One example of the present disclosure relates to an antibody directed against CD4, compositions thereof, and methods employing such compositions for the prevention, treatment, and/or functional cure of HIV infection. In certain examples, the antibody specifically binds to the CDR2 region in domain 1 of CD4. The disclosed antibody exerts potent competitive HIV entry inhibition through its binding to domain 1 of CD4 in both cell-free and cell-to-cell systems. The disclosed antibody also inhibits antigen induced T cell proliferation and cytokine production (IL2 and IFN-gamma) of CD4 positive T cells, which is implicated in the pathogenic cycle of pyroptosis. The disclosed antibody also has the ability to reactivate resting CD4 positive T cells. This property is particularly useful for reactivating latent reservoirs of HIV in resting T cells to make these cells susceptible to treatment with antiretroviral agents. Such high affinity antibodies to CD4 are capable of activating resting HIV infected cells for the release of HIV. Reactivation of HIV infected resting CD4+ T cells allows combinational treatment incorporating antibody of the current invention with HAART in HIV infected patients leading to functional cure.

The present disclosure is directed to methods for the treatment, prevention, and functional cure of HIV infection. In certain examples, the formulations contain antibodies directed against CD4. The present disclosure also includes antiviral agents that can be used in the methods for the treatment, prevention, and functional cure of HIV infection.

In certain examples, disclosed herein are pharmaceutical compositions comprising monoclonal human, humanized or chimeric, anti-CD4 antibodies with the above described binding characteristics that when given, at a dose of about 10 mg/kg or higher on a weekly or biweekly schedule, as a monotherapy, such treatment can reduce viral load down to non-detectable level in treated subjects with no viral load rebound as long the serum antibody level is higher than 10 µg/mL.

In another examples, disclosed herein are pharmaceutical compositions comprising a monoclonal humanized anti-CD4 antibody with the above described binding characteristics as the key ingredient in an adjunct therapy with HAART, that when given, at about 10 mg/kg or higher on a weekly or biweekly schedule, to treatment naïve HIV patients, will lead to functional cure of the patients.

In one aspect, the present invention refers to use of an antibody that specifically binds to the CDR2 region in domain 1 of CD4 in the manufacture of a medicament for treating a subject before or after being exposed to HIV, wherein the antibody comprises: a heavy

chain comprising an amino acid sequence of SEQ ID NO: 10; and a light chain comprising an amino acid sequence of SEQ ID NO: 8. In another aspect, the present invention refers to use of a) a pharmacologically effective amount of an antibody directed against the CDR2 region of domain 1 of CD4 comprising a heavy chain variable region amino acid sequence comprising: CDR1 of SEQ ID NO: 1, CDR2 of SEQ ID NO: 2, and CDR3 of SEQ ID NO: 3; and a heavy chain constant region amino acid sequence of SEQ ID NO: 12; a light chain variable region amino acid sequence comprising: CDR1 of SEQ ID NO: 4, CDR2 of SEQ ID NO: 5, and CDR3 of SEQ ID NO: 6; and a light chain constant region amino acid sequence of SEQ ID NO: 14; and b) a highly active antiretroviral therapy (HAART), in the manufacture of a medicament for treating a subject with HIV infection, wherein the medicament is to be administered to the subject in a treatment regimen. In yet another aspect, the present invention refers to a antibody directed against a CD4 molecule, wherein the antibody comprises: a heavy chain variable region amino acid sequence comprising: CDR1 of SEQ ID NO: 1, CDR2 of SEQ ID NO: 2, and CDR3 of SEQ ID NO: 3; and a heavy chain constant region amino acid sequence of SEQ ID NO: 12; a light chain variable region amino acid sequence comprising: CDR1 of SEQ ID NO: 4, CDR2 of SEQ ID NO: 5, and CDR3 of SEQ ID NO: 6; and a light chain constant region amino acid sequence of SEQ ID NO: 14; and wherein the antibody: a) binds to the CDR2 region in domain 1 of the CD4 molecule; b) competitively inhibits HIV entry into a cell; and c) reduces HIV viral load in an HIV positive patient to less than 50 copies per milliliter of blood without viral load rebound, when the antibody is to be administered to the HIV positive patient in an amount sufficient to fully coat CD4+ cells. In a further aspect, the present invention refers to a composition comprising the antibody as disclosed herein. In another aspect, the present invention refers to a pharmaceutical composition comprising the antibody disclosed herein and a pharmaceutically acceptable carrier. In another aspect, the present invention refers to a pharmaceutical composition comprising the antibody as disclosed herein in phosphate buffer saline (PBS), 20 mM glycine, and 0.05% (v/v) polysorbate 20. In a further aspect, the present invention refers to a pharmaceutical composition comprising the antibody as disclosed herein in phosphate buffer saline (PBS), 20 mM glycine, 0.05% (v/v) polysorbate 20, and 10 mM histidine. In yet another aspect, the present invention refers to a pharmaceutical composition comprising the antibody as disclosed herein in phosphate buffer saline (PBS), 20 mM glycine, 0.05% (v/v) polysorbate 20, and 10 mM histidine. In one aspect, the present invention refers to a pharmaceutical composition comprising about 1.0 mg/mL to about 200.0 mg/mL of the antibody as disclosed herein in phosphate buffer saline (PBS), 20 mM glycine, 0.05% (v/v)

polysorbate 20, and 10 mM histidine. In another aspect, the present invention refers to a pharmaceutical composition comprising about 10.0 mg/mL of the antibody as disclosed herein in phosphate buffer saline (PBS), 20 mM glycine, and 0.05% (v/v) polysorbate 20. In yet another aspect, the present invention refers to a pharmaceutical composition comprising about 10.0 mg/mL of the antibody as disclosed herein in phosphate buffer saline (PBS), 20 mM glycine, 0.05% (v/v) polysorbate 20, and 10 mM histidine. In another aspect, the present invention refers to use of the antibody as disclosed herein in the manufacture of a medicament for treating a subject before or after being exposed to HIV. In one aspect, the present invention refers to use of a) a pharmacologically effective amount of the antibody as disclosed herein; and b) a highly active antiretroviral therapy (HAART) in the manufacture of a medicament for treating a subject with HIV infection, wherein the medicament is to be administered to the subject in a treatment regimen. In another aspect, the present invention refers an *in vitro* method for inhibiting HIV entry into a CD4+ cell, comprising exposing the antibody as disclosed herein to the cell. In a further aspect, the present invention refers to an *in vitro* method for inhibiting gp120 binding to a CD4+ cell, comprising exposing the antibody as disclosed herein to the cell. In another aspect, the present invention refers to an *in vitro* method for activating a resting CD4+ T cell upon crosslinking, comprising exposing the antibody disclosed herein to the cell. In a further aspect, the present invention refers to an *in vitro* method for activating a latent reservoir of HIV in a resting T cell upon crosslinking, comprising exposing the antibody disclosed herein to the cell. In another aspect, the present invention refers to an *in vitro* method for reducing latent HIV reservoirs in a sample of cells infected with HIV, the use comprising a) exposing the antibody as disclosed herein to the sample of cells; and b) exposing HAART to the sample of cells.

CLAIMS

1. Use of an antibody that specifically binds to the CDR2 region in domain 1 of CD4 in the manufacture of a medicament for treating a subject before or after being exposed to HIV, wherein the antibody comprises:

a heavy chain comprising an amino acid sequence of SEQ ID NO: 10; and

a light chain comprising an amino acid sequence of SEQ ID NO: 8.

2. The use according to claim 1, wherein the heavy chain comprises an amino acid sequence of SEQ ID NO: 7.

3. The use according to claim 1, wherein the heavy chain comprises an amino acid sequence of SEQ ID NO: 9.

4. The use according to any one of claims 1 to 3, wherein the antibody is a monoclonal antibody, a polyclonal antibody, or a combination thereof.

5. The use according to any one of claims 1 to 3, wherein the antibody is a humanized monoclonal antibody.

6. The use according to claim 5, wherein the humanized monoclonal antibody is to be administered to the subject prior to exposure to HIV.

7. The use according to claim 5, wherein the humanized monoclonal antibody is to be administered to the subject after exposure to HIV.

8. The use according to claim 7, wherein the humanized monoclonal antibody is to be administered within 48 hours after exposure to HIV.

9. The use according to claim 5, wherein the humanized monoclonal antibody is to be administered to the subject at a dosage of at least about 5 mg/kg body weight.

10. The use according to claim 5, wherein the humanized monoclonal antibody is to be administered to the subject multiple times.

11. The use according to claim 10, wherein the humanized monoclonal antibody is to be administered to the subject in a weekly or bi-weekly interval.

12. The use according to any one of claims 10 or 11, further comprising a step of an antiviral agent to be administered to the subject.

13. The use according to claim 12, wherein the antiviral agent is a highly active antiretroviral therapy (HAART).

14. The use according to claim 13, wherein HAART comprises a nucleoside analogue reverse transcriptase inhibitor in combination with a protease inhibitor or a non-nucleoside reverse transcriptase inhibitor.

15. The use according to claim 13, wherein the humanized monoclonal antibody is to be administered concurrently with HAART.

16. The use according to claim 13, wherein the humanized monoclonal antibody and HAART are to be administered to the subject over the course of a cycle, wherein the cycle comprises:

i) the humanized monoclonal antibody to be administered to the subject for a period of four months in a weekly or bi-weekly interval followed by a two-month treatment holiday; and

ii) HAART to be administered to the subject continuously during the six-month period in (i).

17. The use according to claim 16, wherein the subject is treated over the course of two or more cycles.

18. Use of:

a) a pharmacologically effective amount of an antibody directed against the CDR2 region of domain 1 of CD4 comprising

a heavy chain variable region amino acid sequence comprising:

CDR1 of SEQ ID NO: 1,

CDR2 of SEQ ID NO: 2, and

CDR3 of SEQ ID NO: 3; and

a heavy chain constant region amino acid sequence of SEQ ID NO: 12;

a light chain variable region amino acid sequence comprising:

CDR1 of SEQ ID NO: 4,

CDR2 of SEQ ID NO: 5, and

CDR3 of SEQ ID NO: 6; and

a light chain constant region amino acid sequence of SEQ ID NO: 14; and

b) a highly active antiretroviral therapy (HAART),

in the manufacture of a medicament for treating a subject with HIV infection, wherein the medicament is to be administered to the subject in a treatment regimen.

19. The use according to claim 18, wherein the heavy chain constant region amino acid sequence of SEQ ID NO: 12 has amino acids Met (M), Ser (S), Thr (T), and His (H) at positions 135, 137, 139, and 180, respectively.

20. The use according to claim 18, wherein the heavy chain constant region amino acid sequence of SEQ ID NO: 12 has amino acids Tyr (Y), Thr (T), Glu (E), and His (H) at positions 135, 137, 139, and 180, respectively.

21. The use according to any one of claims 18 to 20, wherein the HAART comprises a nucleoside analogue reverse transcriptase inhibitor in combination with a protease inhibitor or a non-nucleoside reverse transcriptase inhibitor.

22. The use according to any one of claims 18 to 20, wherein the antibody is a monoclonal antibody, a polyclonal antibody, or a combination thereof.

23. The use according to any one of claims 18 to 20, wherein the antibody is a humanized monoclonal antibody.

24. The use according to claim 19, wherein the antibody is a humanized monoclonal antibody comprising:

a heavy chain comprising an amino acid sequence of SEQ ID NO: 7; and

a light chain comprising an amino acid sequence of SEQ ID NO: 8.

25. The use according to claim 20, wherein the humanized monoclonal antibody comprises:

a heavy chain comprising an amino acid sequence of SEQ ID NO: 9; and

a light chain comprising an amino acid sequence of SEQ ID NO: 8

26. The use according to any one of claims 18 to 20, wherein the humanized monoclonal antibody comprises:

a heavy chain comprising an amino acid sequence of SEQ ID NO: 11; and

a light chain comprising an amino acid sequence of SEQ ID NO: 13.

27. The use according to claim 18, wherein the humanized monoclonal antibody comprises:

a heavy chain comprising an amino acid sequence of SEQ ID NO: 10; and

a light chain comprising an amino acid sequence of SEQ ID NO: 8.

28. The use according to any one of claims 23 to 27, wherein the humanized monoclonal antibody is to be administered to the subject at a dosage of at least about 5 mg/kg body weight.

29. The use according to any one of claims 23 to 27, wherein the treatment regimen is to be administered to the subject over the course of a cycle, wherein the cycle comprises:

i) administering the humanized monoclonal antibody to the subject for a period of four months in a weekly or bi-weekly interval followed by a two month treatment holiday; and

ii) administering HAART to the subject continuously during the six-month period in (i).

30. The use according to claim 29, wherein the subject is treated with two or more cycles.

31. An antibody directed against a CD4 molecule, wherein the antibody comprises:

a heavy chain variable region amino acid sequence comprising:

CDR1 of SEQ ID NO: 1,

CDR2 of SEQ ID NO: 2, and

CDR3 of SEQ ID NO: 3; and

a heavy chain constant region amino acid sequence of SEQ ID NO: 12;

a light chain variable region amino acid sequence comprising:

CDR1 of SEQ ID NO: 4,

CDR2 of SEQ ID NO: 5, and

CDR3 of SEQ ID NO: 6; and

a light chain constant region amino acid sequence of SEQ ID NO: 14; and

wherein the antibody:

a) binds to the CDR2 region in domain 1 of the CD4 molecule;
b) competitively inhibits HIV entry into a cell; and
c) reduces HIV viral load in an HIV positive patient to less than 50 copies per milliliter of blood without viral load rebound, when the antibody is to be administered to the HIV positive patient in an amount sufficient to fully coat CD4+ cells.

32. The antibody according to claim 31, wherein the heavy chain constant region amino acid sequence of SEQ ID NO: 12 has amino acids Met (M), Ser (S), Thr (T), and His (H) at positions 135, 137, 139, and 180, respectively.

33. The antibody according to claim 31, wherein the heavy chain constant region amino acid sequence of SEQ ID NO: 12 has amino acids Tyr (Y), Thr (T), Glu (E), and His (H) at positions 135, 137, 139, and 180, respectively.

34. The antibody according to claim 31, wherein the antibody comprises:

a heavy chain variable region comprising an amino acid sequence of SEQ ID NO: 11; and

a light chain variable region comprising an amino acid sequence of SEQ ID NO: 13.

35. The antibody according to claim 31, wherein the antibody comprises:

a heavy chain comprising an amino acid sequence of SEQ ID NO: 10; and

a light chain comprising an amino acid sequence of SEQ ID NO: 8.

36. The antibody according to claim 31, wherein the antibody comprises:

a heavy chain comprising an amino acid sequence of SEQ ID NO: 9; and

a light chain comprising an amino acid sequence of SEQ ID NO: 8.

37. The antibody according to claim 31, wherein the antibody comprises:

a heavy chain comprising an amino acid sequence of SEQ ID NO: 7; and

a light chain comprising an amino acid sequence of SEQ ID NO: 8.

38. The antibody according to any one of claims 31 to 37, wherein the antibody activates a resting CD4+ cells upon crosslinking.

39. The antibody according to any one of claims 31 to 38, wherein the antibody comprises an N-glycosylation site in the Fv region.

40. The antibody according to any one of claims 31 to 39, wherein the antibody comprises an N-glycosylation site in the heavy chain variable region.

41. The antibody according to any one of claims 31 to 40, wherein the antibody comprises an asparagine (Asn) residue located in a CDR of the heavy chain variable region that is bound to a sugar molecule.

42. The antibody according to any one of claims 31 to 41, wherein the antibody is a monoclonal antibody, a polyclonal antibody, or a combination thereof.

43. The antibody according to any one of claims 31 to 42, wherein the antibody is a humanized monoclonal antibody.

44. The antibody according to any one of claims 31 to 43 having an absolute binding affinity (Kd) to membrane-bound CD4 on HPB-ALL cells between 3.1×10^{-11} M to 8.1×10^{-11} M.

45. The antibody according to any one of claims 31 to 43 bound to a CD4 molecule.

46. The antibody according to any one of claims 31 to 43 bound to a CD4 molecule on the surface of a T cell.

47. A composition comprising the antibody according to any one of claims 31 to 43.

48. A pharmaceutical composition comprising the antibody according to any one of claims 31 to 43 and a pharmaceutically acceptable carrier.

49. A pharmaceutical composition comprising the antibody according to any one of claims 31 to 43 in phosphate buffer saline (PBS), 20 mM glycine, and 0.05% (v/v) polysorbate 20.

50. A pharmaceutical composition comprising the antibody according to any one of claims 31 to 43 in phosphate buffer saline (PBS), 20 mM glycine, 0.05% (v/v) polysorbate 20, and 10 mM histidine.

51. A pharmaceutical composition comprising about 1.0 mg/mL to about 200.0 mg/mL of the antibody according to any one of claims 31 to 43 in phosphate buffer saline (PBS), 20 mM glycine, and 0.05% (v/v) polysorbate 20.

52. A pharmaceutical composition comprising about 1.0 mg/mL to about 200.0 mg/mL of the antibody according to any one of claims 31 to 43 in phosphate buffer saline (PBS), 20 mM glycine, 0.05% (v/v) polysorbate 20, and 10 mM histidine.

53. A pharmaceutical composition comprising about 10.0 mg/mL of the antibody according to any one of claims 31 to 43 in phosphate buffer saline (PBS), 20 mM glycine, and 0.05% (v/v) polysorbate 20.

54. A pharmaceutical composition comprising about 10.0 mg/mL of the antibody according to any one of claims 31 to 43 in phosphate buffer saline (PBS), 20 mM glycine, 0.05% (v/v) polysorbate 20, and 10 mM histidine.

55. Use of the antibody according to any one of claims 31 to 43 in the manufacture of a medicament for treating a subject before or after being exposed to HIV.

56. The use of claim 55, wherein the antibody is to be administered to the subject prior to exposure to HIV.

57. The use according to claim 55, wherein the antibody is to be administered to the subject after exposure to HIV.

58. The use according to claim 55, wherein the antibody is to be administered within 48 hours after exposure to HIV.

59. The use according to claim 55, wherein the antibody is to be administered to the subject at a dosage of at least about 5 mg/kg body weight.

60. The use according to claim 55, wherein the antibody is to be administered to the subject multiple times.

61. The use according to claim 60, wherein the antibody is to be administered to the subject in a weekly, bi-weekly, or monthly interval.

62. The use according to claim 60, further comprising a step of an antiviral agent to be administered to the subject.

63. The use according to claim 62, wherein the antiviral agent is a highly active antiretroviral therapy (HAART).

64. The use according to claim 63, wherein HAART comprises a nucleoside analogue reverse transcriptase inhibitor in combination with a protease inhibitor or a non-nucleoside reverse transcriptase inhibitor.

65. The use according to claim 63, wherein the antibody is to be administered concurrently with HAART.

66. The use according to claim 63, wherein the antibody and HAART are to be administered to the subject over the course of a cycle, wherein the cycle comprises:

i) the antibody to be administered to the subject for a first period of time followed by a treatment holiday for a second period of time; and

ii) HAART to be administered to the subject continuously during the first period of time and the second period of time in (i).

67. The use according to claim 63, wherein the antibody and HAART are to be administered to the subject over the course of a cycle, wherein the cycle comprises:

i) the antibody to be administered to the subject for a period of four months in a weekly, bi-weekly, or monthly interval followed by a two month treatment holiday; and

ii) HAART to be administered to the subject continuously during the six-month period in (i).

68. The use according to any one of claims 66 or 67, wherein the subject is treated over the course of two cycles.

69. The use according to claim 63, wherein the antibody is to be administered at a time that is not concurrent with HAART.

70. The use according to claim 63, wherein the antibody and HAART are to be administered to the subject over the course of a cycle, wherein the cycle comprises:

i) the antibody to be administered to the subject for a first period of time followed by a treatment holiday for a second period of time; and

ii) HAART to be administered to the subject during the second period of time and not during the first period of time.

71. The use according to claim 70, wherein the antibody is to be administered in regular intervals during the first time period.

72. The use according to claim 70, wherein the antibody is to be administered in weekly, bi-weekly, or monthly intervals during the first time period.

73. Use of

a) a pharmacologically effective amount of the antibody according to any one of claims 31 to 43; and

b) a highly active antiretroviral therapy (HAART)

in the manufacture of a medicament for treating a subject with HIV infection, wherein the medicament is to be administered to the subject in a treatment regimen.

74. The use of claim 73, wherein the antibody is to be administered to the subject at a dosage of at least about 5 mg/kg body weight.

75. The use according to claim 73, wherein the antibody and HAART are to be administered to the subject over the course of a cycle, wherein the cycle comprises:

i) the antibody to be administered to the subject for a first period of time followed by a treatment holiday for a second period of time; and

ii) HAART to be administered the subject continuously during the first period of time and the second period of time in (i).

76. The use according to claim 73, wherein the antibody and HAART are to be administered to the subject over the course of a cycle, wherein the cycle comprises:

i) the antibody to be administered to the subject for a period of four months in a weekly, bi-weekly, or monthly interval followed by a two-month treatment holiday; and

ii) HAART to be administered to the subject continuously during the six-month period in (i).

77. The use according to any one of claims 75 or 76, wherein the subject is treated over the course of two or more cycles.

78. The use according to claim 73, wherein the antibody and HAART are to be administered to the subject over the course of a cycle, wherein the cycle comprises:

i) the antibody to be administered to the subject for a period of four months in a weekly, bi-weekly, or monthly interval followed by a two-month treatment holiday; and

ii) HAART to be administered to the subject continuously during the six-month period in (i).

79. The use according to claim 73, wherein the antibody in (a) is to be administered at a time that is not concurrent with HAART in (b).

80. The use according to claim 73, wherein the antibody in (a) and HAART in (b) are to be administered to the subject over the course of a cycle, wherein the cycle comprises:

i) the antibody to be administered to the subject for a first period of time followed by a treatment holiday for a second period of time; and

ii) HAART to be administered to the subject during the second period of time and not during the first period of time.

81. The use according to claim 80, wherein the antibody is to be administered in regular intervals during the first time period.

82. The use according to claim 80, wherein the antibody is to be administered in weekly, bi-weekly, or monthly intervals during the first time period.

83. An *in vitro* method for inhibiting HIV entry into a CD4+ cell, comprising exposing the antibody according to any one of claims 31 to 43 to the cell.

84. An *in vitro* method for inhibiting gp120 binding to a CD4+ cell, comprising exposing the antibody according to any one of claims 31 to 43 to the cell.

85. An *in vitro* method for activating a resting CD4+ T cell upon crosslinking, comprising exposing the antibody according to any one of claims 31 to 43 to the cell.

86. An *in vitro* method for activating a latent reservoir of HIV in a resting T cell upon crosslinking, comprising

exposing the antibody according to any one of claims 31 to 43 to the cell.

87. An *in vitro* method for reducing latent HIV reservoirs in a sample of cells infected with HIV comprising

a) exposing the antibody according to any one of claims 31 to 43 to the sample of cells; and

b) exposing HAART to the sample of cells.