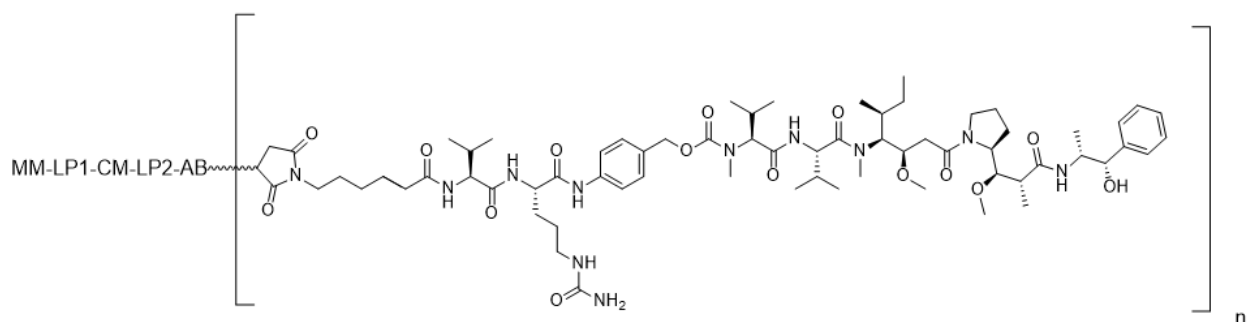


[0006] Accordingly, there is a continued need in the field of antibody-based therapeutics for antibodies that mimic the desirable characteristics of the small molecule prodrug.

SUMMARY OF THE DISCLOSURE

[0007] The disclosure provides conjugated activatable antibodies that specifically bind CD71, also known as transferrin receptor protein 1 (TfR1).

[0008] In an aspect of the disclosure, provided herein is a conjugated activatable antibody comprising the structure of Formula (I) or a salt thereof:



Formula (I)

wherein (i) AB is an antibody that specifically binds to human CD71 and comprises a heavy chain variable region comprising a CDRH1 sequence comprising SEQ ID NO: 9, a CDRH2 sequence comprising SEQ ID NO: 10, and a CDRH3 sequence comprising SEQ ID NO: 11; and a light chain variable region comprising a CDRL1 sequence comprising SEQ ID NO: 12 or SEQ ID NO: 13, a CDRL2 sequence comprising SEQ ID NO: 14, and a CDRL3 sequence comprising SEQ ID NO: 15; (ii) MM is a masking moiety comprising the amino acid sequence of SEQ ID NO: 18, wherein the MM inhibits the binding of the AB to human CD71 when the conjugated activatable antibody is in an uncleaved state; (iii) LP1 is a first linking moiety comprising the amino acid sequence of SEQ ID NO: 207; (iv) CM is a cleavable moiety comprising the sequence of SEQ ID NO: 156, wherein the CM is a polypeptide that functions as a substrate for a protease; and (v) LP2 is a second linking moiety comprising the amino acid sequence of SEQ ID NO: 38; and (b) wherein “n” is 2. In some examples, the conjugated activatable antibody of Formula (I) wherein the AB comprises an IgG1 isotype. In some examples, the conjugated activatable antibody of Formula (I) wherein the AB is an antibody having a heavy chain constant region, and wherein the C-terminal residue of the heavy

chain constant region is not a lysine. In some examples, the conjugated activatable antibody of Formula (I), wherein the N-terminal glutamate on either the heavy chain and/or light chain is optionally either pyroglutamate or post-translationally modified to pyroglutamate.

[0009] In a related example of the disclosure, provided herein is a conjugated activatable antibody comprising the structure of Formula (I) or a salt thereof wherein (i) AB is an antibody that specifically binds to human CD71 and comprises a heavy chain variable region comprising a sequence of SEQ ID NO: 5 and a light chain variable region comprising a sequence of SEQ ID NO: 7; (ii) MM is a masking moiety comprising the amino acid sequence of SEQ ID NO: 18, wherein the MM inhibits the binding of the AB to human CD71 when the conjugated activatable antibody is in an uncleaved state; (iii) LP1 is a first linking moiety comprising the amino acid sequence of SEQ ID NO: 207; (iv) CM is a cleavable moiety comprising the sequence of SEQ ID NO: 156, wherein the CM is a polypeptide that functions as a substrate for a protease; and (v) LP2 is a second linking moiety comprising the amino acid sequence of SEQ ID NO: 38; and (b) wherein “n” is 2. In some examples, the conjugated activatable antibody of Formula (I) wherein the AB comprises an IgG1 isotype. In some examples, the conjugated activatable antibody of Formula (I) wherein the AB is an antibody having a heavy chain constant region, and wherein the C-terminal residue of the heavy chain constant region is not a lysine. In some examples, the conjugated activatable antibody of Formula (I), wherein the N-terminal glutamate on either the heavy chain and/or light chain is optionally either pyroglutamate or post-translationally modified to pyroglutamate.

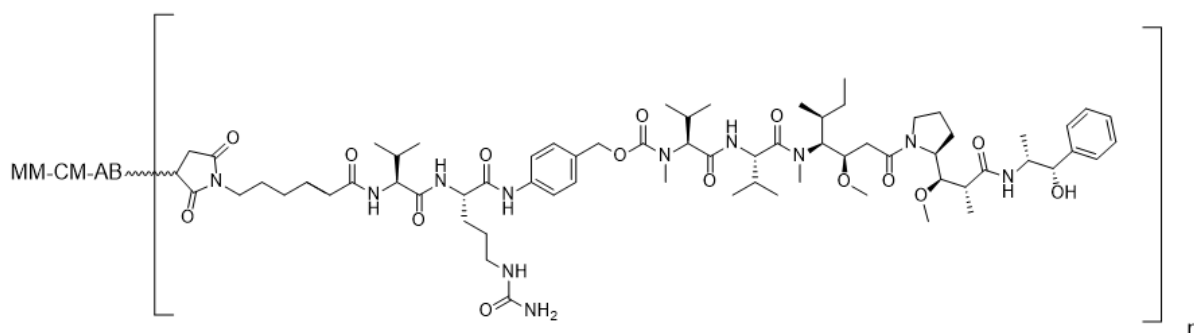
[0010] In a related example of the disclosure, provided herein is a conjugated activatable antibody comprising the structure of Formula (I) or a salt thereof wherein (i) AB is an antibody that specifically binds to human CD71 and comprises a heavy chain comprising a sequence of SEQ ID NO: 167 and a light chain comprising a sequence of SEQ ID NO: 19; (ii) MM is a masking moiety comprising the amino acid sequence of SEQ ID NO: 18, wherein the MM inhibits the binding of the AB to human CD71 when the conjugated activatable antibody is in an uncleaved state; (iii) LP1 is a first linking moiety comprising the amino acid sequence of SEQ ID NO: 207; (iv) CM is a cleavable moiety comprising the sequence of SEQ ID NO: 156, wherein the CM is a polypeptide that functions as a substrate for a protease; and (v) LP2 is a second linking moiety comprising the amino acid sequence of SEQ ID NO: 38; and (b) wherein “n” is 2. In some examples, the conjugated activatable antibody of Formula (I) wherein the AB comprises an IgG1 isotype. In some examples,

the conjugated activatable antibody of Formula (I) wherein the AB is an antibody having a heavy chain constant region, and wherein the C-terminal residue of the heavy chain constant region is not a lysine. In some examples, the conjugated activatable antibody of Formula (I), wherein the N-terminal glutamate on either the heavy chain and/or light chain is optionally either pyroglutamate or post-translationally modified to pyroglutamate.

[0011] In a related example of the disclosure, provided herein is a conjugated activatable antibody comprising the structure of Formula (I) or a salt thereof wherein MM-LP1-CM-LP2-AB is an activatable antibody, wherein the AB is an antibody that specifically binds to human CD71, wherein the activatable antibody comprises a heavy chain comprising a sequence of SEQ ID NO: 167 and a light chain comprising a sequence of SEQ ID NO: 169, and wherein “n” is 2. In some examples, the conjugated activatable antibody of Formula (I) wherein the AB comprises an IgG1 isotype. In some examples, the conjugated activatable antibody of Formula (I) wherein the AB is an antibody having a heavy chain constant region, and wherein the C-terminal residue of the heavy chain constant region is not a lysine. In some examples, the conjugated activatable antibody of Formula (I), wherein the N-terminal glutamate on either the heavy chain and/or light chain is optionally either pyroglutamate or post-translationally modified to pyroglutamate.

[0012] In a related example of the disclosure, provided herein is a conjugated activatable antibody comprising the structure of Formula (I) or a salt thereof wherein MM-LP1-CM-LP2-AB is an activatable antibody, wherein the AB is an antibody that specifically binds to human CD71, wherein the activatable antibody comprises a heavy chain comprising a sequence of SEQ ID NO: 167 and a light chain comprising a sequence of SEQ ID NO: 170 wherein “n” is 2. In some examples, the conjugated activatable antibody of Formula (I) wherein the AB comprises an IgG1 isotype. In some examples, the conjugated activatable antibody of Formula (I) wherein the AB is an antibody having a heavy chain constant region, and wherein the C-terminal residue of the heavy chain constant region is not a lysine. In some examples, the conjugated activatable antibody of Formula (I), wherein the N-terminal glutamate on either the heavy chain and/or light chain is optionally either pyroglutamate or post-translationally modified to pyroglutamate.

[0013] In a related example of the disclosure, provided herein a conjugated activatable antibody comprising the structure of Formula (II) or a salt thereof:



Formula (II)

wherein (i) AB is an antibody that specifically binds to human CD71 and comprises a heavy chain variable region comprising a CDRH1 sequence comprising SEQ ID NO: 9, a CDRH2 sequence comprising SEQ ID NO: 10, and a CDRH3 sequence comprising SEQ ID NO: 11; and a light chain variable region comprising a CDRL1 sequence comprising SEQ ID NO: 12 or SEQ ID NO: 13, a CDRL2 sequence comprising SEQ ID NO: 14, and a CDRL3 sequence comprising SEQ ID NO: 15; (ii) MM is a masking moiety comprising the amino acid sequence of SEQ ID NO: 18, wherein the MM inhibits the binding of the AB to human CD71 when the conjugated activatable antibody is in an uncleaved state; and (iii) CM is a cleavable moiety comprising the sequence of SEQ ID NO: 156, wherein the CM is a polypeptide that functions as a substrate for a protease; and (b) wherein “n” is 2. In some examples, the conjugated activatable antibody of Formula (II) wherein the AB comprises an IgG1 isotype. In some examples, the conjugated activatable antibody of Formula (II) wherein the AB is an antibody having a heavy chain constant region, and wherein the C-terminal residue of the heavy chain constant region is not a lysine. In some examples, the conjugated activatable antibody of Formula (II), wherein the N-terminal glutamate on either the heavy chain and/or light chain is optionally either pyroglutamate or post-translationally modified to pyroglutamate.

[0014] In a related example of the disclosure, provided herein is a conjugated activatable antibody comprising the structure of Formula (II) or a salt thereof wherein (i) AB is an antibody that specifically binds to human CD71 and comprises a heavy chain variable region comprising a sequence of SEQ ID NO: 5 and a light chain variable region comprising a sequence of SEQ ID NO: 7; (ii) MM is a masking moiety comprising the amino acid sequence of SEQ ID NO: 18, wherein the MM inhibits the binding of the AB to human CD71 when the conjugated activatable antibody is in an uncleaved state; and (iii) CM is a cleavable moiety comprising the sequence of SEQ ID NO:

156, wherein the CM is a polypeptide that functions as a substrate for a protease; and (b) wherein “n” is 2. In some examples, the conjugated activatable antibody of Formula (II) wherein the AB comprises an IgG1 isotype. In some examples, the conjugated activatable antibody of Formula (II) wherein the AB is an antibody having a heavy chain constant region, and wherein the C-terminal residue of the heavy chain constant region is not a lysine. In some examples, the conjugated activatable antibody of Formula (II), wherein the N-terminal glutamate on either the heavy chain and/or light chain is optionally either pyroglutamate or post-translationally modified to pyroglutamate.

[0015] In a related example of the disclosure, provided herein is a conjugated activatable antibody comprising the structure of Formula (II) or a salt thereof wherein (i) AB is an antibody that specifically binds to human CD71 and comprises a heavy chain comprising a sequence of SEQ ID NO: 167 and a light chain comprising a sequence of SEQ ID NO: 19; (ii) MM is a masking moiety comprising the amino acid sequence of SEQ ID NO: 18, wherein the MM inhibits the binding of the AB to human CD71 when the conjugated activatable antibody is in an uncleaved state; and (iii) CM is a cleavable moiety comprising the sequence of SEQ ID NO: 156, wherein the CM is a polypeptide that functions as a substrate for a protease; and (b) wherein “n” is 2. In some examples, the conjugated activatable antibody of Formula (II) wherein the AB comprises an IgG1 isotype. In some examples, the conjugated activatable antibody of Formula (II) wherein the AB is an antibody having a heavy chain constant region, and wherein the C-terminal residue of the heavy chain constant region is not a lysine. In some examples, the conjugated activatable antibody of Formula (II), wherein the N-terminal glutamate on either the heavy chain and/or light chain is optionally either pyroglutamate or post-translationally modified to pyroglutamate.

[0016] In a related example of the disclosure, provided herein is a conjugated activatable antibody comprising the structure of Formula (II) or a salt thereof wherein MM-LP1-CM-LP2-AB is an activatable antibody, wherein the AB is an antibody that specifically binds to human CD71, wherein the activatable antibody comprises a heavy chain comprising a sequence of SEQ ID NO: 167 and a light chain comprising a sequence of SEQ ID NO: 169, and wherein “n” is 2. In some examples, the conjugated activatable antibody of Formula (II) wherein the AB comprises an IgG1 isotype. In some examples, the conjugated activatable antibody of Formula (II) wherein the AB is an antibody having a heavy chain constant region, and wherein the C-terminal residue of the heavy

chain constant region is not a lysine. In some examples, the conjugated activatable antibody of Formula (II), wherein the N-terminal glutamate on either the heavy chain and/or light chain is optionally either pyroglutamate or post-translationally modified to pyroglutamate.

[0017] In a related example of the disclosure, provided herein is a conjugated activatable antibody comprising the structure of Formula (II) or a salt thereof wherein MM-LP1-CM-LP2-AB is an activatable antibody, wherein the AB is an antibody that specifically binds to human CD71, wherein the activatable antibody comprises a heavy chain comprising a sequence of SEQ ID NO: 167 and a light chain comprising a sequence of SEQ ID NO: 170, and wherein “n” is 2. In some examples, the conjugated activatable antibody of Formula (II) wherein the AB comprises an IgG1 isotype. In some examples, the conjugated activatable antibody of Formula (II) wherein the AB is an antibody having a heavy chain constant region, and wherein the C-terminal residue of the heavy chain constant region is not a lysine. In some examples, the conjugated activatable antibody of Formula (II), wherein the N-terminal glutamate on either the heavy chain and/or light chain is optionally either pyroglutamate or post-translationally modified to pyroglutamate.

[0018] In another example of the disclosure, provided herein are methods of manufacturing a conjugated activatable antibody comprising the structure of Formula (I) or a salt thereof, wherein (i) AB is an antibody that specifically binds to human CD71 and comprises a heavy chain variable region comprising a CDRH1 sequence comprising SEQ ID NO: 9, a CDRH2 sequence comprising SEQ ID NO: 10, and a CDRH3 sequence comprising SEQ ID NO: 11; and a light chain variable region comprising a CDRL1 sequence comprising SEQ ID NO: 12 or SEQ ID NO: 13, a CDRL2 sequence comprising SEQ ID NO: 14, and a CDRL3 sequence comprising SEQ ID NO: 15; (ii) MM is a masking moiety comprising the amino acid sequence of SEQ ID NO: 18, wherein the MM inhibits the binding of the AB to human CD71 when the conjugated activatable antibody is in an uncleaved state; (iii) LP1 is a first linking moiety comprising the amino acid sequence of SEQ ID NO: 207; (iv) CM is a cleavable moiety comprising the sequence of SEQ ID NO: 156, wherein the CM is a polypeptide that functions as a substrate for a protease; and (v) LP2 is a second linking moiety comprising the amino acid sequence of SEQ ID NO: 38; and (b) wherein “n” is 2; the method comprising (i) reducing an activatable antibody comprising MM-LP1-CM-LP2-AB with a reducing agent; and (ii) conjugating one or more vcMMAE to the reduced activatable antibody. In some examples, the conjugated activatable antibody of Formula (I) wherein the AB comprises an

IgG1 isotype. In some examples, the conjugated activatable antibody of Formula (I) wherein the AB is an antibody having a heavy chain constant region, and wherein the C-terminal residue of the heavy chain constant region is not a lysine. In some examples, the conjugated activatable antibody of Formula (I), wherein the N-terminal glutamate on either the heavy chain and/or light chain is optionally either pyroglutamate or post-translationally modified to pyroglutamate.

[0019] In another example of the disclosure, provided herein are methods of manufacturing a conjugated activatable antibody comprising the structure of Formula (I) or a salt thereof, wherein (i) AB is an antibody that specifically binds to human CD71 and comprises a heavy chain variable region comprising a sequence of SEQ ID NO: 5 and a light chain variable region comprising a sequence of SEQ ID NO: 7; (ii) MM is a masking moiety comprising the amino acid sequence of SEQ ID NO: 18, wherein the MM inhibits the binding of the AB to human CD71 when the conjugated activatable antibody is in an uncleaved state; (iii) LP1 is a first linking moiety comprising the amino acid sequence of SEQ ID NO: 207; (iv) CM is a cleavable moiety comprising the sequence of SEQ ID NO: 156, wherein the CM is a polypeptide that functions as a substrate for a protease; and (v) LP2 is a second linking moiety comprising the amino acid sequence of SEQ ID NO: 38; and (b) wherein “n” is 2; the method comprising (i) reducing an activatable antibody comprising MM-LP1-CM-LP2-AB with a reducing agent; and (ii) conjugating one or more vcMMAE to the reduced activatable antibody. In some examples, the conjugated activatable antibody of Formula (I) wherein the AB comprises an IgG1 isotype. In some examples, the conjugated activatable antibody of Formula (I) wherein the AB is an antibody having a heavy chain constant region, and wherein the C-terminal residue of the heavy chain constant region is not a lysine. In some examples, the conjugated activatable antibody of Formula (I), wherein the N-terminal glutamate on either the heavy chain and/or light chain is optionally either pyroglutamate or post-translationally modified to pyroglutamate.

[0020] In another example of the disclosure, provided herein are methods of manufacturing a conjugated activatable antibody comprising the structure of Formula (I) or a salt thereof, wherein (i) AB is an antibody that specifically binds to human CD71 and comprises a heavy chain comprising a sequence of SEQ ID NO: 167 and a light chain comprising a sequence of SEQ ID NO: 19; (ii) MM is a masking moiety comprising the amino acid sequence of SEQ ID NO: 18, wherein the MM inhibits the binding of the AB to human CD71 when the conjugated activatable antibody is in an

uncleaved state; (iii) LP1 is a first linking moiety comprising the amino acid sequence of SEQ ID NO: 207; (iv) CM is a cleavable moiety comprising the sequence of SEQ ID NO: 156, wherein the CM is a polypeptide that functions as a substrate for a protease; and (v) LP2 is a second linking moiety comprising the amino acid sequence of SEQ ID NO: 38; and (b) wherein “n” is 2; the method comprising (i) reducing an activatable antibody comprising MM-LP1-CM-LP2-AB with a reducing agent; and (ii) conjugating one or more vcMMAE to the reduced activatable antibody. In some examples, the conjugated activatable antibody of Formula (I) wherein the AB comprises an IgG1 isotype. In some examples, the conjugated activatable antibody of Formula (I) wherein the AB is an antibody having a heavy chain constant region, and wherein the C-terminal residue of the heavy chain constant region is not a lysine. In some examples, the conjugated activatable antibody of Formula (I), wherein the N-terminal glutamate on either the heavy chain and/or light chain is optionally either pyroglutamate or post-translationally modified to pyroglutamate.

[0021] In another example of the disclosure, provided herein are methods of manufacturing a conjugated activatable antibody comprising the structure of Formula (I) or a salt thereof wherein MM-LP1-CM-LP2-AB is an activatable antibody, wherein the AB is an antibody that specifically binds to human CD71, wherein the activatable antibody comprises a heavy chain comprising a sequence of SEQ ID NO: 167 and a light chain comprising a sequence of SEQ ID NO: 169; and (b) wherein “n” is 2; the method comprising (i) reducing an activatable antibody comprising MM-LP1-CM-LP2-AB with a reducing agent; and (ii) conjugating one or more vcMMAE to the reduced activatable antibody. In some examples, the conjugated activatable antibody of Formula (I) wherein the AB comprises an IgG1 isotype. In some examples, the conjugated activatable antibody of Formula (I) wherein the AB is an antibody having a heavy chain constant region, and wherein the C-terminal residue of the heavy chain constant region is not a lysine. In some examples, the conjugated activatable antibody of Formula (I), wherein the N-terminal glutamate on either the heavy chain and/or light chain is optionally either pyroglutamate or post-translationally modified to pyroglutamate.

[0022] In another example of the disclosure, provided herein are methods of manufacturing a conjugated activatable antibody comprising the structure of Formula (I) or a salt thereof wherein MM-LP1-CM-LP2-AB is an activatable antibody, wherein the AB is an antibody that specifically binds to human CD71, wherein the activatable antibody comprises a heavy chain comprising a

sequence of SEQ ID NO: 167 and a light chain comprising a sequence of SEQ ID NO: 170; and (b) wherein “n” is 2; the method comprising (i) reducing an activatable antibody comprising MM-LP1-CM-LP2-AB with a reducing agent; and (ii) conjugating one or more vcMMAE to the reduced activatable antibody. In some examples, the conjugated activatable antibody of Formula (I) wherein the AB comprises an IgG1 isotype. In some examples, the conjugated activatable antibody of Formula (I) wherein the AB is an antibody having a heavy chain constant region, and wherein the C-terminal residue of the heavy chain constant region is not a lysine. In some examples, the conjugated activatable antibody of Formula (I), wherein the N-terminal glutamate on either the heavy chain and/or light chain is optionally either pyroglutamate or post-translationally modified to pyroglutamate.

[0023] In another example of the disclosure, provided herein are methods of manufacturing a conjugated activatable antibody comprising the structure of Formula (II) or a salt thereof, wherein (i) AB is an antibody that specifically binds to human CD71 and comprises a heavy chain variable region comprising a CDRH1 sequence comprising SEQ ID NO: 9, a CDRH2 sequence comprising SEQ ID NO: 10, and a CDRH3 sequence comprising SEQ ID NO: 11; and a light chain variable region comprising a CDRL1 sequence comprising SEQ ID NO: 12 or SEQ ID NO:13, a CDRL2 sequence comprising SEQ ID NO: 14, and a CDRL3 sequence comprising SEQ ID NO: 15; (ii) MM is a masking moiety comprising the amino acid sequence of SEQ ID NO: 18, wherein the MM inhibits the binding of the AB to human CD71 when the conjugated activatable antibody is in an uncleaved state; (iii) CM is a cleavable moiety comprising the sequence of SEQ ID NO: 156, wherein the CM is a polypeptide that functions as a substrate for a protease; and (b) wherein “n” is 2; the method comprising (i) reducing an activatable antibody comprising MM-LP1-CM-LP2-AB with a reducing agent; and (ii) conjugating one or more vcMMAE to the reduced activatable antibody. In some examples, the conjugated activatable antibody of Formula (I) wherein the AB comprises an IgG1 isotype. In some examples, the conjugated activatable antibody of Formula (I) wherein the AB is an antibody having a heavy chain constant region, and wherein the C-terminal residue of the heavy chain constant region is not a lysine. In some examples, the conjugated activatable antibody of Formula (I), wherein the N-terminal glutamate on either the heavy chain and/or light chain is optionally either pyroglutamate or post-translationally modified to pyroglutamate.

[0024] In another example of the disclosure, provided herein are methods of manufacturing a conjugated activatable antibody comprising the structure of Formula (II) or a salt thereof, wherein (i) AB is an antibody that specifically binds to human CD71 and comprises a heavy chain variable region comprising a sequence of SEQ ID NO: 5 and a light chain variable region comprising a sequence of SEQ ID NO: 7; (ii) MM is a masking moiety comprising the amino acid sequence of SEQ ID NO: 18, wherein the MM inhibits the binding of the AB to human CD71 when the conjugated activatable antibody is in an uncleaved state; (iii) CM is a cleavable moiety comprising the sequence of SEQ ID NO: 156, wherein the CM is a polypeptide that functions as a substrate for a protease; and (b) wherein “n” is 2; the method comprising (i) reducing an activatable antibody comprising MM-LP1-CM-LP2-AB with a reducing agent; and (ii) conjugating one or more vcMMAE to the reduced activatable antibody. In some examples, the conjugated activatable antibody of Formula (I) wherein the AB comprises an IgG1 isotype. In some examples, the conjugated activatable antibody of Formula (I) wherein the AB is an antibody having a heavy chain constant region, and wherein the C-terminal residue of the heavy chain constant region is not a lysine. In some examples, the conjugated activatable antibody of Formula (I), wherein the N-terminal glutamate on either the heavy chain and/or light chain is optionally either pyroglutamate or post-translationally modified to pyroglutamate.

[0025] In another example of the disclosure, provided herein are methods of manufacturing a conjugated activatable antibody comprising the structure of Formula (II) or a salt thereof, wherein (i) AB is an antibody that specifically binds to human CD71 and comprises a heavy chain comprising a sequence of SEQ ID NO: 167 and a light chain comprising a sequence of SEQ ID NO: 19; (ii) MM is a masking moiety comprising the amino acid sequence of SEQ ID NO: 18, wherein the MM inhibits the binding of the AB to human CD71 when the conjugated activatable antibody is in an uncleaved state; (iii) CM is a cleavable moiety comprising the sequence of SEQ ID NO: 156, wherein the CM is a polypeptide that functions as a substrate for a protease; and (b) wherein “n” is 2; the method comprising (i) reducing an activatable antibody comprising MM-LP1-CM-LP2-AB with a reducing agent; and (ii) conjugating one or more vcMMAE to the reduced activatable antibody. In some examples, the conjugated activatable antibody of Formula (I) wherein the AB comprises an IgG1 isotype. In some examples, the conjugated activatable antibody of Formula (I) wherein the AB is an antibody having a heavy chain constant region, and wherein the C-terminal

residue of the heavy chain constant region is not a lysine. In some examples, the conjugated activatable antibody of Formula (I), wherein the N-terminal glutamate on either the heavy chain and/or light chain is optionally either pyroglutamate or post-translationally modified to pyroglutamate.

[0026] In another example of the disclosure, provided herein are methods of manufacturing a conjugated activatable antibody comprising the structure of Formula (II) or a salt thereof wherein MM-LP1-CM-LP2-AB is an activatable antibody, wherein the AB is an antibody that specifically binds to human CD71, wherein the activatable antibody comprises a heavy chain comprising a sequence of SEQ ID NO: 167 and a light chain comprising a sequence of SEQ ID NO: 169; and (b) wherein “n” is 2; the method comprising (i) reducing an activatable antibody comprising MM-LP1-CM-LP2-AB with a reducing agent; and (ii) conjugating one or more vcMMAE to the reduced activatable antibody. In some examples, the conjugated activatable antibody of Formula (I) wherein the AB comprises an IgG1 isotype. In some examples, the conjugated activatable antibody of Formula (I) wherein the AB is an antibody having a heavy chain constant region, and wherein the C-terminal residue of the heavy chain constant region is not a lysine. In some examples, the conjugated activatable antibody of Formula (I), wherein the N-terminal glutamate on either the heavy chain and/or light chain is optionally either pyroglutamate or post-translationally modified to pyroglutamate.

[0027] In another example of the disclosure, provided herein are methods of manufacturing a conjugated activatable antibody comprising the structure of Formula (II) or a salt thereof wherein MM-LP1-CM-LP2-AB is an activatable antibody, wherein the AB is an antibody that specifically binds to human CD71, wherein the activatable antibody comprises a heavy chain comprising a sequence of SEQ ID NO: 167 and a light chain comprising a sequence of SEQ ID NO: 170; and (b) wherein “n” is 2; the method comprising (i) reducing an activatable antibody comprising MM-LP1-CM-LP2-AB with a reducing agent; and (ii) conjugating one or more vcMMAE to the reduced activatable antibody. In some examples, the conjugated activatable antibody of Formula (I) wherein the AB comprises an IgG1 isotype. In some examples, the conjugated activatable antibody of Formula (I) wherein the AB is an antibody having a heavy chain constant region, and wherein the C-terminal residue of the heavy chain constant region is not a lysine. In some examples, the conjugated activatable antibody of Formula (I), wherein the N-terminal glutamate on either the

heavy chain and/or light chain is optionally either pyroglutamate or post-translationally modified to pyroglutamate.

[0028] In another example of the disclosure, provided herein are pharmaceutical compositions comprising a conjugated activatable antibody of one iteration of Formula (I) or Formula (II). In some examples the pharmaceutical compositions may comprise a pharmaceutically acceptable carrier.

[0029] In another example of the disclosure, provided herein are methods of treating, alleviating a symptom of, or delaying the progression of a cancer in a subject, the method comprising administering a therapeutically effective amount of the conjugated activatable antibody of Formula (I) or Formula (II), or the pharmaceutical composition comprising the conjugated activatable antibody of one iteration of Formula (I) or Formula (II) and a optionally, a pharmaceutically acceptable carrier, to a subject in need thereof for a cancer selected from the group consisting of: gastric cancer, ovarian cancer, esophageal cancer, non-small cell lung cancer, ER+ breast cancer, triple-negative breast cancer, colorectal cancer, melanoma, prostate cancer, multiple myeloma, diffuse large B-cell lymphoma, head and neck small cell carcinoma, pancreatic cancer, mesothelioma, non-Hodgkin's lymphoma, hepatocellular carcinoma, and glioblastoma.

[0030] In another example of the disclosure, provided herein is a conjugated activatable antibody comprising (a) an activatable antibody (AA) comprising in an uncleaved state the structural arrangement from N-terminus to C-terminus as follows: MM-CM-AB, wherein (i) AB is an antibody that specifically binds to mammalian CD71 and comprises the heavy chain variable region sequence of SEQ ID NO: 5 and the light chain variable region sequence of SEQ ID NO: 7; (ii) MM is a masking moiety comprising the amino acid sequence of SEQ ID NO: 18, wherein the MM coupled to the AB inhibits the binding of the AB to CD71 when the conjugated activatable antibody is in an uncleaved state; (iii) CM is a cleavable moiety comprising the sequence of SEQ ID NO: 156 coupled to the AB wherein the CM is a polypeptide that functions as a substrate for a protease; and (b) monomethyl auristatin E (MMAE), wherein the activatable antibody is conjugated to two equivalents of MMAE. In some examples, the conjugated activatable antibody wherein the AB is an antibody having a heavy chain constant region, and wherein the C-terminal residue of the heavy chain constant region is not a lysine. In some examples, the conjugated activatable antibody wherein

the N-terminal glutamate on either the heavy chain and/or light chain is optionally either pyroglutamate or post-translationally modified to pyroglutamate.

[0031] In another example of the disclosure, provided herein is a conjugated activatable antibody having the formula AA-(AG)_p wherein (a) AA is an activatable antibody comprising in an uncleaved state the structural arrangement from N-terminus to C-terminus as follows: MM-CM-AB, wherein (i) AB is an antibody that specifically binds to mammalian CD71 and comprises the heavy chain variable region sequence of SEQ ID NO: 5 and the light chain variable region sequence of SEQ ID NO: 7; (ii) MM is a masking moiety comprising the amino acid sequence of SEQ ID NO: 18, wherein the MM coupled to the AB inhibits the binding of the AB to CD71 when the conjugated activatable antibody is in an uncleaved state; (iii) CM is a cleavable moiety comprising the sequence of SEQ ID NO: 156 coupled to the AB wherein the CM is a polypeptide that functions as a substrate for a protease; and (b) AG is an agent conjugated to the AA, wherein the agent is MMAE and wherein p is 2. In some examples, the conjugated activatable antibody wherein the AB is an antibody having a heavy chain constant region, and wherein the C-terminal residue of the heavy chain constant region is not a lysine. In some examples, the conjugated activatable antibody wherein the N-terminal glutamate on either the heavy chain and/or light chain is optionally either pyroglutamate or post-translationally modified to pyroglutamate.

[0032] In another example of the disclosure, provided herein is a method of manufacturing a conjugated activatable antibody comprising (a) conjugating at least one MMAE to an activatable antibody (AA) thereby producing a composition comprising AA-(MMAE)_p, wherein p is 1 to 8; and (b) enriching the composition for the conjugated activatable antibody species in which p is 2, wherein AA comprises in an uncleaved state comprises the structural arrangement from N-terminus to C-terminus as follows: MM-CM-AB wherein AB is an antibody that specifically binds to mammalian CD71 and comprises the heavy chain variable region sequence of SEQ ID NO: 5 and the light chain variable region sequence of SEQ ID NO: 7; (ii) MM is a masking moiety comprising the amino acid sequence of SEQ ID NO: 18, wherein the MM coupled to the AB inhibits the binding of the AB to CD71 when the conjugated activatable antibody is in an uncleaved state; (iii) CM is a cleavable moiety comprising the sequence of SEQ ID NO: 156 coupled to the AB wherein the CM is a polypeptide that functions as a substrate for a protease; and (b) AG is an agent conjugated to the AA, wherein the agent is MMAE and wherein p is 2. In some examples, the

conjugated activatable antibody wherein the AB is an antibody having a heavy chain constant region, and wherein the C-terminal residue of the heavy chain constant region is not a lysine. In some examples, the conjugated activatable antibody wherein the N-terminal glutamate on either the heavy chain and/or light chain is optionally either pyroglutamate or post-translationally modified to pyroglutamate.

[0033] In another example of the disclosure, provided herein is any conjugated activatable antibody, or a pharmaceutical composition, as disclosed herein, for use as a medicament.

[0034] In another example of the disclosure, provided herein is any conjugated activatable antibody, or a pharmaceutical composition, as disclosed herein, for use in the treatment of cancer, optionally wherein the cancer is selected from the group consisting of: gastric cancer, ovarian cancer, esophageal cancer, non-small cell lung cancer, ER+ breast cancer, triple-negative breast cancer, colorectal cancer, melanoma, prostate cancer, multiple myeloma, diffuse large B-cell lymphoma, head and neck small cell carcinoma, pancreatic cancer, mesothelioma, non-Hodgkin's lymphoma, hepatocellular carcinoma, and glioblastoma.

[0035] In another example of the disclosure, provided herein is a kit comprising at least one activatable antibody as disclosed herein. The kit may further comprise one or more vcMMAE, and/or a reducing agent.

BRIEF DESCRIPTION OF THE DRAWINGS

[0036] FIG. 1, as discussed in Example 5, depicts exemplary immunohistochemical (IHC) assays to determine levels of CD71 expression in various primary and metastatic cancer tissue types. The exemplary results shown in this figure and example showed that CD71 is expressed at high levels in primary tumors of a variety of human cancers.

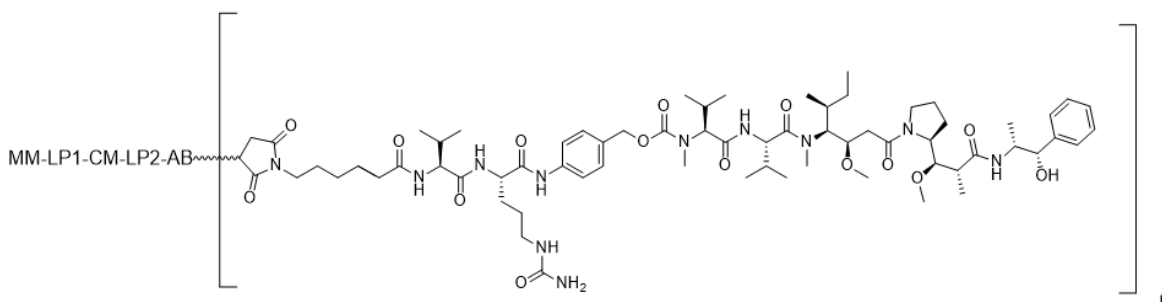
[0037] FIG. 2, as discussed in Example 5, depicts exemplary studies of the expression level of CD71 in multiple patient-derived metastatic cancer samples. The exemplary results shown in this figure and example showed that CD71 is expressed at high levels in metastatic tumors in a variety of human cancers.

[0038] FIGS. 3A and 3B, as discussed in Example 4, depict an exemplary *in vitro* assay of the ability of unconjugated and conjugated anti-CD71 activatable antibodies of the disclosure to bind

CLAIMS

What is claimed:

1. A conjugated activatable antibody comprising the structure of Formula (I) or a salt thereof:



Formula (I)

(a) wherein

- (i) AB is an antibody that specifically binds to human CD71 and comprises
 - i. a heavy chain variable region comprising a CDRH1 sequence comprising SEQ ID NO: 9, a CDRH2 sequence comprising SEQ ID NO: 10, and a CDRH3 sequence comprising SEQ ID NO: 11; and
 - ii. a light chain variable region comprising a CDRL1 sequence comprising SEQ ID NO: 12 or SEQ ID NO: 13, a CDRL2 sequence comprising SEQ ID NO: 14, and a CDRL3 sequence comprising SEQ ID NO: 15;
 - (ii) MM is a masking moiety comprising the amino acid sequence of SEQ ID NO: 18, wherein the MM inhibits the binding of the AB to human CD71 when the conjugated activatable antibody is in an uncleaved state;
 - (iii) LP1 is a first linking moiety comprising the amino acid sequence of SEQ ID NO: 207;
 - (iv) CM is a cleavable moiety comprising the sequence of SEQ ID NO: 156, wherein the CM is a polypeptide that functions as a substrate for a protease; and
 - (v) LP2 is a second linking moiety comprising the amino acid sequence of SEQ ID NO: 38; and
- (b) wherein “n” is 2.

2. The conjugated activatable antibody of claim 1, wherein the AB comprises an IgG1 isotype.

3. The conjugated activatable antibody of claim 1 or 2, wherein the AB is an antibody having a heavy chain constant region, and wherein the C-terminal residue of the heavy chain constant region is not a lysine.

4. The conjugated activatable antibody of any of claims 1-3, wherein the heavy chain variable region comprises a sequence of SEQ ID NO: 5 and the light chain variable region comprises a sequence of SEQ ID NO: 7.

5. The conjugated activatable antibody of any of claims 1-4, comprising a heavy chain comprising a sequence of SEQ ID NO: 167 and a light chain comprising a sequence of SEQ ID NO: 19.

6. The conjugated activatable antibody of any of claims 1-5, comprising a heavy chain variable region comprising a sequence of SEQ ID NO: 5 and a light chain variable region comprising a sequence of SEQ ID NO: 201.

7. The conjugated activatable antibody of claim 6, comprising a heavy chain variable region comprising a sequence of SEQ ID NO: 5 and a light chain variable region comprising a sequence of SEQ ID NO: 202.

8. The conjugated activatable antibody of claim 6, comprising a heavy chain variable region comprising a sequence of SEQ ID NO: 5 and a light chain variable region comprising a sequence of SEQ ID NO: 202, wherein the N-terminal amino acid of SEQ ID NO: 202 is modified to pyroglutamate.

9. The conjugated activatable antibody of any of claims 1-5, comprising a heavy chain comprising a sequence of SEQ ID NO: 167 and a light chain comprising a sequence of SEQ ID NO: 169.

10. The conjugated activatable antibody of claim 9, comprising a heavy chain comprising a sequence of SEQ ID NO: 167 and a light chain comprising a sequence of SEQ ID NO: 170.

11. The conjugated activatable antibody of claim 9, comprising a heavy chain comprising a sequence of SEQ ID NO: 167 and a light chain comprising a sequence of SEQ ID NO: 170, wherein the N-terminal amino acid of SEQ ID NO: 170 is modified to pyroglutamate.