

SUMMARY

The disclosure generally provides binding agents that bind to CLDN18.2, particularly bispecific binding agents that bind to CLDN18.2 and CD3.

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In one aspect, the disclosure provides a binding agent comprising a binding domain with specificity for CLDN18.2 and a binding domain with specificity for CD3, wherein the binding agent comprises at least three polypeptide chains, wherein (i) a first polypeptide chain comprises a variable region of a heavy chain (VH) derived from an immunoglobulin with specificity for CLDN18.2 (VH(CLDN18.2)), (ii) a second polypeptide chain comprises a variable region of a VH derived from an immunoglobulin with specificity for CD3 (VH(CD3)) and a variable region of a light chain (VL) derived from an immunoglobulin with specificity for CD3 (VL(CD3)), and (iii) a third polypeptide chain comprises a VL derived from an immunoglobulin with specificity for CLDN18.2 (VL(CLDN18.2)).

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In one example, the binding agent of the disclosure is a bispecific trimeric binding agent.

In one example, the first polypeptide chain comprises a constant region 1 of a heavy chain (CH1) derived from an immunoglobulin or a functional variant thereof.

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In one example, the first and the second polypeptide chains comprise a constant region 2 of a heavy chain (CH2) derived from an immunoglobulin or a functional variant thereof and a constant region 3 of a heavy chain (CH3) derived from an immunoglobulin or a functional variant thereof.

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In one example, the third polypeptide chain comprises a constant region of a light chain (CL) derived from an immunoglobulin or a functional variant thereof.

In one example, the VH and the CH1, CH2 and CH3 on the first polypeptide chain are arranged, from N-terminus to C-terminus, in the order VH(CLDN18.2)-CH1-CH2-CH3.

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In one example, the VH, the VL, and the CH2 and CH3 on the second polypeptide chain are arranged, from N-terminus to C-terminus, in the order VH(CD3)-VL(CD3)-CH2-CH3, or VL(CD3)-VH(CD3)-CH2-CH3.

In one example, the first polypeptide chain interacts with the second polypeptide chain and with the third polypeptide chain. In one example, the VH(CLDN18.2) and the VL(CLDN18.2) interact to form the binding domain with specificity for CLDN18.2, and the VH(CD3) and the VL(CD3) interact to form the binding domain with specificity for CD3.

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In one example, the CH2 on the first polypeptide chain interacts with the CH2 on the second polypeptide chain and/or the CH3 on the first polypeptide chain interacts with the CH3 on the second polypeptide chain. In one example, the CH1 on the first polypeptide chain interacts with the CL on the third polypeptide chain.

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In one example, the binding agent of the disclosure comprises a further binding domain with specificity for CLDN18.2, wherein the second polypeptide chain further comprises a VH(CLDN18.2) and wherein the binding agent comprises a fourth polypeptide chain identical to the third polypeptide chain.

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In one example, the binding agent of the disclosure is a bispecific tetrameric binding agent.

In one example, the second polypeptide chain further comprises a CH1 derived from an immunoglobulin or a functional variant thereof.

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In one example, the immunoglobulin is IgG1, preferably human IgG1. In one example, in the binding agent described herein, VH(CLDN18.2) and/or VL(CLDN18.2) are derived from IgG1, VH(CD3) and/or VL(CD3) are derived from IgG1, and/or CH1, CH2, CH3, and/or CL are derived from IgG1, wherein IgG1 preferably is human IgG1.

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In one example, the VHs, the VL, and the CH1, CH2 and CH3 on the second polypeptide chain are arranged, from N-terminus to C-terminus, in the order VH(CLDN18.2)-CH1-VH(CD3)-VL(CD3)-CH2-CH3, or VH(CLDN18.2)-CH1-VL(CD3)-VH(CD3)-CH2-CH3.

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In one example, the second polypeptide chain interacts with the fourth polypeptide chain.

In one example, the VH(CLDN18.2) on the second polypeptide chain interacts with the VL(CLDN18.2) on the fourth polypeptide chain to form the further binding domain with specificity for CLDN18.2.

In one example, the CH1 on the second polypeptide chain interacts with the CL on the fourth polypeptide chain.

In one example, the VH(CD3) comprises CDR1, CDR2 and CDR3 of an amino acid sequence selected from the group consisting of SEQ ID NO: 54, 58 and 61.

In one example, the VH(CD3) comprises

(i) a CDR1 comprising the amino acid sequence TYAMN (SEQ ID NO: 43) or a functional variant thereof, a CDR2 comprising the amino acid sequence RIRSKANNYATYYADSVKG (SEQ ID NO: 50) or a functional variant thereof, and a CDR3 comprising the amino acid sequence HGNFGDSYVSWFAY (SEQ ID NO: 45) or a functional variant thereof,

(ii) a CDR1 comprising the amino acid sequence TYAMN (SEQ ID NO: 43) or a functional variant thereof, a CDR2 comprising the amino acid sequence RIRSKYNNYATYYADSVKG (SEQ ID NO: 44) or a functional variant thereof, and a CDR3 comprising the amino acid sequence HGNFGDEYVSWFAY (SEQ ID NO: 51) or a functional variant thereof, or

(iii) a CDR1 comprising the amino acid sequence TYAMN (SEQ ID NO: 43) or a functional variant thereof, a CDR2 comprising the amino acid sequence RIRSKYNNYATYYADSVKG (SEQ ID NO: 44) or a functional variant thereof, and a CDR3 comprising the amino acid sequence HGNFGDSYVSWFAY (SEQ ID NO: 45) or a functional variant thereof.

In one example, the VL(CD3) comprises CDR1, CDR2 and CDR3 of SEQ ID NO: 55.

In one example, the VL(CD3) comprises a CDR1 comprising the amino acid sequence GSSTGAVTTSNYAN (SEQ ID NO: 46) or a functional variant thereof, a CDR2 comprising the amino acid sequence GTNKRAP (SEQ ID NO: 47) or a functional variant thereof, and a CDR3 comprising the amino acid sequence ALWYSNHWV (SEQ ID NO: 48) or a functional variant thereof.

In one example, the VH(CLDN18.2) comprises CDR1, CDR2 and CDR3 of SEQ ID NO: 39.

In one example, the VH(CLDN18.2) comprises a CDR1 comprising the amino acid sequence SYWIN (SEQ ID NO: 32) or a functional variant thereof, a CDR2 comprising the amino acid sequence NIYPSDSYTNYNQKFQG (SEQ ID NO: 33) or a functional variant thereof, and a

CDR3 comprising the amino acid sequence SWRGNSFDY (SEQ ID NO: 34) or a functional variant thereof.

In one example, the VL(CLDN18.2) comprises CDR1, CDR2 and CDR3 of SEQ ID NO: 42.

In one example, the VL(CLDN18.2) comprises a CDR1 comprising the amino acid sequence KSSQSLLNSGNQKNYLT (SEQ ID NO: 35) or a functional variant thereof, a CDR2 comprising the amino acid sequence WASTRES (SEQ ID NO: 36) or a functional variant thereof, and a CDR3 comprising the amino acid sequence QNDYSYPFT (SEQ ID NO: 37) or a functional variant thereof.

In one example,

(i) the VH(CD3) comprises CDR1, CDR2 and CDR3 of SEQ ID NO: 58, the VL(CD3) comprises CDR1, CDR2 and CDR3 of SEQ ID NO: 55, the VH(CLDN18.2) comprises CDR1, CDR2 and CDR3 of SEQ ID NO: 39, and the VL(CLDN18.2) comprises CDR1, CDR2 and CDR3 of SEQ ID NO: 42,

(ii) the VH(CD3) comprises CDR1, CDR2 and CDR3 of SEQ ID NO: 61, the VL(CD3) comprises CDR1, CDR2 and CDR3 of SEQ ID NO: 55, the VH(CLDN18.2) comprises CDR1, CDR2 and CDR3 of SEQ ID NO: 39, and the VL(CLDN18.2) comprises CDR1, CDR2 and CDR3 of SEQ ID NO: 42, or

(iii) the VH(CD3) comprises CDR1, CDR2 and CDR3 of SEQ ID NO: 54, the VL(CD3) comprises CDR1, CDR2 and CDR3 of SEQ ID NO: 55, the VH(CLDN18.2) comprises CDR1, CDR2 and CDR3 of SEQ ID NO: 39, and the VL(CLDN18.2) comprises CDR1, CDR2 and CDR3 of SEQ ID NO: 42.

In one example, the VH(CD3) comprises or consists of an amino acid sequence represented by SEQ ID NO: 58 or a functional variant thereof, the VL(CD3) comprises or consists of an amino acid sequence represented by SEQ ID NO: 55 or a functional variant thereof, the VH(CLDN18.2) comprises or consists of an amino acid sequence represented by SEQ ID NO: 39 or a functional variant thereof, and/or the VL(CLDN18.2) comprises or consists of an amino acid sequence represented by SEQ ID NO: 42 or a functional variant thereof.

In one example, the VH(CD3) comprises or consists of an amino acid sequence represented by SEQ ID NO: 61 or a functional variant thereof, the VL(CD3) comprises or consists of an amino

acid sequence represented by SEQ ID NO: 55 or a functional variant thereof, the VH(CLDN18.2) comprises or consists of an amino acid sequence represented by SEQ ID NO: 39 or a functional variant thereof, and/or the VL(CLDN18.2) comprises or consists of an amino acid sequence represented by SEQ ID NO: 42 or a functional variant thereof.

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In one example, the VH(CD3) comprises or consists of an amino acid sequence represented by SEQ ID NO: 54 or a functional variant thereof, the VL(CD3) comprises or consists of an amino acid sequence represented by SEQ ID NO: 55 or a functional variant thereof, the VH(CLDN18.2) comprises or consists of an amino acid sequence represented by SEQ ID NO: 39 or a functional variant thereof, and/or the VL(CLDN18.2) comprises or consists of an amino acid sequence represented by SEQ ID NO: 42 or a functional variant thereof.

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In one example, on the first polypeptide chain the CH1 is connected to the CH2 by a peptide linker. In one example, the peptide linker comprises the amino acid sequence EPKSCDKTHTCPPCP (SEQ ID NO: 27) or a functional variant thereof.

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In one example, the VH(CD3) or VL(CD3) is connected to the CH2 by a peptide linker. In one example, the VH(CD3) or VL(CD3) is connected to the CH1 by a peptide linker. In one example, the peptide linker comprises the amino acid sequence $(G_4S)_x$ or a functional variant thereof, wherein x is 2, 3, 4, 5 or 6. In one example, the peptide linker comprises the amino acid sequence $(G_4S)_2$ (SEQ ID NO: 26) or a functional variant thereof. In one example, the peptide linker connecting the VH(CD3) or VL(CD3) and the CH2 comprises the amino acid sequence KTHTCPPCP (SEQ ID NO: 21) or a functional variant thereof. In one example, the peptide linker comprises the amino acid sequence $(G_4S)_2$ KTHTCPPCP (SEQ ID NO: 23) or a functional variant thereof. In one example, the peptide linker comprises the amino acid sequence EPKSSDKTHTCPPCP (SEQ ID NO: 22) or a functional variant thereof.

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In one example, the VH(CD3) and the VL(CD3) are connected to one another by a peptide linker. In one example, the peptide linker comprises the amino acid sequence $(GKPGS)_x$ or a functional variant thereof, wherein x is 2, 3, 4, 5 or 6. In one example, the peptide linker comprises the amino acid sequence $(GKPGS)_4$ (SEQ ID NO: 11) or a functional variant thereof.

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In certain examples, the CH1, CH2 and/or CH3 domains of the binding agent of the disclosure comprise one or more amino acid modifications, in particular substitutions and/or deletions, in

positions corresponding to positions of human IgG1 according to EU numbering. In one example, the CH1 on the first and/or the second polypeptide chain comprises an amino acid sequence comprising an aspartic acid residue at position 208 according to EU numbering. In one example, the CH1 on the first polypeptide chain comprises an amino acid sequence comprising an aspartic acid residue at position 208 according to EU numbering and the CH1 on the second polypeptide chain comprises an amino acid sequence comprising an asparagine residue at position 208 according to EU numbering.

Furthermore, in certain examples, the binding agent of the disclosure does not substantially, e.g., not detectably, bind to human FcγRI, IIa, IIb, and/or IIIa. In one example, the CH2 on the first and/or the second polypeptide chain comprises an amino acid sequence comprising one or more of the following: a proline residue at position 233, a valine residue at position 234, an alanine residue at position 235, a deletion at position 236, a lysine residue at position 267 and a glutamic acid residue at position 295 according to EU numbering. In one example, the CH2s on the first and the second polypeptide chain comprise an amino acid sequence comprising a proline residue at position 233, a valine residue at position 234, an alanine residue at position 235, a deletion at position 236 and a lysine residue at position 267 according to EU numbering and wherein the CH2 on the first polypeptide chain further comprises a glutamic acid residue at position 295 according to EU numbering and the CH2 on the second polypeptide chain further comprises a glutamine residue at position 295 according to EU numbering.

In one example, the CH3 on the first and/or the second polypeptide chain comprises an amino acid sequence comprising one or more of the following: a glutamine residue at position 357, a lysine residue at position 364, an aspartic acid residue at position 368, a serine residue at position 370, an aspartic acid residue at position 384, a glutamic acid residue at position 418 and an aspartic acid residue at position 421 according to EU numbering. In one example, the CH3 on the first polypeptide chain comprises an amino acid sequence comprising a glutamic acid residue at position 357, a serine residue at position 364, an aspartic acid residue at position 368, a serine residue at position 370, an aspartic acid residue at position 384, a glutamic acid residue at position 418 and an aspartic acid residue at position 421 according to EU numbering and the CH3 on the second polypeptide chain comprises an amino acid sequence comprising a glutamine residue at position 357, a lysine residue at position 364, a leucine residue at position 368, a lysine residue at position 370, an asparagine residue at position 384, a glutamine residue at position 418 and an asparagine residue at position 421 according to EU numbering.

In one example, the first polypeptide chain comprises an amino acid sequence represented by SEQ ID NO: 28 or a functional variant thereof. In one example, the second polypeptide chain comprises an amino acid sequence represented by SEQ ID NO: 30 or a functional variant thereof. In one example, the second polypeptide chain comprises an amino acid sequence represented by SEQ ID NO: 29 or a functional variant thereof. In one example, the third polypeptide chain comprises an amino acid sequence represented by SEQ ID NO: 31 or a functional variant thereof.

In one example, the first polypeptide chain comprises or consists of an amino acid sequence represented by SEQ ID NO: 73 or a functional variant thereof. In one example, the second polypeptide chain comprises or consists of an amino acid sequence selected from the group consisting of SEQ ID NO: 74, 75, 76 and 77 or a functional variant thereof. In one example, the third polypeptide chain comprises or consists of an amino acid sequence represented by SEQ ID NO: 78 or a functional variant thereof.

Preferably, in one example, (i) the first polypeptide chain comprises or consists of an amino acid sequence represented by SEQ ID NO: 73 or a functional variant thereof, (ii) the second polypeptide chain comprises or consists of an amino acid sequence represented by SEQ ID NO: 75 or a functional variant thereof, and (iii) the third polypeptide chain comprises or consists of an amino acid sequence represented by SEQ ID NO: 78 or a functional variant thereof.

Preferably, in one example, (i) the first polypeptide chain comprises or consists of an amino acid sequence represented by SEQ ID NO: 73 or a functional variant thereof, (ii) the second polypeptide chain comprises or consists of an amino acid sequence represented by SEQ ID NO: 74 or a functional variant thereof, and (iii) the third polypeptide chain comprises or consists of an amino acid sequence represented by SEQ ID NO: 78 or a functional variant thereof.

Preferably, in one example, (i) the first polypeptide chain comprises or consists of an amino acid sequence represented by SEQ ID NO: 73 or a functional variant thereof, (ii) the second polypeptide chain comprises or consists of an amino acid sequence represented by SEQ ID NO: 76 or a functional variant thereof, and

(iii) the third polypeptide chain comprises or consists of an amino acid sequence represented by SEQ ID NO: 78 or a functional variant thereof.

Preferably, in one example, (i) the first polypeptide chain comprises or consists of an amino acid sequence represented by SEQ ID NO: 73 or a functional variant thereof,

(ii) the second polypeptide chain comprises or consists of an amino acid sequence represented by SEQ ID NO: 77 or a functional variant thereof, and

(iii) the third polypeptide chain comprises or consists of an amino acid sequence represented by SEQ ID NO: 78 or a functional variant thereof.

The disclosure also provides a binding agent comprising a binding domain with specificity for CLDN18.2 comprising a variable region of a heavy chain (VH) derived from an immunoglobulin with specificity for CLDN18.2 (VH(CLDN18.2)) and a variable region of a light chain (VL) derived from an immunoglobulin with specificity for CLDN18.2 (VL(CLDN18.2)), wherein the VH(CLDN18.2) comprises a CDR1 comprising the amino acid sequence SYWIN (SEQ ID NO: 32) or a functional variant thereof, a CDR2 comprising the amino acid sequence NIYPSDSYTNYNQKFQG (SEQ ID NO: 33) or a functional variant thereof, and a CDR3 comprising the amino acid sequence SWRGNSFDY (SEQ ID NO: 34) or a functional variant thereof, and the VL(CLDN18.2) comprises a CDR1 comprising the amino acid sequence KSSQSLLNSGNQKNYLT (SEQ ID NO: 35) or a functional variant thereof, a CDR2 comprising the amino acid sequence WASTRES (SEQ ID NO: 36) or a functional variant thereof, and a CDR3 comprising the amino acid sequence QNDYSYPFT (SEQ ID NO: 37) or a functional variant thereof. In one example, the binding agent further comprises a binding domain with specificity for CD3. In one example, the binding domain with specificity for CD3 comprises a VH derived from an immunoglobulin with specificity for CD3 (VH(CD3)) and a VL derived from an immunoglobulin with specificity for CD3 (VL(CD3)), wherein the VH(CD3) comprises CDR1, CDR2 and CDR3 of an amino acid sequence selected from the group consisting of SEQ ID NO: 54, 58 and 61, and the VL(CD3) comprises CDR1, CDR2 and CDR3 of SEQ ID NO: 55.

In another aspect, the disclosure provides a binding agent comprising a binding domain with specificity for CLDN18.2 comprising a variable region of a heavy chain (VH) derived from an immunoglobulin with specificity for CLDN18.2 (VH(CLDN18.2)) and a variable region of a light

chain (VL) derived from an immunoglobulin with specificity for CLDN18.2 (VL(CLDN18.2)), wherein

the VH(CLDN18.2) comprises a CDR1 comprising the amino acid sequence SYWIN (SEQ ID NO: 32), a CDR2 comprising the amino acid sequence NIYPSDSYTNYNQKFQG (SEQ ID NO: 33), and a CDR3 comprising the amino acid sequence SWRGNSFDY (SEQ ID NO: 34), and

the VL(CLDN18.2) comprises a CDR1 comprising the amino acid sequence KSSQSLLNSGNQKNYLT (SEQ ID NO: 35), a CDR2 comprising the amino acid sequence WASTRES (SEQ ID NO: 36), and a CDR3 comprising the amino acid sequence QNDYSYPFT (SEQ ID NO: 37),

wherein optionally the binding agent further comprises a binding domain with specificity for CD3, wherein further optionally the binding domain with specificity for CD3 comprises a VH derived from an immunoglobulin with specificity for CD3 (VH(CD3)) and a VL derived from an immunoglobulin with specificity for CD3 (VL(CD3)), wherein

the VH(CD3) comprises CDR1, CDR2 and CDR3 of an amino acid sequence selected from the group consisting of SEQ ID NO: 54, 58 and 61, and

the VL(CD3) comprises CDR1, CDR2 and CDR3 of SEQ ID NO: 55,

wherein still further optionally the VH(CD3) comprises

(i) a CDR1 comprising the amino acid sequence TYAMN (SEQ ID NO: 43) or a functional variant thereof, a CDR2 comprising the amino acid sequence RIRSKANNYATYYADSVKG (SEQ ID NO: 50) or a functional variant thereof, and a CDR3 comprising the amino acid sequence HGNFGDSYVSWFAY (SEQ ID NO: 45) or a functional variant thereof,

(ii) a CDR1 comprising the amino acid sequence TYAMN (SEQ ID NO: 43) or a functional variant thereof, a CDR2 comprising the amino acid sequence RIRSKYNNYATYYADSVKG (SEQ ID NO: 44) or a functional variant thereof, and a CDR3 comprising the amino acid sequence HGNFGDEYVSWFAY (SEQ ID NO: 51) or a functional variant thereof, or

(iii) a CDR1 comprising the amino acid sequence TYAMN (SEQ ID NO: 43) or a functional variant thereof, a CDR2 comprising the amino acid sequence RIRSKYNNYATYYADSVKG (SEQ ID NO: 44) or a functional variant thereof, and a CDR3 comprising the amino acid sequence HGNFGDSYVSWFAY (SEQ ID NO: 45) or a functional variant thereof, and

the VL(CD3) comprises a CDR1 comprising the amino acid sequence GSSTGAVTTSNYAN (SEQ ID NO: 46) or a functional variant thereof, a CDR2 comprising the amino acid sequence GTNKRAP (SEQ ID NO: 47) or a functional variant thereof, and a CDR3

comprising the amino acid sequence ALWYSNHWV (SEQ ID NO: 48) or a functional variant thereof.

In one example, the functional variant of the amino acid sequence NIYPSDSYTNYNQKFQG
5 (SEQ ID NO: 33) comprises or retains the amino acid residues QG.

In one example, the binding agent is in the format of a full-length antibody or an antibody fragment. In one example, the binding domain with specificity for CLDN18.2 is in the format

of a Fab fragment, a Fab' fragment, a F(ab')₂ fragment, an Fv fragment, or an scFv fragment. In one example, the binding domain with specificity for CD3 is in the format of a Fab fragment, a Fab' fragment, a F(ab')₂ fragment, an Fv fragment, or an scFv fragment.

5 In one example, the binding agent is a bispecific molecule such as a bispecific antibody. In one example, the binding agent is a bispecific single chain antibody. In one example, the binding agent is capable of binding monovalently or bivalently to CLDN18.2. In one example, the binding agent comprises two binding domains with specificity for CLDN18.2. In one example, the binding domain with specificity for CLDN18.2 is in the format of a Fab fragment and the binding domain
10 with specificity for CD3 is in the format of an scFv fragment. In one example, the VH(CLDN18.2) is connected to the VL(CLDN18.2) by a peptide linker and/or the VH(CD3) is connected to the VL(CD3) by a peptide linker such as a peptide linker selected from the group consisting of SEQ ID NO: 2-20.

15 The disclosure also provides a binding agent comprising two binding domains with specificity for CLDN18.2 and a binding domain with specificity for CD3, wherein the binding agent comprises at least four polypeptide chains, wherein the first polypeptide chain comprises or consists of an amino acid sequence represented by SEQ ID NO: 73 or a functional variant thereof, the second polypeptide chain comprises or consists of an amino acid sequence represented by SEQ ID NO:
20 74 or a functional variant thereof, the third polypeptide chain comprises or consists of an amino acid sequence represented by SEQ ID NO: 78 or a functional variant thereof, and the fourth polypeptide chain is identical to the third polypeptide chain. In one example, the polypeptide chains of the binding agent and/or the domains on the polypeptide chains of the binding agent interact with one another as described herein. In one example, the first polypeptide chain interacts
25 with the second polypeptide chain and with the third polypeptide chain, and the second polypeptide chain interacts with the fourth polypeptide chain.

In another aspect, the disclosure provides a binding agent comprising two binding domains with specificity for CLDN18.2 and a binding domain with specificity for CD3, wherein the binding
30 agent comprises four polypeptide chains, wherein (i) the first polypeptide chain comprises or consists of an amino acid sequence represented by SEQ ID NO: 73, (ii) the second polypeptide chain comprises or consists of an amino acid sequence represented by SEQ ID NO: 74, (iii) the third polypeptide chain comprises or consists of an amino acid sequence represented by SEQ ID

NO: 78, and (iv) the fourth polypeptide chain is identical to the third polypeptide chain, wherein the first polypeptide chain interacts with the second polypeptide chain and with the third polypeptide chain, and the second polypeptide chain interacts with the fourth polypeptide chain.

- 5 The disclosure also provides a binding agent comprising a binding domain with specificity for CLDN18.2 and a binding domain with specificity for CD3, wherein the binding agent comprises at least three polypeptide chains, wherein the first polypeptide chain comprises or consists of an amino acid sequence represented by SEQ ID NO: 73 or a functional variant thereof, the second polypeptide chain comprises or consists of an amino acid sequence represented by SEQ ID NO:
10 75 or a functional variant thereof, and the third polypeptide chain comprises or consists of an amino acid sequence represented by SEQ ID NO: 78 or a functional variant thereof. In one

example, the polypeptide chains of the binding agent and/or the domains on the polypeptide chains of the binding agent interact with one another as described herein. In one example, the first polypeptide chain interacts with the second polypeptide chain and with the third polypeptide chain.

5 In another aspect, the disclosure provides a binding agent comprising a binding domain with specificity for CLDN18.2 and a binding domain with specificity for CD3, wherein the binding agent comprises three polypeptide chains, wherein (i) the first polypeptide chain comprises or consists of an amino acid sequence represented by SEQ ID NO: 73, (ii) the second polypeptide chain comprises or consists of an amino acid sequence represented by SEQ ID NO: 75, and (iii)
10 the third polypeptide chain comprises or consists of an amino acid sequence represented by SEQ ID NO: 78, wherein the first polypeptide chain interacts with the second polypeptide chain and with the third polypeptide chain.

The disclosure also provides a binding agent comprising two binding domains with specificity for
15 CLDN18.2 and a binding domain with specificity for CD3, wherein the binding agent comprises at least four polypeptide chains, wherein the first polypeptide chain comprises or consists of an amino acid sequence represented by SEQ ID NO: 73 or a functional variant thereof, the second polypeptide chain comprises or consists of an amino acid sequence represented by SEQ ID NO: 76 or a functional variant thereof, the third polypeptide chain comprises or consists of an amino
20 acid sequence represented by SEQ ID NO: 78 or a functional variant thereof, and the fourth polypeptide chain is identical to the third polypeptide chain. In one example, the polypeptide chains of the binding agent and/or the domains on the polypeptide chains of the binding agent interact with one another as described herein. In one example, the first polypeptide chain interacts with the second polypeptide chain and with the third polypeptide chain, and the second polypeptide
25 chain interacts with the fourth polypeptide chain.

In another aspect, the disclosure provides a binding agent comprising two binding domains with specificity for CLDN18.2 and a binding domain with specificity for CD3, wherein the binding agent comprises four polypeptide chains, wherein (i) the first polypeptide chain comprises or
30 consists of an amino acid sequence represented by SEQ ID NO: 73, (ii) the second polypeptide chain comprises or consists of an amino acid sequence represented by SEQ ID NO: 76, (iii) the third polypeptide chain comprises or consists of an amino acid sequence represented by SEQ ID NO: 78, and (iv) the fourth polypeptide chain is identical to the third polypeptide chain, wherein

the first polypeptide chain interacts with the second polypeptide chain and with the third polypeptide chain, and the second polypeptide chain interacts with the fourth polypeptide chain.

The disclosure also provides a binding agent comprising two binding domains with specificity for CLDN18.2 and a binding domain with specificity for CD3, wherein the binding agent comprises at least four polypeptide chains, wherein the first polypeptide chain comprises or consists of an amino acid sequence represented by SEQ ID NO: 73 or a functional variant thereof, the second polypeptide chain comprises or consists of an amino acid sequence represented by SEQ ID NO: 77 or a functional variant thereof, the third polypeptide chain comprises or consists of an amino acid sequence represented by SEQ ID NO: 78 or a functional variant thereof, and the fourth polypeptide chain is identical to the third polypeptide chain. In one example, the polypeptide chains of the binding agent and/or the domains on the polypeptide chains of the binding agent interact with one another as described herein. In one example, the first polypeptide chain interacts with the second polypeptide chain and with the third polypeptide chain, and the second polypeptide chain interacts with the fourth polypeptide chain.

In another aspect, the disclosure provides a binding agent comprising two binding domains with specificity for CLDN18.2 and a binding domain with specificity for CD3, wherein the binding agent comprises four polypeptide chains, wherein (i) the first polypeptide chain comprises or consists of an amino acid sequence represented by SEQ ID NO: 73, (ii) the second polypeptide chain comprises or consists of an amino acid sequence represented by SEQ ID NO: 77, (iii) the third polypeptide chain comprises or consists of an amino acid sequence represented by SEQ ID NO: 78, and (iv) the fourth polypeptide chain is identical to the third polypeptide chain, wherein the first polypeptide chain interacts with the second polypeptide chain and with the third polypeptide chain, and the second polypeptide chain interacts with the fourth polypeptide chain.

In one example, the VH(CLDN18.2), VL(CLDN18.2), VH(CD3) and/or VL(CD3) are humanized.

In one example, CD3 is expressed on the surface of T cells. In one example, the binding agent of the disclosure binds to the epsilon chain of CD3. In one example, binding of the binding agent to CD3 on T cells results in proliferation and/or activation of the T cells. In one example, proliferation and/or activation of T cells includes proliferation and/or activation of CD4 and/or CD8 T cells, preferably CD107a+ T cells. In one example, said proliferated and/or activated T cells are capable of degranulation. In one example, said activated T cells release cytotoxic factors, e.g., perforins and granzymes, and initiate cytolysis and/or apoptosis of cancer cells.

In one example, CLDN18.2 is expressed in cancer cells. In one example, CLDN18.2 is expressed on the surface of cancer cells. In one example, the binding agent binds to an extracellular portion of CLDN18.2. In one example, the binding agent induces T cell-mediated cytotoxicity against cancer cells expressing CLDN18.2.

In one example, the cancer cells are from a cancer selected from the group consisting of gastric cancer, esophageal cancer, cancer of the gastroesophageal junction (GEJ), pancreatic cancer, lung cancer such as non-small cell lung cancer (NSCLC), breast cancer, ovarian cancer, colon cancer, rectal cancer, colorectal cancer, hepatic cancer, head-neck cancer, cancer of the gallbladder and the metastasis thereof, a Krukenberg tumor, peritoneal metastasis and/or lymph node metastasis.

In some examples, the binding agent of the disclosure or one or more of the polypeptide chains of the binding agent of the disclosure comprise or do not comprise secretion signals such as N-terminal secretion signals, in particular immunoglobulin such as IgG secretion signals such as the sequence MGWSCIILFLVATATGVHS and/or comprise or do not comprise tags, in particular C-terminal tags such as His-tags, in particular the sequence Gly-Gly-Ser-(His)₆ or (His)₆, or a Strep-tag.

In some examples, the binding agent of the disclosure comprises one or more posttranslational modifications. The disclosure also provides a binding agent which is derived from one or more posttranslational modifications of a binding agent described herein. In one example, the one or more posttranslational modifications are selected from pyroglutamylation at the N-terminus of one or more VH(CLDN18.2), pyroglutamylation at the N-terminus of VH(CD3), deletion of lysine at the C-terminus of the first polypeptide chain and deletion of lysine at the C-terminus of the second polypeptide chain.

In another aspect, the disclosure also provides a nucleic acid encoding a polypeptide chain of the binding agent as defined herein, or a vector comprising said nucleic acid. In another aspect, the disclosure also provides a nucleic acid encoding the binding agent as defined herein. In another aspect, the disclosure also provides a set of nucleic acids together encoding the binding agent as defined herein.

In another aspect, the disclosure also provides a vector comprising the nucleic acid or the set of nucleic acids as defined herein. In another aspect, the disclosure also provides a set of vectors comprising the set of nucleic acids as defined herein. In one example, each nucleic acid of the set of nucleic acids is contained in a vector of the set of vectors. In one example, the vector or the set of vectors is capable of expressing the binding agent.

In one example, a nucleic acid is operably linked to any number of regulatory elements (promoters, origin of replication, selectable markers, ribosomal binding sites, inducers, etc.). The vector can be an expression vector and can be extra-chromosomal or integrating vector. In some examples, nucleic acids encoding a polypeptide chain of the binding agent of the disclosure are each contained within a single expression vector. The nucleic acids can be under control of different or the same promoters. In such examples, different vector ratios can be used to drive formation of the binding agent of the disclosure.

In another aspect, the disclosure also provides a host cell comprising said nucleic acid, set of nucleic acids, vector or set of vectors. In one example, the host cell is a mammalian cell, preferably selected from the group consisting of CHO cells, BHK cells, HeLa cells, COS cells, HEK293 cells, HEK293 T cells and the like. In one example, the host cell is a bacterial cell, a yeast cell, a fungal cell, a plant cell or an insect cell.

In another aspect, the disclosure also provides a pharmaceutical composition comprising the binding agent as defined herein, the nucleic acid as defined herein, the set of nucleic acids as defined herein, the vector as defined herein, the set of vectors as defined herein or the host cell as defined herein. In one example, the pharmaceutical composition further comprises a pharmaceutically acceptable carrier and/or excipient.

In another aspect, the disclosure also provides the binding agent as defined herein, the nucleic acid as defined herein, the set of nucleic acids as defined herein, the vector as defined herein, the set of vectors

as defined herein, the host cell as defined herein or the pharmaceutical composition as defined herein for use in therapy.

The disclosure also provides the binding agent of the disclosure, the nucleic acid of the disclosure, the set of nucleic acids of the disclosure, the vector of the disclosure, the set of vectors of the disclosure, the host cell of the disclosure or the pharmaceutical composition of the disclosure for use in treating or preventing cancer.

In another aspect, the disclosure provides the binding agent as defined herein, the nucleic acid as defined herein, the set of nucleic acids as defined herein, the vector as defined herein, the set of vectors as defined herein, the host cell as defined herein or the pharmaceutical composition as defined herein for use in treating or preventing cancer involving cancer cells expressing CLDN18.2, wherein optionally the cancer is selected from the group consisting of gastric cancer, esophageal cancer, cancer of the gastroesophageal junction (GEJ), pancreatic cancer, lung cancer such as non small cell lung cancer (NSCLC), breast cancer, ovarian cancer, colon cancer, rectal cancer, colorectal cancer, hepatic cancer, head-neck cancer, cancer of the gallbladder and the metastasis thereof, a Krukenberg tumor, peritoneal metastasis and/or lymph node metastasis

The disclosure also provides a method of treating or preventing cancer comprising administering the binding agent of the disclosure, the nucleic acid of the disclosure, the set of nucleic acids of the disclosure, the vector of the disclosure, the set of vectors of the disclosure, the host cell of the disclosure or the pharmaceutical composition of the disclosure to a subject in need thereof. In one example, the cancer involves cancer cells expressing CLDN18.2.

The disclosure also provides a use of the binding agent of the disclosure, the nucleic acid of the disclosure, the set of nucleic acids of the disclosure, the vector of the disclosure, the set of vectors of the disclosure, the host cell of the disclosure or the pharmaceutical composition of the disclosure for the preparation of a medicament. In one example, the medicament is for treating or preventing of cancer. In one example, the cancer involves cancer cells expressing CLDN18.2.

In one example, said cancer is selected from the group consisting of gastric cancer, esophageal cancer, cancer of the gastroesophageal junction (GEJ), pancreatic cancer, lung cancer such as non-small cell lung cancer (NSCLC), breast cancer, ovarian cancer, colon cancer, rectal cancer, colorectal cancer, hepatic cancer, head-neck cancer, cancer of the gallbladder and the metastasis thereof, a Krukenberg tumor, peritoneal metastasis and/or lymph node metastasis.

In another aspect, the disclosure provides use of the binding agent as defined herein, the nucleic acid as defined herein, the set of nucleic acids as defined herein, the vector as defined herein, the set of vectors as defined herein, the host cell as defined herein or the pharmaceutical composition as defined herein for the preparation of a medicament for treating or preventing cancer involving cancer cells expressing CLDN18.2, wherein optionally the cancer is selected from the group consisting of gastric cancer, esophageal cancer, cancer of the gastroesophageal junction (GEJ), pancreatic cancer, lung cancer such as non small cell lung cancer (NSCLC), breast cancer, ovarian cancer, colon cancer, rectal cancer, colorectal cancer, hepatic cancer, head-neck cancer, cancer of the gallbladder and the metastasis thereof, a Krukenberg tumor, peritoneal metastasis and/or lymph node metastasis.

The disclosure also provides a binding agent, a nucleic acid, a set of nucleic acids, a vector, a set of vectors, a host cell or a pharmaceutical composition as described herein for use in the methods of treatment described herein. In one example, provided is a method of treating or preventing a disease such as cancer comprising administering the binding agent of the disclosure, the nucleic acid of the disclosure, the set of nucleic acids of the disclosure, the vector of the disclosure, the set of vectors of the disclosure, the host cell of the disclosure or the pharmaceutical composition of the disclosure to a subject having a disease such as a subject having cancer. Preferably, the disease

involves cells such as diseased cells expressing CLDN18.2. Preferably, the disease is cancer and the cancer involves cancer cells expressing CLDN18.2.

According to the disclosure, CLDN18.2 preferably has the amino acid sequence according to SEQ ID NO: 1.

In some examples, the first polypeptide chain does not comprise a VL(CLDN18.2). In some examples, the third polypeptide chain does not comprise a VH(CLDN18.2).

In some examples, the binding domain(s) of the binding agent with specificity for CLDN18.2 are in the format of a Fab fragment. In some examples, the binding domain of the binding agent of the disclosure with specificity for CD3 is in the format of an scFv moiety.

In some examples, the binding agent of the disclosure does not bind to CLDN18.1. Preferably, the binding agent does not bind to CLDN18.1 of human, mouse or cynomolgus. In some examples, the binding agent of the disclosure does not bind to CLDN9, such as human CLDN9.

In some examples, the binding agent of the disclosure binds to CLDN18.2 of more than one species such as CLDN18.2 of human, mouse and cynomolgus.

In some examples, treating a patient with the binding agent described herein results in prolonged survival of said patient. In some examples, the binding agent described herein exhibits one or more immune effector functions. In some examples, said one or more immune effector functions are selected from the group consisting of complement dependent cytotoxicity (CDC), antibody-dependent cell mediated cytotoxicity (ADCC), antibody-dependent cell-mediated phagocytosis (ADCP), induction of apoptosis, and inhibition of proliferation. The binding agents described herein preferably are able to redirect T cells to attack cancer cells and, thus, act through redirected T cell cytotoxicity (RTCC). In some examples, the binding agent is not capable, or not substantially capable, of inducing ADCC. In some examples, the binding agent is not capable, or not substantially capable, of inducing CDC. In some examples, the binding agent is not capable, or not substantially capable, of inducing ADCP. In some examples, the binding agent is capable of reducing, preferably significantly reducing, growth and/or volume of a tumor of a subject such as a patient.

The disclosure also provides methods of producing the binding agent of the disclosure. In one example, a method of producing the binding agent of the disclosure comprises transfecting a host cell with a nucleic acid of the disclosure, a set of nucleic acids of the disclosure, a vector of the disclosure or a set of vectors of the disclosure. In one example, the host cell expresses a nucleic acid encoding the binding agent of the disclosure. In one example, the host cell co-expresses a nucleic acid encoding the first polypeptide chain of the binding agent of the disclosure and a nucleic acid encoding the second polypeptide chain of the binding agent of the disclosure. In one example, the host cell further expresses a nucleic acid encoding the third polypeptide chain of the binding agent of the disclosure. In one example, the host cell further expresses a nucleic acid encoding the fourth polypeptide chain of the binding agent of the disclosure. In one example, said nucleic acid(s) are contained in a vector or in a set of vectors. In one example, the host cell expresses all polypeptide chains of the binding agent of the disclosure. In one example, the host cell after transfection produces the binding agent of the disclosure, preferably when grown under appropriate conditions for binding agent production such as those described herein or known in the art. In one example, the binding agent of the disclosure can be obtained from the host cell.

In another aspect, the disclosure provides a method for producing the binding agent as defined herein, optionally comprising transfecting a host cell with the nucleic acid as defined herein, the set of nucleic acids as defined herein, the vector as defined herein, or the set of vectors as defined herein.

Thus, in one example, a method of producing the binding agent of the disclosure comprises the steps of transfecting a host cell with a nucleic acid encoding the first polypeptide chain of the binding agent of the disclosure, a nucleic acid encoding the second polypeptide chain of the binding agent of the disclosure, a nucleic acid encoding the third polypeptide chain of the binding agent of the disclosure, and optionally a nucleic acid encoding the fourth polypeptide chain of the binding agent of the disclosure, expressing said nucleic acids in the host cell and obtaining the binding agent of the disclosure. In one example, the host cell is a mammalian cell, preferably selected from the group consisting of CHO cells, BHK cells, HeLa cells, COS cells, HEK293 cells,

HEK293 T cells and the like. In one example, the host cell is a bacterial cell, a yeast cell, a fungal cell, a plant cell or an insect cell. In one example, the binding agent is produced in vitro. In one example, the binding agent is produced in vivo, e.g., in a subject to be treated such as a subject having a disease, in particular a disease associated with cells expressing CLDN18.2, e.g. cancer.

- 5 In one example, the first and/or the second polypeptide chain of the binding agent of the disclosure are produced, e.g., in a host cell, and the third polypeptide chain is produced, e.g., in another host cell. In one example, all polypeptide chains of the binding agent of the disclosure are produced in the same host cell.

In one example, the polypeptide chains of the binding agent of the disclosure are linked to one another, e.g., covalently linked. In one example, the polypeptide chains of the binding agent are produced as one polypeptide comprising all polypeptide chains of the binding agent. In one example, at least two polypeptide chains of the binding agent are linked together and are produced as one polypeptide. In one example, the first and the second polypeptide chains are linked together and are produced as one polypeptide and the third polypeptide chain is produced as a separate polypeptide. In one example, the third and first polypeptide chains are linked together and are produced as one polypeptide and the second polypeptide chain is produced as a separate polypeptide. In one example, the second and third polypeptide chains are linked together and are produced as one polypeptide and the first polypeptide chain is produced as a separate polypeptide, or is linked to the fourth polypeptide chain and both are produced together as one polypeptide. Preferably, the polypeptide chains of the binding agent of the disclosure are produced separately, i.e., as separate polypeptides, e.g., in the same or in different cells and interact upon or after production to form the binding agent, e.g., within a cell or not within a cell. Preferably, the polypeptide chains are produced as separate polypeptides, i.e., the first polypeptide chain is produced as one polypeptide, the second polypeptide chain is produced as one polypeptide, the third polypeptide chain is produced as one polypeptide, and optionally the fourth polypeptide chain is produced as one polypeptide, and the polypeptide chains of the disclosure interact to form the binding agent of the disclosure. In one example, administering the binding agent of the disclosure comprises administering the first, second, third, and optionally fourth, polypeptide chain formulated in a carrier such as a lipid nanoparticle, a liposome, a lipoplex etc. In one example, the first and second polypeptide chains are administered together, e.g., formulated in the same or in different carriers, and the third polypeptide chain is administered, e.g., formulated separately, e.g., in a carrier. In one example, the fourth polypeptide chain is further administered, e.g., formulated separately, e.g., in a carrier. In one example, the third and the fourth polypeptide chains are administered together, e.g., formulated in the same or in different carriers. In one example, all peptide chains of the binding agent of the disclosure are formulated together in the same carrier. In one example, each polypeptide chain of the binding agent of the disclosure is formulated in a separate carrier, wherein the carriers may be the same or different.

Other features and advantages of the disclosure will be apparent from the following detailed description and claims.

Claims

1. A binding agent comprising a binding domain with specificity for CLDN18.2 and a binding domain with specificity for CD3,
wherein the binding agent comprises at least three polypeptide chains, wherein
 - (i) a first polypeptide chain comprises a variable region of a heavy chain (VH) derived from an immunoglobulin with specificity for CLDN18.2 (VH(CLDN18.2)),
 - (ii) a second polypeptide chain comprises a VH derived from an immunoglobulin with specificity for CD3 (VH(CD3)) and a variable region of a light chain (VL) derived from an immunoglobulin with specificity for CD3 (VL(CD3)), and
 - (iii) a third polypeptide chain comprises a VL derived from an immunoglobulin with specificity for CLDN18.2 (VL(CLDN18.2)).
2. The binding agent of claim 1, wherein:
 - a) the first polypeptide chain comprises a constant region 1 of a heavy chain (CH1) derived from an immunoglobulin or a functional variant thereof;
 - b) wherein the third polypeptide chain comprises a constant region of a light chain (CL) derived from an immunoglobulin or a functional variant thereof, wherein optionally the CH1 on the first polypeptide chain interacts with the CL on the third polypeptide chain;
 - c) the immunoglobulin is IgG1, optionally human IgG1;
 - d) the first polypeptide chain interacts with the second polypeptide chain and with the third polypeptide chain; and/or
 - e) wherein the VH(CLDN18.2) and the VL(CLDN18.2) interact to form the binding domain with specificity for CLDN18.2, and the VH(CD3) and the VL(CD3) interact to form the binding domain with specificity for CD3.
3. The binding agent of claim 1 or 2, wherein the first and the second polypeptide chains comprise a constant region 2 of a heavy chain (CH2) derived from an immunoglobulin or a functional variant thereof and a constant region 3 of a heavy chain (CH3) derived from an immunoglobulin or a functional variant thereof and wherein optionally:

a) the VH and the CH1, CH2 and CH3 on the first polypeptide chain are arranged, from N-terminus to C-terminus, in the order

VH(CLDN18.2)-CH1-CH2-CH3;

b) the VH, the VL, and the CH2 and CH3 on the second polypeptide chain are arranged, from N-terminus to C-terminus, in the order

VH(CD3)-VL(CD3)-CH2-CH3, or

VL(CD3)-VH(CD3)-CH2-CH3; and/or

c) the CH2 on the first polypeptide chain interacts with the CH2 on the second polypeptide chain and/or the CH3 on the first polypeptide chain interacts with the CH3 on the second polypeptide chain.

4. The binding agent of any one of claims 1 to 3 comprising a further binding domain with specificity for CLDN18.2, wherein the second polypeptide chain further comprises a VH(CLDN18.2) and wherein the binding agent comprises a fourth polypeptide chain identical to the third polypeptide chain.

5. The binding agent of claim 4, wherein:

a) the second polypeptide chain further comprises a CH1 derived from an immunoglobulin or a functional variant thereof, wherein optionally

(i) the immunoglobulin is IgG1 or human IgG1,

(ii) the CH1 on the second polypeptide chain interacts with the CL on the fourth polypeptide chain, and/or

(iii) wherein the VHs, the VL, and the CH1, CH2 and CH3 on the second polypeptide chain are arranged, from N-terminus to C-terminus, in the order

VH(CLDN18.2)-CH1-VH(CD3)-VL(CD3)-CH2-CH3, or

VH(CLDN18.2)-CH1-VL(CD3)-VH(CD3)-CH2-CH3;

b) the second polypeptide chain interacts with the fourth polypeptide chain; and/or

c) VH(CLDN18.2) on the second polypeptide chain interacts with the VL(CLDN18.2) on the fourth polypeptide chain to form the further binding domain with specificity for CLDN18.2.

6. The binding agent of any one of claims 1 to 5, wherein:

a) the VH(CD3) comprises CDR1, CDR2 and CDR3 of an amino acid sequence selected from the group consisting of SEQ ID NO: 54, 58 and 61

b) the VH(CD3) comprises

- (i) a CDR1 comprising the amino acid sequence TYAMN (SEQ ID NO: 43) or a functional variant thereof, a CDR2 comprising the amino acid sequence RIRSKANNYATYYADSVKG (SEQ ID NO: 50) or a functional variant thereof, and a CDR3 comprising the amino acid sequence HGNFGDSYVSWFAY (SEQ ID NO: 45) or a functional variant thereof,
- (ii) a CDR1 comprising the amino acid sequence TYAMN (SEQ ID NO: 43) or a functional variant thereof, a CDR2 comprising the amino acid sequence RIRSKYNNYATYYADSVKG (SEQ ID NO: 44) or a functional variant thereof, and a CDR3 comprising the amino acid sequence HGNFGDEYVSWFAY (SEQ ID NO: 51) or a functional variant thereof, or
- (iii) a CDR1 comprising the amino acid sequence TYAMN (SEQ ID NO: 43) or a functional variant thereof, a CDR2 comprising the amino acid sequence RIRSKYNNYATYYADSVKG (SEQ ID NO: 44) or a functional variant thereof, and a CDR3 comprising the amino acid sequence HGNFGDSYVSWFAY (SEQ ID NO: 45) or a functional variant thereof;
- c) the VL(CD3) comprises CDR1, CDR2 and CDR3 of SEQ ID NO: 55;
- d) the VL(CD3) comprises a CDR1 comprising the amino acid sequence GSSTGAVTTSNYAN (SEQ ID NO: 46) or a functional variant thereof, a CDR2 comprising the amino acid sequence GTNKRAP (SEQ ID NO: 47) or a functional variant thereof, and a CDR3 comprising the amino acid sequence ALWYSNHWV (SEQ ID NO: 48) or a functional variant thereof;
- e) the VH(CLDN18.2) comprises CDR1, CDR2 and CDR3 of SEQ ID NO: 39;
- f) the VH(CLDN18.2) comprises a CDR1 comprising the amino acid sequence SYWIN (SEQ ID NO: 32) or a functional variant thereof, a CDR2 comprising the amino acid sequence NIYPSDSYTNYNQKFQG (SEQ ID NO: 33) or a functional variant thereof, and a CDR3 comprising the amino acid sequence SWRGNSFDY (SEQ ID NO: 34) or a functional variant thereof;
- g) the VL(CLDN18.2) comprises CDR1, CDR2 and CDR3 of SEQ ID NO: 42; and/or
- h) the VL(CLDN18.2) comprises a CDR1 comprising the amino acid sequence KSSQSLNLSGNQKNYLT (SEQ ID NO: 35) or a functional variant thereof, a CDR2 comprising the amino acid sequence WASTRES (SEQ ID NO: 36) or a functional variant thereof, and a CDR3 comprising the amino acid sequence QNDYSPFT (SEQ ID NO: 37) or a functional variant thereof.

7. The binding agent of any one of claims 1 to 6, wherein

- (i) the VH(CD3) comprises CDR1, CDR2 and CDR3 of SEQ ID NO: 58, the VL(CD3) comprises CDR1, CDR2 and CDR3 of SEQ ID NO: 55, the VH(CLDN18.2) comprises CDR1, CDR2 and CDR3 of SEQ ID NO: 39, and the VL(CLDN18.2) comprises CDR1, CDR2 and CDR3 of SEQ ID NO: 42,

(ii) the VH(CD3) comprises CDR1, CDR2 and CDR3 of SEQ ID NO: 61, the VL(CD3) comprises CDR1, CDR2 and CDR3 of SEQ ID NO: 55, the VH(CLDN18.2) comprises CDR1, CDR2 and CDR3 of SEQ ID NO: 39, and the VL(CLDN18.2) comprises CDR1, CDR2 and CDR3 of SEQ ID NO: 42, or

(iii) the VH(CD3) comprises CDR1, CDR2 and CDR3 of SEQ ID NO: 54, the VL(CD3) comprises CDR1, CDR2 and CDR3 of SEQ ID NO: 55, the VH(CLDN18.2) comprises CDR1, CDR2 and CDR3 of SEQ ID NO: 39, and the VL(CLDN18.2) comprises CDR1, CDR2 and CDR3 of SEQ ID NO: 42.

8. The binding agent of any one of claims 1 to 7, wherein

the VH(CD3) comprises or consists of an amino acid sequence represented by SEQ ID NO: 58 or a functional variant thereof,

the VL(CD3) comprises or consists of an amino acid sequence represented by SEQ ID NO: 55 or a functional variant thereof,

the VH(CLDN18.2) comprises or consists of an amino acid sequence represented by SEQ ID NO: 39 or a functional variant thereof, and/or

the VL(CLDN18.2) comprises or consists of an amino acid sequence represented by SEQ ID NO: 42 or a functional variant thereof.

9. The binding agent of any one of claims 1 to 7, wherein

the VH(CD3) comprises or consists of an amino acid sequence represented by SEQ ID NO: 61 or a functional variant thereof,

the VL(CD3) comprises or consists of an amino acid sequence represented by SEQ ID NO: 55 or a functional variant thereof,

the VH(CLDN18.2) comprises or consists of an amino acid sequence represented by SEQ ID NO: 39 or a functional variant thereof, and/or

the VL(CLDN18.2) comprises or consists of an amino acid sequence represented by SEQ ID NO: 42 or a functional variant thereof.

10. The binding agent of any one of claims 1 to 7, wherein

the VH(CD3) comprises or consists of an amino acid sequence represented by SEQ ID NO: 54 or a functional variant thereof,

the VL(CD3) comprises or consists of an amino acid sequence represented by SEQ ID NO: 55 or a functional variant thereof,

the VH(CLDN18.2) comprises or consists of an amino acid sequence represented by SEQ ID NO: 39 or a functional variant thereof, and/or

the VL(CLDN18.2) comprises or consists of an amino acid sequence represented by SEQ ID NO: 42 or a functional variant thereof.

11. The binding agent of any one of claims 3 to 10, wherein on the first polypeptide chain the CH1 is connected to the CH2 by a peptide linker which optionally comprises the amino acid sequence EPKSCDKTHTCPPCP (SEQ ID NO: 27) or a functional variant thereof.

12. The binding agent of any one of claims 3 to 11, wherein the VH(CD3) or VL(CD3) is connected to the CH2 by a peptide linker.

13. The binding agent of any one of claims 5 to 12, wherein the VH(CD3) or VL(CD3) is connected to the CH1 by a peptide linker.

14. The binding agent of claim 12 or 13, wherein the peptide linker comprises the amino acid sequence $(G_4S)_x$ or a functional variant thereof, wherein x is 2, 3, 4, 5 or 6, wherein optionally the peptide linker comprises the amino acid sequence $(G_4S)_2$ (SEQ ID NO: 26) or a functional variant thereof.

15. The binding agent of any one of claims 12 to 14, wherein the peptide linker connecting the VH(CD3) or VL(CD3) and the CH2 comprises the amino acid sequence KTHTCPPCP (SEQ ID NO: 21) or a functional variant thereof.

16. The binding agent of claim 15, wherein the peptide linker comprises the amino acid sequence $(G_4S)_2$ KTHTCPPCP (SEQ ID NO: 23) or a functional variant thereof.

17. The binding agent of claim 15, wherein the peptide linker comprises the amino acid sequence EPKSSDKTHTCPPCP (SEQ ID NO: 22) or a functional variant thereof.

18. The binding agent of any one of claims 1 to 17, wherein the VH(CD3) and the VL(CD3) are connected to one another by a peptide linker, wherein optionally the peptide linker comprises the amino acid sequence $(GKPGS)_x$ or a functional variant thereof, wherein x is 2, 3, 4, 5 or 6, wherein further optionally the peptide linker comprises the amino acid sequence $(GKPGS)_4$ (SEQ ID NO: 11) or a functional variant thereof.

19. The binding agent of any one of claims 2 to 18, wherein the CH1 comprises an amino acid sequence comprising an aspartic acid residue at position 208 according to EU numbering.

20. The binding agent of any one of claims 5 to 19, wherein the CH1 on the first polypeptide chain comprises an amino acid sequence comprising an aspartic acid residue at position 208 according to EU numbering and the CH1 on the second polypeptide chain comprises an amino acid sequence comprising an asparagine residue at position 208 according to EU numbering.
21. The binding agent of any one of claims 1 to 20, which does not substantially bind to human FcγRI, IIa, IIb, and/or IIIa.
22. The binding agent of any one of claims 3 to 21, wherein
- a) the CH2 on the first and/or the second polypeptide chain comprises an amino acid sequence comprising one or more of the following: a proline residue at position 233, a valine residue at position 234, an alanine residue at position 235, a deletion at position 236, a lysine residue at position 267 and a glutamic acid residue at position 295 according to EU numbering, wherein optionally the CH2s on the first and the second polypeptide chain comprise an amino acid sequence comprising a proline residue at position 233, a valine residue at position 234, an alanine residue at position 235, a deletion at position 236 and a lysine residue at position 267 according to EU numbering and wherein the CH2 on the first polypeptide chain further comprises a glutamic acid residue at position 295 according to EU numbering and the CH2 on the second polypeptide chain further comprises a glutamine residue at position 295 according to EU numbering; and/or
 - b) the CH3 on the first and/or the second polypeptide chain comprises an amino acid sequence comprising one or more of the following: a glutamine residue at position 357, a lysine residue at position 364, an aspartic acid residue at position 368, a serine residue at position 370, an aspartic acid residue at position 384, a glutamic acid residue at position 418 and an aspartic acid residue at position 421 according to EU numbering, wherein optionally the CH3 on the first polypeptide chain comprises an amino acid sequence comprising a glutamic acid residue at position 357, a serine residue at position 364, an aspartic acid residue at position 368, a serine residue at position 370, an aspartic acid residue at position 384, a glutamic acid residue at position 418 and an aspartic acid residue at position 421 according to EU numbering and the CH3 on the second polypeptide chain comprises an amino acid sequence comprising a glutamine residue at position 357, a lysine residue at position 364, a leucine residue at position 368, a lysine residue at position 370, an asparagine residue at position 384, a glutamine residue at position 418 and an asparagine residue at position 421 according to EU numbering.
23. The binding agent of any one of claims 1 to 22, wherein

- a) the first polypeptide chain comprises an amino acid sequence represented by SEQ ID NO: 28 or a functional variant thereof;
- b) the second polypeptide chain comprises an amino acid sequence represented by SEQ ID NO: 30 or a functional variant thereof;
- c) the third polypeptide chain comprises an amino acid sequence represented by SEQ ID NO: 31 or a functional variant thereof;
- d) the first polypeptide chain comprises or consists of an amino acid sequence represented by SEQ ID NO: 73 or a functional variant thereof;
- e) the second polypeptide chain comprises or consists of an amino acid sequence selected from the group consisting of SEQ ID NO: 74, 75, 76 and 77 or a functional variant thereof; and/or
- f) the third polypeptide chain comprises or consists of an amino acid sequence represented by SEQ ID NO: 78 or a functional variant thereof.

24. The binding agent of any one of claims 1 to 3, 6, 7, 10 to 12, 15, 17 to 19 and 21 to 23, wherein

- (i) the first polypeptide chain comprises or consists of an amino acid sequence represented by SEQ ID NO: 73 or a functional variant thereof,
- (ii) the second polypeptide chain comprises or consists of an amino acid sequence represented by SEQ ID NO: 75 or a functional variant thereof, and
- (iii) the third polypeptide chain comprises or consists of an amino acid sequence represented by SEQ ID NO: 78 or a functional variant thereof.

25. The binding agent of any one of claims 1 to 8, 11 to 16 and 18 to 23,

- a) wherein
 - (i) the first polypeptide chain comprises or consists of an amino acid sequence represented by SEQ ID NO: 73 or a functional variant thereof,
 - (ii) the second polypeptide chain comprises or consists of an amino acid sequence represented by SEQ ID NO: 74 or a functional variant thereof, and
 - (iii) the third polypeptide chain comprises or consists of an amino acid sequence represented by SEQ ID NO: 78 or a functional variant thereof; or
- b) wherein
 - (i) the first polypeptide chain comprises or consists of an amino acid sequence represented by SEQ ID NO: 73 or a functional variant thereof,
 - (ii) the second polypeptide chain comprises or consists of an amino acid sequence represented by SEQ ID NO: 76 or a functional variant thereof, and

(iii) the third polypeptide chain comprises or consists of an amino acid sequence represented by SEQ ID NO: 78 or a functional variant thereof.

26. The binding agent of any one of claims 1 to 7, 9, 11 to 15 and 17 to 23, wherein

(i) the first polypeptide chain comprises or consists of an amino acid sequence represented by SEQ ID NO: 73 or a functional variant thereof,

(ii) the second polypeptide chain comprises or consists of an amino acid sequence represented by SEQ ID NO: 77 or a functional variant thereof, and

(iii) the third polypeptide chain comprises or consists of an amino acid sequence represented by SEQ ID NO: 78 or a functional variant thereof.

27. The binding agent of any one of claims 1 to 26, wherein the VH(CLDN18.2), VL(CLDN18.2), VH(CD3) and/or VL(CD3) are humanized.

28. A binding agent comprising a binding domain with specificity for CLDN18.2 comprising a variable region of a heavy chain (VH) derived from an immunoglobulin with specificity for CLDN18.2 (VH(CLDN18.2)) and a variable region of a light chain (VL) derived from an immunoglobulin with specificity for CLDN18.2 (VL(CLDN18.2)), wherein

the VH(CLDN18.2) comprises a CDR1 comprising the amino acid sequence SYWIN (SEQ ID NO: 32), a CDR2 comprising the amino acid sequence NIYPSDSYTNYNQKFQG (SEQ ID NO: 33), and a CDR3 comprising the amino acid sequence SWRGNSFDY (SEQ ID NO: 34), and

the VL(CLDN18.2) comprises a CDR1 comprising the amino acid sequence KSSQSLNLSGNQKNYLT (SEQ ID NO: 35), a CDR2 comprising the amino acid sequence WASTRES (SEQ ID NO: 36), and a CDR3 comprising the amino acid sequence QNDYSYPFT (SEQ ID NO: 37),

wherein optionally the binding agent further comprises a binding domain with specificity for CD3, wherein further optionally the binding domain with specificity for CD3 comprises a VH derived from an immunoglobulin with specificity for CD3 (VH(CD3)) and a VL derived from an immunoglobulin with specificity for CD3 (VL(CD3)), wherein

the VH(CD3) comprises CDR1, CDR2 and CDR3 of an amino acid sequence selected from the group consisting of SEQ ID NO: 54, 58 and 61, and

the VL(CD3) comprises CDR1, CDR2 and CDR3 of SEQ ID NO: 55,

wherein still further optionally the VH(CD3) comprises

(i) a CDR1 comprising the amino acid sequence TYAMN (SEQ ID NO: 43) or a functional variant thereof, a CDR2 comprising the amino acid sequence RIRSKANNYATYYADSVKG (SEQ ID NO:

50) or a functional variant thereof, and a CDR3 comprising the amino acid sequence HGNFGDSYVSWFAY (SEQ ID NO: 45) or a functional variant thereof,

(ii) a CDR1 comprising the amino acid sequence TYAMN (SEQ ID NO: 43) or a functional variant thereof, a CDR2 comprising the amino acid sequence RIRSKYNNYATYYADSVKG (SEQ ID NO: 44) or a functional variant thereof, and a CDR3 comprising the amino acid sequence HGNFGDEYVSWFAY (SEQ ID NO: 51) or a functional variant thereof, or

(iii) a CDR1 comprising the amino acid sequence TYAMN (SEQ ID NO: 43) or a functional variant thereof, a CDR2 comprising the amino acid sequence RIRSKYNNYATYYADSVKG (SEQ ID NO: 44) or a functional variant thereof, and a CDR3 comprising the amino acid sequence HGNFGDSYVSWFAY (SEQ ID NO: 45) or a functional variant thereof, and

the VL(CD3) comprises a CDR1 comprising the amino acid sequence GSSTGAVTTSNYAN (SEQ ID NO: 46) or a functional variant thereof, a CDR2 comprising the amino acid sequence GTNKRAP (SEQ ID NO: 47) or a functional variant thereof, and a CDR3 comprising the amino acid sequence ALWYSNHWV (SEQ ID NO: 48) or a functional variant thereof.

29. The binding agent of claim 28, wherein

a) the VH(CLDN18.2) comprises or consists of an amino acid sequence represented by SEQ ID NO: 39 or a functional variant thereof;

b) the VL(CLDN18.2) comprises or consists of an amino acid sequence represented by SEQ ID NO: 42 or a functional variant thereof;

c) the VH(CD3) comprises or consists of an amino acid sequence selected from the group consisting of SEQ ID NO: 54, 58 or 61 or a functional variant thereof, and the VL(CD3) comprises or consists of an amino acid sequence represented by SEQ ID NO: 55 or a functional variant thereof;

d) the binding agent is in the format of a full-length antibody or an antibody fragment;

e) the binding domain with specificity for CLDN18.2 is in the format of a Fab fragment, a Fab' fragment, a F(ab')₂ fragment, an Fv fragment, or an scFv fragment;

f) the binding domain with specificity for CD3 is in the format of a Fab fragment, a Fab' fragment, a F(ab')₂ fragment, an Fv fragment, or an scFv fragment;

g) wherein the binding domain with specificity for CLDN18.2 is in the format of a Fab fragment and the binding domain with specificity for CD3 is in the format of an scFv fragment;

h) the binding agent is a bispecific molecule, wherein optionally the bispecific molecule is a bispecific antibody;

i) wherein the binding agent is capable of binding monovalently or bivalently to CLDN18.2;

j) the VH(CLDN18.2), VL(CLDN18.2), VH(CD3) and/or VL(CD3) are humanized; and/or

k) the VH(CLDN18.2) is connected to the VL(CLDN18.2) by a peptide linker and/or the VH(CD3) is connected to the VL(CD3) by a peptide linker, wherein the peptide linker optionally comprises an amino acid sequence selected from the group consisting of SEQ ID NO: 2-20.

30. A binding agent comprising two binding domains with specificity for CLDN18.2 and a binding domain with specificity for CD3,

wherein the binding agent comprises four polypeptide chains, wherein

(i) the first polypeptide chain comprises or consists of an amino acid sequence represented by SEQ ID NO: 73,

(ii) the second polypeptide chain comprises or consists of an amino acid sequence represented by SEQ ID NO: 74,

(iii) the third polypeptide chain comprises or consists of an amino acid sequence represented by SEQ ID NO: 78, and

(iv) the fourth polypeptide chain is identical to the third polypeptide chain, wherein the first polypeptide chain interacts with the second polypeptide chain and with the third polypeptide chain, and the second polypeptide chain interacts with the fourth polypeptide chain.

31. A binding agent comprising a binding domain with specificity for CLDN18.2 and a binding domain with specificity for CD3,

wherein the binding agent comprises three polypeptide chains, wherein

(i) the first polypeptide chain comprises or consists of an amino acid sequence represented by SEQ ID NO: 73,

(ii) the second polypeptide chain comprises or consists of an amino acid sequence represented by SEQ ID NO: 75, and

(iii) the third polypeptide chain comprises or consists of an amino acid sequence represented by SEQ ID NO: 78,

wherein the first polypeptide chain interacts with the second polypeptide chain and with the third polypeptide chain.

32. A binding agent comprising two binding domains with specificity for CLDN18.2 and a binding domain with specificity for CD3,

wherein the binding agent comprises four polypeptide chains, wherein

(i) the first polypeptide chain comprises or consists of an amino acid sequence represented by SEQ ID NO: 73,

(ii) the second polypeptide chain comprises or consists of an amino acid sequence represented by SEQ ID NO: 76,

(iii) the third polypeptide chain comprises or consists of an amino acid sequence represented by SEQ ID NO: 78, and

(iv) the fourth polypeptide chain is identical to the third polypeptide chain, wherein the first polypeptide chain interacts with the second polypeptide chain and with the third polypeptide chain, and the second polypeptide chain interacts with the fourth polypeptide chain.

33. A binding agent comprising two binding domains with specificity for CLDN18.2 and a binding domain with specificity for CD3,

wherein the binding agent comprises four polypeptide chains, wherein

(i) the first polypeptide chain comprises or consists of an amino acid sequence represented by SEQ ID NO: 73,

(ii) the second polypeptide chain comprises or consists of an amino acid sequence represented by SEQ ID NO: 77,

(iii) the third polypeptide chain comprises or consists of an amino acid sequence represented by SEQ ID NO: 78, and

(iv) the fourth polypeptide chain is identical to the third polypeptide chain, wherein the first polypeptide chain interacts with the second polypeptide chain and with the third polypeptide chain, and the second polypeptide chain interacts with the fourth polypeptide chain.

34. The binding agent of any one of claims 1 to 33, wherein CD3 is expressed on the surface of T cells, wherein optionally binding of the binding agent to CD3 on T cells results in proliferation and/or activation of the T cells.

35. The binding agent of any one of claims 1 to 34, wherein CLDN18.2 is expressed in cancer cells, wherein optionally

a) CLDN18.2 is expressed on the surface of the cancer cells; and/or

b) the binding agent binds to an extracellular portion of CLDN18.2.

36. The binding agent of any one of claims 1 to 35, wherein the binding agent induces T cell-mediated cytotoxicity against cancer cells expressing CLDN18.2.

37. The binding agent of claim 35 or 36, wherein the cancer cells are from a cancer selected from the group consisting of gastric cancer, esophageal cancer, pancreatic cancer, lung cancer such as non small cell lung cancer (NSCLC), breast cancer, ovarian cancer, colon cancer, rectal cancer, colorectal cancer, hepatic cancer, head-neck cancer, cancer of the gallbladder and the metastasis thereof, a Krukenberg tumor, peritoneal metastasis and/or lymph node metastasis.
38. A nucleic acid encoding a polypeptide chain of the binding agent of any one of claims 1 to 27 and 30 to 37, or a vector comprising said nucleic acid.
39. A nucleic acid encoding the binding agent of any one of claims 1 to 37.
40. A set of nucleic acids together encoding the binding agent of any one of claims 1 to 37.
41. A vector comprising the nucleic acid of claim 39 or the set of nucleic acids of claim 40.
42. A set of vectors comprising the set of nucleic acids of claim 40.
43. The vector of claim 41 or the set of vectors of claim 42, which is capable of expressing the binding agent.
44. A host cell comprising the nucleic acid of claim 39, the set of nucleic acids of claim 40, the vector of claim 41 or 43, or the set of vectors of claim 42 or 43.
45. A pharmaceutical composition comprising the binding agent of any one of claims 1 to 37, the nucleic acid of claim 39, the set of nucleic acids of claim 40, the vector of claim 41 or 43, the set of vectors of claim 42 or 43, or the host cell of claim 44.
46. The binding agent of any one of claims 1 to 37, the nucleic acid of claim 39, the set of nucleic acids of claim 40, the vector of claim 41 or 43, the set of vectors of claim 42 or 43, the host cell of claim 44 or the pharmaceutical composition of claim 45 for use in therapy.
47. The binding agent of any one of claims 1 to 37, the nucleic acid of claim 39 the set of nucleic acids of claim 40, the vector of claim 41 or 43, the set of vectors of claim 42 or 43, the host cell of claim 44 or the pharmaceutical composition of claim 45 for use in treating or preventing cancer involving cancer cells expressing CLDN18.2, wherein optionally the cancer is selected from the group consisting of gastric cancer, esophageal cancer, cancer of the gastroesophageal junction (GEJ),

pancreatic cancer, lung cancer such as non small cell lung cancer (NSCLC), breast cancer, ovarian cancer, colon cancer, rectal cancer, colorectal cancer, hepatic cancer, head-neck cancer, cancer of the gallbladder and the metastasis thereof, a Krukenberg tumor, peritoneal metastasis and/or lymph node metastasis.

48. Use of the binding agent of any one of claims 1 to 37, the nucleic acid of claim 39, the set of nucleic acids of claim 40, the vector of claim 41 or 43, the set of vectors of claim 42 or 43, the host cell of claim 44 or the pharmaceutical composition of claim 45 for the preparation of a medicament for treating or preventing cancer involving cancer cells expressing CLDN18.2, wherein optionally the cancer is selected from the group consisting of gastric cancer, esophageal cancer, cancer of the gastroesophageal junction (GEJ), pancreatic cancer, lung cancer such as non small cell lung cancer (NSCLC), breast cancer, ovarian cancer, colon cancer, rectal cancer, colorectal cancer, hepatic cancer, head-neck cancer, cancer of the gallbladder and the metastasis thereof, a Krukenberg tumor, peritoneal metastasis and/or lymph node metastasis.

49. A method for producing the binding agent of any one of claims 1 to 37, optionally comprising transfecting a host cell with the nucleic acid of claim 39, the set of nucleic acids of claim 40, the vector of claim 41 or 43, or the set of vectors of claim 42 or 43.