domain 4 polypeptide may be obtained, for example, by extraction from a natural source (e.g., a fibroblast, neuronal cell, or lymphocyte) by expression of a recombinant nucleic acid encoding an ILY domain 4 polypeptide, or by chemically synthesizing the polypeptide. Purity can be measured by any appropriate method, e.g., by column chromatography, polyacrylamide gel electrophoresis, or HPLC analysis.

A protein is substantially free of naturally associated components when it is separated from those contaminants that accompany it in its natural state. Thus, a protein that is chemically synthesized or produced in a cellular system different from the cell from which it naturally originates will be substantially free from its naturally associated components. Accordingly, substantially pure polypeptides include those derived from eukaryotic organisms but synthesized in *E. coli* or other prokaryotes.

The "percent sequence identity" of two nucleic acid or polypeptide sequences can be readily calculated by known methods, including but not limited to those described in Computational Molecular Biology, Lesk, A. M., ed., Oxford University Press, New York, 1988; Biocomputing: Informatics and Genome Projects, Smith, D. W., ed., Academic Press, New York, 1993; Computer Analysis of Sequence Data, Part I, Griffin, A. M., and Griffin, H. G., eds., Humana Press, New Jersey, 1994; Sequence Analysis in Molecular Biology, von Heinje, Academic Press, 1987; and Sequence Analysis Primer, Gribskov, and Devereux, eds., M. Stockton Press, New York, 1991; and Carillo and Lipman, SIAM J. Applied Math. 48:1073, 1988.

Methods to determine identity are available in publicly available computer programs. Computer program methods to determine identity between two sequences include, but are not limited to, the GCG program package (Devereux et al., Nucleic Acids Research 12:387, 1984), BLASTP, BLASTN, and FASTA (Altschul et al., J. Mol. Biol. 215:403, 1990). The well known Smith Waterman algorithm may also be used to determine identity. The BLAST program is publicly available from NCBI and other sources (BLAST

Manual, Altschul, et al., NCBI NLM NIH Bethesda, Md. 20894). Searches can be performed in URLs such as the following: http://www.ncbi.nlm.nih.gov/BLAST/unfinishedgenome.html; or http://www.tigr.org/cgi-bin/BlastSearch/blast.cgi. These software programs match similar sequences by assigning degrees of homology to various substitutions, deletions, and other modifications. Conservative substitutions typically include substitutions within the following groups: glycine, alanine; valine, isoleucine, leucine; aspartic acid, glutamic acid, asparagine, glutamine; serine, threonine; lysine, arginine; and phenylalanine, tyrosine.

By "hybridize" is meant to form a double-stranded complex containing complementary paired nucleobase sequences, or portions thereof, under various conditions of stringency. (See, e.g., Wahl. and Berger, Methods Enzymol. 152:399 (1987); Kimmel, Methods Enzymol. 152:507 (1987))

By "hybridizes under high stringency conditions" is meant under conditions of stringent salt concentration, stringent temperature, or in the presence of formamide. For example, stringent salt concentration will ordinarily be less than about 750 mM NaCl and 75 mM trisodium citrate, preferably less than about 500 mM NaCl and 50 mM trisodium citrate, and most preferably less than about 250 mM NaCl and 25 mM trisodium citrate. Low stringency hybridization can be obtained in the absence of organic solvent, e.g., formamide, while high stringency hybridization can be obtained in the presence of at least about 35% formamide, and most preferably at least about 50% formamide. Stringent temperature conditions will ordinarily include temperatures of at least about 30° C, more preferably of at least about 37° C, and most preferably of at least about 42° C. Varying additional parameters, such as hybridization time, the concentration of detergent, e.g., sodium dodecyl sulfate (SDS), and the inclusion or exclusion of carrier DNA, are well known to those skilled in the art. Various levels of stringency are accomplished by combining these various conditions as needed. In a preferred embodiment, hybridization will occur at 30° C in 750 mM NaCl, 75 mM trisodium citrate, and 1% SDS. In a more preferred embodiment, hybridization will occur at 37° C in 500 mM NaCl, 50 mM trisodium citrate, 1% SDS, 35% formamide, and

100 μg/ml denatured salmon sperm DNA (ssDNA). In a most preferred embodiment, hybridization will occur at 42° C in 250 mM NaCl, 25 mM trisodium citrate, 1% SDS, 50% formamide, and 200 μg/ml ssDNA. Useful variations on these conditions will be readily apparent to those skilled in the art.

For most applications, washing steps that follow hybridization will also vary in stringency. Wash stringency conditions can be defined by salt concentration and by temperature. As above, wash stringency can be increased by decreasing salt concentration or by increasing temperature. For example, stringent salt concentration for the wash steps will preferably be less than about 30 mM NaCl and 3 mM trisodium citrate, and most preferably less than about 15 mM NaCl and 1.5 mM trisodium citrate. Stringent temperature conditions for the wash steps will ordinarily include a temperature of at least about 25° C, more preferably of at least about 42° C, and most preferably of at least about 68° C. In a preferred embodiment, wash steps will occur at 25° C in 30 mM NaCl, 3 mM trisodium citrate, and 0.1% SDS. In a more preferred embodiment, wash steps will occur at 42° C in 15 mM NaCl, 1.5 mM trisodium citrate, and 0.1% SDS. In a most preferred embodiment, wash steps will occur at 68° C in 15 mM NaCl, 1.5 mM trisodium citrate, and 0.1% SDS. Additional variations on these conditions will be readily apparent to those skilled in the art. Hybridization techniques are well known to those skilled in the art and are described, for example, in Benton and Davis (Science 196:180 (1977)); Grunstein and Hogness (Proc. Natl. Acad. Sci. USA 72:3961 (1975)); Ausubel et al. (Current Protocols in Molecular Biology, Wiley Interscience, New York (2001)); Berger and Kimmel (Guide to Molecular Cloning Techniques, Academic Press, New York, (1987)); and Sambrook et al. (Molecular Cloning: A Laboratory Manual, Cold Spring Harbor Laboratory Press, New York). Preferably, hybridization occurs under physiological conditions. Typically, complementary nucleobases hybridize via hydrogen bonding, which may be Watson-Crick, Hoogsteen or reversed Hoogsteen hydrogen bonding, between complementary nucleobases. For example, adenine and thymine are complementary nucleobases that pair through the formation of hydrogen bonds.

By "therapeutic antibody" is meant a pharmaceutical composition containing an antibody or antibody derivative formulated to treat a pathogenic disease, e.g., HIV or AIDS.

By "HIV treatment regimen" is meant a course of therapy designed to treat HIV infection. The HIV treatment regimens of the invention may include certain dosages of ILY domain 4 administered at certain frequencies. The regimen may further include certain dosages of anti-HIV therapeutic antibodies at certain frequencies. Alternatively, the HIV treatment regimen may not include either ILY domain 4 therapy or anti-HIV antibody therapy.

Brief Description of the Drawings

Figs. 1A and 1B are schematics showing an optimal alignment of the indicated toxin fragments.

Figs. 2A and 2B are histograms showing the amount of hCD59 expressed on the CD59 negative promonocytic cell line U1c (Fig. 2A) and the amount expressed on CD59 positive T CD4+ lymphocytic cell line ACH-2 (Fig. 2B).

Fig. 2C is a graph showing percent virolysis as a function of anti-HIV gp-120 antibody concentration.

Fig. 2D is a graph showing the amount of p24 released in the indicated cells treated with the indicated compound. The abrogation of hCD59 with ILYd4 sensitizes HIV from hCD59 positive cells to complement-mediated virolysis.

Figs. 3A and 3B are graphs showing percent virolysis as a function of concentration of the indicated compound. Viral preparations (20 µl containing 5 ng HIV-1 p24/ml), derived from H9 cells infected with HIV-1MN or HIV-1 chronically infected cell line OM10, were pre-incubated with ILY4 (Fig. 3A) or anti-hCD59 monoclonal Ab (BRIC229, Bristol, Great Britain) (Fig. 3B) at various concentrations as indicated for 30 min at 37°C.

Fig. 3C is a series of histograms showing the level of hCD59 in two cell lines that express hCD59 at a high level. Solid grey curves are stained with

isotype-matched Ab + FITC-labeled secondary Ab. Blank black curves are anti-hCD59 + FITC-labeled secondary Ab.

Fig. 4A is a graph showing percent HIV-1 virolysis in several patients. Two plasma samples from HIV-1-infected were tested and the each sample was repeated once. Open bars, black bars, and gray bars represent ILYd4 treatment, anti-CD59 treatment, and medium alone.

Fig. 4B is a graph showing percent HIV-1 virolysis in samples treated with the indicated compound ILY4 pre-incubation triggers significantly higher complement-mediated virolysis than pretreated with anti-hCD59 antibody or PBS pre-incubation. Pooled data of ILY4 or anti-CD59 Ab treatment experiments from all participants are shown. Horizontal bars represent means of pooled responses.

Fig. 4C is a graph showing percent HIV-1 virolysis in samples treated with the indicated sera and ILYd4.

Fig. 4D is a graph showing the amount of p24 production in cells exposed for 10 days to conditioned medium from virions pretreated with the following conditions: medium alone, anti-CD59 Ab (BRIC 229), rILYd4, and Triton X or originally exposed to heat-inactivated serum. The experiments were repeated twice for each test. The results are represented by mean 6 SD

Fig. 5 is a series of graphs showing percent virolysis in samples treated with the indicated compound of virons isolated from patient serum. HIV-1 primary isolates were derived from six HIV-1–infected patients. PBMCs preincubated with rILYd4 (20 mg/ml), medium only, or anti-hCD59 monoclonal Ab (BRIC 229) were treated with heat-inactivated plasma from 5 HIV-1–positive individuals containing anti–HIV-1 envelope Abs (patients 1–5 shown in Table I) followed by exposure to 10% normal human serum as a source of complement (heat-inactivated normal serum was used as a negative control). Each panel represents the sensitivity of HIV-1 virons derived from one patient to complement-mediated virolysis activated by the endogenous anti–HIV-1 Abs developed in five HIV-1–infected patients who were naive for antiretroviral therapy. Horizontal lines represent the mean. Statistical

significance (p , 0.01 versus medium treatment group) is indicated by an asterisk.

Fig. 6 is a series of graphs showing percent virolysis in samples treated with the indicated compound as induced by anti-HIV-1 antibodies isolated from patients. In the presence of rILYd4, the endogenous anti–HIV-1 Abs lyse the HIV-1 virions through complement-mediated virolysis. In the presence of rILYd4, the endogenous anti–HIV-1 Abs developed in six HIV-1–infected patients are shown to destroying HIV-1 virions through complement-mediated virolysis. Each panel represents the ability of the endogenous anti–HIV-1 Abs developed in one patient to destroy the HIV-1–infected PBMC-derived virions. Horizontal lines represent the mean. Statistical significance (p , 0.01 versus medium treatment group) is indicated by an asterisk.

Detailed Description

The invention features methods of selecting an HIV treatment regimen for an HIV positive subject including a CD59 antagonist (e.g., ILY domain 4 protein) therapy optionally combined with therapeutic anti-HIV antibody therapy. CD59 receptor activity has been associated with decreased sensitivity to antibody (both endogenously produced and therapeutically administered) mediated complement-based lysis. Administration of ILY domain 4 protein is sufficient to inhibit CD59 activity while avoiding the general toxicity associated with full length ILY. Measurement of the concentration of CD59 on immune cells (e.g., T cells) alone, or in combination with measurement of endogenous anti-HIV antibodies, is useful to selecting an appropriate CD59 antagonist (e.g., ILY domain 4) based therapy.

I. Methods of Administration

Therapy according to the invention may be performed alone or in conjunction with another therapy and may be provided at home, the doctor's office, a clinic, a hospital's outpatient department, or a hospital. Treatment optionally begins at a hospital so that the doctor can observe the therapy's effects closely and make any adjustments that are needed, or it may begin on an outpatient basis. The duration of the therapy depends on the type of disease or

disorder being treated, the age and condition of the patient, the stage and type of the patient's disease, and how the patient responds to the treatment.

Routes of administration for the various embodiments include, but are not limited to, topical, transdermal, transcranial, nasal, and systemic administration (such as, intravenous, intramuscular, subcutaneous, inhalation, rectal, buccal, vaginal, intraperitoneal, intraarticular, ophthalmic, otic, or oral administration). As used herein, "systemic administration" refers to all nondermal routes of administration, and specifically excludes topical and transdermal routes of administration.

Dosages

The invention features a method of an HIV treatment regime based on the patient's T-cell CD59 expression and expression of anti-HIV antibodies. ILY domain 4 therapy is indicated with any expression of CD59 on the patient's T-cells. Preferably, the greater the CD59 expression, the greater the dosage of the ILY domain 4 therapy selected. A treatment regimen not including ILY domain 4 therapy is indicated in the absence of detected CD59 expression.

The invention also features the detection of anti-HIV antibodies in order to select an HIV treatment regimen in a patient. In this aspect, therapeutic anti-HIV antibodies are indicated when low levels (including complete absence) of anti-HIV antibodies are detected. In general, greater detection of endogenous antibodies indicates a treatment regime including low dosages of anti-HIV therapeutic antibodies or, if a sufficient amount of antibodies are detected, such detection indicates a treatment regime not including anti-HIV therapeutic antibodies.

In addition, the dosage of peptides (including ILY domain 4 and therapeutic antibodies) of the invention depends on several factors, including: the administration method, the disease to be treated, the severity of the disease, whether the disease is to be treated or prevented, and the age, weight, and health of the person to be treated. Additionally, pharmacogenomic (the effect

of genotype on the pharmacokinetic, pharmacodynamic or efficacy profile of a therapeutic) information about a particular patient may affect dosage used.

Continuous daily dosing with the peptides (including ILY domain 4 and therapeutic antibodies) of the invention may not be required. A therapeutic regimen may require cycles, during which time a drug is not administered, or therapy may be provided on an as needed basis during periods of acute inflammation.

As described above, the peptides of the invention may be administered orally in the form of tablets, capsules, elixirs or syrups, or rectally in the form of suppositories. The peptides may also be administered topically in the form of foams, lotions, drops, creams, ointments, emollients, or gels. Parenteral administration of a compound is suitably performed, for example, in the form of saline solutions or with the compound incorporated into liposomes

These domain 4 ILY polypeptides may be administered concomitantly (in a single or in separate formulations) or within 14 days of a therapeutic antibody.

II. Indications

Therapy may be performed alone or in conjunction with other anti-HIV therapies. The duration of the therapy depends on the age and condition of the patient, the stage and type of the patient's disease, and how the patient responds to the treatment. Therapy may be given in on-and-off cycles that include rest periods so that the patient's body has a chance to recovery from any as yet unforeseen side-effects.

III. Therapeutic antibodies

Methods of developing therapeutic antibodies for use in combination with the ILY domain 4 polypeptide of the invention are well known in the art. An example of such antibodies, for treating HIV, are the humanized antibody hNM-01 (Nakamura et al., Hybridoma, 19:427 (2000)), and the humanized KD-247 antibody (Matsushita et al., Hum Antibodies 14:81-88 (2005)). Other

antibodies (preferably humanized antibodies) can be developed using any epitope of HIV using standard methods.

IV. Selection of Anti-HIV Treatment Regimens

The invention features methods of selecting treatment regimens for an HIV positive subject by measuring the levels of CD59 expression on subject T-cells and, optionally, by measuring the levels of subject anti-HIV antibodies.

HIV patients are able to develop anti-HIV neutralizing antibodies spontaneously and after vaccination. However, HIV virus escapes immune surveillance in most HIV patients leading to a disease progression from HIV infection to AIDS. One explanation for the failure of either natural or vaccine-triggered HIV antibodies is the expression of human CD59 on HIV that allows the virus to escape the antibody-mediated immune response.

Inhibition of human CD59 function with CD59 antagonists (e.g., small molecules, recombinant or synthetic fragments derived from C8 and C9, anti-CD59 antibodies, or ILY domain 4 polypeptides) enhances complement-mediated virolysis of HIV by patient serum carrying anti-HIV antibodies. Therefore, antagonists of human CD59 function represent a useful approach for treatment of HIV/AIDS (see, e.g., International Application No. PCT/US2008/004191, which is hereby incorporated by reference in its entirety).

The therapeutic response of individual patients to a human CD59 antagonist (e.g., ILY domain 4) can be determined by measuring either individually or in combination, the density of human CD59 molecules in infected T-cells, and the level of anti-HIV complement activating antibodies.

Therefore, one aspect of the invention features measuring the density of CD59 on T-cells (e.g., T-cells infected with HIV) by contacting a patient sample containing HIV infected T-cells with CD59 binding agents (e.g., ILY domain 4 protein, fragments derived from C8 and C9, anti-human CD59 antibodies or other human CD59 binding toxins such as vaginolysin) and measuring the binding of these agents to the infected T-cells. This measurement can be achieved using FACS, or other techniques known in the art to measure binding to cell surface molecules.

In another aspect, the invention features the measurement of anti-HIV complement activating antibodies in an HIV positive patent sample. For this assay, the individual patient's heat inactivated serum is used as a source of anti-HIV antibodies and standard normal serum is used as source of complement. Complement-mediated virolysis is measured in the absence and presence of saturating concentrations of ILY domain 4 polpeptides, fragments derived from C8 and C9, neutralizing anti-human CD59 antibodies, or other human CD59 binding toxins such as vaginolysin. Virolysis can be quantified, e.g., by measuring the amount of p24 protein released from the HIV virus. p24 can be measured, for example, by ELISA well known in the art (Saifuddin, et al AIDS Res Hum Retroviruses, 1994, 10 (7): 829-37)(Sullivan, et al, J. Immunol. 1996, 167 (4), 1791-1798).

Based on the density of human CD59 in individual patients T-cells and the levels of anti-HIV complement activating antibodies in their sera, clinicians will be able to either adjust the dose of an anti-human CD59 antagonists like ILY domain 4, and/or if necessary, increase the levels of anti-HIV antibodies by active or passive immunization.

V. Experimental Results

Streptococcus intermedius (SI) is part of the normal human oral micro flora and can cause liver and brain abscesses. ILY secreted by SI specifically binds and lyses CD59 positive human cells. Our results demonstrate that human serum, but not the serum from any other tested species, can neutralize the lytic function of ILY. We have identified an ILY-binding human immunoglobulin (IgG) purified from human serum that exhibits a functional inhibitory effect on ILY-mediated hemolysis *ex vivo* and *in vivo* (see, e.g., International Application No. PCT/US2008/004191, which is hereby incorporated by reference in its entirety).

We have discovered that hCD59 expression in HIV is a critical regulator for protecting HIV from complement-mediated virolysis. The following experiments demonstrate that inhibitors of hCD59, in combination with

antibodies provided by the serum of infected subjects, are sufficient to induce HIV virolysis.

We prepared isolates of HIV from ACH-2 cells that express hCD59 at a high level and of HIV from U1, which lack hCD59 expression. The hCD59 expression of the two cell lines was demonstrated by fluorescent activated cell sorting (FACS) analysis (Fig. 2A and 2B). When exposed to complement and anti-HIV-gp120 antibody, HIV from hCD59 negative cells was sensitive to complement mediated virolysis, while HIV from hCD59 positive cells was resistant to complement-mediated virolysis (Fig. 2C). This result indicates that hCD59 in HIV is a critical regulator for protecting HIV from complement-mediated virolysis.

Pre-incubation of ILYd4 with hCD59 positive HIV from two cell lines expressing hCD59 at high levels blocked hCD59 function and sensitized HIV to anti-HIV gp120/160 antibody dependent complement-mediated virolysis (Fig. 2D) in a dose-dependent manner (Fig. 3A). In contrast, pre-incubation of anti-hCD59 antibody BRIC229 with hCD59 positive HIV from two cell lines expressing hCD59 at high levels did not induce virolysis in a dose dependant manner (Fig. 3B). The level of hCD59 was demonstrated using FACS analysis (Fig. 3C).

Next, we tested whether the anti-HIV antibodies of HIV positive subjects can induce complement mediated virolysis in the presence of an inhibitor of hCD59 (Fig. 4A). Using the sera from HIV patients as a source of anti-HIV antibodies, we demonstrated that ILY4 abrogates hCD59 function and enhances HIV-1 patient antibody-dependent complement-mediated virolysis (Fig. 4B). The sera from several patients induced less complement-mediated virolysis, indicating that these sera may contain lower titers of anti-HIV complement activating antibodies. Preincubation with rILYd4 dramatically increased complement-mediated virolysis of CD59-positive virions exposed to HIV-1 plasma, but not to the control plasma (Fig. 4C). Fig. 4D shows that p24 was undetectable in the supernatant from H9 cells exposed to conditioned medium from Triton X-100 treatment (total lysis), indicating that potentially infective particles were totally lysed and no infectious viral particles remained.

Additionally, we generated primary HIV-1 isolates from six HIV-1–seropositive individuals who were naive for antiretroviral therapy, and we tested whether rILYd4 sensitizes these virions to complement-mediated virolysis. In the same experiment, we assessed the relative potency of endogenous anti–HIV-1 Abs developed by HIV-1–infected patients to promote complement-mediated virolysis of PBMC-derived HIV-1 primary isolates in the presence and absence of rILYd4. To this end, we pretreated the primary HIV-1 isolates with or without rILYd4 and exposed them to heat-inactivated HIV-1 plasma (patients 1 to 5 in Table 2), followed by incubation with pooled normal human serum as a source of complement. The results showed that rILYd4 sensitized each of the six primary HIV-1 isolates to complement-mediated virolysis activated by HIV-1 plasma (Fig. 5). In the presence of rILYd4, each of the five different HIV-1 plasma samples tested significantly increased complement-mediated lysis of each of the six primary HIV-1 isolates (Fig. 6). These effects of rILYd4 were comparable with, albeit much stronger than, those mediated by the anti-hCD59 monoclonal Ab BRIC229 (Figs. 5 and 6). These results confirm that rILYd4 sensitizes HIV-1 to complement-mediated virolysis not only under experimental conditions using cell lines and commercially available Abs, but also of primary HIV-1 isolates sensitized by the endogenous anti–HIV-1 Abs naturally present in the blood of HIV individuals. These results also indicate that inhibition of hCD59 with rILYd4 unprotects HIV-1, unleashing the ability of complement to lyse the virions sensitized by anti–HIV-1 Abs present in the circulation of patients with HIV-1.

Table 1

Patient #	Clinical Stage	CD4+ T cell (count/ul)	Plasma HIV-1 RNA (Copies/ml)	Treatment at Times of Study	Duration HIV-1 Diagnosis (yrs)	Duration of Antivirial Therapy (yrs)	p24 (pg/ml)	Anti HIV-1 env Ab titer
1	Chronic	184	<50	HAART	4	3	Undetectable	2000
2	Acute	319	82	HAART	NA	NA	58.9	10000
3	Chronic	351	17,799	HAART	17	12	153.8	4000
4	Chronic	309	<50	HAART	NA	10	Undetectable	10000
5	Acute	721	5,586	HAART	1	1	82	8000
6	Chronic	674	<50	HAART	16	NA	Undetectable	40000
7	Acute	453	520,909	HAART	1	1	74.6	800
8	Chronic	522	<50	HAART	3	2	Undetectable	800
9	Acute	71	160	HAART	1	1	38	10000
10	Chronic	395	<50	HAART	16	13	Undetectable	4000
11	Chronic	198	9,708	HAART	11	NA	15	1000
12	Chronic	824	<50	HAART	10	8	Undetectable	8000
13	Chronic	670	<50	HAART	9	7	Undetectable	10000
14	Chronic	579	21,990	HAART	17	17	55.5	10000
15	Chronic	296	2,588	HAART	13	13	25.6	8000
16	Chronic	218	152,000	HAART	10	10	129	10000
17	Chronic	362	18,836	HAART	2	1	163.3	10000

Methods

Preparation of HIV

Suspension cell lines were grown in RPMI 1640 (Invitrogen) with 10% fetal bovine serum (Invitrogen), 50 U/mL penicillin, 50 µ g/mL streptomycin (Invitrogen), and 2 mM glutamine (Invitrogen). Cells were treated with 10 ng/mL of PMA (Sigma). After 24 h PMA treatment, supernatant was harvested for measuring HIV-1 p24 by ELISA. Viral preparations (20 µl containing 100 ng HIV-1 p24/ml) derived from the supernatant of PMA-activated ACH-2 or U1 cell cultures.

gp120/160 mediated virolysis

HIV virus was pre-incubated with ILY4 at 20 µg/ml for 30 min at 37°C. After pre-incubation, anti-HIV-1 gp120/160 polyclonal antibodies (Abcom, Cambridge, MA) and complement or heat-inactivated serum were added. HIV-

1 structural protein p24 was then measured by ELISA to determine the extent of virolysis. Treatments with growth medium and Triton X-100 were also included in each experiment to determine background and 100% viral lysis, respectively. Each value represents the mean ± SD of three experiments. Data were compared using the paired two-tailed Student t test.

Native antibody mediated virolysis

Viral preparations (20 µl containing 5 ng HIV-1 p24/ml) derived from OM 10, an HIV-1 chronically infected cell line, were pre-incubated with ILY4 or anti-hCD59 monoclonal Ab (BRIC229, Bristol, Great Britain) at 300 µg/ml for 30 min at 37°C in a 5% CO_2 incubator. After pre-incubation, plasma from HIV-1-infected individuals (1:5 at final dilution) and complement or heat-inactivated serum (1:10 at final dilution) were added. Treatments with growth medium and Triton X-100 were also included in each experiment to determine 0 and 100% viral lysis, respectively. Percentage of virolysis was calculated by measuring the release of HIV-1 p24 caused by complement activation compared to total p24 content released by detergent.

Measurement of HIV-1 p24 in plasma samples from HIV-1–infected patients

Plasma specimens were tested for HIV-1 p24 Ag using the Perkin Elmer HIV-1 ELISA kit as described above. Each plasma sample was treated with the lysis buffer included in the ELISA kit to lyse the viral particles for releasing HIV-1 core protein p24, which was then measured.

Preparation of HIV-1 Isolates from Patients

HIV-1 primary isolates were generated by coculture of PBMCs from HIV-1– infected and healthy donors. PBMCs were prepared from heparinized peripheral blood donated by six HIV-1–seropositive patients naive for antiretroviral therapy (patients 1–6 in Table I) and by HIV-1–seronegative donors. PBMCs from seronegative and seropositive individuals were stimulated separately for 2 days with PHA (5 mg/ml) and cocultured at a 1:3

ratio in the presence of IL-2 (10 ng/ml) in complete RPMI 1640 medium (200 ml per well) in 96-well round- bottom plates. After 7 days of coculture, supernatants were harvested, aliquoted, and stored at -80°C as HIV-1 primary isolate stocks for virolysis assay.

Complement-mediated virolysis activated by anti–HIV-1 Abs in plasma of HIV-1-infected patients

Viral preparations (20 ml; 5 ng HIV-1 p24/ml) derived from the chronically-infected cell line OM10 or from primary HIV-1 isolates were preincubated for 30 min at 37°C with either rILYd4 (20 mg/ml) or neutralizing anti-hCD59 monoclonal Ab (30 mg/ml; BRIC229). After preincubation, heat-inactivated plasma from either HIV-1–infected or healthy individuals (1:5 at final dilution) were individually added as a source of endogenous Abs, followed by exposure to either complement-competent or heat-inactivated human serum diluted in GVB++ buffer. Triton X-100 was used for determining the total virolysis. Experiments were conducted in duplicates and the paired two-tailed Student's t test was used to compare the means 6 SD.

Other Embodiments

Various modifications and variations of the described methods and compositions of the invention will be apparent to those skilled in the art without departing from the scope and spirit of the invention. Although the invention has been described in connection with specific desired embodiments, it should be understood that the invention as claimed should not be unduly limited to such specific embodiments. Indeed, various modifications of the described modes for carrying out the invention that are obvious to those skilled in the fields of medicine, immunology, pharmacology, endocrinology, or related fields are intended to be within the scope of the invention.

All publications mentioned in this specification are herein incorporated by reference to the same extent as if each independent publication was specifically and individually incorporated by reference.

What is claimed is:

1. A method of selecting an ILY domain 4 treatment regimen, said method comprising detecting CD59 expression levels on T-cells obtained from a subject and on the basis of said CD59 expression levels selecting an ILY domain 4 treatment regimen, wherein said treatment regimen comprises a high dosage of ILY domain 4 therapy if elevated levels of CD59 expression are detected; and wherein said treatment regimen comprises a low dosage of ILY domain 4 therapy if low levels of CD59 expression are detected.

2. The method of claim 1 further comprising detecting the anti-HIV antibody level in the serum of said subject, wherein said treatment regimen further comprises administering anti-HIV therapeutic antibodies to said subject if said level in said subject is insufficient.

3. The method of claim 1, wherein said detecting CD59 expression levels comprises: contacting a sample comprising said T-cells obtained from said subject with a CD59 binding agent, and measuring the binding of said CD59 binding agent to said T-cells; whereby elevated binding of said CD59 binding agent to said T-cells indicates elevated CD59 expression levels.

4. The method of claim 3, wherein said CD59 binding agent comprises a protein selected from the group consisting of an anti-CD59 antibody, ILY domain 4, vaginolysin, and fragments thereof.

5. The method of claim3, wherein said CD59 binding agent comprises a protein with at least 95% sequence identity to ILY domain 4.

6. The method of claim 4, wherein said protein is conjugated to a fluorescent marker or radioactive marker.

7. The method of claim 3, wherein said binding is measured using FACS analysis.

8. The method of claim 2, wherein said detecting anti-HIV antibody levels comprises contacting heat-inactivated serum from said HIV positive subject with a source of normal complement in the absence and presence of saturating concentrations of a CD59 binding agent, and measuring the amount of viral p24 protein released from the resulting HIV virolysis, whereby elevated virolysis in the presence of saturating concentrations of CD59 binding agent compared to virolysis in the absence of CD59 binding agent diagnoses said HIV positive subject as having sufficient anti-HIV antibodies.

9. The method of claim 8, wherein said source of complement is serum derived from a non-HIV positive subject.

10. The method of claim 8, wherein said CD59 binding agent comprises a protein selected from the group consisting of an anti-CD59 antibody, ILY domain 4, vaginolysin, and fragments thereof.

11. The method of claim 10, wherein said CD59 binding agent comprises a protein with at least 95% sequence identity to ILY domain 4 protein.

12. A method of selecting an ILY domain 4 treatment regimen, said method comprising providing CD59 expression levels on T-cells obtained from a subject and on the basis of said CD59 expression levels selecting an ILY domain 4 treatment regimen, wherein said treatment regimen comprises a high dosage of ILY domain 4 therapy if elevated levels of CD59 expression are provided; and wherein said treatment regimen comprises a low dosage of ILY domain 4 therapy if low levels of CD59 expression are provided.

13. The method of claim 12 further comprising providing the anti-HIV antibody level in the serum of said subject, wherein said treatment regimen further comprises administering anti-HIV therapeutic antibodies to said subject if said level in said subject is insufficient.

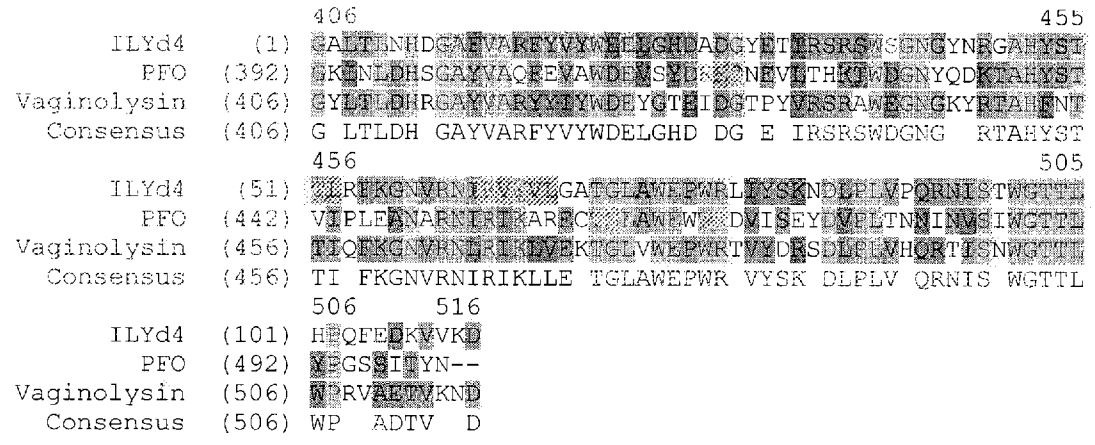
14. A method of selecting an ILY domain 4 treatment regimen, said method comprising detecting the anti-HIV antibody level in the serum of said subject and, on the basis of said anti-HIV antibody level, selecting an ILY domain 4 treatment regimen, wherein said treatment regimen comprises a high dosage of ILY domain 4 therapy if low levels of anti-HIV antibody are detected; and wherein said treatment regimen comprises a low dosage of ILY domain 4 therapy if elevated levels of anti-HIV antibody are detected.

15. A method of selecting an ILY domain 4 treatment regimen, said method comprising providing the anti-HIV antibody level in the serum of said subject and, on the basis of said anti-HIV antibody level, selecting an ILY domain 4 treatment regimen, wherein said treatment regimen comprises a high dosage of ILY domain 4 therapy if low levels of anti-HIV antibody are provided; and wherein said treatment regimen comprises a low dosage of ILY domain 4 therapy if elevated levels of anti-HIV antibody are provided.

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Fig. 1A

Domain 4

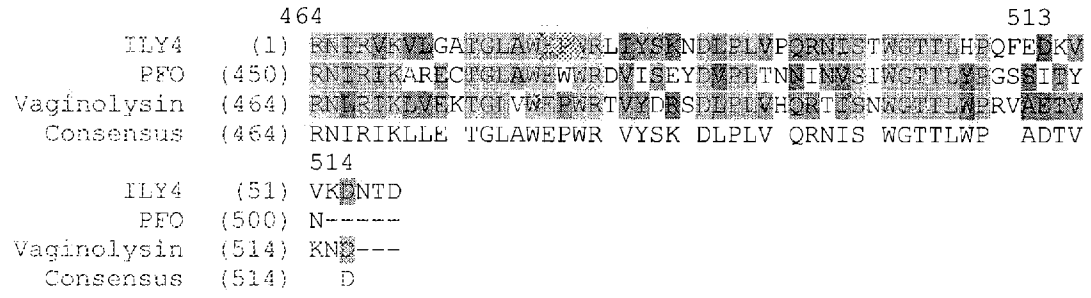


Consensus positions: 84.7%

Identity positions: 34.2%

Fig. 1B

Truncated domain 4



Consensus positions: 83.0%

Identity positions: 39.6%

Fig. 2A

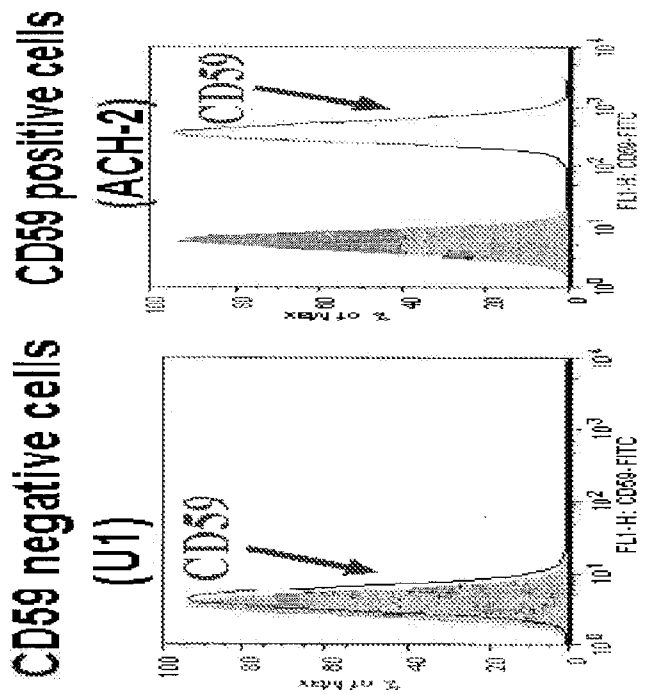


Fig. 2B

Fig. 2C

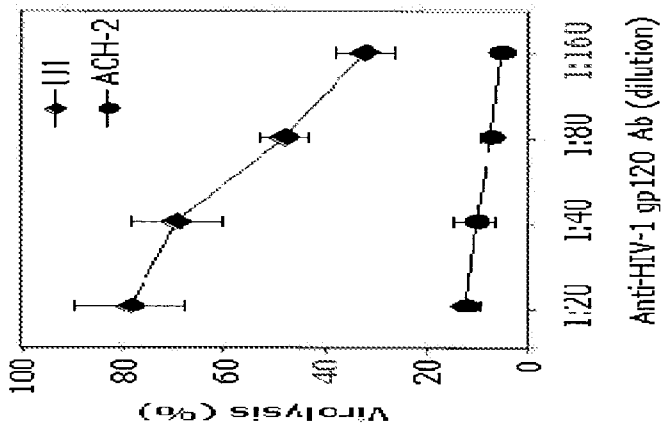


Fig. 2D

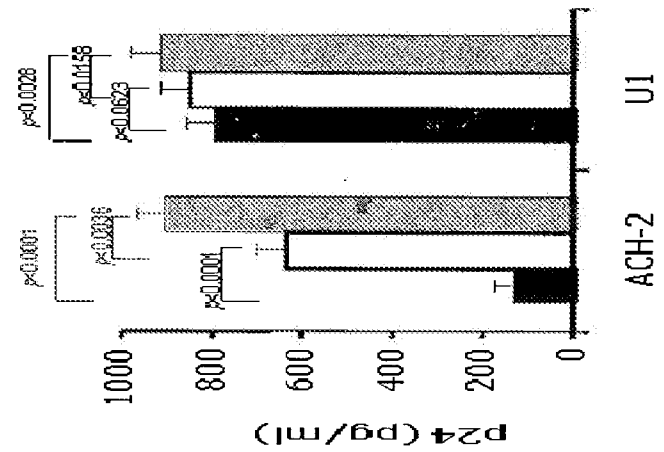


Fig. 3A

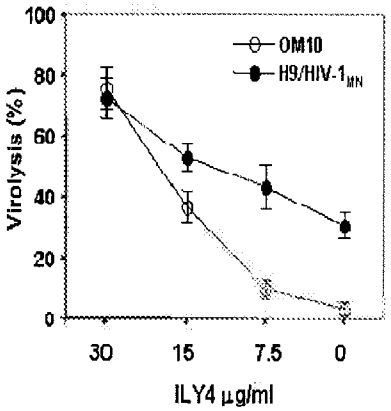


Fig. 3B

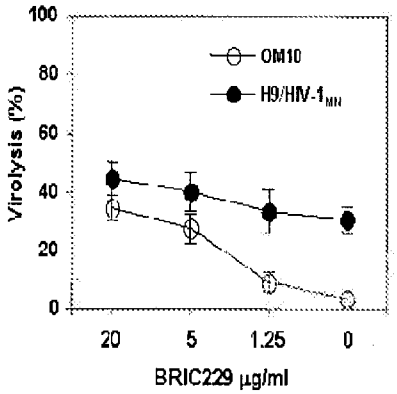


Fig. 3C

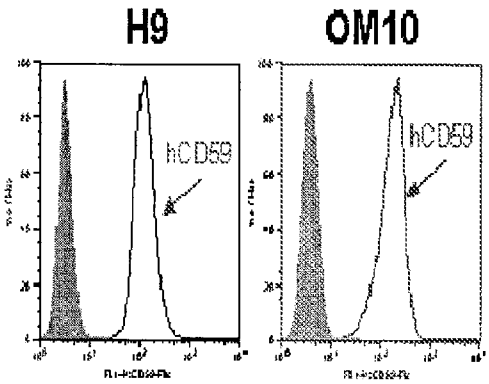


Fig. 4A

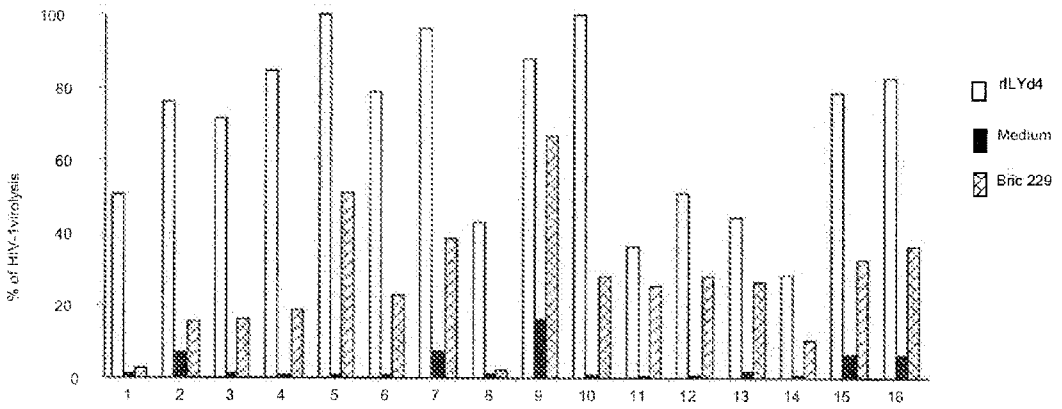


Fig. 4B

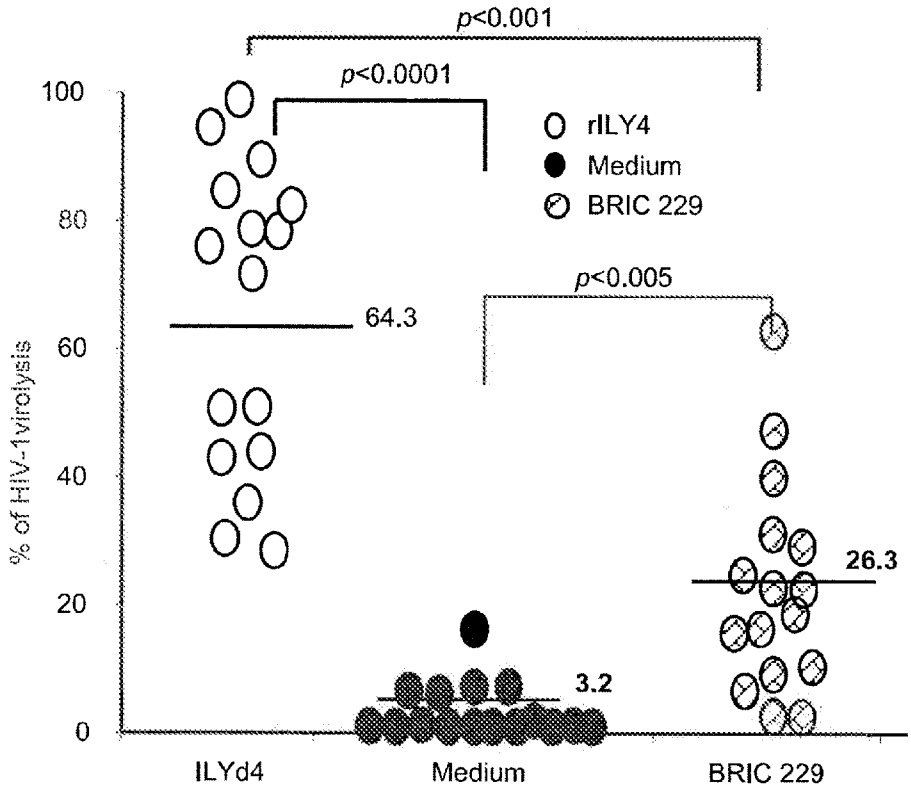

p<0.001
p<0.0001
p<0.005
rILY4
Medium
BRIC 229
64.3
26.3
3.2
% of HIV-1virolysis
100
80
60
40
20
0
ILYd4
Medium
BRIC 229

Fig. 4C

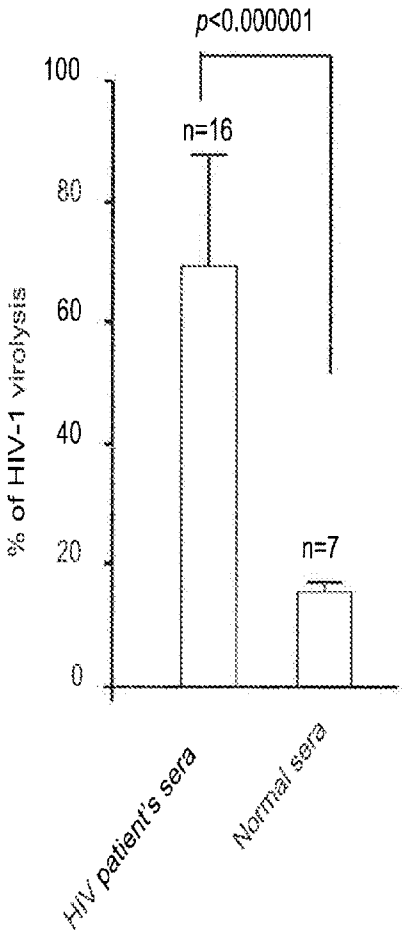

p<0.000001
n=16
n=7
% of HIV-1 virolysis
100
80
60
40
20
0
HIV patient's sera
Normal sera

Fig. 4D

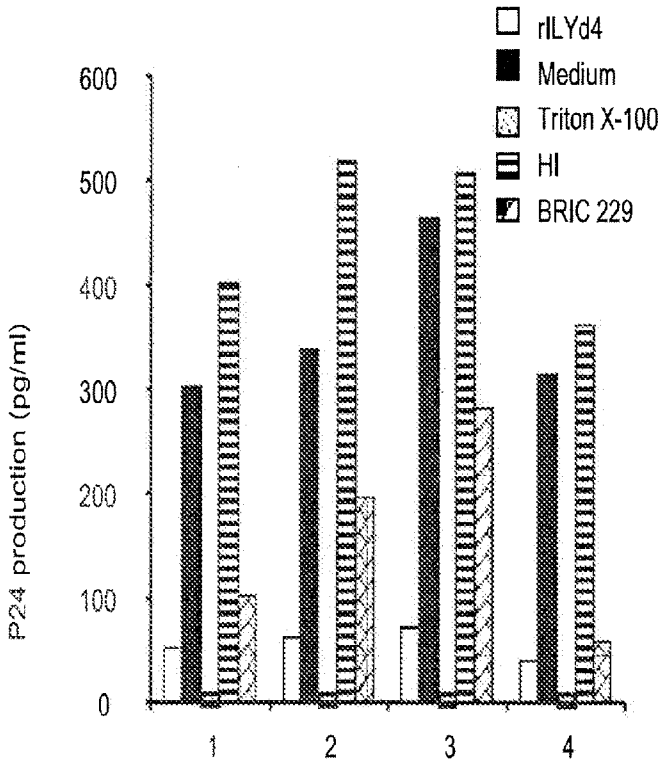

rILYd4
Medium
Triton X-100
HI
BRIC 229
P24 production (pg/ml)
600
500
400
300
200
100
0
1
2
3
4

Fig. 5

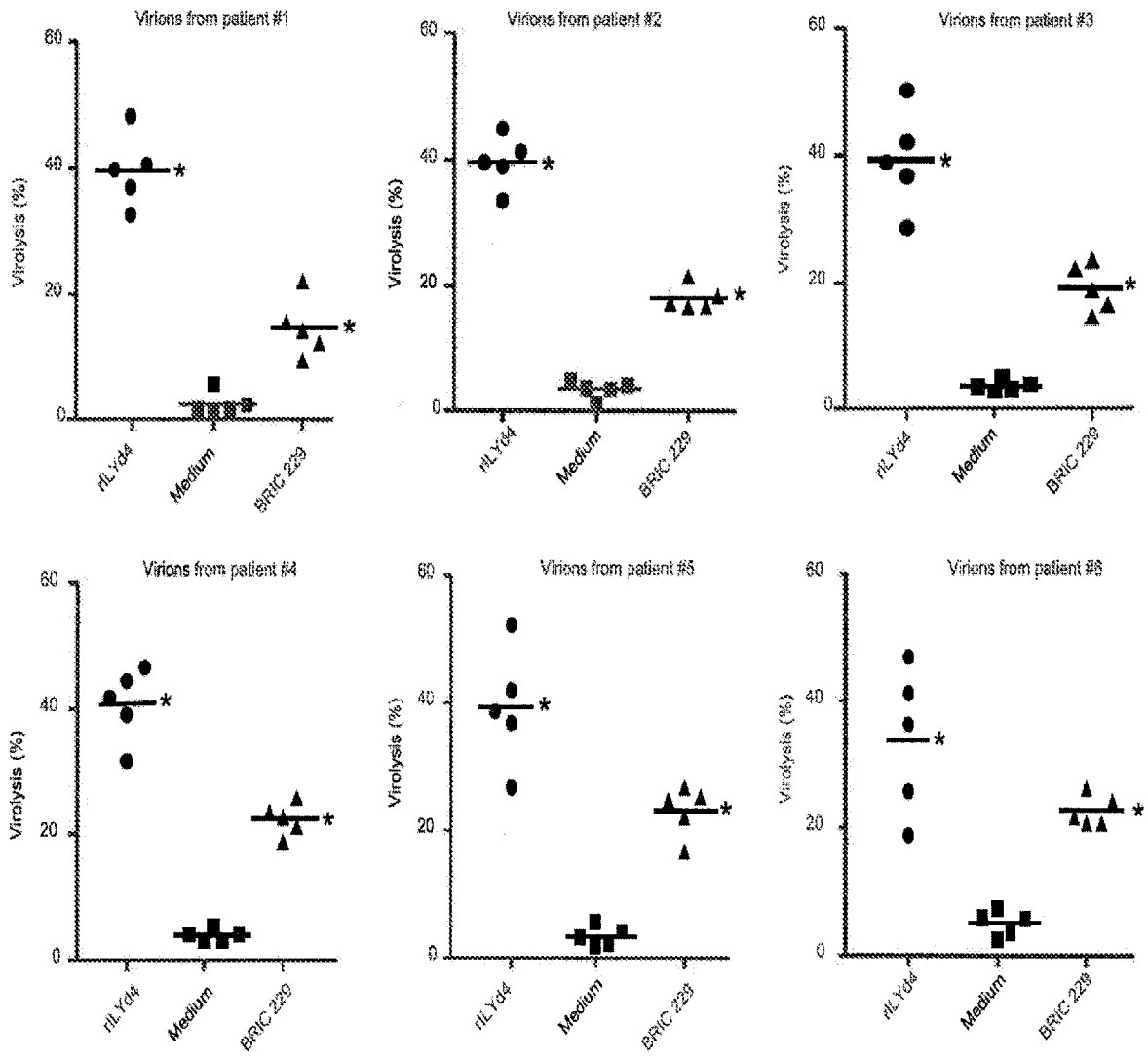
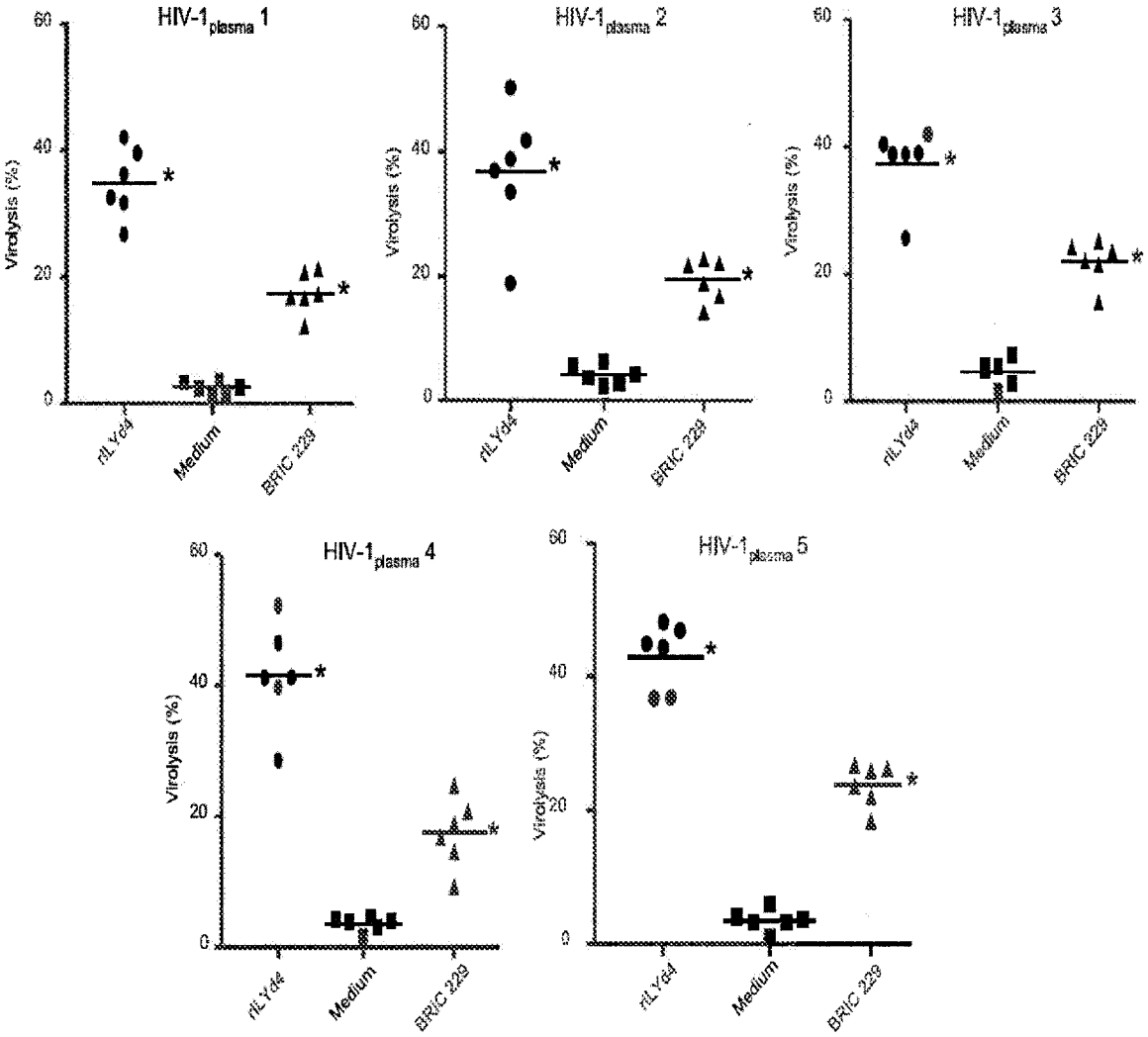

Virions from patient #1
Virions from patient #2
Virions from patient #3
Virions from patient #4
Virions from patient #5
Virions from patient #6
Virolysis (%)
0
20
40
60
rILYd4
Medium
BRIC 229

Fig. 6


HIV-1plasma 1
HIV-1plasma 2
HIV-1plasma 3
HIV-1plasma 4
HIV-1plasma 5
Virolysis (%)
0
20
40
60
rIL Ydd
Medium
BRIC 229

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 09/69694

A. CLASSIFICATION OF SUBJECT MATTER
IPC(8) - C12N 7/00 (2010.01)
USPC - 435/339.1
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)
USPC: 435/339.1

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched
USPC: 435/5,6,91.33,339,337,336,339.1,344.1 (text search)

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
Electronic data bases: PubWEST (EPAB, JPAB, PGPB, USPT); Google Scholar
Search terms: intermedilysin (ILY), domain 4 (ILYd4), CD59, HIV, virolysis

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X ---- Y	HU et al. Domain 4 of ILY sensitizes antibody therapy on cancer and HIV through abrogating human CD59 function. FASEB J. April 2008 Vol 22 No 2-Meeting Abstracts Abstract: lb522. Abstract only.	1, 2, 12-15 ---------- 3-11
Y	FLETCHER et al. New monoclonal antibodies in CD59: use for the analysis of peripheral blood cells from paroxysmal nocturnal haemoglobinuria (PNH) patients and for the quantitation of CD59 on normal and decay accelerating factor (DAF)-deficient erythrocytes. Immunology March 1992 Vol 75 No 3 Pages 507-512. Especially abstract, pg 508 right col para 2, pg 510 table 1 legend.	3-7
Y	SAIFUDDIN et al. Role of virion-associated glycosylphosphatidylinositol-linked proteins CD55 and CD59 in complement resistance of cell line-derived and primary isolates of HIV-1. J Exp Med 1 August 1995 Vol 182 No 2 Pages 501-509. Especially pg 502 left col para 4-6, pg 502 right col para 2.	8-11

☐ Further documents are listed in the continuation of Box C. ☐

* 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
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"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	Date of mailing of the international search report
4 March 2010 (04.03.2010)	12 MAR 2010
Name and mailing address of the ISA/US Mail Stop PCT, Attn: ISA/US, Commissioner for Patents P.O. Box 1450, Alexandria, Virginia 22313-1450 Facsimile No. 571-273-3201	Authorized officer: Lee W. Young PCT Helpdesk: 571-272-4300 PCT OSP: 571-272-7774