

of the three expression vectors was 1:1:1. In Example 36 the preparation of DP47 GS TCB is described by co-transfecting HEK293-EBNA cells with the corresponding expression vectors in a 1:2:1:1 ratio ("vector heavy chain Fc(hole)" : "vector light chain" : "vector light chain CrossFab" : "vector heavy chain Fc(knob)- FabCrossFab"). A similar disclosure is provided in WO 2017/055389 and WO 2016/020309.

WO 2014/033074 discloses a blood brain barrier shuttle. In Example 2 the transient production of a trivalent MAb31-scFab(8D3) is disclosed using three expression plasmids at equimolar plasmid ratio upon transfection.

WO 2017/184831 allegedly discloses site-specific integration and expression of recombinant proteins in eukaryotic cells, especially methods for improved expression of antibodies including bispecific antibodies in eukaryotic cells, particularly Chinese hamster (*Cricetulus griseus*) cell lines, by employing an expression-enhancing locus. The data in this document is presented in an anonymized way, thus, not allowing a conclusion what has actually been shown.

Rajendra, Y., et al. discloses that a single quad vector is a simple, yet effective, alternative approach for generation of stable CHO cell lines and may accelerate cell line generation for clinical hetero-mAb therapeutics (Biotechnol. Prog. 33 (2017) 469-477).

Summary

Herein is reported a recombinant mammalian cell expressing a trivalent antibody, especially a bispecific, trivalent antibodies, such as a T-cell bispecific antibody (TCB). A trivalent antibody is a heteromultimeric polypeptide not naturally expressed by said mammalian cell. More specifically, a trivalent antibody is a heteromultimeric protein consisting of four polypeptides or polypeptide chains: one light chain, which is a full length light chain; a further light chain, which is a domain exchanged light chain; one heavy chain, which is a full length heavy chain; and a further heavy chain, which is an extended heavy chain comprising an addition domain exchanged heavy or light chain Fab fragment. To achieve expression of a trivalent antibody a recombinant nucleic acid comprising multiple different expression cassettes in a specific and defined sequence has been integrated into the genome of a mammalian cell.

Especially, the method according to the current disclosure can be used for the generation of T-cell bispecific antibodies (TCBs). These can have a format as described, e.g. in WO 2013/026831. Those molecules can simultaneously bind to CD3 (first specificity) on T-cells and to an antigen on a target (e.g. tumor) cell (second specificity) and thereby induce killing of target cells.

Herein is also reported a method for generating a recombinant mammalian cell expressing a trivalent antibody, especially a trivalent, bispecific antibody, more specifically a TCB and a method for producing a trivalent antibody, especially a trivalent, bispecific antibody, more specifically a TCB using said recombinant mammalian cell.

In one preferred example the bispecific, trivalent antibody comprises

- a) a first and a second Fab fragment that each specifically bind to a first antigen,
- b) one domain exchanged Fab fragment that specifically binds to a second antigen in which the CH1 and the CL domain are exchanged for each other,
- c) one Fc-region comprising a first heavy chain Fc-region polypeptide and a second heavy chain Fc-region polypeptide,

wherein the C-terminus of CH1 domain of the first Fab fragment is connected to the N-terminus of one of the heavy chain Fc-region polypeptides and the C-terminus of the CL-domain of the domain exchanged Fab fragment is connected to the N-terminus of the other heavy chain Fc-region polypeptide, and

wherein the C-terminus of the CH1 domain of the second Fab fragment is connected to the N-terminus of the VH domain of the first Fab fragment or to the N-terminus of the VH domain of the domain exchanged Fab fragment, and

wherein the first antigen or the second antigen is human CD3.

In one preferred example the bispecific, trivalent antibody comprises

- a) a first and a second Fab fragment that each specifically bind to a first antigen,

b) one domain exchanged Fab fragment that specifically binds to a second antigen in which the VH and the VL domain are exchanged for each other,

c) one Fc-region comprising a first heavy chain Fc-region polypeptide and a second heavy chain Fc-region polypeptide,

5 wherein the C-terminus of CH1 domain of the first Fab fragment is connected to the N-terminus of one of the heavy chain Fc-region polypeptides and the C-terminus of the CH1-domain of the domain exchanged Fab fragment is connected to the N-terminus of the other heavy chain Fc-region polypeptide, and

10 wherein the C-terminus of the CH1 domain of the second Fab fragment is connected to the N-terminus of the VH domain of the first Fab fragment or to the N-terminus of the VL domain of the domain exchanged Fab fragment, and

wherein the first antigen or the second antigen is human CD3.

15 In one preferred example none of the first light chain and the second light chain of the trivalent, bispecific antibody is a common light chain or a universal light chain.

The current disclosure is based, at least in part, on the finding that the sequence of the different expression cassettes required for the expression of the heteromultimeric, trivalent antibody, i.e. the expression cassette organization, as integrated into the genome of a mammalian cell influences the expression yield of the trivalent antibody (e.g. of a TCB).

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The current disclosure is based, at least in part, on the finding that by integrating a nucleic acid encoding the heteromultimeric, trivalent antibody (e.g. a TCB) that has a specific expression cassette organization into the genome of a mammalian cell efficient recombinant expression and production of the trivalent antibody (e.g. of a TCB) can be achieved.

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It has been found that the defined expression cassette sequence can advantageously be integrated into the genome of a mammalian cell by a double recombinase mediated cassette exchange reaction.

The current disclosure provides a method for producing a trivalent antibody (e.g. a TCB) comprising the steps of

30

- a first expression cassette encoding the first heavy chain,
- a second expression cassette encoding the first light chain,
- a third expression cassette encoding the second light chain,
- a fourth expression cassette encoding the second heavy chain,
- 5 - a fifth expression cassette encoding the second light chain, and
- a sixth expression cassette encoding the second light chain.

One aspect according to the current disclosure is a method for producing a trivalent antibody comprising the steps of

- 10 a) cultivating a mammalian cell comprising a deoxyribonucleic acid encoding the trivalent antibody, and

- b) recovering the trivalent antibody from the cell or the cultivation medium,

wherein the deoxyribonucleic acid encoding the trivalent antibody is stably integrated into the genome of the mammalian cell and comprises in 5'- to 3'- direction

15 either (1)

- a first expression cassette encoding the first heavy chain,
- a second expression cassette encoding the first light chain,
- a third expression cassette encoding the first light chain,
- a fourth expression cassette encoding the second heavy chain, and
- 20 - a fifth expression cassette encoding the second light chain,

or (2)

- a first expression cassette encoding the first heavy chain,
- a second expression cassette encoding the first light chain,
- a third expression cassette encoding the first light chain,
- 25 - a fourth expression cassette encoding the second heavy chain,
- a fifth expression cassette encoding the second light chain,
- a sixth expression cassette encoding the second light chain,

or (3)

- a first expression cassette encoding the first heavy chain,
- 30 - a second expression cassette encoding the second heavy chain,
- a third expression cassette encoding the first light chain,

- a fourth expression cassette encoding the second light chain,
- a fifth expression cassette encoding the second light chain,

or (4)

- a first expression cassette encoding the first heavy chain,
- a second expression cassette encoding the second heavy chain,
- a third expression cassette encoding the first light chain,
- a fourth expression cassette encoding the second light chain,
- a fifth expression cassette encoding the first heavy chain,
- a sixth expression cassette encoding the second light chain,

or (5)

- a first expression cassette encoding the first heavy chain,
- a second expression cassette encoding the second heavy chain,
- a third expression cassette encoding the first light chain,
- a fourth expression cassette encoding the second light chain,
- a fifth expression cassette encoding the second heavy chain,
- a sixth expression cassette encoding the second light chain,
- a seventh expression cassette encoding the second light chain,

or (6)

- a first expression cassette encoding the first light chain,
- a second expression cassette encoding the first light chain,
- a third expression cassette encoding the first heavy chain,
- a fourth expression cassette encoding the second heavy chain,
- a fifth expression cassette encoding the second light chain,
- a sixth expression cassette encoding the second light chain.

In one preferred example the first heavy chain comprises in the CH3 domain the mutation T366W (numbering according to Kabat) and the second heavy chain comprises in the CH3 domain the mutations T366S, L368A, and Y407V, or vice versa (numbering according to Kabat). In one example one of the heavy chains further comprises the mutation S354C and the respective other heavy chain comprises the mutation Y349C (numbering according to Kabat). In one example the first heavy chain is an extended heavy chain comprising an additional domain exchanged Fab fragment. In one example the first light chain is a domain exchanged light chain.

In one example the deoxyribonucleic acid comprises a further expression cassette between the first and the second expression cassette encoding the second heavy chain.

In one example

- 5 - the first heavy chain comprises from N- to C-terminus a first heavy chain variable domain, a CH1 domain, a first light chain variable domain, a CH1 domain, a hinge region, a CH2 domain and a CH3 domain,
- the second heavy chain comprises from N- to C-terminus the first heavy chain variable domain, a CH1 domain, a hinge region, a CH2 domain
10 and a CH3 domain,
- the first light chain comprises from N- to C-terminus a second heavy chain variable domain and a CL domain, and
- the second light chain comprises from N- to C- terminus a second light chain variable domain and a CL domain,
- 15 wherein the first heavy chain variable domain and the second light chain variable domain form a first binding site and the second heavy chain variable domain and the first light chain variable domain form a second binding site.

In one example

- the first heavy chain comprises from N- to C-terminus a first heavy chain variable domain, a CH1 domain, a second heavy chain variable domain, a CL domain, a hinge region, a CH2 domain and a CH3 domain,
- 5 - the second heavy chain comprises from N- to C-terminus the first heavy chain variable domain, a CH1 domain, a hinge region, a CH2 domain and a CH3 domain,
- the first light chain comprises from N- to C-terminus a first light chain variable domain and a CH1 domain, and
- 10 - the second light chain comprises from N- to C- terminus a second light chain variable domain and a CL domain,

wherein the first heavy chain variable domain and the second light chain variable domain form a first binding site and the second heavy chain variable domain and the first light chain variable domain form a second binding site.

- 15 In one example the deoxyribonucleic acid is stably integrated into the genome of the mammalian cell at a single site or locus.

The current disclosure provides a deoxyribonucleic acid encoding a trivalent antibody (e.g. a TCB) comprising in 5'- to 3'-direction

either

- 20 1)
- a first expression cassette encoding the first heavy chain,
 - a second expression cassette encoding the first light chain,
 - a third expression cassette encoding the first light chain,
 - a fourth expression cassette encoding the second heavy chain,
 - 25 - a fifth expression cassette encoding the second light chain, and
 - optionally a sixth expression cassette encoding the second light chain,
- or
- 2)
- a first expression cassette encoding the first light chain,
 - 30 - a second expression cassette encoding the first light chain,
 - a third expression cassette encoding the first heavy chain,
 - a fourth expression cassette encoding the second heavy chain,

- a fifth expression cassette encoding the second light chain, and
 - a sixth expression cassette encoding the second light chain,
- wherein the first to third expression cassettes are arranged unidirectional, the fourth to sixth expression cassettes are arranged unidirectional and in opposite direction as the first to third expression cassette;

5

or

3)

- a first expression cassette encoding the first heavy chain,
- a second expression cassette encoding the first light chain,
- a third expression cassette encoding the second light chain,
- a fourth expression cassette encoding the second heavy chain,
- a fifth expression cassette encoding the second light chain, and
- a sixth expression cassette encoding the first light chain,

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or

- a first expression cassette encoding the first heavy chain,
- a second expression cassette encoding the first light chain,
- a third expression cassette encoding the second light chain,
- a fourth expression cassette encoding the second heavy chain,
- a fifth expression cassette encoding the second light chain, and
- a sixth expression cassette encoding the second light chain.

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Another aspect of the current disclosure is a deoxyribonucleic acid encoding a trivalent antibody comprising in 5' - to 3'-direction

either (1)

- a first expression cassette encoding the first heavy chain,
- a second expression cassette encoding the first light chain,
- a third expression cassette encoding the first light chain,
- a fourth expression cassette encoding the second heavy chain, and
- a fifth expression cassette encoding the second light chain,

25

or (2)

- a first expression cassette encoding the first heavy chain,
- a second expression cassette encoding the first light chain,
- a third expression cassette encoding the first light chain,
- 5 - a fourth expression cassette encoding the second heavy chain,
- a fifth expression cassette encoding the second light chain,
- a sixth expression cassette encoding the second light chain,

or (3)

- a first expression cassette encoding the first heavy chain,
- 10 - a second expression cassette encoding the second heavy chain,
- a third expression cassette encoding the first light chain,
- a fourth expression cassette encoding the second light chain,
- a fifth expression cassette encoding the second light chain,

or (4)

- 15 - a first expression cassette encoding the first heavy chain,
- a second expression cassette encoding the second heavy chain,
- a third expression cassette encoding the first light chain,
- a fourth expression cassette encoding the second light chain,
- a fifth expression cassette encoding the first heavy chain,
- 20 - a sixth expression cassette encoding the second light chain,

or (5)

- a first expression cassette encoding the first heavy chain,
- a second expression cassette encoding the second heavy chain,
- a third expression cassette encoding the first light chain,
- 25 - a fourth expression cassette encoding the second light chain,
- a fifth expression cassette encoding the second heavy chain,
- a sixth expression cassette encoding the second light chain,
- a seventh expression cassette encoding the second light chain,

or (6)

- a first expression cassette encoding the first light chain,
- a second expression cassette encoding the first light chain,
- a third expression cassette encoding the first heavy chain,
- 5 - a fourth expression cassette encoding the second heavy chain,
- a fifth expression cassette encoding the second light chain,
- a sixth expression cassette encoding the second light chain.

10 In one preferred example the first heavy chain comprises in the CH3 domain the mutation T366W (numbering according to Kabat) and the second heavy chain comprises in the CH3 domain the mutations T366S, L368A, and Y407V, or vice versa (numbering according to Kabat). In one example one of the heavy chains further comprises the mutation S354C and the respective other heavy chain comprises the mutation Y349C (numbering according to Kabat). In one example the first heavy chain is an extended heavy chain comprising an additional domain
15 exchanged Fab fragment. In one example the first light chain is a domain exchanged light chain.

In one example the deoxyribonucleic acid comprises a further expression cassette between the first and the second expression cassette encoding the second heavy chain.

20 In one example

- the first heavy chain comprises from N- to C-terminus a first heavy chain variable domain, a CH1 domain, a first light chain variable domain, a CH1 domain, a hinge region, a CH2 domain and a CH3 domain,
- 5 - the second heavy chain comprises from N- to C-terminus the first heavy chain variable domain, a CH1 domain, a hinge region, a CH2 domain and a CH3 domain,
- the first light chain comprises from N- to C-terminus a second heavy chain variable domain and a CL domain, and
- 10 - the second light chain comprises from N- to C- terminus a second light chain variable domain and a CL domain,

wherein the first heavy chain variable domain and the second light chain variable domain form a first binding site and the second heavy chain variable domain and the first light chain variable domain form a second binding site.

In one example

- 15 - the first heavy chain comprises from N- to C-terminus a first heavy chain variable domain, a CH1 domain, a second heavy chain variable domain, a CL domain, a hinge region, a CH2 domain and a CH3 domain,
- the second heavy chain comprises from N- to C-terminus the first heavy chain variable domain, a CH1 domain, a hinge region, a CH2 domain
- 20 and a CH3 domain,
- the first light chain comprises from N- to C-terminus a first light chain variable domain and a CH1 domain, and
- the second light chain comprises from N- to C- terminus a second light chain variable domain and a CL domain,

25 wherein the first heavy chain variable domain and the second light chain variable domain form a first binding site and the second heavy chain variable domain and the first light chain variable domain form a second binding site.

The current disclosure provides the use of a deoxyribonucleic acid comprising in 5'-to 3'-direction

30 either

1)

- a first expression cassette encoding the first heavy chain,
- a second expression cassette encoding the first light chain,
- a third expression cassette encoding the first light chain,
- 5 - a fourth expression cassette encoding the second heavy chain,
- a fifth expression cassette encoding the second light chain, and
- optionally a sixth expression cassette encoding the second light chain,

or

2)

- 10 - a first expression cassette encoding the first light chain,
- a second expression cassette encoding the first light chain,
- a third expression cassette encoding the first heavy chain,
- a fourth expression cassette encoding the second heavy chain,
- a fifth expression cassette encoding the second light chain, and
- 15 - a sixth expression cassette encoding the second light chain,

wherein the first to third expression cassettes are arranged unidirectional, the fourth to sixth expression cassettes are arranged unidirectional and in opposite direction as the first to third expression cassette;

or

3)

- 20 - a first expression cassette encoding the first heavy chain,
- a second expression cassette encoding the first light chain,
- a third expression cassette encoding the second light chain,
- a fourth expression cassette encoding the second heavy chain,
- 25 - a fifth expression cassette encoding the second light chain, and
- a sixth expression cassette encoding the first light chain,

or

- a first expression cassette encoding the first heavy chain,
- a second expression cassette encoding the first light chain,
- 30 - a third expression cassette encoding the second light chain,
- a fourth expression cassette encoding the second heavy chain,
- a fifth expression cassette encoding the second light chain, and
- a sixth expression cassette encoding the second light chain,

for the expression of the trivalent antibody (e.g. a TCB) in a mammalian cell.

Another aspect of the current disclosure is the use of a deoxyribonucleic acid comprising in 5'- to 3'-direction

either (1)

- 5 - a first expression cassette encoding the first heavy chain,
- a second expression cassette encoding the first light chain,
- a third expression cassette encoding the first light chain,
- a fourth expression cassette encoding the second heavy chain, and
- a fifth expression cassette encoding the second light chain,

or (2)

- 10 - a first expression cassette encoding the first heavy chain,
- a second expression cassette encoding the first light chain,
- a third expression cassette encoding the first light chain,
- a fourth expression cassette encoding the second heavy chain,
- a fifth expression cassette encoding the second light chain,
- 15 - a sixth expression cassette encoding the second light chain,

or (3)

- a first expression cassette encoding the first heavy chain,
- a second expression cassette encoding the second heavy chain,
- a third expression cassette encoding the first light chain,
- 20 - a fourth expression cassette encoding the second light chain,
- a fifth expression cassette encoding the second light chain,

or (4)

- a first expression cassette encoding the first heavy chain,
- a second expression cassette encoding the second heavy chain,
- 25 - a third expression cassette encoding the first light chain,
- a fourth expression cassette encoding the second light chain,
- a fifth expression cassette encoding the first heavy chain,
- a sixth expression cassette encoding the second light chain,

or (5)

- a first expression cassette encoding the first heavy chain,
- a second expression cassette encoding the second heavy chain,
- a third expression cassette encoding the first light chain,
- 5 - a fourth expression cassette encoding the second light chain,
- a fifth expression cassette encoding the second heavy chain,
- a sixth expression cassette encoding the second light chain,
- a seventh expression cassette encoding the second light chain,

or (6)

- 10 - a first expression cassette encoding the first light chain,
- a second expression cassette encoding the first light chain,
- a third expression cassette encoding the first heavy chain,
- a fourth expression cassette encoding the second heavy chain,
- a fifth expression cassette encoding the second light chain,
- 15 - a sixth expression cassette encoding the second light chain,

for the expression of the trivalent antibody in a mammalian cell.

In one preferred example the first heavy chain comprises in the CH3 domain the mutation T366W (numbering according to Kabat) and the second heavy chain

comprises in the CH3 domain the mutations T366S, L368A, and Y407V, or vice versa (numbering according to Kabat). In one example one of the heavy chains further comprises the mutation S354C and the respective other heavy chain comprises the mutation Y349C (numbering according to Kabat). In one example the first heavy chain is an extended heavy chain comprising an additional domain exchanged Fab fragment. In one example the first light chain is a domain exchanged light chain.

In one example the deoxyribonucleic acid comprises a further expression cassette between the first and the second expression cassette encoding the second heavy chain.

In one example

- the first heavy chain comprises from N- to C-terminus a first heavy chain variable domain, a CH1 domain, a first light chain variable domain, a CH1 domain, a hinge region, a CH2 domain and a CH3 domain,
- the second heavy chain comprises from N- to C-terminus the first heavy chain variable domain, a CH1 domain, a hinge region, a CH2 domain and a CH3 domain,
- the first light chain comprises from N- to C-terminus a second heavy chain variable domain and a CL domain, and
- the second light chain comprises from N- to C- terminus a second light chain variable domain and a CL domain,

wherein the first heavy chain variable domain and the second light chain variable domain form a first binding site and the second heavy chain variable domain and the first light chain variable domain form a second binding site.

In one example

- the first heavy chain comprises from N- to C-terminus a first heavy chain variable domain, a CH1 domain, a second heavy chain variable domain, a CL domain, a hinge region, a CH2 domain and a CH3 domain,

- the second heavy chain comprises from N- to C-terminus the first heavy chain variable domain, a CH1 domain, a hinge region, a CH2 domain and a CH3 domain,
- the first light chain comprises from N- to C-terminus a first light chain variable domain and a CH1 domain, and
- the second light chain comprises from N- to C- terminus a second light chain variable domain and a CL domain,

wherein the first heavy chain variable domain and the second light chain variable domain form a first binding site and the second heavy chain variable domain and the first light chain variable domain form a second binding site.

In one example of the use the deoxyribonucleic acid is integrated into the genome of the mammalian cell.

In one example of the use the deoxyribonucleic acid is stably integrated into the genome of the mammalian cell at a single site or locus.

The disclosure provides a recombinant mammalian cell comprising a deoxyribonucleic acid encoding a trivalent antibody (e.g. a TCB) integrated in the genome of the cell,

wherein the deoxyribonucleic acid encoding the trivalent antibody (e.g. a TCB) comprises in 5'- to 3'-direction

either

1)

- a first expression cassette encoding the first heavy chain,
- a second expression cassette encoding the first light chain,
- a third expression cassette encoding the first light chain,
- a fourth expression cassette encoding the second heavy chain,
- a fifth expression cassette encoding the second light chain, and
- optionally, a sixth expression cassette encoding the second light chain,

or

2)

- a first expression cassette encoding the first light chain,
- a second expression cassette encoding the first light chain,
- a third expression cassette encoding the first heavy chain,

- a fourth expression cassette encoding the second heavy chain,
- a fifth expression cassette encoding the second light chain, and
- a sixth expression cassette encoding the second light chain,

wherein the first to third expression cassettes are arranged unidirectional, the fourth to sixth expression cassettes are arranged unidirectional and in opposite direction as the first to third expression cassette;

or

3)

- a first expression cassette encoding the first heavy chain,
- a second expression cassette encoding the first light chain,
- a third expression cassette encoding the second light chain,
- a fourth expression cassette encoding the second heavy chain,
- a fifth expression cassette encoding the second light chain, and
- a sixth expression cassette encoding the first light chain,

or

- a first expression cassette encoding the first heavy chain,
- a second expression cassette encoding the first light chain,
- a third expression cassette encoding the second light chain,
- a fourth expression cassette encoding the second heavy chain,
- a fifth expression cassette encoding the second light chain, and
- a sixth expression cassette encoding the second light chain.

Another aspect of the disclosure is a recombinant mammalian cell comprising a deoxyribonucleic acid encoding a trivalent antibody integrated in the genome of the cell, wherein the deoxyribonucleic acid encoding the trivalent antibody comprises in 5'- to 3'-direction

either (1)

- a first expression cassette encoding the first heavy chain,
- a second expression cassette encoding the first light chain,
- a third expression cassette encoding the first light chain,
- a fourth expression cassette encoding the second heavy chain, and
- a fifth expression cassette encoding the second light chain,

or (2)

- a first expression cassette encoding the first heavy chain,
- a second expression cassette encoding the first light chain,
- a third expression cassette encoding the first light chain,
- 5 - a fourth expression cassette encoding the second heavy chain,
- a fifth expression cassette encoding the second light chain,
- a sixth expression cassette encoding the second light chain,

or (3)

- a first expression cassette encoding the first heavy chain,
- 10 - a second expression cassette encoding the second heavy chain,
- a third expression cassette encoding the first light chain,
- a fourth expression cassette encoding the second light chain,
- a fifth expression cassette encoding the second light chain,

or (4)

- 15 - a first expression cassette encoding the first heavy chain,
- a second expression cassette encoding the second heavy chain,
- a third expression cassette encoding the first light chain,
- a fourth expression cassette encoding the second light chain,
- a fifth expression cassette encoding the first heavy chain,
- 20 - a sixth expression cassette encoding the second light chain,

or (5)

- a first expression cassette encoding the first heavy chain,
- a second expression cassette encoding the second heavy chain,
- a third expression cassette encoding the first light chain,
- 25 - a fourth expression cassette encoding the second light chain,
- a fifth expression cassette encoding the second heavy chain,
- a sixth expression cassette encoding the second light chain,
- a seventh expression cassette encoding the second light chain,

or (6)

- a first expression cassette encoding the first light chain,
- a second expression cassette encoding the first light chain,
- a third expression cassette encoding the first heavy chain,
- 5 - a fourth expression cassette encoding the second heavy chain,
- a fifth expression cassette encoding the second light chain,
- a sixth expression cassette encoding the second light chain.

10 In one preferred example the first heavy chain comprises in the CH3 domain the mutation T366W (numbering according to Kabat) and the second heavy chain comprises in the CH3 domain the mutations T366S, L368A, and Y407V, or vice versa (numbering according to Kabat). In one example one of the heavy chains further comprises the mutation S354C and the respective other heavy chain comprises the mutation Y349C (numbering according to Kabat). In one example the first heavy chain is an extended heavy chain comprising an additional domain
15 exchanged Fab fragment. In one example the first light chain is a domain exchanged light chain.

In one example the deoxyribonucleic acid comprises a further expression cassette between the first and the second expression cassette encoding the second heavy chain.

20 In one example of the above

- the first heavy chain comprises from N- to C-terminus a first heavy chain variable domain, a CH1 domain, a first light chain variable domain, a CH1 domain, a hinge region, a CH2 domain and a CH3 domain,
- 5 - the second heavy chain comprises from N- to C-terminus the first heavy chain variable domain, a CH1 domain, a hinge region, a CH2 domain and a CH3 domain,
- the first light chain comprises from N- to C-terminus a second heavy chain variable domain and a CL domain, and
- 10 - the second light chain comprises from N- to C- terminus a second light chain variable domain and a CL domain,

wherein the first heavy chain variable domain and the second light chain variable domain form a first binding site and the second heavy chain variable domain and the first light chain variable domain form a second binding site.

In one example of the above

- 15 - the first heavy chain comprises from N- to C-terminus a first heavy chain variable domain, a CH1 domain, a second heavy chain variable domain, a CL domain, a hinge region, a CH2 domain and a CH3 domain,
- the second heavy chain comprises from N- to C-terminus the first heavy chain variable domain, a CH1 domain, a hinge region, a CH2 domain
- 20 and a CH3 domain,
- the first light chain comprises from N- to C-terminus a first light chain variable domain and a CH1 domain, and
- the second light chain comprises from N- to C- terminus a second light chain variable domain and a CL domain,

25 wherein the first heavy chain variable domain and the second light chain variable domain form a first binding site and the second heavy chain variable domain and the first light chain variable domain form a second binding site.

In one example the deoxyribonucleic acid is stably integrated into the genome of the mammalian cell at a single site or locus.

In one example of the above the deoxyribonucleic acid encoding the trivalent antibody (e.g. a TCB) further comprises

- a first recombination recognition sequence located 5' to the first (most 5') expression cassette,
- 5 - a second recombination recognition sequence located 3' to the sixth (most 3') expression cassette, and
- a third recombination recognition sequence located
 - between the first and the second recombination recognition sequence, and
 - 10 - between two of the expression cassettes,and

wherein all recombination recognition sequences are different.

In one example of the above the third recombination recognition sequence is located between the third and the fourth expression cassette.

- 15 In one example of the above the deoxyribonucleic acid encoding the trivalent antibody (e.g. a TCB) comprises a further expression cassette encoding for a selection marker and the expression cassette encoding for the selection marker is located partly 5' and partly 3' to the third recombination recognition sequence, wherein the 5'-located part of said expression cassette comprises the promoter and
- 20 the start-codon and the 3'-located part of said expression cassette comprises the coding sequence without a start-codon and a polyA signal, wherein the start-codon is operably linked to the coding sequence.

The current disclosure provides a composition comprising two deoxyribonucleic acids, which comprise in turn three different recombination recognition sequences

25 and six expression cassettes, wherein

- the first deoxyribonucleic acid comprises in 5'- to 3'-direction,
 - a first recombination recognition sequence,
 - a first expression cassette encoding the first heavy chain,
 - a second expression cassette encoding the first light chain,
 - 30 - a third expression cassette encoding the first light chain, and

- a first copy of a third recombination recognition sequence,

and

- the second deoxyribonucleic acid comprises in 5'- to 3'-direction
 - a second copy of the third recombination recognition sequence,
 - 5 - a fourth expression cassette encoding the second heavy chain,
 - a fifth expression cassette encoding the second light chain,
 - a sixth expression cassette encoding the second light chain, and
 - a second recombination recognition sequence.

10 Another aspect of the current disclosure is a composition comprising two deoxyribonucleic acids, which comprise in turn three different recombination recognition sequences and five to seven expression cassettes, wherein

- the first deoxyribonucleic acid comprises in 5'- to 3'-direction,
 - either (1)
 - a first recombination recognition sequence,
 - 15 - a first expression cassette encoding the first heavy chain,
 - a second expression cassette encoding the first light chain,
 - a third expression cassette encoding the first light chain, and
 - a first copy of a third recombination recognition sequence,
 - or (2)
 - 20 - a first recombination recognition sequence,
 - a first expression cassette encoding the first heavy chain,
 - a second expression cassette encoding the first light chain,
 - a third expression cassette encoding the first light chain, and
 - a first copy of a third recombination recognition sequence,
 - 25 or (3)
 - a first recombination recognition sequence,
 - a first expression cassette encoding the first heavy chain,
 - a second expression cassette encoding the second heavy chain,
 - a third expression cassette encoding the first light chain,
 - 30 - a fourth expression cassette encoding the second light chain, and
 - a first copy of a third recombination recognition sequence,

or (4)

- a first recombination recognition sequence,
- a first expression cassette encoding the first heavy chain,
- a second expression cassette encoding the second heavy chain,
- 5 - a third expression cassette encoding the first light chain,
- a fourth expression cassette encoding the second light chain, and
- a first copy of a third recombination recognition sequence,

or (5)

- a first recombination recognition sequence,
- 10 - a first expression cassette encoding the first heavy chain,
- a second expression cassette encoding the second heavy chain,
- a third expression cassette encoding the first light chain,
- a fourth expression cassette encoding the second light chain, and
- a first copy of a third recombination recognition sequence,

or (6)

- a first recombination recognition sequence,
- a first expression cassette encoding the first light chain,
- a second expression cassette encoding the first light chain,
- a third expression cassette encoding the first heavy chain, and
- 20 - a first copy of a third recombination recognition sequence,

and

- the second deoxyribonucleic acid comprises in 5'- to 3'-direction
either (1)

- a second copy of the third recombination recognition sequence,
- 25 - a fourth expression cassette encoding the second heavy chain,
- a fifth expression cassette encoding the second light chain, and
- a second recombination recognition sequence,

or (2)

- a second copy of the third recombination recognition sequence,
- 30 - a fourth expression cassette encoding the second heavy chain,
- a fifth expression cassette encoding the second light chain,
- a sixth expression cassette encoding the second light chain, and
- a second recombination recognition sequence,

or (3)

- a second copy of the third recombination recognition sequence,
- a fifth expression cassette encoding the second light chain, and
- a second recombination recognition sequence,

5

or (4)

- a second copy of the third recombination recognition sequence,
- a fifth expression cassette encoding the first heavy chain,
- a sixth expression cassette encoding the second light chain, and
- a second recombination recognition sequence,

10

or (5)

- a second copy of the third recombination recognition sequence,
- a fifth expression cassette encoding the second heavy chain,
- a sixth expression cassette encoding the second light chain,
- a seventh expression cassette encoding the second light chain, and
- a second recombination recognition sequence,

15

or (6)

- a second copy of the third recombination recognition sequence,
- a fourth expression cassette encoding the second heavy chain,
- a fifth expression cassette encoding the second light chain,
- a sixth expression cassette encoding the second light chain, and
- a second recombination recognition sequence.

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In one preferred example the first heavy chain comprises in the CH3 domain the mutation T366W (numbering according to Kabat) and the second heavy chain comprises in the CH3 domain the mutations T366S, L368A, and Y407V, or vice versa (numbering according to Kabat). In one example one of the heavy chains further comprises the mutation S354C and the respective other heavy chain comprises the mutation Y349C (numbering according to Kabat). In one example the first heavy chain is an extended heavy chain comprising an additional domain exchanged Fab fragment. In one example the first light chain is a domain exchanged light chain.

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In one example of the above

- the first heavy chain comprises from N- to C-terminus a first heavy chain variable domain, a CH1 domain, a first light chain variable domain, a CH1 domain, a hinge region, a CH2 domain and a CH3 domain,
- 5 - the second heavy chain comprises from N- to C-terminus the first heavy chain variable domain, a CH1 domain, a hinge region, a CH2 domain and a CH3 domain,
- the first light chain comprises from N- to C-terminus a second heavy chain variable domain and a CL domain, and
- 10 - the second light chain comprises from N- to C- terminus a second light chain variable domain and a CL domain,

wherein the first heavy chain variable domain and the second light chain variable domain form a first binding site and the second heavy chain variable domain and the first light chain variable domain form a second binding site.

In one example of the above

- the first heavy chain comprises from N- to C-terminus a first heavy chain variable domain, a CH1 domain, a second heavy chain variable domain, a CL domain, a hinge region, a CH2 domain and a CH3 domain,
- 5 - the second heavy chain comprises from N- to C-terminus the first heavy chain variable domain, a CH1 domain, a hinge region, a CH2 domain and a CH3 domain,
- the first light chain comprises from N- to C-terminus a first light chain variable domain and a CH1 domain, and
- 10 - the second light chain comprises from N- to C- terminus a second light chain variable domain and a CL domain,

wherein the first heavy chain variable domain and the second light chain variable domain form a first binding site and the second heavy chain variable domain and the first light chain variable domain form a second binding site.

- 15 In one example of the above the deoxyribonucleic acid encoding the trivalent antibody (e.g. a TCB) further comprises a further expression cassette encoding for a selection marker.

In one example of the above the expression cassette encoding for a selection marker is located either

- 20 i) 5', or
- ii) 3', or
- iii) partly 5' and partly 3'

to the third recombination recognition sequence.

- 25 In one example of the above the expression cassette encoding for a selection marker is located partly 5' and partly 3' to the third recombination recognition sequences, wherein the 5'-located part of said expression cassette comprises the promoter and a start-codon and the 3'-located part of said expression cassette comprises the coding sequence without a start-codon and a polyA signal.

In one example of the above the 5'-located part of the expression cassette encoding the selection marker comprises a promoter sequence operably linked to a start-codon, whereby the promoter sequence is flanked upstream by (i.e. is positioned downstream to) the third expression cassette and the start-codon is flanked downstream by (i.e. is positioned upstream of) the third recombination recognition sequence; and the 3'-located part of the expression cassette encoding the selection marker comprises a nucleic acid encoding the selection marker lacking a start-codon and is flanked upstream by the third recombination recognition sequence and downstream by the fourth expression cassette.

In one example of the above the start-codon is a transcription start-codon. In one example the start-codon is ATG.

The disclosure provides a recombinant mammalian cell comprising a deoxyribonucleic acid encoding a trivalent antibody (e.g. a TCB) integrated in the genome of the cell,

wherein the deoxyribonucleic acid encoding the trivalent antibody (e.g. a TCB) comprises the following elements:

a first, a second and a third recombination recognition sequence,
a first and a second selection marker, and
a first to sixth expression cassette,

wherein the sequences of said elements in 5'-to-3' direction is

RRS1-1st EC-2nd EC-3rd EC-RRS3-SM1-4th EC-5th EC-6th EC-RRS2

with

RRS = recombination recognition sequence,

EC = expression cassette,

SM = selection marker.

The current disclosure provides a method for producing a recombinant mammalian cell comprising a deoxyribonucleic acid encoding a trivalent antibody (e.g. a TCB) and secreting the trivalent antibody (e.g. a TCB) comprising the following steps:

a) providing a mammalian cell comprising an exogenous nucleotide sequence integrated at a single site within a locus of the genome of the mammalian

one or more recombinases,
wherein the one or more recombinases recognize the recombination
recognition sequences of the first and the second deoxyribonucleic acid;
(and optionally wherein the one or more recombinases perform two
5 recombinase mediated cassette exchanges;)

and

d) selecting for cells expressing the second selection marker and secreting the
trivalent antibody (e.g. a TCB),

10 thereby producing a recombinant mammalian cell comprising a deoxyribonucleic
acid encoding the trivalent antibody (e.g. a TCB) and secreting the trivalent
antibody (e.g. a TCB).

Another aspect of the current disclosure is a method for producing a recombinant
mammalian cell comprising a deoxyribonucleic acid encoding a trivalent antibody
and secreting the trivalent antibody, comprising the following steps:

15 a) providing a mammalian cell comprising an exogenous nucleotide sequence
integrated at a single site within a locus of the genome of the mammalian
cell, wherein the exogenous nucleotide sequence comprises a first and a
second recombination recognition sequence flanking at least one first
selection marker, and a third recombination recognition sequence located
20 between the first and the second recombination recognition sequence, and
all the recombination recognition sequences are different;

b) introducing into the cell provided in a) a composition of two
deoxyribonucleic acids comprising three different recombination
recognition sequences and five to seven expression cassettes, wherein

- 25 - the first deoxyribonucleic acid comprises in 5' - to 3' -direction,
either (1)
- a first recombination recognition sequence,
 - a first expression cassette encoding the first heavy chain,
 - a second expression cassette encoding the first light chain,
 - 30 - a third expression cassette encoding the first light chain, and
 - a first copy of a third recombination recognition sequence,

or (2)

- a first recombination recognition sequence,
- a first expression cassette encoding the first heavy chain,
- a second expression cassette encoding the first light chain,
- a third expression cassette encoding the first light chain, and
- a first copy of a third recombination recognition sequence,

or (3)

- a first recombination recognition sequence,
- a first expression cassette encoding the first heavy chain,
- a second expression cassette encoding the second heavy chain,
- a third expression cassette encoding the first light chain,
- a fourth expression cassette encoding the second light chain, and
- a first copy of a third recombination recognition sequence,

or (4)

- a first recombination recognition sequence,
- a first expression cassette encoding the first heavy chain,
- a second expression cassette encoding the second heavy chain,
- a third expression cassette encoding the first light chain,
- a fourth expression cassette encoding the second light chain, and
- a first copy of a third recombination recognition sequence,

or (5)

- a first recombination recognition sequence,
- a first expression cassette encoding the first heavy chain,
- a second expression cassette encoding the second heavy chain,
- a third expression cassette encoding the first light chain,
- a fourth expression cassette encoding the second light chain, and
- a first copy of a third recombination recognition sequence,

or (6)

- a first recombination recognition sequence,
- a first expression cassette encoding the first light chain,
- a second expression cassette encoding the first light chain,
- a third expression cassette encoding the first heavy chain, and
- a first copy of a third recombination recognition sequence,

and

- the second deoxyribonucleic acid comprises in 5' - to 3'-direction
either (1)

5

- a second copy of the third recombination recognition sequence,
- a fourth expression cassette encoding the second heavy chain,
- a fifth expression cassette encoding the second light chain, and
- a second recombination recognition sequence,

or (2)

10

- a second copy of the third recombination recognition sequence,
- a fourth expression cassette encoding the second heavy chain,
- a fifth expression cassette encoding the second light chain,
- a sixth expression cassette encoding the second light chain, and
- a second recombination recognition sequence,

or (3)

15

- a second copy of the third recombination recognition sequence,
- a fifth expression cassette encoding the second light chain, and
- a second recombination recognition sequence,

or (4)

20

- a second copy of the third recombination recognition sequence,
- a fifth expression cassette encoding the first heavy chain,
- a sixth expression cassette encoding the second light chain, and
- a second recombination recognition sequence,

or (5)

25

- a second copy of the third recombination recognition sequence,
- a fifth expression cassette encoding the second heavy chain,
- a sixth expression cassette encoding the second light chain,
- a seventh expression cassette encoding the second light chain,
and
- a second recombination recognition sequence,

30

or (6)

- a second copy of the third recombination recognition sequence,
- a fourth expression cassette encoding the second heavy chain,
- a fifth expression cassette encoding the second light chain,
- a sixth expression cassette encoding the second light chain, and
- a second recombination recognition sequence,

wherein the first to third recombination recognition sequences of the first and second deoxyribonucleic acids are matching the first to third recombination recognition sequence on the integrated exogenous nucleotide sequence,

wherein the 5'-terminal part and the 3'-terminal part of the expression cassette encoding the one second selection marker when taken together form a functional expression cassette of the one second selection marker;

c) introducing

- i) either simultaneously with the first and second deoxyribonucleic acid of b);

or

- ii) sequentially thereafter

one or more recombinases,

wherein the one or more recombinases recognize the recombination recognition sequences of the first and the second deoxyribonucleic acid; (and optionally wherein the one or more recombinases perform two recombinase mediated cassette exchanges;)

and

- d) selecting for cells expressing the second selection marker and secreting the trivalent antibody,

thereby producing a recombinant mammalian cell comprising a deoxyribonucleic acid encoding the trivalent antibody and secreting the trivalent antibody.

5 In one preferred example of the above the first heavy chain comprises in the CH3 domain the mutation T366W (numbering according to Kabat) and the second heavy chain comprises in the CH3 domain the mutations T366S, L368A, and Y407V, or vice versa (numbering according to Kabat). In one example one of the heavy chains further comprises the mutation S354C and the respective other heavy chain comprises the mutation Y349C (numbering according to Kabat). In one example the first heavy chain is an extended heavy chain comprising an additional domain exchanged Fab fragment.

In one example of the above the first light chain is a domain exchanged light chain.

10 In one example of the above

- the first heavy chain comprises from N- to C-terminus a first heavy chain variable domain, a CH1 domain, a first light chain variable domain, a CH1 domain, a hinge region, a CH2 domain and a CH3 domain,
- 15 - the second heavy chain comprises from N- to C-terminus the first heavy chain variable domain, a CH1 domain, a hinge region, a CH2 domain and a CH3 domain,
- the first light chain comprises from N- to C-terminus a second heavy chain variable domain and a CL domain, and

- the second light chain comprises from N- to C- terminus a second light chain variable domain and a CL domain,

wherein the first heavy chain variable domain and the second light chain variable domain form a first binding site and the second heavy chain variable domain and the first light chain variable domain form a second binding site.

In one example of the above

- the first heavy chain comprises from N- to C-terminus a first heavy chain variable domain, a CH1 domain, a second heavy chain variable domain, a CL domain, a hinge region, a CH2 domain and a CH3 domain,

- the second heavy chain comprises from N- to C-terminus the first heavy chain variable domain, a CH1 domain, a hinge region, a CH2 domain and a CH3 domain,

- the first light chain comprises from N- to C-terminus a first light chain variable domain and a CH1 domain, and

- the second light chain comprises from N- to C- terminus a second light chain variable domain and a CL domain,

wherein the first heavy chain variable domain and the second light chain variable domain form a first binding site and the second heavy chain variable domain and the first light chain variable domain form a second binding site.

In one example of the above the expression cassette encoding the one second selection marker is located partly 5' and partly 3' to the third recombination recognition sequences, wherein the 5'-located part of said expression cassette comprises the promoter and the start-codon and said 3'-located part of the expression cassette comprises the coding sequence of the one second selection marker without a start-codon and a polyA signal.

In one example of the above the 5'-terminal part of the expression cassette encoding the one second selection marker comprises a promoter sequence operably linked to the start-codon, whereby the promoter sequence is flanked upstream by (i.e. is positioned downstream to) the expression cassettes and the start-codon is flanked downstream by (i.e. is positioned upstream of) the third recombination recognition sequence; and the 3'-terminal part of the

expression cassette encoding the one second selection marker comprises the coding sequence of the one second selection marker lacking a start-codon flanked upstream by the third recombination recognition sequence and downstream by the expression cassettes.

5 In one example of the above the start-codon is a translation start-codon. In one example the start-codon is ATG.

In one example of the above the first deoxyribonucleic acid is integrated into a first vector and the second deoxyribonucleic acid is integrated into a second vector.

10 In one example of the above each of the expression cassettes comprise in 5'-to-3' direction a promoter, a coding sequence and a polyadenylation signal sequence optionally followed by a terminator sequence.

In one example of the above

15 i) the first expression cassette comprises in 5'-to-3' direction a promoter, a nucleic acid encoding the first heavy chain, and a polyadenylation signal sequence and optionally a terminator sequence,

ii) the second expression cassette comprises in 5'-to-3' direction a promoter, a nucleic acid encoding the first light chain, and a polyadenylation signal sequence and optionally a terminator sequence,

20 iii) the third expression cassette comprises in 5'-to-3' direction a promoter, a nucleic acid encoding the first light chain, and a polyadenylation signal sequence and optionally a terminator sequence,

iv) the fourth expression cassette comprises in 5'-to-3' direction a promoter, a nucleic acid encoding the second heavy chain, and a polyadenylation signal sequence and optionally a terminator sequence,

25 v) the fifth expression cassette comprises in 5'-to-3' direction a promoter, a nucleic acid encoding the second light chain., and a polyadenylation signal sequence and optionally a terminator sequence,

vi) the sixth expression cassette comprises in 5'-to-3' direction a promoter, a nucleic acid encoding the second light chain, and a polyadenylation signal sequence and optionally a terminator sequence, and

5 vii) the expression cassette encoding the selection marker comprises in 5'-to-3' direction a promoter, a nucleic acid encoding the selection marker, and a polyadenylation signal sequence and optionally a terminator sequence.

In one example of the above the promoter is the human CMV promoter with or without intron A, the polyadenylation signal sequence is the bGH polyA site and the terminator is the hGT terminator.

10 A terminator sequence prevents the generation of very long RNA transcripts by RNA polymerase II, i.e. the read-through into the next expression cassette in the deoxyribonucleic acid according to the disclosure and used in the methods according to the disclosure. That is, the expression of one structural gene of interest is controlled by its own promoter.

15 Thus, by the combination of a polyadenylation signal and a terminator sequence efficient transcription termination is achieved. That is, read-through of the RNA polymerase II is prevented by the presence of double termination signals. The terminator sequence initiates complex resolution and promotes dissociation of RNA polymerase from the DNA template.

20 In one example of the above the promoter is the human CMV promoter with intron A, the polyadenylation signal sequence is the bGH polyadenylation signal sequence and the terminator is the hGT terminator except for the expression cassette of the selection marker, wherein the promoter is the SV40 promoter and the polyadenylation signal sequence is the SV40 polyadenylation signal sequence and a
25 terminator is absent.

In one example of the above the mammalian cell is a CHO cell. In one example the CHO cell is a CHO-K1 cell.

In one example of the above the trivalent antibody is a therapeutic antibody.

30 In one example of the above the trivalent, bispecific (therapeutic) antibody (TCB) comprises

- a first and a second Fab fragment, wherein each binding site of the first and the second Fab fragment specifically bind to the second antigen,
- a third Fab fragment, wherein the binding site of the third Fab fragment specifically binds to the first antigen, and wherein the third Fab fragment comprises a domain crossover such that the variable light chain domain (VL) and the variable heavy chain domain (VH) are replaced by each other, and
- an Fc-region comprising a first Fc-region polypeptide and a second Fc-region polypeptide,

10 wherein the first and the second Fab fragment each comprise a heavy chain fragment and a full length light chain,

wherein the C-terminus of the heavy chain fragment of the first Fab fragment is fused to the N-terminus of the first Fc-region polypeptide,

15 wherein the C-terminus of the heavy chain fragment of the second Fab fragment is fused to the N-terminus of the variable light chain domain of the third Fab fragment and the C-terminus of the heavy chain constant domain 1 of the third Fab fragment is fused to the N-terminus of the second Fc-region polypeptide.

20 In one example of the above at least one selection marker expression cassette is oriented in the opposite direction as the antibody heavy and light chain expression cassettes.

25 In one example of the above the antibody heavy and light chain expression cassettes are arranged with respect to each other unidirectional (i.e. have the same orientation in 3'- to 5'-direction) and at least one of the selection marker expression cassette is arranged bidirectional with respect to the antibody heavy and light chain expression cassettes.

30 In one example of the above the trivalent antibody is an anti-CD3/CD20 bispecific antibody. In one example the anti-CD3/CD20 bispecific antibody is a TCB with CD20 being the second antigen. In one example the bispecific anti-CD3/CD20 antibody is RG6026. Such an antibody is reported in WO 2016/020309, which is incorporated herein by reference in its entirety.

5 In one example of the above the trivalent antibody is an anti-CD3/CEA bispecific antibody. In one example the anti-CD3/CEA bispecific antibody is a TCB with CEA being the second antigen. In one example the bispecific anti-CD3/CEA antibody is RO6958688 or RG7802 or cibisatamab. Such an antibody is reported in WO 2017/055389, which is incorporated herein by reference in its entirety.

In one example of the above none of the first light chain and the second light chain of the trivalent, bispecific antibody is a common light chain or a universal light chain.

10 In one example of the above the second heavy chain variable domain and the first light chain variable domain form a first binding site and the first heavy chain variable domain and the second light chain variable domain form a second binding site.

In one preferred example of the above the first binding site specifically binds to human CD3.

In one preferred example of the above the second binding site specifically binds to human CD3.

15 In one preferred example of the above exactly two deoxyribonucleic acids are comprised or introduced.

20 The individual expression cassettes in the deoxyribonucleic acid according to the disclosure are arranged sequentially. The distance between the end of one expression cassette and the start of the thereafter following expression cassette is only a few nucleotides, which were required for, i.e. result from, the cloning procedure.

25 In one example of the above two directly following expression cassettes are spaced at most 100 bps apart (i.e. from the end of the poly A signal sequence or the terminator sequence, respectively, until the start of the following promoter element are at most 100 basepairs (bps)). In one example two directly following expression cassettes are spaced at most 50 bps apart. In one preferred example two directly following expression cassettes are spaced at most 25 bps apart.