preclinical and clinical testing involve a single, molecule. Thus, therapies based on a single bispecific antibody are facilitated by a less complicated and cost-effective drug development process while providing more efficacious antibody therapies.

Bispecific antibodies based on the IgG format, consisting of 2 heavy and two light chains have been produced by a variety of methods. For instance, bispecific antibodies may be produced by fusing two antibody-secreting cell lines to create a new cell line or by expressing two antibodies in a single cell using recombinant DNA technology. These approaches yield multiple antibody species as the respective heavy chains from each antibody may form monospecific dimers (also called homodimers), which contain two identical paired heavy chains with the same specificity, and bispecific dimers (also called heterodimers) which contain two different paired heavy chains with different specificity. In addition, light chains and heavy chains from each antibody may randomly pair to form inappropriate, non-functional combinations. This problem, known as heavy and light chain miss-pairings, can be solved by choosing antibodies that share a common light chain for expression as bispecific. But even when a common light chain is used, expression of two heavy chains and one common light chain in a single cell will result in 3 different antibody species, i.e. two monospecific 'parental' antibodies and the bispecific antibody so that the bispecific antibody of interest needs to be purified from the resulting antibody mixture. Although technologies have been employed to further increase the percentage of bispecific antibodies in the mixtures of parental and bispecific antibodies and to decrease the percentage of miss-paired heavy and light chains, there remains a need for bispecific formats that eliminate or minimize some of the disadvantages mentioned above.

Taken together, the art provides a variety of technologies and methods for generating monoclonal antibodies, bispecific antibodies, mixtures of monoclonal antibodies, or mixtures of monospecific and bispecific antibodies that can subsequently be used for therapeutic application in patients. However, as discussed above, each of these existing technologies and methods have their drawbacks and limitations.
There is thus a need for improved and/or alternative technologies for producing biological therapeutics in the form of mixtures or bispecific approaches for targeting multiple disease-modifying molecules

SUMMARY

In one aspect, the present disclosure refers to a composition comprising a mixture of at least two different antibodies, wherein one antibody in the mixture comprises a 1st antibody heavy chain comprising at least one substitution of a neutral amino acid residue in the CH3 domain by a positively charged amino acid residue as compared to wildtype, and a 2nd antibody heavy chain comprising at least one substitution of a neutral amino acid residue, as compared to wildtype, in the CH3 domain by a negatively charged amino acid residue, and wherein the positively charged amino acid residue of the first heavy chain interacts with the negatively charged amino acid residue of the second heavy chain at the interface between the first and second heavy chains.

Claims

1. 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 molecule encoding a 1st CH3 domain-comprising polypeptide chain,

a. A second nucleic acid molecule encoding a 2nd CH3 domain-comprising polypeptide chain,

b. A third nucleic acid molecule encoding a 3rd CH3 domain-comprising polypeptide chain, and

c. A fourth nucleic acid molecule encoding a 4th CH3 domain-comprising polypeptide chain,

wherein at least two of said nucleic acid molecules are provided with means for preferential pairing of said 1st and 2nd CH3 domain-comprising polypeptides and said 3rd and 4th CH3 domain-comprising polypeptides, said method further comprising the step of culturing said host cell and allowing for expression of said at least four nucleic acid molecules and harvesting said at least two different Ig-like molecules from the culture.

2. The method of claim 1, further comprising providing said host cell with a nucleic acid molecule encoding a common light chain.

3. The method of claim 1 or 2, wherein said first CH3-domain comprising polypeptide chain comprises the amino acid substitution T366K, and said second CH3-domain comprising polypeptide chain comprises the amino acid substitution L351D.

4. The method of claim 3, wherein said first CH3-domain comprising polypeptide chain further comprises the amino acid substitution L351K.

~~5. The method of claim 3 or 4, wherein said second CH3 domain comprising polypeptide chain further comprises an amino acid substitution selected from the group consisting of Y349E, Y349D and L368E.~~

~~6. The method of claim 5, wherein said second CH3 domain comprising polypeptide chain further comprises the amino acid substitution L368E.~~

~~7. The method of any one of claims 1-6, wherein said third CH3 domain comprising polypeptide chain comprises the amino acid substitutions E356K and D399K, and said fourth CH3 domain comprising polypeptide chain comprises the amino acid substitutions K392D and K409D.~~

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

~~9. The method of claim 8, wherein each of the 4 variable regions of the 4 CH3 domain comprising polypeptide chains recognizes different target epitopes.~~

~~10. The method of claim 8, wherein the variable regions of the 1st and the 2nd CH3 domain comprising polypeptide chains recognize different target epitopes, whereas the variable regions of the 3rd and the 4th CH3 domain comprising polypeptide chains recognize the same target epitopes.~~

~~11. The method of claim 10 wherein the target epitope recognized by the variable regions of the 3rd and 4th CH3 domain comprising polypeptide chain is the same as the target epitope recognized by the variable region of the 1st or the 2nd CH3 domain comprising polypeptide chain.~~

~~12. The method of claim 10 wherein the target epitope recognized by the variable regions of the 3rd and 4th CH3 domain comprising polypeptide chain is different from the target epitope recognized by the variable region of the 1st or the 2nd CH3 domain comprising polypeptide chain.~~

~~13. The method of claim 8, wherein the variable regions of the 1st and the 2nd CH3 domain comprising polypeptide chains recognize the same target epitope, whereas the variable regions of the 3rd and the 4th CH3 domain comprising polypeptide chains recognize a second target epitope which differs from the target epitope recognized by said 1st and 2nd variable regions.~~

~~14. The method of any one of claims 8-13 wherein the target epitopes are located on the same target molecule.~~

~~15. The method of claim 14 wherein the target molecule is a soluble molecule.~~

~~16. The method of claim 14 wherein the target molecule is a membrane-bound molecule.~~

~~17. The method of any one of claims 8-13 wherein the target epitopes are located on different target molecules.~~

~~18. The method of claim 17, wherein the different target molecules are expressed on the same cells.~~

~~19. The method of claim 17, wherein the different target molecules are expressed on different cells.~~

~~20. The method of claim 17, wherein the different target molecules are soluble molecules.~~

~~21. The method of claim 17, wherein one target molecule is a soluble molecule whereas the second target molecule is a membrane bound molecule.~~

~~22. The method according to any one of claims 1-21, wherein said at least two different Ig-like molecules are antibodies.~~

~~23. The method of claim 1, wherein said means for preferential pairing comprises engineered complementary knob-into-hole mutations, disulfide bridges, charge mutations or combinations thereof.~~

~~24. The method of claim 23, wherein said means for preferential pairing are selected from Table B.~~

~~25. The method of claim 1, wherein all 4 of said nucleic acid molecules are provided with means for preferential pairing of said 1st and 2nd CH3 domain comprising polypeptides and said 3rd and 4th CH3-domain comprising polypeptides, wherein said means for preferential pairing of said 1st and 2nd CH3 domain comprising polypeptides are different from those means for preferential pairing of said 3rd and 4th CH3-domain comprising polypeptides.~~

~~26. The method of any one of claims 8-13, 17-19 or 21, wherein at least one of said target epitopes is located on a tumor cell.~~

~~27. The method of any one of claims 8-13, 17-19 or 21, wherein at least one of said target epitopes is located on an effector cell.~~

~~28. The method of claim 27, 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.~~

~~29. The method of claims 27 or 28, wherein said target epitope is located on a CD3, CD16, CD25, CD28, CD64, CD89, NKG2D or a NKp46 molecule.~~

~~30. A mixture of at least two different Ig-like molecules obtainable by the methods according to any one of claims 1-29.~~

~~31. A mixture according to claim 30, wherein said at least two different Ig-like molecules bind to different epitopes on the same antigen and/or to different epitopes on different antigens.~~

~~32. A mixture according to claim 30 or 31, wherein said at least two different Ig-like molecules comprises at least one heterodimeric Ig-like molecule.~~

~~33. A mixture according to any one of claims 30-32, wherein two of said at least two different Ig-like molecules are heterodimeric Ig-like molecules.~~

~~34. A recombinant host cell comprising nucleic acid sequences encoding at least a first, a second, a third and a fourth CH3-domain comprising polypeptide chain, wherein at least two of said nucleic acid sequences are provided with means for preferential pairing of said first and second CH3-domain comprising polypeptides and said third and fourth CH3-domain comprising polypeptides.~~

~~35. A recombinant host cell according to claim 34, wherein said host cell further comprises a nucleic acid sequence encoding a common light chain.~~

~~36. A pharmaceutical composition comprising the at least two different Ig-like molecules of any one of claims 30-33, and a pharmaceutically acceptable carrier.~~

~~37. A pharmaceutical composition according to claim 36, wherein said at least two different Ig-like molecules have been produced by recombinant host cells according to claims 34 or 35.~~

~~38. 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 nucleic acid sequences encoding at least a first, a second, a third and a fourth CH3-domain comprising polypeptide chain, wherein at least two of said nucleic acid sequences are provided with means for preferential pairing of said first and second CH3-domain comprising polypeptides and said third and fourth CH3-domain comprising polypeptides, wherein said nucleic acid sequences are introduced consecutively or concomitantly.~~

~~39. A method according to claim 38, further comprising the step of introducing into said host cell a nucleic acid sequence encoding a common light chain.~~

~~40. A culture of recombinant host cells of claims 34 or 35, or of recombinant host cells obtained by the method according to claims 38 or 39, producing at least two different Ig-like molecules.~~

1. A composition comprising a mixture of at least two different antibodies, wherein one antibody in the mixture comprises a 1st antibody heavy chain comprising at least one substitution of a neutral amino acid residue in the CH3 domain by a positively charged amino acid residue as compared to wildtype, and a 2nd antibody heavy chain comprising at least one substitution of a neutral amino acid residue, as compared to wildtype, in the CH3 domain by a negatively charged amino acid residue, and wherein the positively charged amino acid residue of the first heavy chain interacts with the negatively charged amino acid residue of the second heavy chain at the interface between the first and second heavy chains.

2. The composition of claim 1, wherein the 1st antibody heavy chain of the one antibody comprises a substitution of the amino acid residue at position 366 in the CH3 domain according to the EU numbering system by a lysine (K) residue, and wherein the 2nd antibody heavy chain of the one antibody comprises a substitution of the amino acid residue at position 351 in the CH3 domain according to the EU numbering system by an aspartic acid (D) residue.

3. The composition of claim 2, wherein the CH3 domain of the 1st antibody heavy chain of the one antibody further comprises a substitution of the amino acid residue at position 351 according to the EU numbering system by a lysine (K) residue.

4. The composition of claim 1, wherein the CH3 domain of the 2nd antibody heavy chain of the one antibody further comprises amino acid substitution(s) selected from the group consisting of

(i) a substitution of the amino acid residue at position 349 according to the EU numbering system by a glutamic acid (E) residue;

(ii) a substitution of the amino acid residue at position 349 according to the EU numbering system by an aspartic acid (D) residue;

(iii) a substitution of the amino acid residue at position 368 according to the EU numbering system by a glutamic acid (E) residue;

(iv) a substitution of the amino acid residue at position 349 according to the EU numbering system by an aspartic acid (D) residue, and a substitution of the amino acid residue at position 368 according to the EU numbering system by a glutamic acid (E) residue; and

(v) substitution of the amino acid residues at positions 349 and 355 according to the EU numbering system by aspartic acid (D) residues.

5. The composition of claim 4, wherein the CH3 domain of the 2nd antibody heavy chain of the one antibody comprises the substitution of the amino acid residue at position 368 according to the EU numbering system by a glutamic acid (E) residue.

6. The composition of claim 1, wherein the CH3 domain of the 1st antibody heavy chain of the one antibody comprises substitutions of the amino acid residues at positions 366 and 351 according to the EU numbering system by a lysine (K) residue, and the CH3 domain of the 2nd antibody heavy chain of the one antibody comprises a substitution of the amino acid residue at position 351 according to the EU numbering system by an aspartic acid (D) residue and a substitution of the amino acid residue at position 368 according to the EU numbering system by a glutamic acid (E) residue.

7. The composition of claim 1, wherein a second antibody in the mixture comprises two antibody heavy chains wherein

a. the two antibody heavy chains of the second antibody comprise a wild type CH3 domain;

b. the 1st antibody heavy chain of the second antibody comprises a substitution of the amino acid residues at positions 356 and 399 in the CH3 domain according to the EU numbering system by a lysine (K) residue, and the 2nd antibody heavy chain of the second antibody comprises a substitution of the amino acid residues at positions 392 and 409 in the CH3 according to the EU numbering system by an aspartic acid (D) residue;

c. the 1st antibody heavy chain of the second antibody comprises a substitution of the amino acid residues at positions 356 and 399 in the CH3 domain according to the EU numbering system by a lysine (K) residue, and the 2nd antibody heavy chain of the second antibody comprises a substitution of the amino acid residues at positions 392, 409 and 439 in the CH3 domain according to the EU numbering system by an aspartic acid (D) residue;

d. the 1st antibody heavy chain of the second antibody comprises a substitution of the amino acid residues at positions 392 and 409 in the CH3 domain according to the EU numbering system by an aspartic acid (D) residue and a substitution of the amino acid residue at position 399 in the CH3 domain according to the EU numbering system by a lysine (K) residue, and the 2nd antibody heavy chain of the second antibody comprises a substitution of the amino acid residues at positions 392 and 409 in the CH3 domain according to the EU numbering system by an aspartic acid (D) residue and a substitution of the amino acid residue at position 399 in the CH3 domain according to the EU numbering system by a lysine (K) residue;

e. the 1st antibody heavy chain of the second antibody comprises a substitution of the amino acid residues at position 356 and 357 in the CH3 domain according to the EU numbering system by a lysine (K) residue, and a substitution of the amino acid residues at position 439 and 370 in the CH3 domain according to the EU numbering system by an aspartic acid (D) residue, and the 2nd antibody heavy chain of the second antibody comprises a substitution of the amino acid residues at position 356 and 357 in the CH3 according to the EU numbering system by a lysine (K) residue, and a substitution of the amino acid residues at position 439 and 370 in the CH3 domain according to the EU numbering system by an aspartic acid (D) residue; or

f. the 1st antibody heavy chain of the second antibody comprises a substitution of the amino acid residue at position 366 in the CH3 domain according to the EU numbering system by a tryptophan (W) residue, and the 2nd antibody heavy chain of the second

antibody comprises a substitution of the amino acid residue at position 366 in the CH3 domain according to the EU numbering system by a serine (S) residue, a substitution of the amino acid residue at position 368 in the CH3 domain according to the EU numbering system by an alanine (A) residue and a substitution of the amino acid residue at position 407 in the CH3 domain according to the EU numbering system by a valine (V) residue.

8. The composition of claim 1, wherein the mixture comprises

(a) one antibody wherein the 1st antibody heavy chain of the one antibody comprises at least one substitution of a neutral amino acid residue in the CH3 domain by a positively charged amino acid residue, as compared to wildtype, and the 2nd antibody heavy chain of the one antibody comprises at least one substitution of a neutral amino acid residue in the CH3 domain by a negatively charged amino acid residue, as compared to wildtype; and

(b) a second antibody wherein the 1st antibody heavy chain of the second antibody comprises a substitution of the amino acid residue at position 356 in the CH3 domain according to the EU numbering system by a lysine (K) residue and a substitution of the amino acid residue at position 399 in the CH3 domain according to the EU numbering system by a lysine (K) residue, and the 2nd antibody heavy chain of the second antibody comprises a substitution of the amino acid residue at position 392 in the CH3 domain according to the EU numbering system by an aspartic acid (D) residue and the substitution of the amino acid residue at position 409 in the CH3 domain according to the EU numbering system by an aspartic acid (D) residue.

9. The composition of claim 1, wherein the mixture comprises

(a) one antibody wherein the 1st antibody heavy chain of the one antibody comprises a substitution of the amino acid residue at positions 366 and 351 in the CH3 domain according to the EU numbering system by a lysine (K) residue, and the 2nd antibody heavy chain of the one antibody comprises a substitution of the amino acid residue at position 351 in the CH3 domain according to the EU numbering system by an aspartic acid (D) residue and a substitution of the amino acid at position 368 in the CH3 domain according to the EU numbering system by a glutamic acid (E) residue; and

(b) a second antibody wherein the 1st antibody heavy chain of the second antibody comprises a substitution of the amino acid residue at position 356 in the CH3 domain according to the EU numbering system by a lysine (K) residue and a substitution of the amino acid residue at position 399 in the CH3 domain according to the EU numbering system by a lysine (K) residue, and the 2nd antibody heavy chain of the second antibody comprises a substitution of the amino acid residue at position 392 in the CH3 domain according to the EU numbering system by an aspartic acid (D) residue and the substitution of the amino acid residue at position 409 in the CH3 domain according to the EU numbering system by an aspartic acid (D) residue.

10. The composition of claim 1, wherein at least one antibody in the mixture comprises a common light chain.

11. The composition of claim 1, wherein at least two of the variable regions of the antibody heavy chains of the at least two different antibodies in the mixture recognize different target epitopes.

12. The composition of claim 1, wherein each of the variable regions of the antibody heavy chains of the at least two different antibodies in the mixture recognize different target epitopes.

13. The composition of claim 1, wherein the variable regions of the 1st and the 2nd antibody heavy chains of the one antibody in the mixture recognize different target epitopes, whereas the variable regions of the 1st and the 2nd antibody heavy chains of the second antibody in the mixture recognize the same target epitope.

14. The composition of claim 1, wherein the target epitope recognized by the variable regions of the 1st and the 2nd antibody heavy chains of the second antibody in the mixture is the same as one of the target epitopes recognized by the variable region of the 1st or the 2nd antibody heavy chain of the one antibody in the mixture.

15. The composition of claim 1, wherein the target epitope recognized by the variable regions of the 1st and the 2nd antibody heavy chains of the second antibody in the mixture is different from the target epitope recognized by the variable region of the 1st or the 2nd antibody heavy chain of the one antibody in the mixture.

16. The composition of claim 1, wherein the variable regions of the 1st and the 2nd antibody heavy chains of the one antibody in the mixture recognize the same target epitope, whereas the variable regions of the 1st and the 2nd antibody heavy chains of the second antibody in the mixture recognize a second target epitope which differs from the target epitope recognized by the variable regions of the 1st and the 2nd antibody heavy chains of the one antibody in the mixture.

17. The composition of claim 1, wherein at least one antibody in the mixture is a heterodimeric antibody.

18. The composition of claim 1, wherein at least two different antibodies in the mixture are heterodimeric antibodies.

19. The composition of claim 1, wherein the variable regions of the 1st and 2nd antibody heavy chains of the one antibody recognize the same target epitope.

20. The composition of claim 1, wherein the variable regions of the 1st and 2nd antibody heavy chains of the one antibody recognize different target epitopes.

21. The composition of claim 20, wherein the two different target epitopes are located on the same target molecule.

22. The composition of claim 21, wherein the target molecule is a soluble molecule.

23. The composition of claim 21, wherein the target molecule is a membrane-bound molecule.

24. The composition of claim 20, wherein the two different target epitopes are located on different target molecules.

25. The composition of claim 24, wherein the different target molecules are expressed on the same cells.

26. The composition of claim 24, wherein the different target molecules are expressed on different cells.

27. The composition of claim 24, wherein the different target molecules are soluble molecules.

28. The composition of claim 24, wherein one target molecule is a soluble molecule whereas the second target molecule is a membrane bound molecule.

29. The composition of claim 20, wherein at least one of the target epitopes is located on a tumor cell.

30. The composition of claim 20, wherein at least one of the target epitopes is located on an effector cell.

31. The composition of claim 30, wherein the effector cell is an NK cell, a T cell, a B cell, a monocyte, a macrophage, a dendritic cell or a neutrophilic granulocyte.

32. The composition of claim 30, wherein the target epitope is located on a CD3, CD16, CD25, CD28, CD64, CD89, NKG2D or a NKp46 molecule.

33. The composition of claim 7, wherein the variable regions of the 1st and 2nd antibody heavy chains of the second antibody recognize the same target epitope.

34. The composition of claim 7, wherein the variable regions of the 1st and 2nd antibody heavy chains of the second antibody recognize different target epitopes.

35. The composition of claim 34, wherein the two different target epitopes are located on the same target molecule.

36. The composition of claim 35, wherein the target molecule is a soluble molecule.

37. The composition of claim 35, wherein the target molecule is a membrane-bound molecule.

38. The composition of claim 34, wherein the two different target epitopes are located on different target molecules.

39. The composition of claim 38, wherein the different target molecules are expressed on the same cells.

40. The composition of claim 38, wherein the different target molecules are expressed on different cells.

41. The composition of claim 38, wherein the different target molecules are soluble molecules.

42. The composition of claim 38, wherein one target molecule is a soluble molecule whereas the second target molecule is a membrane bound molecule.

43. The composition of claim 34, wherein at least one of the target epitopes is located on a tumor cell.

44. The composition of claim 34, wherein at least one of the target epitopes is located on an effector cell.

45. The composition of claim 44, wherein the effector cell is an NK cell, a T cell, a B cell, a monocyte, a macrophage, a dendritic cell or a neutrophilic granulocyte.

46. The composition of claim 44, wherein the target epitope is located on a CD3, CD16, CD25, CD28, CD64, CD89, NKG2D or a NKp46 molecule.

47. The composition of claim 7, wherein at least two of the variable regions of the antibody heavy chains of the at least two different antibodies in the mixture recognize different target epitopes.

48. The composition of claim 7, wherein each of the variable regions of the antibody heavy chains of the at least two different antibodies in the mixture recognize different target epitopes.

49. The composition of claim 7, wherein the variable regions of the 1st and the 2nd antibody heavy chains of the one antibody in the mixture recognize different target epitopes, whereas the variable regions of the 1st and the 2nd antibody heavy chains of the second antibody in the mixture recognize the same target epitope.

50. The composition of claim 7, wherein the target epitope recognized by the variable regions of the 1st and the 2nd antibody heavy chains of the second antibody in the mixture is the same as one of the target epitopes recognized by the variable region of the 1st or the 2nd antibody heavy chain of the one antibody in the mixture.

51. The composition of claim 7, wherein the target epitope recognized by the variable regions of the 1st and the 2nd antibody heavy chains of the second antibody in the mixture is different from the target epitope recognized by the variable region of the 1st or the 2nd antibody heavy chain of the one antibody in the mixture.

52. The composition of claim 7, wherein the variable regions of the 1st and the 2nd antibody heavy chains of the one antibody in the mixture recognize the same target epitope, whereas the variable regions of the 1st and the 2nd antibody heavy chains of the second antibody in the mixture recognize a second target epitope which differs from the target epitope recognized by the variable regions of the 1st and the 2nd antibody heavy chains of the one antibody in the mixture.

53. The composition of claim 1 or 7, wherein at least one antibody in the mixture is an IgG antibody.

54. The composition of claim 1 or 7, wherein at least two different antibodies in the mixture are IgG antibodies.

55. The composition of claim 1 or 7, wherein at least one antibody in the mixture is an IgG1 antibody.

56. The composition of claim 1 or 7, wherein at least two different antibodies in the mixture are IgG1 antibodies.