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COMPOSITIONS AND METHODS FOR THE DEPLETION OF CD2+ CELLS

Abstract of the Invention

The invention provides anti-CD2 antibodies, antigen-binding fragments thereof, and antibody-drug conjugates thereof, for use as agents to treat a stem cell disorder, cancer, or autoimmune disease, among other hematological and proliferative diseases. The compositions and methods described herein can be used to deplete populations of CD2+ cells, such as CD2+ cancer cells and CD2+ immune cells, and can be used to prepare a patient for hematopoietic stem cell transplantation.

CLAIMS

1. A method of depleting a population of CD2+ cells in a human patient, the method comprising administering to the patient an effective amount of an anti-CD2 antibody, or antigen-binding fragment thereof .

2. A method of depleting a population of CD2+ cells in a human patient in need of a hematopoietic stem cell transplant, the method comprising administering to the patient an effective amount of an anti-CD2 antibody, or antigen-binding fragment thereof .

3. A method of preventing rejection of a hematopoietic stem cell graft in a human patient in need of a hematopoietic stem cell transplant, the method comprising administering to the patient an effective amount of an anti-CD2 antibody, or antigen-binding fragment thereof, prior to the human patient receiving a transplant comprising hematopoietic stem cells.

4. A method of depleting a population of CD2+ cells in a human patient in need of a hematopoietic stem cell transplant, the method comprising administering to the patient an effective amount of an anti-CD2 antibody, or antigen-binding fragment thereof, prior to the patient receiving a transplant comprising hematopoietic stem cells.

5. A method comprising administering to a human patient a transplant comprising hematopoietic stem cells, wherein the patient has been previously administered an anti-CD2 antibody or antigen-binding fragment thereof, in an amount sufficient to deplete a population of CD2+ cells in the patient.

6. A method comprising:
   a. administering to a human patient an antibody, or antigen-binding fragment thereof, that binds to CD2 in an amount sufficient to deplete a population of CD2+ cells in the patient; and
   b. subsequently administering to the patient a transplant comprising hematopoietic stem cells.

7. The method of any one of claims 1-6, wherein the antibody or antigen-binding fragment thereof is produced by the hybridoma cell line ATCC HB 11423.

8. The method of any one of claims 1-6, wherein the antibody or antigen-binding fragment thereof comprises a heavy chain variable region CDR set (CDR1, CDR2, and CDR3) and a light chain variable region CDR set (CDR1, CDR2, and CDR3) of antibody LO-CD2A produced by the hybridoma cell line having ATCC accession number HB 11423.

9. The method of any one of claims 1-6, wherein the antibody, or antigen-binding fragment thereof, comprises the following complementarity determining regions (CDRs):

a. a CDR-H1 having the amino acid sequence EYYMY (SEQ ID NO: 1);
b. a CDR-H2 having the amino acid sequence RIDPEDGSIDYVEKFKK (SEQ ID NO: 2);
c. a CDR-H3 having the amino acid sequence GKFNYRFAY (SEQ ID NO: 3);
d. a CDR-L1 having the amino acid sequence RSSQSLLHSSGNTYLN (SEQ ID NO: 4);
e. a CDR-L2 having the amino acid sequence LVSKLES (SEQ ID NO: 5); and
f. a CDR-L3 having the amino acid sequence MQFTHYPYT (SEQ ID NO: 6).

10. The method of any one of claims 1-6, wherein the antibody, or antigen-binding fragment thereof, competitively inhibits the binding of CD2 to an antibody or antigen-binding fragment thereof that comprises the following CDRs:

a. a CDR-H1 having the amino acid sequence EYYMY (SEQ ID NO: 1);
b. a CDR-H2 having the amino acid sequence RIDPEDGSIDYVEKFKK (SEQ ID NO: 2);
c. a CDR-H3 having the amino acid sequence GKFNYRFAY (SEQ ID NO: 3);
d. a CDR-L1 having the amino acid sequence RSSQSLLHSSGNTYLN (SEQ ID NO: 4);
e. a CDR-L2 having the amino acid sequence LVSKLES (SEQ ID NO: 5); and
f. a CDR-L3 having the amino acid sequence MQFTHYPYT (SEQ ID NO: 6).

11. The method of any one of claims 1-6, wherein the antibody or antigen-binding fragment thereof is selected from the group consisting of a monoclonal antibody or antigen-binding fragment thereof, a polyclonal antibody or antigen-binding fragment thereof, a humanized antibody or antigen-binding fragment thereof, a bispecific antibody or antigen-binding fragment thereof, an intact antibody, a dual-variable immunoglobulin domain, a single-chain Fv molecule (scFv), a diabody, a triabody, a nanobody, an antibody-like protein scaffold, a Fv fragment, a Fab fragment, a $F(ab')_2$ molecule, and a tandem di-scFv.

12. The method of any one of claims 1-10, wherein the antibody or antigen-binding fragment thereof is a humanized antibody, or antigen-binding fragment thereof.

13. The method of any one of claims 1-12, wherein the antibody has an isotype selected from the group consisting of IgG, IgA, IgM, IgD, and IgE.

14. The method of any one of claims 1-13, wherein the antibody, or antigen-binding fragment thereof, is conjugated to a cytotoxin.

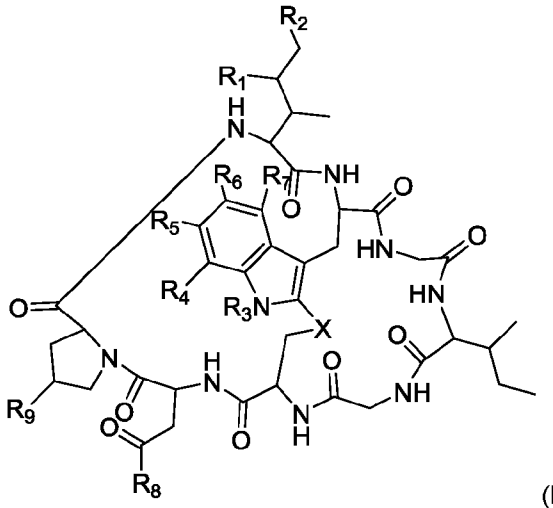
15. The method of claim 14, wherein the cytotoxin is selected from the group consisting of pseudomonas exotoxin A, deBouganin, diphtheria toxin, an amatoxin, saporin, maytansine, a maytansinoid, an auristatin, an anthracycline, a calicheamicin, irinotecan, SN-38, a duocarmycin, a pyrrolobenzodiazepine, a pyrrolobenzodiazepine dimer, an indolinobenzodiazepine, and an indolinobenzodiazepine dimer, or a variant thereof.

16. The method of claim 14, wherein the cytotoxin is an RNA polymerase inhibitor.

17. The method of claim 16, wherein the RNA polymerase inhibitor is an RNA polymerase II inhibitor.

18. The method of claim 17, wherein the RNA polymerase II inhibitor is amatoxin.

19. The method of claim 14, wherein the antibody or antigen-binding fragment thereof conjugated to a cytotoxin is represented by the formula Ab-Am, wherein Ab is the antibody or antigen-binding fragment thereof and Am an amatoxin represented by formula (I)



(I)

wherein R_1 is H, OH, OR_A, or OR_C;

R_2 is H, OH, OR_B, or OR_C;

R_A and R_B, together with the oxygen atoms to which they are bound, combine to form an optionally substituted 5-membered heterocyclolalkyl group;

R_3 is H, R_C, or R_D;

R_4, R_5, R_6, and R_7 are each independently H, OH, OR_C, OR_D, R_C, or R_D;

R_8 is OH, NH_2, OR_C, OR_D, NHR_C, or NR_CR_D;

R_9 is H, OH, OR_C, or OR_D;

X is -S-, -S(O)-, or $-SO_2-$;

R_C is -L-Z;

R_D is optionally substituted C_1-C_6 alkyl, optionally substituted C_1-C_6 heteroalkyl, optionally substituted C_2-C_6 alkenyl, optionally substituted C_2-C_6 heteroalkenyl, optionally substituted C_2-C_6 alkynyl, optionally substituted C_2-C_6 heteroalkynyl, optionally substituted cycloalkyl, optionally substituted heterocycloalkyl, optionally substituted aryl, or optionally substituted heteroaryl;

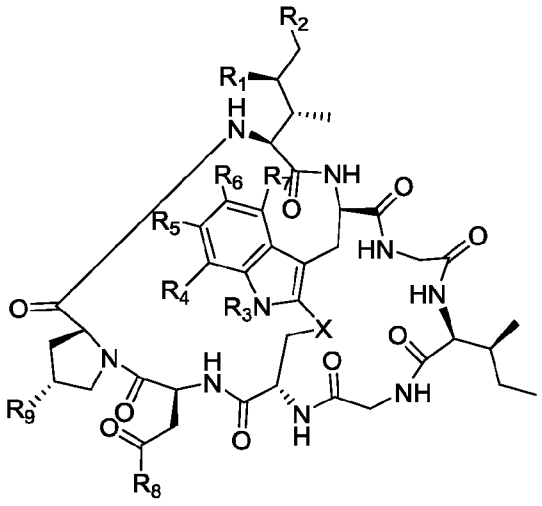
L is optionally substituted C_1-C_6 alkylene, optionally substituted C_1-C_6 heteroalkylene, optionally substituted C_2-C_6 alkenylene, optionally substituted C_2-C_6 heteroalkenylene, optionally substituted C_2-C_6 alkynylene, optionally substituted C_2-C_6 heteroalkynylene, optionally substituted

cycloalkylene, optionally substituted heterocycloalkylene, optionally substituted arylene, or optionally substituted heteroarylene; and

Z is a chemical moiety formed from a coupling reaction between a reactive substituent present on L and a reactive substituent present within the antibody or antigen-binding fragment thereof,

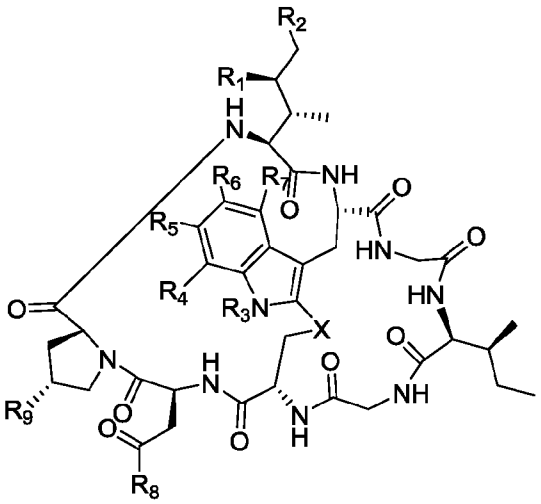
wherein Am comprises exactly one R_C substituent.

20. The method of claim 19, wherein Am is an amatoxin represented by formula (**IA**).



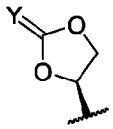
(**IA**)

21. The method of claim 19, wherein Am is an amatoxin represented by formula (**IB**).



(IB)

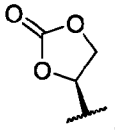
22. The method of claim 20 or 21, wherein R_A and R_B, together with the oxygen atoms to which they are bound, combine to form:



wherein Y is selected from O, S, NR_E, and $CR_ER_{E'}$, and

R_E and $R_{E'}$ are each independently optionally substituted C_1-C_6 alkylene-R_C, optionally substituted C_1-C_6 heteroalkylene-R_C, optionally substituted C_2-C_6 alkenylene-R_C, optionally substituted C_2-C_6 heteroalkenylene-R_C, optionally substituted C_2-C_6 alkynylene-R_C, optionally substituted C_2-C_6 heteroalkynylene-R_C, optionally substituted cycloalkylene-R_C, optionally substituted heterocycloalkylene-R_C, optionally substituted arylene-R_C, or optionally substituted heteroarylene-R_C.

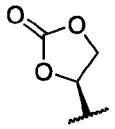
23. The method of claim 22, wherein R_A and R_B, together with the oxygen atoms to which they are bound, combine to form:



24. The method of claim 20 or 21, wherein R_1 is H, OH, or OR_A;

R_2 is H, OH, or OR_B;

R_A and R_B, together with the oxygen atoms to which they are bound, combine to form:



R_3, R_4, R_6, and R_7 are each H;

R_5 is OR_C;

R_8 is OH or NH_2; and

R_9 is H or OH.

25. The method of claim 20 or 21, wherein R_1 and R_2 are each independently H or OH;

R_3 is R_C;

R_4, R_6, and R_7 are each H;

R_5 is H, OH, or OC_1-C_6 alkyl;

R_8 is OH or NH_2; and

R_9 is H or OH.

26. The method of claim 20 or 21, wherein R_1 and R_2 are each independently H or OH;

R_3, R_6, and R_7 are each H;

R_4 and R_5 are each independently H, OH, OR_C, or R_C;

R_8 is OH or NH_2; and

R_9 is H or OH.

27. The method of claim 20 or 21, wherein R_1 and R_2 are each independently H or OH;

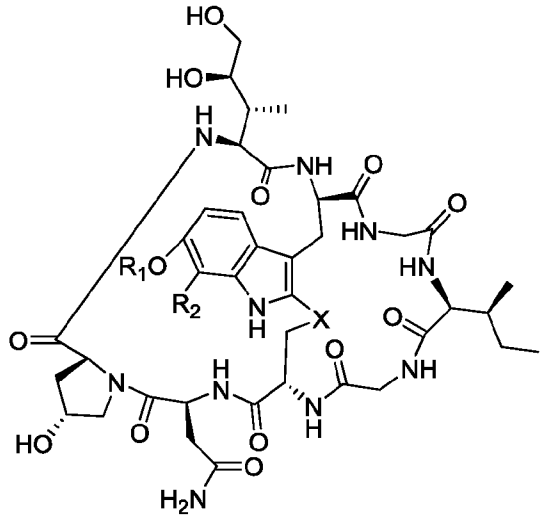
R_3, R_6, and R_7 are each H;

R_4 and R_5 are each independently H or OH;

R_8 is OR_C or NHR_C; and

R_9 is H or OH.

28. The method of claim 14, wherein the antibody or antigen-binding fragment thereof conjugated to a cytotoxin is represented by the formula Ab-Am, wherein Ab is the antibody or antigen-binding fragment thereof and Am is an amatoxin represented by formula (**II**)



(**II**)

wherein X is S, SO, or SO_2;

R_1 is H or a linker covalently bound to the antibody or antigen-binding fragment thereof; and

R_2 is H or a linker covalently bound to the antibody or antigen-binding fragment thereof;

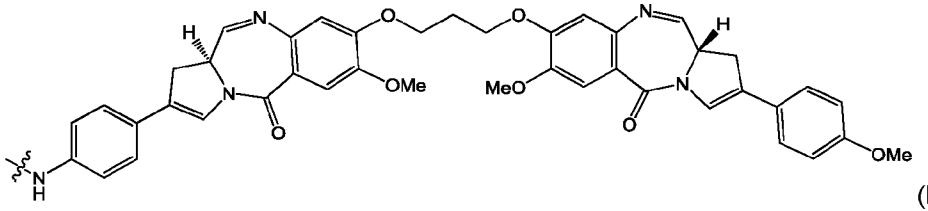
wherein when R_1 is H, R_2 is the linker, and when R_2 is H, R_1 is the linker.

29. The method of claim 14, wherein the cytotoxin is a maytansinoid selected from the group consisting of DM1 and DM4.

30. The method of claim 14, wherein the cytotoxin is an auristatin selected from the group consisting of monomethyl auristatin E and monomethyl auristatin F.

31. The method of claim 14, wherein the cytotoxin is an anthracycline selected from the group consisting of daunorubicin, doxorubicin, epirubicin, and idarubicin.

32. The method of claim 14, wherein the cytotoxin is a pyrrolobenzodiazepine dimer derivative represented by formula (**IV**)



(**IV**).

33. The method of any one of claims 1-32, wherein the antibody or antigen-binding fragment thereof is internalized by an immune cell following administration to the patient.

34. The method of any one of claims 1-33, wherein the antibody or antigen-binding fragment thereof is capable of promoting necrosis of an immune cell.

35. The method of any one of claims 1-34, wherein the antibody or antigen-binding fragment thereof is capable of recruiting one or more complement proteins to an immune cell upon administration to the patient.

36. The method of any one of claims 33-35, wherein the immune cell is selected from the group consisting of a T cell and NK cell.

37. The method of any one of claims 3-35, wherein the transplant comprising hematopoietic stem cells is administered to the patient after the concentration of the antibody or antigen-binding fragment thereof has substantially cleared from the blood of the patient.

38. The method of claim 37, wherein the transplant comprising hematopoietic stem cells is administered to the patient between 1 hour and 7 days after the concentration of the antibody or antigen-binding fragment thereof has substantially cleared from the blood of the patient.

39. The method of claim 37, wherein the transplant comprising hematopoietic stem cells is administered to the patient between 6 hours and 3 days after the concentration of the antibody or antigen-binding fragment thereof has substantially cleared from the blood of the patient.

40. The method of claim 37, wherein the transplant comprising hematopoietic stem cells is administered to the patient between 12 hours and 36 hours after the concentration of the antibody or antigen-binding fragment thereof has substantially cleared from the blood of the patient.

41. The method of claim 37, wherein the transplant comprising hematopoietic stem cells is administered to the patient 24 hours after the concentration of the antibody or antigen-binding fragment thereof has substantially cleared from the blood of the patient.

42. The method of any one of claims 3-35, wherein the hematopoietic stem cells or progeny thereof maintain hematopoietic stem cell functional potential after two or more days following transplantation of the hematopoietic stem cells into the patient.

43. The method of any one of claims 3-42, wherein the hematopoietic stem cells are autologous with respect to the patient.

44. The method of any one of claims 3-42, wherein the hematopoietic stem cells are allogeneic with respect to the patient.

45. The method of claim 44, wherein the hematopoietic stem cells are HLA-matched with respect to the patient.

46. The method of claim 44, wherein the hematopoietic stem cells are HLA-mismatched with respect to the patient.

47. The method of any one of claims 1, 2, and 4-36, wherein the population of CD2+ cells comprises T cells.

48. The method of any one of claims 3-47, wherein the hematopoietic stem cells or progeny thereof are capable of localizing to hematopoietic tissue and/or reestablishing hematopoiesis following transplantation of the hematopoietic stem cells into the patient.

49. The method of any one of claims 3-48, wherein upon transplantation into the patient, the hematopoietic stem cells give rise to recovery of a population of cells selected from the group consisting of megakaryocytes, thrombocytes, platelets, erythrocytes, mast cells, myeoblasts, basophils, neutrophils, eosinophils, microglia, granulocytes, monocytes, osteoclasts, antigen-presenting cells, macrophages, dendritic cells, natural killer cells, T lymphocytes, and B lymphocytes.

50. The method of any one of claims 1-49, wherein the patient is suffering from a stem cell disorder.

51. The method of any one of claims 1-49, wherein the patient is suffering from a hemoglobinopathy disorder.

52. The method of claim 51, wherein the hemoglobinopathy disorder is selected from the group consisting of sickle cell anemia, thalassemia, Fanconi anemia, aplastic anemia, and Wiskott-Aldrich syndrome.

53. The method of claim 51, wherein the hemoglobinopathy disorder is fanconi anemia.

54. The method of claim 51, wherein the hemoglobinopathy disorder is aplastic anemia.

55. The method of claim 51, wherein the hemoglobinopathy disorder is sickle cell anemia.

56. The method of claim 51, wherein the hemoglobinopathy disorder is thalassemia.

57. The method of any one of claims 1-49, wherein the patient is suffering from a myelodysplastic disorder.

58. The method of any one of claims 1-49, wherein the patient is suffering from an immunodeficiency disorder.

59. The method of claim 58, wherein the immunodeficiency disorder is a congenital immunodeficiency.

60. The method of claim 58, wherein the immunodeficiency disorder is an acquired immunodeficiency.

61. The method of claim 60, wherein the acquired immunodeficiency is human immunodeficiency virus or acquired immune deficiency syndrome.

62. The method of any one of claims 1-49, wherein the patient is suffering from a metabolic disorder.

63. The method of claim 62, wherein the metabolic disorder is selected from the group consisting of glycogen storage diseases, mucopolysaccharidoses, Gaucher's Disease, Hurlers Disease, sphingolipidoses, and metachromatic leukodystrophy.

64. The method of any one of claims 1-63, wherein the patient is suffering from cancer.

65. The method of claim 64, wherein the cancer is selected from the group consisting of leukemia, lymphoma, multiple myeloma, and neuroblastoma.

66. The method of claim 64, wherein the cancer is a hematological cancer.

67. The method of claim 64, wherein the cancer is acute myeloid leukemia.

68. The method of claim 64, wherein the cancer is acute lymphoid leukemia.

69. The method of claim 64, wherein the cancer is chronic myeloid leukemia.

70. The method of claim 64, wherein the cancer is chronic lymphoid leukemia.

71. The method of claim 64, wherein the cancer is multiple myeloma.

72. The method of claim 64, wherein the cancer is diffuse large B-cell lymphoma.

73. The method of claim 64, wherein the cancer is non-Hodgkin's lymphoma.

74. The method of any one of claims 1-73, wherein the patient is suffering from a disorder selected from the group consisting of adenosine deaminase deficiency and severe combined immunodeficiency, hyper immunoglobulin M syndrome, Chediak-Higashi disease, hereditary lymphohistiocytosis, osteopetrosis, osteogenesis imperfecta, storage diseases, thalassemia major, systemic sclerosis, systemic lupus erythematosus, multiple sclerosis, and juvenile rheumatoid arthritis.

75. The method of any one of claims 1-74, wherein the patient is suffering from an autoimmune disorder.

76. The method of claim 75, wherein the autoimmune disorder is selected from the group consisting of multiple sclerosis, human systemic lupus, rheumatoid arthritis, inflammatory bowel disease, treating psoriasis, Type 1 diabetes mellitus, acute disseminated encephalomyelitis, Addison's disease, alopecia universalis, ankylosing spondylitisis, antiphospholipid antibody syndrome, aplastic anemia, autoimmune hemolytic anemia, autoimmune hepatitis, autoimmune inner ear disease, autoimmune lymphoproliferative syndrome, autoimmune oophoritis, Balo disease, Behcet's disease, bullous pemphigoid, cardiomyopathy, Chagas' disease, chronic

fatigue immune dysfunction syndrome, chronic inflammatory demyelinating polyneuropathy, Crohn's disease, cicatrical pemphigoid, coeliac sprue-dermatitis herpetiformis, cold agglutinin disease, CREST syndrome, Degos disease, discoid lupus, dysautonomia, endometriosis, essential mixed cryoglobulinemia, fibromyalgia-fibromyositis, Goodpasture' s syndrome, Grave's disease, Guillain-Barre syndrome, Hashimoto' s thyroiditis, Hidradenitis suppurativa, idiopathic and/or acute thrombocytopenic purpura, idiopathic pulmonary fibrosis, IgA neuropathy, interstitial cystitis, juvenile arthritis, Kawasaki's disease, lichen planus, Lyme disease, Meniere disease, mixed connective tissue disease, myasthenia gravis, neuromyotonia, opsoclonus myoclonus syndrome, optic neuritis, Ord's thyroiditis, pemphigus vulgaris, pernicious anemia, polychondritis, polymyositis and dermatomyositis, primary biliary cirrhosis, polyarteritis nodosa, polyglandular syndromes, polymyalgia rheumatica, primary agammaglobulinemia, Raynaud phenomenon, Reiter' s syndrome, rheumatic fever, sarcoidosis, scleroderma, Sjögren's syndrome, stiff person syndrome, Takayasu's arteritis, temporal arteritis, ulcerative colitis, uveitis, vasculitis, vitiligo, vulvodynia, and Wegener's granulomatosis.

77. The method of claim 75, wherein the autoimmune disorder is scleroderma.

78. The method of claim 75, wherein the autoimmune disorder is multiple sclerosis.

79. The method of claim 75, wherein the autoimmune disorder is ulcerative colitis.

80. The method of claim 75, wherein the autoimmune disorder is Crohn’s disease.

81. The method of claim 75, wherein the autoimmune disorder is Type 1 diabetes.

82. The method of any one of claims 50-81, wherein the method treats the disorder or cancer.

83. A method of treating a stem cell disorder in a human patient, the method comprising administering to the patient a therapeutically effective amount of an antibody, or antigen-binding fragment thereof, that binds to CD2.

84. A method of treating a hemoglobinopathy disorder in a human patient, the method comprising administering to the patient a therapeutically effective amount of an antibody, or antigen-binding fragment thereof, that binds to CD2.

85. The method of claim 84, wherein the hemoglobinopathy disorder is selected from the group consisting of sickle cell anemia, thalassemia, Fanconi anemia, aplastic anemia, and Wiskott-Aldrich syndrome.

86. The method of claim 84, wherein the hemoglobinopathy disorder is Fanconi anemia.

87. The method of claim 84, wherein the hemoglobinopathy disorder is aplastic anemia.

88. The method of claim 84, wherein the hemoglobinopathy disorder is sickle cell anemia.

89. The method of claim 84, wherein the hemoglobinopathy disorder is thalassemia.

90. A method of treating a myelodysplastic disorder in a human patient, the method comprising administering to the patient a therapeutically effective amount of an antibody, or antigen-binding fragment thereof, that binds to CD2.

91. A method of treating an immunodeficiency disorder in a human patient, the method comprising administering to the patient a therapeutically effective amount of an antibody, or antigen-binding fragment thereof, that binds to CD2.

92. The method of claim 91, wherein the immunodeficiency disorder is a congenital immunodeficiency.

93. The method of claim 91, wherein the immunodeficiency disorder is an acquired immunodeficiency.

94. The method of claim 93, wherein the acquired immunodeficiency is human immunodeficiency virus or acquired immune deficiency syndrome.

95. A method of treating a metabolic disorder in a human patient, the method comprising administering to the patient a therapeutically effective amount of an antibody, or antigen-binding fragment thereof, that binds to CD2.

96. The method of claim 95, wherein the metabolic disorder is selected from the group consisting of glycogen storage diseases, mucopolysaccharidoses, Gaucher's Disease, Hurlers Disease, sphingolipidoses, and metachromatic leukodystrophy.

97. A method of treating cancer in a human patient, the method comprising administering to the patient a therapeutically effective amount of an antibody or antigen-binding fragment thereof that binds to CD2.

98. The method of claim 97, wherein the cancer is selected from the group consisting of leukemia, lymphoma, multiple myeloma, and neuroblastoma.

99. The method of claim 97, wherein the cancer is a hematological cancer.

100. The method of claim 97, wherein the cancer is acute myeloid leukemia.

101. The method of claim 97, wherein the cancer is acute lymphoid leukemia.

102. The method of claim 97, wherein the cancer is chronic myeloid leukemia.

103. The method of claim 97, wherein the cancer is chronic lymphoid leukemia.

104. The method of claim 97, wherein the cancer is multiple myeloma.

105. The method of claim 97, wherein the cancer is diffuse large B-cell lymphoma.

106. The method of claim 97, wherein the cancer is non-Hodgkin's lymphoma.

107. A method of treating a disorder selected from the group consisting of adenosine deaminase deficiency and severe combined immunodeficiency, hyper immunoglobulin M syndrome, Chediak-Higashi disease, hereditary lymphohistiocytosis, osteopetrosis, osteogenesis imperfecta, storage diseases, thalassemia major, systemic sclerosis, systemic lupus erythematosus, multiple sclerosis, and juvenile rheumatoid arthritis in a human patient, the method comprising administering to the patient a therapeutically effective amount of an antibody or antigen-binding fragment thereof that binds to CD2.

108. A method of treating an autoimmune disorder in a human patient, the method comprising administering to the patient a therapeutically effective amount of an antibody or antigen-binding fragment thereof that binds to CD2

109. The method of claim 108, wherein the autoimmune disorder is selected from the group consisting of multiple sclerosis, human systemic lupus, rheumatoid arthritis, inflammatory bowel disease, treating psoriasis, Type 1 diabetes mellitus, acute disseminated encephalomyelitis, Addison's disease, alopecia universalis, ankylosing spondylitisis, antiphospholipid antibody syndrome, aplastic anemia, autoimmune hemolytic anemia, autoimmune hepatitis, autoimmune inner ear disease, autoimmune lymphoproliferative syndrome, autoimmune oophoritis, Balo disease, Behcet's disease, bullous pemphigoid, cardiomyopathy, Chagas' disease, chronic fatigue immune dysfunction syndrome, chronic inflammatory demyelinating polyneuropathy, Crohn's disease, cicatrical pemphigoid, coeliac sprue-dermatitis herpetiformis, cold agglutinin disease, CREST syndrome, Degos disease, discoid lupus, dysautonomia, endometriosis, essential mixed cryoglobulinemia, fibromyalgia-fibromyositis, Goodpasture' s syndrome, Grave's disease, Guillain-Barre syndrome, Hashimoto' s thyroiditis, Hidradenitis suppurativa, idiopathic and/or acute thrombocytopenic purpura, idiopathic pulmonary fibrosis, IgA neuropathy, interstitial cystitis, juvenile arthritis, Kawasaki's disease, lichen planus, Lyme disease, Meniere disease, mixed connective tissue disease, myasthenia gravis,

neuromyotonia, opsoclonus myoclonus syndrome, optic neuritis, Ord's thyroiditis, pemphigus vulgaris, pernicious anemia, polychondritis, polymyositis and dermatomyositis, primary biliary cirrhosis, polyarteritis nodosa, polyglandular syndromes, polymyalgia rheumatica, primary agammaglobulinemia, Raynaud phenomenon, Reiter' s syndrome, rheumatic fever, sarcoidosis, scleroderma, Sjögren's syndrome, stiff person syndrome, Takayasu's arteritis, temporal arteritis, ulcerative colitis, uveitis, vasculitis, vitiligo, vulvodynia, and Wegener's granulomatosis.

110. The method of claim 108, wherein the autoimmune disorder is scleroderma.

111. The method of claim 108, wherein the autoimmune disorder is multiple sclerosis.

112. The method of claim 108, wherein the autoimmune disorder is ulcerative colitis.

113. The method of claim 108, wherein the autoimmune disorder is Crohn's disease.

114. The method of claim 108, wherein the autoimmune disorder is Type 1 diabetes.

115. The method of any one of claims 83-114, wherein the antibody or antigen-binding fragment thereof is produced by the hybridoma cell line ATCC HB 11423.

116. The method of any one of claims 83-114, wherein the antibody or antigen-binding fragment thereof comprises the following complementarity determining regions (CDRs):

a. a CDR-H1 having the amino acid sequence EYYMY (SEQ ID NO: 1);
b. a CDR-H2 having the amino acid sequence RIDPEDGSIDYVEKFKK (SEQ ID NO: 2);
c. a CDR-H3 having the amino acid sequence GKFNYRFAY (SEQ ID NO: 3);
d. a CDR-L1 having the amino acid sequence RSSQSLLHSSGNTYLN (SEQ ID NO: 4);
e. a CDR-L2 having the amino acid sequence LVSKLES (SEQ ID NO: 5); and
f. a CDR-L3 having the amino acid sequence MQFTHYPYT (SEQ ID NO: 6).

117. The method of any one of claims 83-114, wherein the antibody or antigen-binding fragment thereof competitively inhibits the binding of CD2 to an antibody or antigen-binding fragment thereof that comprises the following CDRs:

a. a CDR-H1 having the amino acid sequence EYYMY (SEQ ID NO: 1);
b. a CDR-H2 having the amino acid sequence RIDPEDGSIDYVEKFKK (SEQ ID NO: 2);
c. a CDR-H3 having the amino acid sequence GKFNYRFAY (SEQ ID NO: 3);
d. a CDR-L1 having the amino acid sequence RSSQSLLHSSGNTYLN (SEQ ID NO: 4);
e. a CDR-L2 having the amino acid sequence LVSKLES (SEQ ID NO: 5); and
f. a CDR-L3 having the amino acid sequence MQFTHYPYT (SEQ ID NO: 6).

118. The method of any one of claims 83-117, wherein the antibody or antigen-binding fragment thereof is selected from the group consisting of a monoclonal antibody,, a polyclonal antibody, a humanized antibody, a bispecific antibody, a dual-variable immunoglobulin domain, a single-chain Fv molecule (scFv), a diabody, a triabody, a nanobody, an antibody-like protein scaffold, a Fv fragment, a Fab fragment, a $F(ab')_2$ molecule, and a tandem di-scFv.

119. The method of claim 83-117, wherein the antibody or antigen-binding fragment thereof is a humanized antibody, or antigen-binding fragment thereof.

120. The method of any one of claims 83-119, wherein the antibody has an isotype selected from the group consisting of IgG, IgA, IgM, IgD, and IgE.

121. The method of any one of claims 83-120, wherein the antibody or antigen-binding fragment thereof is conjugated to a cytotoxin.

122. The method of claim 121, wherein the cytotoxin is selected from the group consisting of pseudomonas exotoxin A, deBouganin, diphtheria toxin, an amatoxin, saporin, maytansine, a maytansinoid, an auristatin, an anthracycline, a calicheamicin, irinotecan, SN-38, a

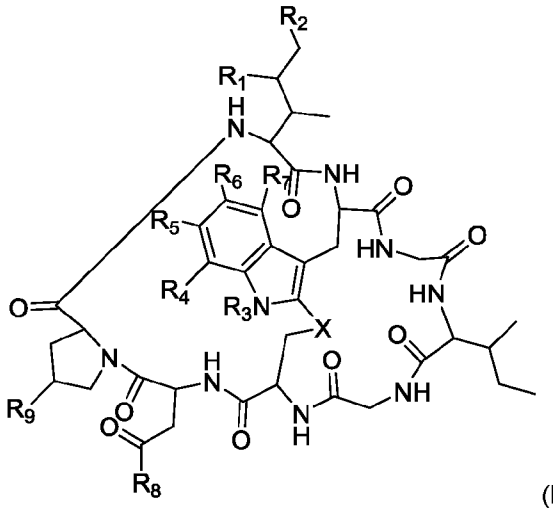
duocarmycin, a pyrrolobenzodiazepine, a pyrrolobenzodiazepine dimer, an indolinobenzodiazepine, and an indolinobenzodiazepine dimer, or a variant thereof.

123. The method of claim 121, wherein the cytotoxin is an RNA polymerase inhibitor.

124. The method of claim 123, wherein the RNA polymerase inhibitor is an RNA polymerase II inhibitor.

125. The method of claim 124, wherein the RNA polymerase II inhibitor is amatoxin.

126. The method of claim 121, wherein the antibody or antigen-binding fragment thereof conjugated to a cytotoxin is represented by the formula Ab-Am, wherein Ab is the antibody or antigen-binding fragment thereof and Am an amatoxin represented by formula (I)



(I)

wherein R_1 is H, OH, OR_A, or OR_C;

R_2 is H, OH, OR_B, or OR_C;

R_A and R_B, together with the oxygen atoms to which they are bound, combine to form an optionally substituted 5-membered heterocyclolalkyl group;

R_3 is H, R_C, or R_D;

R_4, R_5, R_6, and R_7 are each independently H, OH, OR_C, OR_D, R_C, or R_D;

R_8 is OH, NH_2, OR_C, OR_D, NHR_C, or NR_CR_D;

R_9 is H, OH, OR_C, or OR_D;

X is -S-, -S(O)-, or $-SO_2-$;

R_C is -L-Z;

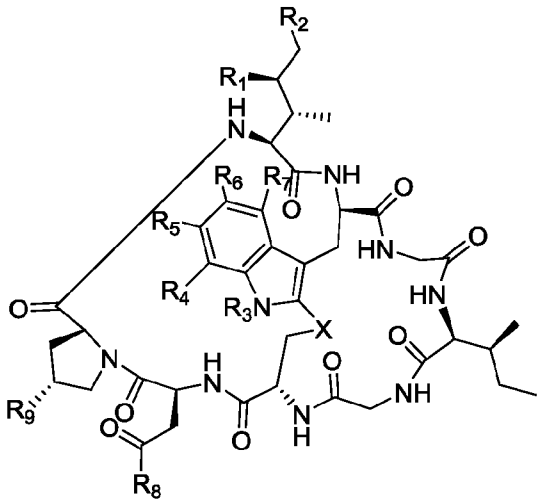
R_D is optionally substituted C_1-C_6 alkyl, optionally substituted C_1-C_6 heteroalkyl, optionally substituted C_2-C_6 alkenyl, optionally substituted C_2-C_6 heteroalkenyl, optionally substituted C_2-C_6 alkynyl, optionally substituted C_2-C_6 heteroalkynyl, optionally substituted cycloalkyl, optionally substituted heterocycloalkyl, optionally substituted aryl, or optionally substituted heteroaryl;

L is optionally substituted C_1-C_6 alkylene, optionally substituted C_1-C_6 heteroalkylene, optionally substituted C_2-C_6 alkenylene, optionally substituted C_2-C_6 heteroalkenylene, optionally substituted C_2-C_6 alkynylene, optionally substituted C_2-C_6 heteroalkynylene, optionally substituted cycloalkylene, optionally substituted heterocycloalkylene, optionally substituted arylene, or optionally substituted heteroarylene; and

Z is a chemical moiety formed from a coupling reaction between a reactive substituent present on L and a reactive substituent present within the antibody or antigen-binding fragment thereof,

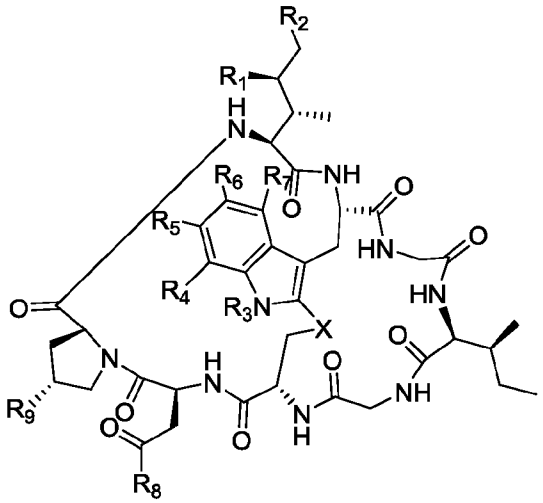
wherein Am comprises exactly one R_C substituent.

127. The method of claim 126, wherein Am is an amatoxin represented by formula (**IA**).



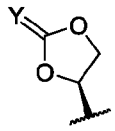
(**IA**)

128. The method of claim 126, wherein Am is an amatoxin represented by formula (**IB**).



(**IB**)

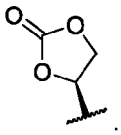
129. The method of claim 127 or 128, wherein R_A and R_B, together with the oxygen atoms to which they are bound, combine to form:



wherein Y is selected from O, S, NR_E, and $CR_ER_{E'}$, and

R_E and $R_{E'}$ are each independently optionally substituted C_1-C_6 alkylene-R_C, optionally substituted C_1-C_6 heteroalkylene-R_C, optionally substituted C_2-C_6 alkenylene-R_C, optionally substituted C_2-C_6 heteroalkenylene-R_C, optionally substituted C_2-C_6 alkynylene-R_C, optionally substituted C_2-C_6 heteroalkynylene-R_C, optionally substituted cycloalkylene-R_C, optionally substituted heterocycloalkylene-R_C, optionally substituted arylene-R_C, or optionally substituted heteroarylene-R_C.

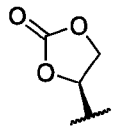
130. The method of claim 129, wherein R_A and R_B, together with the oxygen atoms to which they are bound, combine to form:



131. The method of claim 127 or 128, wherein R_1 is H, OH, or OR_A;

R_2 is H, OH, or OR_B;

R_A and R_B, together with the oxygen atoms to which they are bound, combine to form:



R_3, R_4, R_6, and R_7 are each H;

R_5 is OR_C;

R_8 is OH or NH_2; and

R_9 is H or OH.

132. The method of claim 127 or 128, wherein R_1 and R_2 are each independently H or OH;

R_3 is R_C;

R_4, R_6, and R_7 are each H;

R_5 is H, OH, or OC_1-C_6 alkyl;

R_8 is OH or NH_2; and

R_9 is H or OH.

133. The method of claim 127 or 128, wherein R_1 and R_2 are each independently H or OH;

R_3, R_6, and R_7 are each H;

R_4 and R_5 are each independently H, OH, OR_C, or R_C;

R_8 is OH or NH_2; and

R_9 is H or OH.

134. The method of claim 127 or 128, wherein R_1 and R_2 are each independently H or OH;

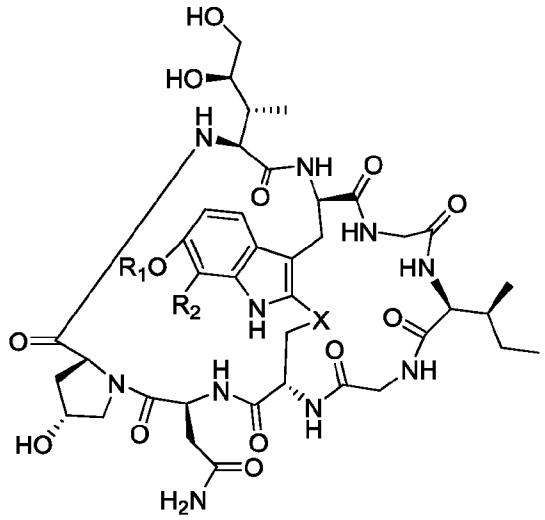
R_3, R_6, and R_7 are each H;

R_4 and R_5 are each independently H or OH;

R_8 is OR_C or NHR_C; and

R_9 is H or OH.

135. The method of claim 121, wherein the antibody or antigen-binding fragment thereof conjugated to a cytotoxin is represented by the formula Ab-Am, wherein Ab is the antibody or antigen-binding fragment thereof and Am is an amatoxin represented by formula (**II**)



(II)

wherein X is S, SO, or SO_2;

R_1 is H or a linker covalently bound to the antibody or antigen-binding fragment thereof; and

R_2 is H or a linker covalently bound to the antibody or antigen-binding fragment thereof;

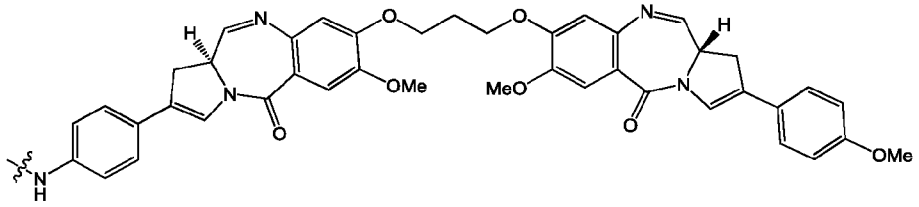
wherein when R_1 is H, R_2 is the linker, and when R_2 is H, R_1 is the linker.

136. The method of claim 121, wherein the cytotoxin is a maytansinoid selected from the group consisting of DM1 and DM4.

137. The method of claim 121, wherein the cytotoxin is an auristatin monomethyl auristatin E or monomethyl auristatin F.

138. The method of claim 121, wherein the cytotoxin is an anthracycline selected from the group consisting of daunorubicin, doxorubicin, epirubicin, and idarubicin.

139. The method of claim 121, wherein the cytotoxin is a pyrrolobenzodiazepine dimer derivative represented by formula (**IV**)



(**IV**).

140. A conjugate represented by the formula Ab-Cy, wherein Ab is an antibody or antigen-binding fragment thereof that binds CD2 and Cy is a cytotoxin.

141. The conjugate of claim 140, wherein the antibody or antigen-binding fragment thereof is produced by the hybridoma cell line ATCC HB 11423.

142. The conjugate of claim 140, wherein the antibody or antigen-binding fragment thereof comprises the following complementarity determining regions (CDRs):

a. a CDR-H1 having the amino acid sequence EYYMY (SEQ ID NO: 1);
b. a CDR-H2 having the amino acid sequence RIDPEDGSIDYVEKFKK (SEQ ID NO: 2);
c. a CDR-H3 having the amino acid sequence GKFNYRFAY (SEQ ID NO: 3);
d. a CDR-L1 having the amino acid sequence RSSQSLLHSSGNTYLN (SEQ ID NO: 4);
e. a CDR-L2 having the amino acid sequence LVSKLES (SEQ ID NO: 5); and
f. a CDR-L3 having the amino acid sequence MQFTHYPYT (SEQ ID NO: 6).

143. The conjugate of claim 140, wherein the antibody or antigen-binding fragment thereof competitively inhibits the binding of CD2 to an antibody or antigen-binding fragment thereof that comprises the following CDRs:

a. a CDR-H1 having the amino acid sequence EYYMY (SEQ ID NO: 1);
b. a CDR-H2 having the amino acid sequence RIDPEDGSIDYVEKFKK (SEQ ID NO: 2);
c. a CDR-H3 having the amino acid sequence GKFNYRFAY (SEQ ID NO: 3);
d. a CDR-L1 having the amino acid sequence RSSQSLLHSSGNTYLN (SEQ ID NO: 4);

e. a CDR-L2 having the amino acid sequence LVSKLES (SEQ ID NO: 5); and

f. a CDR-L3 having the amino acid sequence MQFTHYPYT (SEQ ID NO: 6).

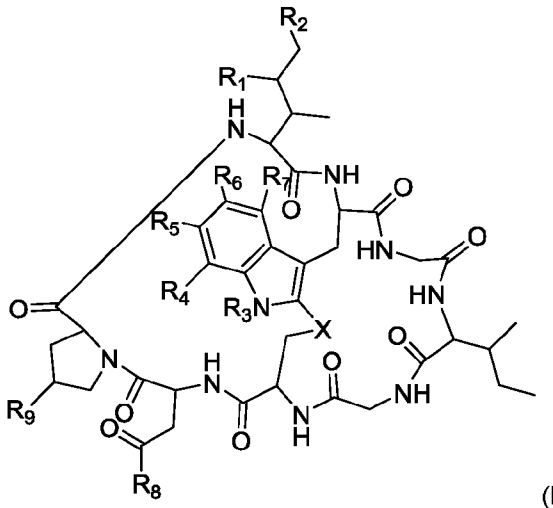
144. The conjugate of any one of claims 140-143, wherein the antibody or antigen-binding fragment thereof is selected from the group consisting of a monoclonal antibody, a polyclonal antibody, a humanized antibody, a bispecific antibody, a dual-variable immunoglobulin domain, a single-chain Fv molecule (scFv), a diabody, a triabody, a nanobody, an antibody-like protein scaffold, a Fv fragment, a Fab fragment, a $F(ab')_2$ molecule, and a tandem di-scFv.

145. The conjugate of any one of claims 140-143, wherein the antibody or antigen-binding fragment thereof is a humanized antibody or antigen-binding fragment thereof.

146. The conjugate of any one of claims 140-145, wherein the antibody has an isotype selected from the group consisting of IgG, IgA, IgM, IgD, and IgE.

147. The conjugate of any one of claims 140-146, wherein Cy is selected from the group consisting of pseudomonas exotoxin A, deBouganin, diphtheria toxin, an amatoxin, saporin, maytansine, a maytansinoid, an auristatin, an anthracycline, a calicheamicin, irinotecan, SN-38, a duocarmycin, a pyrrolobenzodiazepine, a pyrrolobenzodiazepine dimer, an indolinobenzodiazepine, and an indolinobenzodiazepine dimer, or a variant thereof.

148. The conjugate of any one of claims 140-146, wherein Cy is an amatoxin (Am) represented by formula (**I**)



(I)

wherein R_1 is H, OH, OR_A, or OR_C;

R_2 is H, OH, OR_B, or OR_C;

R_A and R_B, together with the oxygen atoms to which they are bound, combine to form an optionally substituted 5-membered heterocyclolalkyl group;

R_3 is H, R_C, or R_D;

R_4, R_5, R_6, and R_7 are each independently H, OH, OR_C, OR_D, R_C, or R_D;

R_8 is OH, NH_2, OR_C, OR_D, NHR_C, or NR_CR_D;

R_9 is H, OH, OR_C, or OR_D;

X is -S-, -S(O)-, or $-SO_2-$;

R_C is -L-Z;

R_D is optionally substituted C_1-C_6 alkyl, optionally substituted C_1-C_6 heteroalkyl, optionally substituted C_2-C_6 alkenyl, optionally substituted C_2-C_6 heteroalkenyl, optionally substituted C_2-C_6 alkynyl, optionally substituted C_2-C_6 heteroalkynyl, optionally substituted cycloalkyl, optionally substituted heterocycloalkyl, optionally substituted aryl, or optionally substituted heteroaryl;

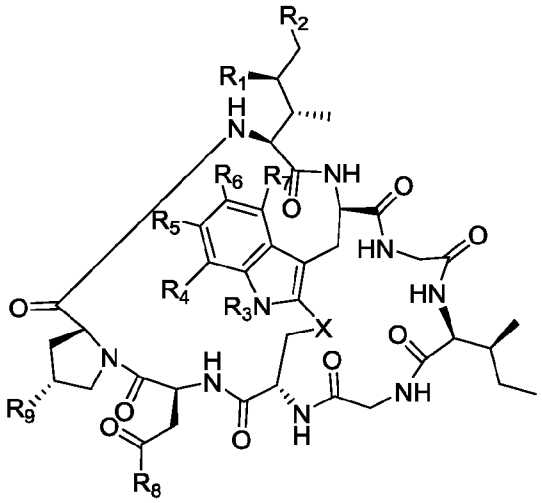
L is optionally substituted C_1-C_6 alkylene, optionally substituted C_1-C_6 heteroalkylene, optionally substituted C_2-C_6 alkenylene, optionally substituted C_2-C_6 heteroalkenylene, optionally substituted C_2-C_6 alkynylene, optionally substituted C_2-C_6 heteroalkynylene, optionally substituted cycloalkylene, optionally substituted heterocycloalkylene, optionally substituted arylene, or optionally substituted heteroarylene; and

Z is a chemical moiety formed from a coupling reaction between a reactive substituent

present on L and a reactive substituent present within the antibody or antigen-binding fragment thereof,

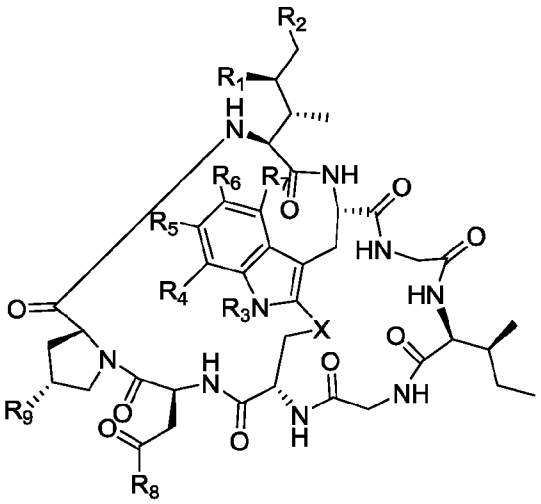
wherein Am comprises exactly one R_C substituent.

149. The conjugate of claim 148, wherein Am is an amatoxin represented by formula (**IA**).



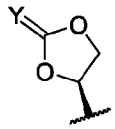
(**IA**)

150. The conjugate of claim 148, wherein Am is an amatoxin represented by formula (**IB**).



(IB)

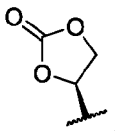
151. The conjugate of claim 149 or 150, wherein R_A and R_B, together with the oxygen atoms to which they are bound, combine to form:



wherein Y is selected from O, S, NR_E, and $CR_ER_{E'}$, and

R_E and $R_{E'}$ are each independently optionally substituted C_1-C_6 alkylene-R_C, optionally substituted C_1-C_6 heteroalkylene-R_C, optionally substituted C_2-C_6 alkenylene-R_C, optionally substituted C_2-C_6 heteroalkenylene-R_C, optionally substituted C_2-C_6 alkynylene-R_C, optionally substituted C_2-C_6 heteroalkynylene-R_C, optionally substituted cycloalkylene-R_C, optionally substituted heterocycloalkylene-R_C, optionally substituted arylene-R_C, or optionally substituted heteroarylene-R_C.

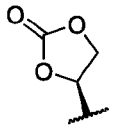
152. The conjugate of claim 151, wherein R_A and R_B, together with the oxygen atoms to which they are bound, combine to form:



153. The conjugate of claim 149 or 150, wherein R_1 is H, OH, or OR_A;

R_2 is H, OH, or OR_B;

R_A and R_B, together with the oxygen atoms to which they are bound, combine to form:



R_3, R_4, R_6, and R_7 are each H;

R_5 is OR_C;

R_8 is OH or NH_2; and

R_9 is H or OH.

154. The conjugate of claim 149 or 150, wherein R_1 and R_2 are each independently H or OH;

R_3 is R_C;

R_4, R_6, and R_7 are each H;

R_5 is H, OH, or OC_1-C_6 alkyl;

R_8 is OH or NH_2; and

R_9 is H or OH.

155. The conjugate of claim 149 or 150, wherein R_1 and R_2 are each independently H or OH;

R_3, R_6, and R_7 are each H;

R_4 and R_5 are each independently H, OH, OR_C, or R_C;

R_8 is OH or NH_2; and

R_9 is H or OH.

156. The conjugate of claim 149 or 150, wherein R_1 and R_2 are each independently H or OH;

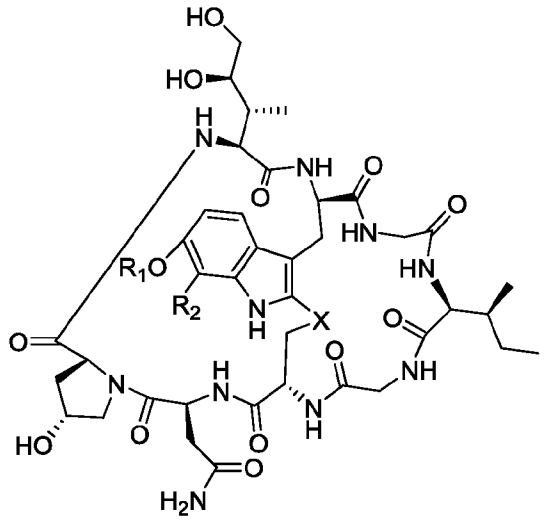
R_3, R_6, and R_7 are each H;

R_4 and R_5 are each independently H or OH;

R_8 is OR_C or NHR_C; and

R_9 is H or OH.

157. The conjugate of any one of claims 140-146, wherein Cy is an amatoxin (Am) represented by formula (II)



(II)

wherein X is S, SO, or SO_2;

R_1 is H or a linker covalently bound to the antibody or antigen-binding fragment thereof; and

R_2 is H or a linker covalently bound to the antibody or antigen-binding fragment thereof;

wherein when R_1 is H, R_2 is the linker, and when R_2 is H, R_1 is the linker.

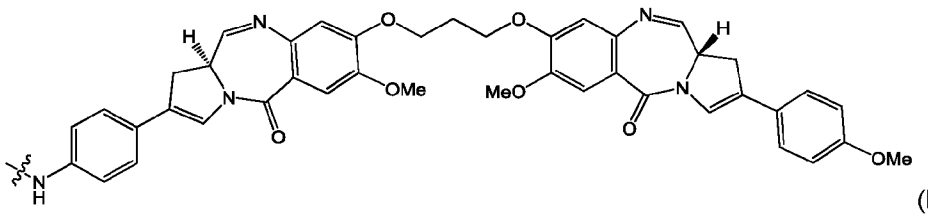
158. The conjugate of any one of claims 140-146, wherein Cy is a maytansinoid selected from the group consisting of DM1 and DM4.

159. The conjugate of any one of claims 140-146, wherein Cy is an auristatin.

160. The conjugate of claim 159, wherein the auristatin is monomethyl auristatin E and monomethyl auristatin F.

161. The conjugate of any one of claims 140-146, wherein Cy is an anthracycline selected from the group consisting of daunorubicin, doxorubicin, epirubicin, and idarubicin.

162. The conjugate of any one of claims 140-146, wherein Cy is a pyrrolobenzodiazepine dimer derivative represented by formula (**IV**)



(**IV**).

163. The method of any one of claims 140-146, wherein the Cy is an RNA polymerase inhibitor.

164. The method of claim 163, wherein the RNA polymerase inhibitor is an RNA polymerase II inhibitor.

165. The method of claim 164, wherein the RNA polymerase II inhibitor is amatoxin.

166. A pharmaceutical composition comprising the conjugate of any one of claims 140-165, and a pharmaceutically acceptable excipient.

167. The pharmaceutical composition of claim 166, wherein the pharmaceutical composition is formulated for transdermal, subcutaneous, intravenous, intramuscular, intraocular, intratumoral, parenteral, intrathecal or intracerebroventricular administration to a human patient.

168. The pharmaceutical composition of claim 166, wherein the pharmaceutical composition is formulated for intravenous administration to a human patient.

COMPOSITIONS AND METHODS FOR THE DEPLETION OF CD2+ CELLS

Background of the Invention

Despite advances in the medicinal arts, there remains a demand for treating pathologies of the hematopoietic system, such as diseases of a particular blood cell, metabolic disorders, cancers, and autoimmune conditions, among others. While hematopoietic stem cells have significant therapeutic potential, a limitation that has hindered their use in the clinic has been the difficulty associated with ensuring engraftment of hematopoietic stem cell transplants in a host. There is currently a need for compositions and methods for treating disorders of the hematopoietic system, such as autoimmune disorders, as well as compositions and methods for promoting the engraftment of exogenous hematopoietic stem cell grafts such that the multi-potency and hematopoietic functionality of these cells is preserved following transplantation.

Summary of the Invention

Provided herein are compositions and methods for the direct treatment of various disorders of the hematopoietic system, metabolic disorders, cancers, and autoimmune diseases, among others. In one aspect, the invention additionally features compositions and methods for conditioning a patient, such as a human patient, prior to receiving hematopoietic stem cell transplant therapy so as to promote the engraftment of hematopoietic stem cell grafts. The patient may be one that is suffering from one or more blood disorders, such as an autoimmune disease, cancer, hemoglobinopathy, or other hematopoietic pathology, and is thus in need of hematopoietic stem cell transplantation. As described herein, hematopoietic stem cells are capable of differentiating into a multitude of cell types in the hematopoietic lineage, and can be administered to a patient in order to populate or re-populate a cell type that is deficient in the patient. In certain aspects, the invention features antibodies and antibody-drug conjugates that bind CD2, as well as methods of administering the same to a patient so as to (i) directly treat a blood disorder, such as an autoimmune disease, by selectively depleting a population of immune cells that express CD2, such as an autoreactive T cell or natural killer (NK) cell, and/or to (ii) deplete a population of T cells or NK cells prior to administration of a hematopoietic stem cell

transplant to the patient, thereby reducing the likelihood of hematopoietic stem cell graft rejection. The former activity enables the direct treatment of a wide range of autoimmune disorders, as CD2 may be expressed by a T cell or NK cell that cross-reacts with, and mounts an inappropriate immune response against, a self antigen. Administration of an anti-CD2 antibody or antigen-binding fragment thereof to a patient in this case can cause depletion of a population of CD2+ autoimmune cells, such as T cells or NK cells that cross-react with one or more self antigens, thereby treating the autoimmune pathology. The latter activity facilitates the generation of an environment that is conducive to hematopoietic stem cell engraftment, as T cells and/or NK cells that cross-react with one or more non-self antigens expressed by a hematopoietic stem cell (e.g., non-self MHC antigens) can mount an immune response against transplanted hematopoietic stem cells and thus promote graft rejection. In this latter case, patients suffering from a disorder such as cancer, an autoimmune disease, or other condition of the hematopoietic system can subsequently be administered a hematopoietic stem cell transplant in order, for instance, to repopulate one or more populations of blood cells that is defective or depleted in the patient. Also provided herein are methods of treating a variety of hematopoietic conditions, such as sickle cell anemia, thalassemia, Fanconi anemia, Wiskott-Aldrich syndrome, adenosine deaminase deficiency-severe combined immunodeficiency, metachromatic leukodystrophy, Diamond-Blackfan anemia and Schwachman-Diamond syndrome, human immunodeficiency virus infection, and acquired immune deficiency syndrome, as well as cancers and autoimmune diseases, among others.

In one aspect, the invention provides a method of depleting a population of CD2+ cells, for instance, in a human patient, such as a population of CD2+ T cells and/or CD2+ NK cells in a human patient, by administering to the patient an effective amount of an antibody, or an antigen-binding fragment thereof, that binds to CD2.

In another aspect, the invention provides a method of depleting a population of CD2+ cells in a human patient in need of a hematopoietic stem cell transplant, such as a population of CD2+ T cells and/or CD2+ NK cells in a human patient in need of hematopoietic stem cell transplant, by administering to the patient an effective amount of an antibody or antigen-binding fragment thereof that binds to CD2, for example, prior to the patient receiving a transplant including hematopoietic stem cells,.

In an additional aspect, provided herein is a method of preventing or reducing the likelihood of rejection of a hematopoietic stem cell graft in a human patient in need of hematopoietic stem cell transplant therapy by administering, prior to the patient receiving a transplant including hematopoietic stem cells, an effective amount of an antibody, or antigen-binding fragment thereof, that binds to CD2.

In another aspect, the invention provides a method of depleting a population of endogenous T cells in a human patient in need of hematopoietic stem cell transplant therapy by administering, prior to the patient receiving a transplant including hematopoietic stem cells, an effective amount of an antibody or antigen-binding fragment thereof, that binds to CD2.

In another aspect, the invention features a method, for example, of treating a human patient in need of a hematopoietic stem cell transplant, including administering to a human patient a transplant including hematopoietic stem cells, wherein the patient has been previously administered an antibody or antigen-binding fragment thereof, that binds to CD2. The antibody or antigen-binding fragment thereof may be administered to the patient in an amount sufficient to deplete a population of CD2+ cells in the patient, such as a population of CD2+ T cells and/or CD2+ NK cells in the human patient.

In an additional aspect, the invention features a method, for example, of treating a human patient in need of a hematopoietic stem cell transplant, including: administering to a human patient an antibody, or antigen-binding fragment thereof, that binds to CD2 in an amount sufficient to deplete a population of CD2+ cells in the patient, such as a population of CD2+ T cells and/or CD2+ NK cells in the patient, and subsequently administering to the patient a transplant including hematopoietic stem cells.

In some embodiments of any of the foregoing aspects, the anti-CD2 antibody or antigen-binding fragment thereof, is produced by the hybridoma cell line ATCC HB 11423. In some embodiments, the anti-CD2 antibody or antigen-binding fragment thereof competitively inhibits the binding of CD2 to an anti-CD2 antibody or antigen-binding fragment thereof produced by the hybridoma cell line ATCC HB 11423.

In some embodiments, the anti-CD2 antibody or antigen-binding fragment thereof contains the following complementarity determining regions (CDRs):

a CDR-H1 having the amino acid sequence EYYMY (SEQ ID NO: 1);

a CDR-H2 having the amino acid sequence RIDPEDGSIDYVEKFKK (SEQ ID NO: 2);

a CDR-H3 having the amino acid sequence GKFNYRFAY (SEQ ID NO: 3);

a CDR-L1 having the amino acid sequence RSSQSLLHSSGNTYLN (SEQ ID NO: 4);

a CDR-L2 having the amino acid sequence LVSKLES (SEQ ID NO: 5); and

a CDR-L3 having the amino acid sequence MQFTHYPYT (SEQ ID NO: 6).

In some embodiments, the antibody or antigen-binding fragment thereof competitively inhibits the binding of CD2 to an antibody or antigen-binding fragment thereof comprising the following CDRs:

a CDR-H1 having the amino acid sequence EYYMY (SEQ ID NO: 1);

a CDR-H2 having the amino acid sequence RIDPEDGSIDYVEKFKK (SEQ ID NO: 2);

a CDR-H3 having the amino acid sequence GKFNYRFAY (SEQ ID NO: 3);

a CDR-L1 having the amino acid sequence RSSQSLLHSSGNTYLN (SEQ ID NO: 4);

a CDR-L2 having the amino acid sequence LVSKLES (SEQ ID NO: 5); and

a CDR-L3 having the amino acid sequence MQFTHYPYT (SEQ ID NO: 6).

In some embodiments, the anti-CD2 antibody, or the antigen-binding fragment thereof, is selected from the group consisting of a monoclonal antibody, a polyclonal antibody or antigen-binding fragment thereof, a humanized antibody, a bispecific antibody , a dual-variable immunoglobulin domain, a single-chain Fv molecule (scFv), a diabody, a triabody, a nanobody, an antibody-like protein scaffold, a Fv fragment, a Fab fragment, a $F(ab')_2$ molecule, and a tandem di-scFv, or antigen-binding fragments thereof. In some embodiments, the antibody has an isotype selected from the group consisting of IgG, IgA, IgM, IgD, and IgE.

In some embodiments, the anti-CD2 antibody, or antigen binding fragment, is conjugated to a cytotoxin. In some embodiments, the cytotoxin is selected from the group consisting of an amatoxin, pseudomonas exotoxin A, deBouganin, diphtheria toxin, saporin, maytansine, a maytansinoid, an auristatin, an anthracycline, a calicheamicin, irinotecan, SN-38, a duocarmycin, a pyrrolobenzodiazepine, a pyrrolobenzodiazepine dimer, an indolinobenzodiazepine, and an indolinobenzodiazepine dimer, or a variant thereof.

In another aspect, the invention provides a method of depleting a population of CD2+ cells in a human patient, such as a population of CD2+ T cells and/or CD2+ NK cells in a human patient, by administering to the patient an effective amount of an antibody, or an antigen binding fragment thereof, that binds CD2.

In an additional aspect, the invention provides a method of depleting a population of CD2+ cells in a human patient in need of a hematopoietic stem cell transplant, such as a population of CD2+ T cells and/or CD2+ NK cells in a human patient in need of hematopoietic stem cell transplant, by administering, prior to the patient receiving a transplant including hematopoietic stem cells, an effective amount of an anti-CD2 antibody, or antigen-binding fragment thereof.

In another aspect, the invention features a method, for example, of treating a human patient in need of a hematopoietic stem cell transplant, including administering to a human patient a transplant including hematopoietic stem cells, wherein the patient has been previously administered an antibody or fragment thereof that binds CD2, in an amount sufficient to deplete a population of CD2+ cells in the patient, such as a population of CD2+ T cells and/or CD2+ NK cells in the human patient.

In an additional aspect, the invention features a method, for example, of treating a human patient in need of a hematopoietic stem cell transplant, including: administering to a human patient an antibody or fragment thereof, that binds CD2, in an amount sufficient to deplete a population of CD2+ cells in the patient, such as a population of CD2+ T cells and/or CD2+ NK cells in the patient, and subsequently administering to the patient a transplant including hematopoietic stem cells.

In some embodiments of any of the preceding four aspects, the antibody or fragment thereof that binds CD2 (e.g., on the surface of a CD2+ T cell or CD2+ NK cell) is covalently bound to an Fc domain, such as a dimeric Fc domain isolated from a human antibody (for example, isolated from an IgG1, IgG2, IgG3, or IgG4 isotype human antibody). In some embodiments, the Fc domain is a monomeric Fc domain containing a single polypeptide strand. In some embodiments, the N-terminus of the antibody or fragment thereof is bound to the Fc domain. In some embodiments, the C-terminus of the antibody or fragment thereof is bound to the Fc domain. The Fc domain may be conjugated to one or more copies of the antibody or fragment thereof. For instance, conjugates that may be used in conjunction with the methods

described herein include dimeric Fc domains in which each polypeptide strand of the Fc domain is conjugated to the antibody or fragment thereof. The Fc domain may in turn be conjugated to a cytotoxin, such as a cytotoxin described herein (for example, an amatoxin, such as α-amanitin, pseudomonas exotoxin A, deBouganin, diphtheria toxin, saporin, maytansine, a maytansinoid, an auristatin, an anthracycline, a calicheamicin, irinotecan, SN-38, a duocarmycin, a pyrrolobenzodiazepine, a pyrrolobenzodiazepine dimer, an indolinobenzodiazepine, and an indolinobenzodiazepine dimer, or a variant thereof).

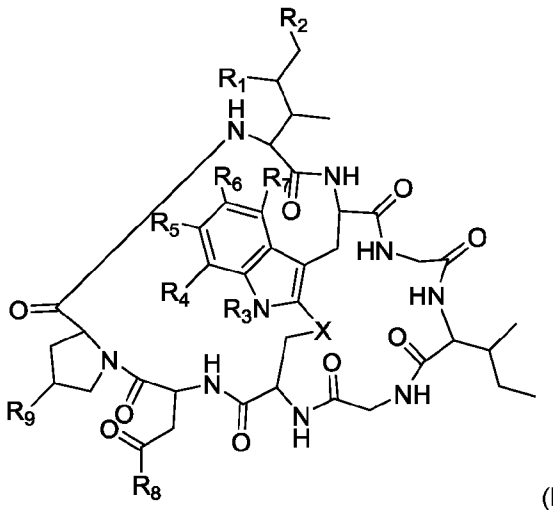
In some embodiments, the antibody or fragment thereof is covalently bound to a cytotoxin, such as a cytotoxin described herein (for example, an amatoxin, such as α-amanitin, pseudomonas exotoxin A, deBouganin, diphtheria toxin, saporin, maytansine, a maytansinoid, an auristatin, an anthracycline, a calicheamicin, irinotecan, SN-38, a duocarmycin, a pyrrolobenzodiazepine, a pyrrolobenzodiazepine dimer, an indolinobenzodiazepine, and an indolinobenzodiazepine dimer, or a variant thereof). In some embodiments, the N-terminus of the antibody or fragment thereof is bound to the cytotoxin. In some embodiments, the C-terminus of the antibody or fragment thereof is bound to the cytotoxin. The cytotoxin may in turn be conjugated to an Fc domain.

In some embodiments, the antibody or fragment thereof is covalently bound to the cytotoxin at one site on the antibody or fragment thereof (for example, the N- or C-terminus of the antibody or fragment thereof) and is covalently bound to an Fc domain at another site on the antibody or fragment thereof (for example, the opposite terminus of the antibody or fragment thereof).

In some embodiments, the Fc domain is a human IgG1 isotype Fc domain. In some embodiments, the Fc domain is a human IgG2 isotype Fc domain. In some embodiments, the Fc domain is a human IgG3 isotype Fc domain. In some embodiments, the Fc domain is a human IgG4 isotype Fc domain.

In some embodiments of any of the above aspects, the cytotoxin is an amatoxin or derivative thereof, such as α-amanitin, β-amanitin, γ-amanitin, ε-amanitin, amanin, amaninamide, amanullin, amanullinic acid, and proamanullin. In some embodiments of any of the above aspects, the cytotoxin is an amatoxin, and the antibody, or the antigen-binding fragment thereof, or antibody conjugated to the cytotoxin is represented by the formula Ab-Am, wherein Ab is the

antibody, antigen-binding fragment thereof, and Am is the amatoxin. In some embodiments, Am is represented by formula (I)



(I)

wherein R_1 is H, OH, OR_A, or OR_C;

R_2 is H, OH, OR_B, or OR_C;

R_A and R_B, together with the oxygen atoms to which they are bound, combine to form an optionally substituted 5-membered heterocyclolalkyl group;

R_3 is H, R_C, or R_D;

R_4 is H, OH, OR_C, OR_D, R_C, or R_D;

R_5 is H, OH, OR_C, OR_D, R_C, or R_D;

R_6 is H, OH, OR_C, OR_D, R_C, or R_D;

R_7 is H, OH, OR_C, OR_D, R_C, or R_D;

R_8 is OH, NH_2, OR_C, OR_D, NHR_C, or NR_CR_D;

R_9 is H, OH, OR_C, or OR_D;

X is -S-, -S(O)-, or $-SO_2-$;

R_C is -L-Z;

R_D is optionally substituted alkyl (e.g., C_1-C_6 alkyl), optionally substituted heteroalkyl (e.g., C_1-C_6 heteroalkyl), optionally substituted alkenyl (e.g., C_2-C_6 alkenyl), optionally substituted heteroalkenyl (e.g., C_2-C_6 heteroalkenyl), optionally substituted alkynyl (e.g., C_2-C_6 alkynyl),

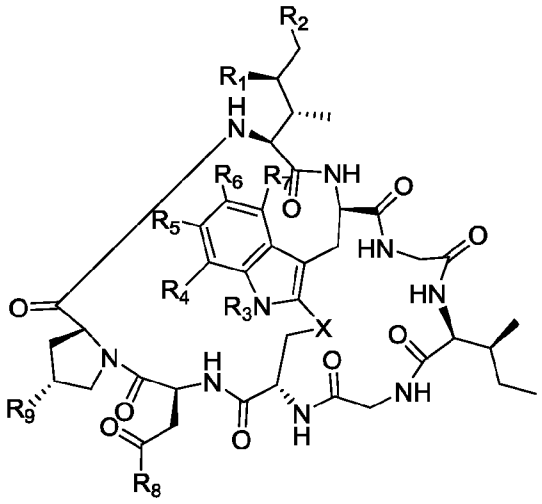
optionally substituted heteroalkynyl (e.g., C_2-C_6 heteroalkynyl), optionally substituted cycloalkyl, optionally substituted heterocycloalkyl, optionally substituted aryl, or optionally substituted heteroaryl;

L is a linker, such as optionally substituted alkylene (e.g., C_1-C_6 alkylene), optionally substituted heteroalkylene (C_1-C_6 heteroalkylene), optionally substituted alkenylene (e.g., C_2-C_6 alkenylene), optionally substituted heteroalkenylene (e.g., C_2-C_6 heteroalkenylene), optionally substituted alkynylene (e.g., C_2-C_6 alkynylene), optionally substituted heteroalkynylene (e.g., C_2-C_6 heteroalkynylene), optionally substituted cycloalkylene, optionally substituted heterocycloalkylene, optionally substituted arylene, or optionally substituted heteroarylene; and

Z is a chemical moiety formed from a coupling reaction between a reactive substituent present on L and a reactive substituent present within an antibody, or an antigen-binding fragment thereof, that binds CD2, such as on the surface of a CD2+ T cell or CD2+ NK cell.

In some embodiments, Am contains exactly one R_C substituent.

In some embodiments, Am is represented by formula (**IA**)



(**IA**)

wherein R_1 is H, OH, OR_A, or OR_C;

R_2 is H, OH, OR_B, or OR_C;

R_A and R_B, together with the oxygen atoms to which they are bound, combine to form an optionally substituted 5-membered heterocyclolalkyl group;

R_3 is H, R_C, or R_D;

R_4 is H, OH, OR_C, OR_D, R_C, or R_D;

R_5 is H, OH, OR_C, OR_D, R_C, or R_D;

R_6 is H, OH, OR_C, OR_D, R_C, or R_D;

R_7 is H, OH, OR_C, OR_D, R_C, or R_D;

R_8 is OH, NH_2, OR_C, OR_D, NHR_C, or NR_CR_D;

R_9 is H, OH, OR_C, or OR_D;

X is -S-, -S(O)-, or $-SO_2-$;

R_C is -L-Z;

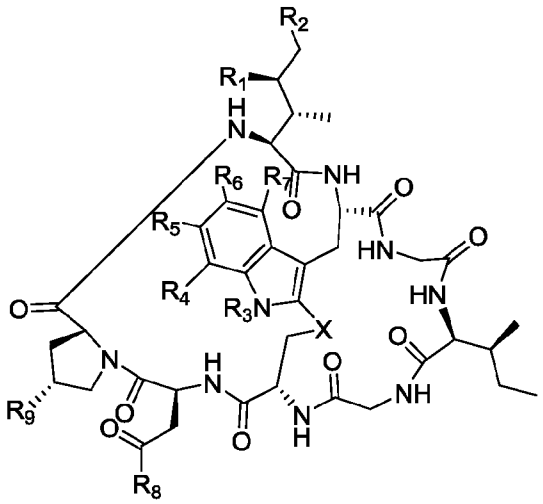
R_D is optionally substituted alkyl (e.g., C_1-C_6 alkyl), optionally substituted heteroalkyl (e.g., C_1-C_6 heteroalkyl), optionally substituted alkenyl (e.g., C_2-C_6 alkenyl), optionally substituted heteroalkenyl (e.g., C_2-C_6 heteroalkenyl), optionally substituted alkynyl (e.g., C_2-C_6 alkynyl), optionally substituted heteroalkynyl (e.g., C_2-C_6 heteroalkynyl), optionally substituted cycloalkyl, optionally substituted heterocycloalkyl, optionally substituted aryl, or optionally substituted heteroaryl;

L is a linker, such as optionally substituted alkylene (e.g., C_1-C_6 alkylene), optionally substituted heteroalkylene (C_1-C_6 heteroalkylene), optionally substituted alkenylene (e.g., C_2-C_6 alkenylene), optionally substituted heteroalkenylene (e.g., C_2-C_6 heteroalkenylene), optionally substituted alkynylene (e.g., C_2-C_6 alkynylene), optionally substituted heteroalkynylene (e.g., C_2-C_6 heteroalkynylene), optionally substituted cycloalkylene, optionally substituted heterocycloalkylene, optionally substituted arylene, or optionally substituted heteroarylene;

Z is a chemical moiety formed from a coupling reaction between a reactive substituent present on L and a reactive substituent present within an antibody, or an antigen-binding fragment thereof, that binds CD2, such as on the surface of a CD2+ T cell or CD2+ NK cell; and

wherein Am contains exactly one R_C substituent.

In some embodiments, Am is represented by formula (**IB**)



(IB)

wherein R_1 is H, OH, OR_A, or OR_C;

R_2 is H, OH, OR_B, or OR_C;

R_A and R_B, together with the oxygen atoms to which they are bound, combine to form an optionally substituted 5-membered heterocyclolalkyl group;

R_3 is H, R_C, or R_D;

R_4 is H, OH, OR_C, OR_D, R_C, or R_D;

R_5 is H, OH, OR_C, OR_D, R_C, or R_D;

R_6 is H, OH, OR_C, OR_D, R_C, or R_D;

R_7 is H, OH, OR_C, OR_D, R_C, or R_D;

R_8 is OH, NH_2, OR_C, OR_D, NHR_C, or NR_CR_D;

R_9 is H, OH, OR_C, or OR_D;

X is -S-, -S(O)-, or $-SO_2-$;

R_C is -L-Z;

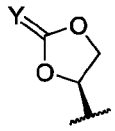
R_D is optionally substituted alkyl (e.g., C_1-C_6 alkyl), optionally substituted heteroalkyl (e.g., C_1-C_6 heteroalkyl), optionally substituted alkenyl (e.g., C_2-C_6 alkenyl), optionally substituted heteroalkenyl (e.g., C_2-C_6 heteroalkenyl), optionally substituted alkynyl (e.g., C_2-C_6 alkynyl), optionally substituted heteroalkynyl (e.g., C_2-C_6 heteroalkynyl), optionally substituted cycloalkyl, optionally substituted heterocycloalkyl, optionally substituted aryl, or optionally substituted heteroaryl;

L is a linker, such as optionally substituted alkylene (e.g., C_1-C_6 alkylene), optionally substituted heteroalkylene (C_1-C_6 heteroalkylene), optionally substituted alkenylene (e.g., C_2-C_6 alkenylene), optionally substituted heteroalkenylene (e.g., C_2-C_6 heteroalkenylene), optionally substituted alkynylene (e.g., C_2-C_6 alkynylene), optionally substituted heteroalkynylene (e.g., C_2-C_6 heteroalkynylene), optionally substituted cycloalkylene, optionally substituted heterocycloalkylene, optionally substituted arylene, or optionally substituted heteroarylene;

Z is a chemical moiety formed from a coupling reaction between a reactive substituent present on L and a reactive substituent present within an antibody, or an antigen-binding fragment thereof, that binds CD2, such as on the surface of a CD2+ T cell or CD2+ NK cell; and

wherein Am contains exactly one R_C substituent.

In some embodiments, R_A and R_B, together with the oxygen atoms to which they are bound, combine to form:



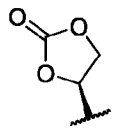
wherein Y is selected from O, S, NR_E, and $CR_ER_{E'}$, and

R_E and $R_{E'}$ are each independently optionally substituted C_1-C_6 alkylene-R_C, optionally substituted C_1-C_6 heteroalkylene-R_C, optionally substituted C_2-C_6 alkenylene-R_C, optionally substituted C_2-C_6 heteroalkenylene-R_C, optionally substituted C_2-C_6 alkynylene-R_C, optionally substituted C_2-C_6 heteroalkynylene-R_C, optionally substituted cycloalkylene-R_C, optionally substituted heterocycloalkylene-R_C, optionally substituted arylene-R_C, or optionally substituted heteroarylene-R_C.

In some embodiments, Am is represented by formula (**IA**) or formula (**IB**), wherein R_1 is H, OH, OR_A, or OR_C;

R_2 is H, OH, OR_B, or OR_C;

R_A and R_B, together with the oxygen atoms to which they are bound, combine to form:



R_3 is H or R_C;

R_4 is H, OH, OR_C, OR_D, R_C, or R_D;

R_5 is H, OH, OR_C, OR_D, R_C, or R_D;

R_6 is H, OH, OR_C, OR_D, R_C, or R_D;

R_7 is H, OH, OR_C, OR_D, R_C, or R_D;

R_8 is OH, NH_2, OR_C, or NHR_C;

R_9 is H or OH; and

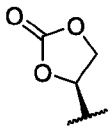
wherein R_C and R_D are each as defined above.

In some embodiments, Am is represented by formula (**IA**) or formula (**IB**),

wherein R_1 is H, OH, OR_A, or OR_C;

R_2 is H, OH, OR_B, or OR_C;

R_A and R_B, together with the oxygen atoms to which they are bound, combine to form:



R_3 is H or R_C;

R_4 and R_5 are each independently H, OH, OR_C, R_C, or OR_D;

R_6 and R_7 are each H;

R_8 is OH, NH_2, OR_C, or NHR_C;

R_9 is H or OH; and

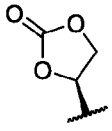
wherein R_C is as defined above.

In some embodiments, Am is represented by formula (**IA**) or formula (**IB**),

wherein R_1 is H, OH, or OR_A;

R_2 is H, OH, or OR_B;

R_A and R_B, together with the oxygen atoms to which they are bound, combine to form:



R_3, R_4, R_6, and R_7 are each H;

R_5 is OR_C;

R_8 is OH or NH_2;

R_9 is H or OH; and

wherein R_C is as defined above.

In some embodiments, Am is represented by formula (**IA**) or formula (**IB**),

wherein R_1 and R_2 are each independently H or OH;

R_3 is R_C;

R_4, R_6, and R_7 are each H;

R_5 is H, OH, or OC_1-C_6 alkyl;

R_8 is OH or NH_2;

R_9 is H or OH; and

wherein R_C is as defined above.

In some embodiments, Am is represented by formula (**IA**) or formula (**IB**),

wherein R_1 and R_2 are each independently H or OH;

R_3, R_6, and R_7 are each H;

R_4 and R_5 are each independently H, OH, OR_C, or R_C;

R_8 is OH or NH_2;

R_9 is H or OH; and

wherein R_C is as defined above.

In some embodiments, Am is represented by formula (**IA**) or formula (**IB**),

wherein R_1 and R_2 are each independently H or OH;

R_3, R_6, and R_7 are each H;

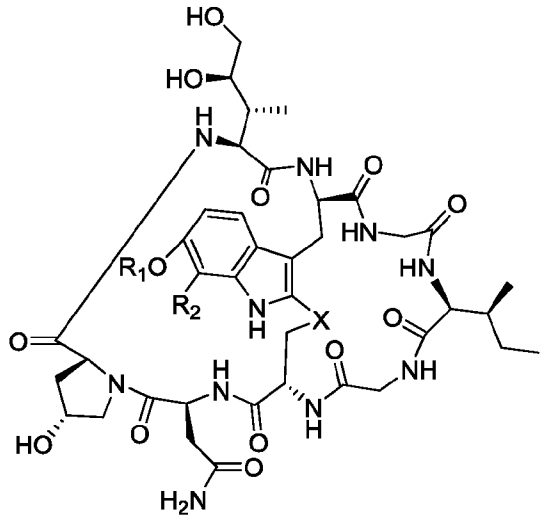
R_4 and R_5 are each independently H or OH;

R_8 is OH, NH_2, OR_C, or NHR_C;

R_9 is H or OH; and

wherein R_C is as defined above.

In some embodiments, Am is represented by formula (**II**)

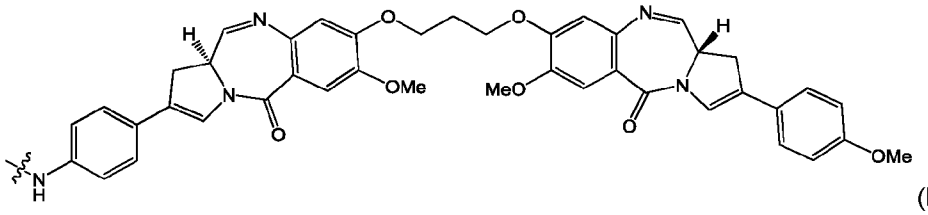


(II)

wherein X is S, SO, or SO_2; R_1 is H or a linker covalently bound to the antibody, or the antigen-binding fragment thereof,; and R_2 is H or a linker covalently bound to the antibody, or the antigen-binding fragment thereof; wherein when R_1 is H, R_2 is the linker, and when R_2 is H, R_1 is the linker.

In some embodiments of any of the above aspects, the cytotoxin is a maytansinoid selected from the group consisting of DM1 and DM4. In some embodiments, the cytotoxin is an auristatin selected from the group consisting of monomethyl auristatin E and monomethyl auristatin F. In some embodiments, the cytotoxin is an anthracycline selected from the group consisting of daunorubicin, doxorubicin, epirubicin, and idarubicin.

In some embodiments, the cytotoxin is a pyrrolobenzodiazepine dimer represented by formula (**IV**):



(IV)

In some embodiments, the cytotoxin is conjugated to the antibody, or the antigen-binding fragment thereof, by way of a maleimidocaproyl linker.

In some embodiments, the cytotoxin is an auristatin selected from the group consisting of monomethyl auristatin E and monomethyl auristatin F.

In some embodiments, the cytotoxin is an anthracycline selected from the group consisting of daunorubicin, doxorubicin, epirubicin, and idarubicin.

In some embodiments, the antibody, or the antigen-binding fragment thereof, is internalized by an immune cell, such as a T cell or NK cell (e.g., a CD2+ T cell or CD2+ NK cell) following administration to the patient. For instance, the antibody, or the antigen-binding fragment thereof, may be internalized by T cells by receptor mediated endocytosis (e.g., upon binding to cell-surface CD2). In some embodiments, a cytotoxin covalently bound to the antibody, or the antigen-binding fragment thereof, may be released intracellularly by chemical cleavage (for instance, by enzymatic or non-specific cleavage of a linker described herein). The cytotoxin may then access its intracellular target (such as RNA polymerase, the mitotic spindle apparatus, nuclear DNA, ribosomal RNA, or topoisomerases, among others) so as to promote the death of an endogenous immune cell (e.g., CD2+ T cell or CD2+ NK cell) prior to hematopoietic stem cell transplantation therapy.

In some embodiments, the antibody, or the antigen-binding fragment thereof, is capable of promoting necrosis of an immune cell, such as a T cell or NK cell (e.g., a CD2+ T cell or CD2+ NK cell). In some embodiments, the antibody, or the antigen-binding fragment thereof, may promote the death of an endogenous immune cell (e.g., CD2+ T cell or CD2+ NK cell) prior to transplantation therapy by recruiting one or more complement proteins, NK cells, macrophages, neutrophils, and/or eosinophils to the immune cell upon administration to the patient.

In some embodiments, an autologous transplant containing hematopoietic stem cells is administered to the patient. For instance, autologous hematopoietic stem cells can be removed from a patient, such as a patient in need of hematopoietic stem cell transplant therapy, and the cells can subsequently be administered to (e.g., infused into) the patient so as to re-populate one or more cell types of the hematopoietic lineage. The withdrawn hematopoietic stem cells may be freshly re-infused into the subject, for instance, following maintenance ex vivo for one or more hours, days, or weeks. For instance, the withdrawn hematopoietic stem cells may re-infused into the patient from 1 hour to about 1 week, from 1 hour to about 72 hours, from about 1 hour to about 48 hours, or from about 1 hour to about 24 hours following withdrawal from the patient. In some embodiments, the withdrawn hematopoietic stem cells are frozen for longer-term storage

prior to re-infusion into the patient. For instance, the withdrawn hematopoietic stem cells may be frozen and cryopreserved for from 1 week to 1 year, or longer, prior to re-infusion into the patient.

In some embodiments, an allogenic transplant containing hematopoietic stem cells is administered to the patient. For instance, allogeneic hematopoietic stem cells can be removed from a donor, such as donor that is HLA-matched with respect to the patient, for instance, a closely related family member of the patient. In some embodiments, the allogenic hematopoietic stem cells are HLA-mismatched with respect to the patient. Following withdrawal of the allogeneic hematopoietic stem cells from a donor, the cells can subsequently be administered to (e.g., infused into) the patient so as to re-populate one or more cell types of the hematopoietic lineage. The withdrawn hematopoietic stem cells may be freshly infused into the subject, for instance, following maintenance ex vivo for one or more hours, days, or weeks. For instance, the withdrawn hematopoietic stem cells may infused into the patient from 1 hour to about 1 week, from 1 hour to about 72 hours, from about 1 hour to about 48 hours, or from about 1 hour to about 24 hours following withdrawal from the donor. In some embodiments, the withdrawn hematopoietic stem cells are frozen for longer-term storage prior to infusion into the patient. For instance, the withdrawn hematopoietic stem cells may be frozen and cryopreserved for from 1 week to 1 year, or longer, prior to infusion into the patient.

In some embodiments, a transplant containing hematopoietic stem cells is administered to the patient after the concentration of the antibody, or the antigen-binding fragment thereof, has substantially cleared from the blood of the patient.

In some embodiments, a transplant containing hematopoietic stem cells is administered to the patient from 1 hour to 7 days (e.g., from 6 hours to 3 days, 12 hours to 36 hours, or about 24 hours) after the concentration of the antibody, or the antigen-binding fragment, has substantially cleared from the blood of the patient.

In some embodiments, the hematopoietic stem cells or progeny thereof maintain hematopoietic stem cell functional potential after two or more days (for example, from about 2 to about 5 days, from about 2 to about 7 days, from about 2 to about 20 days, from about 2 to about 30 days, such as 2 days, 3 days, 4 days, 5 days, 6 days, 7 days, 8 days, 9 days, 10 days, 11 days, 12 days, 13 days, 14 days, 15 days, 16 days, 17 days, 18 days, 19 days, 20 days, 21 days, 22 days, 23 days, 24 days, 25 days, 26 days, 27 days, 28 days, 29 days, 30 days, or more) following transplantation of the hematopoietic stem cells into the patient.

In some embodiments, the hematopoietic stem cells or progeny thereof are capable of localizing to hematopoietic tissue, such as the bone marrow, and/or reestablishing hematopoiesis following transplantation of the hematopoietic stem cells into the patient.

In some embodiments, upon transplantation into the patient, the hematopoietic stem cells give rise to recovery of a population of cells selected from the group consisting of megakaryocytes, thrombocytes, platelets, erythrocytes, mast cells, myeoblasts, basophils, neutrophils, eosinophils, microglia, granulocytes, monocytes, osteoclasts, antigen-presenting cells, macrophages, dendritic cells, natural killer cells, T lymphocytes, and B lymphocytes.

In some embodiments, the patient is suffering from cancer. The cancer can be a blood cancer or a type of leukemia, such as acute myeloid leukemia, acute lymphoid leukemia, chronic myeloid leukemia, or chronic lymphoid leukemia.

In some embodiments, the CD2+ cells comprise cancer cells.

In some embodiments, the antibody or antigen-binding fragment thereof depletes cancer cells in a patient. For example, the antibody or antigen-binding fragment thereof may deplete 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, or substantially all of the cancer cells in a patient.

In some embodiments, the antibody or antigen-binding fragment thereof depletes blood cancer cells (e.g., leukemic cells) in a patient. In some embodiments, the blood cancer cells are acute myeloid leukemic cells, acute lymphoid leukemic cells, chronic myeloid leukemic cells, or chronic lymphoid leukemic cells. In some embodiments, the blood cancer cells are megakaryocytes, thrombocytes, platelets, erythrocytes, mast cells, myeoblasts, basophils, neutrophils, eosinophils, microglia, granulocytes, monocytes, osteoclasts, antigen-presenting cells, macrophages, dendritic cells, natural killer cells, T lymphocytes, or B lymphocytes.

In some embodiments, the population of CD2+ cells comprises immune cells, such as CD2+ T cells and/or CD2+ NK cells.

In some embodiments of any of the above aspects, the method is used to treat one or more disorders, such as by depleting a population of immune cells in a patient, for instance, prior to hematopoietic stem cell transplant therapy so as to prevent or reduce the likelihood of rejection of the hematopoietic stem cell transplant that could otherwise be caused by a population of immune cells that cross-reacts with the hematopoietic stem cell graft, (e.g., by cross-reacting with non-self MHC antigens expressed by the hematopoietic stem cell graft). Following

transplantation, the hematopoietic stem cells may establish productive hematopoiesis, so as to replenish a deficient cell type in the patient or a cell type that is being actively killed or has been killed, for instance, by chemotherapeutic methods. For instance, the patient may be one that is suffering from a stem cell disorder. In some embodiments, the patient is suffering from a hemoglobinopathy disorder, such as sickle cell anemia, thalassemia, Fanconi anemia, aplastic anemia, and Wiskott-Aldrich syndrome. The patient may be suffering from an immunodeficiency disorder, such as a congenital immunodeficiency disorder or an acquired immunodeficiency disorder (e.g., human immunodeficiency virus or acquired immune deficiency syndrome). In some embodiments, the patient is suffering from a metabolic disorder, such as glycogen storage diseases, mucopolysaccharidoses, Gaucher's Disease, Hurlers Disease, sphingolipidoses, and metachromatic leukodystrophy. In some embodiments, the patient is suffering from a disorder selected from the group consisting of adenosine deaminase deficiency and severe combined immunodeficiency, hyper immunoglobulin M syndrome, Chediak-Higashi disease, hereditary lymphohistiocytosis, osteopetrosis, osteogenesis imperfecta, storage diseases, thalassemia major, systemic sclerosis, systemic lupus erythematosus, and juvenile rheumatoid arthritis. In some embodiments, the patient is suffering from an autoimmune disease, such as scleroderma, multiple sclerosis, ulcerative colitis, Crohn's disease, ant Type 1 diabetes. In some embodiments, the patient is suffering from cancer or myeloproliferative disease, such as a hematological cancer. In some embodiments, the patient is suffering from acute myeloid leukemia, acute lymphoid leukemia, chronic myeloid leukemia, chronic lymohoid leukemia, multiple myeloma, diffuse large B-cell lymphoma, or non-Hodgkin's lymphoma. In some embodiments, the patient is suffering from a myelodysplastic disease, such as myelodysplastic syndrome.

In some embodiments of any of the above aspects, the method is used to directly treat a cancer, such as a cancer characterized by CD2+ cells (e.g., a leukemia characterized by CD2+ cells), by administration of an antibody, or the antigen-binding fragment thereof, that depletes a population of CD2+ cancer cells in the patient and/or by administration of an antibody, or the antigen-binding fragment thereof, prior to hematopoietic stem cell transplant therapy so as to prevent or reduce the likelihood of rejection of the hematopoietic stem cell transplant that could otherwise be caused by a population of immune cells that cross-reacts with the hematopoietic stem cell graft (e.g., that cross-reacts with non-self MHC antigens expressed by the

hematopoietic stem cell graft). In the latter case, the transplantation may in turn re-constitute, for example, a population of cells depleted during the process of eradicating cancer cells. The cancer may be a hematological cancer, such as acute myeloid leukemia, acute lymphoid leukemia, chronic myeloid leukemia, chronic lymohoid leukemia, multiple myeloma, diffuse large B-cell lymphoma, or non-Hodgkin's lymphoma.

In some embodiments of any of the above aspects, the method is used to treat an autoimmune disease, such as by administration of an antibody, or the antigen-binding fragment thereof, so as to deplete a population of CD2+ autoimmune cells (e.g., a population of autoreactive, CD2+ T cells and/or NK cells) and/or by administration of an antibody, or an antigen-binding fragment thereof, prior to hematopoietic stem cell transplant therapy so as to prevent or reduce the likelihood of rejection of the hematopoietic stem cell transplant that could otherwise be caused by a population of immune cells that cross-reacts with the hematopoietic stem cell graft (e.g., that cross-reacts with non-self MHC antigens expressed by the hematopoietic stem cell graft). In the latter case, the transplantation may in turn re-constitute, for example, a population of cells depleted during the process of eradicating autoimmune cells. The autoimmune disease may be, for example, scleroderma, multiple sclerosis (MS), human systemic lupus (SLE), rheumatoid arthritis (RA), inflammatory bowel disease (IBD), treating psoriasis, Type 1 diabetes mellitus (Type 1 diabetes), acute disseminated encephalomyelitis (ADEM), Addison's disease, alopecia universalis, ankylosing spondylitisis, antiphospholipid antibody syndrome (APS), aplastic anemia, autoimmune hemolytic anemia, autoimmune hepatitis, autoimmune inner ear disease (AIED), autoimmune lymphoproliferative syndrome (ALPS), autoimmune oophoritis, Balo disease, Behcet's disease, bullous pemphigoid, cardiomyopathy, Chagas' disease, chronic fatigue immune dysfunction syndrome (CFIDS), chronic inflammatory demyelinating polyneuropathy, Crohn's disease, cicatrical pemphigoid, coeliac sprue-dermatitis herpetiformis, cold agglutinin disease, CREST syndrome, Degos disease, discoid lupus, dysautonomia, endometriosis, essential mixed cryoglobulinemia, fibromyalgia-fibromyositis, Goodpasture' s syndrome, Grave's disease, Guillain-Barre syndrome (GBS), Hashimoto' s thyroiditis, Hidradenitis suppurativa, idiopathic and/or acute thrombocytopenic purpura, idiopathic pulmonary fibrosis, IgA neuropathy, interstitial cystitis, juvenile arthritis, Kawasaki's disease, lichen planus, Lyme disease, Meniere disease, mixed connective tissue disease (MCTD), myasthenia gravis, neuromyotonia, opsoclonus myoclonus syndrome (OMS), optic neuritis, Ord's thyroiditis,

pemphigus vulgaris, pernicious anemia, polychondritis, polymyositis and dermatomyositis, primary biliary cirrhosis, polyarteritis nodosa, polyglandular syndromes, polymyalgia rheumatica, primary agammaglobulinemia, Raynaud phenomenon, Reiter' s syndrome, rheumatic fever, sarcoidosis, scleroderma, Sjögren's syndrome, stiff person syndrome, Takayasu's arteritis, temporal arteritis (also known as "giant cell arteritis"), ulcerative colitis, uveitis, vasculitis, vitiligo, vulvodynia ("vulvar vestibulitis"), and Wegener's granulomatosis.

Thus, in some embodiments of any of the above aspects, the invention features a method of treating a hemoglobinopathy disorder, such as sickle cell anemia, thalassemia, Fanconi anemia, aplastic anemia, and Wiskott-Aldrich syndrome. In some embodiments, the invention features a method of treating an immunodeficiency disorder, such as a congenital immunodeficiency disorder or an acquired immunodeficiency disorder (e.g., human immunodeficiency virus or acquired immune deficiency syndrome). In some embodiments, the invention features a method of treating a metabolic disorder, such as glycogen storage diseases, mucopolysaccharidoses, Gaucher's Disease, Hurlers Disease, sphingolipidoses, and metachromatic leukodystrophy. In some embodiments, the invention features a method of treating a disorder selected from the group consisting of adenosine deaminase deficiency and severe combined immunodeficiency, hyper immunoglobulin M syndrome, Chediak-Higashi disease, hereditary lymphohistiocytosis, osteopetrosis, osteogenesis imperfecta, storage diseases, thalassemia major, systemic sclerosis, systemic lupus erythematosus, and juvenile rheumatoid arthritis In some embodiments, the invention features a method of treating an autoimmune disease, such as scleroderma, multiple sclerosis, ulcerative colitis, Crohn's disease, ant Type 1 diabetes. In some embodiments, the invention features a method of treating a cancer or myeloproliferative disease, such as a hematological cancer. In some embodiments, the invention features a method of treating acute myeloid leukemia, acute lymphoid leukemia, chronic myeloid leukemia, chronic lymohoid leukemia, multiple myeloma, diffuse large B-cell lymphoma, or non-Hodgkin's lymphoma. In some embodiments, the patient is suffering from a myelodyplastic disease, such as myelodysplastic syndrome. In these embodiments, the method may include administering to the patient an antibody, or an antigen-binding fragment thereof, or conjugate thereof that binds CD2, such as the antibody, the antigen-binding fragment thereof, or conjugate thereof of any of the aspects or embodiments of the invention. The method may additionally include administering to the patient a hematopoietic stem cell transplant, for instance,

according to the method of any of the aspects or embodiments of the invention.

Similarly, in some embodiments of any of the above aspects, the invention provides a method of treating cancer directly, such as a cancer characterized by CD2+ cells (e.g., a leukemia characterized by CD2+ cells). In these embodiments, the method may include administering to the patient an antibody, an antigen-binding fragment thereof, or conjugate thereof that binds CD2, such as those described herein. The cancer may be a hematological cancer, such as acute myeloid leukemia, acute lymphoid leukemia, chronic myeloid leukemia, chronic lymohoid leukemia, multiple myeloma, diffuse large B-cell lymphoma, or non-Hodgkin's lymphoma.

Additionally, in some embodiments of any of the above aspects, the invention provides a method of treating an autoimmune disease, such as MS, SLE, RA, IBD, psoriasis, Type 1 diabetes, ADEM, Addison's disease, alopecia universalis, ankylosing spondylitisis, APS, aplastic anemia, autoimmune hemolytic anemia, autoimmune hepatitis, AIED, ALPS, autoimmune oophoritis, Balo disease, Behcet's disease, bullous pemphigoid, cardiomyopathy, Chagas' disease, CFIDS, chronic inflammatory demyelinating polyneuropathy, Crohn's disease, cicatrical pemphigoid, coeliac sprue-dermatitis herpetiformis, cold agglutinin disease, CREST syndrome, Degos disease, discoid lupus, dysautonomia, endometriosis, essential mixed cryoglobulinemia, fibromyalgia-fibromyositis, Goodpasture' s syndrome, Grave's disease, GBS, Hashimoto' s thyroiditis, Hidradenitis suppurativa, idiopathic and/or acute thrombocytopenic purpura, idiopathic pulmonary fibrosis, IgA neuropathy, interstitial cystitis, juvenile arthritis, Kawasaki's disease, lichen planus, Lyme disease, Meniere disease, MCTD, myasthenia gravis, neuromyotonia, OMS, optic neuritis, Ord's thyroiditis, pemphigus vulgaris, pernicious anemia, polychondritis, polymyositis and dermatomyositis, primary biliary cirrhosis, polyarteritis nodosa, polyglandular syndromes, polymyalgia rheumatica, primary agammaglobulinemia, Raynaud phenomenon, Reiter' s syndrome, rheumatic fever, sarcoidosis, scleroderma, Sjögren's syndrome, stiff person syndrome, Takayasu's arteritis, temporal arteritis (also known as "giant cell arteritis"), ulcerative colitis, uveitis, vasculitis, vitiligo, vulvodynia ("vulvar vestibulitis"), and Wegener' s granulomatosis. In these embodiments, the method may include administering to the patient an antibody, an antigen-binding fragment thereof, or conjugate thereof that binds CD2, such as those described herein.

In another aspect, the invention features an antibody, or an antigen-binding fragment thereof, that binds CD2, wherein the antibody or antigen-binding fragment thereof is conjugated to a toxin.

In some embodiments, the antibody or antigen-binding fragment thereof is produced by the hybridoma cell line ATCC HB 11423. In some embodiments, the antibody or antigen-binding fragment thereof competitively inhibits the binding of CD2 to an antibody or antigen-binding fragment thereof produced by the hybridoma cell line ATCC HB 11423.

In some embodiments, the antibody or antigen-binding fragment thereof comprises the following CDRs:

a CDR-H1 having the amino acid sequence EYYMY (SEQ ID NO: 1);

a CDR-H2 having the amino acid sequence RIDPEDGSIDYVEKFKK (SEQ ID NO: 2);

a CDR-H3 having the amino acid sequence GKFNYRFAY (SEQ ID NO: 3);

a CDR-L1 having the amino acid sequence RSSQSLLHSSGNTYLN (SEQ ID NO: 4);

a CDR-L2 having the amino acid sequence LVSKLES (SEQ ID NO: 5); and

a CDR-L3 having the amino acid sequence MQFTHYPYT (SEQ ID NO: 6).

In some embodiments, the antibody or antigen-binding fragment thereof competitively inhibits the binding of CD2 to an antibody or antigen-binding fragment thereof that comprises the following CDRs:

a CDR-H1 having the amino acid sequence EYYMY (SEQ ID NO: 1);

a CDR-H2 having the amino acid sequence RIDPEDGSIDYVEKFKK (SEQ ID NO: 2);

a CDR-H3 having the amino acid sequence GKFNYRFAY (SEQ ID NO: 3);

a CDR-L1 having the amino acid sequence RSSQSLLHSSGNTYLN (SEQ ID NO: 4);

a CDR-L2 having the amino acid sequence LVSKLES (SEQ ID NO: 5); and

a CDR-L3 having the amino acid sequence MQFTHYPYT (SEQ ID NO: 6).

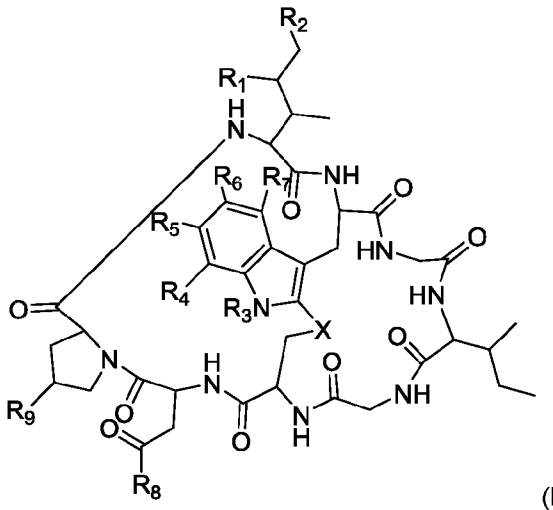
In some embodiments, the anti-CD2 antibody, or antigen-binding fragment thereof, is selected from the group consisting of a monoclonal antibody, a polyclonal antibody,, a humanized antibody or antigen-binding fragment thereof, a bispecific antibody or antigen-binding fragment

thereof, a dual-variable immunoglobulin domain, an scFv, a diabody, a triabody, a nanobody, an antibody-like protein scaffold, a Fv fragment, a Fab fragment, a $F(ab')_2$ molecule, and a tandem di-scFv.

In some embodiments, the anti-CD2 antibody has an isotype selected from the group consisting of IgG, IgA, IgM, IgD, and IgE.

In some embodiments, the antibody, or the antigen-binding fragment thereof, conjugated to the cytotoxin is represented by the formula Ab-Cy, wherein Ab is the anti-CD2 antibody, or antigen-binding fragment thereof, and Cy is the cytotoxin. In some embodiments, the cytotoxin is selected from the group consisting of an amatoxin, pseudomonas exotoxin A, deBouganin, diphtheria toxin, saporin, maytansine, a maytansinoid, an auristatin, an anthracycline, a calicheamicin, irinotecan, SN-38, a duocarmycin, a pyrrolobenzodiazepine, a pyrrolobenzodiazepine dimer, an indolinobenzodiazepine, and an indolinobenzodiazepine dimer, or a variant thereof.

In some embodiments, the cytotoxin is an amatoxin or derivative thereof, such as α-amanitin, β-amanitin, γ-amanitin, ε-amanitin, amanin, amaninamide, amanullin, amanullinic acid, and proamanullin. In some embodiments, the cytotoxin is an amatoxin, and the antibody, or the antigen-binding fragment thereof, conjugated to the cytotoxin is represented by the formula Ab-Am, wherein Ab is the antibody, or the antigen-binding fragment thereof, and Am is the amatoxin. In some embodiments, Am is represented by formula (I)



(I)

wherein R_1 is H, OH, OR_A, or OR_C;

R_2 is H, OH, OR_B, or OR_C;

R_A and R_B, together with the oxygen atoms to which they are bound, combine to form an optionally substituted 5-membered heterocyclolalkyl group;

R_3 is H, R_C, or R_D;

R_4 is H, OH, OR_C, OR_D, R_C, or R_D;

R_5 is H, OH, OR_C, OR_D, R_C, or R_D;

R_6 is H, OH, OR_C, OR_D, R_C, or R_D;

R_7 is H, OH, OR_C, OR_D, R_C, or R_D;

R_8 is OH, NH_2, OR_C, OR_D, NHR_C, or NR_CR_D;

R_9 is H, OH, OR_C, or OR_D;

X is -S-, -S(O)-, or $-SO_2-$;

R_C is -L-Z;

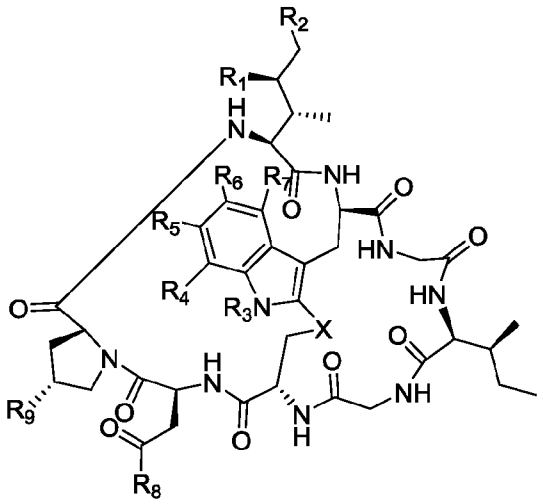
R_D is optionally substituted alkyl (e.g., C_1-C_6 alkyl), optionally substituted heteroalkyl (e.g., C_1-C_6 heteroalkyl), optionally substituted alkenyl (e.g., C_2-C_6 alkenyl), optionally substituted heteroalkenyl (e.g., C_2-C_6 heteroalkenyl), optionally substituted alkynyl (e.g., C_2-C_6 alkynyl), optionally substituted heteroalkynyl (e.g., C_2-C_6 heteroalkynyl), optionally substituted cycloalkyl, optionally substituted heterocycloalkyl, optionally substituted aryl, or optionally substituted heteroaryl;

L is a linker, such as optionally substituted alkylene (e.g., C_1-C_6 alkylene), optionally substituted heteroalkylene (C_1-C_6 heteroalkylene), optionally substituted alkenylene (e.g., C_2-C_6 alkenylene), optionally substituted heteroalkenylene (e.g., C_2-C_6 heteroalkenylene), optionally substituted alkynylene (e.g., C_2-C_6 alkynylene), optionally substituted heteroalkynylene (e.g., C_2-C_6 heteroalkynylene), optionally substituted cycloalkylene, optionally substituted heterocycloalkylene, optionally substituted arylene, or optionally substituted heteroarylene; and

Z is a chemical moiety formed from a coupling reaction between a reactive substituent present on L and a reactive substituent present within an antibody, or an antigen-binding fragment thereof, that binds CD2, such as on the surface of a CD2+ T cell or CD2+ NK cell.

In some embodiments, Am contains exactly one R_C substituent.

In some embodiments, Am is represented by formula (**IA**)



(IA)

wherein R_1 is H, OH, OR_A, or OR_C;

R_2 is H, OH, OR_B, or OR_C;

R_A and R_B, together with the oxygen atoms to which they are bound, combine to form an optionally substituted 5-membered heterocyclolalkyl group;

R_3 is H, R_C, or R_D;

R_4 is H, OH, OR_C, OR_D, R_C, or R_D;

R_5 is H, OH, OR_C, OR_D, R_C, or R_D;

R_6 is H, OH, OR_C, OR_D, R_C, or R_D;

R_7 is H, OH, OR_C, OR_D, R_C, or R_D;

R_8 is OH, NH_2, OR_C, OR_D, NHR_C, or NR_CR_D;

R_9 is H, OH, OR_C, or OR_D;

X is -S-, -S(O)-, or $-SO_2-$;

R_C is -L-Z;

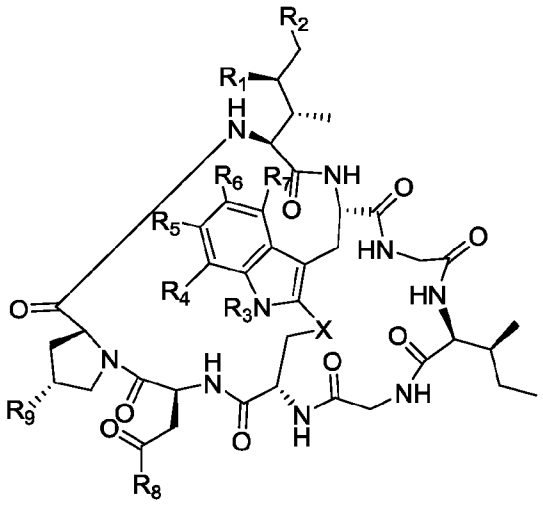
R_D is optionally substituted alkyl (e.g., C_1-C_6 alkyl), optionally substituted heteroalkyl (e.g., C_1-C_6 heteroalkyl), optionally substituted alkenyl (e.g., C_2-C_6 alkenyl), optionally substituted heteroalkenyl (e.g., C_2-C_6 heteroalkenyl), optionally substituted alkynyl (e.g., C_2-C_6 alkynyl), optionally substituted heteroalkynyl (e.g., C_2-C_6 heteroalkynyl), optionally substituted cycloalkyl, optionally substituted heterocycloalkyl, optionally substituted aryl, or optionally substituted heteroaryl;

L is a linker, such as optionally substituted alkylene (e.g., C_1-C_6 alkylene), optionally substituted heteroalkylene (C_1-C_6 heteroalkylene), optionally substituted alkenylene (e.g., C_2-C_6 alkenylene), optionally substituted heteroalkenylene (e.g., C_2-C_6 heteroalkenylene), optionally substituted alkynylene (e.g., C_2-C_6 alkynylene), optionally substituted heteroalkynylene (e.g., C_2-C_6 heteroalkynylene), optionally substituted cycloalkylene, optionally substituted heterocycloalkylene, optionally substituted arylene, or optionally substituted heteroarylene;

Z is a chemical moiety formed from a coupling reaction between a reactive substituent present on L and a reactive substituent present within an antibody, an antigen-binding fragment thereof, that binds CD2, such as on the surface of a CD2+ T cell or CD2+ NK cell; and

wherein Am contains exactly one R_C substituent.

In some embodiments, Am is represented by formula (**IB**)



(**IB**)

wherein R_1 is H, OH, OR_A, or OR_C;

R_2 is H, OH, OR_B, or OR_C;

R_A and R_B, together with the oxygen atoms to which they are bound, combine to form an optionally substituted 5-membered heterocyclolalkyl group;

R_3 is H, R_C, or R_D;

R_4 is H, OH, OR_C, OR_D, R_C, or R_D;

R_5 is H, OH, OR_C, OR_D, R_C, or R_D;

R_6 is H, OH, OR_C, OR_D, R_C, or R_D;

R_7 is H, OH, OR_C, OR_D, R_C, or R_D;

R_8 is OH, NH_2, OR_C, OR_D, NHR_C, or NR_CR_D;

R_9 is H, OH, OR_C, or OR_D;

X is -S-, -S(O)-, or $-SO_2-$;

R_C is -L-Z;

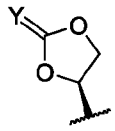
R_D is optionally substituted alkyl (e.g., C_1-C_6 alkyl), optionally substituted heteroalkyl (e.g., C_1-C_6 heteroalkyl), optionally substituted alkenyl (e.g., C_2-C_6 alkenyl), optionally substituted heteroalkenyl (e.g., C_2-C_6 heteroalkenyl), optionally substituted alkynyl (e.g., C_2-C_6 alkynyl), optionally substituted heteroalkynyl (e.g., C_2-C_6 heteroalkynyl), optionally substituted cycloalkyl, optionally substituted heterocycloalkyl, optionally substituted aryl, or optionally substituted heteroaryl;

L is a linker, such as optionally substituted alkylene (e.g., C_1-C_6 alkylene), optionally substituted heteroalkylene (C_1-C_6 heteroalkylene), optionally substituted alkenylene (e.g., C_2-C_6 alkenylene), optionally substituted heteroalkenylene (e.g., C_2-C_6 heteroalkenylene), optionally substituted alkynylene (e.g., C_2-C_6 alkynylene), optionally substituted heteroalkynylene (e.g., C_2-C_6 heteroalkynylene), optionally substituted cycloalkylene, optionally substituted heterocycloalkylene, optionally substituted arylene, or optionally substituted heteroarylene;

Z is a chemical moiety formed from a coupling reaction between a reactive substituent present on L and a reactive substituent present within an antibody, or an antigen-binding fragment thereof, that binds CD2, such as on the surface of a CD2+ T cell or CD2+ NK cell; and

wherein Am contains exactly one R_C substituent.

In some embodiments, R_A and R_B, together with the oxygen atoms to which they are bound, combine to form:



wherein Y is selected from O, S, NR_E, and $CR_ER_{E'}$, and

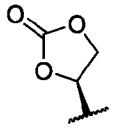
R_E and $R_{E'}$ are each independently optionally substituted C_1-C_6 alkylene-R_C, optionally substituted C_1-C_6 heteroalkylene-R_C, optionally substituted C_2-C_6 alkenylene-R_C, optionally substituted C_2-C_6 heteroalkenylene-R_C, optionally substituted C_2-C_6 alkynylene-R_C, optionally substituted C_2-C_6 heteroalkynylene-R_C, optionally substituted cycloalkylene-R_C, optionally

substituted heterocycloalkylene-R_C, optionally substituted arylene-R_C, or optionally substituted heteroarylene-R_C.

In some embodiments, Am is represented by formula (**IA**) or formula (**IB**), wherein R_1 is H, OH, OR_A, or OR_C;

R_2 is H, OH, OR_B, or OR_C;

R_A and R_B, together with the oxygen atoms to which they are bound, combine to form:



R_3 is H or R_C;

R_4 is H, OH, OR_C, OR_D, R_C, or R_D;

R_5 is H, OH, OR_C, OR_D, R_C, or R_D;

R_6 is H, OH, OR_C, OR_D, R_C, or R_D;

R_7 is H, OH, OR_C, OR_D, R_C, or R_D;

R_8 is OH, NH_2, OR_C, or NHR_C;

R_9 is H or OH; and

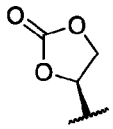
wherein R_C and R_D are each as defined above.

In some embodiments, Am is represented by formula (**IA**) or formula (**IB**),

wherein R_1 is H, OH, OR_A, or OR_C;

R_2 is H, OH, OR_B, or OR_C;

R_A and R_B, together with the oxygen atoms to which they are bound, combine to form:



R_3 is H or R_C;

R_4 and R_5 are each independently H, OH, OR_C, R_C, or OR_D;

R_6 and R_7 are each H;

R_8 is OH, NH_2, OR_C, or NHR_C;

R_9 is H or OH; and

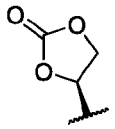
wherein R_C is as defined above.

In some embodiments, Am is represented by formula (**IA**) or formula (**IB**),

wherein R_1 is H, OH, or OR_A;

R_2 is H, OH, or OR_B;

R_A and R_B, together with the oxygen atoms to which they are bound, combine to form:



R_3, R_4, R_6, and R_7 are each H;

R_5 is OR_C;

R_8 is OH or NH_2;

R_9 is H or OH; and

wherein R_C is as defined above.

In some embodiments, Am is represented by formula (**IA**) or formula (**IB**),

wherein R_1 and R_2 are each independently H or OH;

R_3 is R_C;

R_4, R_6, and R_7 are each H;

R_5 is H, OH, or OC_1-C_6 alkyl;

R_8 is OH or NH_2;

R_9 is H or OH; and

wherein R_C is as defined above.

In some embodiments, Am is represented by formula (**IA**) or formula (**IB**),

wherein R_1 and R_2 are each independently H or OH;

R_3, R_6, and R_7 are each H;

R_4 and R_5 are each independently H, OH, OR_C, or R_C;

R_8 is OH or NH_2;

R_9 is H or OH; and

wherein R_C is as defined above.

In some embodiments, Am is represented by formula (**IA**) or formula (**IB**),

wherein R_1 and R_2 are each independently H or OH;

R_3, R_6, and R_7 are each H;

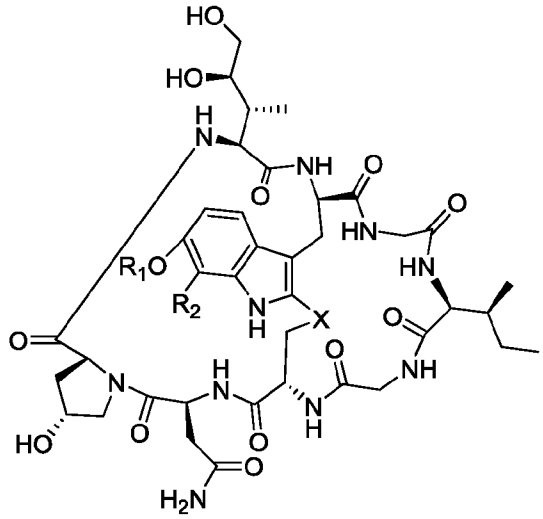
R_4 and R_5 are each independently H or OH;

R_8 is OH, NH_2, OR_C, or NHR_C;

R_9 is H or OH; and

wherein R_C is as defined above.

In some embodiments, Am is represented by formula (**II**)

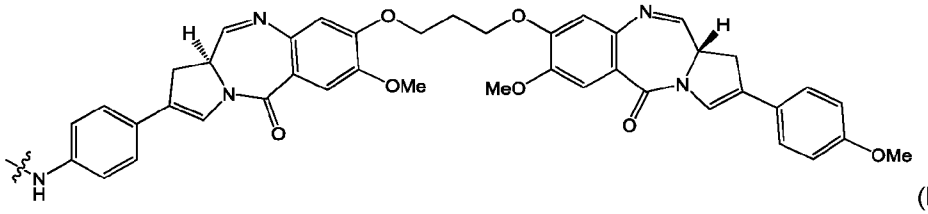


(**II**)

wherein X is S, SO, or SO_2; R_1 is H or a linker covalently bound to the antibody, or the antigen-binding fragment thereof,; and R_2 is H or a linker covalently bound to the antibody, or the antigen-binding fragment thereof; wherein when R_1 is H, R_2 is the linker, and when R_2 is H, R_1 is the linker.

In some embodiments, the cytotoxin is a maytansinoid selected from the group consisting of DM1 and DM4. In some embodiments, the cytotoxin is an auristatin selected from the group consisting of monomethyl auristatin E and monomethyl auristatin F. In some embodiments, the cytotoxin is an anthracycline selected from the group consisting of daunorubicin, doxorubicin, epirubicin, and idarubicin.

In some embodiments, the cytotoxin is a pyrrolobenzodiazepine dimer represented by formula (**IV**):



(**IV**)

In some embodiments, the cytotoxin is conjugated to the antibody, or the antigen-binding fragment thereof, by way of a maleimidocaproyl linker.

In some embodiments, the cytotoxin is an auristatin selected from the group consisting of monomethyl auristatin E and monomethyl auristatin F.

In some embodiments, the cytotoxin is an anthracycline selected from the group consisting of daunorubicin, doxorubicin, epirubicin, and idarubicin.

In another aspect, the invention features a pharmaceutical composition comprising the antibody, or the antigen-binding fragment thereof, of any of the above aspects or embodiments of the invention and a pharmaceutically acceptable excipient.

In some embodiments, the pharmaceutical composition is formulated for administration to a human patient transdermally, subcutaneously, intranasally, intravenously, intramuscularly, intraocularly, intratumorally, parenterally, topically, intrathecally or intracerebroventricularly.

Detailed Description

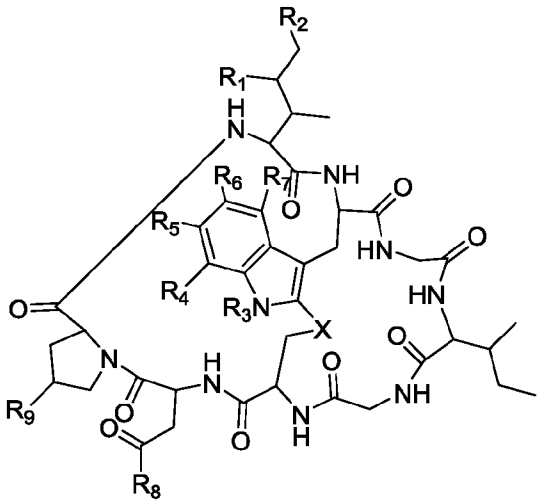
The present invention is based in part on the discovery that antibodies, or antigen-binding fragments thereof, that bind CD2 (also referred to as T cell surface antigen, LFA-2, and LFA-3 receptor) can be used as therapeutic agents to (i) directly treat cancers and autoimmune diseases characterized by CD2+ cells and (ii) promote the engraftment of transplanted hematopoietic stem cells in a patient in need of transplant therapy by depleting populations of immune cells that cross-react with, and mount an immune response against, hematopoietic stem cell grafts (e.g., by cross-reacting with non-self MHC antigens expressed by the hematopoietic stem cell graft). These therapeutic activities can arise, for instance, by the binding of anti-CD2 antibodies, or antigen-binding fragments thereof, to CD2 expressed on the surface of a cell, such as a cancer cell, autoimmune cell, or immune cell that cross-reacts with a non-self hematopoietic stem cell antigen (e.g., a non-self MHC antigen), thereby inducing death of the bound cell. In the case of depleting a population of cancer cells or autoimmune cells, the anti-CD2 antibody, or the antigen-binding fragment thereof, can be used to directly treat a cancer or autoimmune disease, such as a cancer autoimmune disease described herein. In the case of depleting a population of immune cells that cross-react with a non-self hematopoietic stem cell antigen, the anti-CD2 antibody, antigen-binding fragment thereof, can be used to prevent or reduce the likelihood of graft rejection in a patient that is suffering from a stem cell disorder, cancer, or autoimmune

disease and that is undergoing hematopoietic stem cell transplant therapy. In such instances, the depletion of CD2+ immune cells that cross-react with one or more non-self hematopoietic stem cell antigens (e.g., one or more non-self MHC antigens) enables the successful engraftment of transplanted hematopoietic stem cells within the transplant recipient. As the transplanted cells engraft, they can home to hematopoietic tissue, where productive hematopoiesis can then ensue. The transplanted hematopoietic stem cells can subsequently give rise to a population of cells that is deficient or defective in the transplant recipient, such as megakaryocytes, thrombocytes, platelets, erythrocytes, mast cells, myeoblasts, basophils, neutrophils, eosinophils, microglia, granulocytes, monocytes, osteoclasts, antigen-presenting cells, macrophages, dendritic cells, natural killer cells, T lymphocytes, and B lymphocytes. In this way, anti-CD2 antibodies, or the fragments thereof, can be used to promote the successful engraftment of hematopoietic stem cells in a patient, such as human patient suffering from a stem cell disorder described herein.

Definitions

As used herein, the term "about" refers to a value that is within 10% above or below the value being described. For example, the term "about 5 nM" indicates a range of from 4.5 nM to 5.5 nM.

As used herein, the term "amatoxin" refers to a member of the amatoxin family of peptides produced by Amanita phalloides mushrooms, a synthetic amatoxin, a variant amatoxin, or a derivative thereof, such as a variant or derivative thereof capable of inhibiting RNA polymerase II activity. Also included are synthetic amatoxins (see, *e.g.,* US Patent No. 9676702, incorporated by reference herein). Amatoxins useful in conjunction with the compositions and methods described herein include compounds according to formula (**III**), below, such as α-amanitin, β-amanitin, γ-amanitin, ε-amanitin, amanin, amaninamide, amanullin, amanullinic acid, and proamanullin. Formula (**III**) is as follows:



(III)

wherein R_1 is H, OH, or OR_A;

R_2 is H, OH, or OR_B;

R_A and R_B, together with the oxygen atoms to which they are bound, combine to form an optionally substituted 5-membered heterocyclolalkyl group;

R_3 is H or R_D;

R_4 is H, OH, OR_D, or R_D;

R_5 is H, OH, OR_D, or R_D;

R_6 is H, OH, OR_D, or R_D;

R_7 is H, OH, OR_D, or R_D;

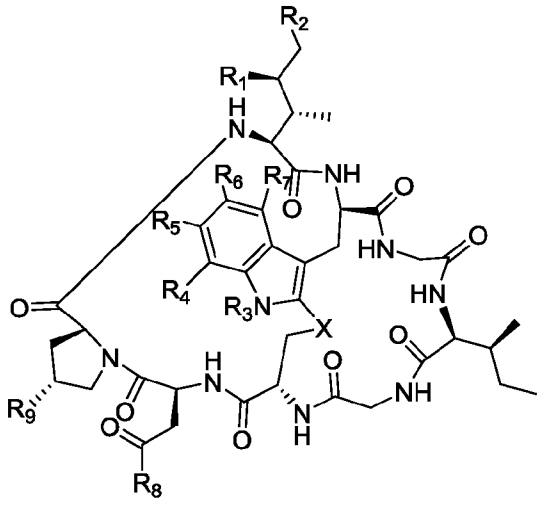
R_8 is OH, NH_2, or OR_D;

R_9 is H, OH, or OR_D;

X is -S-, -S(O)-, or $-SO_2-$; and

R_D is optionally substituted alkyl (e.g., C_1-C_6 alkyl), optionally substituted heteroalkyl (e.g., C_1-C_6 heteroalkyl), optionally substituted alkenyl (e.g., C_2-C_6 alkenyl), optionally substituted heteroalkenyl (e.g., C_2-C_6 heteroalkenyl), optionally substituted alkynyl (e.g., C_2-C_6 alkynyl), optionally substituted heteroalkynyl (e.g., C_2-C_6 heteroalkynyl), optionally substituted cycloalkyl, optionally substituted heterocycloalkyl, optionally substituted aryl, or optionally substituted heteroaryl.

For instance, amatoxins useful in conjunction with the compositions and methods described herein include compounds according to formula (**IIIA**), below:



(**IIIA**)

wherein R_1 is H, OH, or OR_A;

R_2 is H, OH, or OR_B;

R_A and R_B, together with the oxygen atoms to which they are bound, combine to form an optionally substituted 5-membered heterocyclolalkyl group;

R_3 is H or R_D;

R_4 is H, OH, OR_D, or R_D;

R_5 is H, OH, OR_D, or R_D;

R_6 is H, OH, OR_D, or R_D;

R_7 is H, OH, OR_D, or R_D;

R_8 is OH, NH_2, or OR_D;

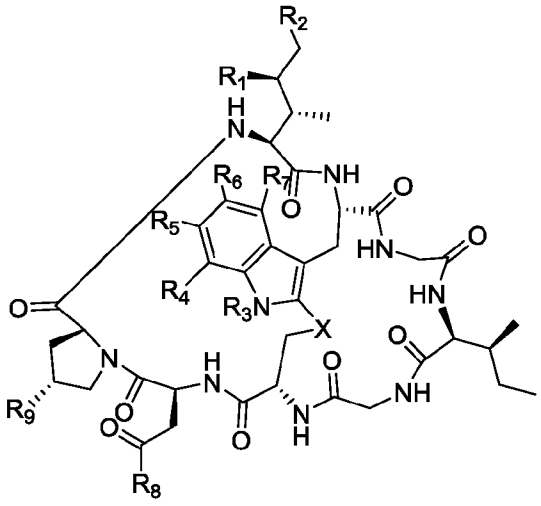
R_9 is H, OH, or OR_D;

X is -S-, -S(O)-, or $-SO_2-$; and

R_D is optionally substituted alkyl (e.g., C_1-C_6 alkyl), optionally substituted heteroalkyl (e.g., C_1-C_6 heteroalkyl), optionally substituted alkenyl (e.g., C_2-C_6 alkenyl), optionally substituted heteroalkenyl (e.g., C_2-C_6 heteroalkenyl), optionally substituted alkynyl (e.g., C_2-C_6 alkynyl), optionally substituted heteroalkynyl (e.g., C_2-C_6 heteroalkynyl), optionally substituted cycloalkyl, optionally substituted heterocycloalkyl, optionally substituted aryl, or optionally substituted

heteroaryl.

Amatoxins useful in conjunction with the compositions and methods described herein also include compounds according to formula (**IIIB**), below:



(**IIIB**)

wherein R_1 is H, OH, or OR_A;

R_2 is H, OH, or OR_B;

R_A and R_B, together with the oxygen atoms to which they are bound, combine to form an optionally substituted 5-membered heterocyclolalkyl group;

R_3 is H or R_D;

R_4 is H, OH, OR_D, or R_D;

R_5 is H, OH, OR_D, or R_D;

R_6 is H, OH, OR_D, or R_D;

R_7 is H, OH, OR_D, or R_D;

R_8 is OH, NH_2, or OR_D;

R_9 is H, OH, or OR_D;

X is -S-, -S(O)-, or $-SO_2-$; and

R_D is optionally substituted alkyl (e.g., C_1-C_6 alkyl), optionally substituted heteroalkyl (e.g., C_1-C_6 heteroalkyl), optionally substituted alkenyl (e.g., C_2-C_6 alkenyl), optionally substituted heteroalkenyl (e.g., C_2-C_6 heteroalkenyl), optionally substituted alkynyl (e.g., C_2-C_6 alkynyl), optionally substituted heteroalkynyl (e.g., C_2-C_6 heteroalkynyl), optionally substituted cycloalkyl,

optionally substituted heterocycloalkyl, optionally substituted aryl, or optionally substituted heteroaryl.

As described herein, amatoxins may be conjugated to an antibody, or an antigen-binding fragment thereof, for instance, by way of a linker moiety. Exemplary methods of amatoxin conjugation and linkers useful for such processes are described in the section entitled "Linkers for chemical conjugation," as well as in Table 1, below. Exemplary linker-containing amatoxins useful for conjugation to an antibody, an antigen-binding fragment, in accordance with the compositions and methods described herein are shown in structural formulas (**I**), (**IA**), (**IB**), and (**II**), recited herein.

As used herein, the term "antibody" refers to an immunoglobulin molecule that specifically binds to, or is immunologically reactive with, a particular antigen. Examples of antibodies include polyclonal, monoclonal, genetically engineered, and otherwise modified forms of antibodies, including but not limited to chimeric antibodies, humanized antibodies, heteroconjugate antibodies (e.g., bi- tri- and quad-specific antibodies, diabodies, triabodies, and tetrabodies), and antigen binding fragments of antibodies, including, for example, Fab', $F(ab')_2$, Fab, Fv, rIgG, and scFv fragments. As used herein, the Fab and $F(ab')_2$ fragments refer to antibody fragments that lack the Fc fragment of an intact antibody. Examples of these antibody fragments are described herein.

The term "antigen-binding fragment," as used herein, refers to a molecule other than an intact antibody that comprises a portion of an intact antibody and that binds the antigen to which the intact antibody binds. The antigen-binding function of an antibody can be performed by fragments of a full-length antibody. The antibody fragments can be, for example, a Fv, Fab, Fab', $F(ab')_2$, scFv, diabody, a triabody, single chain antibody molecules (e.g., scFv), an affibody, a nanobody, an aptamer, or a domain antibody. Examples of binding fragments encompassed of the term "antigen-binding fragment" of an antibody include, but are not limited to: (i) a Fab fragment, a monovalent fragment consisting of the V_L, V_H, C_L, and C_H1 domains; (ii) a $F(ab')_2$ fragment, a bivalent fragment comprising two Fab fragments linked by a disulfide bridge at the hinge region; (iii) a Fd fragment consisting of the V_H and C_H1 domains; (iv) a Fv fragment consisting of the V_L and V_H domains of a single arm of an antibody, (v) a dAb including V_H and V_L domains; (vi) a dAb fragment that consists of a V_H domain (see, e.g., Ward et al., Nature 341:544-546, 1989); (vii) a dAb which consists of a V_H or a V_L domain; (viii) an isolated

complementarity determining region (CDR); and (ix) a combination of two or more (e.g., two, three, four, five, or six) isolated CDRs which may optionally be joined by a synthetic linker. Furthermore, although the two domains of the Fv fragment, V_L and V_H, are coded for by separate genes, they can be joined, using recombinant methods, by a linker that enables them to be made as a single protein chain in which the V_L and V_H regions pair to form monovalent molecules (known as single chain Fv (scFv); see, for example, Bird et al., Science 242:423-426, 1988 and Huston et al., Proc. Natl. Acad. Sci. USA 85:5879-5883, 1988). These antibody fragments can be obtained using conventional techniques known to those of skill in the art, and the fragments can be screened for utility in the same manner as intact antibodies. Antigen-binding fragments can be produced by recombinant DNA techniques, enzymatic or chemical cleavage of intact immunoglobulins, or, in certain cases, by chemical peptide synthesis procedures known in the art.

As used herein, the term "anti-CD2 antibody" refers to an antibody that specifically binds to CD2. An antibody "which binds" an antigen of interest, *i.e.,* CD2, is one capable of binding that antigen with sufficient affinity such that the antibody is useful in targeting a cell expressing the antigen. In a preferred embodiment, the antibody specifically binds to human CD2 (hCD2).

As used herein, the term "bispecific antibody" refers to, a hybrid antibody having two different antigen binding sites. Bispecific antibodies are a species of multispecific antibody and may be produced by a variety of methods including, but not limited to, fusion of hybridomas or linking of Fab' fragments. See, e.g., Songsivilai and Lachmann, 1990, Clin. Exp. Immunol. 79:315-321; Kostelny et al., 1992, J. Immunol. 148:1547-1553. The two binding sites of a bispecific antibody will bind to two different epitopes, which may reside on the same or different protein targets. For instance, one of the binding specificities can be directed towards a T cell surface antigen, such as CD2, the other can be for a different T cell surface antigen or another cell surface protein, such as a receptor or receptor subunit involved in a signal transduction pathway that potentiates cell growth, among others.

As used herein, the term "complementarity determining region" (CDR) refers to a hypervariable region found both in the light chain and the heavy chain variable domains of an antibody. The more highly conserved portions of variable domains are referred to as framework regions (FRs). The amino acid positions that delineate a hypervariable region of an antibody can vary, depending on the context and the various definitions known in the art. Some positions within a variable domain may be viewed as hybrid hypervariable positions in that these positions

can be deemed to be within a hypervariable region under one set of criteria while being deemed to be outside a hypervariable region under a different set of criteria. One or more of these positions can also be found in extended hypervariable regions. The antibodies described herein may contain modifications in these hybrid hypervariable positions. The variable domains of native heavy and light chains each comprise four framework regions that primarily adopt a β-sheet configuration, connected by three CDRs, which form loops that connect, and in some cases form part of, the β-sheet structure. The CDRs in each chain are held together in close proximity by the framework regions in the order FR1-CDR1-FR2-CDR2-FR3-CDR3-FR4 and, with the CDRs from the other antibody chains, contribute to the formation of the target binding site of antibodies (see Kabat et al., Sequences of Proteins of Immunological Interest, National Institute of Health, Bethesda, MD., 1987). As used herein, numbering of immunoglobulin amino acid residues is performed according to the immunoglobulin amino acid residue numbering system of Kabat et al., unless otherwise indicated.

As used herein, the terms "condition" and "conditioning" refer to processes by which a patient is prepared for receipt of a transplant containing hematopoietic stem cells. Such procedures promote the engraftment of a hematopoietic stem cell transplant (for instance, as inferred from a sustained increase in the quantity of viable hematopoietic stem cells within a blood sample isolated from a patient following a conditioning procedure and subsequent hematopoietic stem cell transplantation. According to the methods described herein, a patient may be conditioned for hematopoietic stem cell transplant therapy by administration to the patient of an antibody or antigen-binding fragment thereof capable of binding an antigen expressed by T cells, such as CD2. As described herein, the antibody may be covalently conjugated to a cytotoxin so as to form an antibody-drug conjugate. Administration of an antibody, antigen-binding fragment thereof, or antibody-drug conjugate capable of binding one or more of the foregoing antigens to a patient in need of hematopoietic stem cell transplant therapy can promote the engraftment of a hematopoietic stem cell graft, for example, by selectively depleting endogenous immune cells, such as CD2+ T cells (e.g., CD4+ and/or CD8+ T cells) and/or CD2+ NK cells that cross-react with one or more non-self antigens expressed by a hematopoietic stem cell (e.g., one or more non-self MHC antigens). This selective depletion of immune cells in turn prevents or reduces the likelihood of graft rejection following transplantation of an exogenous (for instance, an autologous, allogeneic, or syngeneic) hematopoietic stem cell graft.

As used herein, the term "conjugate" refers to a compound formed by the chemical bonding of a reactive functional group of one molecule, such as an antibody or antigen-binding fragment thereof, with an appropriately reactive functional group of another molecule, such as a cytotoxin described herein. Conjugates may include a linker between the two molecules bound to one another. Examples of linkers that can be used for the formation of a conjugate include peptide-containing linkers, such as those that contain naturally occurring or non-naturally occurring amino acids, such as D-amino acids. Linkers can be prepared using a variety of strategies described herein and known in the art. Depending on the reactive components therein, a linker may be cleaved, for example, by enzymatic hydrolysis, photolysis, hydrolysis under acidic conditions, hydrolysis under basic conditions, oxidation, disulfide reduction, nucleophilic cleavage, or organometallic cleavage (see, for example, Leriche et al., Bioorg. Med. Chem., 20:571-582, 2012).

As used herein, the term "coupling reaction" refers to a chemical reaction in which two or more substituents suitable for reaction with one another react so as to form a chemical moiety that joins (e.g., covalently) the molecular fragments bound to each substituent. Coupling reactions include those in which a reactive substituent bound to a fragment that is a cytotoxin, such as a cytotoxin known in the art or described herein, reacts with a suitably reactive substituent bound to a fragment that is an antibody, antigen-binding fragment thereof, or antibody, such as an antibody, antigen-binding fragment thereof, or antibody specific for CD2 known in the art or described herein. Examples of suitably reactive substituents include a nucleophile/electrophile pair (e.g., a thiol/haloalkyl pair, an amine/carbonyl pair, or a thiol/α,β-unsaturated carbonyl pair, among others), a diene/dienophile pair (e.g., an azide/alkyne pair, among others), and the like. Coupling reactions include, without limitation, thiol alkylation, hydroxyl alkylation, amine alkylation, amine condensation, amidation, esterification, disulfide formation, cycloaddition (e.g., [4+2] Diels-Alder cycloaddition, [3+2] Huisgen cycloaddition, among others), nucleophilic aromatic substitution, electrophilic aromatic substitution, and other reactive modalities known in the art or described herein.

As used herein, "CRU (competitive repopulating unit)" refers to a unit of measure of long-term engrafting stem cells, which can be detected after in-vivo transplantation.

As used herein, the term "donor" refers to a human or animal from which one or more cells are isolated prior to administration of the cells, or progeny thereof, into a recipient. The one or more cells may be, for example, a population of hematopoietic stem cells.

As used herein, the term "diabody" refers to a bivalent antibody containing two polypeptide chains, in which each polypeptide chain includes V_H and V_L domains joined by a linker that is too short (e.g., a linker composed of five amino acids) to allow for intramolecular association of V_H and V_L domains on the same peptide chain. This configuration forces each domain to pair with a complementary domain on another polypeptide chain so as to form a homodimeric structure. Accordingly, the term "triabody" refers to trivalent antibodies comprising three peptide chains, each of which contains one V_H domain and one V_L domain joined by a linker that is exceedingly short (e.g., a linker composed of 1-2 amino acids) to permit intramolecular association of V_H and V_L domains within the same peptide chain. In order to fold into their native structures, peptides configured in this way typically trimerize so as to position the V_H and V_L domains of neighboring peptide chains spatially proximal to one another (see, for example, Holliger et al., Proc. Natl. Acad. Sci. USA 90:6444-48, 1993).

As used herein, a "dual variable domain immunoglobulin" ("DVD-Ig") refers to an antigen binding protein that combines the target-binding variable domains of two antibodies by way of linkers to create a tetravalent, dual-targeting single agent (see, for example, Gu et al., Meth. Enzymol., 502:25-41, 2012).

As used herein, the term "endogenous" describes a substance, such as a molecule, cell, tissue, or organ (e.g., a hematopoietic stem cell or a cell of hematopoietic lineage, such as a megakaryocyte, thrombocyte, platelet, erythrocyte, mast cell, myeoblast, basophil, neutrophil, eosinophil, microglial cell, granulocyte, monocyte, osteoclast, antigen-presenting cell, macrophage, dendritic cell, natural killer cell, T lymphocyte (e.g., a CD4+ or CD8+ T lymphocyte), or B lymphocyte) that is found naturally in a particular organism, such as a human patient, for instance, a human patient undergoing hematopoietic stem cell transplant therapy as described herein.

As used herein, the term "engraftment potential" is used to refer to the ability of hematopoietic stem and progenitor cells to repopulate a tissue, whether such cells are naturally circulating or are provided by transplantation. The term encompasses all events surrounding or leading up to engraftment, such as tissue homing of cells and colonization of cells within the tissue of interest. The engraftment efficiency or rate of engraftment can be evaluated or quantified using any clinically acceptable parameter as known to those of skill in the art and can include, for example, assessment of competitive repopulating units (CRU); incorporation or

expression of a marker in tissue(s) into which stem cells have homed, colonized, or become engrafted; or by evaluation of the progress of a subject through disease progression, survival of hematopoietic stem and progenitor cells, or survival of a recipient. Engraftment can also be determined by measuring white blood cell counts in peripheral blood during a post-transplant period. Engraftment can also be assessed by measuring recovery of marrow cells by donor cells in a bone marrow aspirate sample.

As used herein, the term “excipient” refers to a substance formulated alongside the active ingredient of a medication. They may be included, for example, for the purpose of long-term stabilization, or to confer a therapeutic enhancement on the active ingredient in the final dosage form.

As used herein, the term “exogenous” describes a substance, such as a molecule, cell, tissue, or organ (e.g., a T cell, hematopoietic stem cell, or a cell of hematopoietic lineage, such as a megakaryocyte, thrombocyte, platelet, erythrocyte, mast cell, myeoblast, basophil, neutrophil, eosinophil, microglial cell, granulocyte, monocyte, osteoclast, antigen-presenting cell, macrophage, dendritic cell, natural killer cell, T lymphocyte, or B lymphocyte) that is not found naturally in a particular organism, such as a human patient. Exogenous substances include those that are provided from an external source to an organism or to cultured matter extracted therefrom.

As used herein, the term “framework region” or “FW region” includes amino acid residues that are adjacent to the CDRs of an antibody or antigen-binding fragment thereof. FW region residues may be present in, for example, human antibodies, humanized antibodies, monoclonal antibodies, antibody fragments, Fab fragments, single chain antibody fragments, scFv fragments, antibody domains, and bispecific antibodies, among others.

The terms "full length antibody," "intact antibody," and "whole antibody" are used herein interchangeably to refer to an antibody generally comprising at least two full-length heavy chains and two full-length light chains, but in some instances may include fewer chains such as antibodies naturally occurring in camelids which may comprise only heavy chains.

As used herein, the term “hematopoietic stem cells” (“HSCs”) refers to immature blood cells having the capacity to self-renew and to differentiate into mature blood cells comprising diverse lineages including but not limited to granulocytes (e.g., promyelocytes, neutrophils, eosinophils, basophils), erythrocytes (e.g., reticulocytes, erythrocytes), thrombocytes (e.g.,

megakaryoblasts, platelet producing megakaryocytes, platelets), monocytes (e.g., monocytes, macrophages), dendritic cells, microglia, osteoclasts, and lymphocytes (e.g., NK cells, B cells and T cells). In addition, HSCs also refer to long term repopulating HSCs (LT-HSC) and short term repopulating HSCs (ST-HSC). LT-HSCs and ST-HSCs are differentiated, based on functional potential and on cell surface marker expression. For example, human HSCs are CD34+, CD38-, CD45RA-, CD90+, CD49F+, and lin- (negative for mature lineage markers, including CD2, CD3, CD4, CD7, CD8, CD10, CD11B, CD19, CD20, CD56, and CD235A). In mice, bone marrow LT-HSCs are CD34-, SCA-1+, C-kit+, CD135-, Slamfl/CD150+, CD48-, and lin- (negative for mature lineage markers, including Ter119, CD11b, Gr1, CD3, CD4, CD8, B220, and IL7ra), whereas ST-HSCs are CD34+, SCA-1+, C-kit+, CD135-, Slamfl/CD150+, and lin- (negative for mature lineage markers, including Ter119, CD11b, Gr1, CD3, CD4, CD8, B220, and IL7ra). In addition, ST-HSCs are less quiescent and more proliferative than LT-HSCs under homeostatic conditions. However, LT-HSC have greater self-renewal potential (i.e., they survive throughout adulthood, and can be serially transplanted through successive recipients), whereas ST-HSCs have limited self-renewal (i.e., they survive for only a limited period of time, and do not possess serial transplantation potential). Any of these HSCs can be used in the methods described herein. ST-HSCs are particularly useful because they are highly proliferative and thus, can more quickly give rise to differentiated progeny.

As used herein, the term "hematopoietic stem cell functional potential" refers to the functional properties of hematopoietic stem cells which include 1) multi-potency (which refers to the ability to differentiate into multiple different blood lineages including, but not limited to, granulocytes (e.g., promyelocytes, neutrophils, eosinophils, basophils), erythrocytes (e.g., reticulocytes, erythrocytes), thrombocytes (e.g., megakaryoblasts, platelet producing megakaryocytes, platelets), monocytes (e.g., monocytes, macrophages), dendritic cells, microglia, osteoclasts, and lymphocytes (e.g., NK cells, B cells and T cells), 2) self-renewal (which refers to the ability of hematopoietic stem cells to give rise to daughter cells that have equivalent potential as the mother cell, and further that this ability can repeatedly occur throughout the lifetime of an individual without exhaustion), and 3) the ability of hematopoietic stem cells or progeny thereof to be reintroduced into a transplant recipient whereupon they home to the hematopoietic stem cell niche and re-establish productive and sustained hematopoiesis.

As used herein, the terms "Major histocompatibility complex antigens" ("MHC", also referred to as "human leukocyte antigens" ("HLA") in the context of humans) refer to proteins expressed on the cell surface that confer a unique antigenic identity to a cell. MHC/HLA antigens are target molecules that are recognized by T cells and NK cells as being derived from the same source of hematopoietic stem cells as the immune effector cells ("self") or as being derived from another source of hematopoietic reconstituting cells ("non-self"). Two main classes of HLA antigens are recognized: HLA class I and HLA class II. HLA class I antigens (A, B, and C in humans) render each cell recognizable as "self," whereas HLA class II antigens (DR, DP, and DQ in humans) are involved in reactions between lymphocytes and antigen presenting cells. Both have been implicated in the rejection of transplanted organs. An important aspect of the HLA gene system is its polymorphism. Each gene, MHC class I (A, B and C) and MHC class II (DP, DQ and DR) exists in different alleles. HLA alleles are designated by numbers and subscripts. For example, two unrelated individuals may carry class I HLA-B, genes B5, and Bw41, respectively. Allelic gene products differ in one or more amino acids in the α and/or β domain(s). Large panels of specific antibodies or nucleic acid reagents are used to type HLA haplotypes of individuals, using leukocytes that express class I and class II molecules. The genes commonly used for HLA typing are the six MHC Class I and Class II proteins, two alleles for each of HLA- A; HLA-B and HLA-DR. The HLA genes are clustered in a "super-locus" present on chromosome position 6p21, which encodes the six classical transplantation HLA genes and at least 132 protein coding genes that have important roles in the regulation of the immune system as well as some other fundamental molecular and cellular processes. The complete locus measures roughly 3.6 Mb, with at least 224 gene loci. One effect of this clustering is that "haplotypes", i.e. the set of alleles present on a single chromosome, which is inherited from one parent, tend to be inherited as a group. The set of alleles inherited from each parent forms a haplotype, in which some alleles tend to be associated together. Identifying a patient's haplotypes can help predict the probability of finding matching donors and assist in developing a search strategy, because some alleles and haplotypes are more common than others and they are distributed at different frequencies in different racial and ethnic groups.

As used herein, the term "HLA-matched" refers to a donor-recipient pair in which none of the HLA antigens are mismatched between the donor and recipient, such as a donor providing a hematopoietic stem cell graft to a recipient in need of hematopoietic stem cell transplant therapy.

HLA-matched (i.e., where all of the 6 alleles are matched) donor-recipient pairs have a decreased risk of graft rejection, as endogenous T cells and NK cells are less likely to recognize the incoming graft as foreign, and are thus less likely to mount an immune response against the transplant.

As used herein, the term "HLA-mismatched" refers to a donor-recipient pair in which at least one HLA antigen, in particular with respect to HLA-A, HLA-B and HLA-DR, is mismatched between the donor and recipient, such as a donor providing a hematopoietic stem cell graft to a recipient in need of hematopoietic stem cell transplant therapy. In some embodiments, one haplotype is matched and the other is mismatched. HLA-mismatched donor-recipient pairs may have an increased risk of graft rejection relative to HLA-matched donor-recipient pairs, as endogenous T cells and NK cells are more likely to recognize the incoming graft as foreign in the case of an HLA-mismatched donor-recipient pair, and such T cells and NK cells are thus more likely to mount an immune response against the transplant.

As used herein, the term “human antibody” refers to an antibody in which substantially every part of the protein (for example, all CDRs, framework regions, C_L, C_H domains (e.g., C_H1, C_H2, C_H3), hinge, and V_L and V_H domains) is substantially non-immunogenic in humans, with only minor sequence changes or variations. A human antibody can be produced in a human cell (for example, by recombinant expression) or by a non-human animal or a prokaryotic or eukaryotic cell that is capable of expressing functionally rearranged human immunoglobulin (such as heavy chain and/or light chain) genes. When a human antibody is a single chain antibody, it can include a linker peptide that is not found in native human antibodies. For example, an Fv can contain a linker peptide, such as two to about eight glycine or other amino acid residues, which connects the variable region of the heavy chain and the variable region of the light chain. Such linker peptides are considered to be of human origin. Human antibodies can be made by a variety of methods known in the art including phage display methods using antibody libraries derived from human immunoglobulin sequences. Human antibodies can also be produced using transgenic mice that are incapable of expressing functional endogenous immunoglobulins, but which can express human immunoglobulin genes (see, for example, PCT Publication Nos. WO1998/24893; WO1992/01047; WO1996/34096; WO1996/33735; U.S. Patent Nos. 5,413,923; 5,625,126; 5,633,425; 5,569,825; 5,661,016; 5,545,806; 5,814,318; 5,885,793; 5,916,771; and 5,939,598).

As used herein, the term "humanized" antibody refers to a chimeric antibody generally comprising amino acid seqeunces from non-human CDRs and human framework regions. In one embodiment, a humanized antibody is a human antibody (recipient antibody) in which residues from the CDRs of the recipient are replaced by residues from the CDRs of a non-human species (donor antibody) such as mouse, rat, rabbit, or nonhuman primate having the desired specificity, affinity, and/or capacity. In general, a humanized antibody contains substantially all of at least one, and typically two, variable domains, in which all or substantially all of the CDR regions correspond to those of a non-human immunoglobulin. All or substantially all of the FW regions may also be those of a human immunoglobulin sequence. The humanized antibody can also comprise at least a portion of an immunoglobulin constant region (Fc), typically that of a human immunoglobulin consensus sequence. Methods of antibody humanization are known in the art and have been described, for example, in Riechmann et al., Nature 332:323-327, 1988; U.S. Patent Nos: 5,530,101; 5,585,089; 5,693,761; 5,693,762; and 6,180,370.

As used herein, the term "immune cell" refers to a cell of the immune system that participates in the mounting and maintaining of an innate or adaptive immune response. Immune cells include lymphocytes that contain a receptor that specifically binds, and mounts an immune response against, an antigen of interest, such as a self antigen in the case of an autoimmune cell. Exemplary immune cells include mast cells, basophils, neutrophils, eosinophils, microglia, granulocytes, monocytes, antigen-presenting cells, macrophages, dendritic cells, natural killer cells, T lymphocytes, and B lymphocytes.

As used herein, patients that are "in need of" a hematopoietic stem cell transplant include patients that exhibit a defect or deficiency in one or more blood cell types, as well as patients having a stem cell disorder. Hematopoietic stem cells generally exhibit 1) multi-potency, and can thus differentiate into multiple different blood lineages including, but not limited to, granulocytes (e.g., promyelocytes, neutrophils, eosinophils, basophils), erythrocytes (e.g., reticulocytes, erythrocytes), thrombocytes (e.g., megakaryoblasts, platelet producing megakaryocytes, platelets), monocytes (e.g., monocytes, macrophages), dendritic cells, microglia, osteoclasts, and lymphocytes (e.g., NK cells, B cells and T cells), 2) self-renewal, and can thus give rise to daughter cells that have equivalent potential as the mother cell, and 3) the ability to be reintroduced into a transplant recipient whereupon they home to the hematopoietic stem cell niche and re-establish productive and sustained hematopoiesis. Hematopoietic stem cells can

thus be administered to a patient defective or deficient in one or more cell types of the hematopoietic lineage in order to re-constitute the defective or deficient population of cells in vivo. For example, the patient may be suffering from cancer, and the deficiency may be caused by administration of a chemotherapeutic agent or other medicament that depletes, either selectively or non-specifically, the cancerous cell population. Additionally or alternatively, the patient may be suffering from a non-malignant hemoglobinopathy that may cause a defect or deficiency in one or more blood cell types, such as sickle cell anemia, thalassemia, Fanconi anemia, and Wiskott-Aldrich syndrome. The subject may be one that is suffering from adenosine deaminase severe combined immunodeficiency (ADA SCID), HIV/AIDS, metachromatic leukodystrophy, Diamond-Blackfan anemia, and Schwachman-Diamond syndrome. The subject may have or be affected by an inherited blood disorder (e.g., sickle cell anemia) or an autoimmune disorder. Additionally or alternatively, the subject may have or be affected by a malignancy, such as a malignancy selected from the group consisting of hematologic cancers (e.g., leukemia, lymphoma, multiple myeloma, or myelodysplastic syndrome) and neuroblastoma. In some embodiments, the subject has or is otherwise affected by a metabolic disorder. For example, the subject may suffer or otherwise be affected by a metabolic disorder selected from the group consisting of glycogen storage diseases, mucopolysaccharidoses, Gaucher's Disease, Hurlers Disease, sphingolipidoses, metachromatic leukodystrophy, or any other diseases or disorders which may benefit from the treatments and therapies disclosed herein and including, without limitation, severe combined immunodeficiency, Wiscott-Aldrich syndrome, hyper immunoglobulin M (IgM) syndrome, Chediak-Higashi disease, hereditary lymphohistiocytosis, osteopetrosis, osteogenesis imperfecta, storage diseases, thalassemia major, sickle cell disease, systemic sclerosis, systemic lupus erythematosus, multiple sclerosis, juvenile rheumatoid arthritis and those diseases, or disorders described in "Bone Marrow Transplantation for Non-Malignant Disease," ASH Education Book, 1:319-338 (2000), the disclosure of which is incorporated herein by reference in its entirety as it pertains to pathologies that may be treated by administration of hematopoietic stem cell transplant therapy. Additionally or alternatively, a patient “in need of” a hematopoietic stem cell transplant may be one that is or is not suffering from one of the foregoing pathologies, but nonetheless exhibits a reduced level (e.g., as compared to that of an otherwise healthy subject) of one or more endogenous cell types within the hematopoietic lineage, such as megakaryocytes, thrombocytes, platelets, erythrocytes, mast cells, myeoblasts, basophils,

neutrophils, eosinophils, microglia, granulocytes, monocytes, osteoclasts, antigen-presenting cells, macrophages, dendritic cells, natural killer cells, T lymphocytes, and B lymphocytes. One of skill in the art can readily determine whether one's level of one or more of the foregoing cell types, or other blood cell type, is reduced with respect to an otherwise healthy subject, for instance, by way of flow cytometry and fluorescence activated cell sorting (FACS) methods, among other procedures, known in the art.

The term "monoclonal antibody" or "mAb" refers to an antibody obtained from a population of substantially homogeneous antibodies, *i.e.,* the individual antibodies comprising the population are identical and/or bind to the same epitope, except for possible variant antibodies, *e.g.*, naturally occurring mutations or variants arising during production of a monoclonal antibody preparation, where such variants may be present in minor amounts. In contrast to polyclonal antibody preparations that typically include different antibodies directed against different determinants (epitopes), each mAb is directed against a single determinant on the antigen. The modifier "monoclonal" is not to be construed as requiring production of the antibody by any particular method.

As used herein, the term "pharmaceutically acceptable" refers to those compounds, materials, compositions and/or dosage forms, which are suitable for contact with the tissues of a subject, such as a mammal (e.g., a human) without excessive toxicity, irritation, allergic response and other problem complications commensurate with a reasonable benefit/risk ratio.

As used herein, the term "pharmaceutical composition" means a mixture containing a therapeutic compound to be administered to a subject, such as a mammal, e.g., a human, in order to prevent, treat or control a particular disease or condition affecting the mammal, such as an autoimmune disorder, cancer, or blood disorder, among others, e.g., as described herein.

As used herein, the term "recipient" refers to a patient that receives a transplant, such as a transplant containing a population of hematopoietic stem cells. The transplanted cells administered to a recipient may be, e.g., autologous, syngeneic, or allogeneic cells.

As used herein, the term "rejection" in the context of a transplant, such as a hematopoietic stem cell graft, refers to the process by which a recipient mounts an immune response against an incoming transplant, thereby reducing the ability of the transplanted matter (e.g., hematopoietic stem cells) to persist in the recipient. Rejection of a transplanted graft, such as a hematopoietic stem cell graft, can be quantified, for instance, by measuring the quantity or

concentration of transplanted cells in various samples isolated from a patient at distinct time points following transplantation. A finding that the quantity or concentration of transplanted cells in samples isolated from the patient diminishes over time, for instance, by 20%, 25%, 30%, 35%, 40%, 56%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, or more, indicates that the patient is suffering from graft rejection. Conversely, a finding that the quantity or concentration of transplanted cells in samples isolated from the patient remains stable over time, for instance, by being diminished by less than 20%, 15%, 10%, 5%, or fewer, indicates that the patient is not suffering from graft rejection. Alternatively, graft rejection can be quantified by measuring the quantity or concentration of immune cells, such as T cells and/or NK cells, that cross-react with MHC antigens expressed by the transplanted cells in various samples isolated from a patient at distinct time points following transplantation. A finding that the quantity or concentration of immune cells, such as T cells and/or NK cells, that cross-react with MHC antigens expressed by the transplanted cells in samples isolated from the patient increases over time, for instance, by 10%, 15%, 20%, 25%, 30%, 35%, 40%, 56%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 100%, 200%, 300%, or more, indicates that the patient is suffering from graft rejection. Conversely, a finding that the quantity or concentration of immune cells, such as T cells and/or NK cells, that cross-react with MHC antigens expressed by the transplanted cells in samples isolated from the patient diminishes over time, for instance, by 20%, 25%, 30%, 35%, 40%, 56%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, or more, indicates that the patient is not suffering from graft rejection.

As used herein, the term “sample” refers to a specimen (e.g., blood, blood component (e.g., serum or plasma), urine, saliva, amniotic fluid, cerebrospinal fluid, tissue (e.g., placental or dermal), pancreatic fluid, chorionic villus sample, and cells) taken from a subject.

As used herein, the term “scFv” refers to a single chain Fv antibody in which the variable domains of the heavy chain and the light chain from an antibody have been joined to form one chain. scFv fragments contain a single polypeptide chain that includes the variable region of an antibody light chain (V_L) (e.g., CDR-L1, CDR-L2, and/or CDR-L3) and the variable region of an antibody heavy chain (V_H) (e.g., CDR-H1, CDR-H2, and/or CDR-H3) separated by a linker. The linker that joins the V_L and V_H regions of a scFv fragment can be a peptide linker composed of proteinogenic amino acids. Alternative linkers can be used so as to increase the resistance of the scFv fragment to proteolytic degradation (for example, linkers containing D-amino acids), in order

to enhance the solubility of the scFv fragment (for example, hydrophilic linkers such as polyethylene glycol-containing linkers or polypeptides containing repeating glycine and serine residues), to improve the biophysical stability of the molecule (for example, a linker containing cysteine residues that form intramolecular or intermolecular disulfide bonds), or to attenuate the immunogenicity of the scFv fragment (for example, linkers containing glycosylation sites). It will also be understood by one of ordinary skill in the art that the variable regions of the scFv molecules described herein can be modified such that they vary in amino acid sequence from the antibody molecule from which they were derived. For example, nucleotide or amino acid substitutions leading to conservative substitutions or changes at amino acid residues can be made (e.g., in CDR and/or framework residues) so as to preserve or enhance the ability of the scFv to bind to the antigen recognized by the corresponding antibody.

The terms "specific binding" or "specifically binds" in reference to the interaction of an antibody, or antibody fragment, with a second chemical species, means that the interaction is dependent upon the presence of a particular structure (e.g., an antigenic determinant or epitope) on the chemical species; for example, an antibody recognizes and binds to a specific protein structure rather than to proteins generally. If an antibody is specific for epitope "A", the presence of a molecule containing epitope A (or free, unlabeled A), in a reaction containing labeled "A" and the antibody, will reduce the amount of labeled A bound to the antibody.

As used herein, the terms “subject” and “patient” refer to a mammal, such as a human, that receives treatment for a particular disease or condition as described herein. For instance, a patient, such as a human patient, may be one that is suffering from an autoimmune disease described herein, and may be administered an anti-CD2 antibody or antibody-drug conjugate described herein so as to (i) deplete a population of autoimmune cells (e.g., a population of autoimmune CD2+ T cells and/or NK cells) and/or (ii) deplete a population of CD2+ immune cells (e.g., CD2+ T cells and/or NK cells that cross-react with a non-self antigen expressed by hematopoietic stem cells (e.g., a non-self MHC antigen), thereby preventing or reducing the likelihood of graft rejection prior to hematopoietic stem cell transplant therapy.

As used herein, the phrase “substantially cleared from the blood” refers to a point in time following administration of a therapeutic agent (such as an anti-CD2 antibody, or an antigen-binding fragment thereof) to a patient when the concentration of the therapeutic agent in a blood sample isolated from the patient is such that the therapeutic agent is not detectable by

conventional means (for instance, such that the therapeutic agent is not detectable above the noise threshold of the device or assay used to detect the therapeutic agent). A variety of techniques known in the art can be used to detect antibodies, or antibody fragments, such as ELISA-based detection assays known in the art or described herein. Additional assays that can be used to detect antibodies, and antibody fragments, include immunoprecipitation techniques and immunoblot assays, among others known in the art.

As used herein, the phrase "stem cell disorder" broadly refers to any disease, disorder, or condition that may be treated or cured by conditioning a subject's target tissues, for instance, by ablating an endogenous T cell population in a target tissue,) and/or by engrafting or transplanting stem cells in a subject's target tissues. For example, Type I diabetes patients have been shown to be cured by hematopoietic stem cell transplant and may benefit from conditioning in accordance with the compositions and methods described herein. Additional disorders that can be treated using the compositions and methods described herein include, without limitation, sickle cell anemia, thalassemias, Fanconi anemia, Wiskott-Aldrich syndrome, ADA SCID, HIV/AIDS, metachromatic leukodystrophy, Diamond-Blackfan anemia, and Schwachman-Diamond syndrome. The subject may have or be affected by an inherited blood disorder (e.g., sickle cell anemia) or an autoimmune disorder. Additionally or alternatively, the subject may have or be affected by a malignancy, such as a malignancy selected from the group consisting of hematologic cancers (e.g., leukemia, lymphoma, multiple myeloma, or myelodysplastic syndrome) and neuroblastoma. In some embodiments, the subject has or is otherwise affected by a metabolic disorder. For example, the subject may suffer or otherwise be affected by a metabolic disorder selected from the group consisting of glycogen storage diseases, mucopolysaccharidoses, Gaucher's Disease, Hurlers Disease, sphingolipidoses, metachromatic leukodystrophy, or any other diseases or disorders which may benefit from the treatments and therapies disclosed herein and including, without limitation, severe combined immunodeficiency, Wiscott-Aldrich syndrome, hyper immunoglobulin M (IgM) syndrome, Chediak-Higashi disease, hereditary lymphohistiocytosis, osteopetrosis, osteogenesis imperfecta, storage diseases, thalassemia major, sickle cell disease, systemic sclerosis, systemic lupus erythematosus, multiple sclerosis, juvenile rheumatoid arthritis and those diseases, or disorders described in "Bone Marrow Transplantation for Non-Malignant Disease," ASH Education Book, 1:319-338

(2000), the disclosure of which is incorporated herein by reference in its entirety as it pertains to pathologies that may be treated by administration of hematopoietic stem cell transplant therapy.

As used herein, the term "transfection" refers to any of a wide variety of techniques commonly used for the introduction of exogenous DNA into a prokaryotic or eukaryotic host cell, such as electroporation, lipofection, calcium- phosphate precipitation, DEAE- dextran transfection and the like.

As used herein, the terms "treat" or "treatment" refer to therapeutic treatment, in which the object is to prevent or slow down (lessen) an undesired physiological change or disorder or to promote a beneficial phenotype in the patient being treated. Beneficial or desired clinical results include, but are not limited to, a reduction in the quantity of autoimmune cells present in a sample isolated from the patient, such as a population of CD2+ T cells and/or NK cells that cross-react with a self antigen in the case of treating an autoimmune disorder directly, or a non-self antigen expressed by hematopoietic stem cells (e.g., a non-self MHC antigen) prior to hematopoietic stem cell transplantation in the case of treating an autoimmune disease by administration an anti-CD2 antibody, antigen-binding fragment thereof, and a hematopoietic stem cell graft. Additional beneficial results include an increase in the cell count or relative concentration of hematopoietic stem cells in a patient in need of a hematopoietic stem cell transplant following conditioning therapy and subsequent administration of an exogenous hematopoietic stem cell graft to the patient. Beneficial results of therapy described herein may also include an increase in the cell count or relative concentration of one or more cells of hematopoietic lineage, such as a megakaryocyte, thrombocyte, platelet, erythrocyte, mast cell, myeoblast, basophil, neutrophil, eosinophil, microglial cell, granulocyte, monocyte, osteoclast, antigen-presenting cell, macrophage, dendritic cell, natural killer cell, T lymphocyte, or B lymphocyte, following conditioning therapy and subsequent hematopoietic stem cell transplant therapy.

As used herein, the terms "variant" and "derivative" are used interchangeably and refer to naturally-occurring, synthetic, and semi-synthetic analogues of a compound, peptide, protein, or other substance described herein. A variant or derivative of a compound, peptide, protein, or other substance described herein may retain or improve upon the biological activity of the original material.

As used herein, the term "vector" includes a nucleic acid vector, such as a plasmid, a DNA vector, a plasmid, a RNA vector, virus, or other suitable replicon. Expression vectors

described herein may contain a polynucleotide sequence as well as, for example, additional sequence elements used for the expression of proteins and/or the integration of these polynucleotide sequences into the genome of a mammalian cell. Certain vectors that can be used for the expression of antibodies and antibody fragments of the invention include plasmids that contain regulatory sequences, such as promoter and enhancer regions, which direct gene transcription. Other useful vectors for expression of antibodies and antibody fragments contain polynucleotide sequences that enhance the rate of translation of these genes or improve the stability or nuclear export of the mRNA that results from gene transcription. These sequence elements may include, for example, 5' and 3' untranslated regions and a polyadenylation signal site in order to direct efficient transcription of the gene carried on the expression vector. The expression vectors described herein may also contain a polynucleotide encoding a marker for selection of cells that contain such a vector. Examples of a suitable marker include genes that encode resistance to antibiotics, such as ampicillin, chloramphenicol, kanamycin, and nourseothricin.

As used herein, the term "alkyl" refers to a straight- or branched-chain alkyl group having, for example, from 1 to 20 carbon atoms in the chain. Examples of alkyl groups include methyl, ethyl, n-propyl, isopropyl, butyl, isobutyl, sec-butyl, tert-butyl, pentyl, isopentyl, tert-pentyl, hexyl, isohexyl, and the like.

As used herein, the term "alkylene" refers to a straight- or branched-chain divalent alkyl group. The divalent positions may be on the same or different atoms within the alkyl chain. Examples of alkylene include methylene, ethylene, propylene, isopropylene, and the like.

As used herein, the term "heteroalkyl" refers to a straight or branched-chain alkyl group having, for example, from 1 to 20 carbon atoms in the chain, and further containing one or more heteroatoms (e.g., oxygen, nitrogen, or sulfur, among others) in the chain.

As used herein, the term "heteroalkylene" refers to a straight- or branched-chain divalent heteroalkyl group. The divalent positions may be on the same or different atoms within the heteroalkyl chain. The divalent positions may be one or more heteroatoms.

As used herein, the term "alkenyl" refers to a straight- or branched-chain alkenyl group having, for example, from 2 to 20 carbon atoms in the chain. Examples of alkenyl groups include vinyl, propenyl, isopropenyl, butenyl, tert-butylenyl, hexenyl, and the like.

As used herein, the term "alkenylene" refers to a straight- or branched-chain divalent

alkenyl group. The divalent positions may be on the same or different atoms within the alkenyl chain. Examples of alkenylene include ethenylene, propenylene, isopropenylene, butenylene, and the like.

As used herein, the term "heteroalkenyl" refers to a straight- or branched-chain alkenyl group having, for example, from 2 to 20 carbon atoms in the chain, and further containing one or more heteroatoms (e.g., oxygen, nitrogen, or sulfur, among others) in the chain.

As used herein, the term "heteroalkenylene" refers to a straight- or branched-chain divalent heteroalkenyl group. The divalent positions may be on the same or different atoms within the heteroalkenyl chain. The divalent positions may be one or more heteroatoms.

As used herein, the term "alkynyl" refers to a straight- or branched-chain alkynyl group having, for example, from 2 to 20 carbon atoms in the chain. Examples of alkynyl groups include propargyl, butynyl, pentynyl, hexynyl, and the like.

As used herein, the term "alkynylene" refers to a straight- or branched-chain divalent alkynyl group. The divalent positions may be on the same or different atoms within the alkynyl chain.

As used herein, the term "heteroalkynyl" refers to a straight- or branched-chain alkynyl group having, for example, from 2 to 20 carbon atoms in the chain, and further containing one or more heteroatoms (e.g., oxygen, nitrogen, or sulfur, among others) in the chain.

As used herein, the term "heteroalkynylene" refers to a straight- or branched-chain divalent heteroalkynyl group. The divalent positions may be on the same or different atoms within the heteroalkynyl chain. The divalent positions may be one or more heteroatoms.

As used herein, the term "cycloalkyl" refers to a monocyclic, or fused, bridged, or spiro polycyclic ring structure that is saturated and has, for example, from 3 to 12 carbon ring atoms. Examples of cycloalkyl groups include cyclopropyl, cyclobutyl, cyclopentyl, cyclohexyl, cycloheptyl, cyclooctyl, bicyclo[3.1.0]hexane, and the like.

As used herein, the term "cycloalkylene" refers to a divalent cycloalkyl group. The divalent positions may be on the same or different atoms within the ring structure. Examples of cycloalkylene include cyclopropylene, cyclobutylene, cyclopentylene, cyclohexylene, and the like.

As used herein, the term "heterocyloalkyl" refers to a monocyclic, or fused, bridged, or spiro polycyclic ring structure that is saturated and has, for example, from 3 to 12 ring atoms per ring structure selected from carbon atoms and heteroatoms selected from, e.g., nitrogen, oxygen,

and sulfur, among others. The ring structure may contain, for example, one or more oxo groups on carbon, nitrogen, or sulfur ring members.

As used herein, the term "heterocycloalkylene" refers to a divalent heterocyclolalkyl group. The divalent positions may be on the same or different atoms within the ring structure.

As used herein, the term "aryl" refers to a monocyclic or multicyclic aromatic ring system containing, for example, from 6 to 19 carbon atoms. Aryl groups include, but are not limited to, phenyl, fluorenyl, naphthyl, and the like. The divalent positions may be one or more heteroatoms.

As used herein, the term "arylene" refers to a divalent aryl group. The divalent positions may be on the same or different atoms.

As used herein, the term "heteroaryl" refers to a monocyclic heteroaromatic, or a bicyclic or a tricyclic fused-ring heteroaromatic group. Heteroaryl groups include pyridyl, pyrrolyl, furyl, thienyl, imidazolyl, oxazolyl, isoxazolyl, thiazolyl, isothiazolyl, pyrazolyl, 1,2,3-triazolyl, 1,2,4-triazolyl, 1,2,3-oxadiazolyl, 1,2,4-oxadia-zolyl, 1,2,5-oxadiazolyl, 1,3,4-oxadiazolyl, 1,3,4-triazinyl, 1,2,3-triazinyl, benzofuryl, [2,3-dihydro]benzofuryl, isobenzofuryl, benzothienyl, benzotriazolyl, isobenzothienyl, indolyl, isoindolyl, 3H-indolyl, benzimidazolyl, imidazo[1,2-a]pyridyl, benzothiazolyl, benzoxazolyl, quinolizinyl, quinazolinyl, pthalazinyl, quinoxalinyl, cinnolinyl, napthyridinyl, pyrido[3,4-b]pyridyl, pyrido[3,2-b]pyridyl, pyrido[4,3-b]pyridyl, quinolyl, isoquinolyl, tetrazolyl, 5,6,7,8-tetrahydroquinolyl, 5,6,7,8-tetrahydroisoquinolyl, purinyl, pteridinyl, carbazolyl, xanthenyl, benzoquinolyl, and the like.

As used herein, the term "heteroarylene" refers to a divalent heteroaryl group. The divalent positions may be on the same or different atoms. The divalent positions may be one or more heteroatoms.

Unless otherwise constrained by the definition of the individual substituent, the foregoing chemical moieties, such as "alkyl", "alkylene", "heteroalkyl", "heteroalkylene", "alkenyl", "alkenylene", "heteroalkenyl", "heteroalkenylene", "alkynyl", "alkynylene", "heteroalkynyl", "heteroalkynylene", "cycloalkyl", "cycloalkylene", "heterocyclolalkyl", heterocycloalkylene", "aryl," "arylene", "heteroaryl", and "heteroarylene" groups can optionally be substituted with, for example, from 1 to 5 substituents selected from the group consisting of alkyl, alkenyl, alkynyl, cycloalkyl, heterocycloalkyl, alkyl aryl, alkyl heteroaryl, alkyl cycloalkyl, alkyl heterocycloalkyl, amino, ammonium, acyl, acyloxy, acylamino, aminocarbonyl, alkoxycarbonyl, ureido, carbamate, aryl, heteroaryl, sulfinyl, sulfonyl, alkoxy, sulfanyl, halogen, carboxy, trihalomethyl, cyano,

hydroxy, mercapto, nitro, and the like. The substitution may include situations in which neighboring substituents have undergone ring closure, such as ring closure of vicinal functional substituents, to form, for instance, lactams, lactones, cyclic anhydrides, acetals, hemiacetals, thioacetals, aminals, and hemiaminals, formed by ring closure, for example, to furnish a protecting group.

Anti-CD2 Antibodies

The present invention is based in part on the discovery that anti-CD2 antibodies, or antigen-binding fragments thereof, can be used to treat cancers and autoimmune diseases directly, for instance, due to the ability of such agents to kill CD2+ cancer cells (e.g., CD2+ leukemic cells) and CD2+ autoimmune cells (e.g., CD2+ autoimmune T cells and/or NK cells).

The invention is additionally based in part on the discovery that antibodies, or antigen-binding fragments thereof, capable of binding CD2 can be used as therapeutic agents to promote the engraftment of transplanted hematopoietic stem cells in a patient in need of transplant therapy by preventing or reducing the likelihood of immune cell-mediated graft rejection. For instance, anti-CD2 antibodies, and antigen binding fragments, can bind cell-surface CD2 expressed by immune cells such as T cells or NK cells that cross-react with, and mount an immune response against, one or more non-self hematopoietic stem cell antigens, such as one or more non-self MHC antigens expressed by the hematopoietic stem cells. The binding of such antibodies, and antigen-binding fragments, to hematopoietic stem cell-specific CD2+ immune cells can induce death of the bound immune cell, for instance, by antibody-dependent cell-mediated cytotoxicity or by the action of a cytotoxic agent that is conjugated to the antibody, or the antigen-binding fragment thereof. The depletion of a population of CD2+ immune cells that cross-react with non-self hematopoietic stem cells can thus facilitate the engraftment of hematopoietic stem cell transplants in a patient in need thereof by attenuating the ability of the recipient's immune system to mount an immune response against the incoming graft. In this way, a patient suffering from a stem cell disorder, cancer, autoimmune disease, or other blood disorder described herein can be treated, as a hematopoietic stem cell transplant can be provided to a subject in order to repopulate a lineage of cells that is defective and/or deficient in the subject. The subject may be deficient in a population of cells due to, for instance, chemotherapy that has

been administered to the subject with the aim of eradicating cancerous cells but that has, in the process, depleted healthy hematopoietic cells as well.

For example, the invention thus provides compositions and methods of promoting the engraftment of transplanted hematopoietic stem cells by administration of an antibody, or an antigen-binding fragment thereof, capable of binding an antigen expressed by T cells. This administration can cause the selective depletion of a population of endogenous T cells, such as CD4+ and CD8+ T cells. This selective depletion of T cells can, in turn, prevent graft rejection following transplantation of an exogenous (for instance, an autologous, allogeneic, or syngeneic) hematopoietic stem cell graft. For instance, the selective depletion of CD4+ and/or CD8+ T cells using an anti-CD2 antibody, antigen-binding fragment, antibody-drug conjugate, or antibody-drug conjugate as described herein can attenuate a T cell-mediated immune response that may occur against a transplanted hematopoietic stem cell graft. The invention is based in part on the discovery that antibodies, and antigen-binding fragments thereof, capable of binding CD2 can be administered to a patient in need of hematopoietic stem cell transplant therapy in order to promote the survival and engraftment potential of transplanted hematopoietic stem cells.

Engraftment of hematopoietic stem cell transplants due to the administration of anti-CD2 antibodies, or antigen-binding fragments thereof, can manifest in a variety of empirical measurements. For instance, engraftment of transplanted hematopoietic stem cells can be evaluated by assessing the quantity of competitive repopulating units (CRU) present within the bone marrow of a patient following administration of an antibody or antigen-binding fragment thereof capable of binding CD2 and subsequent administration of a hematopoietic stem cell transplant. Additionally, one can observe engraftment of a hematopoietic stem cell transplant by incorporating a reporter gene, such as an enzyme that catalyzes a chemical reaction yielding a fluorescent, chromophoric, or luminescent product, into a vector with which the donor hematopoietic stem cells have been transfected and subsequently monitoring the corresponding signal in a tissue into which the hematopoietic stem cells have homed, such as the bone marrow. One can also observe hematopoietic stem cell engraftment by evaluation of the quantity and survival of hematopoietic stem and progenitor cells, for instance, as determined by fluorescence activated cell sorting (FACS) analysis methods known in the art. Engraftment can also be determined by measuring white blood cell counts in peripheral blood during a post-transplant

period, and/or by measuring recovery of marrow cells by donor cells in a bone marrow aspirate sample.

The sections that follow provide a description of antibodies, or antigen-binding fragments thereof, that can be administered to a patient in need of hematopoietic stem cell transplant therapy in order to promote engraftment of hematopoietic stem cell grafts, as well as methods of administering such therapeutics to a patient prior to hematopoietic stem cell transplantation.

Exemplary Antibodies

T cells and NK cells have been shown to express CD2, which is a cell adhesion molecule and specific marker for such lymphocytes. For instance, CD2 interacts with other adhesion molecules, such as lymphocyte function-associated antigen-3 (LFA-3/CD58), to potentiate T cell activation. Antibodies and antigen-binding fragments thereof capable of binding CD2 may suppress T cell activation and T cell-mediated immune responses against hematopoietic stem cell grafts, for example, by inhibiting the interaction between CD2 and LFA-3. Antibodies and antigen-binding fragments thereof that bind to this cell-surface antigen can be identified using techniques known in the art and described herein, including immunization, computational modeling techniques, and in vitro selection methods, such as the phage display and cell-based display platforms described below.

Anti-CD2 antibodies that can be used in conjunction with the compositions and methods described herein include those that have one or more, or all, of the following CDRs:

a. a CDR-H1 having the amino acid sequence EYYMY (SEQ ID NO: 1);
b. a CDR-H2 having the amino acid sequence RIDPEDGSIDYVEKFKK (SEQ ID NO: 2);
c. a CDR-H3 having the amino acid sequence GKFNYRFAY (SEQ ID NO: 3);
d. a CDR-L1 having the amino acid sequence RSSQSLLHSSGNTYLN (SEQ ID NO: 4);
e. a CDR-L2 having the amino acid sequence LVSKLES (SEQ ID NO: 5); and
f. a CDR-L3 having the amino acid sequence MQFTHYPYT (SEQ ID NO: 6).

Antibodies and antigen-binding fragments thereof containing the foregoing CDR sequences are described, e.g., in US Patent No. 6,849,258, the disclosure of which is incorporated herein by reference as it pertains to anti-CD2 antibodies and antigen-binding

fragments thereof. The antibodies and fragments thereof disclosed in US Patent Nos. 5,730,979; 5,817,311; 5,951,983; and 7,592,006; such as LO-CD2a, BTI-322, and antibodies produced by the hybridoma cell line deposited as ATCC Deposit No. HB 11423 (e.g., antibodies or antigen-binding fragments thereof containing one or more, or all, of the CDR sequences of antibody LO-CD2a isolated from the hybridoma cell line deposited as ATCC Deposit No. HB 11423) can be used in conjunction with the compositions and methods disclosed herein. Exemplary antibodies that may be used in conjunction with the compositions and methods described herein include humanized antibodies containing one or more, or all, of the CDR sequences of an antibody isolated from the hybridoma cell line deposited as ATCC Deposit No. HB 11423, such as MEDI-507. MEDI-507 is a humanized anti-CD2 monoclonal antibody that contains the CDR-H and CDR-L sequences of (a) through (f) above, and is described in Branco et al., Transplantation 68:1588-1596 (1999). MEDI-507 is additionally described in US Patent Application Publication Nos. 2011/0280869 and 2011/0091453, the disclosures of each of which are incorporated herein by reference as they pertain to anti-CD2 antibodies and antigen-binding fragments thereof, such as the anti-CD2 antibody MEDI-507.

The disclosures of the foregoing scientific journal article and US Patents are incorporated herein by reference as they pertain to anti-CD2 antibodies and antigen-binding fragments thereof.

Other anti-CD2 antibodies that can be used in conjunction with the compositions and methods described herein include, for instance, anti-CD2 antibodies that are described in US Patent Nos. 6,541,611 and 7,250,167, the disclosures of each of which are incorporated herein by reference as they pertain to anti-CD2 antibodies and antigen-binding fragments thereof, such as the anti-CD2 antibody LO-CD2b and antibodies produced by the hybridoma cell line deposited as ATCC Deposit No. PTA-802. Exemplary antibodies that may be used in conjunction with the compositions and methods described herein include humanized antibodies containing one or more, or all, of the CDR sequences of an antibody isolated from the hybridoma cell line deposited as ATCC Deposit No. PTA-802.

Other anti-CD2 antibodies that can be used in conjunction with the compositions and methods described herein include, for instance, anti-CD2 antibodies that are described in US Patent Nos. 5,795,572 and 5,807,734, the disclosures of each of which are incorporated herein by reference as they pertains to anti-CD2 antibodies and antigen-binding fragments thereof, such as the anti-CD2 antibody produced by hybridoma cell line deposited as ATCC Deposit No. HB

69277. For instance, anti-CD2 antibodies and antigen-binding fragments thereof that may be used in conjunction with the compositions and methods described herein include those that contain a hinge region having an amino acid sequence of EPKSSDKTHTSPPSP (SEQ ID NO: 7), such as scFv fragments containing a hinge region having the amino acid sequence of EPKSSDKTHTSPPSP (SEQ ID NO: 7). The incorporation of a hinge region having the amino acid sequence of SEQ ID NO: 7 can be beneficial, as this hinge motif has been mutated relative to wild-type hinge region sequences so as to eliminate potentially reactive cysteine residues that may promote undesirable oxidative dimerization of a single-chain antibody fragment, such as a scFv fragment.

Other anti-CD2 antibodies that can be used in conjunction with the compositions and methods described herein include, for instance, anti-CD2 antibodies that are described in US Patent No. 6,764,688, such as the anti-CD2 antibody TS2/18 and antibodies produced by hybridoma cell line deposited as ATCC Deposit No. HB-195. The disclosure of US Patent No. 6,764,688 is incorporated herein by reference as it pertains to anti-CD2 antibodies and antigen-binding fragments thereof.

Other anti-CD2 antibodies that can be used in conjunction with the compositions and methods described herein include, for instance, anti-CD2 antibodies that are described in US Patent Nos. 6,162,432, 6,558,662, 7,408,039, 7,332,157, 7,638,121, 7,939,062, and 7,115,259, US Patent Application Publication No. 2006/0084107, 2014/0369974, 2002/0051784, and 2013/0183322, and PCT Publication No. WO1992/016563, the disclosures of each of which are incorporated herein by reference as they pertain to anti-CD2 antibodies and antigen binding fragments thereof.

Antibodies and fragments thereof for use in conjunction with the methods described herein include variants of those antibodies described above, such as antibody fragments that contain or lack an Fc domain, as well as humanized variants of non-human antibodies described herein and antibody-like protein scaffolds (e.g., ^{10}Fn3 domains) containing one or more, or all, of the CDRs or equivalent regions thereof of an antibody, or an antibody fragment, described herein. Exemplary antigen-binding fragments of the foregoing antibodies include a dual-variable immunoglobulin domain, a single-chain Fv molecule (scFv), a diabody, a triabody, a nanobody, an antibody-like protein scaffold, a Fv fragment, a Fab fragment, a $F(ab')_2$ molecule, and a tandem di-scFv, among others.

Methods of Identifying Anti-CD2 Antibodies

Methods for high throughput screening of libraries of antibodies, or antibody fragments, that bind CD2 can be used to identify and affinity mature agents useful for conditioning a patient (e.g., a human patient) in need of hematopoietic stem cell therapy and/or for directly treating a cancer or autoimmune disease as described herein. Such methods include in vitro display techniques known in the art, such as phage display, bacterial display, yeast display, mammalian cell display, ribosome display, mRNA display, and cDNA display, among others. The use of phage display to isolate antibodies, or antigen-binding fragments, that bind biologically relevant molecules has been reviewed, for example, in Felici et al., Biotechnol. Annual Rev. 1:149-183, 1995; Katz, Annual Rev. Biophys. Biomol. Struct. 26:27-45, 1997; and Hoogenboom et al., Immunotechnology 4:1-20, 1998, the disclosures of each of which are incorporated herein by reference as they pertain to in vitro display techniques. Randomized combinatorial peptide libraries have been constructed to select for polypeptides that bind cell surface antigens as described in Kay, Perspect. Drug Discovery Des. 2:251-268, 1995 and Kay et al., Mol. Divers. 1:139-140, 1996, the disclosures of each of which are incorporated herein by reference as they pertain to the discovery of antigen-binding molecules. Proteins, such as multimeric proteins, have been successfully phage-displayed as functional molecules (see, for example, EP 0349578; EP 4527839; and EP 0589877, as well as Chiswell and McCafferty, Trends Biotechnol. 10:80-84 1992, the disclosures of each of which are incorporated herein by reference as they pertain to the use of in vitro display techniques for the discovery of antigen-binding molecules. In addition, functional antibody fragments, such as Fab and scFv fragments, have been expressed in in vitro display formats (see, for example, McCafferty et al., Nature 348:552- 554, 1990; Barbas et al., Proc. Natl. Acad. Sci. USA 88:7978-7982, 1991; and Clackson et al., Nature 352:624-628, 1991, the disclosures of each of which are incorporated herein by reference as they pertain to in vitro display platforms for the discovery of antigen-binding molecules). These techniques, among others, can be used to identify and improve the affinity of antibodies, or antibody fragments, that bind CD2 that can in turn be used to deplete CD2+ T cells and/or NK cells in a patient (e.g., a human patient) in need of hematopoietic stem cell transplant therapy and/or suffering from cancer or an autoimmune disease described herein.

Additional techniques can be used to identify antibodies, and antigen-binding fragments thereof, that bind CD2 on the surface of a cell (e.g., a T cell or NK cell) and that are internalized by the cell, for instance, by receptor-mediated endocytosis. For example, the in vitro display techniques described above can be adapted to screen for antibodies, and antigen-binding fragments thereof, that bind CD2 on the surface of a T cell or NK cell and that are subsequently internalized. Phage display represents one such technique that can be used in conjunction with this screening paradigm. To identify anti-CD2 antibodies, and fragments thereof, that bind CD2 and are subsequently internalized by T cells and/or NK cells, one of skill in the art can use the phage display techniques described in Williams et al., Leukemia 19:1432-1438, 2005, the disclosure of which is incorporated herein by reference in its entirety. For example, using mutagenesis methods known in the art, recombinant phage libraries can be produced that encode antibodies, antibody fragments, such as scFv fragments, Fab fragments, diabodies, triabodies, and ^{10}Fn3 domains, among others, or antibodies that contain randomized amino acid cassettes (e.g., in one or more, or all, of the CDRs or equivalent regions thereof or an antibody or antibody fragment). The framework regions, hinge, Fc domain, and other regions of the antibodies or antibody fragments may be designed such that they are non-immunogenic in humans, for instance, by virtue of having human germline antibody sequences or sequences that exhibit only minor variations relative to human germline antibodies.

Using phage display techniques described herein or known in the art, phage libraries containing randomized antibodies, or antibody fragments, covalently bound to the phage particles can be incubated with CD2 antigen, for instance, by first incubating the phage library with blocking agents (such as, for instance, milk protein, bovine serum albumin, and/or IgG so as to remove phage encoding antibodies, or fragments thereof, that exhibit non-specific protein binding and phage that encode antibodies or fragments thereof that bind Fc domains, and then incubating the phage library with a population of T cells or NK cells that are CD2+. The phage library can be incubated with the T cells or NK cells for a time sufficient to allow CD2-specific antibodies, or antigen-binding fragments thereof, to bind cell-surface CD2 and to subsequently be internalized by the T cells or NK cells (e.g., from 30 minutes to 6 hours at 4° C, such as 1 hour at 4° C). Phage containing antibodies, or fragments thereof, that do not exhibit sufficient affinity for CD2 so as to permit binding to, and internalization by, T cells or NK cells can subsequently be removed by washing the cells, for instance, with cold (4° C) 0.1 M glycine buffer at pH 2.8. Phage bound

to antibodies, or fragments thereof, that have been internalized by the T cells and/or NK cells can be identified, for instance, by lysing the cells and recovering internalized phage from the cell culture medium. The phage can then be amplified in bacterial cells, for example, by incubating bacterial cells with recovered phage in 2xYT medium using methods known in the art. Phage recovered from this medium can then be characterized, for instance, by determining the nucleic acid sequence of the gene(s) encoding the antibodies, or fragments thereof, inserted within the phage genome. The encoded antibodies, fragments thereof, can subsequently be prepared de novo by chemical synthesis (for instance, of antibody fragments, such as scFv fragments) or by recombinant expression (for instance, of full-length antibodies).

The internalizing capacity of the prepared antibodies, or fragments thereof, can be assessed, for instance, using radionuclide internalization assays known in the art. For example, anti-CD2 antibodies, or fragments thereof, identified using in vitro display techniques described herein or known in the art can be functionalized by incorporation of a radioactive isotope, such as ^{18}F, ^{75}Br, ^{77}Br, ^{122}I, ^{123}I, ^{124}I, ^{125}I, ^{129}I, ^{131}I, ^{211}At, ^{67}Ga, ^{111}In, ^{99}Tc, ^{169}Yb, ^{186}Re, ^{64}Cu, ^{67}Cu, ^{177}Lu, ^{77}As, ^{72}As, ^{86}Y, ^{90}Y, ^{89}Zr, ^{212}Bi, ^{213}Bi, or ^{225}Ac. For instance, radioactive halogens, such as ^{18}F, ^{75}Br, ^{77}Br, ^{122}I, ^{123}I, ^{124}I, ^{125}I, ^{129}I, ^{131}I, ^{211}At, can be incorporated into antibodies, or fragments thereof, using beads, such as polystyrene beads, containing electrophilic halogen reagents (e.g., Iodination Beads, Thermo Fisher Scientific, Inc., Cambridge, MA). Radiolabeled antibodies, or fragments thereof, can be incubated with T cells and/or NK cells for a time sufficient to permit internalization (e.g., from 30 minutes to 6 hours at 4° C, such as 1 hour at 4° C). The cells can then be washed to remove non-internalized antibodies, or fragments thereof, (e.g., using cold (4° C) 0.1 M glycine buffer at pH 2.8). Internalized antibodies, or fragments thereof, can be identified by detecting the emitted radiation (e.g., γ-radiation) of the resulting T cells and/or NK cells in comparison with the emitted radiation (e.g., γ-radiation) of the recovered wash buffer.

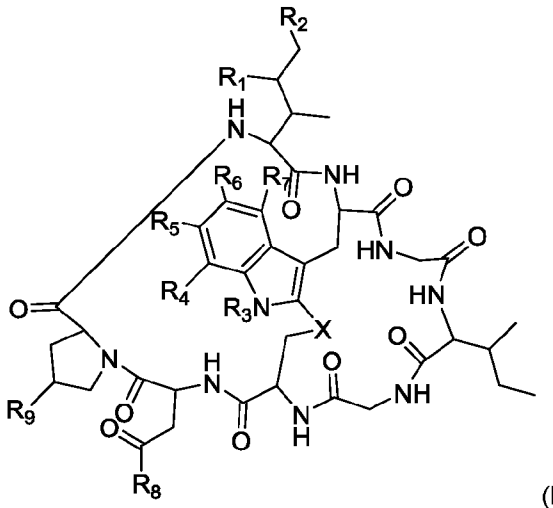
Antibody-Drug Conjugates

Cytotoxins

Antibodies, and antigen-binding fragments thereof, described herein (e.g., antibodies, antigen-binding fragments, that recognize and bind CD2) can be conjugated to a cytotoxin, such as pseudomonas exotoxin A, deBouganin, diphtheria toxin, an amatoxin, such as α-amanitin, saporin, maytansine, a maytansinoid, an auristatin, an anthracycline, a calicheamicin, irinotecan,

SN-38, a duocarmycin, a pyrrolobenzodiazepine, a pyrrolobenzodiazepine dimer, an indolinobenzodiazepine, and an indolinobenzodiazepine dimer, or a variant thereof, or another cytotoxic compound described herein or known in the art in order to (i) directly treat a cancer or autoimmune disease described herein or (ii) deplete endogenous immune cells so as to prevent or reduce the likelihood of rejection of hematopoietic stem cells upon transplantation into a patient (e.g., a human patient) in need of hematopoietic stem cell transplant therapy. In some embodiments, the cytotoxic molecule is conjugated to an internalizing antibody, or antigen-binding fragment thereof, such that following the cellular uptake of the antibody, or antigen-binding fragment, the cytotoxin may access its intracellular target and kill endogenous T cells and/or NK cells. Suitable cytotoxins suitable for use with the compositions and methods described herein include DNA-intercalating agents, (e.g., anthracyclines), agents capable of disrupting the mitotic spindle apparatus (e.g., vinca alkaloids, maytansine, maytansinoids, and derivatives thereof), RNA polymerase inhibitors (e.g., an amatoxin, such as α-amanitin, and derivatives thereof), agents capable of disrupting protein biosynthesis (e.g., agents that exhibit rRNA N-glycosidase activity, such as saporin and ricin A-chain), among others known in the art.

In some embodiments, the cytotoxin is an amatoxin or a derivative thereof, such as α-amanitin, β-amanitin, γ-amanitin, ε-amanitin, amanin, amaninamide, amanullin, amanullinic acid, and proamanullin. For instance, the antibodies, and antigen-binding fragments, described herein may be bound to an amatoxin so as to form a conjugate represented by the formula Ab-Am, wherein Ab is the antibody, or antigen-binding fragment thereof, and Am is an amatoxin. In some embodiments, Am is represented by formula (**I**)



(I)

wherein R_1 is H, OH, OR_A, or OR_C;

R_2 is H, OH, OR_B, or OR_C;

R_A and R_B, together with the oxygen atoms to which they are bound, combine to form an optionally substituted 5-membered heterocyclolalkyl group;

R_3 is H, R_C, or R_D;

R_4 is H, OH, OR_C, OR_D, R_C, or R_D;

R_5 is H, OH, OR_C, OR_D, R_C, or R_D;

R_6 is H, OH, OR_C, OR_D, R_C, or R_D;

R_7 is H, OH, OR_C, OR_D, R_C, or R_D;

R_8 is OH, NH_2, OR_C, OR_D, NHR_C, or NR_CR_D;

R_9 is H, OH, OR_C, or OR_D;

X is -S-, -S(O)-, or $-SO_2-$;

R_C is -L-Z;

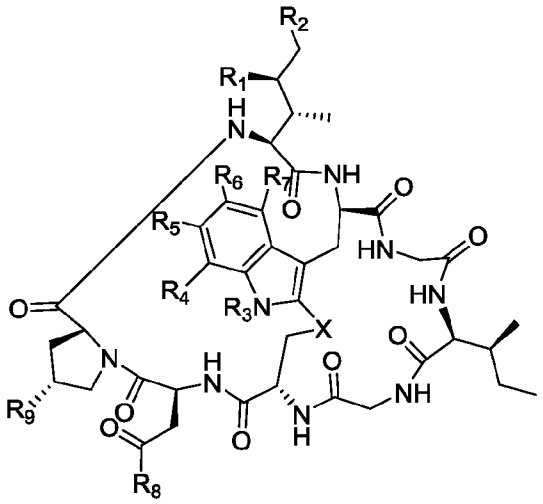
R_D is optionally substituted alkyl (e.g., C_1-C_6 alkyl), optionally substituted heteroalkyl (e.g., C_1-C_6 heteroalkyl), optionally substituted alkenyl (e.g., C_2-C_6 alkenyl), optionally substituted heteroalkenyl (e.g., C_2-C_6 heteroalkenyl), optionally substituted alkynyl (e.g., C_2-C_6 alkynyl), optionally substituted heteroalkynyl (e.g., C_2-C_6 heteroalkynyl), optionally substituted cycloalkyl, optionally substituted heterocycloalkyl, optionally substituted aryl, or optionally substituted heteroaryl;

L is a linker, such as optionally substituted alkylene (e.g., C_1-C_6 alkylene), optionally substituted heteroalkylene (C_1-C_6 heteroalkylene), optionally substituted alkenylene (e.g., C_2-C_6 alkenylene), optionally substituted heteroalkenylene (e.g., C_2-C_6 heteroalkenylene), optionally substituted alkynylene (e.g., C_2-C_6 alkynylene), optionally substituted heteroalkynylene (e.g., C_2-C_6 heteroalkynylene), optionally substituted cycloalkylene, optionally substituted heterocycloalkylene, optionally substituted arylene, or optionally substituted heteroarylene; and

Z is a chemical moiety formed from a coupling reaction between a reactive substituent present on L and a reactive substituent present within an antibody, or antigen-binding fragment thereof, that binds CD2.

In some embodiments, Am contains exactly one R_C substituent.

In some embodiments, Am is represented by formula (**IA**)



(**IA**)

wherein R_1 is H, OH, OR_A, or OR_C;

R_2 is H, OH, OR_B, or OR_C;

R_A and R_B, together with the oxygen atoms to which they are bound, combine to form an optionally substituted 5-membered heterocyclolalkyl group;

R_3 is H, R_C, or R_D;

R_4 is H, OH, OR_C, OR_D, R_C, or R_D;

R_5 is H, OH, OR_C, OR_D, R_C, or R_D;

R_6 is H, OH, OR_C, OR_D, R_C, or R_D;

R_7 is H, OH, OR_C, OR_D, R_C, or R_D;

R_8 is OH, NH_2, OR_C, OR_D, NHR_C, or NR_CR_D;

R_9 is H, OH, OR_C, or OR_D;

X is -S-, -S(O)-, or $-SO_2-$;

R_C is -L-Z;

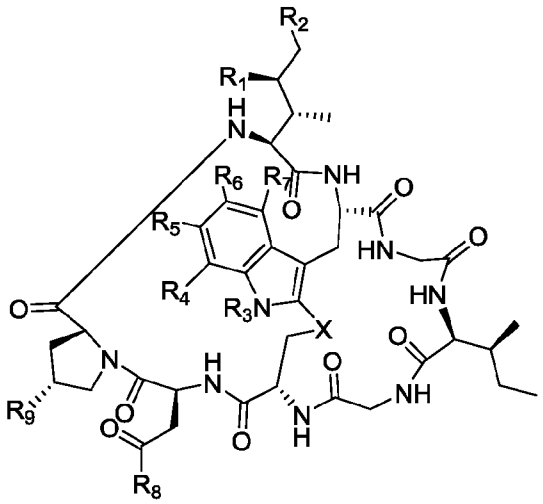
R_D is optionally substituted alkyl (e.g., C_1-C_6 alkyl), optionally substituted heteroalkyl (e.g., C_1-C_6 heteroalkyl), optionally substituted alkenyl (e.g., C_2-C_6 alkenyl), optionally substituted heteroalkenyl (e.g., C_2-C_6 heteroalkenyl), optionally substituted alkynyl (e.g., C_2-C_6 alkynyl), optionally substituted heteroalkynyl (e.g., C_2-C_6 heteroalkynyl), optionally substituted cycloalkyl, optionally substituted heterocycloalkyl, optionally substituted aryl, or optionally substituted heteroaryl;

L is a linker, such as optionally substituted alkylene (e.g., C_1-C_6 alkylene), optionally substituted heteroalkylene (C_1-C_6 heteroalkylene), optionally substituted alkenylene (e.g., C_2-C_6 alkenylene), optionally substituted heteroalkenylene (e.g., C_2-C_6 heteroalkenylene), optionally substituted alkynylene (e.g., C_2-C_6 alkynylene), optionally substituted heteroalkynylene (e.g., C_2-C_6 heteroalkynylene), optionally substituted cycloalkylene, optionally substituted heterocycloalkylene, optionally substituted arylene, or optionally substituted heteroarylene;

Z is a chemical moiety formed from a coupling reaction between a reactive substituent present on L and a reactive substituent present within an antibody, or antigen-binding fragment thereof, that binds CD2; and

wherein Am contains exactly one R_C substituent.

In some embodiments, Am is represented by formula (**IB**)



(IB)

wherein R_1 is H, OH, OR_A, or OR_C;

R_2 is H, OH, OR_B, or OR_C;

R_A and R_B, together with the oxygen atoms to which they are bound, combine to form an optionally substituted 5-membered heterocyclolalkyl group;

R_3 is H, R_C, or R_D;

R_4 is H, OH, OR_C, OR_D, R_C, or R_D;

R_5 is H, OH, OR_C, OR_D, R_C, or R_D;

R_6 is H, OH, OR_C, OR_D, R_C, or R_D;

R_7 is H, OH, OR_C, OR_D, R_C, or R_D;

R_8 is OH, NH_2, OR_C, OR_D, NHR_C, or NR_CR_D;

R_9 is H, OH, OR_C, or OR_D;

X is -S-, -S(O)-, or $-SO_2-$;

R_C is -L-Z;

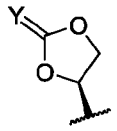
R_D is optionally substituted alkyl (e.g., C_1-C_6 alkyl), optionally substituted heteroalkyl (e.g., C_1-C_6 heteroalkyl), optionally substituted alkenyl (e.g., C_2-C_6 alkenyl), optionally substituted heteroalkenyl (e.g., C_2-C_6 heteroalkenyl), optionally substituted alkynyl (e.g., C_2-C_6 alkynyl), optionally substituted heteroalkynyl (e.g., C_2-C_6 heteroalkynyl), optionally substituted cycloalkyl, optionally substituted heterocycloalkyl, optionally substituted aryl, or optionally substituted heteroaryl;

L is a linker, such as optionally substituted alkylene (e.g., C_1-C_6 alkylene), optionally substituted heteroalkylene (C_1-C_6 heteroalkylene), optionally substituted alkenylene (e.g., C_2-C_6 alkenylene), optionally substituted heteroalkenylene (e.g., C_2-C_6 heteroalkenylene), optionally substituted alkynylene (e.g., C_2-C_6 alkynylene), optionally substituted heteroalkynylene (e.g., C_2-C_6 heteroalkynylene), optionally substituted cycloalkylene, optionally substituted heterocycloalkylene, optionally substituted arylene, or optionally substituted heteroarylene;

Z is a chemical moiety formed from a coupling reaction between a reactive substituent present on L and a reactive substituent present within an antibody, or antigen-binding fragment thereof, that binds CD2; and

wherein Am contains exactly one R_C substituent.

In some embodiments, R_A and R_B, together with the oxygen atoms to which they are bound, combine to form:



wherein Y is selected from O, S, NR_E, and $CR_ER_{E'}$, and

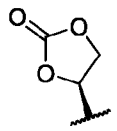
R_E and $R_{E'}$ are each independently optionally substituted C_1-C_6 alkylene-R_C, optionally substituted C_1-C_6 heteroalkylene-R_C, optionally substituted C_2-C_6 alkenylene-R_C, optionally substituted C_2-C_6 heteroalkenylene-R_C, optionally substituted C_2-C_6 alkynylene-R_C, optionally substituted C_2-C_6 heteroalkynylene-R_C, optionally substituted cycloalkylene-R_C, optionally substituted heterocycloalkylene-R_C, optionally substituted arylene-R_C, or optionally substituted heteroarylene-R_C.

In some embodiments, Am is represented by formula (**IA**) or formula (**IB**),

wherein R_1 is H, OH, OR_A, or OR_C;

R_2 is H, OH, OR_B, or OR_C;

R_A and R_B, together with the oxygen atoms to which they are bound, combine to form:



R_3 is H or R_C;

R_4 is H, OH, OR_C, OR_D, R_C, or R_D;

R_5 is H, OH, OR_C, OR_D, R_C, or R_D;

R_6 is H, OH, OR_C, OR_D, R_C, or R_D;

R_7 is H, OH, OR_C, OR_D, R_C, or R_D;

R_8 is OH, NH_2, OR_C, or NHR_C;

R_9 is H or OH; and

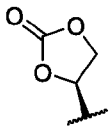
wherein R_C and R_D are each as defined above.

In some embodiments, Am is represented by formula (**IA**) or formula (**IB**),

wherein R_1 is H, OH, OR_A, or OR_C;

R_2 is H, OH, OR_B, or OR_C;

R_A and R_B, together with the oxygen atoms to which they are bound, combine to form:



R_3 is H or R_C;

R_4 and R_5 are each independently H, OH, OR_C, R_C, or OR_D;

R_6 and R_7 are each H;

R_8 is OH, NH_2, OR_C, or NHR_C;

R_9 is H or OH; and

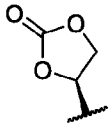
wherein R_C is as defined above.

In some embodiments, Am is represented by formula (**IA**) or formula (**IB**),

wherein R_1 is H, OH, or OR_A;

R_2 is H, OH, or OR_B;

R_A and R_B, together with the oxygen atoms to which they are bound, combine to form:



R_3, R_4, R_6, and R_7 are each H;

R_5 is OR_C;

R_8 is OH or NH_2;

R_9 is H or OH; and

wherein R_C is as defined above. Such amatoxin conjugates are described, for example,

in US Patent Application Publication No. 2016/0002298, the disclosure of which is incorporated herein by reference in its entirety.

In some embodiments, Am is represented by formula (**IA**) or formula (**IB**),

wherein R_1 and R_2 are each independently H or OH;

R_3 is R_C;

R_4, R_6, and R_7 are each H;

R_5 is H, OH, or OC_1-C_6 alkyl;

R_8 is OH or NH_2;

R_9 is H or OH; and

wherein R_C is as defined above. Such amatoxin conjugates are described, for example, in US Patent Application Publication No. 2014/0294865, the disclosure of which is incorporated herein by reference in its entirety.

In some embodiments, Am is represented by formula (**IA**) or formula (**IB**),

wherein R_1 and R_2 are each independently H or OH;

R_3, R_6, and R_7 are each H;

R_4 and R_5 are each independently H, OH, OR_C, or R_C;

R_8 is OH or NH_2;

R_9 is H or OH; and

wherein R_C is as defined above. Such amatoxin conjugates are described, for example, in US Patent Application Publication No. 2015/0218220, the disclosure of which is incorporated herein by reference in its entirety.

In some embodiments, Am is represented by formula (**IA**) or formula (**IB**),

wherein R_1 and R_2 are each independently H or OH;

R_3, R_6, and R_7 are each H;

R_4 and R_5 are each independently H or OH;

R_8 is OH, NH_2, OR_C, or NHR_C;

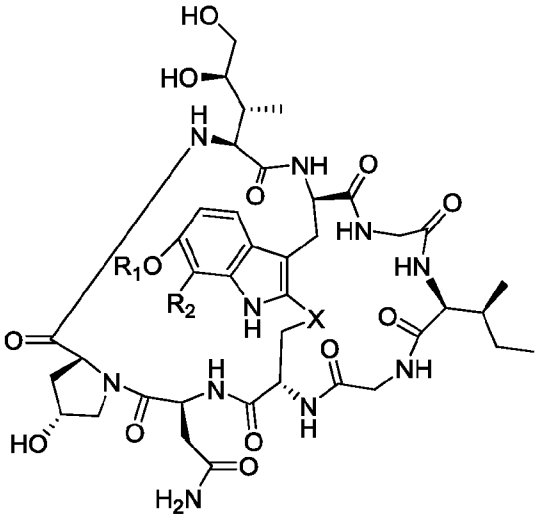
R_9 is H or OH; and

wherein R_C is as defined above. Such amatoxin conjugates are described, for example, in US Patent Nos. 9,233,173 and 9,399,681, as well as in US 2016/0089450, the disclosures of each of which are incorporated herein by reference in their entirety.

Additional amatoxins that may be used for conjugation to an antibody, or antigen-binding

fragment thereof, in accordance with the compositions and methods described herein are described, for example, in WO 2016/142049; WO 2016/071856; and WO 2017/046658, the disclosures of each of which are incorporated herein by reference in their entirety.

In some embodiments, Am is represented by formula (**II**),



(II)

wherein X is S, SO, or SO_2;

R_1 is H or a linker covalently bound to the antibody or antigen-binding fragment thereof;

R_2 is H or a linker covalently bound to the antibody or antigen-binding fragment thereof; and

wherein when R_1 is H, R_2 is the linker, and when R_2 is H, R_1 is the linker.

Antibodies, and antigen-binding fragments, for use with the compositions and methods described herein can be conjugated to an amatoxin, such as α-amanitin or a variant thereof, using conjugation techniques known in the art or described herein. For instance, antibodies, and antigen-binding fragments thereof, that recognize and bind CD2 can be conjugated to an amatoxin, such as α-amanitin or a variant thereof, as described in US 2015/0218220, the disclosure of which is incorporated herein by reference as it pertains, for example, to amatoxins, such as α-amanitin and variants thereof, as well as covalent linkers that can be used for covalent conjugation.

Exemplary antibody- drug conjugates useful in conjunction with the methods described herein may be formed by the reaction of an antibody, or an antigen-binding fragment thereof, with

an amatoxin that is conjugated to a linker containing a substituent suitable for reaction with a reactive residue on the antibody, or the antigen-binding fragment thereof. Amatoxins that are conjugated to a linker containing a substituent suitable for reaction with a reactive residue on the antibody, or antigen-binding fragment thereof, described herein include, without limitation, 7'C-(4-(6-(maleimido)hexanoyl)piperazin-1-yl)-amatoxin; 7'C-(4-(6-(maleimido)hexanamido)piperidin-1-yl)-amatoxin; 7'C-(4-(6-(6-(maleimido)hexanamido)hexanoyl)piperazin-1-yl)-amatoxin; 7'C-(4-(4-((maleimido)methyl)cyclohexanecarbonyl)piperazin-1-yl)-amatoxin; 7'C-(4-(6-(4-((maleimido)methyl)cyclohexanecarboxamido)hexanoyl)piperazin-1-yl)-amatoxin; 7'C-(4-(2-(6-(maleimido)hexanamido)ethyl)piperidin-1-yl)-amatoxin; 7'C-(4-(2-(6-(6-(maleimido)hexanamido)hexanamido)ethyl)piperidin-1-yl)-amatoxin; 7'C-(4-(2-(4-((maleimido)methyl)cyclohexanecarboxamido)ethyl)piperidin-1-yl)-amatoxin; 7'C-(4-(2-(6-(4-((maleimido)methyl)cyclohexanecarboxamido)hexanamido)ethyl)piperidin-1-yl)-amatoxin; 7'C-(4-(2-(3-carboxypropanamido)ethyl)piperidin-1-yl)-amatoxin; 7'C-(4-(2-(2-bromoacetamido)ethyl)piperidin-1-yl)-amatoxin; 7'C-(4-(2-(3-(pyridin-2-yldisulfanyl)propanamido)ethyl)piperidin-1-yl)-amatoxin; 7'C-(4-(2-(4-(maleimido)butanamido)ethyl)piperidin-1-yl)-amatoxin; 7'C-(4-(2-(maleimido)acetyl)piperazin-1-yl)-amatoxin; 7'C-(4-(3-(maleimido)propanoyl)piperazin-1-yl)-amatoxin; 7'C-(4-(4-(maleimido)butanoyl)piperazin-1-yl)-amatoxin; 7'C-(4-(2-(6-(4-((maleimido)methyl)cyclohexanecarboxamido)hexanamido)ethyl)piperidin-1-yl)-amatoxin; 7'C-(3-((6-(maleimido)hexanamido)methyl)pyrrolidin-1-yl)-amatoxin; 7'C-(3-((6-(6-(maleimido)hexanamido)hexanamido)methyl)pyrrolidin-1-yl)-amatoxin; 7'C-(3-((4-((maleimido)methyl)cyclohexanecarboxamido)methyl)pyrrolidin-1-yl)-amatoxin; 7'C-(3-((6-((4-(maleimido)methyl)cyclohexanecarboxamido)hexanamido)methyl)pyrrolidin-1-yl)-amatoxin; 7'C-(4-(2-(6-(2-(aminooxy)acetamido)hexanamido)ethyl)piperidin-1-yl)-amatoxin; 7'C-(4-(2-(4-(2-(aminooxy)acetamido)butanamido)ethyl)piperidin-1-yl)-amatoxin; 7'C-(4-(4-(2-(aminooxy)acetamido)butanoyl)piperazin-1-yl)-amatoxin; 7'C-(4-(6-(2-(aminooxy)acetamido)hexanoyl)piperazin-1-yl)-amatoxin; 7'C-((4-(6-(maleimido)hexanamido)piperidin-1-yl)methyl)-amatoxin; 7'C-((4-(2-(6-(maleimido)hexanamido)ethyl)piperidin-1-yl)methyl)-amatoxin; 7'C-((4-(6-(maleimido)hexanoyl)piperazin-1-yl)methyl)-amatoxin; (R)-7'C-((3-((6-(maleimido)hexanamido)methyl)pyrrolidin-1-yl)methyl)-amatoxin; (S)-7'C-((3-((6-

(maleimido)hexanamido)methyl)pyrrolidin-1-yl)methyl)-amatoxin; 7'C-((4-(2-(6-(6-(maleimido)hexanamido)hexanamido)ethyl)piperidin-1-yl)methyl)-amatoxin; 7'C-((4-(2-(4-((maleimido)methyl)cyclohexanecarboxamido)ethyl)piperidin-1-yl)methyl)-amatoxin; 7'C-((4-(2-(6-(4-((maleimido)methyl)cyclohexanecarboxamido)hexanamido)ethyl)piperidin-1-yl)methyl)-amatoxin; 7'C-((4-(2-(6-(maleimido)hexanamido)ethyl)piperazin-1-yl)methyl)-amatoxin; 7'C-((4-(2-(6-(6-(maleimido)hexanamido)hexanamido)ethyl)piperazin-1-yl)methyl)-amatoxin; 7'C-((4-(2-(4-((maleimido)methyl)cyclohexanecarboxamido)ethyl)piperazin-1-yl)methyl)-amatoxin; 7'C-((4-(2-(6-(4-((maleimido)methyl)cyclohexanecarboxamido)hexanamido)ethyl)piperazin-1-yl)methyl)-amatoxin; 7'C-((3-((6-(6-(maleimido)hexanamido)hexanamido)-S-methyl)pyrrolidin-1-yl)methyl)-amatoxin; 7'C-((3-((6-(6-(maleimido)hexanamido)hexanamido)-R-methyl)pyrrolidin-1-yl)methyl)-amatoxin; 7'C-((3-((4-((maleimido)methyl)cyclohexanecarboxamido)-S-methyl)pyrrolidin-1-yl)methyl)-amatoxin; 7'C-((3-((4-((maleimido)methyl)cyclohexanecarboxamido)-R-methyl)pyrrolidin-1-yl)methyl)-amatoxin; 7'C-((3-((6-(4-((maleimido)methyl)cyclohexanecarboxamido)hexanamido)methyl)pyrrolidin-1-yl)methyl)-amatoxin; 7'C-((4-(2-(3-carboxypropanamido)ethyl)piperazin-1-yl)methyl)-amatoxin; 7'C-((4-(6-(6-(maleimido)hexanamido)hexanoyl)piperazin-1-yl)methyl)-amatoxin; 7'C-((4-(6-(4-((maleimido)methyl)cyclohexanecarboxamido)hexanoyl)piperazin-1-yl)methyl)-amatoxin; 7'C-((4-(2-(maleimido)acetyl)piperazin-1-yl)methyl)-amatoxin; 7'C-((4-(3-(maleimido)propanoyl)piperazin-1-yl)methyl)-amatoxin; 7'C-((4-(4-(maleimido)butanoyl)piperazin-1-yl)methyl)-amatoxin; 7'C-((4-(2-(2-(maleimido)acetamido)ethyl)piperidin-1-yl)methyl)-amatoxin; 7'C-((4-(2-(4-(maleimido)butanamido)ethyl)piperidin-1-yl)methyl)-amatoxin; 7'C-((4-(2-(6-(4-((maleimido)methyl)cyclohexanecarboxamido)hexanamido)ethyl)piperidin-1-yl)methyl)-amatoxin; 7'C-((3-((6-(maleimido)hexanamido)methyl)azetidin-1-yl)methyl)-amatoxin; 7'C-((3-(2-(6-(maleimido)hexanamido)ethyl)azetidin-1-yl)methyl)-amatoxin; 7'C-((3-((4-((maleimido)methyl)cyclohexanecarboxamido)methyl)azetidin-1-yl)methyl)-amatoxin; 7'C-((3-(2-(4-((maleimido)methyl)cyclohexanecarboxamido)ethyl)azetidin-1yl)methyl)-amatoxin; 7'C-((3-(2-(6-(4-((maleimido)methyl)cyclohexanecarboxamido)hexanamido)ethyl)azetidin-1-yl)methyl)-amatoxin; 7'C-(((2-(6-(maleimido)-N-methylhexanamido)ethyl)(methyl)amino)methyl)-amatoxin; 7'C-(((4-(6-(maleimido)-N-methylhexanamido)butyl(methyl)amino)methyl)-amatoxin; 7'C-((2-(2-(6-(maleimido)hexanamido)ethyl)aziridin-1-yl)methyl)-amatoxin; 7'C-((2-(2-(6-(4-((maleimido)methyl)cyclohexanecarboxamido)hexanamido)ethyl)aziridin-1-yl)methyl)-amatoxin;

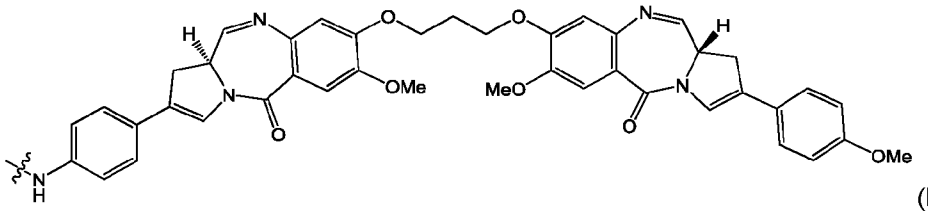
7'C-((4-(6-(6-(2-(aminooxy)acetamido)hexanamido)hexanoyl)piperazin-1-yl)methyl)-amatoxin; 7'C-((4-(1-(aminooxy)-2-oxo-6,9,12,15-tetraoxa-3-azaheptadecan-17-oyl)piperazin-1-yl)methyl)-amatoxin; 7'C-((4-(2-(2-(aminooxy)acetamido)acetyl)piperazin-1-yl)methyl)-amatoxin; 7'C-((4-(3-(2-(aminooxy)acetamido)propanoyl)piperazin-1-yl)methyl)-amatoxin; 7'C-((4-(4-(2-(aminooxy)acetamido)butanoyl)piperazin-1-yl)methyl)-amatoxin; 7'C-((4-(2-(6-(2-(aminooxy)acetamido)hexanamido)ethyl)piperidin-1-yl)methyl)-amatoxin; 7'C-((4-(2-(2-(2-(aminooxy)acetamido)acetamido)ethyl)piperidin-1-yl)methyl)-amatoxin; 7'C-((4-(2-(4-(2-(aminooxy)acetamido)butanamido)ethyl)piperidin-1-yl)methyl)-amatoxin; 7'C-((4-(20-(aminooxy)-4,19-dioxo-6,9,12,15-tetraoxa-3,18-diazaicosyl)piperidin-1-yl)methyl)-amatoxin; 7'C-(((2-(6-(2-(aminooxy)acetamido)-N-methylhexanamido)ethyl)(methyl)amino)methyl)-amatoxin; 7'C-(((4-(6-(2-(aminooxy)acetamido)-N-methylhexanamido)butyl)(methyl)amino)methyl)-amatoxin; 7'C-((3-((6-(4-((maleimido)methyl)cyclohexanecarboxamido)hexanamido)methyl)pyrrolidin-1-yl)-S-methyl)-amatoxin; 7'C-((3-((6-(4-((maleimido)methyl)cyclohexanecarboxamido)hexanamido)-R-methyl)pyrrolidin-1-yl)methyl)-amatoxin; 7'C-((4-(2-(2-bromoacetamido)ethyl)piperazin-1-yl)methyl)-amatoxin; 7'C-((4-(2-(2-bromoacetamido)ethyl)piperidin-1-yl)methyl)-amatoxin; 7'C-((4-(2-(3-(pyridine-2-yldisulfanyl)propanamido)ethyl)piperidin-1-yl)methyl)-amatoxin; 6'O-(6-(6-(maleimido)hexanamido)hexyl)-amatoxin; 6'O-(5-(4-((maleimido)methyl)cyclohexanecarboxamido)pentyl)-amatoxin; 6'O-(2-((6-(maleimido)hexyl)oxy)-2-oxoethyl)-amatoxin; 6'O-((6-(maleimido)hexyl)carbamoyl)-amatoxin; 6'O-((6-(4-((maleimido)methyl)cyclohexanecarboxamido)hexyl)carbamoyl)-amatoxin; 6'O-(6-(2-bromoacetamido)hexyl)-amatoxin; 7'C-(4-(6-(azido)hexanamido)piperidin-1-yl)-amatoxin; 7'C-(4-(hex-5-ynoylamino)piperidin-1-yl)-amatoxin; 7'C-(4-(2-(6-(maleimido)hexanamido)ethyl)piperazin-1-yl)-amatoxin; 7'C-(4-(2-(6-(6-(maleimido)hexanamido)hexanamido)ethyl)piperazin-1-yl)-amatoxin; 6'O-(6-(6-(11,12-didehydro-5,6-dihydro-dibenz[b,f]azocin-5-yl)-6-oxohexanamido)hexyl)-amatoxin; 6'O-(6-(hex-5-ynoylamino)hexyl)-amatoxin; 6'O-(6-(2-(aminooxy)acetylamido)hexyl)-amatoxin; 6'O-((6-aminooxy)hexyl)-amatoxin; and 6'O-(6-(2-iodoacetamido)hexyl)-amatoxin. The foregoing linkers, among others useful in conjunction with the compositions and methods described herein, are described, for example, in US Patent Application Publication No. 2015/0218220, the disclosure of which is incorporated herein by reference in its entirety.

Additional cytotoxins that can be conjugated to antibodies, and antigen-binding fragments

thereof, that recognize and bind CD2 for use in directly treating a cancer, autommine condition, or for conditioning a patient (e.g., a human patient) in preparation for hematopoietic stem cell transplant therapy include, without limitation, 5-ethynyluracil, abiraterone, acylfulvene, adecypenol, adozelesin, aldesleukin, altretamine, ambamustine, amidox, amifostine, aminolevulinic acid, amrubicin, amsacrine, anagrelide, anastrozole, andrographolide, angiogenesis inhibitors, antarelix, anti-dorsalizing morphogenetic protein-1, antiandrogen, prostatic carcinoma, antiestrogen, antineoplaston, antisense oligonucleotides, aphidicolin glycinate, apoptosis gene modulators, apoptosis regulators, apurinic acid, asulacrine, atamestane, atrimustine, axinastatin 1, axinastatin 2, axinastatin 3, azasetron, azatoxin, azatyrosine, baccatin III derivatives, balanol, batimastat, BCR/ABL antagonists, benzochlorins, benzoylstaurosporine, beta lactam derivatives, beta-alethine, betaclamycin B, betulinic acid, bFGF inhibitors, bicalutamide, bisantrene, bisaziridinylspermine, bisnafide, bistratene A, bizelesin, breflate, bleomycin A2, bleomycin B2, bropirimine, budotitane, buthionine sulfoximine, calcipotriol, calphostin C, camptothecin derivatives (e.g., 10-hydroxy-camptothecin), capecitabine, carboxamide-amino-triazole, carboxyamidotriazole, carzelesin, casein kinase inhibitors, castanospermine, cecropin B, cetrorelix, chlorins, chloroquinoxaline sulfonamide, cicaprost, cis-porphyrin, cladribine, clomifene and analogues thereof, clotrimazole, collismycin A, collismycin B, combretastatin A4, combretastatin analogues, conagenin, crambescidin 816, crisnatol, cryptophycin 8, cryptophycin A derivatives, curacin A, cyclopentanthraquinones, cycloplatam, cypemycin, cytarabine ocfosfate, cytolytic factor, cytostatin, dacliximab, decitabine, dehydrodidemnin B, 2'deoxycoformycin (DCF), deslorelin, dexifosfamide, dexrazoxane, dexverapamil, diaziquone, didemnin B, didox, diethylnorspermine, dihydro-5-azacytidine, dihydrotaxol, dioxamycin, diphenyl spiromustine, discodermolide, docosanol, dolasetron, doxifluridine, droloxifene, dronabinol, duocarmycin SA, ebselen, ecomustine, edelfosine, edrecolomab, eflornithine, elemene, emitefur, epothilones, epithilones, epristeride, estramustine and analogues thereof, etoposide, etoposide 4'-phosphate (also referred to as etopofos), exemestane, fadrozole, fazarabine, fenretinide, filgrastim, finasteride, flavopiridol, flezelastine, fluasterone, fludarabine, fluorodaunorunicin hydrochloride, forfenimex, formestane, fostriecin, fotemustine, gadolinium texaphyrin, gallium nitrate, galocitabine, ganirelix, gelatinase inhibitors, gemcitabine, glutathione inhibitors, hepsulfam, homoharringtonine (HHT), hypericin, ibandronic acid, idoxifene, idramantone, ilmofosine, ilomastat, imidazoacridones, imiquimod, immunostimulant peptides, iobenguane, iododoxorubicin, ipomeanol, irinotecan, iroplact, irsogladine, isobengazole, jasplakinolide, kahalalide F, lamellarin-N triacetate, lanreotide, leinamycin,

lenograstim, lentinan sulfate, leptolstatin, letrozole, lipophilic platinum compounds, lissoclinamide 7, lobaplatin, lometrexol, lonidamine, losoxantrone, loxoribine, lurtotecan, lutetium texaphyrin, lysofylline, masoprocol, maspin, matrix metalloproteinase inhibitors, menogaril, merbarone, meterelin, methioninase, metoclopramide, MIF inhibitor, ifepristone, miltefosine, mirimostim, mithracin, mitoguazone, mitolactol, mitomycin and analogues thereof, mitonafide, mitoxantrone, mofarotene, molgramostim, mycaperoxide B, myriaporone, N-acetyldinaline, N-substituted benzamides, nafarelin, nagrestip, napavin, naphterpin, nartograstim, nedaplatin, nemorubicin, neridronic acid, nilutamide, nisamycin, nitrullyn, octreotide, okicenone, onapristone, ondansetron, oracin, ormaplatin, oxaliplatin, oxaunomycin, paclitaxel and analogues thereof, palauamine, palmitoylrhizoxin, pamidronic acid, panaxytriol, panomifene, parabactin, pazelliptine, pegaspargase, peldesine, pentosan polysulfate sodium, pentostatin, pentrozole, perflubron, perfosfamide, phenazinomycin, picibanil, pirarubicin, piritrexim, podophyllotoxin, porfiromycin, purine nucleoside phosphorylase inhibitors, raltitrexed, rhizoxin, rogletimide, rohitukine, rubiginone B1, ruboxyl, safingol, saintopin, sarcophytol A, sargramostim, sobuzoxane, sonermin, sparfosic acid, spicamycin D, spiromustine, stipiamide, sulfinosine, tallimustine, tegafur, temozolomide, teniposide, thaliblastine, thiocoraline, tirapazamine, topotecan, topsentin, triciribine, trimetrexate, veramine, vinorelbine, vinxaltine, vorozole, zeniplatin, and zilascorb, among others.

In some embodiments, the cytotoxin is a pyrrolobenzodiazepine dimer represented by formula (**IV**):



(**IV**)

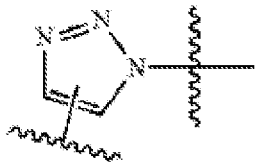
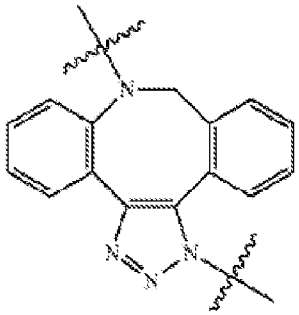
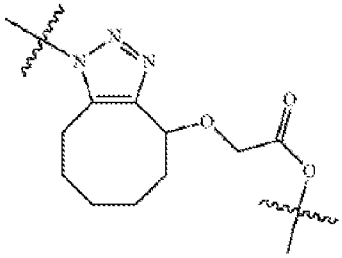
A variety of linkers can be used to conjugate antibodies, and antigen-binding fragments, described herein that recognize and bind CD2, with a cytotoxic molecule. Linkers include those that may be cleaved, for instance, by enzymatic hydrolysis, photolysis, hydrolysis under acidic conditions, hydrolysis under basic conditions, oxidation, disulfide reduction, nucleophilic cleavage, or organometallic cleavage (see, for example, Leriche et al., Bioorg. Med. Chem., 20:571-582, 2012, the disclosure of which is incorporated herein by reference as it pertains to

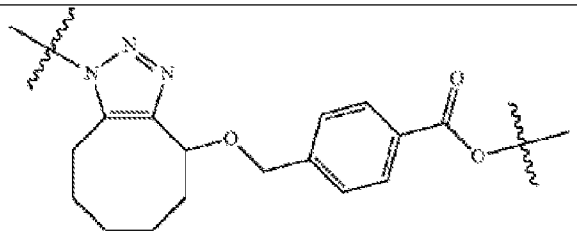
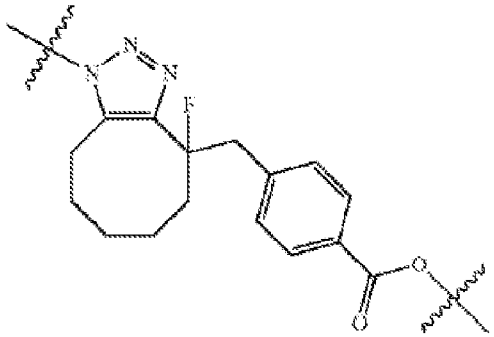
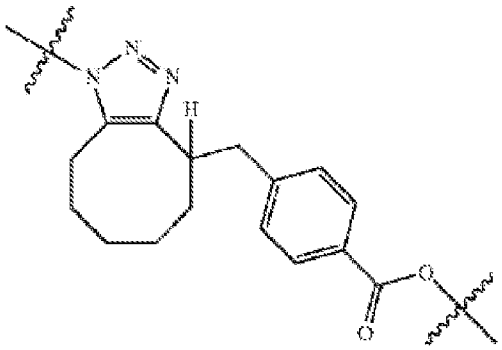
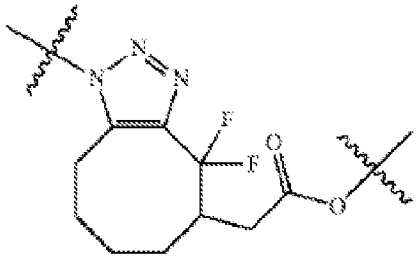
linkers suitable for covalent conjugation). Examples of linkers useful for the synthesis of drug-antibody conjugates include those that contain electrophiles, such as Michael acceptors (e.g., maleimides), activated esters, electron-deficient carbonyl compounds, and aldehydes, among others, suitable for reaction with nucleophilic substituents present within antibodies or antigen-binding fragments, such as amine and thiol moieties. For instance, linkers suitable for the synthesis of drug-antibody conjugates include, without limitation, succinimidyl 4-(N-maleimidomethyl)-cyclohexane-L-carboxylate (SMCC), N- succinimidyl iodoacetate (SIA), sulfo-SMCC, *m*-maleimidobenzoyl-*N*-hydroxysuccinimidyl ester (MBS), sulfo-MBS, and succinimidyl iodoacetate, among others described, for instance, Liu et al., 18:690-697, 1979, the disclosure of which is incorporated herein by reference as it pertains to linkers for chemical conjugation. Additional linkers include the non-cleavable maleimidocaproyl linkers, which are particularly useful for the conjugation of microtubule-disrupting agents such as auristatins, are described by Doronina et al., Bioconjugate Chem. 17:14-24, 2006, the disclosure of which is incorporated herein by reference as it pertains to linkers for chemical conjugation. Additional linkers suitable for the synthesis of drug-antibody conjugates as described herein include those capable of releasing a cytotoxin by a 1,6-elimination process, such as *p*-aminobenzyl alcohol (PABC), 6-maleimidohexanoic acid, pH-sensitive carbonates, and other reagents described in Jain et al., Pharm. Res. 32:3526-3540, 2015, the disclosure of which is incorporated herein by reference in its entirety.

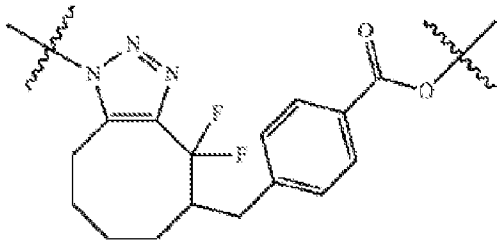
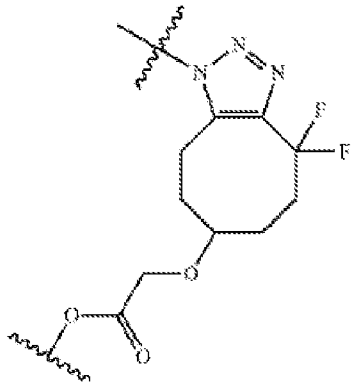
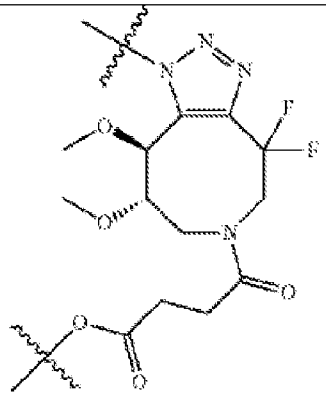
Linkers that can be used to conjugate an antibody, or an antigen-binding fragment thereof, to a cytotoxic agent include those that are covalently bound to the cytotoxic agent on one end of the linker and, on the other end of the linker, contain a chemical moiety formed from a coupling reaction between a reactive substituent present on the linker and a reactive substituent present within the antibody, or an antigen-binding fragment thereof, that binds CD2. Reactive substituents that may be present within an antibody, or an antigen-binding fragment thereof, that binds CD2 include, without limitation, hydroxyl moieties of serine, threonine, and tyrosine residues; amino moieties of lysine residues; carboxyl moieties of aspartic acid and glutamic acid residues; and thiol moieties of cysteine residues, as well as propargyl, azido, haloaryl (e.g., fluoroaryl), haloheteroaryl (e.g., fluoroheteroaryl), haloalkyl, and haloheteroalkyl moieties of non-naturally occurring amino acids. Linkers useful in conjunction with the antibody-drug conjugates described herein include, without limitation, linkers containing chemical moieties formed by

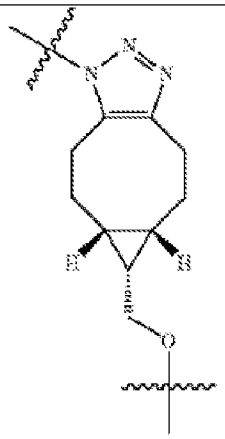
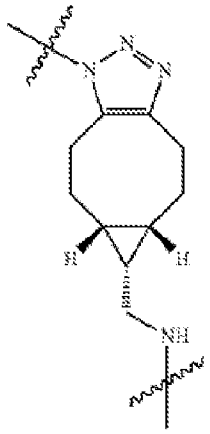
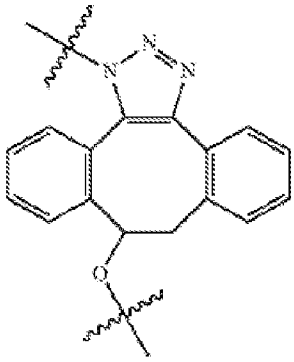
coupling reactions as depicted in Table 1, below. Curved lines designate points of attachment to the antibody, or antigen-binding fragment, and the cytotoxic molecule, respectively.

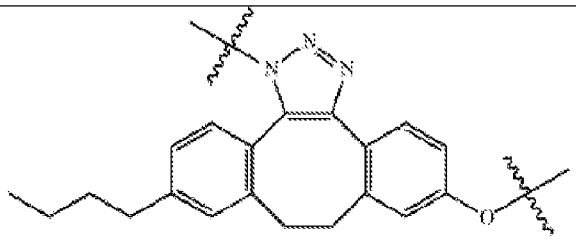
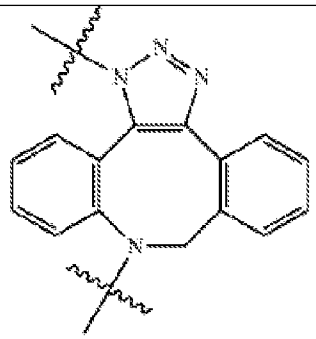
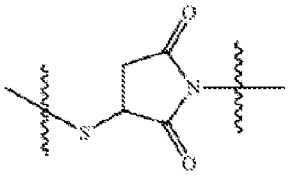
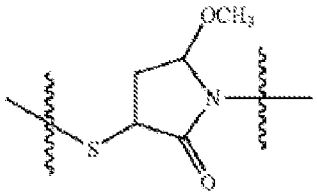
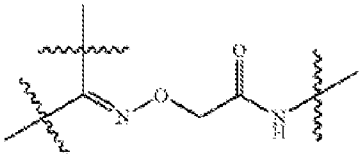
Table 1. Exemplary chemical moieties formed by coupling reactions in the formation of antibody-drug conjugates

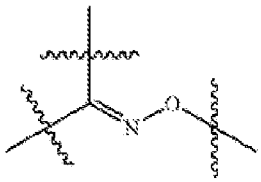
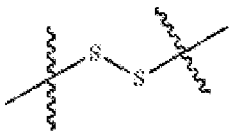
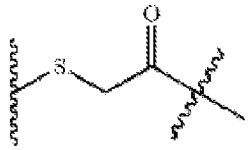
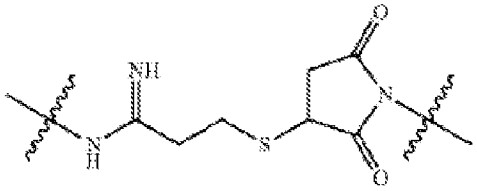
Exemplary Coupling Reactions	**Chemical Moiety Formed by Coupling Reactions**
[3+2] Cycloaddition	
[3+2] Cycloaddition	
[3+2] Cycloaddition, Esterification	

[3+2] Cycloaddition, Esterification	
[3+2] Cycloaddition, Esterification	
[3+2] Cycloaddition, Esterification	
[3+2] Cycloaddition, Esterification	

[3+2] Cycloaddition, Esterification	
[3+2] Cycloaddition, Esterification	
[3+2] Cycloaddition, Esterification	

[3+2] Cycloaddition, Esterification	
[3+2] Cycloaddition, Esterification	
[3+2] Cycloaddition, Esterification	

[3+2] Cycloaddition, Etherification	
[3+2] Cycloaddition	
Michael addition	
Michael addition	
Imine condensation, Amidation	

Imine condensation	
Disulfide formation	
Thiol alkylation	
Condensation, Michael addition	

Methods of Treatment

As described herein, hematopoietic stem cell transplant therapy can be administered to a subject in need of treatment so as to populate or re-populate one or more blood cell types. Hematopoietic stem cells generally exhibit multi-potency, and can thus differentiate into multiple different blood lineages including, but not limited to, granulocytes (e.g., promyelocytes, neutrophils, eosinophils, basophils), erythrocytes (e.g., reticulocytes, erythrocytes), thrombocytes (e.g., megakaryoblasts, platelet producing megakaryocytes, platelets), monocytes (e.g., monocytes, macrophages), dendritic cells, microglia, osteoclasts, and lymphocytes (e.g., NK cells, B-cells and T-cells). Hematopoietic stem cells are additionally capable of self-renewal, and can thus give rise to daughter cells that have equivalent potential as the mother cell, and also

feature the capacity to be reintroduced into a transplant recipient whereupon they home to the hematopoietic stem cell niche and re-establish productive and sustained hematopoiesis.

Hematopoietic stem cells can thus be administered to a patient defective or deficient in one or more cell types of the hematopoietic lineage in order to re-constitute the defective or deficient population of cells in vivo, thereby treating the pathology associated with the defect or depletion in the endogenous blood cell population. The compositions and methods described herein can thus be used to treat a non-malignant hemoglobinopathy (e.g., a hemoglobinopathy selected from the group consisting of sickle cell anemia, thalassemia, Fanconi anemia, aplastic anemia, and Wiskott-Aldrich syndrome). Additionally or alternatively, the compositions and methods described herein can be used to treat an immunodeficiency, such as a congenital immunodeficiency. Additionally or alternatively, the compositions and methods described herein can be used to treat an acquired immunodeficiency (e.g., an acquired immunodeficiency selected from the group consisting of HIV and AIDS). The compositions and methods described herein can be used to treat a metabolic disorder (e.g., a metabolic disorder selected from the group consisting of glycogen storage diseases, mucopolysaccharidoses, Gaucher's Disease, Hurlers Disease, sphingolipidoses, and metachromatic leukodystrophy).

Additionally or alternatively, the compositions and methods described herein can be used to treat a malignancy or proliferative disorder, such as a hematologic cancer, myeloproliferative disease. In the case of cancer treatment, the compositions and methods described herein may be administered to a patient prior to hematopoietic stem cell transplantation therapy in order to deplete a population of immune cells that cross-react with, and mount an immune response against, non-self hematopoietic stem cells. This serves to prevent or reduce the likelihood of rejection of the transplanted hematopoietic stem cell grafts, allowing the transplanted hematopoietic stem cells to home to a stem cell niche and establish productive hematopoiesis. This, in turn, can re-constitute a population of cells depleted during cancer cell eradication, such as during systemic chemotherapy. Exemplary hematological cancers that can be treated using the compositions and methods described herein include, without limitation, acute myeloid leukemia, acute lymphoid leukemia, chronic myeloid leukemia, chronic lymphoid leukemia, multiple myeloma, diffuse large B-cell lymphoma, and non-Hodgkin's lymphoma, as well as other cancerous conditions, including neuroblastoma.

Additional diseases that can be treated with the compositions and methods described herein include, without limitation, adenosine deaminase deficiency and severe combined immunodeficiency, hyper immunoglobulin M syndrome, Chediak-Higashi disease, hereditary lymphohistiocytosis, osteopetrosis, osteogenesis imperfecta, storage diseases, thalassemia major, systemic sclerosis, systemic lupus erythematosus, multiple sclerosis, and juvenile rheumatoid arthritis.

The antibodies, or antigen-binding fragments thereof, and conjugates described herein may be used to induce solid organ transplant tolerance. For instance, the compositions and methods described herein may be used to deplete or ablate a population of immune cells prior to hematopoietic stem cell transplantation. Following such depletion of cells from the target tissues, a population of stem or progenitor cells from an organ donor (e.g., hematopoietic stem cells from the organ donor) may be administered to the transplant recipient, and following the engraftment of such stem or progenitor cells, a temporary or stable mixed chimerism may be achieved, thereby enabling long-term transplant organ tolerance without the need for further immunosuppressive agents. The likelihood of rejection of the transplanted graft can be reduced, or rejection may be prevented altogether, by administration of the anti-CD2 antibody, or antigen-binding fragment thereof. In this way, the compositions and methods described herein may be used to induce transplant tolerance in a solid organ transplant recipient (e.g., a kidney transplant, lung transplant, liver transplant, and heart transplant, among others). The compositions and methods described herein are well-suited for use in connection the induction of solid organ transplant tolerance, for instance, because a low percentage temporary or stable donor engraftment is sufficient to induce long-term tolerance of the transplanted organ.

In addition, the compositions and methods described herein can be used to treat cancers directly, such as cancers characterized by cells that are CD2+. For instance, the compositions and methods described herein can be used to treat leukemia, particularly in patients that exhibit CD2+ leukemic cells. By depleting CD2+ cancerous cells, such as leukemic cells, the compositions and methods described herein can be used to treat various cancers directly. Exemplary cancers that may be treated in this fashion include hematological cancers, such as acute myeloid leukemia, acute lymphoid leukemia, chronic myeloid leukemia, chronic lymphoid leukemia, multiple myeloma, diffuse large B-cell lymphoma, and non-Hodgkin's lymphoma,

In addition, the compositions and methods described herein can be used to treat autoimmune disorders. For instance, an antibody, or antigen-binding fragment thereof, can be administered to a subject, such as a human patient suffering from an autoimmune disorder, so as to kill a CD2+ immune cell. The CD2+ immune cell may be an autoreactive lymphocyte, such as a T-cell that expresses a T-cell receptor that specifically binds, and mounts an immune response against, a self antigen. By depleting self-reactive, CD2+ cells, the compositions and methods described herein can be used to treat autoimmune pathologies, such as those described below. Additionally or alternatively, the compositions and methods described herein can be used to treat an autoimmune disease by depleting a population of endogenous hematopoietic stem cells prior to hematopoietic stem cell transplantation therapy, in which case the transplanted cells can home to a niche created by the endogenous cell depletion step and establish productive hematopoiesis. This, in turn, can re-constitute a population of cells depleted during autoimmune cell eradication.

Autoimmune diseases that can be treated using the compositions and methods described herein include, without limitation, psoriasis, psoriatic arthritis, Type 1 diabetes mellitus (Type 1 diabetes), rheumatoid arthritis (RA), human systemic lupus (SLE), multiple sclerosis (MS), inflammatory bowel disease (IBD), lymphocytic colitis, acute disseminated encephalomyelitis (ADEM), Addison's disease, alopecia universalis, ankylosing spondylitisis, antiphospholipid antibody syndrome (APS), aplastic anemia, autoimmune hemolytic anemia, autoimmune hepatitis, autoimmune inner ear disease (AIED), autoimmune lymphoproliferative syndrome (ALPS), autoimmune oophoritis, Balo disease, Behcet's disease, bullous pemphigoid, cardiomyopathy, Chagas' disease, chronic fatigue immune dysfunction syndrome (CFIDS), chronic inflammatory demyelinating polyneuropathy, Crohn's disease, cicatrical pemphigoid, coeliac sprue-dermatitis herpetiformis, cold agglutinin disease, CREST syndrome, Degos disease, discoid lupus, dysautonomia, endometriosis, essential mixed cryoglobulinemia, fibromyalgia-fibromyositis, Goodpasture' s syndrome, Grave's disease, Guillain-Barre syndrome (GBS), Hashimoto' s thyroiditis, Hidradenitis suppurativa, idiopathic and/or acute thrombocytopenic purpura, idiopathic pulmonary fibrosis, IgA neuropathy, interstitial cystitis, juvenile arthritis, Kawasaki's disease, lichen planus, Lyme disease, Meniere disease, mixed connective tissue disease (MCTD), myasthenia gravis, neuromyotonia, opsoclonus myoclonus syndrome (OMS), optic neuritis, Ord's thyroiditis, pemphigus vulgaris, pernicious anemia, polychondritis, polymyositis and dermatomyositis, primary biliary cirrhosis, polyarteritis nodosa,

polyglandular syndromes, polymyalgia rheumatica, primary agammaglobulinemia, Raynaud phenomenon, Reiter' s syndrome, rheumatic fever, sarcoidosis, scleroderma, Sjögren's syndrome, stiff person syndrome, Takayasu's arteritis, temporal arteritis (also known as "giant cell arteritis"), ulcerative colitis, collagenous colitis, uveitis, vasculitis, vitiligo, vulvodynia ("vulvar vestibulitis"), and Wegener' s granulomatosis.

Antibody drug conjugates comprising anti-CD2 antibodies, or antigen-binding fragments thereof, can also be used in combination with CAR T therapy. Specifically, an efffective amount of an anti-CD2 antibody drug conjugate can be administered to a patient in need thereof prior to CAR T treatment in order to deplete native T cells. Depletion of native T cells expressing CD2 using the methods and compositions described herein can provide for more effective transfer of enginereed T cells used in CAR T therapy.

Routes of Administration and Dosing

Antibodies, or antigen-binding fragments thereof, described herein can be administered to a patient (e.g., a human patient in need of hematopoietic stem cell transplant therapy) in a variety of dosage forms. For instance, antibodies, or antigen-binding fragments thereof, described herein can be administered to a patient in need of hematopoietic stem cell transplant therapy and/or suffering from cancer or an autoimmune disease in the form of an aqueous solution, such as an aqueous solution containing one or more pharmaceutically acceptable excipients. Exemplary pharmaceutically acceptable excipients for use with the compositions and methods described herein are viscosity-modifying agents. The aqueous solution may be sterilized using techniques known in the art.

The antibodies, and antigen-binding fragments, described herein may be administered by a variety of routes, such as orally, transdermally, subcutaneously, intranasally, intravenously, intramuscularly, intraocularly, or parenterally. The most suitable route for administration in any given case will depend on the particular antibody or antigen-binding fragment administered, the patient, pharmaceutical formulation methods, administration methods (e.g., administration time and administration route), the patient's age, body weight, sex, severity of the diseases being treated, the patient's diet, and the patient's excretion rate.

The effective dose of an antibody, or an antigen-binding fragment thereof, described herein can range, for example from about 0.001 to about 100 mg/kg of body weight per single

(e.g., bolus) administration, multiple administrations, or continuous administration, or to achieve an optimal serum concentration (e.g., a serum concentration of 0.0001-5000 μg/mL) of the antibody, or an antigen-binding fragment thereof. The dose may be administered one or more times (e.g., 2-10 times) per day, week, or month to a subject (e.g., a human) undergoing conditioning therapy in preparation for receipt of a hematopoietic stem cell transplant. The antibody or antigen-binding fragment thereof can be administered to the patient at a time that optimally promotes engraftment of the exogenous hematopoietic stem cells, for instance, at a time that optimally depletes CD2+ T cells or NK cells that cross-react with a non-self hematopoietic stem cell antigen (e.g., a non-self MHC antigen expressed by the hematopoietic stem cells) prior to hematopoietic stem cell transplantation. For example, anti-CD2 antibodies, and antigen-binding fragments thereof, may be administered to a patient undergoing hematopoietic stem cell transplant therapy from 1 hour to 1 week (e.g., 1 hour, 2 hours, 3 hours, 4 hours, 5 hours, 6 hours, 7 hours, 8 hours, 9 hours, 10 hours, 11 hours, 12 hours, 13 hours, 14 hours, 15 hours, 16 hours, 17 hours, 18 hours, 19 hours, 20 hours, 21 hours, 22 hours, 23 hours, 24 hours, 2 days, 3 days, 4 days, 5 days, 6 days, or 7 days) or more prior to administration of the exogenous hematopoietic stem cell transplant. The half-life of the antibody may be between 1 hour and 24 hours (e.g., 1 hour, 2 hours, 3 hours, 4 hours, 5 hours, 6 hours, 7 hours, 8 hours, 9 hours, 10 hours, 11, hours, 12 hours, 13 hours, 14 hours, 15 hours, 16 hours, 17 hours, 18 hours, 19 hours, 20 hours, 21 hours, 22 hours, 23 hours, or 24 hours).

Examples

The following examples are put forth so as to provide those of ordinary skill in the art with a description of how the compositions and methods described herein may be used, made, and evaluated, and are intended to be purely exemplary of the invention and are not intended to limit the scope of what the inventors regard as their invention.

Example 1. Administration of an anti-CD2 antibody to a human patient in preparation for hematopoietic stem cell transplant therapy

According to the methods disclosed herein, a physician of skill in the art can condition a patient, such as a human patient, so as to promote the engraftment of exogenous hematopoietic stem cell grafts prior to hematopoietic stem cell transplant therapy. To this end, a physician of

skill in the art can administer to the human patient an antibody, or antigen-binding fragment thereof, capable of binding CD2, such as an anti-CD2 antibody described herein. The antibody, or fragment thereof, may be covalently conjugated to a toxin, such as a cytotoxic molecule described herein or known in the art, or an Fc domain. For instance, an anti-CD2 antibody, or antigen-binding fragment thereof, can be covalently conjugated to a cytotoxin, such as pseudomonas exotoxin A, deBouganin, diphtheria toxin, an amatoxin, such as α-amanitin, saporin, maytansine, a maytansinoid, an auristatin, an anthracycline, a calicheamicin, irinotecan, SN-38, a duocarmycin, a pyrrolobenzodiazepine, a pyrrolobenzodiazepine dimer, an indolinobenzodiazepine, an indolinobenzodiazepine dimer, or a variant thereof. This conjugation can be performed using covalent bond-forming techniques described herein or known in the art. The antibody, antigen-binding fragment thereof, or antibody-drug conjugate can subsequently be administered to the patient, for example, by intravenous administration, prior to transplantation of exogenous hematopoietic stem cells (such as autologous, syngeneic, or allogeneic hematopoietic stem cells) to the patient.

The anti-CD2 antibody, antigen-binding fragment thereof, or antibody-drug conjugate, can be administered in an amount sufficient to reduce the quantity of endogenous T cells, such as bone marrow resident T cells, for example, by 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 95%, or more prior to hematopoietic stem cell transplant therapy. The reduction in T cell count can be monitored using conventional techniques known in the art, such as by FACS analysis of cells expressing characteristic T cell surface antigens in a blood sample withdrawn from the patient at varying intervals during conditioning therapy. For instance, a physician of skill in the art can withdraw a bone marrow sample from the patient at various time points during conditioning therapy and determine the extent of endogenous T cell reduction by conducting a FACS analysis to elucidate the relative concentrations of T cells in the sample using antibodies that bind to T cell marker antigens. According to some embodiments, when the concentration of T cells has reached a minimum value in response to conditioning therapy with an anti-CD2 antibody, an antigen-binding fragment thereof, or antibody-drug conjugate,, the physician may conclude the conditioning therapy, and may begin preparing the patient for hematopoietic stem cell transplant therapy.

The anti-CD2 antibody, antigen-binding fragment thereof, or antibody-drug conjugate, can be administered to the patient in an aqueous solution containing one or more

pharmaceutically acceptable excipients, such as a viscosity-modifying agent. The aqueous solution may be sterilized using techniques described herein or known in the art. The antibody, antigen-binding fragment thereof, or antibody-drug conjugate, can be administered to the patient at a dosage of, for example, from 0.001 mg/kg to 100 mg/kg prior to administration of a hematopoietic stem cell graft to the patient. The antibody, antigen-binding fragment thereof, or antibody-drug conjugate, can be administered to the patient at a time that optimally promotes engraftment of the exogenous hematopoietic stem cells, for instance, from 1 hour to 1 week (e.g., 1 hour, 2 hours, 3 hours, 4 hours, 5 hours, 6 hours, 7 hours, 8 hours, 9 hours, 10 hours, 11 hours, 12 hours, 13 hours, 14 hours, 15 hours, 16 hours, 17 hours, 18 hours, 19 hours, 20 hours, 21 hours, 22 hours, 23 hours, 24 hours, 2 days, 3 days, 4 days, 5 days, 6 days, or 7 days) or more prior to administration of the exogenous hematopoietic stem cell transplant.

Following the conclusion of conditioning therapy, the patient may then receive an infusion (e.g., an intravenous infusion) of exogenous hematopoietic stem cells, such as from the same physician that performed the conditioning therapy or from a different physician. The physician may administer the patient an infusion of autologous, syngeneic, or allogeneic hematopoietic stem cells, for instance, at a dosage of from 1×10^3 to 1×10^9 hematopoietic stem cells/kg. The physician may monitor the engraftment of the hematopoietic stem cell transplant, for example, by withdrawing a blood sample from the patient and determining the increase in concentration of hematopoietic stem cells or cells of the hematopoietic lineage (such as megakaryocytes, thrombocytes, platelets, erythrocytes, mast cells, myeoblasts, basophils, neutrophils, eosinophils, microglia, granulocytes, monocytes, osteoclasts, antigen-presenting cells, macrophages, dendritic cells, natural killer cells, T lymphocytes, and B lymphocytes) following administration of the transplant. This analysis may be conducted, for example, from 1 hour to 6 months, or more, following hematopoietic stem cell transplant therapy (e.g., 1 hour, 2 hours, 3 hours, 4 hours, 5 hours, 6 hours, 7 hours, 8 hours, 9 hours, 10 hours, 11 hours, 12 hours, 13 hours, 14 hours, 15 hours, 16 hours, 17 hours, 18 hours, 19 hours, 20 hours, 21 hours, 22 hours, 23 hours, 24 hours, 2 days, 3 days, 4 days, 5 days, 6 days, 7 days, 2 weeks, 3 weeks, 4 weeks, 5 weeks, 6 weeks, 7 weeks, 8 weeks, 9 weeks, 10 weeks, 11 weeks, 12 weeks, 13 weeks, 14 weeks, 15 weeks, 16 weeks, 17 weeks, 18 weeks, 19 weeks, 20 weeks, 21 weeks, 22 weeks, 23 weeks, 24 weeks, or more). A finding that the concentration of hematopoietic stem cells or cells of the hematopoietic lineage has increased (e.g., by 1%, 2%, 3%, 4%, 5%, 6%, 7%,

8%, 9%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 100%, 200%, 500%, or more) following the transplant therapy relative to the concentration of the corresponding cell type prior to transplant therapy provides one indication that treatment with the anti-CD2 antibody, antigen-binding fragment thereof, antibody-drug conjugate, has successfully promoted engraftment of the transplanted hematopoietic stem cell graft.

Example 2. Generating anti-CD2 antibodies by phage display

An exemplary method for in vitro evolution of anti-CD2 antibodies for use with the compositions and methods described herein is phage display. Phage display libraries can be created by making a designed series of mutations or variations within a coding sequence for the CDRs of an antibody or the analogous regions of an antibody-like scaffold (e.g., the BC, CD, and DE loops of ^{10}Fn3 domains). The template antibody-encoding sequence into which these mutations are introduced may be, for example, a naive human germline sequence. These mutations can be performed using standard mutagenesis techniques known in the art. Each mutant sequence thus encodes an antibody corresponding to the template save for one or more amino acid variations. Retroviral and phage display vectors can be engineered using standard vector construction techniques known in the art. P3 phage display vectors along with compatible protein expression vectors can be used to generate phage display vectors for antibody diversification.

The mutated DNA provides sequence diversity, and each transformant phage displays one variant of the initial template amino acid sequence encoded by the DNA, leading to a phage population (library) displaying a vast number of different but structurally related amino acid sequences. Due to the well-defined structure of antibody hypervariable regions, the amino acid variations introduced in a phage display screen are expected to alter the binding properties of the binding peptide or domain without significantly altering its overall molecular structure.

In a typical screen, a phage library may be contacted with and allowed to bind CD2 or an epitope thereof. To facilitate separation of binders and non-binders, it is convenient to immobilize the target on a solid support. Phage bearing a CD2-binding moiety can form a complex with the target on the solid support, whereas non-binding phage remain in solution and can be washed away with excess buffer. Bound phage can then liberated from the target by changing the buffer

to an extreme pH (pH 2 or pH 10), changing the ionic strength of the buffer, adding denaturants, or other known means.

The recovered phage can then be amplified through infection of bacterial cells, and the screening process can be repeated with the new pool that is now depleted in non-binding antibodies and enriched for antibodies that bind CD2. The recovery of even a few binding phage is sufficient to amplify the phage for a subsequent iteration of screening. After a few rounds of selection, the gene sequences encoding the antibodies or antigen-binding fragments thereof derived from selected phage clones in the binding pool are determined by conventional methods, thus revealing the peptide sequence that imparts binding affinity of the phage to the target. During the panning process, the sequence diversity of the population diminishes with each round of selection until desirable peptide-binding antibodies remain. The sequences may converge on a small number of related antibodies or antigen-binding fragments thereof. An increase in the number of phage recovered at each round of selection is an indication that convergence of the library has occurred in a screen.

Example 3. Producing humanized anti-CD2 antibodies

Non-human antibodies that bind CD2 can be humanized, for instance, according to the following procedure. Consensus human antibody heavy chain and light chain sequences are known in the art (see e.g., the "VBASE" human germline sequence database; Kabat et al. Sequences of Proteins of Immunological Interest, Fifth Edition, U.S. Department of Health and Human Services, NIH Publication No. 91 -3242, 1991; Tomlinson et al., J. Mol. Biol. 227:776-798, 1992; and Cox et al.. Eur. J. Immunol. 24:827- 836, 1994, the disclosures of each of which are incorporated herein by reference as they pertain to consensus human antibody heavy chain and light chain sequences. Using established procedures, one of skill in the art can identify the variable domain framework residues and CDRs of a consensus antibody sequence (e.g., by sequence alignment). One can substitute one or more CDRs of the heavy chain and/or light chain variable domains of consensus human antibody with one or more corresponding CDRs of a non-human antibody that binds CD2 in order to produce a humanized antibody. This CDR exchange can be performed using gene editing techniques described herein or known in the art.

One example of a variable domain of a consensus human antibody contains the heavy chain variable domain

EVQLVESGGGLVQPGGSLRLSCAAS**GFTFSDYAMS**WVRQAPGKGLEWVA**VISENGSDTYYAD SVKG**RFTISRDDSKNTLYLQMNSLRAEDTAVYYCAR**DRGGAVSYFDVW**GQGTLVTVSS (SEQ ID NO: 8) and the light chain variable domain DIQMTQSPSSLSASVGDRVTITC**RASQDVSSYLAW**YQQKPGKAPKLLIY**AASSLES**GVPSRFSG SGSGTDFTLTISSLQPEDFATYYC**QQYNSLPYT**FGQGTKVEIKRT (SEQ ID NO: 9), identified in US Patent No. 6,054,297, the disclosure of which is incorporated herein by reference as it pertains to human antibody consensus sequences. The CDRs in the above sequences are shown in bold.

To produce humanized antibodies, one can recombinantly express a polynucleotide encoding the above consensus sequence in which one or more variable region CDRs have been replaced with one or more variable region CDR sequences of a non-human antibody that binds CD2. As the affinity of the antibody for CD2 is determined primarily by the CDR sequences, the resulting humanized antibody is expected to exhibit an affinity for CD2 that is about the same as that of the non-human antibody from which the humanized antibody was derived. Methods of determining the affinity of an antibody for a target antigen include, for instance, ELISA-based techniques described herein and known in the art, as well as surface plasmon resonance, fluorescence anisotropy, and isothermal titration calorimetry, among others.

Example 4. Direct treatment of an autoimmune disease by administration of an anti-CD2 antibody, or antigen-binding fragment thereof

Using the compositions and methods described herein, one of skill in the art can administer to a subject suffering from an autoimmune disorder an anti-CD2 antibody, or antigen-binding fragment thereof, in a quantity sufficient to treat the autoimmune pathology. For instance, the subject may be suffering from scleroderma, multiple sclerosis, ulcerative colitis, Chrohn's disease, and/or Type 1 diabetes. To ameliorate one or more of these conditions, a physician of skill in the art can prescribe and administer to the subject an anti-CD2 antibody, or fragment thereof, such as an antibody, or fragment thereof, that is bound to a cytotoxic agent. The antibody, or fragment thereof, may be conjugated to a cytotoxic agent using conjugation techniques and linkers detailed above. A variety of cytotoxic agents can be conjugated to an anti-CD2 antibody, or antigen-binding fragment thereof, in order to deplete a population of endogenous, autoreactive CD2+ T cells or NK cells in a subject. For instance, the antibody or

antigen-binding fragment thereof may be conjugated to an amatoxin or another cytotoxin moiety described herein.

In preparation for therapy, the physician may assess the quantity or concentration of autoreactive T cells and/or NK cells in a sample isolated from a subject. This may be done, for instance, using FACS analysis techniques known in the art. One of skill in the art may then administer to the subject an antibody, or fragment thereof, either alone or conjugated to a cytotoxin, so as to deplete the population of autoreactive T cells and/or NK cells. To evaluate the efficacy of the therapy, the physician may determine the quantity or concentration of autoreactive T cells and/or NK cells in a sample isolated from the patient at a time subsequent to the administration of the anti-CD2 antibody, or fragment thereof. A determination that the quantity or concentration of autoreactive T cells and/or NK cells in a sample isolated from the subject following therapy relative to the quantity or concentration of T cells or NK cells prior to therapy provides an indication that the patient is responding to the anti-CD2 antibody, or fragment thereof.

Other Embodiments

All publications, patents, and patent applications mentioned in this specification are incorporated herein by reference to the same extent as if each independent publication or patent application was specifically and individually indicated to be incorporated by reference.

While the invention has been described in connection with specific embodiments thereof, it will be understood that it is capable of further modifications and this application is intended to cover any variations, uses, or adaptations of the invention following, in general, the principles of the invention and including such departures from the invention that come within known or customary practice within the art to which the invention pertains and may be applied to the essential features hereinbefore set forth, and follows in the scope of the claims.

Other embodiments are within the claims.

Doc Code: **TR.PROV**
Document Description: Provisional Cover Sheet (SB16)

PTO/SB/16 (11-08)
Approved for use through 05/31/2015. OMB 0651-0032
U.S. Patent and Trademark Office: U.S. DEPARTMENT OF COMMERCE

Provisional Application for Patent Cover Sheet

This is a request for filing a PROVISIONAL APPLICATION FOR PATENT under 37 CFR 1.53(c)

Inventor(s)

Inventor 1 — Remove

Given Name	Middle Name	Family Name	City	State	Country

All Inventors Must Be Listed – Additional Inventor Information blocks may be generated within this form by selecting the **Add** button. — Add

Title of Invention	COMPOSITIONS AND METHODS FOR THE DEPLETION OF CD2+ CELLS
Attorney Docket Number (if applicable)	M103034 1050US.P1 (0004.8

Correspondence Address

Direct all correspondence to (select one):

◉ The address corresponding to Customer Number	○ Firm or Individual Name
Customer Number	26158

The invention was made by an agency of the United States Government or under a contract with an agency of the United States Government.

- ● No.
- Yes, the invention was made by an agency of the United States Government. The U.S. Government agency name is:
- Yes, the invention was under a contract with an agency of the United States Government. The name of the U.S. Government agency and Government contract number are:

Doc Code: **TR.PROV**
Document Description: Provisional Cover Sheet (SB16)

PTO/SB/16 (11-08)
Approved for use through 05/31/2015. OMB 0651-0032
U.S. Patent and Trademark Office: U.S. DEPARTMENT OF COMMERCE

Entity Status
Applicant asserts small entity status under 37 CFR 1.27 or applicant certifies micro entity status under 37 CFR 1.29

○	Applicant asserts small entity status under 37 CFR 1.27
○	Applicant certifies micro entity status under 37 CFR 1.29. Applicant must attach form PTO/SB/15A or B or equivalent.
◉	No

Warning

Petitioner/applicant is cautioned to avoid submitting personal information in documents filed in a patent application that may contribute to identity theft. Personal information such as social security numbers, bank account numbers, or credit card numbers (other than a check or credit card authorization form PTO-2038 submitted for payment purposes) is never required by the USPTO to support a petition or an application. If this type of personal information is included in documents submitted to the USPTO, petitioners/applicants should consider redacting such personal information from the documents before submitting them to USPTO. Petitioner/applicant is advised that the record of a patent application is available to the public after publication of the application (unless a non-publication request in compliance with 37 CFR 1.213(a) is made in the application) or issuance of a patent. Furthermore, the record from an abandoned application may also be available to the public if the application is referenced in a published application or an issued patent (see 37 CFR1.14). Checks and credit card authorization forms PTO-2038 submitted for payment purposes are not retained in the application file and therefore are not publicly available.

Signature

Please see 37 CFR 1.4(d) for the form of the signature.

Signature	/Kevin A. Fiala/			Date (YYYY-MM-DD)	2017-11-29
First Name	Kevin	Last Name	Fiala	Registration Number (If appropriate)	68113

This collection of information is required by 37 CFR 1.51. The information is required to obtain or retain a benefit by the public which is to file (and by the USPTO to process) an application. Confidentiality is governed by 35 U.S.C. 122 and 37 CFR 1.11 and 1.14. This collection is estimated to take 8 hours to complete, including gathering, preparing, and submitting the completed application form to the USPTO. Time will vary depending upon the individual case. Any comments on the amount of time you require to complete this form and/or suggestions for reducing this burden, should be sent to the Chief Information Officer, U.S. Patent and Trademark Office, U.S. Department of Commerce, P.O. Box 1450, Alexandria, VA 22313-1450. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. **This form can only be used when in conjunction with EFS-Web. If this form is mailed to the USPTO, it may cause delays in handling the provisional application.**

Privacy Act Statement

The Privacy Act of 1974 (P.L. 93-579) requires that you be given certain information in connection with your submission of the attached form related to a patent application or paten. Accordingly, pursuant to the requirements of the Act, please be advised that : (1) the general authority for the collection of this information is 35 U.S.C. 2(b)(2); (2) furnishing of the information solicited is voluntary; and (3) the principal purpose for which the information is used by the U.S. Patent and Trademark Office is to process and/or examine your submission related to a patent application or patent. If you do not furnish the requested information, the U.S. Patent and Trademark Office may not be able to process and/or examine your submission, which may result in termination of proceedings or abandonment of the application or expiration of the patent.

The information provided by you in this form will be subject to the following routine uses:

1. The information on this form will be treated confidentially to the extent allowed under the Freedom of Information Act (5 U.S.C. 552) and the Privacy Act (5 U.S.C 552a). Records from this system of records may be disclosed to the Department of Justice to determine whether disclosure of these records is required by the Freedom of Information Act.
2. A record from this system of records may be disclosed, as a routine use, in the course of presenting evidence to a court, magistrate, or administrative tribunal, including disclosures to opposing counsel in the course of settlement negotiations.
3. A record in this system of records may be disclosed, as a routine use, to a Member of Congress submitting a request involving an individual, to whom the record pertains, when the individual has requested assistance from the Member with respect to the subject matter of the record.
4. A record in this system of records may be disclosed, as a routine use, to a contractor of the Agency having need for the information in order to perform a contract. Recipients of information shall be required to comply with the requirements of the Privacy Act of 1974, as amended, pursuant to 5 U.S.C. 552a(m).
5. A record related to an International Application filed under the Patent Cooperation Treaty in this system of records may be disclosed, as a routine use, to the International Bureau of the World Intellectual Property Organization, pursuant to the Patent Cooperation Treaty.
6. A record in this system of records may be disclosed, as a routine use, t o a n other federal agency for purposes of National Security review (35 U.S.C. 181) and for review pursuant to the Atomic Energy Act (42 U.S.C. 218(c)).
7. A record from this system of records may be disclosed, as a routine use, to the Administrator, General Services, or his/her designee, during an inspection of records conducted by GSA as part of that agency's responsibility to recommend improvements in records management practices and programs, under authority of 44 U.S.C. 2904 and 2906. Such disclosure shall be made in accordance with the GSA regulations governing inspection of records for this purpose, and any other relevant (i.e., GSA or Commerce) directive. Such disclosure shall not be used to make determinations about individuals.
8. A record from this system of records may be disclosed, as a routine use, to the public after either publication of the application pursuant to 35 U.S.C. 122(b) or issuance of a patent pursuant to 35 U.S.C. 151. Further, a record may be disclosed, subject to the limitations of 37 CFR 1.14, as a routine use, to the public if the record was filed in an application which became abandoned or in which the proceedings were terminated and which application is referenced by either a published application, an application open to public inspection or an issued patent.
9. A record from this system of records may be disclosed, as a routine use, to a Federal, State, or local law enforcement agency, if the USPTO becomes aware of a violation or potential violation of law or regulation.

PTO/AIA/14 (11-15)
Approved for use through 04/30/2017. OMB 0651-0032
U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE

Application Data Sheet 37 CFR 1.76	Attorney Docket Number	M103034 1050US.P1 (0004.8
	Application Number	
Title of Invention	COMPOSITIONS AND METHODS FOR THE DEPLETION OF CD2+ CELLS	

The application data sheet is part of the provisional or nonprovisional application for which it is being submitted. The following form contains the bibliographic data arranged in a format specified by the United States Patent and Trademark Office as outlined in 37 CFR 1.76.
This document may be completed electronically and submitted to the Office in electronic format using the Electronic Filing System (EFS) or the document may be printed and included in a paper filed application.

Secrecy Order 37 CFR 5.2:

☐ Portions or all of the application associated with this Application Data Sheet may fall under a Secrecy Order pursuant to 37 CFR 5.2 (Paper filers only. Applications that fall under Secrecy Order may not be filed electronically.)

Inventor Information:

Inventor 1 — Remove

Legal Name

Prefix	Given Name	Middle Name	Family Name	Suffix
	*		*	

Residence Information (Select One) ● US Residency Non US Residency Active US Military Service

City		State/Province		Country of Residence	

Mailing Address of Inventor:

Address 1	
Address 2	
City	State/Province
Postal Code	Country i

All Inventors Must Be Listed - Additional Inventor Information blocks may be generated within this form by selecting the **Add** button. Add

Correspondence Information:

Enter either Customer Number or complete the Correspondence Information section below.
For further information see 37 CFR 1.33(a).

☐ **An Address is being provided for the correspondence Information of this application.**

Customer Number	26158		
Email Address	bostondocket@wcsr.com	Add Email	Remove Email

Application Information:

Title of the Invention	COMPOSITIONS AND METHODS FOR THE DEPLETION OF CD2+ CELLS		
Attorney Docket Number	M103034 1050US.P1 (0004.8	Small Entity Status Claimed	☐
Application Type	Provisional		
Subject Matter	Utility		
Total Number of Drawing Sheets (if any)	0	Suggested Figure for Publication (if any)	n/a

PTO/AIA/14 (11-15)
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Application Data Sheet 37 CFR 1.76	Attorney Docket Number	M103034 1050US.P1 (0004.8
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Filing By Reference:

Only complete this section when filing an application by reference under 35 U.S.C. 111(c) and 37 CFR 1.57(a). Do not complete this section if application papers including a specification and any drawings are being filed. Any domestic benefit or foreign priority information must be provided in the appropriate section(s) below (i.e., "Domestic Benefit/National Stage Information" and "Foreign Priority Information").

For the purposes of a filing date under 37 CFR 1.53(b), the description and any drawings of the present application are replaced by this reference to the previously filed application, subject to conditions and requirements of 37 CFR 1.57(a).

Application number of the previously filed application	Filing date (YYYY-MM-DD)	Intellectual Property Authority or Country

Publication Information:

☐ Request Early Publication (Fee required at time of Request 37 CFR 1.219)

☐ **Request Not to Publish.** I hereby request that the attached application not be published under 35 U.S.C. 122(b) and certify that the invention disclosed in the attached application **has not and will not** be the subject of an application filed in another country, or under a multilateral international agreement, that requires publication at eighteen months after filing.

Representative Information:

Representative information should be provided for all practitioners having a power of attorney in the application. Providing this information in the Application Data Sheet does not constitute a power of attorney in the application (see 37 CFR 1.32).
Either enter Customer Number or complete the Representative Name section below. If both sections are completed the customer Number will be used for the Representative Information during processing.

Please Select One:	● Customer Number	US Patent Practitioner	○ Limited Recognition (37 CFR 11.9)
Customer Number	26158		

Domestic Benefit/National Stage Information:

This section allows for the applicant to either claim benefit under 35 U.S.C. 119(e), 120, 121, 365(c), or 386(c) or indicate National Stage entry from a PCT application. Providing benefit claim information in the Application Data Sheet constitutes the specific reference required by 35 U.S.C. 119(e) or 120, and 37 CFR 1.78.
When referring to the current application, please leave the "Application Number" field blank.

Prior Application Status			Remove
Application Number	Continuity Type	Prior Application Number	Filing or 371(c) Date (YYYY-MM-DD)

Additional Domestic Benefit/National Stage Data may be generated within this form by selecting the **Add** button. Add

PTO/AIA/14 (11-15)
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Application Data Sheet 37 CFR 1.76	Attorney Docket Number	M103034 1050US.P1 (0004.8
	Application Number	
Title of Invention	COMPOSITIONS AND METHODS FOR THE DEPLETION OF CD2+ CELLS	

Foreign Priority Information:

This section allows for the applicant to claim priority to a foreign application. Providing this information in the application data sheet constitutes the claim for priority as required by 35 U.S.C. 119(b) and 37 CFR 1.55. When priority is claimed to a foreign application that is eligible for retrieval under the priority document exchange program (PDX)[i] the information will be used by the Office to automatically attempt retrieval pursuant to 37 CFR 1.55(i)(1) and (2). Under the PDX program, applicant bears the ultimate responsibility for ensuring that a copy of the foreign application is received by the Office from the participating foreign intellectual property office, or a certified copy of the foreign priority application is filed, within the time period specified in 37 CFR 1.55(g)(1).

Remove

Application Number	Country[i]	Filing Date (YYYY-MM-DD)	Access Code[i] (if applicable)

Additional Foreign Priority Data may be generated within this form by selecting the **Add** button. Add

Statement under 37 CFR 1.55 or 1.78 for AIA (First Inventor to File) Transition Applications

☐ This application (1) claims priority to or the benefit of an application filed before March 16, 2013 and (2) also contains, or contained at any time, a claim to a claimed invention that has an effective filing date on or after March 16, 2013.
NOTE: By providing this statement under 37 CFR 1.55 or 1.78, this application, with a filing date on or after March 16, 2013, will be examined under the first inventor to file provisions of the AIA.

PTO/AIA/14 (11-15)
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	Application Number	
Title of Invention	COMPOSITIONS AND METHODS FOR THE DEPLETION OF CD2+ CELLS	

Authorization or Opt-Out of Authorization to Permit Access:

When this Application Data Sheet is properly signed and filed with the application, applicant has provided written authority to permit a participating foreign intellectual property (IP) office access to the instant application-as-filed (see paragraph A in subsection 1 below) and the European Patent Office (EPO) access to any search results from the instant application (see paragraph B in subsection 1 below).

Should applicant choose not to provide an authorization identified in subsection 1 below, applicant **must opt-out** of the authorization by checking the corresponding box A or B or both in subsection 2 below.

NOTE: This section of the Application Data Sheet is **ONLY** reviewed and processed with the **INITIAL** filing of an application. After the initial filing of an application, an Application Data Sheet cannot be used to provide or rescind authorization for access by a foreign IP office(s). Instead, Form PTO/SB/39 or PTO/SB/69 must be used as appropriate.

1. Authorization to Permit Access by a Foreign Intellectual Property Office(s)

A. Priority Document Exchange (PDX) - Unless box A in subsection 2 (opt-out of authorization) is checked, the undersigned hereby **grants the USPTO authority** to provide the European Patent Office (EPO), the Japan Patent Office (JPO), the Korean Intellectual Property Office (KIPO), the State Intellectual Property Office of the People's Republic of China (SIPO), the World Intellectual Property Organization (WIPO), and any other foreign intellectual property office participating with the USPTO in a bilateral or multilateral priority document exchange agreement in which a foreign application claiming priority to the instant patent application is filed, access to: (1) the instant patent application-as-filed and its related bibliographic data, (2) any foreign or domestic application to which priority or benefit is claimed by the instant application and its related bibliographic data, and (3) the date of filing of this Authorization. See 37 CFR 1.14(h)(1).

B. Search Results from U.S. Application to EPO - Unless box B in subsection 2 (opt-out of authorization) is checked, the undersigned hereby **grants the USPTO authority** to provide the EPO access to the bibliographic data and search results from the instant patent application when a European patent application claiming priority to the instant patent application is filed. See 37 CFR 1.14(h)(2).

The applicant is reminded that the EPO's Rule 141(1) EPC (European Patent Convention) requires applicants to submit a copy of search results from the instant application without delay in a European patent application that claims priority to the instant application.

2. Opt-Out of Authorizations to Permit Access by a Foreign Intellectual Property Office(s)

☐ A. Applicant **DOES NOT** authorize the USPTO to permit a participating foreign IP office access to the instant application-as-filed. If this box is checked, the USPTO will not be providing a participating foreign IP office with any documents and information identified in subsection 1A above.

☐ B. Applicant **DOES NOT** authorize the USPTO to transmit to the EPO any search results from the instant patent application. If this box is checked, the USPTO will not be providing the EPO with search results from the instant application.

NOTE: Once the application has published or is otherwise publicly available, the USPTO may provide access to the application in accordance with 37 CFR 1.14.

PTO/AIA/14 (11-15)
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U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE

Application Data Sheet 37 CFR 1.76	Attorney Docket Number	M103034 1050US.P1 (0004.8
	Application Number	
Title of Invention	COMPOSITIONS AND METHODS FOR THE DEPLETION OF CD2+ CELLS	

Applicant Information:

Providing assignment information in this section does not substitute for compliance with any requirement of part 3 of Title 37 of CFR to have an assignment recorded by the Office.

Applicant 1 Remove

If the applicant is the inventor (or the remaining joint inventor or inventors under 37 CFR 1.45), this section should not be completed. The information to be provided in this section is the name and address of the legal representative who is the applicant under 37 CFR 1.43; or the name and address of the assignee, person to whom the inventor is under an obligation to assign the invention, or person who otherwise shows sufficient proprietary interest in the matter who is the applicant under 37 CFR 1.46. If the applicant is an applicant under 37 CFR 1.46 (assignee, person to whom the inventor is obligated to assign, or person who otherwise shows sufficient proprietary interest) together with one or more joint inventors, then the joint inventor or inventors who are also the applicant should be identified in this section.

Clear

Assignee	Legal Representative under 35 U.S.C. 117	Joint Inventor
● Person to whom the inventor is obligated to assign.	Person who shows sufficient proprietary interest	

If applicant is the legal representative, indicate the authority to file the patent application, the inventor is:

Name of the Deceased or Legally Incapacitated Inventor:

If the Applicant is an Organization check here. ☒

Organization Name	Magenta Therapeutics, Inc.		
Mailing Address Information For Applicant:			
Address 1	50 Hampshire Street, 8th Floor		
Address 2			
City	Cambridge	**State/Province**	MA
Country	US	Postal Code	02139
Phone Number		Fax Number	
Email Address			

Additional Applicant Data may be generated within this form by selecting the Add button. Add

Assignee Information including Non-Applicant Assignee Information:

Providing assignment information in this section does not substitute for compliance with any requirement of part 3 of Title 37 of CFR to have an assignment recorded by the Office.

PTO/AIA/14 (11-15)
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Application Data Sheet 37 CFR 1.76	Attorney Docket Number	M103034 1050US.P1 (0004.8
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Title of Invention	COMPOSITIONS AND METHODS FOR THE DEPLETION OF CD2+ CELLS	

Assignee 1

Complete this section if assignee information, including non-applicant assignee information, is desired to be included on the patent application publication. An assignee-applicant identified in the "Applicant Information" section will appear on the patent application publication as an applicant. For an assignee-applicant, complete this section only if identification as an assignee is also desired on the patent application publication.

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Mailing Address Information For Assignee including Non-Applicant Assignee:

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NOTE: This Application Data Sheet must be signed in accordance with 37 CFR 1.33(b). **However, if this Application Data Sheet is submitted with the INITIAL filing of the application and either box A or B is not checked in subsection 2 of the "Authorization or Opt-Out of Authorization to Permit Access" section, then this form must also be signed in accordance with 37 CFR 1.14(c).**

This Application Data Sheet must be signed by a patent practitioner if one or more of the applicants is a **juristic entity** (e.g., corporation or association). If the applicant is two or more joint inventors, this form must be signed by a patent practitioner, **all** joint inventors who are the applicant, or one or more joint inventor-applicants who have been given power of attorney (e.g., see USPTO Form PTO/AIA/81) on behalf of **all** joint inventor-applicants.

See 37 CFR 1.4(d) for the manner of making signatures and certifications.

Signature	/Kevin A. Fiala/			Date (YYYY-MM-DD)	2017-11-29
First Name	Kevin	Last Name	Fiala	Registration Number	68113

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Application Data Sheet 37 CFR 1.76	Attorney Docket Number	M103034 1050US.P1 (0004.8
	Application Number	
Title of Invention	COMPOSITIONS AND METHODS FOR THE DEPLETION OF CD2+ CELLS	

This collection of information is required by 37 CFR 1.76. The information is required to obtain or retain a benefit by the public which is to file (and by the USPTO to process) an application. Confidentiality is governed by 35 U.S.C. 122 and 37 CFR 1.14. This collection is estimated to take 23 minutes to complete, including gathering, preparing, and submitting the completed application data sheet form to the USPTO. Time will vary depending upon the individual case. Any comments on the amount of time you require to complete this form and/or suggestions for reducing this burden, should be sent to the Chief Information Officer, U.S. Patent and Trademark Office, U.S. Department of Commerce, P.O. Box 1450, Alexandria, VA 22313-1450. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. **SEND TO: Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450.**

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EFS ID:	31078057
Application Number:	62592169
International Application Number:	
Confirmation Number:	9527
Title of Invention:	COMPOSITIONS AND METHODS FOR THE DEPLETION OF CD2+ CELLS
First Named Inventor/Applicant Name:	* .
Customer Number:	26158
Filer:	Kevin A. Fiala
Filer Authorized By:	
Attorney Docket Number:	M1030341050US.P1(0004.8)
Receipt Date:	29-NOV-2017
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Payment information:

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Payment was successfully received in RAM	$260
RAM confirmation Number	113017INTEFSW00004669090528
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1		M103034_1050USP1_CD2_App In_FINAL_Final.pdf	1141435 9bc16b7e8ea990c37171d7f7882dce927a08d37e	yes	130

	Multipart Description/PDF files in .zip description		
	Document Description	Start	End
	Abstract	130	130
	Claims	96	129
	Specification	1	95

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2	Provisional Cover Sheet (SB16)	ProvCoverSheetM1030341050.PDF	1506112 c5d7650738d133d5cb50367d653d4126ef363803	no	3

Warnings:

Information:

3	Application Data Sheet	AppDataSheetM1030341050.PDF	1913723 8da498072c9bb3fb4dd4f7a1717a9f01a74cb668	no	8

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4	Fee Worksheet (SB06)	fee-info.pdf	29862 1ca10dcd22bb0905969d28e3a9c680dfc09c769c	no	2

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