

CLAIMS

1. An antibody or fusion protein comprising a heavy chain constant region in which position 295 by EU numbering is occupied by cysteine, wherein the antibody or fusion protein is conjugated to a drug or label via the cysteine at position 295.

~~2. The antibody or fusion protein of claim 1, conjugated to a drug or label via the cysteine at position 295.~~

32. The antibody or fusion protein of claim 1, in which position 239 by EU numbering is occupied by a cysteine.

43. The antibody or fusion protein of claim 32, conjugated to a drug or label via the cysteines at positions 295 and 239.

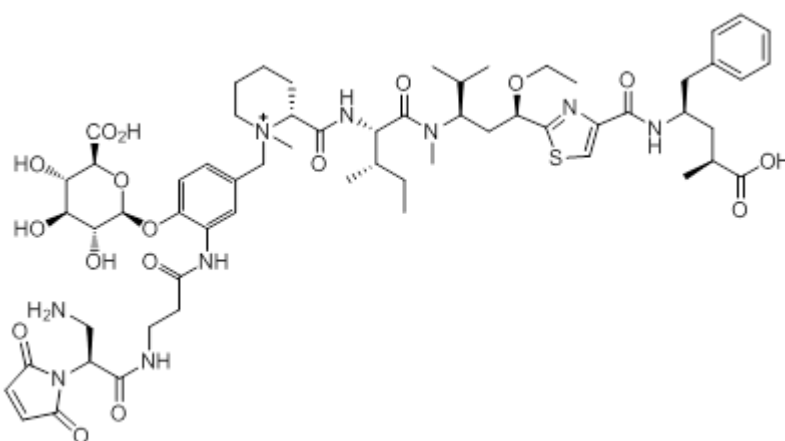
54. The antibody of claim 43 that is a heterodimer comprising two heavy chains and two light chains, wherein one molecule of the antibody is conjugated to four molecules of the drug via conjugation to the cysteine at position 295 and 239 in both heavy chains.

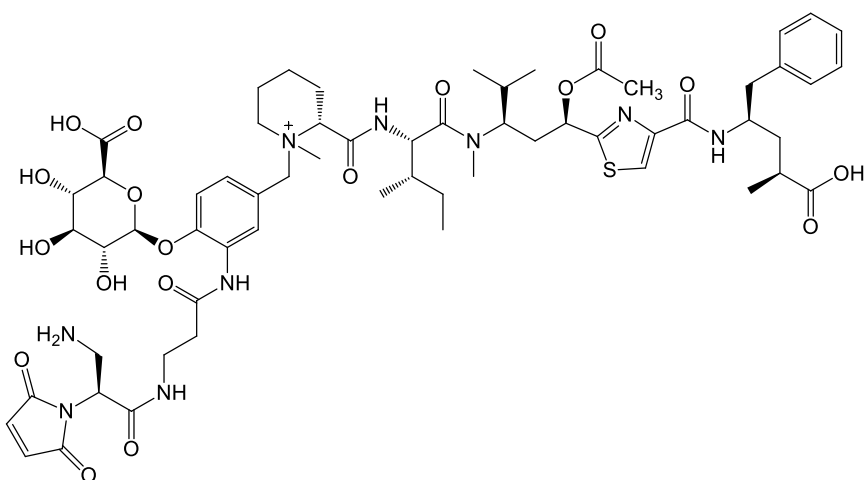
65. The antibody or fusion protein of any ~~preceding claim~~ one of claims 1-4, wherein the constant region has an isotype, which is human IgG1, IgG2, IgG3 or IgG4.

76. The antibody or fusion protein of any ~~preceding claim~~ one of claims 1-5, wherein the drug is a tubulysin.

87. The antibody or fusion protein of claim 76, wherein the drug is conjugated to the antibody or fusion protein via a glucuronide linker.

98. The antibody or fusion protein of claim 76, wherein the antibody or fusion protein is conjugated to a compound having a structure shown below providing the tubulysin and glucuronide linker





~~409.~~ The antibody or fusion protein of any one of ~~claim~~claims 1-~~65~~, wherein the drug is MMAE, MMAF, or a minor groove binder.

~~4410.~~ The antibody or fusion protein of any ~~preceding claim~~one of claims 1-9, wherein the heavy chain constant region has the sequence of any of SEQ ID NOS. 5-12 provided the C-terminal lysine can be absent.

~~4211.~~ The antibody or fusion protein of claim 1, wherein antibody is conjugated to the drug via a cleavable linker.

~~4312.~~ A pharmaceutical composition comprising the antibody or fusion protein of any ~~preceding claim~~one of claims 1-11.

~~14.~~ ~~An antibody or fusion protein comprising a heavy chain constant region in which position 239 by EU numbering is occupied by cysteine, which cysteine is conjugated to tubulysin M.~~

~~4513.~~ A method of producing an antibody or fusion protein comprising a heavy chain constant region in which positions 239 and 295 by EU numbering are occupied by cysteines, the method comprising:

culturing a cell engineered to encode the antibody or fusion protein, wherein the antibody or fusion protein is expressed; and

purifying the antibody or fusion protein.

~~4614.~~ The method of claim ~~4513~~, further comprising conjugating the antibody or fusion protein to a drug via the cysteines at position 239 and 295.

~~4715.~~ The method of claim ~~4513~~, further comprising contacting the antibody or fusion protein with a reducing agent that inhibits formation of disulfide bonds between cysteines at positions 239 and 295.

~~4816.~~ The method of claim ~~4715~~, wherein the antibody or fusion protein is contacted with the reducing agent by including the reducing agent in a medium in which the antibody or fusion protein is cultured.

~~19~~17. The method of claim ~~17~~15 or ~~18~~16, wherein the reducing agent is dithiothreitol, beta-mercaptoethanol or tris(2-carboxyethyl)phosphine.

~~20~~18. The method of claim ~~18~~16, wherein the reducing agent is dithiothreitol at a concentration of 0.1 to 2 mM.