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 heterodimeric antibody comprising two heavy chains with CH3 domains, wherein the CH3 domain of the first heavy chain comprises at least one substitution of a neutral amino acid residue by a positively charged amino acid residue as compared to wildtype, and the CH3 domain of the second heavy chain comprises at least one substitution of a neutral amino acid residue by a negatively charged amino acid residue as compared to wildtype, and wherein the at least one positively charged amino acid residue substituted in the CH3 domain of said first heavy

chain interacts with the at least one negatively charged amino acid residue substituted in the CH3 domain of said second heavy chain in the interface between said first and second heavy chains.

In one aspect, the present disclosure refers to a heterodimeric antibody comprising two heavy chains with CH3 domains wherein,

(i) the CH3 domain of the first heavy chain comprises a substitution of the amino acid residue at position 366 as compared to wildtype and according to the EU numbering system, by a positively charged amino acid residue, and the CH3 domain of the second heavy chain comprises substitutions of the amino acid residues at positions 351 and 368 as compared to wildtype and according to the EU numbering system, by negatively charged amino acid residues; or

(ii) the CH3 domain of the first heavy chain comprises a substitution of the amino acid residue at position 366 as compared to wildtype and according to the EU numbering system, by a positively charged amino acid residue, and the CH3 domain of the second heavy chain comprises substitutions of the amino acid residues at positions 349 and 351 as compared to wildtype and according to the EU numbering system, by negatively charged amino acid residues; or

(iii) the CH3 domain of the first heavy chain comprises a substitution of the amino acid residue at position 366 as compared to wildtype and according to the EU numbering system, by a positively charged amino acid residue, and the CH3 domain of the second heavy chain comprises substitutions of the amino acid residues at positions 349, 351 and 368 as compared to wildtype and according to the EU numbering system, by negatively charged amino acid residues; or

(iv) the CH3 domain of the first heavy chain comprises a substitution of the amino acid residue at position 366 as compared to wildtype and according to the EU numbering system, by a positively charged amino acid residue, and the CH3 domain of the second heavy chain comprises substitutions of the amino acid residues at positions 349 and 351 and 355 as compared to wildtype and according to the EU numbering system, by negatively charged amino acid residues; or

(v) the CH3 domain of the first heavy chain comprises substitutions of the amino acids at positions 351 and 366 as compared to wildtype and according to the EU numbering system, by positively charged residues, and the CH3 domain of the second heavy chain comprises substitutions of the amino acid residues at positions 351 and 368 as compared to wildtype and according to the EU numbering system, by negatively charged amino acid residues; or

(vi) the CH3 domain of the first heavy chain comprises substitutions of the amino acids at positions 351 and 366 as compared to wildtype and according to the EU numbering system, by positively charged residues, and the CH3 domain of the second heavy chain comprises substitutions of the amino acid residues at positions 349, 351 and 355 as compared to wildtype and according to the EU numbering system, by negatively charged residues; or

(vii) the CH3 domain of the first heavy chain comprises substitutions of the amino acids at positions 351 and 366 as compared to wildtype and according to the EU numbering system, by positively charged residues, and the CH3 domain of the second heavy chain comprises substitutions of the amino acid residues at positions 349 and 351 as compared to wildtype and according to the EU numbering system, by negatively charged amino acid residues; or

(viii) the CH3 domain of the first heavy chain comprises substitutions of the amino acids at positions 351 and 366 as compared to wildtype and according to the EU numbering system, by positively charged residues, and the CH3 domain of the second heavy chain comprises substitutions of the amino acid residues at positions 349 and 351 and 368 as compared to wildtype and according to the EU numbering system, by negatively charged amino acid residues.

In one aspect, the present disclosure refers to a pharmaceutical composition comprising the heterodimeric antibody disclosed herein and a pharmaceutically acceptable carrier.

Claims

1. A heterodimeric antibody comprising two heavy chains with CH3 domains, wherein the CH3 domain of the first heavy chain comprises at least one substitution of a neutral amino acid residue by a positively charged amino acid residue as compared to wildtype, and the CH3 domain of the second heavy chain comprises at least one substitution of a neutral amino acid residue by a negatively charged amino acid residue as compared to wildtype, and wherein the at least one positively charged amino acid residue substituted in the CH3 domain of said first heavy chain interacts with the at least one negatively charged amino acid residue substituted in the CH3 domain of said second heavy chain in the interface between said first and second heavy chains.

2. The heterodimeric antibody of claim 1, wherein the at least one positively charged amino acid residue substitution in the CH3 domain of the first heavy chain is lysine (K).

3. The heterodimeric antibody of claim 1, wherein the CH3 domain of the first heavy chain comprises a substitution of the amino acid residue at position 366 as compared to wildtype and according to the EU numbering system, by a positively charged amino acid residue, and the CH3 domain of the second heavy chain comprises a substitution of the amino acid residue at position 351 as compared to wildtype and according to the EU numbering system, by a negatively charged residue.

4. The heterodimeric antibody of claim 3, wherein the CH3 domain of the first heavy chain further comprises a substitution, as compared to wildtype, of the amino acid residue at position 351 by a positively charged amino acid residue.

5. The heterodimeric antibody of claims 1 or 3, wherein the CH3 domain of the second heavy chain further comprises amino acid substitution(s), as compared to wildtype, 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 negatively charged amino acid residue;

(ii) a substitution of the amino acid residue at position 368, according to the EU numbering system, by negatively charged amino acid residue;

(iii) a substitution of the amino acid residue at position 355, according to the EU numbering system, by a negatively charged amino acid residue;

(iv) a substitution of the amino acid residues at position 349 and 368, according to the EU numbering system, by negatively charged amino acid residues;

(v) a substitution of the amino acid residues at positions 349 and 355, according to the EU numbering system, by negatively charged amino acid residues.

6. The heterodimeric antibody of claim 5, wherein the CH3 domain of the first heavy chain further comprises a substitution, as compared to wildtype, of the amino acid residue at position 351 by a positively charged amine acid residue.

7. The heterodimeric antibody of claim 1, wherein the CH3 domain of the first heavy chain comprises amino acid substitutions at positions 366 and 351 as compared to wildtype

and according to the EU numbering system, by positively charged amino acid residues, and the CH3 domain of the second heavy chain comprises amino acid substitutions at positions 351 and 368 as compared to wildtype and according to the EU numbering system, by negatively charged amino acid residues.

8. A heterodimeric antibody comprising two heavy chains with CH3 domains wherein,

(i) the CH3 domain of the first heavy chain comprises a substitution of the amino acid residue at position 366 as compared to wildtype and according to the EU numbering system, by a positively charged amino acid residue, and the CH3 domain of the second heavy chain comprises substitutions of the amino acid residues at positions 351 and 368 as compared to wildtype and according to the EU numbering system, by negatively charged amino acid residues; or

(ii) the CH3 domain of the first heavy chain comprises a substitution of the amino acid residue at position 366 as compared to wildtype and according to the EU numbering system, by a positively charged amino acid residue, and the CH3 domain of the second heavy chain comprises substitutions of the amino acid residues at positions 349 and 351 as compared to wildtype and according to the EU numbering system, by negatively charged amino acid residues; or

(iii) the CH3 domain of the first heavy chain comprises a substitution of the amino acid residue at position 366 as compared to wildtype and according to the EU numbering system, by a positively charged amino acid residue, and the CH3 domain of the second heavy chain comprises substitutions of the amino acid residues at positions 349, 351 and 368 as compared to wildtype and according to the EU numbering system, by negatively charged amino acid residues; or

(iv) the CH3 domain of the first heavy chain comprises a substitution of the amino acid residue at position 366 as compared to wildtype and according to the EU numbering system, by a positively charged amino acid residue, and the CH3 domain of the second heavy chain comprises substitutions of the amino acid residues at positions 349 and 351 and 355 as compared to wildtype and according to the EU numbering system, by negatively charged amino acid residues; or

(v) the CH3 domain of the first heavy chain comprises substitutions of the amino acids at positions 351 and 366 as compared to wildtype and according to the EU numbering system, by positively charged residues, and the CH3 domain of the second heavy chain comprises substitutions of the amino acid residues at positions 351 and 368 as compared to wildtype and according to the EU numbering system, by negatively charged amino acid residues; or

(vi) the CH3 domain of the first heavy chain comprises substitutions of the amino acids at positions 351 and 366 as compared to wildtype and according to the EU numbering system, by positively charged residues, and the CH3 domain of the second heavy chain comprises substitutions of the amino acid residues at positions 349, 351 and 355 as compared to wildtype and according to the EU numbering system, by negatively charged residues; or

(vii) the CH3 domain of the first heavy chain comprises substitutions of the amino acids at positions 351 and 366 as compared to wildtype and according to the EU numbering system, by positively charged residues, and the CH3 domain of the second heavy chain comprises substitutions of the amino acid residues at positions 349 and 351 as

compared to wildtype and according to the EU numbering system, by negatively charged amino acid residues; or

(viii) the CH3 domain of the first heavy chain comprises substitutions of the amino acids at positions 351 and 366 as compared to wildtype and according to the EU numbering system, by positively charged residues, and the CH3 domain of the second heavy chain comprises substitutions of the amino acid residues at positions 349 and 351 and 368 as compared to wildtype and according to the EU numbering system, by negatively charged amino acid residues.

9. The heterodimeric antibody of claim 8, wherein the positively charged amino acid substitutions in the CH3 domain of the first heavy chain are lysine (K).

10. The heterodimeric antibody of claim 1, wherein the antibody further comprises a common light chain.

11. The heterodimeric antibody of claim 10, wherein the common light chain is a germline light chain.

12. The heterodimeric antibody of claim 10, wherein the common light chain is a rearranged germline human kappa light chain.

13. The heterodimeric antibody of claim 12, wherein the common light chain is the rearranged germline human kappa light chain IgVK1-39/JK or IgVK3-20/JK.

14. The heterodimeric antibody of claim 1, wherein the heterodimeric antibody is human IgG.

15. The heterodimeric antibody of claim 14, wherein the heterodimeric antibody is human IgG1.

16. The heterodimeric antibody of claim 1, wherein each variable region of the first and second heavy chains binds to different epitopes.

17. The heterodimeric antibody of claim 16, wherein the different epitopes are located on the same target molecule.

18. The heterodimeric antibody of claim 17, wherein the target molecule is a soluble molecule.

19. The heterodimeric antibody of claim 17, wherein the target molecule is a membrane-bound molecule.

20. The heterodimeric antibody of claim 16, wherein the different epitopes are located on different target molecules.

21. The heterodimeric antibody of claim 20, wherein the different target molecules are expressed on the same cells.

22. The heterodimeric antibody of claim 20, wherein the different target molecules are expressed on different cells.

23. The heterodimeric antibody of claim 20, wherein the different target molecules are soluble molecules.

24. The heterodimeric antibody of claim 20, wherein one target molecule is a soluble molecule and a second target molecule is a membrane bound molecule.

25. The heterodimeric antibody of claim 16, wherein at least one of said epitopes is located on a tumor cell.

26. The heterodimeric antibody of claim 16, wherein at least one of said epitopes is located on an effector cell.

27. The heterodimeric antibody of claim 26, 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.

28. The heterodimeric antibody of claim 16, wherein at least one of said epitopes is located on a CD3, CD16, CD25, CD28, CD64, CD89, NKG2D or a NKp46 molecule.

29. A pharmaceutical composition comprising the heterodimeric antibody of claim 1 and a pharmaceutically acceptable carrier.

30. The heterodimeric antibody of claim 1, wherein the at least one negatively charged amino acid residue substitution in the CH3 domain of the second heavy chain is aspartic acid (D) or glutamic acid (E).

31. The heterodimeric antibody of claim 3, wherein the CH3 domain of the second heavy chain further comprises a substitution of the amino acid residue at position 368 as compared to wildtype and according to the EU numbering system, by a negatively charged amino acid residue.