Summary

In one aspect, there is provided a method for producing at least two different monospecific antibodies from a single host cell, wherein each of said two antibodies comprises two CH3 domains that are capable of forming an interface, said method comprising providing in said cell a) a first nucleic acid encoding a first CH3 domain-comprising polypeptide chain, and b) a second nucleic acid encoding a second CH3 domain-comprising polypeptide chain, wherein said first CH3 domain-comprising polypeptide chain comprises negatively charged amino acid residues at amino acid positions 392 and 409 and a positively charged amino acid residue at amino acid position 399 according to the EU numbering system,

and wherein said second CH3 domain-comprising polypeptide chain comprises:

- either a wildtype CH3 domain, or
- positively charged amino acid residues at amino acid positions 356 and 357 and negatively charged amino acid residues at amino acid positions 370 and 439 according to the EU numbering system,

said method further comprising the step of culturing said host cell and allowing for expression of said nucleic acids and harvesting said at least two different antibodies from the culture. In another aspect, there is provided a method for producing at least two different monospecific antibodies from a single host cell, wherein each of said two antibodies comprises two CH3 domains that are capable of forming an interface, said method comprising providing in said cell a) a first nucleic acid encoding a first CH3 domain-comprising polypeptide chain, and b) a second nucleic acid encoding a second CH3 domain-comprising polypeptide chain, wherein said first CH3 domain-comprising polypeptide chain comprises:

- either a wildtype CH3 domain, or
- negatively charged amino acid residues at amino acid positions 392 and 409 and a positively charged amino acid residue at amino acid position 399 according to the EU numbering system,

and wherein said second CH3 domain-comprising polypeptide chain comprises positively charged amino acid residues at amino acid positions 356 and 357 and negatively charged amino acid residues at amino acid positions 370 and 439 according to the EU numbering system, said method further comprising the step of culturing said host cell and allowing for expression of said nucleic acids and harvesting said at least two different antibodies from the culture. Disclosed is a method for producing at least two different Ig-like molecules from a single host cell, wherein each of said two Ig-like molecules comprises two CH3 domains that are capable of forming an interface, said method comprising providing in said cell a) A first nucleic acid encoding a first CH3 domain-comprising polypeptide chain, b) A second nucleic acid encoding a second CH3 domain-comprising polypeptide chain, c) A third nucleic acid encoding a third CH3 domain-comprising polypeptide chain, and d) A fourth nucleic acid encoding a fourth CH3 domain-comprising polypeptide chain, wherein said first CH3 domain-comprising polypeptide chain comprises a lysine (K) residue at amino acid position 366 and a lysine (K) residue at amino acid position 351, and wherein said second CH3 domain-comprising polypeptide chain comprises an aspartic acid (D) residue at amino acid position 351 and a glutamic acid (E) residue at amino acid position 368, and wherein said third and fourth CH3 domain-comprising polypeptide chains comprise a wild type CH3 domain, said method further comprising the step of culturing said host cell and allowing for expression of said at least four nucleic acids and harvesting said at least two different Ig-like molecules from the culture. Disclosed is a method for producing at least two different Ig-like molecules from a single host cell, wherein each of said two Ig-like molecules comprises two CH3 domains that are capable of forming an interface, said method comprising providing in said cell a) A first nucleic acid encoding a first CH3 domain-comprising polypeptide chain, b) A second nucleic acid encoding a second CH3 domain-comprising polypeptide chain, c) A third nucleic acid encoding a third CH3 domain-comprising polypeptide chain, and d) A fourth nucleic acid

encoding a fourth CH3 domain-comprising polypeptide chain, wherein said first CH3 domain-comprising polypeptide chain comprises a lysine (K) residue at amino acid position 366 and a lysine (K) residue at amino acid position 351, and wherein said second CH3 domain-comprising polypeptide chain comprises an aspartic acid (D) residue at amino acid position 351 and a glutamic acid (E) residue at amino acid position 368, and wherein said third and/or said fourth CH3 domain-comprising polypeptide chain comprises one or more amino acid substitutions selected from Table B, said method further comprising the step of culturing said host cell and allowing for expression of said at least four nucleic acids and harvesting said at least two different Ig-like molecules from the culture. Disclosed is a method for producing at least two different Ig-like molecules from a single host cell, wherein each of said two Ig-like molecules comprises two CH3 domains that are capable of forming an interface, said method comprising providing in said cell a) A first nucleic acid encoding a first CH3 domain-comprising polypeptide chain, b) A second nucleic acid encoding a second CH3 domain-comprising polypeptide chain, and c) A third nucleic acid encoding a third CH3 domain-comprising polypeptide chain, wherein said first CH3 domain-comprising polypeptide chain comprises a lysine (K) residue at amino acid position 366 and a lysine (K) residue at amino acid position 351, and wherein said second CH3 domain-comprising polypeptide chain comprises an aspartic acid (D) residue at amino acid position 351 and a glutamic acid (E) residue at amino acid position 368, and wherein said third CH3 domain-comprising polypeptide chain comprises an aspartic acid (D) residue at amino acid position 351 and a glutamic acid (E) residue at amino acid position 368, said method further comprising the step of culturing said host cell and allowing for expression of said at least four nucleic acids and harvesting said at least two different Ig-like molecules from the culture. Disclosed is a method for producing at least two different Ig-like molecules from a single host cell, wherein each of said two Ig-like molecules comprises two CH3 domains that are capable of forming an

interface, said method comprising providing in said cell a) A first nucleic acid encoding a first CH3 domain-comprising polypeptide chain, b) A second nucleic acid encoding a second CH3 domain-comprising polypeptide chain, c) A third nucleic acid encoding a third CH3 domain-comprising polypeptide chain, and wherein said first CH3 domain-comprising polypeptide chain comprises a lysine (K) residue at amino acid position 366 and a lysine (K) residue at amino acid position 351, and wherein said second CH3 domain-comprising polypeptide chain comprises an aspartic acid (D) residue at amino acid position 351 and a glutamic acid (E) residue at amino acid position 368, and wherein said third CH3 domain-comprising polypeptide chain comprises a lysine (K) residue at amino acid position 366 and a lysine (K) residue at amino acid position 351, said method further comprising the step of culturing said host cell and allowing for expression of said at least four nucleic acids and harvesting said at least two different Ig-like molecules from the culture. In a further aspect, there is provided a mixture of at least two different monospecific antibodies obtainable by the methods according to the present invention. In a further aspect, there is provided a recombinant host cell comprising one or more nucleic acids encoding at least a first and a second CH3 domain-comprising polypeptide chain, wherein said first CH3 domain-comprising polypeptide chain comprises negatively charged amino acid residues at amino acid positions 392 and 409 and a positively charged amino acid residue at amino acid position 399 according to the EU numbering system, and wherein said second CH3 domain-comprising polypeptide chain comprises:

- either a wildtype CH3 domain, or
- positively charged amino acid residues at amino acid positions 356 and 357 and negatively charged amino acid residues at amino acid positions 370 and 439 according to the EU numbering system. In a further aspect, there is provided a recombinant host cell comprising one or more nucleic acids encoding at least a first and a second CH3 domain-comprising polypeptide chain, wherein said first CH3 domain-comprising polypeptide chain comprises:

- either a wildtype CH3 domain, or
- negatively charged amino acid residues at amino acid positions 392 and 409 and a positively charged amino acid residue at amino acid position 399 according to the EU numbering system,

and wherein said second CH3 domain-comprising polypeptide chain comprises positively charged amino acid residues at amino acid positions 356 and 357 and negatively charged amino acid residues at amino acid positions 370 and 439 according to the EU numbering system. Disclosed is a recombinant host cell comprising one or more nucleic acids encoding at least a first, a second, a third and a fourth CH3 domain-comprising polypeptide chain, wherein said first CH3 domain-comprising polypeptide chain comprises a lysine (K) residue at amino acid position 366 and a lysine (K) residue at amino acid position 351, and wherein said second CH3 domain-comprising polypeptide chain comprises an aspartic acid (D) residue at amino acid position 351 and a glutamic acid (E) residue at amino acid position 368, and wherein said third and fourth CH3 domain-comprising polypeptide chains comprise a wild type CH3 domain. Disclosed is a recombinant host cell comprising one or more nucleic acids encoding at least a first, a second, a third and a fourth CH3 domain-comprising polypeptide chain, wherein said first CH3 domain-comprising polypeptide chain comprises a lysine (K) residue at amino acid position 366 and a lysine (K) residue at amino acid position 351, and wherein said second CH3 domain-comprising polypeptide chain comprises an aspartic acid (D) residue at amino acid position 351 and a glutamic acid (E) residue at amino acid position 368, and wherein said third and/or said fourth CH3 domain-comprising polypeptide chain comprises one or more amino acid substitutions selected from Table B. Disclosed is a recombinant host cell comprising one or more nucleic acids encoding at least a first, a second and a third CH3 domain-comprising polypeptide chain, wherein said first CH3 domain-comprising polypeptide chain comprises a lysine (K) residue at amino acid position 366 and a lysine (K) residue at amino acid

position 351, and wherein said second CH3 domain-comprising polypeptide chain comprises an aspartic acid (D) residue at amino acid position 351 and a glutamic acid (E) residue at amino acid position 368, and wherein said third CH3 domain-comprising polypeptide chain comprises an aspartic acid (D) residue at amino acid position 351 and a glutamic acid (E) residue at amino acid position 368. Disclosed is a recombinant host cell comprising one or more nucleic acids encoding at least a first, a second and a third CH3 domain-comprising polypeptide chain, wherein said first CH3 domain-comprising polypeptide chain comprises a lysine (K) residue at amino acid position 366 and a lysine (K) residue at amino acid position 351, and wherein said second CH3 domain-comprising polypeptide chain comprises an aspartic acid (D) residue at amino acid position 351 and a glutamic acid (E) residue at amino acid position 368, and wherein said third CH3 domain-comprising polypeptide chain comprises a lysine (K) residue at amino acid position 366 and a lysine (K) residue at amino acid position 351. In a further aspect, there is provided a pharmaceutical composition comprising the at least two different monospecific antibodies of any one of the present invention, and a pharmaceutically acceptable carrier. In a further aspect, there is provided a method for making a host cell for production of at least two different monospecific antibodies, the method comprising the step of introducing into said host cell one or more nucleic acids encoding at least a first and a second CH3 domain-comprising polypeptide chain, wherein said first CH3 domain-comprising polypeptide chain comprises negatively charged amino acid residues at amino acid positions 392 and 409 and positively charged amino acid residue at amino acid position 399 according to the EU numbering system, and wherein said second CH3 domain-comprising polypeptide chain comprises:

- either a wildtype CH3 domain, or
- positively charged amino acid residues at amino acid positions 356 and 357 and negatively charged amino acid residues at amino acid positions 370 and 439 according to the EU numbering system. In a further aspect, there is provided a method for making a host cell for

production of at least two different monospecific antibodies, the method comprising the step of introducing into said host cell one or more nucleic acids encoding at least a first and a second CH3 domain-comprising polypeptide chain, wherein said first CH3 domain-comprising polypeptide chain comprises:

- either a wildtype CH3 domain, or
- negatively charged amino acid residues at amino acid positions 392 and 409 and a positively charged amino acid residue at amino acid position 399 according to the EU numbering system,

and wherein said second CH3 domain-comprising polypeptide chain comprises positively charged amino acid residues at amino acid positions 356 and 357 and negatively charged amino acid residues at amino acid positions 370 and 439 according to the EU numbering system. Disclosed is a method for making a host cell for production of at least two different Ig-like molecules, the method comprising the step of introducing into said host cell one or more nucleic acids encoding at least a first, a second, a third and a fourth CH3 domain-comprising polypeptide chain, wherein said first CH3 domain-comprising polypeptide chain comprises a lysine (K) residue at amino acid position 366 and a lysine (K) residue at amino acid position 351, and wherein said second CH3 domain-comprising polypeptide chain comprises an aspartic acid (D) residue at amino acid position 351 and a glutamic acid (E) residue at amino acid position 368, and wherein said third and fourth CH3 domain-comprising polypeptide chains comprise a wild type CH3 domain. Disclosed is a method for making a host cell for production of at least two different Ig-like molecules, the method comprising the step of introducing into said host cell one or more nucleic acids encoding at least a first, a second, a third and a fourth CH3 domain-comprising polypeptide chain, wherein said first CH3 domain-comprising polypeptide chain comprises a lysine (K) residue at amino acid position 366 and a lysine (K) residue at amino acid position 351, and wherein said second CH3 domain-comprising polypeptide chain comprises an aspartic acid (D) residue at amino acid position 351 and a glutamic acid (E)

residue at amino acid position 368, and wherein said third and/or said fourth CH3 domain-comprising polypeptide chain comprises one or more amino acid substitutions selected from Table B. Disclosed is a method for making a host cell for production of at least two different Ig-like molecules, the method comprising the step of introducing into said host cell one or more nucleic acids encoding at least a first, a second and a third CH3 domain-comprising polypeptide chain, wherein said first CH3 domain-comprising polypeptide chain comprises a lysine (K) residue at amino acid position 366 and a lysine (K) residue at amino acid position 351, and wherein said second CH3 domain-comprising polypeptide chain comprises an aspartic acid (D) residue at amino acid position 351 and a glutamic acid (E) residue at amino acid position 368, and wherein said third CH3 domain-comprising polypeptide chain comprises an aspartic acid (D) residue at amino acid position 351 and a glutamic acid (E) residue at amino acid position 368. Disclosed is a method for making a host cell for production of at least two different Ig-like molecules, the method comprising the step of introducing into said host cell one or more nucleic acids encoding at least a first, a second and a third CH3 domain-comprising polypeptide chain, wherein said first CH3 domain-comprising polypeptide chain comprises a lysine (K) residue at amino acid position 366 and a lysine (K) residue at amino acid position 351, and wherein said second CH3 domain-comprising polypeptide chain comprises an aspartic acid (D) residue at amino acid position 351 and a glutamic acid (E) residue at amino acid position 368, and wherein said third CH3 domain-comprising polypeptide chain comprises a lysine (K) residue at amino acid position 366 and a lysine (K) residue at amino acid position 351. In yet a further aspect, there is provided a culture of recombinant host cells of the present invention, or of recombinant host cells obtained by the method according to the present invention, producing at least two different monospecific antibodies.

Claims

1. A method for producing at least two different monospecific antibodies from a single host cell, wherein each of said two antibodies comprises two CH3 domains that are capable of forming an interface, said method comprising providing in said cell

a) a first nucleic acid encoding a first CH3 domain-comprising polypeptide chain, and

b) a second nucleic acid encoding a second CH3 domain-comprising polypeptide chain,

wherein said first CH3 domain-comprising polypeptide chain comprises negatively charged amino acid residues at amino acid positions 392 and 409 and a positively charged amino acid residue at amino acid position 399 according to the EU numbering system,

and wherein said second CH3 domain-comprising polypeptide chain comprises:

- either a wildtype CH3 domain, or
- positively charged amino acid residues at amino acid positions 356 and 357 and negatively charged amino acid residues at amino acid positions 370 and 439 according to the EU numbering system,

said method further comprising the step of culturing said host cell and allowing for expression of said nucleic acids and harvesting said at least two different antibodies from the culture.

2. A method for producing at least two different monospecific antibodies from a single host cell, wherein each of said two antibodies comprises two CH3 domains that are capable of forming an interface, said method comprising providing in said cell

a) a first nucleic acid encoding a first CH3 domain-comprising polypeptide chain, and

b) a second nucleic acid encoding a second CH3 domain-comprising polypeptide chain,

wherein said first CH3 domain-comprising polypeptide chain comprises:

- either a wildtype CH3 domain, or

- negatively charged amino acid residues at amino acid positions 392 and 409 and a positively charged amino acid residue at amino acid position 399 according to the EU numbering system,

and wherein said second CH3 domain-comprising polypeptide chain comprises positively charged amino acid residues at amino acid positions 356 and 357 and negatively charged amino acid residues at amino acid positions 370 and 439 according to the EU numbering system,

said method further comprising the step of culturing said host cell and allowing for expression of said nucleic acids and harvesting said at least two different antibodies from the culture.

3. The method of claim 1 or 2, wherein said first CH3 domain-comprising polypeptide chain comprises an aspartic acid (D) residue at amino acid position 392 and a lysine (K) residue at amino acid position 399 and an aspartic acid (D) residue at amino acid position 409 according to the EU numbering system.

4. The method of any one of claims 1-3, wherein said second CH3 domain-comprising polypeptide chain comprises a lysine (K) residue at amino acid position 356 and a lysine (K) residue at amino acid position 357 and an aspartic acid (D) residue at amino acid position 439 and an aspartic acid (D) residue at amino acid position 370 according to the EU numbering system.

5. The method of any one of claims 1-4, wherein the formation of bispecific antibodies is reduced to below 5%.

6. The method of any one of claims 1-5, wherein the formation of bispecific antibodies is reduced to below 3%.

7. The method of any one of claims 1-6, further comprising providing said host cell with a nucleic acid encoding a common light chain.

8. The method of any one of claims 1-7, wherein each of the encoded CH3 domain-comprising polypeptide chains further comprises a variable region recognizing an epitope.

9. The method of claim 8, wherein the epitope recognized by the variable region of the second encoded CH3 domain-comprising polypeptide chain is different from the epitope recognized by the variable region of the first encoded CH3 domain-comprising polypeptide chain.

10. The method of any one of claims 8-9, wherein the epitopes are located on the same target.

11. The method of claim 10, wherein the target is soluble.

12. The method of claim 10, wherein the target is membrane-bound.

13. The method of any one of claims 8-9, wherein the epitopes are located on different targets.

14. The method of claim 13, wherein the different targets are expressed on the same cells.

15. The method of claim 13, wherein the different targets are expressed on different cells.

16. The method of claim 13, wherein the different targets are soluble.

17. The method of claim 13, wherein one target is soluble whereas the second target is membrane bound.

18. The method of any one of claims 8-17, wherein at least one of said epitopes is located on a tumor cell.

19. The method of any one of claims 8-18, wherein at least one of said epitopes is located on an effector cell.

20. The method of claim 19, wherein said effector cell is an NK cell, a T cell, a B cell, a monocyte, a macrophage, a dendritic cell or a neutrophilic granulocyte.

21. The method of claims 19 or 20, wherein said epitope is located on a CD3, CD16, CD25, CD28, CD64, CD89, NKG2D or a NKp46 molecule.

22. A mixture of at least two different monospecific antibodies obtainable by the methods according to any one of claims 1-21.

23. A mixture according to claim 22, wherein said at least two different monospecific antibodies bind to different epitopes on the same antigen and/or to different epitopes on different antigens.

24. A recombinant host cell comprising one or more nucleic acids encoding at least a first and a second CH3 domain-comprising polypeptide chain, wherein said first CH3 domain-comprising polypeptide chain comprises negatively charged amino acid residues at amino acid positions 392 and 409 and a positively charged amino acid residue at amino acid position 399 according to the EU numbering system,
and wherein said second CH3 domain-comprising polypeptide chain comprises:
- either a wildtype CH3 domain, or
- positively charged amino acid residues at amino acid positions 356 and 357 and negatively charged amino acid residues at amino acid positions 370 and 439 according to the EU numbering system.

25. A recombinant host cell comprising one or more nucleic acids encoding at least a first and a second CH3 domain-comprising polypeptide chain, wherein said first CH3 domain-comprising polypeptide chain comprises:

- either a wildtype CH3 domain, or
- negatively charged amino acid residues at amino acid positions 392 and 409 and a positively charged amino acid residue at amino acid position 399 according to the EU numbering system,

and wherein said second CH3 domain-comprising polypeptide chain comprises positively charged amino acid residues at amino acid positions 356 and 357 and negatively charged

amino acid residues at amino acid positions 370 and 439 according to the EU numbering system.

26. A recombinant host cell according to claim 24 or 25, wherein said first CH3 domain-comprising polypeptide chain comprises an aspartic acid (D) residue at amino acid position 392 and a lysine (K) residue at amino acid position 399 and an aspartic acid (D) residue at amino acid position 409 according to the EU numbering system.

27. A recombinant host cell according to any one of claims 24-26, wherein said second CH3 domain-comprising polypeptide chain comprises a lysine (K) residue at amino acid position 356 and a lysine (K) residue at amino acid position 357 and an aspartic acid (D) residue at amino acid position 439 and an aspartic acid (D) residue at amino acid position 370 according to the EU numbering system.

28. A recombinant host cell according to any one of claims 24-27, wherein said host cell further comprises a nucleic acid encoding a common light chain.

29. A pharmaceutical composition comprising the at least two different monospecific antibodies of any one of claims 22-23, and a pharmaceutically acceptable carrier.

30. A pharmaceutical composition according to claim 29, wherein said at least two different monospecific antibodies have been produced by recombinant host cells according to any one of claims 24-28.

31. A method for making a host cell for production of at least two different monospecific antibodies, the method comprising the step of introducing into said host cell one or more nucleic acids encoding at least a first and a second CH3 domain-comprising polypeptide chain,
wherein said first CH3 domain-comprising polypeptide chain comprises negatively charged amino acid residues at amino acid positions 392 and 409 and positively charged amino acid residue at amino acid position 399 according to the EU numbering system, and wherein said second CH3 domain-comprising polypeptide chain comprises:
- either a wildtype CH3 domain, or

- positively charged amino acid residues at amino acid positions 356 and 357 and negatively charged amino acid residues at amino acid positions 370 and 439 according to the EU numbering system.

32. A method for making a host cell for production of at least two different monospecific antibodies, the method comprising the step of introducing into said host cell one or more nucleic acids encoding at least a first and a second CH3 domain-comprising polypeptide chain,
wherein said first CH3 domain-comprising polypeptide chain comprises:

- either a wildtype CH3 domain, or
- negatively charged amino acid residues at amino acid positions 392 and 409 and a positively charged amino acid residue at amino acid position 399 according to the EU numbering system,

and wherein said second CH3 domain-comprising polypeptide chain comprises positively charged amino acid residues at amino acid positions 356 and 357 and negatively charged amino acid residues at amino acid positions 370 and 439 according to the EU numbering system.

33. A method according to claim 31 or 32, wherein said first CH3 domain-comprising polypeptide chain comprises an aspartic acid (D) residue at amino acid position 392 and a lysine (K) residue at amino acid position 399 and an aspartic acid (D) residue at amino acid position 409 according to the EU numbering system.

34. A method according to any one of claims 31-33, wherein said second CH3 domain-comprising polypeptide chain comprises a lysine (K) residue at amino acid position 356 and a lysine (K) residue at amino acid position 357 and an aspartic acid (D) residue at amino acid position 439 and an aspartic acid (D) residue at amino acid position 370 according to the EU numbering system.

35. A method according to any one of claims 32-34, further comprising the step of introducing into said host cell a nucleic acid encoding a common light chain.

36. A culture of recombinant host cells of any one of claims 24-28, or of recombinant host cells obtained by the method according to any one of claims 31-35, producing at least two different monospecific antibodies.

37. The method according to any one of claims 1-21 or 31-35, or the mixture according to claim 22 or 23, or the recombinant host cell according to any one of claims 24-28, or the pharmaceutical composition according to claim 29 or 30, or the culture according to claim 36, wherein said antibodies are IgG antibodies, preferably human IgG, most preferably human IgG1.