Claims

1. A humanized anti-α2 integrin antibody comprising a heavy chain variable region, a light chain variable region, a human light chain constant region and a variant human IgG1 heavy constant region, whereas the variant human IgG1 heavy chain constant region comprises at least one amino acid modification relative to the human IgG1 heavy chain constant region of the parent humanized anti-α2 integrin antibody, and whereas the antibody exhibits altered effector function compared to the parent humanized anti-α2 integrin antibody.

2. The humanized anti-α2 integrin antibody of claim 1, wherein the variant human IgG1 heavy chain constant region is an isotypic variant comprising the CH1 from human IgG1, the hinge from human IgG1 and the Fc region from human IgG3.

3. The humanized anti-α2 integrin antibody of claim 1, wherein the variant human IgG1 heavy chain constant region is an isotypic variant comprising the CH1 from human IgG3, the hinge from human IgG1 and the Fc region from human IgG3.

4. The humanized anti-α2 integrin antibody of claim 1, wherein the variant human IgG1 heavy chain constant region comprises a variant human IgG1 Fc region which comprises at least one amino acid modification relative to the human IgG Fc region of the parent humanized anti-α2 integrin antibody.

5. The humanized anti-α2 integrin antibody of claim 4, wherein the amino acid modification comprises an amino acid substitution at amino acid position selected from the group consisting of 269, 298, and 324, wherein the amino acid position of each group member is indicated utilizing the numbering system set forth in Kabat.

6. The humanized anti-α2 integrin antibody of claim 4, wherein the amino acid modification comprises an amino acid substitution selected from the group consisting of E269D, S298A, and S324N, wherein the amino acid position of each group member is indicated utilizing the numbering system set forth in Kabat.

7. The humanized anti-α2 integrin antibody of claim 4, wherein the amino acid modification comprises a combination of amino acid substitutions at amino acid position selected from the group consisting of 269/298, 269/324, 298/324, and 269/298/324, wherein the amino acid position of each group member is indicated utilizing the numbering system set forth in Kabat.

8. The humanized anti-α2 integrin antibody of claim 4, wherein the amino acid modification comprises a combination of amino acid substitutions selected from the group

consisting of E269D/S298A, E269D/S324N, S298A/S324N, and E269D/S298A/S324N, wherein the amino acid position of each group member is indicated utilizing the numbering system set forth in Kabat.

9. The humanized anti-α2 integrin antibody of any one of claims 1-8, wherein said effector function is complement dependent cytotoxicity (CDC) and/or antibody dependent cell mediated cytotoxicity (ADCC).

10. The humanized anti-α2 integrin antibody of any one of claims 1-4, wherein the humanized anti-α2 integrin antibody exhibits improved complement dependent cytotoxicity (CDC) as compared to the parent humanized anti-α2 integrin antibody.

11. The humanized anti-α2 integrin antibody of claim 10, wherein the humanized anti-α2 integrin antibody exhibits antibody dependent cell mediated cytotoxicity (ADCC) equivalent to the parent humanized anti-α2 integrin antibody.

12. The humanized anti-α2 integrin antibody of claim 4, wherein the amino acid modification is an amino acid substitution selected from the group consisting of S324N, S298A/S324N, and E269D/S298A/S324N, whereas the antibody exhibits improved complement dependent cytotoxicity (CDC) as compared to the parent humanized anti-α2 integrin antibody.

13. The humanized anti-α2 integrin antibody of claim 12, wherein the humanized anti-α2 integrin antibody exhibits antibody dependent cell mediated cytotoxicity (ADCC) equivalent to the parent humanized anti-α2 integrin antibody.

14. The humanized anti-α2 integrin antibody of claim 4, wherein the amino acid modification is an amino acid substitution selected from the group consisting of E269D, S298A, S298A/S324N, and E269D/S298A/S324N, whereas the antibody exhibits improved antibody dependent cell mediated cytotoxicity (ADCC) as compared to the parent humanized anti-α2 integrin antibody.

15. The humanized anti-α2 integrin antibody of claim 4, wherein the amino acid modification is amino acid substitution S298A/S324N or E269D/S298A/S324N, whereas the antibody exhibits improved complement dependent cytotoxicity (CDC) and improved antibody dependent cell mediated cytotoxicity (ADCC) as compared to the parent humanized anti-α2 integrin antibody.

16. The humanized anti-α2 integrin antibody of claim 2, wherein the humanized anti-α2 integrin antibody comprises a variant human IgG1 heavy chain constant region comprising SEQ ID NO: 35.

17. The humanized anti-α2 integrin antibody of claim 3, wherein the humanized anti-α2 integrin antibody comprises a variant human IgG1 heavy chain constant region comprising SEQ ID NO: 36.

18. The humanized anti-α2 integrin antibody of claim 4, wherein the humanized anti-α2 integrin antibody comprises a variant human IgG1 Fc region selected from the group consisting of SEQ ID NOs: 37-43.

19. The humanized antibody of any one of claims 1-18, wherein the heavy chain variable region comprises HCDR1 comprising the amino acid sequence GFSLTNYGIH (SEQ ID NO:1), HCDR2 comprising the amino acid sequence VIWARGFTNYNSALMS (SEQ ID NO:2) and HCDR3 comprising the amino acid sequence ANDGVYYAMDY (SEQ ID NO:3).

20. The humanized antibody of any one of claims 1-18, wherein the light chain variable region comprises LCDR1 comprising the amino acid sequence SAQSSVNYIH (SEQ ID NO:4), LCDR2 comprising the amino acid sequence DTSKLAS (SEQ ID NO:5) and LCDR3 comprising the amino acid sequence QQWTTNPLT (SEQ ID NO:6).

21. The humanized antibody of any one of claims 1-18, wherein the heavy chain variable region comprises HCDR1 comprising the amino acid sequence GFSLTNYGIH (SEQ ID NO:1), HCDR2 comprising the amino acid sequence VIWARGFTNYNSALMS (SEQ ID NO:2) and HCDR3 comprising the amino acid sequence ANDGVYYAMDY (SEQ ID NO:3); and/or

wherein the light chain variable region comprises LCDR1 comprising the amino acid sequence SAQSSVNYIH (SEQ ID NO:4), LCDR2 comprising the amino acid sequence DTSKLAS (SEQ ID NO:5) and LCDR3 comprising the amino acid sequence QQWTTNPLT (SEQ ID NO:6).

22. The humanized antibody of any one of claims 1-18, wherein the heavy chain variable region comprises the amino acid sequence of SEQ ID NO: 7.

23. The humanized anti-α2 integrin antibody of claim 22, wherein the heavy chain variable region comprises the amino acid sequence of SEQ ID NO: 7 in which (a) position 71 is Lys, (b) position 73 is Asn, (c) position 78 is Val, or (d) any combination of (a)-(c).

24. The humanized anti-α2 integrin antibody of any one of claims 1-18, wherein the heavy chain variable region comprises an amino acid sequence selected from the group consisting of SEQ ID NOs: 8-19.

25. The humanized anti-α2 integrin antibody of any one of claims 1-18, wherein the heavy chain variable region comprises the amino acid sequence of SEQ ID NO: 17.

26. The humanized anti-α2 integrin antibody of any one of claims 22-25, wherein the heavy chain variable region further comprises a FW4 region comprising the amino acid sequence WGQGTLVTVSS (SEQ ID NO:20).

27. The humanized anti-α2 integrin antibody of any one of claims 1-18, wherein the light chain variable region comprises the amino acid sequence of SEQ ID NO: 21.

28. The humanized anti-α2 integrin antibody of claim 27, wherein the light chain variable region comprises the amino acid sequence of SEQ ID NO: 21 in which (a) position 2 is Phe, (b) position 45 is Lys, (c) position 48 is Tyr, or (d) any combination of (a)-(c).

29. The humanized anti-α2 integrin antibody of any one of claims 1-18, wherein the light chain variable region comprises an amino acid sequence selected from the group consisting of SEQ ID NOs: 22-33.

30. The humanized anti-α2 integrin antibody of any one of claims 1-18, wherein the light chain variable region comprises the amino acid sequence of SEQ ID NO: 30.

31. The humanized anti-α2 integrin antibody of any one of claims 27-30, wherein the light chain variable region the further comprises a FW4 region comprising the amino acids sequence FGQGTKVEIK (SEQ ID NO: 34).

32. The humanized anti-α2 integrin antibody of claim 1, wherein the antibody comprises a heavy chain comprising SEQ ID NO: 47 and a light chain comprising SEQ ID NO: 56.

33. The humanized anti-α2 integrin antibody of claim 1, wherein the antibody comprises a heavy chain comprising SEQ ID NO: 48 and a light chain comprising SEQ ID NO: 56.

34. The humanized anti-α2 integrin antibody of claim 1, wherein the antibody comprises a heavy chain selected from the group consisting of SEQ ID NO: 49-55 and a light chain comprising SEQ ID NO: 56.

35. An isolated nucleic acid encoding a humanized anti-α2β1 integrin antibody of any one of claims 1-34.

36. A vector comprising the nucleic acid of claim 35.

37. A host cell comprising the nucleic acid of claim 35 or the vector of claim 36.

38. A composition comprising the humanized anti-α2 integrin antibody of any one of claims 1-34 and a pharmaceutically acceptable carrier.

39. A method of treating an α2β1 integrin-associated disorder in a subject, the method comprising administering to the subject a therapeutically effective amount of the anti-α2 integrin antibody of any one of claims 1-34 or the composition of claim 38.

40. The method of claim 39, wherein the α2β1 integrin-associated disorder is selected from inflammatory disease, autoimmune disease and a disease characterized by abnormal or increased angiogenesis.

41. The method of claim 39, wherein the α2β1 integrin-associated disorder is selected from inflammatory bowel disease, Crohn's disease, ulcerative colitis, reactions to transplant, optical neuritis, spinal cord trauma, rheumatoid arthritis, systemic lupus erythematosus (SLE), diabetes mellitus, multiple sclerosis, Reynaud's syndrome, experimental autoimmune encephalomyelitis, Sjorgen's syndrome, scleroderma, juvenile onset diabetes, diabetic retinopathy, age related macular degeneration, cardiovascular disease, psoriasis, cancer as well as infections that induce an inflammatory response.

42. The method of claim 39, wherein the α2β1 integrin-associated disorder is selected from multiple sclerosis, rheumatoid arthritis, optical neuritis and spinal cord trauma.

43. The method of claim 39, wherein the α2β1 integrin-associated disorder is cancer selected from the group consisting of squamous cell cancer, lung cancer including small-cell lung cancer, non-small cell lung cancer, adenocarcinoma of the lung, and squamous carcinoma of the lung, cancer of the peritoneum, hepatocellular cancer, gastric or stomach cancer including gastrointestinal cancer, pancreatic cancer, glioblastoma, cervical cancer, ovarian cancer, liver cancer, bladder cancer, hepatoma, breast cancer, colon cancer, colorectal cancer, endometrial or uterine carcinoma, salivary gland carcinoma, kidney or renal cancer, liver cancer, prostate cancer, vulval cancer, thyroid cancer, hepatic carcinoma and various types of head and neck cancer, as well as B-cell lymphoma including low grade/follicular non-Hodgkin's lymphoma (NHL); small lymphocytic (SL) NHL; intermediate grade/ follicular NHL; intermediate grade diffuse NHL; high grade immunoblastic NHL; high grade lymphoblastic NHL; high grade small non-cleaved cell NHL; bulky disease NHL; mantle cell lymphoma; AIDS-related lymphoma; Waldenstrom's Macroglobulinemia; chronic lymphocytic leukemia (CLL); acute lymphoblastic leukemia (ALL); hairy cell leukemia; chronic myeloblastic leukemia; and post-transplant lymphoproliferative disorder (PTLD), as well as abnormal vascular

proliferation associated with phakomatoses, edema such as that associated with brain tumors, Meigs' syndrome, melanoma, mesothelioma, multiple myeloma, fibrosarcoma, osteosarcoma, and epidermoid carcinoma.

44. The method of claim 39, wherein the $\alpha 2\beta 1$ integrin-associated disorder is cancer selected from the group consisting of non-small cell lung cancer, pancreatic cancer, glioblastoma, cervical cancer, ovarian cancer, liver cancer, breast cancer, colon cancer, colorectal cancer, kidney cancer, prostate cancer, metastatic prostate cancer, mesothelioma, fibrosarcoma, osteosarcoma, and epidermoid carcinoma, metastatic colorectal, metastatic prostate and metastatic breast cancer.

45. The method of claim 39, wherein the $\alpha 2\beta 1$ integrin-associated disorder is cancer selected from the group consisting of pancreatic cancer, breast cancer, colon cancer, colorectal cancer, non-small cell lung cancer, fibrosarcoma, metastatic colorectal, prostate cancer, metastatic prostate cancer and metastatic breast cancer.

46. The method of claim 39, wherein the method is not associated with (a) platelet activation, (b) platelet aggregation, (c) a decrease in circulating platelet count, (d) bleeding complications, or (e) any combination of (a) to (d).

47. A method for inhibiting leukocyte binding to collagen comprising administering to a subject an amount of an anti-$\alpha 2\beta 1$ integrin antibody of any one of claims 1-34 or the composition of claim 38 effective to inhibit the binding of the leukocytes to collagen.

48. Use of the humanized anti-$\alpha 2$ integrin antibody of any one of claims 1-34 or the composition of claim 38 as a medicament.

49. Use of the humanized anti-$\alpha 2$ integrin antibody of any one of claims 1-34 or the composition of claim 38 for the treatment of an $\alpha 2\beta 1$ integrin-associated disorder.

50. A humanized anti-$\alpha 2$ integrin antibody of any one of claims 1-34 or the composition of claim 38 for use in a method for the treatment of an $\alpha 2\beta 1$ integrin-associated disorder.

51. Use of the humanized anti-$\alpha 2$ integrin antibody of any one of claims 1-34 or the composition of claim 38 for the preparation of a medicament for the treatment of an $\alpha 2\beta 1$ integrin-associated disorder.

52. A kit comprising the humanized anti-α2 integrin antibody of any one of claim 1-34 or the composition of claim 38 and instructions for the treatment of an α2β1 integrin-associated disorder.

53. An antibody comprising a variant human IgG Fc region which comprises amino acid substitution S324N replacing serine at amino acid position 324 of the parent antibody with asparagine, whereas the antibody exhibits improved complement dependent cytotoxicity (CDC) and improved antibody dependent cell mediated cytotoxicity (ADCC) as compared to the parent antibody.

54. The antibody of claim 53, wherein the antibody further comprises amino acid substitution E269D replacing glutamate at amino acid position 269 of the parent antibody with aspartate and substitution S298A replacing serine at amino acid position 298 of the parent antibody with alanine.

55. The antibody of claim 53 or 54, wherein the antibody is selected from the group consisting of a chimeric antibody, a humanized antibody and a fully human antibody.

56. The antibody of claim 53 or 54, wherein the antibody is a humanized anti-α2 integrin antibody.

CLAIMS

1. A humanized anti-α2 integrin antibody comprising:

(a) a light chain comprising a light chain variable region (V_L) and a human light chain constant region (C_L), and

(b) a heavy chain comprising a heavy chain variable region (V_H) and a variant human IgG1 heavy chain constant region (C_H) comprising a first constant domain (CH1), a hinge region, and an Fc region comprising a second constant domain (CH2) and a third constant domain (CH3),

wherein the variant C_H comprises at least one amino acid modification relative to the human IgG1 heavy chain constant region of a parent humanized anti-α2 integrin antibody,

wherein the at least one amino acid modification comprises amino acid substitution S324N replacing serine at amino acid position 324 of the parent antibody with asparagine, wherein the antibody further comprises amino acid substitution E269D replacing glutamate at amino acid position 269 of the parent antibody with aspartate, and substitution S298A replacing serine at amino acid position 298 of the parent antibody with alanine,

wherein the numbering of the amino acid positions is that of the EU index as in Kabat, and

wherein the antibody exhibits improved complement dependent cytotoxicity (CDC) or improved CDC and antibody dependent cellular cytotoxicity (ADCC) compared to the parent antibody.

2. The antibody of claim 1, wherein the variant C_H comprises the variant Fc region of SEQ ID NO: 43.

3. The antibody of claim 1, wherein the heavy chain variable region comprises an HCDR1 comprising the amino acid sequence GFSLTNYGIH (SEQ ID NO:1), an HCDR2 comprising the amino acid sequence VIWARGFTNYNSALMS (SEQ ID NO:2) and an HCDR3 comprising the amino acid sequence ANDGVYYAMDY (SEQ ID NO:3).

4. The antibody of claim 1, wherein the light chain variable region comprises an LCDR1 comprising the amino acid sequence SAQSSVNYIH (SEQ ID NO:4), an LCDR2 comprising the amino acid sequence DTSKLAS (SEQ ID NO:5) and an LCDR3 comprising the amino acid sequence QQWTTNPLT (SEQ ID NO:6).

5. The antibody of claim 1, wherein the heavy chain variable region comprises an amino acid sequence selected from the group consisting of SEQ ID NOs:8-19.

6. The antibody of claim 5, wherein the heavy chain variable region further comprises a framework 4 region (FW4) comprising the amino acid sequence WGQGTLVTVSS (SEQ ID NO:20).

7. The antibody of claim 1, wherein the light chain variable region comprises an amino acid sequence selected from the group consisting of SEQ ID NOs:22-33.

8. The antibody of claim 7, wherein the light chain variable region further comprises an FR4 comprising the amino acid sequence FGQGTKVEIK (SEQ ID NO: 34).

9. The antibody of claim 1, wherein the antibody comprises a heavy chain/light chain combination of:

SEQ ID NO:55 and SEQ ID NO:56.

10. A composition comprising the humanized anti-α2 integrin antibody of claim 1 and a pharmaceutically acceptable carrier.

11. A kit comprising the humanized anti-α2 integrin antibody of claim 1 and instructions for the treatment of an α2β1 integrin-associated disorder.

12. An antibody comprising a variant human IgG Fc region which comprises amino acid substitution S324N replacing serine at amino acid position 324 of the parent antibody with

asparagine, wherein the antibody further comprises amino acid substitution E269D replacing glutamate at amino acid position 269 of the parent antibody with aspartate, and substitution S298A replacing serine at amino acid position 298 of the parent antibody with alanine, wherein the numbering of the amino acid positions is that of the EU index as in Kabat, wherein the antibody exhibits improved CDC and ADCC as compared to the parent antibody.

13. The antibody of claim 12, wherein the antibody is selected from the group consisting of a chimeric antibody, a humanized antibody, and a fully human antibody.

14. The antibody of claim 12, wherein the antibody is a humanized anti-α2 integrin antibody.