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BRIEF SUMMARY

The present disclosure is directed to antibodies, compositions, and methods for the treatment and sustained virologic remission of HIV infection in HAART stabilized patients as a replacement for HAART. One example of the present disclosure describes antibodies directed against CD4, compositions thereof, and methods of making and employing such compositions for the treatment and sustained virologic remission of HIV infection in HAART stabilized patients in the subsequent absence of other treatments, including cART.

In one aspect, the present invention refers to an antibody directed against a CD4 molecule, wherein the antibody specifically binds to an extracellular region of the CD4 molecule, and wherein when the antibody is bound to the CD4 molecule on the surface of a CD4+ cell, the antibody: a) competitively inhibits HIV entry into the CD4+ cell; b) activates latent HIV reservoirs in a resting CD4+ cell infected with HIV; d) reduces levels of cellular HIV DNA; and e) provides sustained virologic remission of HIV infection without viral load rebound.

In certain examples, the antibodies specifically bind to extracellular sites on CD4. In specific examples, the antibodies specifically bind to CD4 on sites at or near the CDR2 region in domain 1. The disclosed antibodies exert competitive HIV entry inhibition through its binding to CD4 in both cell-free and cell-to-cell systems. The disclosed antibodies also have the ability to reactivate resting CD4 positive T cells with or without crosslinking, which can lead to an increase in TNF- α production. Such antibodies include, but are not limited to monoclonal antibodies (Mabs) including B4, M2, and dB4C7 (e.g., Wang, C.Y. 1999; Lynn. S. and Wang, C.Y. 2009); Leu3a (Than, S. et al., 1997), ST4 (Briant, L,1999); and polyclonal antibodies including anti-HIV RC, CDR2 region of domain 1 in CD4 (Wang, C.Y., WO2016/043788).

In certain examples, the antibodies are directed against, and specifically bind to, CD4 and, functionally, have the ability to (1) block HIV entry, in both cell-free and cell-to-cell transmission modes and (2) reactivate HIV infected resting CD4 T-cells. In specific examples, the antibodies reactivate HIV infected resting CD4 T-cells, in vitro with or without crosslinking, as manifested in an increase in TNF production, HIV p24 release, or T-cell activation via Lck Kinsase phosphorylation. In certain examples, treating HIV patients with the disclosed antibodies results in (1) a reduction in regulatory T cells (Tregs); (2) an increase in blood CD8+ cell counts; (3) an expansion of HIV specific CD8+ cells upon in vitro stimulation by HIV specific antigen(s); and/or (4) a reduction in HIV DNA level in blood cells.

The present disclosure is also directed to pharmaceutical compositions comprising the anti-CD4 antibodies (e.g., monoclonal human, humanized, chimeric, etc.) having the above described functional properties as well as methods employing such pharmaceutical compositions for the treatment and sustained virologic remission of HIV infection. Specific examples relate to methods of making and/or using the pharmaceutical compositions for the treatment and sustained virologic remission of HIV infection in HAART stabilized patients in the subsequent absence of cART.

In one 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 yet another aspect, the present invention refers to a pharmaceutical composition comprising the antibody disclosed herein in phosphate buffer saline (PBS), 20 mM glycine, and 0.05% (v/v) polysorbate 20.

In certain examples, the disclosed pharmaceutical compositions comprising the disclosed antibodies are prepared and administered to a patient to reduce the viral load to a non-detectable level with no viral load rebound. In some examples, pharmaceutical compositions are prepared and administered to a patient at a dose of about 10 mg/kg or higher on a weekly, biweekly, or even longer schedule. In some examples, the pharmaceutical compositions are administered as a monotherapy or in combination with another therapy, such as HAART. In some examples, the viral load is reduced to a non-detectable level with no viral load rebound when the serum antibody level in the treated patient is about 10 µg/mL or higher. In certain examples, the pharmaceutical composition is given at a dose of about 10 mg/kg or higher on a weekly or biweekly or even longer schedule, as a monotherapy, which leads to a reduction in viral load down to non-detectable level in treated subjects with no viral load rebound as long as the serum antibody level is higher than 10 µg/mL.

The disclosed pharmaceutical compositions comprising antibodies directed against CD4 can be used in HIV treatment as (1) a monotherapy, when administered alone; (2) a combinatorial therapy, when administered as an adjunct to other treatment methods (e.g., cART); or (3) a monotherapy in drug substitution treatment cycles with other treatment methods (e.g., cART) given intermitantly.

In one aspect, the present invention refers to use of the antibody disclosed herein in the manufacture of a medicament for treating a subject exposed to HIV. In another aspect, the present invention refers to use of a treatment regimen comprising a) a pharmacologically effective amount of the antibody disclosed herein; and b) a highly active antiretroviral therapy (HAART), in the manufacture of a medicament for treating a subject with HIV infection. In yet another aspect, the present invention refers to a method for inhibiting HIV entry into a CD4+ cell, comprising exposing the antibody disclosed herein to the cell. In a further aspect, the present invention refers to a method for inhibiting gp120 binding to a CD4+ cell, comprising exposing the antibody disclosed herein to the cell. In yet another aspect, the present invention refers to a method for activating a resting CD4+ T cell, comprising exposing the antibody disclosed herein to the cell. In a further aspect, the present invention refers to a method for activating a latent reservoir of HIV in a resting T cell, comprising exposing the antibody disclosed herein to the cell. In yet another aspect, the present invention refers to a method for reducing latent HIV reservoirs

in a sample of cells infected with HIV, comprising a) exposing the antibody disclosed herein to the sample of cells; and b) exposing HAART to the sample of cells.

The cellular and immunological characteristics achieved with the disclosed antibodies and treatment methods resemble those of an elite controller or long-term nonprogressor (LTNP). That is, the disclosed antibodies and treatment methods are capable of achieving a sustained virologic remission of HIV infection in the subsequent absence of cART, a revolution in the treatment of HIV infection.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1A. A graph illustrating a competitive HIV entry inhibition mechanism. The graph shows theoretical results obtained in a competitive HIV entry inhibition model, where HIV envelope protein gp120 and an inhibitor (e.g., antibody drug) compete for binding on the same portion of a common target surface molecule (i.e., CDR2 of CD4 domain 1). In this model, 100% inhibition of HIV binding/entry can be achieved when the concentration of the inhibitor reaches a certain threshold.

Figure 1B. HIV-1 entry inhibition from a panel of over 850 Env pseudotype HIV viruses collected over a 10 year period using mAb B4. MAb B4 offers both breadth and potency in HIV entry inhibition with nearly 100% maximum percent inhibition (MPI) in all 850 Env pseudotype viruses with IC_{50} clustered around two concentrations one between 0.01 to 1 $\mu\text{g/mL}$ and the second one around 10 $\mu\text{g/mL}$.

Figure 2A. A graph illustrating a non-competitive HIV entry inhibition mechanism. The graph shows theoretical results obtained in a non-competitive HIV entry inhibition model, where HIV and an inhibitor bind to different sites on the same target molecule (e.g. domain 2 of CD4 for TMB-355). In this non-competitive inhibition model, HIV binding/entry can be reduced by the inhibitor, but complete inhibition is not achieved regardless of the concentration of the inhibitor. Resistance of HIV to the antibody drug is reflected as a “plateau” in % inhibition regardless of drug concentration.

Figure 2B. HIV-1 entry inhibition results from a panel of 118 diverse HIV-1 Env pseudovirus strains covering 11 clades using TMB-355 (Pace, G., et al., 2011). For each virus, black lines indicate maximum percent inhibition (MPI) when treated at TMB-355 concentrations up to 10 $\mu\text{g/mL}$ (left Y axis); and grey lines indicate the corresponding IC_{50} (right Y axis). TMB-355 neutralized 92% of the viral strains with $\geq 50\%$ inhibition and only neutralized 31% of the viral strains with $\geq 95\%$ inhibition.

Figure 3. Bar graph showing virus reactivation in resting PBMCs (as measured by HIV-1 p24 gag production) induced by the following stimuli: unstimulated (lane 1), PHA (lane 2), inactivated HIV (iHIV) lysate (lane 3), monoclonal antibody directed at CDR2 region of CD4 domain 1 (lane 4), monoclonal antibody directed at CDR3 region of CD4 domain (lane 5), monoclonal antibody directed at CD4 domains 1/2 (lane 6), iHIV in the presence of soluble

24. A pharmaceutical composition comprising the antibody of claim 1 in phosphate buffer saline (PBS), 20 mM glycine, 0.05% (v/v) polysorbate 20, and 10 mM histidine.
25. A pharmaceutical composition comprising about 1.0 mg/mL to about 200.0 mg/mL of the antibody of claim 1 in phosphate buffer saline (PBS), 20 mM glycine, and 0.05% (v/v) polysorbate 20.
26. A pharmaceutical composition comprising about 1.0 mg/mL to about 200.0 mg/mL of the antibody of claim 1 in phosphate buffer saline (PBS), 20 mM glycine, 0.05% (v/v) polysorbate 20, and 10 mM histidine.
27. A pharmaceutical composition comprising about 10.0 mg/mL of the antibody of claim 1 in phosphate buffer saline (PBS), 20 mM glycine, and 0.05% (v/v) polysorbate 20.
28. A pharmaceutical composition comprising about 10.0 mg/mL of the antibody of claim 1 in phosphate buffer saline (PBS), 20 mM glycine, 0.05% (v/v) polysorbate 20, and 10 mM histidine.
29. A pharmaceutical composition comprising the antibody of claim 12 and a pharmaceutically acceptable carrier.
30. A pharmaceutical composition comprising the antibody of claim 16 and a pharmaceutically acceptable carrier.
31. Use of the antibody of claim 1 in the manufacture of a medicament for treating a subject exposed to HIV, wherein the subject is to be administered a pharmacologically effective amount of.
32. The use of claim 31, wherein the antibody is to be administered to the subject prior to exposure to HIV.
33. The use according to claim 31, wherein the antibody is to be administered to the subject after exposure to HIV.
34. The use according to claim 31, wherein the antibody is to be administered within 48 hours after exposure to HIV.

35. The use according to claim 31, wherein the antibody is to be administered to the subject at a dosage of at least about 5 mg/kg body weight.
36. The use according to claim 35, wherein the antibody is to be administered to the subject multiple times.
37. The use according to claim 36, wherein the antibody is to be administered to the subject in a weekly, bi-weekly, or monthly interval.
38. The use according to claim 36, further comprising a step of an antiviral agent to be administered to the subject.
39. The use according to claim 38, wherein the antiviral agent is a highly active antiretroviral therapy (HAART).
40. The use according to claim 39, wherein HAART comprises a nucleoside analogue reverse transcriptase inhibitor in combination with a protease inhibitor or a non-nucleoside reverse transcriptase inhibitor.
41. The use according to claim 39, wherein the antibody is to be administered concurrently with HAART.
42. The use according to claim 39, 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).
43. The use according to claim 39, 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).
44. The use according to claim 42, wherein the subject is treated over the course of two cycles.

45. The use according to claim 43, wherein the subject is treated over the course of two cycles.
46. The use according to claim 39, wherein the antibody is to be administered at a time that is not concurrent with HAART.
47. The use according to claim 39, 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.
48. The use according to claim 47, wherein the antibody is to be administered in regular intervals during the first time period.
49. The use according to claim 47, wherein the antibody is to be administered in weekly, bi-weekly, or monthly intervals during the first time period.
50. Use of a treatment regimen comprising:
- a) a pharmacologically effective amount of the antibody of claim 1; and
 - b) a highly active antiretroviral therapy (HAART),
- in the manufacture of a medicament for treating a subject with HIV infection.
51. The use of claim 50, wherein the antibody is to be administered to the subject at a dosage of at least about 5 mg/kg body weight.
52. The use according to claim 50, 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).
53. The use according to claim 50, 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).
54. The use according to claim 52, wherein the subject is treated over the course of two or more cycles.
55. The use according to claim 53, wherein the subject is treated over the course of two or more cycles.
56. The use according to claim 53, 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).
57. The use according to claim 50, wherein the antibody in (a) is to be administered at a time that is not concurrent with HAART in (b).
58. The use according to claim 50, 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 the subject during the second period of time and not during the first period of time.
59. The use according to claim 58, wherein the antibody is to be administered in regular intervals during the first time period.
60. The use according to claim 58, wherein the antibody is to be administered in weekly, bi-weekly, or monthly intervals during the first time period.
61. A method for inhibiting HIV entry into a CD4⁺ cell, comprising exposing the antibody of claim 1 to the cell.
62. A method for inhibiting gp120 binding to a CD4⁺ cell, comprising exposing the antibody of claim 1 to the cell.

63. A method for activating a resting CD4+ T cell, comprising exposing the antibody of claim 1 to the cell.

64. A method for activating a latent reservoir of HIV in a resting T cell, comprising exposing the antibody of claim 1 to the cell.

65. A method for reducing latent HIV reservoirs in a sample of cells infected with HIV, comprising

- a) exposing the antibody of claim 1 to the sample of cells; and
- b) exposing HAART to the sample of cells.