Claims

1. An antibody with pH dependent binding which specifically binds an antigen with higher affinity at pH 7.4 than at pH 6.0, wherein the K_D ratio and/or the k_{off} ratio at pH 6.0/pH 7.4 and at 25°C is more than, or ranges between 3, 4, 8, 10, 16, or more, and wherein the antigen is soluble and the antibody has reduced plasma clearance in vivo when exposed to said antigen as compared to an antibody without pH dependent binding that has a similar affinity for the antigen at pH 7.4, but has a comparable K_D and/or k_{off} ratio at pH 6.0/pH 7.4 that is less than 2.

2. An antibody with pH dependent binding which specifically binds an antigen with higher affinity at pH 7.4 than at pH 6.0, wherein the K_D ratio and/or the k_{off} ratio at pH 6.0/pH 7.4 and at 25°C is more than, or ranges between, 2, 3, 4, 8, 10, 16, or more, and wherein the antigen is a receptor and the receptor has reduced clearance in vivo when exposed to said antibody as compared to an antibody that has similar affinity for the receptor at pH 7.4, but has a comparable K_D and/or k_{off} ratio at pH 6.0/pH 7.4 that is less than 2.

3. The antibody of claim 2, wherein said antibody with pH dependent binding is an antibody drug conjugate, mediates antibody dependent cell-mediated cytotoxicity (ADCC), and/or complement-dependent cytotoxicity (CDC).

4. The antibody as claimed in claim 3, wherein the antibody is an agonist antibody.

5. An antibody with pH dependent binding which specifically binds an antigen with higher affinity at pH 7.4 than at pH 6.0, wherein the K^D ratio and/or the k^{off} ratio at pH 6.0/pH 7.4 and at 25°C is more than, or ranges between, 2, 3, 4, 8, 10, 16, or more, wherein the antigen is IgE, DKK1 or C5, and wherein the decrease in the in vivo amount of non-antibody bound antigen is prolonged when exposed to said antibody as compared to an antibody without pH dependent binding that has a similar affinity for the antigen at pH 7.4, but has a comparable K_D and/or k_{off} ratio at pH 6.0/pH 7.4 that is less than 2.

6. The antibody as claimed in of any one of claims 1-5, wherein the K_D ratio or k_{off} ratio at pH 6.0/pH 7.4 is more than, or ranges between, 20, 30, 40 or 100 or more.

7. The antibody as claimed in of any one of claims 1 -6, wherein the binding of the antibody to the antigen at pH 7.4 has a K_D of about 0.01 nM to about 100 nM.

8. The antibody as claimed in claim 7, wherein the binding of the antibody to the antigen at pH 7.4 has a K_D of about 0.1 nM to about 10 nM.

9. The antibody as claimed in of one any of claims 1-8 , wherein the binding of the antibody to the antigen at pH 7.4 has a k_{off} of about 1x10E-4 s-1 to about 1x10E-1 s-1.

10. The antibody as claimed in claim 9, wherein the binding of the antibody to the antigen at pH 7.4 has a koff of about 1x10E-3 s-1 to about 1x10E-1 s-1.

11. The antibody as claimed in any one of claims 1 and 6-10, wherein the antigen is PCSK9.

12. The antibody as claimed in claim 5, wherein the K_D ranges between 1.0 nM to about 10 nM or between 1.0 nM to about 100 nM.

13. Use of an antibody in the preparation of a medicament to be used for extending the interval dosing and/or decreasing the therapeutic dose during the treatment of a patient with a therapeutic antibody, wherein the medicament comprises a therapeutically effective amount of the antibody of any of claims 1-12 and 17-23.

14. A method of making an antibody as claimed in any one of claims 1-12 and 17-23, the antibody having a prolonged half-life and/or pharmacodynamic effect by regulating antibody binding affinity in a pH dependent manner, said method comprising selecting for antibody CDR histidine residues or other residues that optimize the microenvironment affecting pKa, such that antibody antigen binding has a K_D ratio and/or koff ratio at pH 6.0/pH7.4 that is more than, or ranges between, 2, 3, 4, 8, 10, 16, or more.

15. An antibody library enriched for histidines in CDR residues or other residues that optimize the microenvironment affecting pKa.

16. The method of claim 14, further comprising mutagenizing the antibody to achieve antibody affinity with a KD at pH 7.4 of at least 100 nM.

17. An isolated antibody which specifically binds to PCSK9 and comprises a heavy chain variable region (VH) complementary determining region one (CDR1), a VH CDR2, and a VH CDR3 from the VH amino acid sequence shown in SEQ ID NO: 4 or 5 or a variant thereof having one, two, three or more conservative amino acid substitutions in CDR1 , CDR2, and/or CDR3.

18. The isolated antibody of claim 17, further comprising the light chain variable region (VL) CDR1 , CDR2, and CDR3 of the VL amino acid sequence shown in SEQ ID NO: 3 or a variant thereof having one, two, three or more conservative amino acid substitutions in CDR1 , CDR2, and/or CDR3.

19. The isolated antibody of claim 18 , wherein the VH CDR1 has the amino acid sequence shown in SEQ ID NO:6, the VH CDR2 has the amino acid sequence shown in SEQ ID NO: 7, the VH CDR3 has the amino acid sequence shown in SEQ ID NO: 8 or 9, the VL CDR1 has the amino acid sequence shown in SEQ ID NO: 10, the VL CDR2 has the amino acid sequence shown in SEQ ID NO: 11, and the VL CDR3 has the amino acid sequence shown in SEQ ID NO: 12 or a variant thereof having one, two, three or more conservative amino acid substitutions in CDR1 , CDR2, and/or CDR3.

20. The isolated antibody of claim 19, wherein the VH CDR3 has the amino acid sequence of SEQ ID NO: 9.

21. The isolated antibody of claim 20, wherein the VH region comprises SEQ ID NO: 4 or SEQ ID NO: 5 and the VL region comprises SEQ ID NO: 3.

22. The isolated antibody of claim 21, wherein the VH region comprises SEQ ID NO: 5.

23. An isolated antibody or antigen-binding portion thereof, encoded by the plasmid deposited at the ATCC and having ATCC Accession No. PTA-10547, or PTA-10548, and/or PTA-10549.

24. A pharmaceutical composition comprising a therapeutically effective amount of the antibody of any of claims 1-23.

25. A host cell that recombinantly produces the antibody of any of claims 1-23.

26. An isolated nucleic acid encoding the antibody of any of claims 1-23.

27. An isolated plasmid as deposited at the ATCC and having ATCC Accession No. PTA-10547, or PTA-10548, and/or PTA-10549.

28. Use of the antibody of any one of claims 12 and 17-23 in the preparation of a medicament for reducing a level of LDL-cholesterol in blood of a subject in need thereof.

Claims

1. An antibody with pH dependent binding which specifically binds an antigen with higher affinity at pH 7.4 than at pH 6.0, wherein the K_D ratio and/or the k_{off} ratio at pH 6.0/pH 7.4 and at 25°C is more than, or ranges between 3, 4, 8, 10, 16, or more, and wherein the antigen is soluble and the antibody has reduced plasma clearance in vivo when exposed to said antigen as compared to an antibody without pH dependent binding that has a similar affinity for the antigen at pH 7.4, but has a comparable K_D and/or k_{off} ratio at pH 6.0/pH 7.4 that is less than 2.

2. An antibody with pH dependent binding which specifically binds an antigen with higher affinity at pH 7.4 than at pH 6.0, wherein the K^D ratio and/or the k^{off} ratio at pH 6.0/pH 7.4 and at 25°C is more than, or ranges between, 2, 3, 4, 8, 10, 16, or more, wherein the antigen is IgE, DKK1 or C5, and wherein the decrease in the in vivo amount of non-antibody bound antigen is prolonged when exposed to said antibody as compared to an antibody without pH dependent binding that has a similar affinity for the antigen at pH 7.4, but has a comparable K_D and/or k_{off} ratio at pH 6.0/pH 7.4 that is less than 2.

3. The antibody as claimed in of any one of claims 1-2, wherein the K_D ratio or k_{off} ratio at pH 6.0/pH 7.4 is more than, or ranges between, 20, 30, 40 or 100 or more.

4. The antibody as claimed in of any one of claims 1 -3 wherein the binding of the antibody to the antigen at pH 7.4 has a K_D of about 0.01 nM to about 100 nM.

5. The antibody as claimed in claim 4, wherein the binding of the antibody to the antigen at pH 7.4 has a K_D of about 0.1 nM to about 10 nM.

6. The antibody as claimed in of one any of claims 1-5, wherein the binding of the antibody to the antigen at pH 7.4 has a k_{off} of about 1x10E-4 s-1 to about 1x10E-1 s-1.

7. The antibody as claimed in claim 6, wherein the binding of the antibody to the antigen at pH 7.4 has a koff of about 1x10E-3 s-1 to about 1x10E-1 s-1.

8. The antibody as claimed in any one of claims 1 and 3-7, wherein the antigen is PCSK9.

9. The antibody as claimed in claim 2, wherein the K_D ranges between 1.0 nM to about 10 nM or between 1.0 nM to about 100 nM.

10. Use of an antibody in the preparation of a medicament to be used for extending the interval dosing and/or decreasing the therapeutic dose during the treatment of a patient with a therapeutic antibody, wherein the medicament comprises a therapeutically effective amount of the antibody of any of claims 1-9 and 14-20.

11. A method of making an antibody as claimed in any one of claims 1-9 and 14-20, the antibody having a prolonged half-life and/or pharmacodynamic effect by regulating antibody binding affinity in a pH dependent manner, said method comprising selecting for

antibody CDR histidine residues or other residues that optimize the microenvironment affecting pKa, such that antibody antigen binding has a K_D ratio and/or koff ratio at pH 6.0/pH7.4 that is more than, or ranges between, 2, 3, 4, 8, 10, 16, or more.

12. An antibody library enriched for histidines in CDR residues or other residues that optimize the microenvironment affecting pKa.

13. The method of claim 11, further comprising mutagenizing the antibody to achieve antibody affinity with a KD at pH 7.4 of at least 100 nM.

14. An isolated antibody which specifically binds to PCSK9 and comprises a heavy chain variable region (VH) complementary determining region one (CDR1), a VH CDR2, and a VH CDR3 from the VH amino acid sequence shown in SEQ ID NO: 4 or 5 or a variant thereof having one, two, three or more conservative amino acid substitutions in CDR1 , CDR2, and/or CDR3.

15. The isolated antibody of claim 14, further comprising the light chain variable region (VL) CDR1 , CDR2, and CDR3 of the VL amino acid sequence shown in SEQ ID NO: 3 or a variant thereof having one, two, three or more conservative amino acid substitutions in CDR1 , CDR2, and/or CDR3.

16. The isolated antibody of claim 15, wherein the VH CDR1 has the amino acid sequence shown in SEQ ID NO:6, the VH CDR2 has the amino acid sequence shown in SEQ ID NO: 7, the VH CDR3 has the amino acid sequence shown in SEQ ID NO: 8 or 9, the VL CDR1 has the amino acid sequence shown in SEQ ID NO: 10, the VL CDR2 has the amino acid sequence shown in SEQ ID NO: 11, and the VL CDR3 has the amino acid sequence shown in SEQ ID NO: 12 or a variant thereof having one, two, three or more conservative amino acid substitutions in CDR1 , CDR2, and/or CDR3.

17. The isolated antibody of claim 16, wherein the VH CDR3 has the amino acid sequence of SEQ ID NO: 9.

18. The isolated antibody of claim 17, wherein the VH region comprises SEQ ID NO: 4 or SEQ ID NO: 5 and the VL region comprises SEQ ID NO: 3.

19. The isolated antibody of claim 18, wherein the VH region comprises SEQ ID NO: 5.

20. An isolated antibody or antigen-binding portion thereof, encoded by the plasmid deposited at the ATCC and having ATCC Accession No. PTA-10547, or PTA-10548, and/or PTA-10549.

21. A pharmaceutical composition comprising a therapeutically effective amount of the antibody of any of claims 1-20.

22. A host cell that recombinantly produces the antibody of any of claims 1-20.

23. An isolated nucleic acid encoding the antibody of any of claims 1-20.

24. An isolated plasmid as deposited at the ATCC and having ATCC Accession No. PTA-10547, or PTA-10548, and/or PTA-10549.

25. Use of the antibody of any one of claims 9 and 14-20 in the preparation of a medicament for reducing a level of LDL-cholesterol in blood of a subject in need thereof.