CLAIMS

1. Use of a bispecific antibody construct in the manufacture of a medicament for treating glioblastoma or glioma, wherein said antibody construct comprises a first binding domain which binds to human and macaque epidermal growth factor receptor VIII (EGFRVIII) on the surface of a target cell and a second binding domain which binds to human CD3 on the surface of a T cell, wherein the first binding domain comprises a heavy chain variable region (VH) comprising the amino acid sequence of SEQ ID NO: 157, and a light chain variable region (VL) comprising the amino acid sequence of SEQ ID NO: 158.

2. The use of claim 1, wherein said antibody construct is in a format selected from the group consisting of $(scFv)_2$, diabodies, and oligomers thereof.

3. The use of claim 1 or 2, wherein said second binding domain binds to human CD3 epsilon and to *Callithrix jacchus, Saguinus Oedipus* or *Saimiri sciureus* CD3 epsilon, and comprises:

(i) a VL region comprising: a CDR-L1 comprising the amino acid of SEQ ID NO: 11, a CDR-L2 comprising the amino acid of SEQ ID NO:12, and a CDR-L3 comprising the amino acid of SEQ ID NO:13; and a VH region comprising: a CDR-H1 comprising the amino acid of SEQ ID NO: 14, a CDR-H2 comprising the amino acid of SEQ ID NO:15, and a CDR-H3 comprising the amino acid of SEQ ID NO:16;

(ii) a VL region comprising: a CDR-L1 comprising the amino acid of SEQ ID NO: 20, a CDR-L2 comprising the amino acid of SEQ ID NO:21, and a CDR-L3 comprising the amino acid of SEQ ID NO:22; and a VH region comprising: a CDR-H1 comprising the amino acid of SEQ ID NO: 23, a CDR-H2 comprising the amino acid of SEQ ID NO:24, and a CDR-H3 comprising the amino acid of SEQ ID NO:25;

(iii) a VL region comprising: a CDR-L1 comprising the amino acid of SEQ ID NO: 29, a CDR-L2 comprising the amino acid of SEQ ID NO:30, and a CDR-L3 comprising the amino acid of SEQ ID NO:31; and a VH region comprising: a CDR-H1 comprising the amino acid of SEQ ID NO: 32, a CDR-H2 comprising the amino acid of SEQ ID NO:33, and a CDR-H3 comprising the amino acid of SEQ ID NO:34;

(iv) a VL region comprising: a CDR-L1 comprising the amino acid of SEQ ID NO: 38, a CDR-L2 comprising the amino acid of SEQ ID NO:39, and a CDR-L3 comprising the amino acid of SEQ ID NO:40; and a VH region comprising: a CDR-H1 comprising the amino acid of SEQ ID NO: 41, a CDR-H2 comprising the amino acid of SEQ ID NO:42, and a CDR-H3 comprising the amino acid of SEQ ID NO:43;

(v) a VL region comprising: a CDR-L1 comprising the amino acid of SEQ ID NO: 47, a CDR-L2 comprising the amino acid of SEQ ID NO:48, and a CDR-L3 comprising the amino acid of SEQ ID NO:49; and a VH region comprising: a CDR-H1 comprising the amino acid of SEQ ID NO: 50, a CDR-H2 comprising the amino acid of SEQ ID NO:51, and a CDR-H3 comprising the amino acid of SEQ ID NO:52;

(vi) a VL region comprising: a CDR-L1 comprising the amino acid of SEQ ID NO: 56, a CDR-L2 comprising the amino acid of SEQ ID NO:57, and a CDR-L3 comprising the amino acid of SEQ ID NO:58; and a VH region comprising: a CDR-H1 comprising the amino acid of SEQ ID NO: 59, a CDR-H2 comprising the amino acid of SEQ ID NO:60, and a CDR-H3 comprising the amino acid of SEQ ID NO:61;

(vii) a VL region comprising: a CDR-L1 comprising the amino acid of SEQ ID NO: 65, a CDR-L2 comprising the amino acid of SEQ ID NO:66, and a CDR-L3 comprising the amino acid of SEQ ID NO:67; and a VH region comprising: a CDR-H1 comprising the amino acid of SEQ ID NO: 68, a CDR-H2 comprising the amino acid of SEQ ID NO:69, and a CDR-H3 comprising the amino acid of SEQ ID NO:70;

(viii) a VL region comprising: a CDR-L1 comprising the amino acid of SEQ ID NO: 74, a CDR-L2 comprising the amino acid of SEQ ID NO:75, and a CDR-L3 comprising the amino acid of SEQ ID NO:76; and a VH region comprising: a CDR-H1 comprising the amino acid of SEQ ID NO: 77, a CDR-H2 comprising the amino acid of SEQ ID NO:78, and a CDR-H3 comprising the amino acid of SEQ ID NO:79;

(ix) a VL region comprising: a CDR-L1 comprising the amino acid of SEQ ID NO: 83, a CDR-L2 comprising the amino acid of SEQ ID NO:84, and a CDR-L3 comprising the amino acid of SEQ ID NO:85; and a VH region comprising: a CDR-H1 comprising the amino acid of SEQ ID NO: 86, a CDR-H2 comprising the amino acid of SEQ ID NO:87, and a CDR-H3 comprising the amino acid of SEQ ID NO:88;

(x) a VL region comprising: a CDR-L1 comprising the amino acid of SEQ ID NO: 92, a CDR-L2 comprising the amino acid of SEQ ID NO:93, and a CDR-L3 comprising the amino acid of SEQ ID NO:94; and a VH region comprising: a CDR-H1 comprising the amino acid of SEQ ID NO: 95, a CDR-H2 comprising the amino acid of SEQ ID NO:96, and a CDR-H3 comprising the amino acid of SEQ ID NO:97;

or

(xi) a VH region comprising amino acid sequence of SEQ ID NO: 101, and a VL region comprising amino acid sequence of SEQ ID NO: 102.

4. The use of any one of claims 1-3, wherein said antibody construct comprises:

(a) a polypeptide comprising in the following order starting from the N-terminus:
(i) a polypeptide comprising the amino acid sequence of SEQ ID NO: 159;
(ii) a peptide linker comprising the amino acid sequence selected from any one of SEQ ID NOs: 1-9;
(iii) a polypeptide comprising the amino acid sequence selected from the group consisting of: SEQ ID NO: 19, SEQ ID NO: 28, SEQ ID NO: 37, SEQ ID NO: 46, SEQ ID NO: 55, SEQ ID NO: 64, SEQ ID NO: 73, SEQ ID NO: 82, SEQ ID NO: 91, SEQ ID NO: 100, and SEQ ID NO: 103; and
(iv) optionally, a His-tag comprising the amino acid sequence of SEQ ID NO: 10;

(b) a polypeptide comprising in following order starting from the N-terminus:
(i) a polypeptide comprising the amino acid sequence of SEQ ID NO: 159;
(ii) a peptide linker comprising the amino acid sequence selected from any one of SEQ ID NOs: 1-9;
(iii) a polypeptide comprising the amino acid sequence selected from the group consisting of: SEQ ID NO: 19, SEQ ID NO: 28, SEQ ID NO: 37, SEQ ID NO: 46, SEQ ID NO: 55, SEQ ID NO: 64, SEQ ID NO: 73, SEQ ID NO: 82, SEQ ID NO: 91, SEQ ID NO: 100, and SEQ ID NO: 103;
(iv) optionally, a peptide linker comprising the amino acid sequence selected from any one of SEQ ID NOs: 1-9;
(v) a polypeptide comprising the amino acid sequence of SEQ ID NO:134; and
(vi) optionally, a His-tag comprising the amino acid sequence of SEQ ID NO: 10;

(c) a polypeptide comprising in the following order starting from the N-terminus:

(i) a polypeptide comprising the amino acid sequence QRFVTGHFGGLX$_1$PANG (SEQ ID NO: 135) whereas X_1 is Y or H;

(ii) a polypeptide comprising the amino acid sequence of: SEQ ID NO: 159;

(iii) a peptide linker comprising the amino acid sequence selected from any one of SEQ ID NOs: 1-9;

(iv) a polypeptide comprising the amino acid sequence selected from the group consisting of: SEQ ID NO: 19, SEQ ID NO: 28, SEQ ID NO: 37, SEQ ID NO: 46, SEQ ID NO: 55, SEQ ID NO: 64, SEQ ID NO: 73, SEQ ID NO: 82, SEQ ID NO: 91, SEQ ID NO: 100, and SEQ ID NO: 103;

(v) a polypeptide comprising the amino acid sequence QRFVTGHFGGLHPANG (SEQ ID NO: 137) or QRFCTGHFGGLHPCNG (SEQ ID NO: 139); and

(vi) optionally, a His-tag comprising the amino acid sequence of SEQ ID NO: 10;

(d) a first polypeptide comprising in the following order starting from the N-terminus:

(i) a polypeptide comprising the amino acid sequence selected from the group consisting of: SEQ ID NO: 17, SEQ ID NO: 26, SEQ ID NO: 35, SEQ ID NO: 44, SEQ ID NO: 53, SEQ ID NO: 62, SEQ ID NO: 71, SEQ ID NO: 80, SEQ ID NO: 89, SEQ ID NO: 98, and SEQ ID NO: 101;

(ii) a peptide linker comprising the amino acid sequence of SEQ ID NO: 8;

(iii) a polypeptide comprising the amino acid of SEQ ID NO: 158; and

(iv) a polypeptide comprising the amino acid sequence of SEQ ID NO: 140;

and a second polypeptide comprising in the following order starting from the N-terminus:

(i) a polypeptide comprising the amino acid sequence of SEQ ID NO: 157;

(ii) a peptide linker comprising the amino acid sequence of SEQ ID NO: 8;

(iii) a polypeptide comprising the amino acid sequence selected from the group consisting of: SEQ ID NO: 18, SEQ ID NO: 27, SEQ ID NO: 36, SEQ ID NO: 45, SEQ ID NO: 54, SEQ ID NO: 63, SEQ ID NO: 72, SEQ ID NO: 81, SEQ ID NO: 90, SEQ ID NO: 99, and SEQ ID NO: 102, and a serine residue at the C-terminus; and

(iv) a polypeptide comprising the amino acid sequence of SEQ ID NO: 141;

(e) a first polypeptide comprising in the following order starting from the N-terminus:

(i) a polypeptide comprising the amino acid sequence selected from the group consisting of: SEQ ID NO: 17, SEQ ID NO: 26, SEQ ID NO: 35, SEQ ID NO: 44,

SEQ ID NO: 53, SEQ ID NO: 62, SEQ ID NO: 71, SEQ ID NO: 80, SEQ ID NO: 89, SEQ ID NO: 98, and SEQ ID NO: 101;

(ii) a peptide linker comprising the amino acid sequence of SEQ ID NO: 8;

(iii) a polypeptide comprising the amino acid sequence of SEQ ID NO: 158; and

(iv) a polypeptide comprising the amino acid sequence of SEQ ID NO: 142;

and a second polypeptide comprising in the following order starting from the N-terminus:

(i) a polypeptide comprising the amino acid sequence of SEQ ID NO: 157;

(ii) a peptide linker comprising the amino acid sequence of SEQ ID NO: 8;

(iii) a polypeptide comprising the amino acid sequence selected from the group consisting of: SEQ ID NO: 18, SEQ ID NO: 27, SEQ ID NO: 36, SEQ ID NO: 45, SEQ ID NO: 54, SEQ ID NO: 63, SEQ ID NO: 72, SEQ ID NO: 81, SEQ ID NO: 90, SEQ ID NO: 99, and SEQ ID NO: 102, and a serine residue at the C-terminus; and

(iv) a polypeptide comprising the amino acid sequence of SEQ ID NO: 143;

(f) a first polypeptide comprising in the following order starting from the N-terminus:

(i) a polypeptide comprising the amino acid sequence of SEQ ID NO: 159;

(ii) a peptide linker comprising the amino acid sequence selected from any one of SEQ ID NOs: 1-9;

(iii) a polypeptide comprising the amino acid sequence selected from the group consisting of: SEQ ID NO: 19, SEQ ID NO: 28, SEQ ID NO: 37, SEQ ID NO: 46, SEQ ID NO: 55, SEQ ID NO: 64, SEQ ID NO: 73, SEQ ID NO: 82, SEQ ID NO: 91, SEQ ID NO: 100, and SEQ ID NO: 103; and

(iv) a polypeptide comprising the amino acid sequence of SEQ ID NO: 144;

and a second polypeptide comprising the amino acid sequence of SEQ ID NO: 145;

(g) a first polypeptide comprising in the following order starting from the N-terminus:

(i) a polypeptide comprising the amino acid sequence of SEQ ID NO: 159; and

(ii) a polypeptide comprising the amino acid sequence of SEQ ID NO: 146;

and a second polypeptide comprising in the following order starting from the N-terminus:

(i) a polypeptide comprising the amino acid sequence selected from the group consisting of: SEQ ID NO: 19, SEQ ID NO: 28, SEQ ID NO: 37, SEQ ID NO: 46, SEQ ID NO: 55, SEQ ID NO: 64, SEQ ID NO: 73, SEQ ID NO: 82, SEQ ID NO: 91, SEQ ID NO: 100, and SEQ ID NO: 103; and

(ii) a polypeptide comprising the amino acid sequence of SEQ ID NO: 147;

(h) a first polypeptide comprising in the following order starting from the N-terminus:

(i) a polypeptide comprising the amino acid sequence of SEQ ID NO: 159; and

(ii) a polypeptide comprising the amino acid sequence of SEQ ID NO: 148;

and a second polypeptide comprising in the following order starting from the N-terminus:

(i) a polypeptide comprising the amino acid sequence selected from the group consisting of: SEQ ID NO: 19, SEQ ID NO: 28, SEQ ID NO: 37, SEQ ID NO: 46, SEQ ID NO: 55, SEQ ID NO: 64, SEQ ID NO: 73, SEQ ID NO: 82, SEQ ID NO: 91, SEQ ID NO: 100, and SEQ ID NO: 103; and

(ii) a polypeptide comprising the amino acid sequence of SEQ ID NO: 149;

(i) a polypeptide comprising in the following order starting from the N-terminus:

(i) a polypeptide comprising the amino acid sequence of SEQ ID NO: 159;

(ii) a peptide linker comprising the amino acid sequence selected from any one of SEQ ID NOs: 1-9;

(iii) a polypeptide comprising the amino acid sequence selected from the group consisting of: SEQ ID NO: 19, SEQ ID NO: 28, SEQ ID NO: 37, SEQ ID NO: 46, SEQ ID NO: 55, SEQ ID NO: 64, SEQ ID NO: 73, SEQ ID NO: 82, SEQ ID NO: 91, SEQ ID NO: 100, and SEQ ID NO: 103; and

(iv) a polypeptide comprising the amino acid sequence of SEQ ID NO: 150;

or

(j) a polypeptide comprising in the following order starting from the N-terminus:

(i) a polypeptide comprising the amino acid sequence of SEQ ID NO: 159;

(ii) a peptide linker comprising the amino acid sequence selected from any one of SEQ ID NOs: 1-9;

(iii) a polypeptide comprising the amino acid sequence selected from the group consisting of: SEQ ID NO: 19, SEQ ID NO: 28, SEQ ID NO: 37, SEQ ID NO: 46, SEQ ID NO: 55, SEQ ID NO: 64, SEQ ID NO: 73, SEQ ID NO: 82, SEQ ID NO: 91, SEQ ID NO: 100, and SEQ ID NO: 103;

(iv) a peptide linker having an amino acid sequence selected from the group consisting of: SEQ ID NOs: 1, 2, 4, 5, 6, 8 and 9; and

(v) the third domain comprising the amino acid sequence selected from any one of SEQ ID NOs: 181-188.

5. The use of any one of claims 1-4, wherein said antibody construct comprises a polypeptide comprising the amino acid sequence of SEQ ID NO: 160.

6. Use of a polypeptide in the manufacture of a medicament for treating glioblastoma or glioma, said polypeptide comprising:

(a) a first binding domain that binds to human and macaque Epidermal Growth Factor receptor VIII (EGFRVIII), and comprises: a heavy chain variable region (VH) comprising the amino acid sequence of SEQ ID NO: 157, and a light chain variable region (VL) comprising the amino acid sequence of SEQ ID NO: 158; and

(b) a second binding domain that binds to human CD3, and comprises: a VL region comprising: a CDR-L1 comprising the amino acid of SEQ ID NO: 92, a CDR-L2 comprising the amino acid of SEQ ID NO:93, and a CDR-L3 comprising the amino acid of SEQ ID NO:94; and a VH region comprising: a CDR-H1 comprising the amino acid of SEQ ID NO: 95, a CDR-H2 comprising the amino acid of SEQ ID NO:96, and a CDR-H3 comprising the amino acid of SEQ ID NO:97.

7. The use of claim 6, wherein said polypeptide comprises (a) a first binding domain that binds to human and macaque EGFRVIII, and comprises: a VH comprising the amino acid sequence of SEQ ID NO: 157, and a VL comprising the amino acid sequence of SEQ ID NO: 158; and (b) a second binding domain which binds to human CD3, and comprises a VH comprising the amino acid sequence of SEQ ID NO: 98, and a VL comprising the amino acid sequence of SEQ ID NO: 99.

8. The use of claim 6 or 7, wherein said polypeptide comprises (a) a first domain comprising the amino acid sequence of SEQ ID NO:159; and (b) a second domain comprising the amino acid sequence of SEQ ID NO:100.

9. The use of any one of claims 6-8, wherein said polypeptide further comprises a His-tag comprising the amino acid sequence of SEQ ID NO: 10.

10. The use of any one of claims 6-8, wherein said polypeptide comprises the amino acid sequence as set forth in SEQ ID NO:189.

11. The use of any one of claims 6-8, wherein said polypeptide comprises the amino acid sequence as set forth in SEQ ID NO:190.

12. Use of a polypeptide in the manufacture of a medicament for treating glioblastoma or glioma, said polypeptide comprising an amino acid sequence of SEQ ID NO: 160.

13. The use of claim 12, wherein said polypeptide further comprises a His-tag comprising the amino acid sequence of SEQ ID NO: 10.

14. The use of claim 12 or 13, wherein said polypeptide comprises in the following order starting from the N-terminus: an amino acid sequence as set forth in SEQ ID NO: 160, and a His-tag as set forth in SEQ ID NO: 10.

15. The use of claim 12, wherein said polypeptide further comprises a single-chain Fc (scFc) comprising an amino acid sequence selected from any one of SEQ Nos. 181-188.

16. The use of claim 15, wherein said polypeptide comprises the amino acid sequence as set forth in SEQ ID NO:189.

17. The use of claim 15, wherein said polypeptide the amino acid sequence as set forth in SEQ ID NO:190.

18. The use of any one of claims 1-5, or the use of any one of claims 6-17, wherein said medicament is to be administered intravenously (IV).

19. The use of any one of claims 1-5, or the use of any one of claims 6-17, wherein said medicament is to be administered by continuous IV.