ANTI-Ly6E ANTIBODIES, IMMUNOCONJUGATES, AND USES THEREOF

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This application claims the benefit of priority of US Provisional Application Nos. 63/337,945, filed May 3, 2022, and 63/492,297, filed March 27, 2023, each of which is incorporated by reference herein in their entirety for any purpose.

SEQUENCE LISTING

[0002] The present application contains a Sequence Listing, which has been submitted electronically in XML format. Said XML copy, created on April 14, 2023, is named "2023-04-14_01146-0114-00PCT_ST26.xml" and is 46,932 bytes in size. The information in the electronic format of the sequence listing is incorporated herein by reference herein in its entirety.

FIELD

[0003] The present invention relates to anti-Ly6E antibodies and immunoconjugates, and methods of using the same.

BACKGROUND

[0004] Lymphocyte antigen 6 complex, locus E (Ly6E), also known as retinoic acid induced gene E (RIG-E) and stem cell antigen 2 (SCA-2). It is a GPI linked, 131 amino acid length, ~8.4kDa protein of unknown function with no known binding partners. It was initially identified as a transcript expressed in immature thymocyte, thymic medullary epithelial cells in mice. Mao *et al.,* "RIG-E, a human homolog of the murine Ly-6 family, is induced by retinoic acid during the differentiation of acute promyelocytic leukemia cell," *Proc. Natl. Acad. Sci. U.S.A.* 93:5910-5914 (1996).

[0005] There is a need in the art for agents that target Ly6E for the diagnosis and treatment of Ly6E-associated conditions, such as cancer. The invention fulfills that need and provides other benefits.

SUMMARY

[0006] The disclosure provides anti-Ly6E antibodies, immunoconjugates, and methods of using the same.

[0007] In one aspect, the disclosure provides an isolated antibody that binds to Ly6E, wherein the antibody comprises (i) HVR-L1 comprising the amino acid sequence of SEQ ID NO:7, (ii) HVR-L2 comprising the amino acid sequence of SEQ ID NO:8, (iii) HVR-L3 comprising the amino acid sequence of SEQ ID NO:9, (iv) HVR-H1 comprising the amino acid sequence of SEQ ID NO:10, v) HVR-H2 comprising the amino acid sequence of SEQ ID NO:11, and (vi) HVR-H3 comprising the amino acid sequence of SEQ ID NO:12.

[0008] In some examples, the antibody is a monoclonal antibody. In some examples, the antibody is a humanized or chimeric antibody. In some examples, the antibody is an antibody fragment.

[0009] In some examples, the antibody comprises (a) a VH sequence having at least 95% sequence identity to SEQ ID NO:32; (b) a VL sequence having at least 95% sequence identity to the amino acid sequence of SEQ ID NO:31; or (c) a VH sequence as in (a) and a VL sequence as in (b). In some examples, the antibody comprises a VH sequence of SEQ ID NO:32 and a VL sequence of SEQ ID NO: 31.

[0010] In one aspect, the disclosure provides an isolated antibody comprising a VH sequence SEQ ID NO:32 and a VL sequence of SEQ ID NO:31.

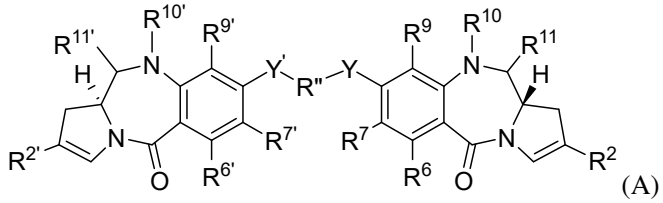
[0011] In some examples, the antibody is an IgG1, IgG2a, IgG2b, IgG3, or IgG4 antibody.

[0012] In some examples, the antibody comprises a heavy chain comprising an amino acid sequence selected from SEQ ID NOs: 5 and a light chain comprising an amino acid sequence selected from SEQ ID NOs: SEQ ID NO:3, SEQ ID NO:17, SEQ ID NO: 19, or SEQ ID NO: 29. In some examples, the antibody comprises a heavy chain comprising the amino acid sequence of SEQ ID NO: 5 and a light chain comprising the amino acid sequence of SEQ ID NO: 3. In one aspect, the disclosure provides an isolated antibody that binds to Ly6E, wherein the antibody comprises a heavy chain comprising the amino acid sequence of SEQ ID NO: 5 and a light chain comprising the amino acid sequence of SEQ ID NO: 3.

[0013] In some examples, the antibody is a multispecific antibody. In some examples, the multispecific antibody is a bispecific antibody. In some examples, the multispecific antibody binds Ly6E and CD3.

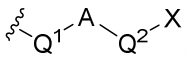
[0014] In one aspect, the disclosure provides an isolated nucleic acid encoding the antibody as disclosed herein. In one aspect, the disclosure provides an expression vector comprising the nucleic acid as disclosed herein. In some examples, the disclosure provides an expression vector comprising the nucleic acid encoding the antibody. In one aspect, the disclosure provides a host cell comprising the nucleic acid as disclosed herein or the expression vector as disclosed herein. In one aspect, the disclosure provides a host cell that expresses the antibody as disclosed herein. In one aspect, the disclosure provides a method of producing an antibody comprising culturing the host cell as disclosed herein so that the antibody is produced.

[0015] In one aspect, the disclosure provides an immunoconjugate comprising the antibody as disclosed herein and a cytotoxic agent. In some examples, the immunoconjugate has the formula Ab-(L-D)p, wherein: (a) Ab is an antibody provided herein; (b) L is a linker; (c) D is a pyrrolobenzodiazepine; and (d) p ranges from 1-8. In some examples, D is a pyrrolobenzodiazepine of Formula A:



(A)

wherein:

R^2 is of formula II: 

(II), wherein A is a C_{5-7} aryl group, X is selected from the group consisting of: OH, SH, CO_2H, COH, N=C=O, NHR^N, and $(OC_2H_4)_mOCH_3$, wherein R^N is

selected from the group consisting of H and C_{1-4} alkyl, and wherein m is an integer from 1 to 3, and either:

(i) Q^1 is a single bond, and Q^2 is selected from a single bond and -Z-$(CH_2)_n$-, wherein Z is a single bond, O, S, or NH and n is an integer from 1 to 3; or

(ii) Q^1 is -CH=CH-, and Q^2 is a single bond;

$R^{2'}$ is a C_{5-10} aryl group, optionally substituted by one or more substituents selected from the group consisting of halo, nitro, cyano, ether, C_{1-7} alkyl, C_{3-7} heterocyclyl, and bis-oxy-C_{1-3} alkylene;

R^6 and R^9 are independently selected from H, R, OH, OR, SH, SR, NH_2, NHR, NRR', nitro, Me_3Sn and halo;

R^7 is selected from the group consisting of H, R, OH, OR, SH, SR, NH_2, NHR, NHRR', nitro, Me_3Sn, and halo;

where R and R' are independently selected from optionally substituted C_{1-12} alkyl, C_{3-20} heterocyclyl and C_{5-20} aryl groups;

either:

(a) R^{10} is H, and R^{11} is OH or OR^A, wherein R^A is C_{1-4} alkyl;

(b) R^{10} and R^{11} form a nitrogen-carbon double bond between the nitrogen and carbon atoms to which they are bound; or

(c) R^{10} is H and R^{11} is SO_ZM, wherein z is 2 or 3 and M is a monovalent pharmaceutically acceptable cation;

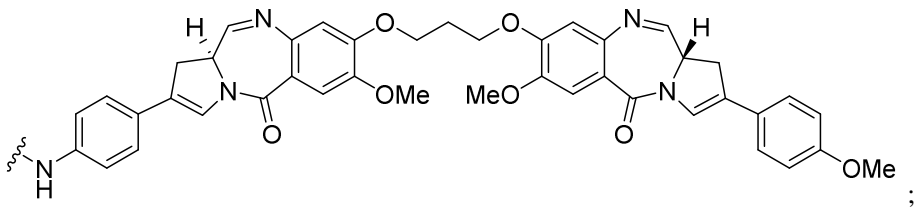
R" is a C_{3-12} alkylene group, which chain may be interrupted by one or more heteroatoms independently selected from the group consisting of O, S, and NH, and/or one or more aromatic rings independently selected from the group consisting of benzene or pyridine;

Y is O, S, or NH;

$R^{6'}$, $R^{7'}$, and $R^{9'}$ are selected from the same groups as R^6, R^7 and R^9 respectively, and $R^{10'}$ and $R^{11'}$ are the same as R^{10} and R^{11} respectively, wherein if R^{11} and $R^{11'}$ are SO_ZM, M may represent a divalent pharmaceutically acceptable cation; and

the point of attachment to the linker L is through R^2 or $R^{2'}$.

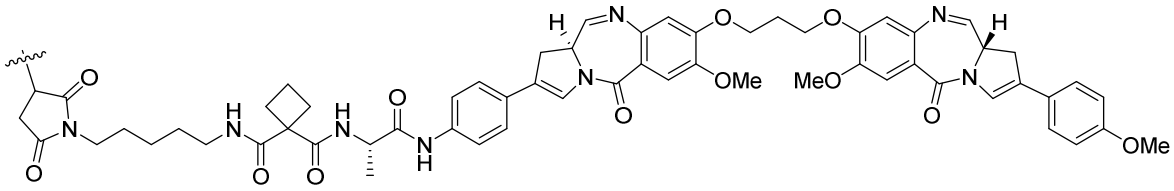
[0016] In some examples, D has the structure:



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wherein the sqiggly line indicates the point of attachment to the linker L.

[0017] In some examples, (L-D) has the structure:



wherein the squiggly line indicates the point of attachment to the protein.

[0018] In some examples, p ranges from 1.5-5 or 1.5-6 or 1.5-4 or 2-3.

[0019] In one aspect, the disclosure provides a pharmaceutical formulation comprising the immunoconjugate as disclosed herein and a pharmaceutically acceptable carrier. In some examples, the pharmaceutical formulation further comprises an additional therapeutic agent.

[0020] In some examples, the discosure provides a method of treating an individual having an Ly6E-positive cancer, the method comprising administering to the individual an effective amount of the immunoconjugate or the pharmaceutical formulation. In one aspect, the disclosure provides use of the immunoconjugate as disclosed herein or the pharmaceutical formulation as disclosed herein, in the manufacture of a medicament for treating an individual having an Ly6E-positive cancer. In some examples, the Ly6E-positive cancer is selected from a breast cancer, pancreatic cancer, colon cancer, colorectal cancer, melanoma, ovarian cancer, non-small cell lung cancer, or gastric cancer. breast cancer

[0021] In some examples, the method further comprises administering an additional therapeutic agent to the individual. In some examples, the additional therapeutic agent is a platinum complex.

[0022] In some examples, the disclosure provides a method of inhibiting proliferation of an Ly6E-positive cell, the method comprising exposing the cell to the immunoconjugate under conditions permissive for binding of the immunoconjugate to Ly6E on the surface of the cell, thereby inhibiting proliferation of the cell. In one aspect, the disclosure provides an *in vitro* method of inhibiting proliferation of an Ly6E-positive cell, the method comprising exposing the cell to the immunoconjugate as disclosed herein under conditions permissive for binding of the immunoconjugate to Ly6E on the surface of the cell, thereby inhibiting proliferation of the cell. In some examples, the cell is a breast, pancreatic, colon, colorectal, melanoma, ovarian non-small cell lung or gastric cancer cell.

[0023] In some examples, the disclosure provides an antibody provided herein conjugated to a label. In some examples, the label is a positron emitter. In some examples, the positron emitter is 89Zr.

[0024] In one aspect, the disclosure provides a method of detecting human Ly6E in a biological sample comprising contacting the biological sample with the anti-Ly6E antibody as disclosed herein under conditions permissive for binding of the anti-Ly6E antibody to a naturally occurring human Ly6E, and detecting whether a complex is formed between the anti-Ly6E antibody and a naturally occurring human Ly6E in the biological sample. In some examples, the biological sample is a breast cancer sample, a pancreatic cancer sample, a colon cancer sample, a colorectal cancer sample, melanoma cancer sample, ovarian cancer sample, a non-small cell lung cancer sample, or a gastric cancer sample.

[0025] In some examples, the disclosure provides a method for detecting a Ly6E-positive cancer comprising: (i) administering a labeled anti-Ly6E antibody to a subject having or suspected of having a Ly6E-positive cancer, wherein the labeled anti-Ly6E antibody comprises an anti-Ly6E antibody provided herein; and (ii) detecting the labeled anti-Ly6E antibody in the subject, wherein detection of the labeled anti-Ly6E antibody indicates a Ly6E-positive cancer in the subject. In some examples, the labeled anti-Ly6E antibody comprises an anti-Ly6E antibody conjugated to a positron emitter. In some examples, the positron emitter is ^{89}Zr.