

CD123 Antibodies and Conjugates Thereof

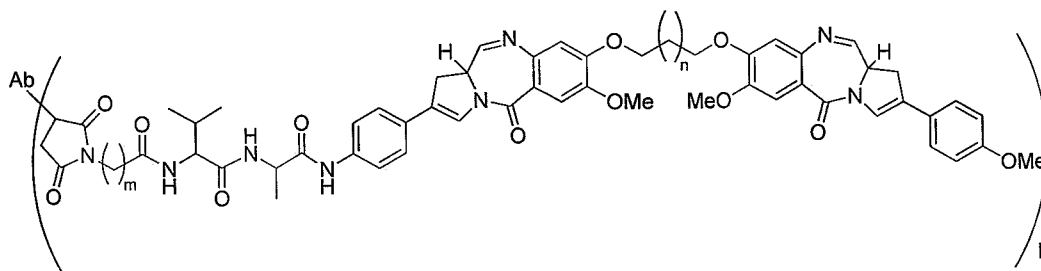
BACKGROUND

[0001] CD123 is the 70kD protein transmembrane alpha chain of the IL-3 receptor and is also referred to as IL3R-alpha. CD123 is known to be expressed on primary AML samples and has been reported on a number of malignant cells. The present invention provides CD123 antibodies and conjugates thereof.

SUMMARY

[0002] Provided herein are anti-CD123 antibodies and CD123 directed antibody-drug conjugates. In particular, provided herein are CD123 directed pyrrolobenzodiazepine ("PBD") antibody-drug conjugates and methods of using such conjugates to treat CD123 expressing disorders. Preferred anti-CD123 antibodies are chimeric or humanized forms of the murine 7G3 antibody (Sun et al., *Blood*, 1996, 87(1):83-92). The murine 7G3 antibody comprises a heavy chain variable region having the amino acid sequence set forth in SEQ ID NO:8 and a light chain variable region having the amino acid sequence set forth in SEQ ID NO:9. Preferred humanized 7G3 antibodies for use herein are antibodies constructed using the human germline sequence hIGHv1-2 and J exon J_H-1 for the heavy chain variable region and the human germline sequence hIGKv4-1 and J exon J_K-2 for the light chain variable regions. Particularly preferred humanized 7G3 antibodies comprise the heavy chain variable region set forth in SEQ ID NO:1 and the light chain variable region set forth in SEQ ID NO:2.

[0002a] In one aspect, there is provided an anti-CD123 antibody-drug conjugate compound having the formula:



or a pharmaceutically salt, solvate, or solvate of the salt; wherein

the subscript n is 1 or 3;

the subscript m is 2 to 5

Ab is an anti-CD123 intact antibody or antigen binding fragment thereof comprising a heavy chain variable region having the amino acid sequence set forth in SEQ ID NO:1; and a light chain variable region having the amino acid sequence set forth in SEQ ID NO:2;

the subscript p is an integer from 1 to 4.

[0003] Antibodies for use in the present disclosure can be intact antibodies or antigen binding fragments thereof. The humanized 7G3 antibody can have a mature heavy chain variable region that is fused to a heavy chain constant region and a mature light chain variable region that is fused to a light chain constant region. The heavy chain constant region can be a naturally occurring or mutant form of a human constant region (e.g., SEQ ID NO:5, a heavy chain IgG1 constant region with cysteine substituting for serine at

position 239, (S239C) or SEQ ID NO:6) . The heavy chain constant region can be of IgG1 isotype. An exemplary light chain constant region amino acid sequence is set forth in SEQ ID NO:7

[0003a] In another aspect, there is provided an anti-CD123 intact antibody or antigen binding fragment thereof comprising a heavy chain variable region having the amino acid sequence set forth in SEQ ID NO:1; and a light chain variable region having the amino acid sequence set forth in SEQ ID NO:2.

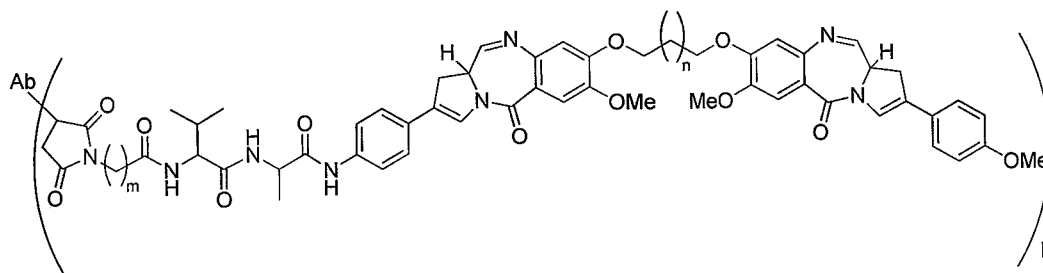
[0003b] In another aspect, there is provided an anti-CD123 intact antibody or antigen binding fragment thereof comprising a heavy chain variable region having the amino acid sequence set forth in SEQ ID NO:1 provided that H20 is occupied by M or V, H38 is occupied by K or R, H48 is occupied by I, H66 is occupied by K or R, H67 is occupied by A, H69 is occupied by L, H71 is occupied by V, H73 is occupied by R, H81 is occupied by E or H, H82A is occupied by S or N, and H93 is occupied by T; and a light chain variable region having the amino acid sequence set forth in SEQ ID NO:2 provided that L2 is occupied by F, L19 is occupied by A or V, L21 is occupied by I or M, L22 is occupied by N or S, L38 is occupied by L.

[0004] The chimeric or humanized 7G3 antibodies described herein are conjugated to drug-linkers, including PBD drug-linkers to provide CD123 antibody-drug conjugates. Attachment can be via conventional or site specific conjugation methods. An exemplary attachment is via an engineered cysteine at position 239 of the heavy chain constant region, according to the EU index as set forth in Kabat. The CD123 directed antibody-drug conjugates are used to treat CD123 expressing disease, including CD123 expressing cancers, such as AML.

[0004a] In another aspect, there is provided use of a pharmaceutical composition described herein for the manufacture of a medicament for treating a cancer that expresses CD123.

[0004b] In another aspect, there is provided a pharmaceutical composition comprising the intact antibody or antigen binding fragment thereof described herein.

[0004c] In another aspect, there is provided use of a pharmaceutical composition comprising a population of anti-CD123 antibody-drug conjugate molecules having the formula:



or a pharmaceutically salt, solvate, or solvate of the salt; wherein

the subscript n is 1 to 3;

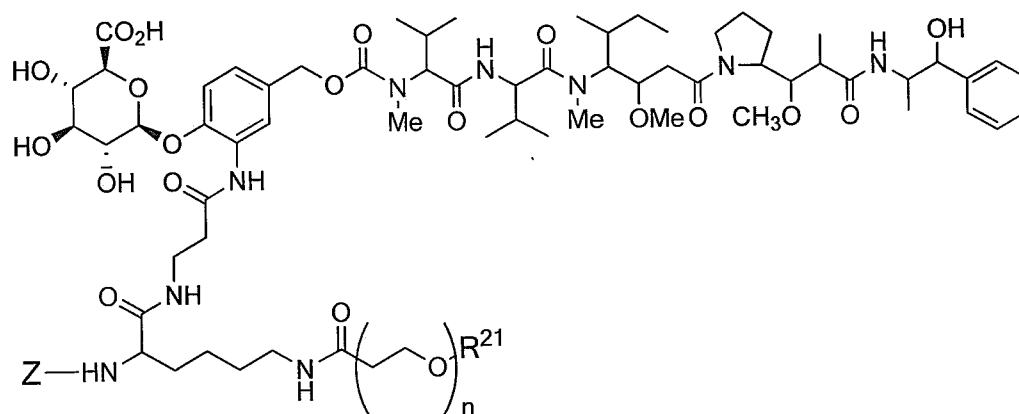
the subscript m is 2 to 5

Ab is an anti-CD123 intact antibody or antigen binding fragment thereof comprising a heavy chain variable region having the amino acid sequence set forth in SEQ ID NO:1; and a light chain variable region having the amino acid sequence set forth in SEQ ID NO:2;

the subscript p is an integer from 1 to 4; and the average drug load of the composition is about 2, for the manufacture of a medicament for treating a cancer that expresses CD123.

[0005] In other embodiments, the chimeric or humanized 7G3 antibodies described herein are conjugated to drug-linkers, including glucuronide-pegylated MMAE drug-linkers to provide CD123 antibody-drug conjugates.

[0006] In a further embodiment, the drug-linker attached to the humanized 7G3 antibody has the formula:

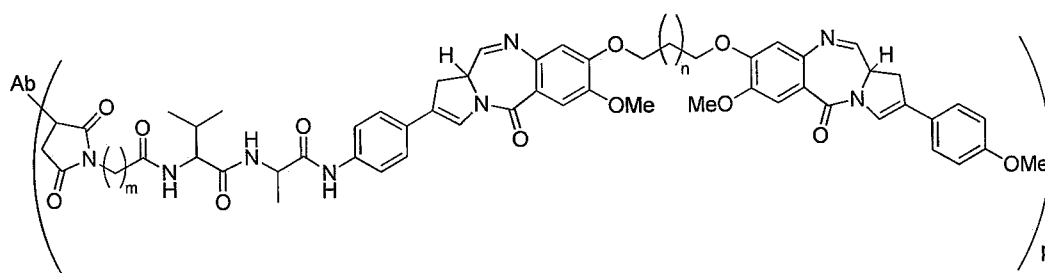


or a pharmaceutically acceptable salt thereof wherein Z represents an organic moiety having a reactive site capable of reacting with a functional group on the antibody to form a covalent attachment thereto, n ranges from 8 to 36, R^{PR} is hydrogen or a protecting group, R^{21} is a capping unit for the polyethylene glycol moiety.

[0007] In some embodiments of this disclosure, the value n can range from 8 to 14. In other embodiment of this disclosure, the value n ranges from 10 to 12. In a further embodiment of this disclosure, the value of n is 12. In another embodiment, R^{21} is $-CH_3$ or $-CH_2CH_2CO_2H$.

[0008] In another embodiment, any of the disclosed pegylated-MMAE antibody-drug conjugates has a p value of 8. In another embodiment, the drug-linker is attached to the antibody via the cysteine residues of the interchain disulfide bonds of the antibody.

[0008a] In another aspect, there is provided an antibody-drug conjugate composition comprising a population of anti-CD123 antibody-drug conjugate molecules having the formula:



or a pharmaceutically salt, solvate, or solvate of the salt; wherein

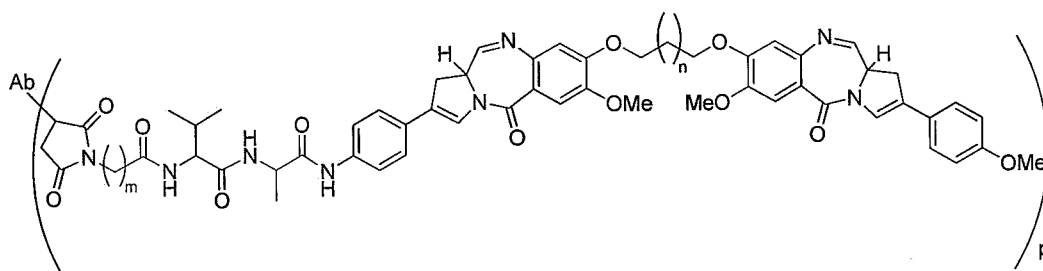
the subscript n is 1 to 3;

the subscript m is 2 to 5

Ab is an anti-CD123 intact antibody or antigen binding fragment thereof comprising a heavy chain variable region having the amino acid sequence set forth in SEQ ID NO:1; and a light chain variable region having the amino acid sequence set forth in SEQ ID NO:2; and

the subscript p is an integer from 1 to 4; and the average drug load of the composition is about 2.

[0008b] In another aspect, there is provided a pharmaceutical composition comprising a population of anti-CD123 antibody-drug conjugate molecules having the formula:



or a pharmaceutically salt, solvate, or solvate of the salt; wherein

the subscript n is 1 to 3;

the subscript m is 2 to 5

Ab is an anti-CD123 intact antibody or antigen binding fragment thereof comprising a heavy chain variable region having the amino acid sequence set forth in SEQ ID NO:1; and a light chain variable region having the amino acid sequence set forth in SEQ ID NO:2;

the subscript p is an integer from 1 to 4; and the average drug load of the composition is about 2.

[0008c] In another aspect, there is provided an isolated polynucleotide comprising a sequence encoding a heavy chain variable region having the amino acid sequence set forth in SEQ ID NO:1 and a light chain variable region having the amino acid sequence set forth in SEQ ID NO:2 or an antibody or antigen binding fragment thereof that binds to a CD123 protein.

[0008d] In another aspect, there is provided an isolated vector comprising the polynucleotide described herein.

[0008e] In another aspect, there is provided an isolated host cell comprising the vector described herein.

[0008f] In another aspect, there is provided a method of making an anti-CD123 antibody or antigen binding fragment thereof, wherein the method comprises:

a) culturing the host cell described herein under conditions suitable for expression of the polynucleotide encoding the antibody or antigen binding fragment thereof; and

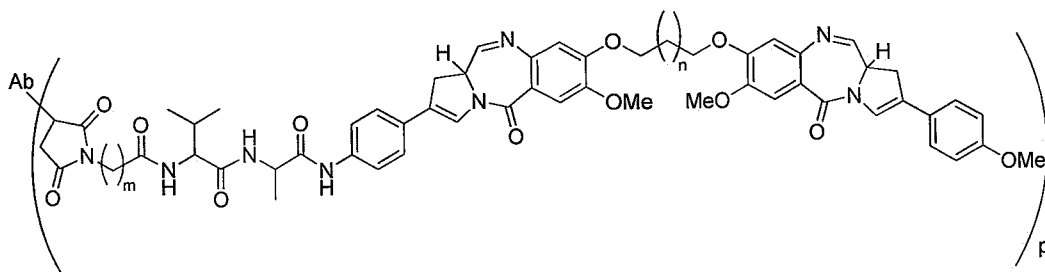
b) isolating the antibody or antigen binding fragment thereof.[0008g] In another aspect, there is provided a method of making an anti-CD123 antibody-drug conjugate, wherein the method comprises:

a) culturing the host cell described herein under conditions suitable for expression of the polynucleotide encoding the antibody or antigen binding fragment thereof;

b) isolating the antibody or antigen binding fragment thereof; and

c) conjugating a cytotoxic agent to the antibody or antigen binding fragment thereof.

[0008h] In another aspect, there is provided a pharmaceutical composition comprising a population of anti-CD123 antibody-drug conjugate molecules having the formula:



or a pharmaceutically salt, solvate, or solvate of the salt; wherein

the subscript n is 1 to 3;

the subscript m is 2 to 5

Ab is an anti-CD123 intact antibody or antigen binding fragment thereof comprising a heavy chain variable region having the amino acid sequence set forth in SEQ ID NO:3; and a light chain variable region having the amino acid sequence set forth in SEQ ID NO:4 and attachment to Ab is via a sulfur atom or an engineered cysteine residue at

position 239 of the heavy chain constant region, according to the EU index system of numbering;

the subscript p is 1 or 2;

and the average drug load of the composition is about 2.

BRIEF DESCRIPTION OF THE FIGURES

[0009] **Figure 1** shows the result of an in vitro cytotoxicity assay testing the humanized 7G3ec SGD-1910 antibody-drug conjugate against a MDR+-positive AML cell line, KG-1, that expresses low copies of CD123 in comparison to CD33. Despite the low copy number, the h7G3ec SGD-1910 antibody-drug conjugate showed potent activity.

[0010] **Figure 2** shows the result of an in vitro cytotoxicity assay testing the humanized 7G3ec SGD-1910 antibody-drug conjugate against a MDR+-positive AML cell line, Kasumi-1, that expresses low copies of CD123 in comparison to CD33. Despite the low copy number, the h7G3ec SGD-1910 antibody-drug conjugate showed potent activity.

[0011] **Figure 3** shows the results of a AML xenograft model, THP-1, showing that the humanized 7G3ec SGD-1910 antibody-drug conjugate displayed potent activity despite low copy number. Activity was comparable to CD33 antibody-drug conjugates despite lower copy number.

[0012] **Figure 4** shows the results of a AML xenograft model, KG1-INV, showing that the humanized 7G3ec SGD-1910 antibody-drug conjugate displayed potent activity and activity comparable to a CD33 antibody-drug conjugate despite lower copy number.

[0013] **Figure 5** shows the amino acid sequences for the heavy chain variable region of the murine 7G3 antibody and the humanized vHA, vHB, and vHC heavy chain and selected human germline acceptor variable region sequences.

[0014] **Figure 6** shows the amino acid sequences for the light chain variable region of the murine 7G3 antibody and the humanized vLA, and vLB, light chain and selected human germline acceptor variable region sequences.

33. The antibody of claim 32 wherein the heavy chain variable region is fused to a heavy chain constant region; and the light chain variable region is fused to a light chain constant region.
34. The antibody of claim 32 comprising a heavy chain having the amino acid sequence set forth in SEQ ID NO:3 and a light chain having the amino acid sequence set forth in SEQ ID NO:4.
35. Use of a pharmaceutical composition of any one of claims 11 to 21 for the manufacture of a medicament for treating a cancer that expresses CD123.
36. The use of claim 35 wherein the cancer is acute myeloid leukemia (AML).
37. The use of claim 35 wherein the cancer is myelodysplastic syndrome (MDS), B-cell acute lymphoblastic leukemia (B-ALL), hairy cell leukemia, Fanconi anemia, Blastic plasmacytoid dendritic cell neoplasm (BPDCN), Hodgkin's disease, Immature T-cell acute lymphoblastic leukemia (Immature T-ALL), Burkitt's lymphoma, Follicular lymphoma, chronic lymphocytic leukemia (CLL), or mantle cell lymphoma.
38. The intact antibody or antigen binding fragment of any one of claims 32-34 conjugated to a cytotoxic agent.
39. The intact antibody or antigen binding fragment of claim 38 wherein the cytotoxic agent is a maytansinoid, auristatin, pyrrolo[1,4]benzodiazepine, indolinobenzodiazepine, or oxazolidinobenzodiazepine.
40. A pharmaceutical composition comprising the intact antibody or antigen binding fragment thereof of claim 38 or 39.
41. An anti-CD123 intact antibody or antigen binding fragment thereof comprising a heavy chain variable region having the amino acid sequence set forth in SEQ ID NO:1 provided that H20 is occupied by M or V, H38 is occupied by K or R, H48 is occupied by I, H66 is occupied by K or R, H67 is occupied by A, H69 is occupied by L, H71 is occupied by V, H73 is occupied by R, H81 is occupied by E or H, H82A is occupied by S or N, and H93 is occupied by T; and a light chain variable region having the amino acid sequence set forth in SEQ ID NO:2 provided that L2 is occupied by F, L19 is occupied by A or V, L21 is occupied by I or M, L22 is occupied by N or S, L38 is occupied by L.

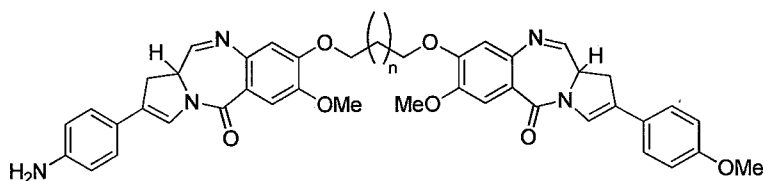
42. The intact antibody or antigen binding fragment thereof of claim 41 that is HALA, HALB, HBLA, HBLB, or HCLB.

43. The intact antibody of claim 41 or 42 wherein the heavy chain variable region is fused to a heavy chain constant region; and the light chain variable region is fused to a light chain constant region.

44. The intact antibody or antigen binding fragment of any one of claims 41-43 conjugated to a cytotoxic agent.

45. The intact antibody or antigen binding fragment of claim 44 wherein the cytotoxic agent is a maytansinoid, auristatin, pyrrolo[1,4]benzodiazepine, indolinobenzodiazepine, or oxazolidinobenzodiazepine.

46. The intact antibody or antigen binding fragment of claim 45 wherein the cytotoxic agent is



or a pharmaceutically acceptable salt, solvate, or solvate of the salt thereof; wherein the subscript n is 1 or 3.

47. A pharmaceutical composition comprising the intact antibody or antigen binding fragment thereof of claim 44 or 45 or 46.

48. An isolated polynucleotide comprising a sequence encoding a heavy chain variable region having the amino acid sequence set forth in SEQ ID NO:1 and a light chain variable region having the amino acid sequence set forth in SEQ ID NO:2 or an antibody or antigen binding fragment thereof that binds to a CD123 protein.

49. An isolated vector comprising the polynucleotide of claim 48.

50. An isolated host cell comprising the vector of claim 49.

51. The host cell of claim 50, wherein the host cell is a CHO cell.

52. A method of making an anti-CD123 antibody or antigen binding fragment thereof, wherein the method comprises:

a) culturing the host cell of claim 50 under conditions suitable for expression of the polynucleotide encoding the antibody or antigen binding fragment thereof; and

b) isolating the antibody or antigen binding fragment thereof.

53. The method of claim 52, wherein the host cell is a CHO cell.

54. A method of making an anti-CD123 antibody-drug conjugate, wherein the method comprises:

a) culturing the host cell of claim 50 under conditions suitable for expression of the polynucleotide encoding the antibody or antigen binding fragment thereof;

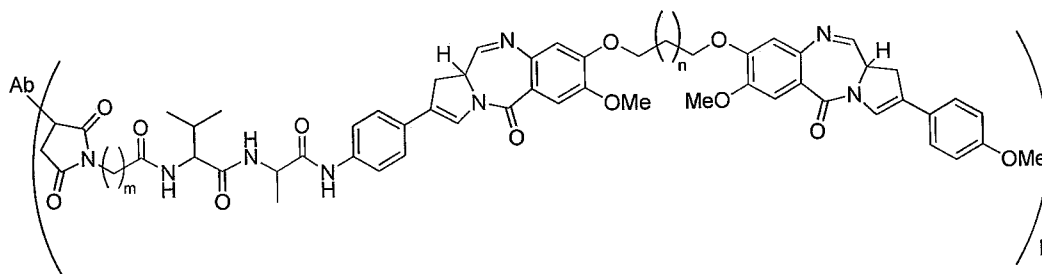
b) isolating the antibody or antigen binding fragment thereof; and

c) conjugating a cytotoxic agent to the antibody or antigen binding fragment thereof.

55. The method of claim 54, wherein the host cell is a CHO cell.

56. The method of claim 54, wherein the cytotoxic agent is a maytansinoid, auristatin, pyrrolo[1,4]benzodiazepine, indolinobenzodiazepine, or oxazolidinobenzodiazepine.

57. A pharmaceutical composition comprising a population of anti-CD123 antibody-drug conjugate molecules having the formula:



or a pharmaceutically salt, solvate, or solvate of the salt; wherein

60. The use of claim 58 wherein the cancer is myelodysplastic syndrome (MDS), B-cell acute lymphoblastic leukemia (B-ALL), hairy cell leukemia, Fanconi anemia, Blastic plasmacytoid dendritic cell neoplasm (BPDCN), Hodgkin's disease, Immature T-cell acute lymphoblastic leukemia (Immature T-ALL), Burkitt's lymphoma, Follicular lymphoma, chronic lymphocytic leukemia (CLL), or mantle cell lymphoma.