

WHAT IS CLAIMED IS:

1. An isolated IgM-derived binding molecule comprising at least one variant IgM-derived heavy chain, wherein the at least one variant IgM-derived heavy chain comprises a variant IgM heavy chain constant region associated with a binding domain that specifically binds to a target, wherein at least one asparagine(N)-linked glycosylation motif of the variant IgM heavy chain constant region is mutated to prevent glycosylation at that motif, and wherein an N-linked glycosylation motif comprises the amino acid sequence N-X₁-S/T, wherein N is asparagine, X₁ is any amino acid except proline, and S/T is serine or threonine.
2. The IgM-derived binding molecule of claim 1, wherein the variant IgM heavy chain constant region is derived from a human IgM heavy chain constant region comprising five N-linked glycosylation motifs N-X₁-S/T starting at amino acid positions corresponding to amino acid 46 (motif N1), amino acid 209 (motif N2), amino acid 272 (motif N3), amino acid 279 (motif N4), and amino acid 440 (motif N5) of SEQ ID NO: 1 (alleleIGHM*03) or SEQ ID NO: 2 (alleleIGHM*04).
3. The IgM-derived binding molecule of claim 2, wherein at least one, at least two, at least three, or at least four of the N-X₁-S/T motifs comprises an amino acid insertion, deletion, or substitution that prevents glycosylation at that motif.
4. The IgM-derived binding molecule of claim 3, comprising an amino acid insertion, deletion, or substitution at motif N1, motif N2, motif N3, motif N5, or any combination of two or more, three or more, or all four of motifs N1, N2, N3, or N5, wherein the amino acid insertion, deletion, or substitution prevents glycosylation at that motif.
5. The IgM-derived binding molecule of claim 4, comprising an amino acid substitution at an amino acid position corresponding to amino acid N46, N209, N272, or N440 of SEQ ID NO: 1 or SEQ ID NO: 2 wherein the substituted amino acid is any amino acid, an amino acid substitution at an amino acid position corresponding to amino acid S48, S211, S274, or S442 of SEQ ID NO: 1 or SEQ ID NO: 2 wherein the substituted amino acid is any amino acid except threonine, or any combination of two or more, three or more, or four or more of the amino acid substitutions.
6. The IgM-derived binding molecule of claim 5, comprising an amino acid substitution corresponding to N46X₂, N46A, N46D, N46Q, N46K, S48X₃, S48A, N229X₂, N229A, N229D, N229Q, N229K, S231X₃, S231A, N272X₂, N272A, N272D, N272Q,

N272K, S274X₃, S274A, N440X₂, N440A, N440D, N449Q, N449K, S242X₃, or S424A of SEQ ID NO: 1 or SEQ ID NO: 2, or any combination of two or more, three or more, or four or more of the amino acid substitutions, wherein X₂ is any amino acid and X₃ is any amino acid except threonine.

7. The IgM-derived binding molecule of any one of claims 1 to 6, wherein the variant IgM heavy chain constant region is a variant human IgM constant region comprising the amino acid sequence SEQ ID NO: 3, SEQ ID NO: 4, SEQ ID NO: 5, SEQ ID NO: 6, SEQ ID NO: 7, SEQ ID NO: 8, SEQ ID NO: 11, SEQ ID NO: 12, SEQ ID NO: 13, SEQ ID NO: 14, SEQ ID NO: 15, SEQ ID NO: 16, SEQ ID NO: 17, or SEQ ID NO: 18.

8. The IgM-derived binding molecule of any one of claims 1 to 7, wherein the variant IgM heavy chain constant region is mutated to introduce at least one new asparagine(N)-linked glycosylation motif into the variant IgM heavy chain constant region, wherein the at least one new asparagine(N)-linked glycosylation motif is introduced at a site in the variant IgM heavy chain constant region that is not naturally glycosylated in an IgM antibody.

9. The IgM-derived binding molecule of claim 8, wherein the new asparagine(N)-linked glycosylation motif is at a position in the variant IgM heavy chain constant region that corresponds to the position of an asparagine(N)-linked glycosylation motif present in a different immunoglobulin isotype.

10. The IgM-derived binding molecule of claim 9, wherein the different immunoglobulin isotype is a human immunoglobulin isotype selected from the group consisting of human IgG1, human IgG2, human IgG3, human IgG4, human IgA1, human IgA2, human IgD, and human IgE.

11. The IgM-derived binding molecule of any one of claims 1 to 10, wherein the target is a target epitope, a target antigen, a target cell, a target organ, or a target virus.

12. The IgM-derived binding molecule of any one of claims 1 to 11, which is a pentameric or a hexameric IgM 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 variant IgM constant region, and two immunoglobulin light chains each comprising a light chain variable domain (VL) situated amino terminal to an immunoglobulin light chain constant region, and wherein the VH and VL combine to form an antigen-binding domain that specifically binds to the target.

13. The IgM-derived binding molecule of claim 12, wherein the five or six IgM binding units are identical.

14. The IgM-derived binding molecule of claim 12 or claim 13, which is pentameric, and further comprises a J-chain, or functional fragment thereof, or a functional variant thereof.

15. The IgM-derived binding molecule of claim 14, wherein the J-chain is a mature human J-chain comprising the amino acid sequence SEQ ID NO: 20 or a functional fragment thereof, or a functional variant thereof.

16. The IgM-derived binding molecule of claim 14 or claim 15, wherein the J-chain is a functional variant J-chain comprising one or more single amino acid substitutions, deletions, or insertions relative to a reference J-chain identical to the variant J-chain except for the one or more single amino acid substitutions, deletions, or insertions, and wherein the IgM-derived binding molecule exhibits an increased serum half-life upon administration to a subject animal relative to a reference IgM-derived binding molecule that is identical except for the one or more single amino acid substitutions, deletions, or insertions in the variant J-chain, and is administered in the same way to the same animal species.

17. The IgM-derived binding molecule of claim 16, wherein the variant J-chain or functional fragment thereof comprises one, two, three, or four single amino acid substitutions, deletions, or insertions relative to the reference J-chain.

18. The IgM-derived binding molecule of claim 16 or claim 17, wherein the variant J-chain or functional fragment thereof comprises an amino acid substitution at the amino acid position corresponding to amino acid Y102 of the wild-type mature human J-chain (SEQ ID NO: 20).

19. The IgM-derived binding molecule of claim 18, wherein the amino acid corresponding to Y102 of SEQ ID NO: 20 is substituted with alanine (A).

20. The IgM-derived binding molecule of claim 19, wherein the J-chain is the variant human J-chain J*, which comprises the amino acid sequence SEQ ID NO: 21.

21. The IgM-derived binding molecule of any one of claims 16 to 19, wherein the variant J-chain or functional fragment thereof comprises an a mutation within the asparagine(N)-linked glycosylation motif N-X₁-S/T starting at the amino acid position corresponding to amino acid 49 (motif N6) of the mature human J-chain (SEQ ID NO: 20), wherein N is asparagine, X₁ is any amino acid except proline, and S/T is serine or threonine, and wherein the mutation prevents glycosylation at that motif.

22. The IgM-derived binding molecule of claim 21, wherein the variant J-chain or functional fragment thereof comprises an amino acid substitution at the amino acid position corresponding to amino acid N49 or amino acid S51 SEQ ID NO: 20 wherein the amino acid corresponding to S51 is not substituted with threonine (T), or wherein the variant J-chain comprises amino acid substitutions at the amino acid positions corresponding to both amino acids N49 and S51 of SEQ ID NO: 20.

23. The IgM-derived binding molecule of claim 22, wherein the position corresponding to N49 of SEQ ID NO: 20 is substituted with alanine (A), glycine (G), threonine (T), serine (S) or aspartic acid (D).

24. The IgM-derived binding molecule of claim 23, wherein the position corresponding to N49 of SEQ ID NO: 20 is substituted with alanine (A).

25. The IgM-derived binding molecule of claim 24, wherein the J-chain is a variant human J-chain and comprises the amino acid sequence SEQ ID NO: 22.

26. The IgM-derived binding molecule of claim 23, wherein the position corresponding to N49 of SEQ ID NO: 20 is substituted with aspartic acid (D).

27. The IgM-derived binding molecule of claim 26, wherein the J-chain is a variant human J-chain and comprises the amino acid sequence SEQ ID NO: 23.

28. The IgM-derived binding molecule of any one of claims 14 to 27, wherein the J-chain or fragment or variant thereof is a modified J-chain further comprising a heterologous moiety, wherein the heterologous moiety is fused or conjugated to the J-chain or fragment or variant thereof.

29. The IgM-derived binding molecule of claim 28, wherein the heterologous moiety is a polypeptide fused to the J-chain or fragment or variant thereof.

30. The IgM-derived binding molecule of claim 29, wherein the heterologous polypeptide is fused to the J-chain or fragment or variant thereof via a peptide linker.

31. The IgM-derived binding molecule of claim 30, wherein the peptide linker comprises at least 5 amino acids, but no more than 25 amino acids.

32. The IgM-derived binding molecule of claim 30 or claim 31, wherein the peptide linker consists of GGGGSGGGGSGGGGS (SEQ ID NO: 29).

33. The IgM-derived binding molecule of any one of claims 29 to 32, 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.

34. The IgM-derived binding molecule of any one of claims 29 to 33, wherein the heterologous polypeptide comprises a binding domain.

35. The IgM-derived binding molecule of claim 34, wherein the binding domain of the heterologous polypeptide is an antibody or antigen-binding fragment thereof.

36. The IgM-derived binding molecule of claim 35, wherein the antigen-binding fragment is a scFv fragment.

37. The IgM-derived binding molecule of claim 36, wherein the heterologous scFv fragment specifically binds to CD3ε.

38. The IgM-derived binding molecule of claim 37, wherein the modified J-chain comprises the amino acid sequence SEQ ID NO: 24 (V15J), SEQ ID NO: 25 (V15J*), SEQ ID NO: 26 (V15J N49D), or SEQ ID NO: 55 (SJ*) or SEQ ID NOs: 20, 21, 22, or 23 fused via a peptide linker to an anti-CD3ε scFv comprising HCDR1, HCDR2, HCDR3, LCDR1, LCDR2, and LCDR3 amino acid sequences comprising SEQ ID NO: 48, SEQ ID NO: 49, SEQ ID NO: 50, SEQ ID NO: 52, SEQ ID NO: 53, and SEQ ID NO: 54; SEQ ID NO: 57, SEQ ID NO: 59, SEQ ID NO: 62, SEQ ID NO: 65, SEQ ID NO: 67, and SEQ ID NO: 69; SEQ ID NO: 57, SEQ ID NO: 59, SEQ ID NO: 62, SEQ ID NO: 65, SEQ ID NO: 67, and SEQ ID NO: 70; SEQ ID NO: 58, SEQ ID NO: 60, SEQ ID NO: 63, SEQ ID NO: 66, SEQ ID NO: 68, and SEQ ID NO: 71; SEQ ID NO: 58, SEQ ID NO: 61, SEQ ID NO: 63, SEQ ID NO: 66, SEQ ID NO: 68, and SEQ ID NO: 72; SEQ ID NO: 58, SEQ ID NO: 61, SEQ ID NO: 64, SEQ ID NO: 66, SEQ ID NO: 68, and SEQ ID NO: 73, respectively.

39. A polynucleotide comprising a nucleic acid sequence that encodes the at least one variant IgM-derived heavy chain of any one of claims 1 to 37.

40. A composition comprising the polynucleotide of claim 39.

41. The composition of claim 40, further comprising a nucleic acid sequence that encodes a light chain polypeptide subunit.

42. The composition of claim 41, wherein the nucleic acid sequence encoding the at least one variant IgM-derived heavy chain and the nucleic acid sequence encoding the light chain polypeptide subunit are on separate vectors.

43. The composition of claim 41, wherein the nucleic acid sequence encoding the at least one variant IgM-derived heavy chain and the nucleic acid sequence encoding the light chain polypeptide subunit are on a single vector.

44. The composition of any one of claims 41 to 43, further comprising a nucleic acid sequence that encodes a J-chain, or functional fragment thereof, or a functional variant thereof.

45. The composition of claim 44, wherein the nucleic acid sequence encoding the at least one variant IgM-derived heavy chain, the nucleic acid sequence encoding the light chain polypeptide subunit, and the nucleic acid sequence encoding the J-chain are on a single vector.

46. The composition of claim 44, wherein the nucleic acid sequence encoding the at least one variant IgM-derived heavy chain, the nucleic acid sequence encoding the light chain polypeptide subunit, and the nucleic acid sequence encoding the J-chain are each on separate vectors.

47. The vector or vectors of any one of claims 42, 43, 45, or 46.

48. A host cell comprising the polynucleotide of claim 39, the composition of any one of claims 40 to 46, or the vector or vectors of claim 47, wherein the host cell can express the IgM-derived binding molecule of any one of claims 1 to 37.

49. A method of producing the IgM-derived binding molecule of any one of claims 1 to 37, comprising culturing the host cell of claim 48, and recovering the constant region or antibody.