Title: NOVEL HIV-2 ISOLATE

Abstract: The invention provides a novel strain of HIV-2 capable of causing immunodeficiency. The invention also provides compositions comprising the nucleic acids and polypeptides characteristic of this HIV-2 virus, antibodies specific for this HIV-2 virus, methods of using these compositions, and methods of detecting HIV-2 virus.
NOVEL HIV-2 ISOLATE

DESCRIPTION OF THE INVENTION

[001] This invention provides a new strain of HIV-2 virus, nucleic acids and polypeptides derived from the virus, methods of detecting HIV-2 virus infection, and methods of treating a patient infected with HIV-2. This application claims the benefit of priority to U.S. Provisional Patent Application Nos. 61/14,807, filed November 14, 2008, and 61/163,190, filed March 25, 2009, the entirety of which are incorporated by reference.

[002] Substantial progress has been made in our understanding of acquired immune deficiency syndrome (AIDS). The principal causative agent of AIDS is human immunodeficiency virus (HIV), a non-transforming retrovirus with a tropism for CD4 T-helper cells. AIDS is characterized by a progressive depletion of the CD4 T cell population with a concomitant increasing susceptibility to the opportunistic infections that are characteristic of the disease. Epidemiological studies indicate that HIV-1 is the etiological agent responsible for the majority of AIDS cases and these infections are widely spread throughout the world.

[003] A second type of HIV, HIV-2, has been isolated from patients in West Africa, but has not appreciably spread beyond this area. The incidence of HIV-2 infection has declined over the last 16-20 years (Hamel et al., AIDS Res Hum Retroviruses, 23:1 189-96 (2007); Van der Loeff et al., M J Epidemiol 35:1322-28 (2006)). There are at least 8 known subtypes of HIV-2, referred to as subtypes A-H. The majority of human HIV-2 infections are caused by subtypes A and B, which are known as the epidemic subtypes. Only a small percentage of individuals infected with HIV-2 subtypes A and B develop the immunodeficiency characteristic of AIDS (Marlink et al., Science 265:1587-90 (1994)). Infections with the non-epidemic HIV-2 subtypes C-G are generally known only as single person infections, and have not been shown to lead to immunodeficiency (Gao et al., J Virol 68:7433-47 (1994); Chen et al., J Virol 71:2953-60 (1997)). There is also only one known
case of a person infected with HIV-2 subtype H, and this virus caused immunodeficiency in the infected man from the Ivory Coast (Damond et al., *AIDS Res Hum Retroviruses*, 20:666-72 (2004)).

[004] HIV-2 subtype F was discovered 16 years ago in an individual from Sierra Leone (Chen et al., *J Virol* 71:2953-60 (1997)). Despite repeated attempts, live virus was never isolated from the infected patient and the patient remained healthy during the time of observation.

[005] We have now identified a new strain of HIV-2. It is accordingly a primary object of the invention to provide a novel strain of Subtype F HIV-2, nucleic acids and polypeptides derived from this virus, and methods of detecting the presence of this novel virus in patients and cell culture.

[006] In one embodiment, the invention provides an isolated HIV-2 virus comprising the virus deposited at the American Type Culture Collection ("ATCC") (ATCC, 10801 University Boulevard. Manassas, Virginia 201 10-2209) as HIV-2NWK08F. In some embodiments, this HIV-2 virus comprises the nucleotide sequence of SEQ ID NO:1 or SEQ ID NO:3. In an additional embodiment, the HIV-2 virus is a variant of HIV-2NWK08F, wherein the variant comprises a nucleotide sequence with 95% homology to SEQ ID NO:1 or SEQ ID NO:3.

[007] In one embodiment, the invention provides a nucleic acid comprising a nucleotide sequence of SEQ ID NO:1 or SEQ ID NO:4, or fragments thereof. This nucleic acid may comprise at least 6 contiguous nucleotides of SEQ ID NO:1 or SEQ ID NO:4. In some embodiments, the nucleic acid contains a detectable label.

[008] In other embodiments, the invention provides a polypeptide comprising an amino acid sequence of SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:5, or SEQ ID NO:6, or fragments thereof. This polypeptide may comprise at least 6 contiguous amino acids of SEQ
ID NO:2, SEQ ID NO:3, SEQ ID NO:5, or SEQ ID NO:6. In particular embodiments the polypeptide encodes the Env, Pol, Gag, or Nef protein of HIV-2NWK08F.

[009] In another embodiment, the invention provides an antibody that specifically binds to a polypeptide derived from the HIV-2NWK08F virus. In some embodiments, the antibody specifically binds to an Env, Pol, Gag, or Nef polypeptide of HIV-2NWK08F, but does not bind to Env polypeptides from other HIV-I or HIV-2 virus strains.

[010] In another embodiment, the antibody specifically binds to an Env, Pol, Gag, or Nef polypeptide of HIV-2 subtype F, but does not bind to Env polypeptides from other HIV-I or HIV-2 virus strains or subtypes.

[011] The invention also provides compositions and methods for detecting HIV-2NWK08F nucleic acids and polypeptides. In one embodiment, the invention provides a composition for detecting HIV-2NWK08F nucleic acids comprising at least 6 contiguous nucleotides of SEQ ID NO:1 or SEQ ID NO:4. In another embodiments, the invention provides a method for detecting HIV-2NWK08F nucleic acids in a biological sample comprising

(a) contacting the biological sample with a composition comprising a nucleotide sequence of HIV-2NWK08F; and

(b) detecting the hybridization of the HIV-2NWK08F nucleotide sequence to the nucleic acid of the biological sample.

[012] In some embodiments, the methods for detecting HIV-2NWK08F nucleic acids involve the amplification of HIV-2NWK08F nucleic acids prior to or during the detection step.

[013] In one embodiment, the invention provides a method of detecting HIV-2NWK08F nucleic acids in a biological sample comprising

(a) contacting the biological sample with primers derived from HIV-2NWK08F capable of amplifying an HIV-2NWK08F genome;
(b) amplifying the HIV-2NWK08F nucleic acid; and

(c) detecting the presence of amplified HIV-2NWK08F nucleic acid.

[014] In another embodiment, the invention provides a composition for detecting HIV-2NWK08F polypeptides comprising an antibody specific for those polypeptides. In an exemplary embodiment, the invention provides a method for detecting HIV-2NWK08F polypeptides in a biological sample comprising

(a) contacting the biological sample with a composition comprising at least one anti-HIV-2NWK08F antibody; and

(b) detecting an immunological complex formed between the polypeptide and the antibody used.

[015] The invention also provides compositions and methods of detecting antibodies specific for HIV-2NWK08F. In one embodiment, the invention provides compositions for detecting antibodies specific for HIV-2NWK08F comprising at least 6 contiguous amino acids of SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:5, or SEQ ID NO:6. In another embodiment, the invention provides a method for detecting antibodies against HIV-2NWK08F virus in a biological sample comprising:

(a) contacting the biological sample with a composition comprising at least one antigen of the HIV-2NWK08F virus; and

(b) detecting an immunological complex formed between the anti-HIV-2NWK08F antibodies and the antigen used.

[016] In another embodiment, the invention provides a method for the preparation of hybridomas which produce monoclonal antibodies specific for the HIV-2NWK08F Env of Gag polypeptide, comprising the following the steps:

(a) immunizing a mammal with a polypeptide from the HIV-2NWK08F Env or Gag polypeptide or immunogenic fragment thereof;

(b) isolating immunized splenocytes from said mammal;
(c) fusing the immunized splenocytes with a myeloma cell line to produce hybridomas;
(d) selecting for the hybridomas by culturing in selective media;
(e) clonally expanding the hybridomas in appropriate culture media; and,
(f) identifying and characterizing those hybridomas that produce monoclonal antibodies specific for HIV-2NWK08F Env, Pol, Gag, or Nef polypeptide.

[017] In yet another embodiment, the invention provides methods of treating patients infected with HIV-2NWK08F by administering anti-viral drugs.

[018] Additional objects and advantages of the invention will be set forth in part in the description which follows, and in part will be obvious from the description, or may be learned by practice of the invention. The objects and advantages of the invention will be realized and attained by means of the elements and combinations particularly pointed out in the appended claims.

[019] It is to be understood that both the foregoing general description and the following detailed description are exemplary and explanatory only and are not restrictive of the invention, as claimed.

**BRIEF DESCRIPTION OF THE DRAWINGS**

[020] Figure 1 is a phylogenetic tree showing the relationship between HIV-2NWK08F env gene sequences to other HIV and SIV strains.

[021] Figure 2 is a phylogenetic tree showing the relationship between HIV-2NWK08F gag gene sequences to other HIV and SIV strains.

**BRIEF DESCRIPTION OF THE SEQUENCES**

[022] The instant application contains a Sequence Listing which has been submitted via EFS-Web and is hereby incorporated by reference in its entirety. Said ASCII copy, created on November 12, 2009, is named 09932000.txt, and is 25,235 bytes in size.
SEQ ED NO: 1 is a partial nucleotide sequence of the env and nef genes of HIV-2NWK08F.

SEQ ED NO:2 is a partial amino acid sequence of the Env polypeptide of HTV-2NWK08F.

SEQ ED NO:3 is a partial amino acid sequence of the Nef polypeptide of HIV-2NWK08F.

SEQ ED NO:4 is the full nucleotide sequence of gag gene of HTV-2NWK08F and a partial nucleotide sequence of the 5'-LTR and the pol gene of HTV-2NWK08F.

SEQ ED NO:5 is the full amino acid sequence of the Gag polypeptide of HTV-2NWK08F.

SEQ ED NO:6 is a partial amino acid sequence of the Nef polypeptide of HTV-2NWK08F.

SEQ ID NOs:7-20 are primers derived from the env/ nef genes of HTV-2 subtype F virus.

SEQ ID NOs:21-36 are primers derived from the gag/pol gene of HIV-2 subtype F virus.

SEQ ED NOs:37-40 are primers derived from the env gene of HTV-2 subtype F virus.

SEQ ID NOs:41-44 are primers derived from the gag gene of HTV-2 subtype F virus.

SEQ ED NOs:45 and 46 are primers derived from the env gene of HTV-2NWK08F for use in a real time PCR assay for HTV-2NWK08F viral load.

SEQ ID NO:47 is a probe derived from the env gene of HTV-2NWK08F for use in detection of the PCR products in a real time PCR assay for HTV-2NWK08F viral load.
DESCRIPTION OF THE EMBODIMENTS

[035]  The invention is based, in part, on the discovery of a novel strain of HIV-2 in a patient in Newark, New Jersey. The invention is further based, in part, on the isolation of an HIV-2 virus, referred to as HTV-2NWK08F, from the patient. The invention is also based, in part, on the nucleic acid sequence of this virus, and in particular, the nucleic acid sequence of the genes encoding the Gag and Env polypeptides of this virus.

[036]  Patient X, a 68 year old man, moved from his homeland of Sierra Leone to New Jersey in 2007. During the immigration process, he tested positive in a general screen for antibodies to HIV. Upon further investigation, Patient X repeatedly tested positive for antibodies in an assay that detected antibodies against either HIV-1 or HIV-2. However, the patient's blood tested negative for HIV-1 by Western blot and PCR assays. His HIV-1 viral load was below the lower limit of the assay (a quantitative RT-PCR assay from LabCorp). Interestingly, the patient tested positive for HIV-2 antibodies, but a PCR assay for HTV-2 proviral DNA was negative, indicating that the patient may have been infected with an unidentified strain of HIV-2.

[037]  Patient X had a CD4 T-cell count of 338 cells/μl and a CD4:CD8 ratio of 0.52. These results are indicative of CD4 T-cell lymphopenia. The combination of the results from the HTV testing and the patient's reduced CD4 T-cell count suggested that Patient X was actively infected with an unrecognized strain of HTV-2.

[038]  Co-culture of Patient X's peripheral blood mononuclear cells (PBMCs) with PHA-stimulated normal donor PMBCs or CEM-X-174 cells resulted in viral isolation. Portions of the env and gag genes of the provirus produced by these cells were successfully amplified by PCR with primers derived from an HIV-2 subtype F strain (Chen et al., J Virol 71:3953-3960 (1997), incorporated by reference herein). The amplified regions were sequenced and a real-time PCR protocol was developed with primers derived from the env gene of the new virus to quantify viral load. Two recent studies of HIV-2 infected
individuals found the median proviral load to be ~300 copies per $10^6$ PBMC (Gottleib et al., *AIDS* 22: 1379-80 (2008); Popper et al., *J Virol* 74:1554-57 (2000)). In contrast, Patient X had a proviral load of 6,100 copies per $10^6$ PBMC.

[039] The sequenced regions of the new virus were subjected to phylogenetic comparisons to existing HTV strains (Posada, et al., *Syst Biol* 50:580-601 (2001); Swofford, *PAUP*. Phylogenetic analysis using parsimony (*and other methods), version 4. Sinauer Associates, Sunderland, Mass (1999)). HTV-2NWK08F clustered significantly with four other viruses, all from Sierra Leone. Two of the viruses (strains of HTV-2 subtypes E and F) have not been known to cause immune suppression, nor have they been shown to be transmitted from person to person. The other 2 viruses were simian immunodeficiency viruses (SFVs) found in sooty mangabey monkeys in Sierra Leone, documenting transmission from monkey to humans in the area. It is unknown how Patient X acquired the virus. Since he denies exposure to monkeys and has not received blood or blood product transfusions, he most likely contracted the virus from another person.

[040] Accordingly, the invention provides a new strain of HIV-2 virus, the nucleotide sequence of the virus, viral proteins and fragments thereof, methods of detecting HTV-2 virus infection, and methods of treating a patient infected with HIV-2.

VIRUSES

[041] The invention provides an isolated virus, known as HIV-2NWK08F. HTV-2NWK08F was deposited with the American Type Culture Collection ("ATCC") (ATCC, 10801 University Boulevard, Manassas, Va. 20110-2209) according to the provisions of the Budapest Treaty. All restrictions on the availability to the public of the above ATCC deposit will be irrevocably removed upon the granting of a patent on this application. In another embodiment, the invention provides variants of this virus comprising a nucleotide sequence of SEQ ID NO:1 or SEQ ID NO:3 or a sequence substantially homologous to SEQ ID NO:1.
or SEQ ID NO:3, i.e., at least about 85%, about 90% or about 95% homologous at the nucleotide level. In yet another embodiment, the invention provides variants of the virus that encodes polypeptides comprising an amino acid sequence of SEQ ED NO:2 or SEQ ID NO:4 a sequence substantially homologous to SEQ ID NO:2 or SEQ DD NO:4, i.e., at least about 85%, about 90% or about 95% homologous at the amino acid level. The invention also provides cells or mammals (including humans) infected with an isolated HTV-2NWK08F virus or variant.

[042] The invention also relates to HTV-2 variants characterized by the nucleotide sequences of the HTV-2NWK08F virus deposited at the ATCC, as well as related variants that comprise a nucleotide sequence that is substantially homologous to the sequences of the virus deposited at the ATCC as HTV-2NWK08F, i.e., at least about 85%, 90% or 95% homologous at the nucleotide level. The sequences of HTV-2NWK08F and its variants disclosed herein characterize a new HIV-2 virus that is part of a subclass of HTV-2 viruses, currently known to be found primarily in Sierra Leone.

[043] "Purified" or "isolated" HTV isolate refers to a preparation of HIV virus particles which has been isolated from the cellular constituents with which the virus is normally associated, and from other types of viruses which may be present in the infected tissue. The techniques for isolating viruses are known to those of skill in the art, and include, for example, centrifugation and affinity chromatography.

[044] An HTV "particle" is an entire virion, as well as particles which are intermediates in virion formation. HIV particles generally have one or more HTV proteins associated with the HTV nucleic acid.

NUCLEIC ACIDS

[045] The invention provides a nucleic acid comprising a nucleotide sequence of HIV-2NWK08F. In some embodiments, the nucleic acid is RNA. In other embodiments, the
- nucleic acid is DNA. In particular embodiments, the nucleic acid is isolated viral RNA or proviral DNA. In other embodiments, the nucleic acid is a cDNA molecule. In other embodiments, the nucleic acid is an oligonucleotide. The nucleic acid may be naturally-occurring, non-naturally occurring, recombinantly produced, or synthetic.

[046] The nucleic acid molecules of the present invention may be used, e.g., (1) to produce HTV-2NWK08F polypeptides; (2) as probes in nucleic acid hybridization assays; (3) as primers for reactions involving the synthesis of HTV-2NWK08F nucleic acid; (4) as binding partners for separating HTV-2NWK08F viral nucleic acid from other constituents which may be present; (5) as a component of a partial or complete HTV-2NWK08F virion; and (6) as anti-sense nucleic acid for preventing the transcription or translation of viral nucleic acid.

[047] In some embodiments, the nucleic acid comprises the entire viral genome. In other embodiments, the invention provides a nucleic acid comprising sequences that encode the Env, Nef, Gag, or Pol protein of HIV-2NWK08F. In particular embodiments, the invention provides a nucleic acid comprising all or a portion of SEQ ID NO: 1 or SEQ ID NO:4 or their complements. In particular embodiments, the invention provides a nucleic acid that encodes all or a portion of the amino acid sequence of SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:5, or SEQ ID NO:6.

Table 1: HIV-2NWK08F Sequences

<table>
<thead>
<tr>
<th>Env/Nef Nucleotide Sequence</th>
<th>SEQ ID NO: 1</th>
</tr>
</thead>
<tbody>
<tr>
<td>TACCTTCATGGACAAATTCGAGGAGAATTTTATATTTGTAATAATGAAATTGTGTTCTCT</td>
<td></td>
</tr>
<tr>
<td>AAATTGGTAGAGGAGAATAATATCTATGGAAGATTTGTACACTCAAAACCAGAGA</td>
<td></td>
</tr>
<tr>
<td>GAAGACAGAAGGGAATATATGCTTGGCCTGCCATCAAGGCAAATCTATATACTTGGCACAA</td>
<td></td>
</tr>
<tr>
<td>AGTAGGAAATTGTGTACCTGCCATCAAGGAAAGTAACTACTGAACAGGTAGGATATAA</td>
<td></td>
</tr>
<tr>
<td>ACACAGCAATTGCAAAACTGACTGGACAGGACAAATGAAACTAATATCATGAGT</td>
<td></td>
</tr>
<tr>
<td>TGGAGAGGGGTCTTTGTGCTAGGGTTCTTGGGATTTCTCGCAACGGCAGGTTCTGCAATGGG</td>
<td></td>
</tr>
<tr>
<td>CGCAGCGTTCCACCTTGGGATTTCTCGCAACGGCAGGTTCTGCAATGGG</td>
<td></td>
</tr>
<tr>
<td>ACAGCAAGCGCCGAGCTTGGGATTTCTGCAATGGG</td>
<td></td>
</tr>
<tr>
<td>GGGAGGCTTGGGATTTCTGCAACGGCAGGTTCTGCAATGGG</td>
<td></td>
</tr>
<tr>
<td>GACCACTTAACACTGAGGATTTCTGCAATGGG</td>
<td></td>
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<tr>
<td>AATGACTTAACACTGAGGATTTCTGCAATGGG</td>
<td></td>
</tr>
<tr>
<td>AGACTTCTACAGAAAATCTAGAGGACAGAGCAGTACCTTGGGATTTCTGCAATGGG</td>
<td></td>
</tr>
<tr>
<td>AAATATGTAAGCTCAAAATTGGCAAACAGCTGAGGATTTCTGCAATGGG</td>
<td></td>
</tr>
</tbody>
</table>
Env Amino Acid Sequence: Encoded by nucleotides 8-1417 of SEQ ID NO:1

MWTNCRGEFLYCKJy-NWFLNWVEDRNITHGRWSTQKPAEKQKRNYVPCHIRQIINTWHKVGKNVYLPPREG
NLTCNSSVTHIANIDWTSNDNITNMSAEVAELYRELGDLHYKLVEITIPGLAPTEVKYRSSATPRKNRKG
VFVLGLFLPLATAGSAMAGAASLTLAQSRTLLAGYIVQQQQQQLLDAVKVRQEQLLRLTVGWTKNLQTRVTAIM
EYKLKDQAOLNSWCAFRQVCHTVTPWPNLTQPKWDNMWTQEWKVDFLTENITELLEQAQIQEQKMK
YELQLNSWDLVGFVNLDSWITTYQGYVVLWQWVIILRISYIYQMILRLRKYGRPFSPPSSPYYYQIQH
YRRDQELPIDREDIEEAGKGNRSWPQIEYHIHLRIQLRLRTLWSNCRDLTYKSFQTLHQLTSAAA
TATRDPIRTEASYSITYGWYQYFPEALAAAMQTAGETLASAGGELWATLGRISSEQ ID NO: 1

Nef Amino Acid Sequence: Encoded by nucleotides 1314-1417 of SEQ ID NO:1

MGGNTSSKPKRQCRLEIRLQLQARGASYQQLWEG (SEQ ID NO:3)

5' LTR/Gag/Pol Nucleotide Sequence: SEQ ID NO:4

5' LTR: nucleotides 1-827 of SEQ ID NO:4

Gag polypeptide: nucleotides 1046-2560 of SEQ ID NO:4

Env polypeptide: nucleotides 2218-2263 of SEQ ID NO:4
In some embodiments, the invention provides a nucleic acid that hybridizes under stringent conditions to the nucleotide sequence of SEQ ID NO: 1 or SEQ ID NO: 4. In certain embodiments, stringent conditions include, but are not limited to (1) wash in aqueous prehybridization buffer (6X SSC, 5X Denhardt's reagent, 0.5% SDS) at 68°C; (2) hybridization of the probe in aqueous hybridization buffer (6X SSC, 5X Denhardt's reagent, 0.5% SDS, 1 µg/ml poly(A), 100 µg/ml salmon sperm DNA) at 68°C; and (3) wash in 2X SSC, 0.5% SDS at room temperature. Alternatively, stringent conditions include, but are not limited to (1) wash in formamide prehybridization buffer (6X SSC, 5X Denhardt's reagent, 0.5% SDS, 1 µg/ml poly(A), 0.5 µg/ml salmon sperm DNA) at 42°C; (2) hybridization of the probe in formamide hybridization buffer (6X SSC, 5X Denhardt's reagent, 0.5% SDS, 50% formamide, 1 µg/ml poly(A), 100 µg/ml salmon sperm DNA) at 68°C; and (3) wash in 2X SSC, 0.5% SDS at room temperature.
poly(A), 100 µg/ml salmon sperm DNA) at 42°C; and (3) wash in 2X SSC, 0.5% SDS at room temperature. Additional variations on these stringent conditions are know to one of skill the art and may be found, for example, in Chapter 6 of Sambrook & Russell, Molecular Cloning: A Laboratory Manual, CSHL Press, 2001, which is incorporated by reference herein. In exemplary embodiments, the invention provides a nucleic acid that is at least about 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical to the nucleotide sequence of SEQ ID NO:1 or SEQ ID NO:4, or fragments thereof.

[049] In some embodiments, the nucleic acid that hybridizes under stringent conditions is a probe or a primer. The probe or the primer maybe used to detect the presence of HTV-2NWK08F or other HIV strains in a biological sample. Exemplary primers are set forth below in Tables 2 and 3.

<table>
<thead>
<tr>
<th>Table 2: HIV-2NWK08F Env-Nef Primers</th>
</tr>
</thead>
<tbody>
<tr>
<td>Primer</td>
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<tr>
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<tr>
<td>1</td>
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</tbody>
</table>
Table 3: HIV-2NWK08F LTR-Gag Primers

<table>
<thead>
<tr>
<th>Primer</th>
<th>Primer Sequence</th>
<th>PCR Fragment Size</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 Outer Forward</td>
<td>5'-AGA AGG CTA GCC GCA AGA GG-3' SEQ ID NO:21</td>
<td>511 bp</td>
</tr>
<tr>
<td>Outer Reverse</td>
<td>5'-TAC CTT CAC GTC CCG CTC AG-3' SEQ ID NO:22</td>
<td></td>
</tr>
<tr>
<td>Inner Forward</td>
<td>5'-GAC ACA GCA GGG ACT TTC CA-3' SEQ ID NO:23</td>
<td></td>
</tr>
<tr>
<td>Inner Reverse</td>
<td>5'-TTC CTC CGT CGC GGT TGG TT-3' SEQ ID NO:24</td>
<td></td>
</tr>
<tr>
<td>2 Outer Forward</td>
<td>5'-ACT CCT GAG TAC GGC TGA GT-3' SEQ ID NO:25</td>
<td>318 bp</td>
</tr>
<tr>
<td>Outer Reverse</td>
<td>5'-CAA CAG GTC TTC TGC CAA TC-3' SEQ ID NO:26</td>
<td></td>
</tr>
<tr>
<td>Inner Forward</td>
<td>5'-GTC TGA GTG AAG GCA GTA AG-3' SEQ ID NO:27</td>
<td></td>
</tr>
<tr>
<td>Inner Reverse</td>
<td>5'-TCT GCC AAT CCG AAT CTG TC-3' SEQ ID NO:28</td>
<td></td>
</tr>
<tr>
<td>3 Outer Forward</td>
<td>5'-TGG GAG ATG GGC GCG AGA AAC TCC GTC-3' SEQ ID NO:29</td>
<td>809 bp</td>
</tr>
<tr>
<td>Outer Reverse</td>
<td>5'-TCC ACA TTT CCA GCA GCC CTG TCT TCT-3' SEQ ID NO:30</td>
<td></td>
</tr>
<tr>
<td>Inner Forward</td>
<td>5'-AGG GAA GAA AGC AGA TGA ATT AGA A-3' SEQ ID NO:31</td>
<td></td>
</tr>
<tr>
<td>Inner Reverse</td>
<td>5'-GCA TTT TGA ATC AGC AGT GTT TGA GTC ATC CA-3' SEQ ID NO:32</td>
<td></td>
</tr>
<tr>
<td>4 Outer Forward</td>
<td>5'-ACG CAC AGC ATC CAA G-3' SEQ ID NO:33</td>
<td>545 bp</td>
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<tr>
<td>Outer Reverse</td>
<td>5'-CTT GAG CCA TGG GGA AAT TG-3' SEQ ID NO:34</td>
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<tr>
<td>Inner Forward</td>
<td>5'-GGA GAT GGA TTC AGC TAG GA-3' SEQ ID NO:35</td>
<td></td>
</tr>
<tr>
<td>Inner Reverse</td>
<td>5'-GGG GCT TCT TTC CCC ATG GAC C-3' SEQ ID NO:36</td>
<td></td>
</tr>
</tbody>
</table>

[050] In exemplary embodiments, the nucleic acid comprises at least six nucleic acids derived from the HTV-2NWK08F genome. In particular embodiments, the nucleic acid comprises at least 6, 8, 10, 12, 15, 20, 25, 30, 40, or 50 consecutive nucleotides derived from the HTV-2NWK08F genome. In some embodiments, the nucleic acid comprises at least 6, 8, 10, 12, 15, 20, 25, 30, 40, or 50 consecutive nucleotides of SEQ ID NO:1 or SEQ ID NO:4. In some embodiments, the nucleic acid is no longer than about 50, 75, 100, or 200 nucleotides. In another embodiment, the nucleic acids of the invention encode a viral protein or fragment thereof, wherein the viral protein or fragment possesses the biological activity associated with the protein.

[051] In some embodiments, the nucleic acids of the invention are attached to a detectable label. Examples of detectable labels include, but are not limited to, chromogens, radioisotopes, chemiluminescent compounds, visible or fluorescent particles, and enzymes.
In the case of enzymes labels (e.g., alkaline phosphatase or horseradish peroxidase), addition of a chromo-, fluoro-, or lumogenic substrate results in the generation of a detectable signal.

The nucleic acids of the invention may be used to differentially detect HTV-2NWK08F from other subtype F HTLV-2 viruses or from other HTV-I, HIV-2, or SIV strains. Alternatively, the nucleic acids of the invention may be used to detect any HTV or SIV strain. One of skill in the art would understand how to design primers and probes so as to preferentially detect HIV-2NWK08F or to detect all HTV and SIV strains. Briefly, one could examine the sequences of a number of HTV and SIV strains and select regions of high or low homology. If the primers and probes are to preferentially detect HIV-2NWK08F, they are derived from regions of the genome with low homology to other strains and subtypes. Examples of regions with low homology include regions encoding the Env polypeptide. If the primers and probes are to detect all or some other HTV or SIV strains, they are derived from regions of the genome with high homology to other strains and subtypes. Examples of regions with high homology between strains include regions encoding the viral reverse transcriptase.

As used herein, a nucleic acid with a designated sequence or "derived from" a designated source refers to a nucleotide sequence that is homologous (i.e., identical) to or complementary to the designated sequence or source, or a portion thereof.

The nucleic acids of the invention may be produced by any method known to one of skill in the art. In exemplary embodiments, the nucleic acids are isolated from cells infected with HTV-2NWK08F.

Methods for isolating viral RNA or DNA are well known in the art. An exemplary method for extracting viral RNA or DNA from infected cells is described in Laure et al., Lancet 2(8610):538-41 (1988), the contents of which are incorporated by reference herein. Because infected cells contain copies of reverse transcribed viral DNA while whole virions contain only viral DNA, the methods for extracting the two nucleic acids differ.
slightly. To extract viral DNA, infected cells are separated from other blood constituents by centrifugation in a gradient, such as, e.g., a Ficoll gradient. The cells are lysed in an appropriate lysis buffer (e.g., 10 mM Tris pH 8, 10 mM EDTA, 10 mM NaCl, 0.5% SDS, 100 μg/mg proteinase K) for approximately 2 hours at 60°C. The DNA is then extracted with phenol and precipitated with ethanol. To isolate viral RNA, the same procedure is carried out on the serum, plasma, or blood leukocytes of infected patients or on the supernatant of infected cell cultures. Once extracted, the RNA may be transformed from single stranded RNA to double stranded DNA by performing a reverse transcriptase reaction, such as that described in U.S. Patent No. 6,020,123, incorporated by reference herein.

[056] The nucleic acids of the invention may also be produced recombinantly. Briefly, the nucleic acid is inserted into a vector and amplified in a host organism. Suitable vectors can be chosen or constructed, containing appropriate regulatory sequences. Vectors may be plasmids or viral, e.g., phage or phagemid, as appropriate. Many known techniques and protocols for manipulation of nucleic acid in preparation of nucleic acid constructs, mutagenesis, sequencing, introduction of DNA and/or vectors into cells, gene expression, and analysis of proteins are described in detail in Sambrook & Russell, *Molecular Cloning: A Laboratory Manual*, CSHL Press, 2001, the contents of which are incorporated by reference herein.

[057] Alternatively, the nucleic acid may be amplified via the polymerase chain reaction using any of the exemplary primers disclosed herein or other primers derived from the nucleotide sequence of HIV-2NWK08F or another HTV virus (e.g., a different HIV-2 subtype F virus, a different HTV-2 subtype virus, or an HTV-1 or SIV virus). Methods for practicing nucleic acid amplification are described in Sambrook & Russell, *Molecular Cloning: A Laboratory Manual*, CSHL Press, 2001. Finally, the nucleic acids of the invention may be chemically synthesized using well known methods available from numerous commercial vendors, including, by not limited to, Applied Biosystems and Integrated DNA

**POLYPEPTIDES**

[058] The invention also provides amino acid sequences of HTV-2NWK08F polypeptides. The polypeptides of the invention are useful for detecting the presence of HTV-2NWK08F-specific antibodies in patient serum. The polypeptides of the invention are also useful for generating HIV-2NWK08F-specific antibodies. The polypeptides may be naturally occurring, recombinantly produced, or synthetic.

[059] In exemplary embodiments, the polypeptides of the invention comprise at least six consecutive amino acids derived from a polypeptide encoded by the HIV-2NWK08F genome. In particular embodiments, the polypeptide comprises at least 6, 8, 6, 8, 10, 12, 15, 20, 25, 30, 40, or 50 consecutive amino acids derived from HTV-2NWK08F. In some embodiments, the polypeptide comprises at least 6, 8, 6, 8, 10, 12, 15, 20, 25, 30, 40, or 50 consecutive amino acids of SEQ ID NO:2, SEQ ED NO:3, SEQ ID NO:5, or SEQ ID NO:6. In some embodiments, the polypeptide is no longer than about 10, 11, 12, 13, 14, 15, 25, 50, 75, 100, 200, 300, 400, or 1000 amino acids. In other embodiments, the polypeptides comprises an entire viral protein, or a fragment thereof, wherein the viral protein or fragment possesses the biological activity associated with that protein.

[060] An amino acid sequence derived from a designated polypeptide or source means that the amino acid sequence is homologous (i.e., identical) to the sequence of the designated polypeptide, or a portion thereof. An amino acid sequence from a designated nucleic acid sequence refers to a polypeptide having an amino acid sequence identical to that of a polypeptide encoded in the sequence, or a portion thereof.
The term "polypeptide" refers to a polymer of amino acids and does not refer to a specific length; thus, peptides, oligopeptides, and proteins are included within the definition of polypeptide. This term does not exclude post-expression modifications of the polypeptide, for example, glycosylation, acetylation, phosphorylation, pegylation, addition of a lipid moiety, or the addition of any organic or inorganic molecule. Included within the definition are, for example, polypeptides containing one or more analogs of an amino acid (including, for example, unnatural amino acids) and polypeptides with substituted linkages, as well as other modifications known in the art, both naturally occurring and non-naturally occurring.

In some embodiments, the polypeptides of the invention are derived from HTV-2NWK08F Gag or Env polypeptides or fragments thereof. In some embodiments, the polypeptides of the invention are immunogenic. In particular embodiments, the polypeptides of the invention comprise SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:5, or SEQ ID NO:6, or fragments thereof.

The polypeptides of the invention may be prepared by any method known to one of skill in the art, including those described in Sambrook & Russell, Molecular Cloning: A Laboratory Manual, CSHL Press, 2001, the contents of which are incorporated by reference herein. The polypeptides may be recombinantly expressed and purified. For example, DNA encoding the desired polypeptide may be ligated into an expression vector for any convenient host, either eukaryotic or prokaryotic. The polypeptide is expressed in a culture of the host cells and then isolated from lysed cells or the culture medium and purified.

Alternatively, the polypeptides of the invention may be isolated from infected cell cultures and purified. The purification of the polypeptides may be performed by techniques known in the art, such as, e.g., differential extraction, salt fractionation, centrifugation, and ion exchange, affinity, or size exclusion chromatography.
Polypeptides of the invention may also be produced by chemical synthesis. For example, the polypeptides may be prepared with solid-phase synthesis such as that described in Bodansky et al., *The Practice of Peptide Synthesis*, Springer-Verlag, 1994.

**ANTIBODIES**

The invention also provides HIV-2NWK08F-specific antibodies. The antibodies may be generated against the entire virion or against a specific polypeptide, including both whole proteins and peptide fragments. The antibodies of the invention are useful for the detection of HIV-2NWK08F infection in patients or cell culture, for the detection of HIV-2NWK08F antigens or virions, and for the treatment of patients infected with HIV-2NWK08F or similar viruses. The antibodies of the invention may also be used as therapeutics for the treatment of HTV infection. In a non-limiting example, antibodies specific for the HTV-2NWK08FEnv protein may be used to prevent the binding of the Env protein to cells.

The term "antibody" as used herein includes polyclonal antibodies, monoclonal antibodies, antibody compositions with polyepitope specificities, bispecific antibodies, diabodies, humanized antibodies, and other purified preparations of antibodies and recombinant antibodies. The antibodies can be whole antibodies of any isotype, e.g., IgG, IgA, IgE, IgM, etc, or fragments thereof, which bind the antigen of interest. In a specific example of an antibody used in the present invention, the antibody is an IgG antibody. Antibodies can be fragmentated using conventional or other techniques and the fragments screened for binding to an antigen of interest. Generally, an antibody fragment comprises the antigen-binding and/or the variable region of an intact antibody.

The term "antibody fragment" includes segments of proteolytically cleaved or recombinantly prepared portions of an antibody molecule that can selectively bind to a selected protein. Non-limiting examples of such proteolytic and/or recombinant fragments include...
include Fab, F(ab\(\gamma\))\(_2\), Fab\(\gamma\), Fv, and single chain antibodies (scFv) containing a V[L] and/or V[H] domain joined by a peptide linker, domain antibodies (dAbs), Nanobodies® (antibody-derived biological therapeutic agents that contain the unique structural and functional properties of naturally-occurring heavy-chain antibodies), and UniBodies (antibodies lacking the hinge region). The scFvs may be covalently or noncovalently linked to form antibodies having two or more binding sites.

[069] The term "antigen" refers to any part of a virus that an antibody may specifically bind to. This includes polypeptides and modifying groups, such as glycoproteins, that may be attached to the polypeptides. It may include the whole virus, part of a virus, a whole protein, or part of a protein. An antibody may only bind to a part of the antigen.

[070] The term "epitope" refers to a portion of an antigen that is responsible for specific interactions with the antibody. An epitope may be a peptide of at least about 6, 8, 10, 11, 12, 13, or 15 amino acids in length.

[071] Antibodies specific for polypeptides of the invention can be prepared by any technique known to one of skill in the art. For example, the antibodies of the invention can be prepared via traditional hybridoma techniques (Kohler and Milstein, Nature 256:495-499 (1975), incorporated by reference herein), recombinant DNA methods (U.S. Patent No. 4,816,567, incorporated by reference herein), or phage display techniques using antibody libraries (Clackson et al., Nature 352:624-628 (1991); Marks et al., J Mol Biol 222:581-597 (1991), both of which are incorporated by reference herein). For various other antibody production techniques, see Harlow et al., Antibodies: A Laboratory Manual, CSHL Press, 1988, the contents of which are incorporated by reference herein.

**VIRAL DETECTION ASSAYS**

[072] The nucleic acids, polypeptides, and antibodies of the invention are useful for the detection of the presence of HTV-2 in a biological sample. These assays may be used to
detect the presence of HIV infection in a patient or to determine whether cultured cells have been infected with the virus. The presence of HIV-2NWK08F nucleic acids, polypeptides, or antibodies in a patient or in a cell culture is indicative of HIV infection. Due to the homology between different subtypes and strains of HTV, detection of HTV-2NWK08F nucleic acids, polypeptides, and antibodies may also be used to detect other HTV subtypes and strains. Alternatively, detection of HIV-2NWK08F nucleic acids, polypeptides, or antibodies in a patient or cell culture may be used to determine that the patient or cells are infected with HIV-2NWK08F, and not a different strain or subtype of HIV.

[073] All of the viral detection assays of the invention involve a step of providing a biological sample suspected of being infected with HIV or of containing HIV nucleic acids, polypeptides or anti-HIV antibodies. Accordingly, the first step in the viral detection assays is the identification of an appropriate patient, mammal, or infected cell culture and providing a biological sample from that source.

[074] "Biological sample" refers to a sample of tissue or fluid isolated from an individual, including but not limited to, for example, whole blood and components thereof, dried blood, plasma, serum, spinal fluid, lymph fluid, the external sections of the skin, respiratory, intestinal, and genitourinary tracts, tears, saliva, urine, milk, blood cells, tumors, organs, and also samples of in vitro cell culture constituents, including, but not limited to, putatively virally infected cells, cells putatively expressing viral polypeptides, cell components, conditioned medium resulting from the growth of cells in cell culture medium, and cell free supernatant of one of the tissues or fluids listed above.

**NUCLEIC ACID-BASED ASSAYS**

[075] Nucleic acid probes and primers are useful in identification of the virus, further characterization of the viral genome, and detection of the virus in diseased individuals. Nucleic acid-based assays are a preferred method for measuring HIV viral load in infected patients. In nucleic acid-based assays, an essential step is the provision of both
the detecting nucleic acid, such as a primer or a probe and the nucleic acid to be detected, such as viral RNA or DNA. Methods for preparing both the detecting nucleic acid and the nucleic acid to be detected are provided herein.

[076] Methods for selecting probes and primers and determining the specificity of these nucleic acids are known to those of skill in the art. The sequence and length of the probe or primer will depend on the proposed use. If the probe or primer is to be used to differentially detect the presence of HFV-2NWK08F, the sequence of the probe or primer will be specific to this virus, i.e., derived from an area of the viral genome that is not conserved between virus subtypes or strains. Viral genes that show low levels of homology between subtypes and strains include the envelope gene. If the probe or primer is to be used to detect all HIV-2 viruses or all HTV viruses in general, it will be derived from an area of the viral genome that is conserved between virus subtypes or strains. Viral genes that show high levels of homology between subtypes and strains include certain regulatory proteins, such as, e.g., the genes encoding the viral reverse transcriptase.

[077] For diagnostic use of probes, the biological sample to be analyzed, such as blood or serum, may be treated, if desired, to extract the nucleic acids contained therein. The resulting nucleic acid from the sample may be subjected to gel electrophoresis or other size separation techniques; alternatively, the nucleic acid sample may be dot blotted without size separation. The probes are then labeled. Suitable labels, and methods for labeling probes are known in the art, and include, for example, radioactive labels incorporated by nick translation or kinasing, biotin, fluorescent probes, and chemiluminescent probes. The nucleic acids extracted from the sample are then treated with the labeled probe under hybridization conditions of suitable stringencies. Usually high stringency conditions are desirable in order to prevent false positives. The stringency of hybridization is determined by a number of factors during hybridization and during the washing procedure, including temperature, ionic strength, length of time, and concentration of formamide. These factors are outlined in, for

[078] If the HTV-2NWK08F genome sequences are present in serum of infected individuals at relatively low levels, detection may require that amplification techniques be used in hybridization assays. Such techniques are known in the art. A particularly desirable technique may first involve amplification of the target HTV-2NWK08F sequences in serum, plasma, or cells approximately 10,000-fold, i.e., to approximately $10^6$ sequences/ml. This may be accomplished, for example, by polymerase chain reactions (PCR). The amplified sequences may then be detected using a hybridization assay. These hybridization assays, which should detect sequences at the level of $10^6$ copies/ml, utilize nucleic acid multimers which bind to single-stranded analyte nucleic acid, and which also bind to a multiplicity of single-stranded labeled oligonucleotides. A suitable solution phase sandwich assay may be used with labeled polynucleotide probes.

[079] The probes and primers of the invention may be used to quantitate viral nucleic acid levels by real-time PCR. Real-time PCR refers to a polymerase chain reaction that is monitored, usually by fluorescence, over time during the amplification process, to measure a parameter related to the extent of amplification of a particular sequence, such as the extent of hybridization of a probe to amplified target sequences. The initial nucleic acids of the reaction are synthesized from the RNA template using reverse transcriptase. Then the DNA generated within a PCR reaction is detected on a cycle by cycle basis during the PCR reaction. The amount of DNA increases with the amount of template sequences present in the original sample. When enough amplification products are made, a threshold is reached at which the PCR products are detected.

[080] The HTV-2NWK08F probes and primers are a length that allows the detection of unique viral sequences by hybridization. These nucleic acids can be prepared using routine methods, including automated oligonucleotide synthetic methods. The nucleic acids
may be a complement to any unique portion of the HIV-2NWK08F genome. For use as probes, complete complementarity is desirable, though it may be unnecessary as the length of the fragment is increased. Primers used in the methods of the invention include oligonucleotides of sufficient length and appropriate sequence to provide specific initiation of polymerization of a HIV or HF/-2NWK08F nucleic acid in a polymerase chain reaction (PCR). Conditions and reagents for performing nucleic acid amplification utilizing PCR and other nucleic acid amplification techniques are known to one of skill in the art and may be found in, e.g., Sambrook & Russell, Molecular Cloning: A Laboratory Manual, CSHL Press, 2001. The nucleic acids that make up the probes or primers of the invention may be at least about, e.g., 6, 8, 10, 15, 20, 25, 30, 40, or 50 nucleotides in length. The nucleic acids may have a maximum length of about, e.g., 50, 75, 100, or 200 nucleotides.

[081] The probes and primers of the invention can be packaged into diagnostic kits. Diagnostic kits include the probe and/or primer nucleic acids, which may be labeled; alternatively, the probe or primer nucleic acids may be unlabeled and the ingredients for labeling may be included in the kit in separate containers. The kit may also contain other suitably packaged reagents and materials needed for the particular amplification and/or hybridization protocol, for example, standards, enzymes, nucleotide triphosphates, wash buffers, as well as instructions for conducting the test.

IMMUNOASSAYS

[082] Immunoassays may be used to detect anti-HFV-2NWK08F antibodies in patient serum or to detect HTV-2NWK08F-specific antigens in patient serum. Immunoassays can be used to test for the presence of HIV antibodies or antigens in blood, oral mucosal transudate (OMT) fluid, saliva, and urine, or cell-free supernatants of these fluids. Immunoassays are currently more commonly used for diagnosing HTV infection than nucleic acid assays.
HIV immunoassays include, but are not limited to, Western blot assays and enzyme-linked immunosorbent assays (ELISA), such as those described at http://hivinsite.ucsf.edu/InSite?page=kb-02-02-01, accessed October 28, 2008, the contents of which are incorporated by reference herein. An immunoassay for viral antigen may use, for example, a monoclonal antibody directed towards a viral epitope, a combination of monoclonal antibodies directed towards epitopes of one viral polypeptide, monoclonal antibodies directed towards epitopes of different viral polypeptides, polyclonal antibodies directed towards the same viral antigen, polyclonal antibodies directed towards different viral antigens, or a combination of monoclonal and polyclonal antibodies.

An immunoassay for viral antibodies may use, for example, a HFV-2NWK08F viral antigen, such as, e.g., fragments of the Env, Pol, Gag, or Nef polypeptides.

Imunoassay protocols may be based, for example, upon competition, or direct reaction, or sandwich type assays. Protocols may also, for example, use solid supports, or may be by immunoprecipitation. Most assays involve the use of labeled antibody or polypeptide. The labels may be, for example, fluorescent, chemiluminescent, radioactive, or dye molecules. Assays which amplify the signals from the probe are also known. Examples of which are assays which utilize biotin and avidin, and enzyme-labeled and mediated immunoassays, such as ELISA assays.

Typically, an immunoassay for anti-HIV-2NWK08F antibody will involve selecting and preparing the test sample, such as a biological sample, and then incubating it with an antigenic (i.e., epitope-containing) HIV-2NWK08F polypeptide under conditions that allow antigen-antibody complexes to form. Such conditions are well known in the art. The polypeptide may be bound to a solid support. Examples of solid supports that can be used are nitrocellulose in membrane or microtiter well form, polyvinylchloride in sheets or microtiter wells, polystyrene latex, in beads or microtiter plates, polyvinylidine fluoride (known as Immobilon™), diazotized paper, nylon membranes, activated beads, and Protein A beads.
certain embodiments, these assays are performed using either Immulon™ microtiter plates (Dynatech) or specular-finished 0.25-inch polystyrene beads (available from Precision Plastic Ball). The solid support is typically washed after separating it from the test sample.

[087] In another embodiment, the test sample is incubated with antigen in solution under conditions that will precipitate any antigen-antibody complexes that are formed, as is known in the art. The precipitated complexes are then separated from the test sample by, for example, centrifugation. The complexes formed comprising anti-HIV-2NWK08F antibody are then detected by any of a number of techniques. Depending on the type of assay, the complexes can be detected with labeled anti-xenogeneic Ig or by measuring the amount of bound, labeled competing antibody.

[088] In immunoassays where HTV-2NWK08F polypeptides are the analyte, the test sample, typically a biological sample, is incubated with anti-HIV-2NWK08F antibodies again under conditions that allow the formation of antigen-antibody complexes. Various formats can be employed, such as the "sandwich" assay. In this assay, antibody is bound to a solid support, incubated with a test sample, washed, incubated with a labeled anti-analyte antibody, and washed again. Analyte is detected by determining if the second antibody is bound to the support. In a competitive format, which can be either heterogeneous or homogeneous, a test sample is usually incubated with and antibody and a labeled, competing antigen either sequentially or simultaneously. These and other formats are well known in the art.

**TREATMENT OF INFECTED PATIENTS**

[089] This invention also provides methods of treating patients infected with HTV-2NWK08F. Methods of treating HIV infections are known in the art. Patients infected with HFV-2NWK08F can be treated by any known anti-HTV therapeutic, such as those described in Panel on Antiretroviral Guidelines for Adults and Adolescents. Guidelines for the use of antiretroviral agents in HTV-I-infected adults and adolescents. Department of Health and

[090] In addition, the HIV-2NWK08F virus, nucleic acids, and proteins of the invention may be used to develop new therapeutic antibodies and vaccines for the treatment of HrV-2 infections. The vaccines may be derived from the whole virus, the virion, viral proteins, or viral nucleic acids.

[091] Other embodiments of the invention will be apparent to those skilled in the art from consideration of the specification and practice of the invention disclosed herein. It is intended that the specification and examples be considered as exemplary only, with a true scope and spirit of the invention being indicated by the following claims.

EXAMPLES

Example 1—Identification of Infected Patient

[092] Patient X, a 68 year old man from Sierra Leone, tested positive for antibodies to HTV in a test that detects the presence of HIV-I or HIV-2 antibodies. Subsequent testing for HTV-1 antibodies in a Western blot showed that the patient was not infected with HTV-1. His HTV-1 viral load was tested in an Real-time Polymerase Chain Reaction (RT-PCR) assay (LabCorp) and was below the lower limit of the assay. HTV-2 viral DNA testing performed at
two commercial laboratories (Viromed; Focus Technologies) was negative, as was an HTV-2 Western blot. HTV-2 viral DNA was finally detected with the use of primers derived from HTV-2 subtype F (as described in Example 3).

Example 2—Isolation and Propagation of HTV

[093] Normal donor PBMCs were stimulated with PHA at 5 µg/ml for three days and then re-suspended in RPMI supplemented with 10% fetal bovine serum and 20 units/ml of IL-2. To isolate the virus, these stimulated normal donor PBMCs were then co-cultured with PBMCs from Patient X. The culture was split every 3-4 days and supernatant was collected for p27 measurement by EIA for SIV p27 (Zeptometrix), which detected the p27 protein of HTV-2NWK08F. PBMCs were collected at each time point and their DNA was extracted.

[094] This co-culturing process was performed with three different populations of normal donor PBMCs. HIV-2NWK08F virus replicated each time, with the peak p27 concentration exceeding 10 µg/ml in each co-culture (tested with Zeptometrix SIV p27 kit).

Example 3—Amplification and Sequencing of Viral RNA

[095] Portions of the gag and env genes were amplified as described in Chen et al., J Virol 71:3953-3960 (1997). Briefly, DNA was extracted from each PBMC culture on day 13 using a DNA extraction kit (Qiagen, Valencia, California). Nested primers were used to amplify a 438-bp env fragment. Traditional polymerase chain reaction (PCR) was performed in a Tpersonal thermocycler (Biometra, Hannover, Germany) using a PCR Master Mix Kit (Applied Biosystems, Foster City, California). These reactions were performed in a 50 µl volume which contained approximately 350 ng of DNA and 20 pmol of each primer. The first round of PCR had an initial activation step at 95°C for 5 minutes followed by 30 cycles of 95°C for 20 seconds, 45°C for 1.5 minutes, and 72°C for 2 minutes with env outer primer pair EF-I and ER-I. The second round of PCR was performed using inner primers EF-2 and
ER-2. This round consisted of 30 cycles of 94°C for 20 seconds, 55°C for 1.5 minutes, and 72°C for 2 minutes. Additionally, both rounds of PCR consisted of an extension step at 72°C for 8 minutes.

EF-I: GGCTGGGATAGTGCAGCAACAGCAACAG (SEQ ID NO:37)
ER-1: GGGAGGGGAAGAGAACACTGGCCTATA (SEQ ID NO:38)
EF-2: TGTGGACGTGGTCAAGAGACAAC (SEQ ID NO:39)
ER-2: AAGCGGGAGGGGAAGAGAACACTGGCC (SEQ ID NO:40)

[096] The same procedure was used to amplify a 826 bp gag fragment using the following primers:

GF-I: TGGGAGATGGGCGCGAGAACTCCGTC (SEQ ID NO:41)
GR-I: TCCACATTTCCAGCAGCCCTGTCTTCT (SEQ ID NO:42)
GF-2: AGGGAAGAAAGCAGATGAATTAGAA (SEQ ID NO:43)
GR-2: GCATTTTGAATCAGCAGTGTTTGAGTCATCCA (SEQ ID NO:44)

[097] The gag and env PCR products were cloned and sequenced as described in Chen et al., J Virol 70:3617-27 (1996).

Example 4-Phylogenetic Analysis

[098] Gag and Env nucleotide sequence alignments were obtained from the Los Alamos National Laboratory HTV Sequence Database (http://hiv-web.lanl.gov). Newly derived HTV-2NK08F sequences were aligned using the CLUSTAL W profile alignment option. The resulting alignments were adjusted manually when necessary. Regions of ambiguous alignment and all gap-containing sites were excluded.

[099] Phylogenetic trees were inferred from the nucleotide sequence alignments by the neighbor-joining method, using the HKY85 model of nucleotide substitution implemented in PAUP*. The reliability of the branching order was assessed by performing
1,000 bootstrap replicates, again using neighbor joining and the HKY85 model. Phylogenetic trees were also inferred by the maximum likelihood method, using PAUP* with models inferred from the alignment by the use of Modeltest (Posada, et al., *Syst Biol* 50:580601 (2001); Swofford, PAUP*. Phylogenetic analysis using parsimony (*and other methods), version 4. Sinauer Associates, Sunderland, Mass (1999)). These trees are shown in Figures 1 and 2.

**Example 5—Detection of anti-HTV antibodies in patient serum**

[0100] A sample of Patient X's blood was sent to LabCorp® of Burlington, NC for detection of HTV antibodies. The assay used to assess the presence of HTV-I antibodies was Protocol Number 005462, a Western blotting procedure that detects antibodies to Gp41, Gpl20, Gpl60, pl8, p24, p31, p40, p51, p55, and p64. This Western blot for HTV-I antibodies was negative. HTV-2 antibodies were positively detected using Protocol Number 163550, an Enzyme Immunoassay (EIA) that differentially detects antibodies to HTV-2, but not HIV-I.

**Example 6—Real Time Reverse Transcription-PCR for BTV-2 proviral load**

[0101] Proviral DNA from PBMCs was isolated using a DNA extraction kit from QIAGEN®. The DNA quality was confirmed with a spectrophotometer. 48 ng and 480 ng of DNA was subsequently quantified with real time PCR using the following primers and probe derived from the BTV-2NWK08F *env* gene.

<table>
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<th>Primer Type</th>
<th>Primer Sequence</th>
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<td>AAGAATTGGTTGCGATTGACAGTCT</td>
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<tr>
<td>Reverse Primer</td>
<td>TGCACACCCCATGAATTTA</td>
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</tr>
<tr>
<td>Probe</td>
<td>ACTAAAAACCTCCAGACACGCGTCACTGC</td>
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[0102] The PCR was performed in TaqMan Universal PCR Master Mix on an Applied Biosystems 7300 Real time PCR System (Applied Biosystems, Foster City, CA). The real time PCR conditions were as described in Table 4.
Table 4

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</tr>
<tr>
<td></td>
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</tbody>
</table>

[0103] The real time PCR data were analyzed using the 2-ΔΔCT method according to the manufacturer's directions. Patient X had a proviral load of 6,100 copies per 10^6 PBMC.

Example 7–Measuring patient T-cell counts

[0104] Patient X's blood was sent to LabCorp® for measurement of CD4 and CD8 T cell levels. The CD4 T cell levels and CD4:CD8 ratios were determined by Protocols 505008 and 505271, respectively. Patient X had a CD4 T-cell count of 338 cells/µl and a CD4:CD8 ratio of 0.52.
### INDICATIONS RELATING TO DEPOSITED MICROORGANISM OR OTHER BIOLOGICAL MATERIAL

(PCT Rule 13WJ)

<table>
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<tr>
<th>A.</th>
<th>The indications made below relate to the deposited microorganism or other biological material referred to in the description on page 2, line , Paragraph 6.</th>
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| B. IDENTIFICATION OF DEPOSIT | Further deposits are identified on an additional sheet □ |

Name of depositary institution

| ATCC Patent Depository |

Address of depositary institution (including postal code and country)

| 10801 University Blvd. |
| Manassas, Virginia 20110-2209 |
| United States of America |

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C. ADDITIONAL INDICATIONS (leave blank if not applicable) This information is continued on an additional sheet □

D. DESIGNATED STATES FOR WHICH INDICATIONS ARE MADE (if the indications are not for all designated States)

E. SEPARATE FURNISHING OF INDICATIONS (leave blank if not applicable)

The indications listed below will be submitted to the International Bureau later (specify the general nature of the indications e.g., “Accession Number of Deposit”)

Accession Number of Deposit

* For receiving Office use only

□ This sheet was received with the international application

Authorized officer

Form PCT/RO/1 34 (July 1998; reprint January 2004)
WHAT IS CLAIMED IS:

1. An HIV-2 virus comprising the virus deposited as HIV2-NWK08F.
2. An HIV-2NWK08F virus comprising the nucleotide sequence of SEQ ID NO:1 or SEQ ID NO:4.
3. An HIV-2NWK08F variant comprising a nucleotide sequence with 85% homology to SEQ ID NO:1 or SEQ ID NO:4.
4. A nucleic acid comprising a nucleotide sequence of SEQ ID NO:1 or SEQ ID NO:4 or a fragment thereof.
5. A nucleic acid comprising a nucleotide sequence that is at least about 85% homologous to a SEQ ID NO:1 or SEQ ID NO:4.
6. A nucleic acid that encodes an amino acid sequence of SEQ ID NO:1 or SEQ ID NO:4, or a fragment thereof.
7. The nucleic acid of claim 4 comprising at least 6 contiguous nucleotides of SEQ ID NO:1 or SEQ ID NO:4.
8. A polypeptide comprising the amino acid sequence of SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:5, or SEQ ID NO:6, or a fragment thereof.
9. A polypeptide comprising an amino acid sequence that is at least about 85% homologous to SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:5, or SEQ ID NO:6.
10. A peptide fragment of the polypeptide of claim 8 comprising at least 6 contiguous amino acids of SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:5, or SEQ ID NO:6.
11. A peptide fragment of the polypeptide of claim 8 comprising at least 10 contiguous amino acids of SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:5, or SEQ ID NO:6.
12. An antibody that specifically binds to a polypeptide of claim 8.
13. An HIV-2 Env polypeptide comprising the amino acid sequence of SEQ ID NO:2.
14. An HIV-2 Nef polypeptide comprising the amino acid sequence of SEQ ID NO:3.

15. An HIV-2 Gag polypeptide comprising the amino acid sequence of SEQ ID NO:5.


17. An antibody that specifically binds to a polypeptide of any one of claims 13-16, but does not bind to Env proteins from other HIV-I or HIV-2 virus strains.

18. A composition for detecting HIV-2NWK08F nucleic acids comprising at least 6 contiguous nucleotides of SEQ ID NO:1 or SEQ ID NO:4.

19. A composition for detecting antibodies specific for HIV-2NWK08F comprising at least 10 contiguous amino acids of SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:5, or SEQ ID NO:6.

20. A composition for detecting HIV-2NWK08F polypeptides comprising an antibody of claim 12.

21. A method for detecting HIV-2NWK08F nucleic acids in a biological sample comprising

   (a) contacting the biological sample with a composition comprising a nucleotide sequence of claim 18;

   (b) detecting the hybridization of said nucleotide sequence to the nucleic acid of the biological sample.

22. A method of detecting HIV-2NWK08F nucleic acids in a biological sample comprising

   (a) contacting the biological sample with primers derived from HIV-2NWK08F capable of amplifying an HIV-2NWK08F genome;

   (b) amplifying the HIV-2NWK08F nucleic acid; and
(c) detecting the presence of amplified HIV-2NWK08F nucleic acid.

23. A method for detecting anti-HIV-2NWK08F antibodies in a biological sample comprising:

(a) contacting the biological sample with a composition comprising at least one antigen of the HIV-2NWK08F virus of claim 1; and

(b) detecting an immunological complex formed between the anti-HIV-2NWK08F antibodies and the antigen used.

24. A method for detecting HIV-2NWK08F antigens in a biological sample comprising:

(a) contacting the biological sample with a composition comprising at least one anti-HIV-2NWK08F-antibody of claim 12; and

(b) detecting an immunological complex formed between the antigens and the antibody used.

25. The method of any one of claims 21 to 24, wherein the biological sample is selected from the group consisting of cells, blood, urine, hair, saliva, and serum.

26. The method of any one of claims 21 to 24, wherein the biological sample is a fluid or a tissue.

27. The method of any one of claims 21 to 24, wherein the biological sample is a cell free supernatant derived from a biological fluid.

28. A method for the preparation of hybridomas which produce monoclonal antibodies specific for an HIV-2NWK08F polypeptide, comprising the following the steps:

(a) immunizing a mammal with a polypeptide of claim 11 or immunogenic fragment thereof;

(b) isolating immunized splenocytes from said mammal;

(c) fusing the immunized splenocytes with a myeloma cell line to produce hybridomas;
(d) selecting for the hybridomas by culturing in selective media;

(e) clonally expanding the hybridomas in appropriate culture media; and,

(f) identifying and characterizing those hybridomas that produce
monoclonal antibodies specific for an HIV-2NWK08F polypeptide.