

265:10506-10513 (1989). There remains a need in the art to identify and characterize modified human IgM antibodies with modified CDC activity.

SUMMARY

[0005] In one aspect, this disclosure provides a modified human IgM constant region that includes one or more amino acid substitutions relative to a wild-type human IgM constant region, where at least one amino acid substitution is at position L310 of SEQ ID NO: 1, position P311 of SEQ ID NO: 1, position P313 of SEQ ID NO: 1, position K315 of SEQ ID NO: 1, or any combination thereof~~a position in the C μ 3 domain ranging from T302 of SEQ ID NO: 1 to K322 of SEQ ID NO: 1~~, and where a modified IgM antibody that includes the modified IgM constant region and a heavy chain variable region specific for a target antigen and an immunoglobulin light chain variable domain (VL) exhibits reduced complement-dependent cytotoxicity (CDC) of cells expressing the target antigen relative to a corresponding wild-type human IgM antibody.

[0005a] In another aspect, the present disclosure provides modified human IgM antibody comprising the modified human IgM constant region as described herein, and a heavy chain variable region (VH) situated amino terminal to the modified human IgM constant region, wherein the modified human IgM antibody specifically binds to a target antigen and exhibits reduced complement dependent cytotoxicity (CDC) of cells expressing the target antigen relative to a corresponding wild-type human IgM antibody.

[0006] In certain examples, the modified human IgM constant region includes at least one amino acid substitution is at amino acid T302, C303, T304, V305, T306, H307, T308, D309, L310, P311, S312, P313, L314, K315, Q316, T317, I318, S319, R320, P321, and/or K322 of SEQ ID NO: 1. For example, the modified human IgM constant region can include at least one amino acid substitution is at position L310 of SEQ ID NO: 1, position P311 of SEQ ID NO: 1, position P313 of SEQ ID NO: 1, position K315 of SEQ ID NO: 1, or any combination thereof.

[0007] In certain examples at least one amino acid substitution can be at position L310 of SEQ ID NO: 1, e.g., L310 of SEQ ID NO: 1 can be substituted with alanine (L310A), serine (L310S), aspartic acid (L310D) or glycine (L310G). In certain examples L310 of SEQ ID NO: 1 can be substituted with alanine (L310A), for example, the modified IgM constant region can include SEQ ID NO: 15. In certain examples L310 of SEQ ID NO: 1 can be substituted with aspartic acid (L310D), for example, the modified IgM constant region can include SEQ ID NO: 23.

WHAT IS CLAIMED IS:

1. A modified human IgM constant region comprising one or more amino acid substitutions relative to a wild-type human IgM constant region, wherein at least one amino acid substitution is at position L310 of SEQ ID NO: 1, position P311 of SEQ ID NO: 1, position P313 of SEQ ID NO: 1, position K315 of SEQ ID NO: 1, or any combination thereof~~a position in the C μ 3 domain ranging from T302 of SEQ ID NO: 1 to K322 of SEQ ID NO: 1~~, and wherein a modified IgM antibody comprising the modified IgM constant region and a heavy chain variable region specific for a target antigen and an immunoglobulin light chain variable domain (VL) exhibits reduced complement-dependent cytotoxicity (CDC) of cells expressing the target antigen relative to a corresponding wild-type human IgM antibody.

~~2. The modified human IgM constant region of claim 1, wherein the at least one amino acid substitution is at amino acid T302, C303, T304, V305, T306, H307, T308, D309, L310, P311, S312, P313, L314, K315, Q316, T317, I318, S319, R320, P321, and/or K322 of SEQ ID NO: 1.~~

23. The modified human IgM constant region of claim 21, wherein:

(a) the amino acid substitution is at position L310 of SEQ ID NO: 1, and wherein L310 of SEQ ID NO: 1 is substituted with alanine (L310A), serine (L310S), aspartic acid (L310D) or glycine (L310G);

(b) the amino acid substitution is at position P311 of SEQ ID NO: 1, and wherein P311 of SEQ ID NO: 1 is substituted with alanine (P311A), serine (P311S), or glycine (P311G);

(c) the amino acid substitution is at position P313 of SEQ ID NO: 1, and wherein P313 of SEQ ID NO: 1 is substituted with alanine (P313A), serine (P313S), or glycine (P313G); and/or

(d) the amino acid substitution is at position K315 of SEQ ID NO: 1, and wherein K315 of SEQ ID NO: 1 is substituted with alanine (K315A), serine (K315S), aspartic acid (K315D), glutamine (K315Q), or glycine (P313G).

43. The modified human IgM constant region of claim 2-1 or claim 32, comprising SEQ ID NO: 15, SEQ ID NO: 23, SEQ ID NO: 2, SEQ ID NO: 3, SEQ ID NO: 16, SEQ ID NO: 24, SEQ ID NO: 25.

54. The modified human IgM constant region of claim 2-1 or claim 32, comprising:

(a) amino acid substitutions at positions P311 and P313 of SEQ ID NO: 1, wherein P311 of SEQ ID NO: 1 is substituted with alanine (P311A), serine (P311S), or glycine (P311G), and wherein P313 of SEQ ID NO: 1 is substituted with alanine (P313A), serine (P313S), or glycine (P313G);

(b) amino acid substitutions at positions P311 and P313 of SEQ ID NO: 1, wherein P311 of SEQ ID NO: 1 is substituted with alanine (P311A) and P313 of SEQ ID NO: 1 is substituted with serine (P313S);

(c) amino acid substitutions at positions L310 and K315 of SEQ ID NO: 1, wherein L310 is substituted with alanine (L310A) or serine (L310S) and K315 is substituted with alanine (K315A) or serine (K315S);

(d) amino acid substitutions at positions L310 and P311 of SEQ ID NO: 1, wherein L310 is substituted with alanine (L310A) and P311 is substituted with alanine (P311A);

(e) amino acid substitutions at positions L310 and P313 of SEQ ID NO: 1, wherein L310 is substituted with alanine (L310A) and P313 is substituted with serine (P313S);

(f) amino acid substitutions at positions P311 and K315 of SEQ ID NO: 1, wherein P311 is substituted with alanine (P311A) and K315 is substituted with alanine (K315A); or

(g) comprising amino acid substitutions at positions P313 and K315 of SEQ ID NO: 1, wherein P313 is substituted with serine (P313S) and K315 is substituted with alanine (K315A).

65. The modified human IgM constant region of claim 54, comprising SEQ ID NO: 4, SEQ ID NO: 17, SEQ ID NO: 18, SEQ ID NO: 19, SEQ ID NO: 20, SEQ ID NO: 21, or SEQ ID NO: 22.

76. A modified human IgM antibody comprising the modified human IgM constant region of any one of claims 1 to 65, and a heavy chain variable region (VH) situated amino terminal to the modified human IgM constant region, wherein the modified human IgM antibody specifically binds to a target antigen and exhibits reduced complement-

dependent cytotoxicity (CDC) of cells expressing the target antigen relative to a corresponding wild-type human IgM antibody.

87. The modified human IgM antibody of claim 76, which is a pentameric or a hexameric antibody comprising five or six bivalent IgM binding units, respectively, wherein each binding unit comprises two IgM heavy chains each comprising a VH situated amino terminal to the modified human IgM constant region, and two immunoglobulin light chains each comprising a light chain variable domain (VL) situated amino terminal to a human immunoglobulin light chain constant region.

98. The modified human IgM antibody of claim 87, which is a pentameric antibody, and further comprises a J-chain, or functional fragment thereof, or a functional variant thereof.

109. The modified human IgM antibody of claim 98, wherein J-chain or fragment thereof comprises amino acids 23 to 158 of SEQ ID NO: 7 or a functional fragment thereof, or a functional variant thereof.

104. The modified human IgM antibody of claim 9-8 or 109, wherein the J-chain or fragment or variant thereof is a modified J-chain further comprising a heterologous polypeptide, wherein the heterologous polypeptide is directly or indirectly fused to the J-chain or fragment or variant thereof.

121. The modified human IgM antibody of claim 140, wherein the heterologous polypeptide is fused to the J-chain or fragment thereof via a peptide linker.

123. The modified human IgM antibody of claim 1211, wherein the peptide linker comprises at least 5 amino acids, but no more than 25 amino acids or wherein the peptide linker consists of GGGGSGGGGSGGGGS (SEQ ID NO: 12).

134. The modified human IgM antibody of any one of claims 140 to 132, wherein the heterologous polypeptide is fused to the N-terminus of the J-chain or fragment or variant thereof, the C-terminus of the J-chain or fragment or variant thereof, or to both the N-terminus and C-terminus of the J-chain or fragment or variant thereof.

~~145~~. The modified human IgM antibody of any one of claims ~~44-10~~ to ~~4413~~, wherein the heterologous polypeptide comprises a binding domain.

~~156~~. The modified human IgM antibody of claim ~~4514~~, wherein the binding domain of the heterologous polypeptide is an antibody or antigen-binding fragment thereof.

~~167~~. The modified human IgM antibody of claim ~~4615~~, wherein the antigen-binding fragment comprises an Fab fragment, an Fab' fragment, an F(ab')₂ fragment, an Fd fragment, an Fv fragment, a single-chain Fv (scFv) fragment, a disulfide-linked Fv (sdFv) fragment, or any combination thereof.

~~4817~~. The modified human IgM antibody of any one of claims ~~45-14~~ to ~~4716~~, wherein the heterologous polypeptide can specifically bind to CD3ε.

~~4918~~. The modified human IgM antibody of any one of claims ~~7-6~~ to ~~4817~~, wherein the maximum CDC activity achieved by a target-specific IgM antibody comprising the modified human IgM constant region in a dose-response assay is reduced by at least 5%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 95%, or 100% relative to a corresponding wild-type IgM antibody identical except for the modified human IgM constant region.

~~2019~~. The modified human IgM antibody of any one of claims ~~7-6~~ to ~~4817~~, wherein the antibody concentration of a target-specific IgM antibody comprising the modified human IgM constant region effecting 50% CDC activity (EC₅₀) is increased by at least 2-fold, at least 5-fold, at least 10-fold, at least 20-fold, and least 30-fold, at least 40-fold, at least 50-fold, at least 60-fold, at least 70-fold, at least 80-fold, at least 90-fold, or at least 100-fold relative to a corresponding wild-type IgM antibody identical except for the modified human IgM constant region.

~~204~~. A polynucleotide comprising a nucleic acid sequence that encodes the modified human IgM constant region of any one of claims 1 to ~~65~~, or a heavy chain polypeptide subunit of the modified human IgM antibody of any one of claims ~~7-6~~ to ~~2019~~.

221. A composition comprising the polynucleotide of claim 240.

2322. The composition of claim 2221, further comprising a nucleic acid sequence that encodes a light chain polypeptide subunit comprising a human antibody light chain constant region or fragment thereof fused to the C-terminal end of a VL.

2423. The composition of claim 2322, wherein the nucleic acid sequence encoding the heavy chain polypeptide subunit and the nucleic acid sequence encoding the light chain polypeptide subunit are on separate vectors or are on a single vector.

2524. The composition of any one of claims 22-21 to 2423, further comprising a nucleic acid sequence that encodes a J-chain, or functional fragment thereof, or a functional variant thereof.

2625. The composition of claim 2524, wherein the nucleic acid sequence encoding the heavy chain polypeptide subunit, the nucleic acid sequence encoding the light chain polypeptide subunit, and the nucleic acid sequence encoding the J-chain are on a single vector or are each on separate vectors.

2726. The vector or vectors of claim 24-23 to 2625.

2827. A host cell comprising the polynucleotide of claim 2420, the composition of any one of claims 22-21 to 2625, or the vector or vectors of claim 2726, wherein the host cell can express the modified human IgM constant region of any one of claims 1 to 65, or the modified human IgM antibody of any one of claims 7-6 to 20-19 or a functional fragment thereof.

2928. A method of producing the modified human IgM constant region of any one of claims 1 to 65, or the modified human IgM antibody of any one of claims 7-6 to 2019, comprising culturing the host cell of claim 2827, and recovering the constant region or antibody.