the stability-contributing component between the parent antibody and the stable framework.

[0017] The solution for this problem that has been provided by the present invention, i.e. novel chimeric human antibody light chain frameworks, comprising framework regions I to III from Vκ and framework region IV from Vλ, with advantageous properties, such as high stability and reduced aggregation propensity, has so far not been achieved or suggested by the prior art.

SUMMARY

[0018] The present disclosure describes novel chimeric human antibody light chain frameworks, comprising framework regions I to III from Vκ and framework region IV from Vλ, with advantageous properties, such as high stability, reduced aggregation propensity and minimal immunogenic potential.

[0019] Thus, in a ~~first~~ one aspect, the present ~~invention~~ disclosure refers to an isolated antibody or ~~functional~~ antigen-binding fragment thereof comprising a VH domain and a VL domain comprising (i) human Vκ framework regions I to III; (ii) CDR domains CDR1, CDR2 and CDR3; and (iii) a framework region IV, which is a Vλ-based sequence, which has ~~one or two mutations, particularly~~ one mutation~~,~~ compared to the closest human Vλ germ line sequence for framework region IV selected from the list of SEQ ID NO. 16 to 22; provided that the sequence FGQGTKLTVLG (SEQ ID No. 15) is excluded. In one example, there is described an antibody VL domain comprising (i) human Vκ framework regions I to III; (ii) CDR domains CDR1, CDR2 and CDR3; and (iii) a framework region IV, which is selected from a. a human Vλ germ line sequence for framework region IV, particularly a Vλ germ line sequence selected from the list of: SEQ ID NO. 16 to 22 ; b. a Vλ-based sequence, which is (bi) a consensus Vλ sequence from human Vλ germ line sequences for framework region IV, particularly SEQ ID NO. 17; or (bii) a consensus Vλ sequence from rearranged human Vλ sequences for framework region IV, particularly a Vλ consensus sequence selected from the list of: SEQ ID NO. 16 and 17; and c. a Vλ-based sequence, which has one or two mutations, particularly one mutation, compared to the closest human Vλ germ line sequence for framework region IV; provided that if, in case of b. or c, framework region IV has the sequence FGQGTKLTVLG (SEQ ID No. 15)

(w) said human Vκ framework regions I to III are different from the framework regions as found in the list of clones: FW1.4gen (SEQ ID NO: 4), 375-FW1.4opt, 435-FW1.4opt, 509-FW1.4opt, 511-FW1.4opt, 534-FW1.4opt, 567-FW1.4opt, 578-FW1.4opt, 1-FW1.4opt, 8-FW1.4opt, 15-FW1.4opt, 19-FW1.4opt, 34-FW1.4opt, 35-FW1.4opt, 42-FW1.4opt, and 43-FW1.4opt;

(x) said human Vκ framework regions I to III are different from a sequence obtainable by permutation from the sequences of the framework regions as found in the list of clones: FW1.4gen (SEQ ID NO: 4), 375-FW1.4opt, 435-FW1.4opt, 509-FW1.4opt, 511-FW1.4opt, 534-FW1.4opt, 567-FW1.4opt, 578-FW1.4opt, 1-FW1.4opt, 8-FW1.4opt, 15-FW1.4opt, 19-FW1.4opt, 34-FW1.4opt, 35-FW1.4opt, 42-FW1.4opt, and 43-FW1.4opt;

(y) said human Vκ framework regions I to III are different from a sequence obtainable by mutation of the sequence FW1.4gen (SEQ ID NO: 4) at one or more of positions 15, 22, 48, 57, 74, 87, 88, 90, 92, 95, 97 and 99 (AHo numbering); or

(z) said human Vκ framework regions I to III comprise not more than five mutations compared to the respective regions in the human Vκ sequence with SEQ ID No: 8, particularly less than five, less than four, less than three, particularly only one or no mutation compared to the human Vκ sequence with SEQ ID No: 8.

[0020] In one example, there is disclosed an isolated antibody or functional fragment thereof comprising an antibody VL domain according to the present disclosure.

[0021] In one aspect, the present disclosure refers to a pharmaceutical composition comprising the isolated antibody or ~~functional~~ antibody-binding fragment thereof of the present disclosure, and optionally a pharmaceutically acceptable carrier and/or excipient.

[0022] In one aspect, the present disclosure refers to a nucleic acid sequence or a collection of nucleic acid sequences encoding ~~the antibody VL domain of any one of the present disclosure, or~~ the isolated antibody or antibody-binding ~~functional~~ fragment thereof of the

present disclosure, and/or to a nucleic acid sequence or nucleic acid sequences obtainable by the method disclosed herein.

[0023] In another aspect, the present disclosure refers to a vector or a collection of vectors comprising the nucleic acid sequence or a collection of nucleic acid sequences as disclosed herein.

[0024] In a one, the present disclosure refers to a host cell, particularly an expression host cell, comprising the nucleic acid sequence or the collection of nucleic acid sequences as disclosed herein, or the vector or collection of vectors as disclosed herein.

[0025] In another aspect, the present disclosure refers to a method for producing ~~the antibody VL domain as disclosed herein, or~~ the isolated antibody or antibody-binding ~~functional~~ fragment thereof as disclosed herein, comprising the step of expressing the nucleic acid sequence or the collection of nucleic acid sequences as disclosed herein, or the vector or collection of vectors as disclosed herein, or the host cell, particularly an expression host cell, as disclosed herein.

[0026] In one aspect, the present disclosure refers to a method for generating a humanized rabbit antibody comprising the steps of:

a) immunization of rabbits with an antigen of interest;
b) isolating at least one antibody of interest; and
c) cloning of the VL CDR regions of said at least one antibody of interest into a nucleic acid sequence encoding an antibody VL domain of the isolated antibody or antibody-binding fragment thereof according to the present ~~invention~~disclosure.

[0027] In another aspect, the present disclosure refers to ~~A~~ a method for generating a nucleic acid sequence encoding ~~an antibody VL domain of the isolated antibody or functional fragment thereof of any one of claims 1 to 8, or~~ one or more nucleic acid sequences encoding the isolated antibody or antibody-binding ~~functional~~ fragment ~~thereof of any one of claims 1 to 8~~as disclosed herein, comprising combining in one or more steps nucleic acid sequences encoding (i) human Vκ framework regions I to III; (ii) CDR domains CDR1, CDR2 and CDR3, and (iii) a framework region IV, which is a Vλ-based sequence, which has ~~one or two mutations, particularly~~ one mutation~~,~~ compared to the closest human Vλ germ line sequence for framework region IV selected from the list of SEQ ID NO. 16 to 22; particularly using one of the following methods: replacing in a nucleic acid construct, particularly in a recombinant vector, comprising a nucleic acid sequence encoding a human or humanized Vκ domain the Vκ framework region IV by a framework region IV, which is a Vλ-based sequence, which has ~~one or two mutations, particularly~~ one mutation~~,~~ compared to the closest human Vλ germ line sequence for framework region IV selected from the list of SEQ ID NO. 16 to 22; inserting in one or more steps into a nucleic acid construct, particularly into a recombinant vector, comprising a nucleic acid sequence encoding a framework region IV one or more nucleic acid sequences encoding (i) human Vκ framework regions I to III; and (ii) CDR domains CDR1, CDR2 and CDR3, wherein said framework region IV is a Vλ-based sequence, which has ~~one or two mutations, particularly~~ one mutation~~,~~ compared to the closest human Vλ germ line sequence for framework region IV selected from the list of SEQ ID NO. 16 to 22; mutating in a nucleic acid sequence encoding a human or humanized Vκ domain the nucleic acid sequence encoding framework region IV to generate a framework region IV, which is a Vλ-based sequence, which has ~~one or two mutations, particularly~~ one mutation~~,~~ compared to the closest human Vλ germ line sequence for framework region IV selected from the list of SEQ ID NO. 16 to 22; or replacing in one or more steps in a nucleic acid construct, particularly in a recombinant vector, comprising a nucleic acid sequence encoding a light chain domain comprising human Vκ framework regions I to III, CDR domains CDR1, CDR2 and CDR3, and a framework region IV, one or more of the nucleic acid sequences encoding said CDR domains by nucleic acid sequence(s) encoding the corresponding CDR domain(s) from an antibody of interest, wherein said framework region IV is a Vλ-based sequence, which has one ~~or two mutations, particularly one~~ mutation~~,~~ compared to the closest human Vλ germ line sequence for framework

region IV selected from the list of SEQ ID NO. 16 to 22; in each of i. to iv. Provided that the sequence FGQGTKLTVLG (SEQ ID No. 15) is excluded as said Vλ-based sequence. In one example there is disclosed a method for generating a nucleic acid sequence encoding an antibody VL domain according to the present ~~invention~~disclosure, or one or more nucleic acid sequences encoding an isolated antibody or functional fragment thereof according to the present ~~invention~~disclosure, comprising combining

CLAIMS

1. An isolated antibody or antigen-binding ~~functional~~ fragment thereof comprising a VH domain and a VL domain comprising (i) human Vκ framework regions I to III; (ii) CDR domains CDR1, CDR2 and CDR3; and (iii) a framework region IV, which is

   a Vλ-based sequence, which has ~~one or two mutations, particularly~~ one mutation~~,~~ compared to the closest human Vλ germ line sequence for framework region IV selected from the list of SEQ ID NO. 16 to 22;

   provided that the sequence FGQGTKLTVLG (SEQ ID No. 15) is excluded.

2. The isolated antibody or antigen-binding ~~functional~~ fragment thereof of claim 1, wherein the amino acid residue in position AHo101 in framework region III is ~~an amino acid residue present at that position in a human Vλ consensus sequence, particularly wherein said amino acid residue is different from phenylalanine, more particularly wherein said amino acid residue is~~ glutamic acid.

3. The isolated antibody or antigen-binding ~~functional~~ fragment thereof of claim 1 or 2, wherein said Vκ framework regions I to III are from the Vκ1 family.

4. The isolated antibody or antigen-binding ~~functional~~ fragment thereof of claim 3, wherein said Vκ framework regions I to III are the framework regions present in a sequence selected from SEQ ID NO: 2 and SEQ ID NO: 8, particularly SEQ ID NO: 8.

5. The isolated antibody or antigen-binding ~~functional~~ fragment thereof of any one of claims 1 to 4, wherein said CDR domains CDR1, CDR2 and CDR3 are independently selected from (i) CDR domains CDR1, CDR2 and CDR3 from a parental non-human antibody with specificity for an antigen of interest, particularly from a parental rabbit antibody or from a parental rodent antibody, particularly a parental mouse or rat antibody; (ii) CDR domains CDR1, CDR2 and CDR3 from a parental human or humanized antibody comprising a Vκ domain, particularly from an antibody approved for therapy or otherwise being commercialized; (iii) CDR domains CDR1, CDR2 and CDR3 derived from the CDR domains according to (i) or (ii), particularly CDR domains obtained by optimizing one or more of the CDR

domains according to (i) or (ii); and (iv) a CDR domain to be replaced by one or more CDR domains according to (i), (ii) and/or (iii).

6. The isolated antibody or antigen-binding ~~functional~~ fragment thereof of any one of claims 1 to 5, wherein the framework regions I to IV are a combination of framework regions as found in a sequence selected from: SEQ ID NO: 8, SEQ ID NO: 9, SEQ ID NO: 11, SEQ ID NO: 12, and SEQ ID NO: 13.

7. The isolated antibody or antigen-binding ~~functional~~ fragment thereof of any one of claims 1 to 6, comprising a VH domain belonging to a VH domain subfamily selected from VH1A, VH1B, VH2, VH3, VH4, VH5, and VH6, particularly to a VH domain subfamily VH3 or VH4, particularly to the VH domain subfamily VH3.

8. The isolated antibody or antigen-binding ~~functional~~ fragment thereof of any one of claims 1 to 7, which is selected from the group consisting of: an IgG antibody, a Fab fragment, an scFv fragment, a single-chain diabody (scDb), a tandem scDb (Tandab), a linear dimeric scDb (LD-scDb), a circular dimeric scDb (CD-scDb), a bispecific T-cell engager (BiTE; tandem di-scFv), a tandem tri-scFv, a tri(a)body, bispecific Fab2, di-miniantibody, tetrabody, scFv-Fc-scFv fusion, di-diabody, DVD-Ig, IgG-scFab, scFab-dsscFv, Fv2-Fc, IgG-scFv fusions, such as bsAb (scFv linked to C-terminus of light chain), Bs1Ab (scFv linked to N-terminus of light chain), Bs2Ab (scFv linked to N-terminus of heavy chain), Bs3Ab (scFv linked to C-terminus of heavy chain), Ts1Ab (scFv linked to N-terminus of both heavy chain and light chain), Ts2Ab (dsscFv linked to C-terminus of heavy chain), and Knob-into-Holes (KiHs) (bispecific IgGs prepared by the KiH technology) and DuoBodies (bispecific IgGs prepared by the Duobody technology)

9. A pharmaceutical composition comprising the isolated antibody or antigen-binding ~~functional~~ fragment thereof of any one of claims 1 to 8, and optionally a pharmaceutically acceptable carrier and/or excipient.

10. A nucleic acid sequence or a collection of nucleic acid sequences (i) encoding ~~the antibody VL domain of the isolated antibody or functional fragment thereof of any one of claims 1 to 8, or~~ the isolated antibody or antigen-binding ~~functional~~ fragment thereof of any one of claims 1 to 8; or (ii) obtainable by the method of claim 17.

11. A vector or a collection of vectors comprising the nucleic acid sequence or a collection of nucleic acid sequences of claim 10.

12. A host cell, particularly an expression host cell, comprising the nucleic acid sequence or the collection of nucleic acid sequences of claim 10, or the vector or collection of vectors of claim 11.

13. A method for producing ~~the antibody VL domain of the isolated antibody or functional fragment thereof of any one of claims 1 to 8, or~~ the isolated antibody or antigen-binding ~~functional~~ fragment thereof of any one of claims 1 to 8, comprising the step of expressing the nucleic acid sequence or the collection of nucleic acid sequences of claim 10, or the vector or collection of vectors of claim 11, or the host cell, particularly an expression host cell, of claim 12.

14. A method for generating a humanized rabbit or rodent antibody comprising the steps of:

    a. immunization of rabbits or rodents with an antigen of interest;

    b. isolating at least one antibody of interest; and

    c. cloning of the VL CDR regions of said at least one antibody of interest into a nucleic acid sequence encoding an antibody VL domain of the isolated antibody or antigen-binding ~~functional~~ fragment thereof of any one of claims 1 to 8.

15. The method of claim 14, further comprising one or more of the steps of:

    aa. clonal isolation of affinity matured memory B-cells that interact with the antigen of interest, particularly by using fluorescence activated cell-sorting;

    ab. cultivation of single B cells in a co-cultivation system that does not require immortalization of single B cell clones;

    ac. screening of B cell culture supernatants in a cell-based ELISA to identify at least one antibody binding to the antigen of interest; and/or

    ad. cloning of the VH CDR regions of at least one antibody into a nucleic acid sequence encoding a human antibody VH domain.

16. A method for generating a nucleic acid sequence encoding ~~an antibody VL domain of the isolated antibody or functional fragment thereof of any one of claims 1 to 8, or~~ one or more nucleic acid sequences encoding the isolated antibody or antigen-binding ~~functional~~ fragment thereof of any one of claims 1 to 8, comprising combining in one or more steps nucleic acid sequences encoding (i) human Vκ framework regions I to III; (ii) CDR domains CDR1, CDR2 and CDR3, and (iii) a framework region IV, which is

a Vλ-based sequence, which has ~~one or two mutations, particularly~~ one mutation~~,~~ compared to the closest human Vλ germ line sequence for framework region IV selected from the list of SEQ ID NO. 16 to 22;

particularly using one of the following methods:

i. replacing in a nucleic acid construct, particularly in a recombinant vector, comprising a nucleic acid sequence encoding a human or humanized Vκ domain the Vκ framework region IV by a framework region IV, which is

a Vλ-based sequence, which has ~~one or two mutations, particularly~~ one mutation~~,~~ compared to the closest human Vλ germ line sequence for framework region IV selected from the list of SEQ ID NO. 16 to 22;

ii. inserting in one or more steps into a nucleic acid construct, particularly into a recombinant vector, comprising a nucleic acid sequence encoding a framework region IV one or more nucleic acid sequences encoding (i) human Vκ framework regions I to III; and (ii) CDR domains CDR1, CDR2 and CDR3, wherein said framework region IV is

a Vλ-based sequence, which has ~~one or two mutations, particularly~~ one mutation~~,~~ compared to the closest human Vλ germ line sequence for framework region IV selected from the list of SEQ ID NO. 16 to 22;

iii. mutating in a nucleic acid sequence encoding a human or humanized Vκ domain the nucleic acid sequence encoding framework region IV to generate a framework region IV, which is

a Vλ-based sequence, which has ~~one or two mutations, particularly~~ one mutation~~,~~ compared to the closest human Vλ germ line sequence for framework region IV selected from the list of SEQ ID NO. 16 to 22; or

iv. replacing in one or more steps in a nucleic acid construct, particularly in a recombinant vector, comprising a nucleic acid sequence encoding a light chain domain comprising

human Vκ framework regions I to III, CDR domains CDR1, CDR2 and CDR3, and a framework region IV, one or more of the nucleic acid sequences encoding said CDR domains by nucleic acid sequence(s) encoding the corresponding CDR domain(s) from an antibody of interest, wherein said framework region IV is

a Vλ-based sequence, which has ~~one or two mutations, particularly~~ one mutation~~,~~ compared to the closest human Vλ germ line sequence for framework region IV selected from the list of SEQ ID NO. 16 to 22;

in each of i. to iv. provided that the sequence FGQGTKLTVLG (SEQ ID No. 15) is excluded as said Vλ-based sequence.