

SUMMARY

[0008] The present disclosure is directed to optimized HIV-1 gp41-Binding Molecules having reduced immunogenicity. More specifically, the disclosure relates to optimized gp41-Binding Molecules that comprise a gp41-binding Variable Light Chain (VL) Domain and/or a gp41-binding Variable Heavy Chain (VH) Domain that has/have been optimized to reduce the immunogenicity of such Domain(s) upon administration to a recipient subject. The disclosure particularly pertains to gp41-Binding Molecules that are multispecific gp41-Binding Molecules (including bispecific diabodies (including DART® diabodies), BiTE®, bispecific antibodies, trivalent binding molecules (including TRIDENT™ molecules), *etc.*) that comprise: (i) such optimized gp41-binding Variable Domain(s) and (ii) a domain capable of binding to an epitope of a molecule present on the surface of an effector cell. The disclosure is also directed to pharmaceutical compositions that comprise any of such gp41-Binding Molecules, and to methods involving the use of any of such gp41-Binding Molecules in the treatment of HIV-1 infection.

[0009] In one aspect, the present disclosure refers to a gp41-Binding Molecule comprising a Variable Light Chain (VL) Domain and a Variable Heavy Chain (VH) Domain, wherein the VL Domain comprises the amino acid sequence of **SEQ ID NO:57** and/or the VH Domain comprises the amino acid sequence of **SEQ ID NO:58**.

[0010] The disclosure further relates to the example of the above-indicated gp41-Binding Molecule, wherein the gp41-Binding Molecule comprises:

- (a) the VL Domain that comprises the amino acid sequence of **SEQ ID NO:57** and
- (b) the VH Domain that comprises the amino acid sequence of **SEQ ID NO:58**.

[0011] The disclosure further relates to the examples of the above-indicated gp41-Binding Molecule, wherein the molecule is an antibody or comprises a gp41 epitope-binding portion thereof.

[0012] The disclosure further relates to the example of the above-indicated gp41-Binding Molecules, wherein the molecule is:

- (a) a bispecific antibody; or
- (b) a diabody, the diabody being a covalently bonded complex that comprises two, three, four or five polypeptide chains; or

- (c) a trivalent binding molecule, the trivalent binding molecule being a covalently bonded complex that comprises three, four, five, or more than five polypeptide chains.

[0013] The disclosure further relates to the example of the above-indicated gp41-Binding Molecules, wherein the molecule is the diabody and comprises an Albumin-Binding Domain (ABD).

[0014] The disclosure further relates to the example of the above-indicated gp41-Binding Molecules, wherein the molecule comprises an Fc Region. The disclosure further relates to the example of such gp41-Binding Molecules, wherein the Fc Region is a variant Fc Region that comprises:

- (a) one or more amino acid modifications that reduces the affinity of the variant Fc Region for an FcγR; and/or
- (b) one or more amino acid modifications that enhances the serum half-life of the variant Fc Region.

[0015] The disclosure further relates to the example of such gp41-Binding Molecules, wherein the modifications that reduce the affinity of the variant Fc Region for an FcγR comprise the substitution of L234A; L235A; or L234A and L235A, wherein the numbering is that of the EU index as in Kabat.

[0016] The disclosure further relates to the example of such gp41-Binding Molecules, wherein the modifications that enhance the serum half-life of the variant Fc Region comprise the substitution of M252Y; M252Y and S254T; M252Y and T256E; M252Y, S254T and T256E; or K288D and H435K, wherein the numbering is that of the EU index as in Kabat.

[0017] The disclosure further relates to the example of the above-indicated gp41-Binding Molecules, wherein the molecule is bispecific and comprises one epitope-binding site capable of immunospecific binding to an epitope of gp41 and one epitope-binding site capable of immunospecific binding to an epitope of a molecule present on the surface of an effector cell.

[0018] The disclosure further relates to the example of the above-indicated gp41-Binding Molecules, wherein the molecule is bispecific and comprises two epitope-binding

sites capable of immunospecific binding to an epitope of gp41 and two epitope-binding sites capable of immunospecific binding to an epitope of a molecule present on the surface of an effector cell.

[0019] The disclosure further relates to the example of the above-indicated gp41-Binding Molecules, wherein the molecule is trispecific and comprises:

- (a) one epitope-binding site capable of immunospecific binding to an epitope of gp41;
- (b) one epitope-binding site capable of immunospecific binding to an epitope of a first molecule present on the surface of an effector cell; and
- (c) one epitope-binding site capable of immunospecific binding to an epitope of a second molecule present on the surface of an effector cell.

[0020] The disclosure further relates to the example of such gp41-Binding Molecules, wherein the first molecule present on the surface of an effector cell is CD3 and the second molecule present on the surface of an effector cell is CD8.

[0021] The disclosure further relates to the example of the above-indicated gp41-Binding Molecules, wherein the molecule is trispecific and comprises:

- (a) one epitope-binding site capable of immunospecific binding to a first epitope of gp41;
 - (b) one epitope-binding site capable of immunospecific binding to a second epitope of gp41 or an epitope of a different HIV-1 protein; and
 - (c) one epitope-binding site capable of immunospecific binding to an epitope a molecule present on the surface of an effector cell;
- wherein the first and second epitopes of gp41 are different.

[0022] The disclosure further relates to the example of the above-indicated gp41-Binding Molecules, wherein the molecule is capable of simultaneously binding to gp41 and the molecule present on the surface of an effector cell. The disclosure further relates to the example of such gp41-Binding Molecules, wherein the molecule present on the surface of an effector cell is CD2, CD3, CD8, CD16, TCR, NKp46, or NKG2D.

[0023] The disclosure further relates to the example of the above-indicated gp41-Binding Molecules, wherein the effector cell is a cytotoxic T-cell, or a Natural Killer (NK) cell.

[0024] The disclosure further relates to the example of the above-indicated gp41-Binding Molecules, wherein the molecule mediates coordinated binding of a cell expressing gp41 and a cytotoxic T cell.

[0025] The disclosure further relates to the example of such gp41-Binding Molecules, wherein the molecule comprises a first polypeptide chain, a second polypeptide chain and a third polypeptide chain, and wherein:

- I) (a) the first polypeptide chain comprises **SEQ ID NO:113**;
(b) the second polypeptide chain comprises **SEQ ID NO:114**; and
(c) the third polypeptide chain comprises **SEQ ID NO:112**;
or
- II) (a) the first polypeptide chain comprises **SEQ ID NO:115**;
(b) the second polypeptide chain comprises **SEQ ID NO:116**; and
(c) the third polypeptide chain comprises **SEQ ID NO:112**.

[0026] The disclosure further relates to the example of such gp41-Binding Molecules, wherein the molecule comprises a first polypeptide chain, a second polypeptide chain, a third polypeptide chain, and a fourth polypeptide chain and wherein:

- (a) the first polypeptide chain comprises **SEQ ID NO:113**;
- (b) the second polypeptide chain comprises **SEQ ID NO:114**;
- (c) the third polypeptide chain comprises **SEQ ID NO:117**; and
- (d) the fourth polypeptide chain comprises **SEQ ID NO:118**.

[0027] The disclosure further relates to the example of such gp41-Binding Molecules, wherein the molecule comprises a first polypeptide chain, a second polypeptide chain, a third polypeptide chain, and a fourth polypeptide chain and wherein:

- I) (a) the first polypeptide chain comprises **SEQ ID NO:113**;
(b) the second polypeptide chain comprises **SEQ ID NO:114**;
(c) the third polypeptide chain comprises **SEQ ID NO:119**; and
(d) the fourth polypeptide chain comprises **SEQ ID NO:120**;
or
- II) (a) the first polypeptide chain comprises **SEQ ID NO:115**;
(b) the second polypeptide chain comprises **SEQ ID NO:116**;
(c) the third polypeptide chain comprises **SEQ ID NO:119**; and
(d) the fourth polypeptide chain comprises **SEQ ID NO:120**.

[0028] In one aspect, the present disclosure refers to a pharmaceutical composition that comprises an effective amount of the gp41-Binding Molecule as disclosed herein and a pharmaceutically acceptable carrier.

[0029] In one aspect, the present disclosure refers to use of gp41-Binding Molecule as disclosed herein, or the pharmaceutical composition as disclosed herein, in the manufacture of a medicament for the treatment or prevention of an HIV-1 infection in a subject. The disclosure further relates to a method to treat or prevent HIV-1 infection in a subject in need thereof comprising administering to the subject a composition comprising any one of the above-indicated gp41-Binding Molecules or the above-indicated pharmaceutical composition in a therapeutically effective amount.

[0030] The disclosure further relates to the example of such method to treat or prevent HIV-1 infection that further comprises administering a latency-activating agent (such as vorinostat, romidepsin, panobinostat, disulfiram, JQ1, bryostatin, PMA, inonomecin, or any combination thereof).

BRIEF DESCRIPTION OF THE DRAWINGS:

[0031] **Figures 1A-1B** provide a schematic of a representative covalently bonded diabody having two Epitope Binding Domains composed of two polypeptide chains, each having an E-coil or K-coil Heterodimer-Promoting Domain (alternative Heterodimer-Promoting Domains are provided below). A cysteine residue may be present in a linker (**Figure 1A**) and/or in the Heterodimer-Promoting Domain (**Figure 1B**). VL and VH Domains that recognize the same epitope are shown using the same shading or fill pattern. The wavy line (WWW) in this and all of the Figures providing schematic presentations of binding molecule domains represents one or more optional Heterodimer-Promoting Domains, that is/are preferably present.

[0032] **Figure 2** provides a schematic of a representative covalently bonded diabody molecule having two Epitope Binding Domains composed of two polypeptide chains, each having a CH2 and CH3 Domain, such that the associated chains form all or part of an Fc Region. VL and VH Domains that recognize the same epitope are shown using the same shading or fill pattern.

[0033] **Figures 3A-3C** provide schematics showing representative covalently bonded tetravalent diabodies having four Epitope Binding Domains composed of two pairs of polypeptide chains (*i.e.*, four polypeptide chains in all). One polypeptide chain of each pair possesses a CH2 and CH3 Domain, such that the associated chains form all or part of an Fc

WHAT IS CLAIMED IS:

- Claim 1. A gp41-Binding Molecule comprising a Variable Light Chain (VL) Domain and a Variable Heavy Chain (VH) Domain, wherein the VL Domain comprises the amino acid sequence of **SEQ ID NO:57** and/or said VH Domain comprises the amino acid sequence of **SEQ ID NO:58**.
- Claim 2. The gp41-Binding Molecule of claim 1, wherein said gp41-Binding Molecule comprises:
- (a) said VL Domain that comprises the amino acid sequence of **SEQ ID NO:57** and
 - (b) said VH Domain that comprises the amino acid sequence of **SEQ ID NO:58**.
- Claim 3. The gp41-Binding Molecule of any one of claims 1-2, wherein said molecule is an antibody or comprises a gp41 epitope-binding portion thereof.
- Claim 4. The gp41-Binding Molecule of any one of claims 1-3, wherein said molecule is:
- (a) a bispecific antibody; or
 - (b) a diabody, said diabody being a covalently bonded complex that comprises two, three, four or five polypeptide chains; or
 - (c) a trivalent binding molecule, said trivalent binding molecule being a covalently bonded complex that comprises three, four, five, or more than five polypeptide chains.
- Claim 5. The gp41-Binding Molecule of claim 4, wherein said molecule is said diabody and comprises an Albumin-Binding Domain (ABD).
- Claim 6. The gp41-Binding Molecule of any one of claims 1-5, wherein said molecule comprises an Fc Region.
- Claim 7. The gp41-Binding Molecule of claim 6, wherein said Fc Region is a variant Fc Region that comprises:
- (a) one or more amino acid modifications that reduces the affinity of the variant Fc Region for an FcγR; and/or

- (b) one or more amino acid modifications that enhances the serum half-life of the variant Fc Region.

Claim 8. The gp41-Binding Molecule of claim 7, wherein said modifications that reduce the affinity of the variant Fc Region for an FcγR comprise the substitution of L234A; L235A; or L234A and L235A, wherein said numbering is that of the EU index as in Kabat.

Claim 9. The gp41-Binding Molecule of any one of claims 7 or 8, wherein said modifications that enhance the serum half-life of the variant Fc Region comprise the substitution of M252Y; M252Y and S254T; M252Y and T256E; M252Y, S254T and T256E; or K288D and H435K, wherein said numbering is that of the EU index as in Kabat.

Claim 10. The gp41-Binding Molecule of any one of claims 1-9, wherein said molecule is bispecific and comprises:

- (a) one epitope-binding site capable of immunospecific binding to an epitope of gp41; and
- (b) one epitope-binding site capable of immunospecific binding to an epitope of a molecule present on the surface of an effector cell.

Claim 11. The gp41-Binding Molecule of any one of claims 1-9, wherein said molecule is bispecific and comprises:

- (a) two epitope-binding sites capable of immunospecific binding to an epitope of gp41; and
- (b) two epitope-binding sites capable of immunospecific binding to an epitope of a molecule present on the surface of an effector cell.

Claim 12. The gp41-Binding Molecule of any one of claims 1-9, wherein said molecule is trispecific and comprises:

- (a) one epitope-binding site capable of immunospecific binding to an epitope of gp41;

- (b) one epitope-binding site capable of immunospecific binding to an epitope of a first molecule present on the surface of an effector cell; and
- (c) one epitope-binding site capable of immunospecific binding to an epitope of a second molecule present on the surface of an effector cell.

Claim 13. The gp41-Binding Molecule of any one of claims 1-9, wherein said molecule is trispecific and comprises:

- (a) one epitope-binding site capable of immunospecific binding to a first epitope of gp41;
- (b) one epitope-binding site capable of immunospecific binding to a second epitope of gp41 or an epitope of a different HIV-1 protein; and
- (c) one epitope-binding site capable of immunospecific binding to an epitope a molecule present on the surface of an effector cell;

wherein said first and second epitopes of gp41 are different.

Claim 14. The gp41-Binding Molecule of any one of claims 10-13, wherein said molecule is capable of simultaneously binding to gp41 and said molecule present on the surface of an effector cell.

Claim 15. The gp41-Binding Molecule of any one of claims 10-14, wherein said molecule present on the surface of an effector cell is CD2, CD3, CD8, CD16, TCR, NKp46, or NKG2D.

Claim 16. The gp41-Binding Molecule of any one of claims 10-15, wherein said effector cell is a cytotoxic T-cell, or a Natural Killer (NK) cell.

Claim 17. The gp41-Binding Molecule of claim 15, wherein said molecule present on the surface of an effector cell is CD3.

Claim 18. The gp41-Binding Molecule of claim 12, wherein said first molecule present on the surface of an effector cell is CD3 and said second molecule present on the surface of an effector cell is CD8.

- Claim 19. The gp41-Binding Molecule of any one of claims 10-18, wherein said molecule mediates coordinated binding of a cell expressing gp41 and a cytotoxic T cell.
- Claim 20. The gp41-Binding Molecule of any one of claims 1-4 and 6-9 wherein said molecule comprises a first polypeptide chain, a second polypeptide chain and a third polypeptide chain, and wherein:
- I) (a) said first polypeptide chain comprises **SEQ ID NO:113**;
 - (b) said second polypeptide chain comprises **SEQ ID NO:114**;
 - and
 - (c) said third polypeptide chain comprises **SEQ ID NO:112**;
 - or
 - II) (a) said first polypeptide chain comprises **SEQ ID NO:115**;
 - (b) said second polypeptide chain comprises **SEQ ID NO:116**;
 - and
 - (c) said third polypeptide chain comprises **SEQ ID NO:112**.
- Claim 21. The gp41-Binding Molecule of any one of claims 1-4 and 6-9, wherein said molecule comprises a first polypeptide chain, a second polypeptide chain, a third polypeptide chain, and a fourth polypeptide chain and wherein:
- (a) said first polypeptide chain comprises **SEQ ID NO:113**;
 - (b) said second polypeptide chain comprises **SEQ ID NO:114**;
 - (c) said third polypeptide chain comprises **SEQ ID NO:117**; and
 - (d) said fourth polypeptide chain comprises **SEQ ID NO:118**.
- Claim 22. The gp41-Binding Molecule of any one of claims 1-4 and 6-9, wherein said molecule comprises a first polypeptide chain, a second polypeptide chain, a third polypeptide chain, and a fourth polypeptide chain and wherein:
- I) (a) said first polypeptide chain comprises **SEQ ID NO:113**;
 - (b) said second polypeptide chain comprises **SEQ ID NO:114**;
 - (c) said third polypeptide chain comprises **SEQ ID NO:119**; and
 - (d) said fourth polypeptide chain comprises **SEQ ID NO:120**;
 - or
 - II) (a) said first polypeptide chain comprises **SEQ ID NO:115**;
 - (b) said second polypeptide chain comprises **SEQ ID NO:116**;

- (c) said third polypeptide chain comprises **SEQ ID NO:119**; and
- (d) said fourth polypeptide chain comprises **SEQ ID NO:120**.

- Claim 23. A pharmaceutical composition that comprises an effective amount of the gp41-Binding Molecule of any one of claims 1-22 and a pharmaceutically acceptable carrier.
- Claim 24. Use of gp41-Binding Molecule of any one of claims 1-22, or the pharmaceutical composition of claim 23, in the manufacture of a medicament for the treatment or prevention of an HIV-1 infection in a subject.
- Claim 25. The use of claim 24, further comprising a latency-activating agent for use in combination with said medicament.
- Claim 26. The use of claim 25, wherein said latency-activating agent is vorinostat, romidepsin, panobinostat, disulfiram, JQ1, bryostatin, PMA, ionomycin, or any combination thereof.