CLAIMS

1. A protein construct comprising a mutated Fab fragment consisting of:
- the VH and VL domains of an antibody of interest;
- a CH1 domain which is derived from the CH1 domain of an immunoglobulin by substitution of the leucine residue at position 124 of said CH1 domain with a glutamine and substitution of the serine residue at position 188 of said CH1 domain with a valine residue; and
- a CL domain which is derived from the CL domain of an immunoglobulin by substitution of the valine residue at position 133 of said CL domain with a threonine residue and substitution of the serine residue at position 176 of said CL domain with a valine residue,
wherein the sequence position numbers used herein for the CH1 and CL domains refer to Kabat numbering.

2. The protein construct of claim 1 that comprises, or consists of, a multispecific antigen-binding fragment comprising at least two Fab fragments with different CH1 and CL domains, wherein each Fab fragment recognizes a different epitope of interest, and said Fab fragments are tandemly arranged in any order, the C-terminal end of the CH1 domain of a first Fab fragment being linked to the N-terminal end of the VH domain of the following Fab fragment through a polypeptide linker,
wherein at least one Fab fragment consists of a mutated Fab fragment as defined in claim 1.

3. The protein construct of claim 2, wherein one Fab fragment consists of a mutated Fab fragment as defined in claim 1 and another Fab fragment consists of:

- the VH and VL domains of an antibody of interest;
- a CH1 domain which is derived from the CH1 domain of an immunoglobulin by substitution of the threonine residue at position 192 of said CH1 domain with an aspartic acid and
- a CL domain which is derived from the CL domain of an immunoglobulin by substitution of the asparagine residue at position 137 of said CL domain with a lysine residue and substitution of the serine residue at position 114 of said CL domain with an alanine residue

4. The protein construct of claim 2 or 3, that is a multispecific antibody having two identical antigen-binding arms, each consisting of a multispecific antigen-binding fragment as defined in claim 2.

5. The protein construct of claim 4, which has an immunoglobulin-like structure, comprising:
- two identical antigen-binding arms each consisting of a multispecific antigen-binding fragment as defined in claim 1;
- the dimerized CH2 and CH3 domains of an immunoglobulin;
- the hinge region of an IgA, IgG, or IgD, linking the C-terminal ends of CH1 domains of the antigen-binding arms to the N-terminal ends of the CH2 domains.

6. The protein construct of claim 5, which is preferably a bispecific antibody, and comprises at least two heavy chains and four light chains,

wherein each heavy chain comprises

a. a Fc region of an immunoglobulin comprising Hinge-CH2-CH3 domains,
b. which Fc region is linked to Fab heavy chain CH1-VH of antibody 1 (Ab1) by said Hinge domain,
c. which in turn is linked to Fab heavy chain CH1-VH of antibody 2 (Ab2) by a polypeptide linker sequence, and the polypeptide linker sequence links the N-terminus of said Fab heavy chain VH domain of Ab1 with the C-terminus of said CH1 domain of Ab2,

and the four light chains comprise Fab light chains CL-VL of Ab1 and Fab light chains CL-VL of Ab2 associated with their cognate heavy chain domains;

wherein Ab1 and Ab2 recognize different epitopes,

wherein the Fab CH1 domain of one or the other of Ab1 or Ab2 is a mutated domain that derives from the CH1 domain of an immunoglobulin by substitution of the leucine residue at position 124 of said CH1 domain with a glutamine and substitution of the serine residue at position 188 of said CH1 domain with a valine residue, and the cognate CL domain is a mutated domain that derives from the CL domain of an immunoglobulin by substitution of the valine residue at position 133 of said CL domain with a threonine residue and substitution of the serine residue at position 176 of said CL domain with a valine residue,

wherein the sequence position numbers used herein for the CH1 and CL domains refer to Kabat numbering.

7. The protein construct of claim 6, wherein the Fab CH1 domain of one of Ab1 or Ab2 is a mutated domain that derives from the CH1 domain of an immunoglobulin by substitution of the threonine residue at position 192 of said CH1 domain with an aspartic acid and the cognate CL domain is a mutated domain that derives from the CL domain of an immunoglobulin by substitution of the asparagine residue at position 137 of said CL domain

with a lysine residue and substitution of the serine residue at position 114 of said CL domain with an alanine residue,
wherein the sequence position numbers used herein for the CH1 and CL domains refer to Kabat numbering.

8. The protein construct of claim 7, wherein the Fab CH1 domain of Ab1 is a mutated domain that derives from the CH1 domain of an immunoglobulin by substitution of the threonine residue at position 192 of said CH1 domain with an aspartic acid and the cognate CL domain is a mutated domain that derives from the CL domain of an immunoglobulin by substitution of the asparagine residue at position 137 of said CL domain with a lysine residue and substitution of the serine residue at position 114 of said CL domain with an alanine residue, and wherein the Fab CH1 domain of Ab2 is a mutated domain that derives from the CH1 domain of an immunoglobulin by substitution of the leucine residue at position 124 of said CH1 domain with a glutamine and substitution of the serine residue at position 188 of said CH1 domain with a valine residue, and the cognate CL domain is a mutated domain that derives from the CL domain of an immunoglobulin by substitution of the valine residue at position 133 of said CL domain with a threonine residue and substitution of the serine residue at position 176 of said CL domain with a valine residue.

9. The protein construct of claim 7, wherein the Fab CH1 domain of Ab2 is a mutated domain that derives from the CH1 domain of an immunoglobulin by substitution of the threonine residue at position 192 of said CH1 domain with an aspartic acid and the cognate CL domain is a mutated domain that derives from the CL domain of an immunoglobulin by substitution of the asparagine residue at position 137 of said CL domain with a lysine residue and substitution of the serine residue at position 114 of said CL domain with an alanine residue, and wherein the Fab CH1 domain of Ab1 is a mutated domain that derives from the CH1 domain of an immunoglobulin by substitution of the leucine residue at position 124 of said CH1 domain with a glutamine and substitution of the serine residue at position 188 of said CH1 domain with a valine residue, and the cognate CL domain is a mutated domain that derives from the CL domain of an immunoglobulin by substitution of the valine residue at position 133 of said CL domain with a threonine residue and substitution of the serine residue at position 176 of said CL domain with a valine residue.

10. The protein construct of any of claims 2 to 9, wherein the polypeptide linker sequence comprises or consists of amino acid sequence

$EPKX_1CDKX_2HX_3X_4PPX_5PAPELLGGPX_6X_7PPX_8PX_9PX_{10}GG$ (SEQ ID NO:1), wherein X_1, X_2, X_3, X_4, X_5, X_6, X_7, X_8, X_9, X_{10}, identical or different, are any amino acid.

11. The protein construct of claim 10, wherein the polypeptide linker sequence comprises or consists of a sequence selected from the group consisting of

EPKSCDKTHTSPPAPAPELLGGPGGPPGPGPGGG (SEQ ID NO: 2);
EPKSCDKTHTSPPAPAPELLGGPAAPPAPAPAGG (SEQ ID NO: 3);
EPKSCDKTHTSPPAPAPELLGGPAAPPGPAPGGG (SEQ ID NO: 4);
EPKSCDKTHTCPPCPAPELLGGPSTPPTPSPSGG (SEQ ID NO:5) and
EPKSCDKTHTSPPSPAPELLGGPSTPPTPSPSGG (SEQ ID NO:6).

12. The protein construct of any of claims 1 to 11, which recognizes both EGFR and HER2/neu.

13. The protein construct of claim 12, which is a multispecific antibody, preferably a bispecific antibody, wherein Ab1 and Ab2, being different, independently are selected from the group consisting of cetuximab or a mutated derivative thereof having at least 90% sequence identity with the native sequence of cetuximab, on the one hand, and trastuzumab, or a mutated derivative thereof having at least 90% sequence identity with the native sequence of trastuzumab, on the other hand.

14. The protein construct of any of claims 1 to 11, which recognizes both CD38 and PD-L1.

15. The protein construct of claim 14, which is a multispecific antibody, preferably a bispecific antibody, wherein Ab1 and Ab2, being different, independently are selected from the group consisting of daratumumab or a mutated derivative thereof having at least 90% sequence identity with the native sequence of daratumumab, on the one hand, and atezolizumab, or a mutated derivative thereof having at least 90% sequence identity with the native sequence of atezolizumab, on the other hand.

16. The protein construct of claim 14, which is a bispecific antibody which comprises, preferably consists of, a) two heavy chains, each comprising, preferably consisting of, SEQ ID NO:7 and b) four light chains, two comprising, preferably consisting of, SEQ ID NO:15, the two others comprising, preferably consisting of, SEQ ID NO: 18.

17. A polypeptide which comprises, preferably consists of, a heavy chain of the antigen-binding fragment or a heavy chain of the multispecific antibody as defined in any of claims 2 to 16.

18. A polynucleotide comprising a sequence encoding the polypeptide of claim 17.

19. A host cell transfected with an expression vector comprising the polynucleotide of claim 18.

20. The host cell of claim 19, further transformed with at least two polynucleotides encoding two different light chains: a first light chain pairing specifically with a first VH/CH1 region of said heavy chain; a second light chain pairing specifically with a second VH/CH1 region of said heavy chain.

21. A method for producing an antigen-binding fragment or a multispecific antibody as defined in any of claims 2 to 16, said method comprising the following steps: a) culturing in suitable medium and culture conditions a host cell expressing an antibody heavy chain as defined in any of claims 2 to 16, and an antibody light chain as defined in any of claims any of claims 2 to 16; and b) recovering said produced antibodies from the culture medium or from said cultured cells.

22. The multispecific antigen-binding fragment or the multispecific antibody as defined in any of claims 2 to 16, for use as a medicament.