HIV phylogenic classifications are normally based either on nucleotide sequences derived from multiple sub genomic regions (*gag, pol* and *env*) of the same isolates, or on full-length genome sequence analysis. A phylogenic analysis of HIV-1 near-full length sequences revealed that HIV-1 subtype B was most closely related genetically to HIV subtype D (Figure 1). Phylogenic analyses of HIV-1 Gag and Pol protein sequences also showed that HIV-1 subtype B was most closely related genetically to HIV subtype D (Figure 2).

Nevertheless, HIV-1-NDK, a subtype D virus, is significantly more cytopathic for CD4+ lymphocytes than the HIV-1-BRU prototype, a subtype B virus. This may be due to enhanced fusogenicity and infectivity of subtype D viruses. De Mareuil et al., J. Virol. 66: 6797 (1992). Phenotypic analysis of recombinant viruses indicated that 75 amino acids from the N-terminal part of HIV-1-NDK matrix (MA) protein, together with the HIV-1-NDK envelope glycoprotein, are responsible for enhanced fusogenicity of HIV-1-NDK in CD4+ lymphocytes as well as for enhanced infectivity of HIV-1-NDK in some CD4- cell lines. *Id.*

There is a need in the art for lentiviral packaging constructs producing higher titers of packaged lentiviral vectors, in order to reduce injection volumes, increase dosages, reduce the cost of vaccination, and increase the number of patients that could be treated with one batch. The current invention fulfills this need.

Brief Summary

The *gag-pol* gene of subtype B of HIV-1 in a lentiviral packaging plasmid (construct p8.74) was replaced by the *gag-pol* gene of a subtype D HIV-1 to generate construct pThV-GP-N. The constructs were used for lentiviral vector production. Approximately 2-fold higher titers were obtained using the pThV-GP-N plasmid as compared to construct p8.74. Thus, the Gag-Pol of a subtype D virus increases the titer of lentiviral vector particles relative to a Gag-Pol of a subtype B virus.

In one aspect, there is provided a replication-defective lentiviral packaging vector lacking a Ψ site, encoding the HIV-1 Gag-Pol protein of HIV-1 NDK and not encoding a functional HIV-1 Env. In one ~~aspect~~example, there is ~~provided~~ disclosed a replication-defective lentiviral packaging vector lacking a Ψ site and encoding a subtype D HIV-1 Gag-Pol protein.

In another aspect, there is provided a method for generating a packaging vector, the method comprising inserting a nucleotide sequence encoding the HIV-1 Gag-Pol protein of HIV-1 NDK into a lentiviral plasmid under the control of a non-HIV promoter to generate the packaging vector, said packaging vector lacking a Ψ site and not encoding a functional HIV-1 Env. In one example, there is disclosed a method for generating a packaging vector, the

method comprising inserting a nucleotide sequence encoding a subtype D HIV-1 Gag-Pol protein into a plasmid under the control of a non-HIV promoter to generate the packaging vector.

In another aspect, there is provided an in vitro method for generating a lentiviral vector particle, the method comprising administering a packaging vector encoding the HIV-1 Gag-Pol protein of HIV-1 NDK to a cell with a lentiviral vector, said packaging vector lacking a Ψ site and not encoding a functional HIV-1 Env. In one example, there is disclosed an *in vitro* method for generating a lentiviral vector particle, the method comprising administering a packaging vector encoding a subtype D HIV-1 Gag-Pol protein to a cell with a lentiviral vector.

In another aspect, there is provided a cell comprising the vector as described herein.

In another aspect, there is provided a lentiviral vector particle comprising the HIV-1 Gag-Pol protein of HIV-1 NDK and VSV Env glycoproteins. In one example, there is disclosed a lentiviral vector particle comprising subtype D HIV-1 Gag and Pol proteins.

Encompassed herein is a lentiviral packaging vector comprising a subtype D *gag-pol* sequence, particularly from HIV-1 NDK. In one example, the lentiviral packaging vector comprises the nucleotide sequence of SEQ ID NO:1. In another example, the lentiviral packaging vector encodes the amino acid sequence of SEQ ID NO:2.

The nucleotide sequence of SEQ ID NO 1 is:

atgggtgcgagagcgtcagtattaagcgggggaaaattagatacatgggaaagaattcggttac
ggccaggaggaaagaaaaaatatgcactaaaacatttgatatgggcaagcagggagctagaacg

Claims

1. A replication-defective lentiviral packaging vector lacking a Ψ site, ~~and~~ encoding the HIV-1 Gag-Pol protein of HIV-1 NDK and not encoding a functional HIV-1 Env.~~a subtype D HIV-1 Gag-Pol protein.~~

~~2. The vector of claim 1, wherein the HIV-1 Gag-Pol protein is the HIV-1 Gag-Pol protein of HIV-1 NDK.~~

~~3~~2. The vector of claim ~~2~~1, wherein the vector encodes the amino acid sequence of SEQ ID NO: 2.

~~4~~3. The vector of any one of claims 1 to ~~3~~2, wherein the vector is a plasmid comprising a nucleotide sequence encoding a subtype D HIV-1 Gag-Pol protein.

~~5~~4. A method for generating a packaging vector, the method comprising inserting a nucleotide sequence encoding the HIV-1 Gag-Pol protein of HIV-1 NDK~~a subtype D HIV-1 Gag-Pol protein~~ into a lentiviral plasmid under the control of a non-HIV promoter to generate the packaging vector, said packaging vector lacking a Ψ site and not encoding a functional HIV-1 Env.

~~6. The method of claim 5, wherein the HIV-1 Gag-Pol protein is the HIV-1 Gag-Pol protein of HIV-1 NDK.~~

~~7~~5. The method of claim ~~6~~4, wherein the vector encodes the amino acid sequence of SEQ ID NO: 2.

~~8~~6. An *in vitro* method for generating a lentiviral vector particle, the method comprising administering a packaging vector encoding the HIV-1 Gag-Pol protein of HIV-1 NDK~~a subtype D HIV-1 Gag-Pol protein~~ to a cell with a lentiviral vector, said packaging vector lacking a Ψ site and not encoding a functional HIV-1 Env.

~~9~~7. The method of claim ~~8~~6, wherein the packaging vector and the lentiviral vector are plasmids.

~~10~~8. The method of claim ~~8~~6, wherein the cell transiently expresses the HIV-1 Gag-Pol protein of HIV-1 NDK.~~subtype D HIV-1 Gag and Pol proteins.~~

~~11~~9. The method of claim ~~8~~6, wherein the packaging vector is integrated into the genome of the cell.

~~12. The method of claim 8, wherein the HIV-1 Gag-Pol protein is the HIV-1 Gag-Pol protein of HIV-1 NDK.~~

~~13~~10. The method of claim ~~12~~6, wherein the packaging vector encodes the amino acid sequence of SEQ ID NO: 2.

~~14~~11. The method of claim ~~8~~6, further comprising selecting for a packaging vector that packages a higher titer of the lentiviral vector than a same packaging vector encoding a subtype B HIV-1 Gag-Pol protein.

~~15~~12. A cell comprising the vector of any one of claim~~s~~ 1 or 2.~~-3.~~

~~16~~13. The cell of claim ~~15~~12, wherein the vector is integrated into the genome of the cell.

~~17~~14. A lentiviral vector particle comprising the HIV-1 Gag-Pol protein of HIV-1 NDK and VSV Env glycoproteins.~~subtype D HIV-1 Gag and Pol proteins.~~

~~18. The lentiviral vector particle of claim 17, wherein the Gag and Pol proteins are HIV-1 NDK Gag and Pol proteins.~~

~~19~~15. The ~~method~~ lentiviral vector particle of claim ~~18~~14, wherein the Gag and Pol proteins are Gag and Pol proteins produced by expressing a nucleic acid sequence that encodes the amino acid sequence of SEQ ID NO: 2.