

thereof. In one aspect, the present disclosure refers to an IgM antibody or IgM-like antibody with enhanced serum half-life comprising five bivalent antibody binding units or variants or fragments thereof and a variant J-chain or functional fragment thereof, wherein each binding unit comprises two IgM heavy chain constant regions or multimerizing fragments or variants thereof, each associated with an antigen-binding domain or subunit thereof, wherein the variant J-chain or functional fragment thereof comprises one, two, three, or four single amino acid substitutions, deletions, or insertions relative to a reference J-chain identical to the variant J-chain except for the one, two, three, or four single amino acid substitutions, deletions, or insertions, and wherein the variant J-chain can affect serum half-life of the IgM antibody or IgM-like antibody; and wherein the IgM antibody or IgM-like antibody exhibits an increased serum half-life upon administration to a subject animal relative to a reference IgM antibody or IgM-like antibody that is identical except for the one or more single amino acid substitutions, deletions, or insertions in the variant J-chain, and is to be administered in the same way to the same animal species.

[0008a] In another aspect, the present disclosure refers to an IgM antibody or IgM-like antibody with enhanced serum half-life comprising five or six bivalent antibody binding units or variants or fragments thereof, wherein each binding unit comprises two variant IgM heavy chain constant regions or multimerizing fragments thereof, each associated with an antigen-binding domain or subunit thereof, wherein the variant IgM heavy chain constant regions or multimerizing fragments thereof each comprise one, two, three, or four single amino acid substitutions, deletions, or insertions relative to a reference IgM heavy chain constant region identical to the variant IgM heavy chain constant regions except for the one, two, three, or four single amino acid substitutions, deletions, or insertions, and wherein the variant IgM heavy chain constant regions can affect serum half-life of the IgM antibody or IgM-like antibody; and wherein the IgM antibody or IgM-like antibody exhibits an increased serum half-life upon administration to a subject animal relative to a reference IgM antibody or IgM-like antibody that is identical except for the one or more single amino acid substitutions, deletions, or insertions in the IgM heavy chain constant regions, and is to be administered in the same way to the same animal species.

[0008b] In another aspect, the present disclosure refers to a composition comprising the IgM antibody or IgM-like antibody as described herein, and a pharmaceutically acceptable carrier. In yet another aspect, the present disclosure refers to a variant J-chain 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: 10, SEQ ID NO: 23, or SEQ ID NO: 24, wherein the variant J-chain can affect the serum half-life of an IgM antibody or IgM-like antibody comprising the variant J-chain. In yet another aspect, the present disclosure refers to an isolated polynucleotide comprising a nucleic acid encoding a subunit polypeptide of the IgM or IgM-like antibody as described herein wherein the subunit polypeptide comprises (a) an IgM or IgM-like heavy chain constant region or multimerizing fragment thereof, (b) an antibody light chain, or (c) a J-chain, a modified J-chain, or functional fragment or variant thereof, or (d) any combination thereof. In yet another aspect, the present disclosure refers to an expression vector comprising the polynucleotide as described herein. In yet another aspect, the present disclosure refers to a host cell comprising the polynucleotide as described herein or the expression vector as described herein.

[0008c] In a further aspect, the present disclosure refers to a method of identifying variant J-chains that can increase the serum half-life of pentameric IgM antibodies or pentameric IgM-like antibodies comprising the variant J-chains, the method comprising: 1(a) testing pentameric IgM antibodies or pentameric IgM-like antibodies comprising variant J-chains or fragments thereof for increased serum half-life in a subject animal relative to a reference pentameric IgM antibody or pentameric IgM-like antibody, wherein the variant J-chains or fragments thereof comprise defined amino acid insertions, deletions, or substitutions, and wherein the reference pentameric IgM antibody or pentameric IgM-like antibody comprises a J-chain or fragment thereof identical to the variant J-chains except for the defined amino acid insertions, deletions or substitutions; and (b) recovering those variant J-chains or fragments thereof that confer increased serum half-life upon the pentameric IgM antibodies or pentameric IgM-like antibodies relative to reference pentameric IgM antibody or pentameric IgM-like antibody; or 2)(a) testing pentameric IgM antibodies or pentameric IgM-like antibodies comprising variant J-chains or fragments thereof for their level of binding to the Fc alpha-mu receptor (Fc α μ R), the polymeric Ig receptor (pIgR), or both the Fc α μ R and the pIgR, wherein the variant J-chains or fragments thereof comprise defined amino acid insertions, deletions, or substitutions; and (b) recovering those variant J-chains or fragments thereof that confer reduced Fc α μ R binding ability, reduced pIgR binding ability, or reduced Fc α μ R and pIgR binding ability upon the pentameric IgM antibodies or pentameric IgM-like antibodies relative to a reference pentameric IgM antibody or pentameric IgM-like antibody comprising a J-chain or fragment thereof identical to the variant J-chains except for the defined amino acid insertions, deletions or substitutions.

[0008d] In a further aspect, the present disclosure refers to a method of identifying variant IgM heavy chain constant regions that can increase the serum half-life of an IgM antibody or IgM-like antibody comprising the variant IgM heavy chain constant regions, comprising: (1)(a) testing IgM antibodies or IgM-like antibodies comprising variant IgM heavy chain constant regions for increased serum half-life in a subject animal relative to a reference pentameric IgM antibody or pentameric IgM-like antibody, wherein the variant IgM heavy chain constant regions comprise defined amino acid insertions, deletions, or substitutions, and wherein the reference IgM antibody or IgM-like antibody comprises IgM heavy chain constant regions identical to the variant IgM heavy chain constant regions except for the defined amino acid insertions, deletions or substitutions; and (b) recovering those IgM antibodies or IgM-like antibodies comprising variant IgM heavy chain constant regions that confer increased serum half-life upon the IgM antibodies or IgM-like antibodies comprising the variant IgM heavy chain constant regions relative to the reference IgM antibody or IgM-like antibody; or (2)(a) testing IgM antibodies or IgM-like antibodies comprising variant IgM heavy chain constant regions for their level of binding to the Fc alpha-mu receptor (Fc α μ R), Fc mu receptor (Fc μ R), the polymeric Ig receptor (pIgR), any combination of two of the receptors, or all three of the receptors, wherein the variant IgM heavy chain constant regions comprise defined amino acid insertions, deletions, or substitutions; and (b) recovering those IgM antibodies or IgM-like antibodies comprising variant IgM heavy chain constant regions that confer reduced Fc α μ R binding ability, reduced Fc μ R binding ability, reduced pIgR binding ability, reduced ability to bind to any two of the receptors, or reduced ability to bind to all three of the receptors, upon the IgM antibodies or IgM-like antibodies comprising the variant IgM heavy chain constant regions relative to a reference IgM antibody or IgM-like antibody comprising IgM heavy chain constant regions identical to the variant IgM heavy chain constant regions except for the defined amino acid insertions, deletions or substitutions.

[0008e] In certain examples the variant J-chain or functional fragment thereof includes 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. The provided variant J-chain can affect serum half-life of the provided IgM antibody or IgM-like antibody. For example, in certain examples the IgM antibody or IgM-like antibody exhibits an increased serum half-life upon administration to a subject animal relative to a reference IgM antibody or IgM-like antibody 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. In certain examples, the variant J-chain or functional fragment thereof includes one, two, three, or four single amino acid substitutions, deletions, or insertions relative to the reference J-chain.

5 **[0009]** In certain examples, the variant J-chain or functional fragment thereof includes an amino acid substitution at the amino acid position corresponding to amino acid Y102 of the wild-type human J-chain (SEQ ID NO: 2). For example, the amino acid corresponding to Y102 of SEQ ID NO: 2 can be substituted with alanine (A), serine (S) or arginine (R). In certain examples, the amino acid corresponding to Y102 of SEQ ID NO: 2 is substituted
10 with alanine (A). In certain examples, the J-chain is a variant human J-chain and includes the amino acid sequence SEQ ID NO: 3. In certain examples, the amino acid corresponding to Y102 of SEQ ID NO: 2 is substituted with serine (S). In certain examples, the J-chain is a variant human J-chain and includes the amino acid sequence SEQ ID NO: 4. In certain examples, the amino acid corresponding to Y102 of SEQ ID NO: 2 is substituted with
15 arginine (R). In certain examples, the J-chain is a variant human J-chain and includes the amino acid sequence SEQ ID NO: 5. In certain examples, the variant J-chain or functional fragment thereof includes an amino acid substitution at the amino acid position corresponding to amino acid T103 of the wild-type human J-chain (SEQ ID NO: 2). For example, the amino acid corresponding to T103 of SEQ ID NO: 2 can be substituted with
20 alanine (A). In certain examples, the J-chain is a variant human J-chain and includes the amino acid sequence SEQ ID NO: 6. In certain examples, the variant J-chain or functional fragment thereof includes an amino acid substitution at the amino acid position corresponding to amino acid N49 or amino acid S51 of the human J-chain (SEQ ID NO: 2), where S51 is not substituted with threonine (T), or where the J-chain includes amino acid substitutions at the amino acid positions corresponding to both amino acids N49 and
25 S51 of the human J-chain (SEQ ID NO: 2). For example, the position corresponding to N49 of SEQ ID NO: 2 can be substituted with alanine (A), glycine (G), threonine (T), serine (S) or aspartic acid (D). In certain examples, the position corresponding to N49 of SEQ ID NO: 2 is substituted with alanine (A). In certain examples, the J-chain is a variant
30 human J-chain and includes the amino acid sequence SEQ ID NO: 7. In another example, the position corresponding to S51 of SEQ ID NO: 2 can be substituted with alanine (A) or glycine (G), for example, the position corresponding to S51 of SEQ ID NO: 2 is substituted

with alanine (A). In certain examples, the J-chain is a variant human J-chain and includes the amino acid sequence SEQ ID NO: 8.

[0010] In certain examples, an IgM antibody or IgM-like antibody that includes a variant J-chain as provided above, the IgM heavy chain constant regions or multimerizing fragments thereof can be variant IgM heavy chain constant regions including one or more single amino acid substitutions, deletions, or insertions relative to a reference IgM heavy chain constant region identical to the variant IgM heavy chain constant regions except for the one or more single amino acid substitutions, deletions, or insertions. According to these examples, the variant IgM heavy chain constant regions can affect serum half-life of the IgM antibody or IgM-like antibody such that the IgM antibody or IgM-like antibody exhibits a further increased serum half-life upon administration to a subject animal relative to a reference IgM antibody or IgM-like antibody that is identical except for the one or more single amino acid substitutions, deletions, or insertions in the IgM heavy chain constant regions, and is administered in the same way to the same animal species. In certain examples, the further increase in serum half-life is additive.

[0011] This disclosure further provides an IgM antibody or IgM-like antibody with enhanced serum half-life, where the IgM antibody or IgM-like antibody includes five or six bivalent antibody binding units or variants or fragments thereof, and where each binding unit includes two variant IgM heavy chain constant regions or multimerizing fragments thereof, each associated with an antigen-binding domain or subunit thereof. According to these examples, the variant IgM heavy chain constant regions or multimerizing fragments thereof each include one or more single amino acid substitutions, deletions, or insertions relative to a reference IgM heavy chain constant region identical to the variant IgM heavy chain constant regions except for the one or more single amino acid substitutions, deletions, or insertions. Also, according to these examples, the variant IgM heavy chain constant regions can affect serum half-life of the IgM antibody or IgM-like antibody. In certain examples, the IgM antibody or IgM-like antibody exhibits an increased serum half-life upon administration to a subject animal relative to a reference IgM antibody or IgM-like antibody that is identical except for the one or more single amino acid substitutions, deletions, or insertions in the IgM heavy chain constant regions, and is administered in the same way to the same animal species. In certain examples, the variant IgM heavy chain constant regions include one, two, three, or four single amino acid

substitutions, deletions, or insertions relative to the reference IgM heavy chain constant region.

[0012] In certain examples, the variant IgM heavy chain constant regions include an amino acid substitution at the amino acid position corresponding to amino acid R344 of the wild-type human IgM constant region SEQ ID NO: 12, for example, the amino acid corresponding to R344 of SEQ ID NO: 12 can be substituted with alanine (A). In certain examples, the variant IgM heavy chain constant regions are variant human IgM heavy chain constant regions and include the amino acid sequence SEQ ID NO: 31. In certain examples, the variant IgM heavy chain constant regions include an amino acid substitution at the amino acid position corresponding to amino acid E345 of the wild-type human IgM constant region SEQ ID NO: 12, for example, the amino acid corresponding to E345 of SEQ ID NO: 12 can be substituted with alanine (A). In certain examples, the variant IgM heavy chain constant regions are variant human IgM heavy chain constant regions and include the amino acid sequence SEQ ID NO: 32. In certain examples, the variant IgM heavy chain constant regions include an amino acid substitution at the amino acid position corresponding to amino acid S401 of the wild-type human IgM constant region SEQ ID NO: 12, for example, the amino acid corresponding to S401 of SEQ ID NO: 12 can be substituted with alanine (A). In certain examples, the variant IgM heavy chain constant regions are variant human IgM heavy chain constant regions and include the amino acid sequence SEQ ID NO: 13. In certain examples, the variant IgM heavy chain constant regions include an amino acid substitution at the amino acid position corresponding to amino acid E402 of the wild-type human IgM constant region SEQ ID NO: 12, for example, the amino acid corresponding to E402 of SEQ ID NO: 12 can be substituted with alanine (A). In certain examples, the variant IgM heavy chain constant regions are variant human IgM heavy chain constant regions and include the amino acid sequence SEQ ID NO: 14. In certain examples, the variant IgM heavy chain constant regions include an amino acid substitution at the amino acid position corresponding to amino acid E403 of the wild-type human IgM constant region SEQ ID NO: 12, for example, the amino acid corresponding to E403 of SEQ ID NO: 12 can be substituted with alanine (A). In certain examples, the variant IgM heavy chain constant regions are variant human IgM heavy chain constant regions and include the amino acid sequence SEQ ID NO: 34.

[0013] In an IgM antibody or IgM-like antibody as provided herein the increased serum half-life can include an increased alpha half-life ($t_{1/2\alpha}$), an increased beta half-life ($t_{1/2\beta}$),

or an increased $t_{1/2\alpha}$ and an increased $t_{1/2\beta}$. Likewise, an IgM antibody or IgM-like antibody as provided herein can further exhibit an increased peak plasma concentration (C_{max}), an increased area under the curve (AUC), a modified clearance time, or any combination thereof relative to the reference antibody.

5 **[0014]** In certain examples, the IgM heavy chain constant regions or multimerizing fragments or variants thereof of an IgM antibody or IgM-like antibody as provided herein each include a $C_{\mu 4}$ domain and an IgM tailpiece (tp) domain, and can further include a $C_{\mu 3}$ domain, a $C_{\mu 2}$ domain, a $C_{\mu 1}$ domain, or any combination thereof.

10 **[0015]** In certain examples, the antigen-binding domain of an IgM antibody or IgM-like antibody as provided herein can be a single-chain Fv (ScFv) fragment, or a single domain variable region (VHH). In certain examples, the antigen-binding domain subunit of an IgM antibody or IgM-like antibody as provided herein can be a heavy chain variable region (VH).

15 **[0016]** In certain examples, each binding unit of an IgM antibody or IgM-like antibody as provided herein can be further includes two light chain constant regions or fragments or variants thereof each associated with an antigen-binding domain or subunit thereof for example the antigen-binding domain can be a scFv fragment or the antigen-binding domain subunit can be a VL.

20 **[0017]** In certain examples, the J-chain or functional fragment or variant thereof of an IgM antibody or IgM-like antibody as provided herein can further includes one or more heterologous polypeptides directly or indirectly fused to the J-chain or functional fragment or variant thereof. In certain examples, the one or more heterologous polypeptides can be fused to the J-chain or fragment thereof via a peptide linker. Exemplary linkers can include at least 5 amino acids, but no more than 25 amino acids, and can consist of GGGGS (SEQ ID NO: 25), GGGGSGGGGS (SEQ ID NO: 26), GGGGSGGGGSGGGGS (SEQ ID NO: 27), GGGGSGGGGSGGGGSGGGGS (SEQ ID NO: 28), or GGGGSGGGGSGGGGSGGGGSGGGGS (SEQ ID NO: 29). In certain examples, the one or more heterologous polypeptides can be 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 fused to both the N-terminus and C-terminus of the J-chain or fragment or variant thereof. Where two or more heterologous polypeptides are fused to the J-chain, the heterologous polypeptides can be the same or different. In certain examples, the at least one heterologous polypeptide can be a binding domain, for example, an antibody or antigen-

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binding fragment thereof, for example, a Fab fragment, a Fab' fragment, a F(ab')₂ fragment, a Fd fragment, a Fv fragment, a single-chain Fv (scFv) fragment, a disulfide-linked Fv (sdFv) fragment, or any combination thereof. In certain examples, antigen-binding fragment is a scFv fragment. In certain examples, the at least one heterologous polypeptide can specifically bind to CD3ε. In certain examples, the J-chain can be a variant of the modified J-chain of SEQ ID NO: 9 (V15J), *e.g.*, the amino acid sequence of SEQ ID NO: 10 (V15J-Y102A), the amino acid sequence of SEQ ID NO: 23 (V15J-T103A), or the amino acid sequence of SEQ ID NO: 24 (V15J-N49A).

[0018] This disclosure further provides a composition including the IgM antibody or IgM-like antibody as provided herein, and a pharmaceutically acceptable carrier.

[0019] This disclosure further provides a J-chain or functional fragment thereof that includes one or more single amino acid substitutions, deletions, or insertions relative to a reference J-chain that is identical except for the one or more single amino acid substitutions, deletions, or insertion, where the variant J-chain can affect the serum half-life of an IgM antibody or IgM-like antibody that includes the variant J-chain. In certain examples, the variant J-chain includes 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: 10, SEQ ID NO: 23, SEQ ID NO: 24, or any combination thereof.

[0020] This disclosure further provides an isolated polynucleotide including a nucleic acid encoding a subunit polypeptide of an IgM or IgM-like antibody as provided herein, where the subunit polypeptide includes (a) an IgM or IgM-like heavy chain constant region or multimerizing fragment thereof, (b) an antibody light chain, or (c) a J-chain, a modified J-chain, or functional fragment or variant thereof, or (d) any combination thereof. In certain examples, the subunit polypeptide includes an IgM or IgM-like heavy chain constant region or multimerizing fragment thereof, for example, the subunit polypeptide can include the amino acid sequence SEQ ID NO: 12, SEQ ID NO: 13, SEQ ID NO: 14, SEQ ID NO: 15, SEQ ID NO: 31, SEQ ID NO: 32, or SEQ ID NO: 34. In certain examples, the subunit polypeptide includes an antibody light chain. In certain examples, the subunit polypeptide can include a J-chain, a modified J-chain, or any functional fragment or variant thereof as provided herein, for example, the subunit polypeptide can include the amino acid sequence SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 3, SEQ ID NO: 4, SEQ ID NO: 5, SEQ ID NO: 7, SEQ ID NO: 8, SEQ ID NO: 9, SEQ ID NO: 10, SEQ ID NO: 11, SEQ ID NO: 23, or SEQ ID NO: 24. The provided polynucleotide can include one,

two, three, or more nucleic acid sequences encoding two, three or more of the subunit polypeptides. Further provided is an expression vector that includes the provided polynucleotide. Further provided is a host cell that includes the provided polynucleotide or the provided expression vector.

5 **[0021]** This disclosure further provides a method for identifying variant J-chains that can increase the serum half-life of pentameric IgM antibodies or pentameric IgM-like antibodies that include the variant J-chains. The method includes testing pentameric IgM antibodies or pentameric IgM-like antibodies that include variant J-chains or fragments thereof for increased serum half-life in a subject animal relative to a reference pentameric
10 IgM antibody or pentameric IgM-like antibody, where the variant J-chains or fragments thereof include defined amino acid insertions, deletions, or substitutions, and where the reference pentameric IgM antibody or pentameric IgM-like antibody includes a J-chain or fragment thereof identical to the variant J-chains except for the defined amino acid insertions, deletions or substitutions; and recovering those variant J-chains or fragments
15 thereof that confer increased serum half-life upon the pentameric IgM antibodies or pentameric IgM-like antibodies relative to the reference pentameric IgM antibody or pentameric IgM-like antibody.

[0022] This disclosure further provides a method of identifying variant J-chains that can increase the serum half-life of pentameric IgM antibodies or pentameric IgM-like
20 antibodies including the variant J-chains. The method includes testing pentameric IgM antibodies or pentameric IgM-like antibodies that include variant J-chains or fragments thereof for their level of binding to the Fc alpha-mu receptor (Fc α μ R), the polymeric Ig receptor (pIgR), or both the Fc α μ R and the pIgR, where the variant J-chains or fragments thereof include defined amino acid insertions, deletions, or substitutions; and recovering
25 those variant J-chains or fragments thereof that confer reduced Fc α μ R binding ability, reduced pIgR binding ability, or reduced Fc α μ R and pIgR binding ability upon the pentameric IgM antibodies or pentameric IgM-like antibodies relative to a reference pentameric IgM antibody or pentameric IgM-like antibody that includes a J-chain or fragment thereof identical to the variant J-chains except for the defined amino acid
30 insertions, deletions or substitutions. This method can further include testing pentameric IgM antibodies or pentameric IgM-like antibodies that include the recovered variant J-chains or fragments thereof for increased serum half-life in a subject animal relative to a reference pentameric IgM antibody or pentameric IgM-like antibody that includes a J-

chain or fragment thereof identical to the recovered variant J-chains or fragments thereof except for the defined amino acid insertions, deletions or substitutions.

[0023] This disclosure further provides a method for identifying variant IgM heavy chain constant regions that can increase the serum half-life of an IgM antibody or IgM-like antibody that includes the variant IgM heavy chain constant regions. The method includes testing IgM antibodies or IgM-like antibodies that include variant IgM heavy chain constant regions for increased serum half-life in a subject animal relative to a reference pentameric IgM antibody or pentameric IgM-like antibody, where the variant IgM heavy chain constant regions include defined amino acid insertions, deletions, or substitutions, and where the reference IgM antibody or IgM-like antibody includes IgM heavy chain constant regions identical to the variant IgM heavy chain constant regions except for the defined amino acid insertions, deletions or substitutions; and recovering those IgM antibodies or IgM-like antibodies including variant IgM heavy chain constant regions that confer increased serum half-life upon the IgM antibodies or IgM-like antibodies including the variant IgM heavy chain constant regions relative to the reference IgM antibody or IgM-like antibody.

[0024] This disclosure further provides a method for identifying variant IgM heavy chain constant regions that can increase the serum half-life of an IgM antibody or IgM-like antibody including the variant IgM heavy chain constant regions. The method includes testing IgM antibodies or IgM-like antibodies including variant IgM heavy chain constant regions for their level of binding to the Fc alpha-mu receptor (Fc α μ R), Fc mu receptor (Fc μ R), the polymeric Ig receptor (pIgR), any combination of two of the receptors, or all three of the receptors, where the variant IgM heavy chain constant regions include defined amino acid insertions, deletions, or substitutions; and recovering those IgM antibodies or IgM-like antibodies including variant IgM heavy chain constant regions that confer reduced Fc α μ R binding ability, reduced Fc μ R binding ability, reduced pIgR binding ability, reduced ability to bind to any two of the receptors, or reduced ability to bind to all three of the receptors, upon the IgM antibodies or IgM-like antibodies including the variant IgM heavy chain constant regions relative to a reference IgM antibody or IgM-like antibody that includes IgM heavy chain constant regions identical to the variant IgM heavy chain constant regions except for the defined amino acid insertions, deletions or substitutions. This method can further include testing the recovered IgM antibodies or IgM-like antibodies that include the variant IgM heavy chain constant regions for increased serum half-life in a subject animal relative to a reference IgM antibody or IgM-

like antibody that includes IgM heavy chain constant regions identical to the variant IgM heavy chain constant regions except for the defined amino acid insertions, deletions or substitutions.

BRIEF DESCRIPTION OF THE DRAWINGS/FIGURES

5 **[0025]** **Figure 1A-1B** shows an alignment of the human IgM heavy chain constant region amino acid sequence (SEQ ID NO: 12) with those of mouse (GenBank: CAC20701.1, SEQ ID NO: 16), cynomolgus monkey (GenBank: EHH62210.1, SEQ ID NO: 30), rhesus monkey (GenBank: AAD02420.1, SEQ ID NO: 17), chimpanzee (GenBank: PNI10622.1, SEQ ID NO: 18), and Sumatran orangutan (GenBank: PNJ04968.1, SEQ ID NO: 19). The
10 amino acids corresponding to amino acids R345, E346, S401, E402, and E403 of SEQ ID NO: 12 are boxed.

15 **[0026]** **Figure 2** is a table summarizing the binding of human and mouse immunoglobulin receptors to anti-CD20/anti-CD3 bispecific IGMs comprising various mutations in the J-chain and/or in the IgM heavy chain constant region measured by ELISA. “WT” signifies anti-CD20 1.5.3 antibodies with the IgM constant regions and V15J regions as described in PCT Publication No. WO 2016/141303 prior to introduction of the alanine substitutions. The percentages indicate the extent of receptor binding relative to the “WT” starting antibody. The percentages indicate the level of receptor binding relative to an IgM pentamer comprising wild-type IgM heavy chain constant regions (“IgM,” SEQ ID NO:
20 12) and a modified J-chain comprising a wild-type human J-chain fused at its N-terminus to an scFv that binds to CD3 (“VJ” or “V15J,” SEQ ID NO: 9), which is designated as 100%.

25 **[0027]** **Figures 3A-3E** are graphs showing the binding of various anti-CD20/anti-CD3 bispecific IgMs with amino acid substitution mutations at a position in the J-chain corresponding to amino acid Y102 of SEQ ID NO: 2, to pIgR. Each graph compares a

WHAT IS CLAIMED IS:

1. An IgM antibody or IgM-like antibody with enhanced serum half-life comprising five bivalent antibody binding units or variants or fragments thereof and a variant J-chain or functional fragment thereof,

wherein each binding unit comprises two IgM heavy chain constant regions or multimerizing fragments or variants thereof, each associated with an antigen-binding domain or subunit thereof,

wherein the variant J-chain or functional fragment thereof comprises one, two, three, or four single amino acid substitutions, deletions, or insertions relative to a reference J-chain identical to the variant J-chain except for the one, two, three, or four single amino acid substitutions, deletions, or insertions, and wherein the variant J-chain can affect serum half-life of the IgM antibody or IgM-like antibody; and

wherein the IgM antibody or IgM-like antibody exhibits an increased serum half-life upon administration to a subject animal relative to a reference IgM antibody or IgM-like antibody that is identical except for the one or more single amino acid substitutions, deletions, or insertions in the variant J-chain, and is to be administered in the same way to the same animal species.

2. The IgM antibody or IgM-like antibody of claim 1, 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 human J-chain (SEQ ID NO: 2).

3. The IgM antibody or IgM-like antibody of claim 2, wherein the amino acid corresponding to Y102 of SEQ ID NO: 2 is substituted with alanine (A), serine (S) or arginine (R).

4. The IgM antibody or IgM-like antibody of claim 3, wherein the amino acid corresponding to Y102 of SEQ ID NO: 2 is substituted with alanine (A).

5. The IgM antibody or IgM-like antibody of claim 4, wherein the J-chain is a variant human J-chain and comprises the amino acid sequence SEQ ID NO: 3.

6. The IgM antibody or IgM-like antibody of claim 1, wherein the variant J-chain or functional fragment thereof comprises an amino acid substitution at the amino acid position corresponding to amino acid T103, N49, or S51 of the wild-type human J-chain (SEQ ID NO: 2).

7. The IgM antibody or IgM-like antibody of any one of claims 1 to 6, wherein the IgM heavy chain constant regions or multimerizing fragments thereof are variant IgM

heavy chain constant regions comprising one, two, three, or four single amino acid substitutions, deletions, or insertions relative to a reference IgM heavy chain constant region identical to the variant IgM heavy chain constant regions except for the one, two, three, or four single amino acid substitutions, deletions, or insertions, wherein the variant IgM heavy chain constant regions can affect serum half-life of the IgM antibody or IgM-like antibody, and wherein the IgM antibody or IgM-like antibody exhibits a further increased serum half-life upon administration to a subject animal relative to a reference IgM antibody or IgM-like antibody that is identical except for the one or more single amino acid substitutions, deletions, or insertions in the IgM heavy chain constant regions, and is to be administered in the same way to the same animal species.

8. An IgM antibody or IgM-like antibody with enhanced serum half-life comprising five or six bivalent antibody binding units or variants or fragments thereof, wherein each binding unit comprises two variant IgM heavy chain constant regions or multimerizing fragments thereof, each associated with an antigen-binding domain or subunit thereof,

wherein the variant IgM heavy chain constant regions or multimerizing fragments thereof each comprise one, two, three, or four single amino acid substitutions, deletions, or insertions relative to a reference IgM heavy chain constant region identical to the variant IgM heavy chain constant regions except for the one, two, three, or four single amino acid substitutions, deletions, or insertions, and wherein the variant IgM heavy chain constant regions can affect serum half-life of the IgM antibody or IgM-like antibody; and

wherein the IgM antibody or IgM-like antibody exhibits an increased serum half-life upon administration to a subject animal relative to a reference IgM antibody or IgM-like antibody that is identical except for the one or more single amino acid substitutions, deletions, or insertions in the IgM heavy chain constant regions, and is to be administered in the same way to the same animal species.

9. The IgM antibody or IgM-like antibody of claim 7 or claim 8, wherein the variant IgM heavy chain constant regions comprise an amino acid substitution at the amino acid position corresponding to amino acid R344, E345, S401, E402, or E403 of the wild-type human IgM constant region SEQ ID NO: 12.

10. The IgM antibody or IgM-like antibody of claim 9, wherein the amino acid corresponding to R344, E345, S401, E402, or E403 of SEQ ID NO: 12 is substituted with alanine (A).

11. The IgM antibody or IgM-like antibody of claim 10, wherein the variant IgM heavy chain constant regions are variant human IgM heavy chain constant region and comprise the amino acid sequence SEQ ID NO: 31, SEQ ID NO: 32, SEQ ID NO: 13, SEQ ID NO: 14, or SEQ ID NO: 34.

12. The IgM antibody or IgM-like antibody of any one of claims 1 to 11, wherein the increased serum half-life comprises an increased alpha half-life ($t_{1/2\alpha}$), an increased beta half-life ($t_{1/2\beta}$), or an increased $t_{1/2\alpha}$ and an increased $t_{1/2\beta}$ and/or wherein the IgM antibody or IgM-like antibody further exhibits an increased peak plasma concentration (C_{max}), an increased area under the curve (AUC), a modified clearance time, or any combination thereof relative to the reference antibody.

13. The IgM antibody or IgM-like antibody of any one of claims 1 to 12, wherein the IgM heavy chain constant regions or multimerizing fragments or variants thereof each comprise a $C_{\mu 4}$ domain and an IgM tailpiece (tp) domain.

14. The IgM antibody or IgM-like antibody of claim 13, wherein the IgM heavy chain constant regions or multimerizing fragments or variants thereof each further comprise a $C_{\mu 3}$ domain, a $C_{\mu 2}$ domain, a $C_{\mu 1}$ domain, or any combination thereof.

15. The IgM antibody or IgM-like antibody of any one of claims 1 to 14, wherein the antigen-binding domain subunit comprises a heavy chain variable region (VH).

16. The IgM antibody or IgM-like antibody of any one of claims 1 to 15, wherein each binding unit further comprises two light chain constant regions or fragments or variants thereof each associated with an antigen-binding domain or subunit thereof.

17. The IgM antibody or IgM-like antibody of any one of claims 1 to 16, wherein the J-chain or functional fragment or variant thereof further comprises one or more heterologous polypeptides directly or indirectly fused to the J-chain or functional fragment or variant thereof.

18. The IgM antibody or IgM-like antibody of claim 17, wherein the one or more heterologous polypeptides is/are fused to the J-chain or fragment thereof via a peptide linker comprising at least 5 amino acids, but no more than 25 amino acids.

19. The IgM antibody or IgM-like antibody of claim 17 or claim 18, wherein the one or more heterologous polypeptides is/are 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 wherein heterologous polypeptides are fused to both the N-terminus and C-terminus of the J-chain or fragment or variant thereof, wherein the heterologous polypeptides can be the same or different.

20. The IgM antibody or IgM-like antibody of claims 17 to 19, wherein at least one heterologous polypeptide comprises a binding domain comprising an antibody or antigen-binding fragment thereof.

21. The IgM antibody or IgM-like antibody of claim 20, wherein the antigen-binding fragment is a scFv fragment.

22. The IgM antibody or IgM-like antibody of claim 20 or 21, wherein at least one heterologous polypeptide can specifically bind to CD3ε.

23. A composition comprising the IgM antibody or IgM-like antibody of any one of claims 1 to 22, and a pharmaceutically acceptable carrier.

24. A variant J-chain 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: 10, SEQ ID NO: 23, or SEQ ID NO: 24, wherein the variant J-chain can affect the serum half-life of an IgM antibody or IgM-like antibody comprising the variant J-chain.

25. An isolated polynucleotide comprising a nucleic acid encoding a subunit polypeptide of the IgM or IgM-like antibody of any one of claims 1 to 22, wherein the subunit polypeptide comprises (a) an IgM or IgM-like heavy chain constant region or multimerizing fragment thereof, (b) an antibody light chain, or (c) a J-chain, a modified J-chain, or functional fragment or variant thereof, or (d) any combination thereof.

26. An expression vector comprising the polynucleotide of claim 25.

27. A host cell comprising the polynucleotide of claim 25 or the expression vector of claim 26.

28. A method of identifying variant J-chains that can increase the serum half-life of pentameric IgM antibodies or pentameric IgM-like antibodies comprising the variant J-chains, the method comprising:

1(a) testing pentameric IgM antibodies or pentameric IgM-like antibodies comprising variant J-chains or fragments thereof for increased serum half-life in a subject animal relative to a reference pentameric IgM antibody or pentameric IgM-like antibody, wherein the variant J-chains or fragments thereof comprise defined amino acid insertions, deletions, or substitutions, and wherein the reference pentameric IgM antibody or pentameric IgM-like antibody comprises a J-chain or fragment thereof identical to the variant J-chains except for the defined amino acid insertions, deletions or substitutions; and

(b) recovering those variant J-chains or fragments thereof that confer increased serum half-life upon the pentameric IgM antibodies or pentameric IgM-like antibodies relative to reference pentameric IgM antibody or pentameric IgM-like antibody; or

(2)(a) testing pentameric IgM antibodies or pentameric IgM-like antibodies comprising variant J-chains or fragments thereof for their level of binding to the Fc alpha-mu receptor (Fc α μ R), the polymeric Ig receptor (pIgR), or both the Fc α μ R and the pIgR, wherein the variant J-chains or fragments thereof comprise defined amino acid insertions, deletions, or substitutions; and

(b) recovering those variant J-chains or fragments thereof that confer reduced Fc α μ R binding ability, reduced pIgR binding ability, or reduced Fc α μ R and pIgR binding ability upon the pentameric IgM antibodies or pentameric IgM-like antibodies relative to a reference pentameric IgM antibody or pentameric IgM-like antibody comprising a J-chain or fragment thereof identical to the variant J-chains except for the defined amino acid insertions, deletions or substitutions.

29. A method of identifying variant IgM heavy chain constant regions that can increase the serum half-life of an IgM antibody or IgM-like antibody comprising the variant IgM heavy chain constant regions, comprising:

(1)(a) testing IgM antibodies or IgM-like antibodies comprising variant IgM heavy chain constant regions for increased serum half-life in a subject animal relative to a reference pentameric IgM antibody or pentameric IgM-like antibody, wherein the variant IgM heavy chain constant regions comprise defined amino acid insertions, deletions, or substitutions, and wherein the reference IgM antibody or IgM-like antibody comprises IgM heavy chain constant regions identical to the variant IgM heavy chain constant regions except for the defined amino acid insertions, deletions or substitutions; and

(b) recovering those IgM antibodies or IgM-like antibodies comprising variant IgM heavy chain constant regions that confer increased serum half-life upon the IgM antibodies or IgM-like antibodies comprising the variant IgM

heavy chain constant regions relative to the reference IgM antibody or IgM-like antibody; or

(2)(a) testing IgM antibodies or IgM-like antibodies comprising variant IgM heavy chain constant regions for their level of binding to the Fc alpha-mu receptor (Fc $\alpha\mu$ R), Fc mu receptor (Fc μ R), the polymeric Ig receptor (pIgR), any combination of two of the receptors, or all three of the receptors, wherein the variant IgM heavy chain constant regions comprise defined amino acid insertions, deletions, or substitutions; and

(b) recovering those IgM antibodies or IgM-like antibodies comprising variant IgM heavy chain constant regions that confer reduced Fc $\alpha\mu$ R binding ability, reduced Fc μ R binding ability, reduced pIgR binding ability, reduced ability to bind to any two of the receptors, or reduced ability to bind to all three of the receptors, upon the IgM antibodies or IgM-like antibodies comprising the variant IgM heavy chain constant regions relative to a reference IgM antibody or IgM-like antibody comprising IgM heavy chain constant regions identical to the variant IgM heavy chain constant regions except for the defined amino acid insertions, deletions or substitutions.