	single variable domains	single variable domains	single variable domains	single variable domains	single variable domains	single variable domains	single variable domains
12B04-V2 single variable domain	12B04-V2 + 1B-1B6-V1 single variable domains	12B04-V2 + 1B-1B6-V2 single variable domains	12B04-V2 + 1B-1B6-V3 single variable domains	12B04-V2 + 1B-1C7-V1 single variable domains	12B04-V2 + 1B-1C7-V2 single variable domains	12B04-V2 + 12B04-V1 single variable domains	12B04-V2 + 12B04-V2 single variable domains

Note: in the table, (X) + (Y) indicates the combination of the third antigen-binding moiety and the first antigen-binding moiety of the multispecific antibody. For example, "1B-1B6-V1 + 1B-1C7-V1 single variable domains" indicates that the third antigen-binding moiety of the multispecific antibody comprises the amino acid sequence of the 1B-1B6-V1 single variable domain, while the first antigen-binding moiety comprises the amino acid sequence of the 1B-1C7-V1 single variable domain; the amino acid sequences of the single variable domains referred to in the table are shown in Table S1.

In the present disclosure, the first antigen-binding moiety may be derived from a camelid or be humanized. The third antigen-binding moiety may be derived from a camelid or be humanized. Humanization may reduce the immunogenicity, and in some embodiments, both the first antigen-binding moiety and the third antigen-binding moiety are humanized. In some embodiments, both the first antigen-binding moiety and the third antigen-binding moiety are derived from a camelid.

In the multispecific antibody of the present disclosure, the second antigen-binding moiety provides the ability to target EGFR. The second antigen-binding moiety may be a Fab, an scFv, or an ScFab (single-chain Fab). In some embodiments, the second antigen-binding moiety is a Fab that binds to EGFR.

In some embodiments, the second antigen-binding moiety is murine, chimeric, or humanized.

In some embodiments, the second antigen-binding moiety comprises a heavy chain variable region and a light chain variable region, wherein the heavy chain variable region comprises: an HCDR1 comprising the amino acid sequence set forth in SEQ ID NO: 63, an HCDR2 comprising the amino acid sequence set forth in SEQ ID NO: 64, and an HCDR3 comprising the amino acid sequence set forth in SEQ ID NO: 65, and the light chain variable region comprises: an LCDR1 comprising the amino acid sequence set forth in SEQ ID NO: 66, an LCDR2 comprising the amino acid sequence set forth in SEQ ID NO: 67, and an LCDR3 comprising the amino acid sequence set forth in SEQ ID NO: 68.

In one specific embodiment, the heavy chain variable region comprises the HCDR1, the HCDR2, and the HCDR3 of the variable region set forth in SEQ ID NO: 69, and the light chain variable region comprises the LCDR1, the LCDR2, and the LCDR3 of the variable region set forth in SEQ ID NO: 70.

In some embodiments, the second antigen-binding moiety comprises a heavy chain variable region having an amino acid sequence having at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identity to the amino acid sequence set forth in SEQ ID NO: 69, and a light chain variable region having an amino acid sequence having at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identity to the amino acid sequence set forth in SEQ ID NO: 70. In some embodiments, the heavy chain variable region of the second antigen-binding moiety comprises the amino acid sequence set forth in SEQ ID NO: 69, and the light chain variable region thereof comprises the amino acid sequence set forth in SEQ ID NO: 70. In some embodiments, for the second antigen-binding moiety, the amino acid sequence of the heavy chain variable region is set forth in SEQ ID NO: 69, and the amino acid sequence of the light chain variable region is set forth in SEQ ID NO: 70.

The multispecific antibody of the present disclosure may have no Fc domain, and the first antigen-binding moiety, the second antigen-binding moiety, and the third antigen-binding moiety are connected by suitable linkers.

The multispecific antibody of the present disclosure may have an Fc domain, and the Fc domain may prolong the half-life, provide the Fc domain-related effector, and the like.

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comprising the amino acid sequence set forth in SEQ ID NO: 64, and an HCDR3 comprising the amino acid sequence set forth in SEQ ID NO: 65, and the light chain variable region comprises an LCDR1 comprising the amino acid sequence set forth in SEQ ID NO: 66, an LCDR2 comprising the amino acid sequence set forth in SEQ ID NO: 67, and an LCDR3 comprising the amino acid sequence set forth in SEQ ID NO: 68.

Embodiment 20. The multispecific antibody according to any one of embodiments 1-18, wherein the second antigen-binding moiety comprises a heavy chain variable region and a light chain variable region; the heavy chain variable region comprises the HCDR1, the HCDR2, and the HCDR3 of the variable region set forth in SEQ ID NO: 69, and the light chain variable region comprises the LCDR1, the LCDR2, and the LCDR3 of the variable region set forth in SEQ ID NO: 70.

Embodiment 21. The multispecific antibody according to any one of embodiments 1-20, wherein the second antigen-binding moiety comprises a heavy chain variable region having an amino acid sequence having at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identity to the amino acid sequence set forth in SEQ ID NO: 69, and a light chain variable region having an amino acid sequence having at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identity to the amino acid sequence set forth in SEQ ID NO: 70.

Embodiment 22. The multispecific antibody according to embodiment 21, wherein the heavy chain variable region of the second antigen-binding moiety comprises the amino acid sequence set forth in SEQ ID NO: 69, and the light chain variable region thereof comprises the amino acid sequence set forth in SEQ ID NO: 70.

Embodiment 23. The multispecific antibody according to any one of embodiments 1-22, wherein the second antigen-binding moiety is murine, chimeric, or humanized.

Embodiment 24. The multispecific antibody according to any one of embodiments 1-23, wherein the third antigen-binding moiety and the first antigen-binding moiety are fused to each other, optionally via a peptide linker.

Embodiment 25. The multispecific antibody according to embodiment 24, wherein the third antigen-binding moiety, at the C-terminus, is fused to the N-terminus of the first antigen-binding moiety.

Embodiment 26. The multispecific antibody according to any one of embodiments 1-25, further comprising: (iv) an Fc domain composed of two Fc polypeptides.

Embodiment 27. The multispecific antibody according to embodiment 26, wherein the first antigen-binding moiety, at the C-terminus, is fused to the N-terminus of one of the Fc polypeptides of the Fc domain, the second antigen-binding moiety is a Fab, and the second antigen-binding moiety, at the C-terminus of the Fab heavy chain, is fused to the N-terminus of the other Fc polypeptide of the Fc domain.

Embodiment 28. The multispecific antibody according to embodiment 26 or 27, wherein the Fc domain is an IgG Fc domain, preferably an IgG1 Fc domain.

Embodiment 29. The multispecific antibody according to embodiment 28, wherein the IgG Fc domain is a human IgG Fc domain, preferably a human IgG1 Fc domain.

Embodiment 30. The multispecific antibody according to any one of embodiments 26-29, wherein the Fc domain comprises an amino acid substitution that promotes the association of the two Fc polypeptides of the Fc domain.

Embodiment 31. The multispecific antibody according to embodiment 30, wherein according to the EU numbering, one of the Fc polypeptides of the Fc domain comprises amino acid substitutions 354C and 366Y/W, and the other Fc polypeptide comprises amino acid substitutions 349C, 366S, 368A, and 407T/V.

Embodiment 32. The multispecific antibody according to any one of embodiments 26-31, wherein the Fc domain comprises an amino acid substitution that reduces or eliminates the binding of the CH3 region of one Fc polypeptide in the Fc domain to protein A.

Embodiment 33. The multispecific antibody according to embodiment 32, wherein according to the EU numbering, the Fc domain comprises amino acid substitution(s): (a) 435R or (b) 435R and 436F that occur only in one of the Fc polypeptides.

Embodiment 34. The multispecific antibody according to any one of embodiments 26-29, wherein according to