

CLAIMS

WHAT IS CLAIMED IS:

1. A dimeric protein comprising two monomers, wherein each monomer comprises:
 - (1) a first NKG2D peptide or variant thereof;
 - (2) a second NKG2D peptide or variant thereof;
 - (3) a first peptide linker connecting said first NKG2D peptide or variant thereof and said second NKG2D peptide or variant thereof; and
 - (4) a fragment crystallizable region (Fc region) of an immunoglobulin (Ig).
2. The dimeric protein of claim 1, wherein said first NKG2D peptide or variant thereof and said second NKG2D peptide or variant thereof have identical amino acid sequence.
3. The dimeric protein of claim 1, wherein said first NKG2D peptide or variant thereof and said second NKG2D peptide or variant thereof have different amino acid sequence.
4. The dimeric protein of any one of the preceding claims, wherein said first NKG2D peptide or variant thereof comprises an amino acid sequence having at least 85% identity to SEQ ID NO: 1, 2, 3 or 4.
5. The dimeric protein of any one of the preceding claims, wherein said first NKG2D peptide or variant thereof comprises an amino acid sequence of SEQ ID NO: 1, 2, 3 or 4.
6. The dimeric protein of any one of the preceding claims, wherein said second NKG2D peptide or variant thereof comprises an amino acid sequence having at least 85% identity to SEQ ID NO: 1, 2, 3 or 4.
7. The dimeric protein of any one of the preceding claims, wherein said second NKG2D peptide or variant thereof comprises an amino acid sequence of SEQ ID NO: 1, 2, 3 or 4.

8. The dimeric protein of any one of the preceding claims, wherein said first peptide linker is a glycine-serine peptide linker.

9. The dimeric protein of any one of the preceding claims, wherein said peptide linker is represented by the formula (GGGS)_n (SEQ ID NO: 9), wherein n is 1, 2, 3, 4, or 5.

10. The dimeric protein of any one of the preceding claims, wherein said peptide linker is (GGGS)₂ (SEQ ID NO: 10)

11. The dimeric protein of any one of the preceding claims, wherein said Fc region comprises an Fc region of human immunoglobulin G (IgG).

12. The dimeric protein of any one of the preceding claims, wherein said Fc region comprises an Fc region of human IgG1.

13. The dimeric protein of any one of the preceding claims, wherein said Fc region comprises an amino acid sequence having at least 85% identity to SEQ ID NO: 5, 6, 63, 64, 65, or 68.

14. The dimeric protein of any one of the preceding claims, wherein said Fc region comprises an amino acid sequence of SEQ ID NO: 5, 6, 63, 64, 65, or 68.

15. The dimeric protein of any one of claims 1-14, wherein said monomer comprises, from N-terminus to C-terminus, said first NKG2D peptide or variant thereof, said first peptide linker, said second NKG2D peptide or variant thereof and said Fc region.

16. The dimeric protein of claim 15, wherein said second NKG2D peptide or variant thereof is directly fused with said Fc region without a peptide linker.

17. The dimeric protein of claim 15, wherein said second NKG2D peptide or variant thereof is linked with said Fc region via a second peptide linker.

18. The dimeric protein of any one of claims 1-14, wherein said monomer comprises, from N-terminus to C-terminus, said Fc region, said first NKG2D peptide or variant thereof, said first peptide linker, and said second NKG2D peptide or variant thereof.

19. The dimeric protein of claim 18, wherein said Fc region is directly fused with said first NKG2D peptide or variant thereof without a peptide linker.

20. The dimeric protein of claim 19, wherein said Fc region is linked with said first NKG2D peptide or variant thereof via a second peptide linker.

21. The dimeric protein of any one of the preceding claims, wherein said monomer further comprising one or two additional NKG2D peptides or variants thereof.

22. The dimeric protein of any one of the preceding claims, wherein said monomer comprises an amino acid sequence having at least 85% identity to any one of SEQ ID NOs: 17, 19, 21, 23, 25, 27, 29, 31, 33, 35, 37, 39, 41, 43, 45, 47, 49, 51, 53, 55, 57, 59, 61, and 69.

23. The dimeric protein of any one of the preceding claims, wherein said monomer comprises an amino acid sequence of any one of SEQ ID NOs: 17, 19, 21, 23, 25, 27, 29, 31, 33, 35, 37, 39, 41, 43, 45, 47, 49, 51, 53, 55, 57, 59, 61, and 69.

24. The dimeric protein of any one of the preceding claims, wherein said monomer is encoded by a nucleic acid sequence comprising or consisting of a nucleic acid sequence having at least 85% identity to any one of SEQ ID NOs: 18, 20, 22, 24, 26, 28, 30, 32, 34, 36, 38, 40, 42, 44, 46, 48, 50, 52, 54, 56, 58, 60, 62, and 70.

25. The dimeric protein of any one of the preceding claims, wherein said monomer is encoded by a nucleic acid sequence comprising or consisting of any one of SEQ ID NOs: 18, 20, 22, 24, 26, 28, 30, 32, 34, 36, 38, 40, 42, 44, 46, 48, 50, 52, 54, 56, 58, 60, 62, and 70.

26. The dimeric protein of any one of the preceding claims, wherein said two monomers are covalently linked.

27. The dimeric protein of any one of the preceding claims, wherein said two monomers are linked through a Cys-Cys bridge.

28. The dimeric protein of any one of the preceding claims, further comprising a drug moiety.

29. An isolated polynucleotide comprising a nucleic acid sequence comprising or consisting of any one of SEQ ID NOs: 18, 20, 22, 24, 26, 28, 30, 32, 34, 36, 38, 40, 42, 44, 46, 48, 50, 52, 54, 56, 58, 60, 62, and 70.

30. A vector comprising said isolated polynucleotide of claim 29.

31. A composition comprising a dimeric protein of any one of claims 1-28, a polynucleotide of claim 29, or a vector of claim 30.

32. The composition of claim 31, further comprising a pharmaceutically acceptable carrier.

33. A cell comprising a dimeric protein of any one of claims 1-28, a polynucleotide of claim 29, a vector of claim 30, or a composition of any one of claims 31-32.

34. The cell of claim 33, wherein said cell is a Chinese hamster ovary (CHO) cell.

35. The cell of any one of claims 33-34, wherein said cell is a modified CHO cell.

36. The cell of any one of claims 33-35, wherein said modified CHO cell comprises an inactivated matriptase gene or a knock-out of matriptase gene.

37. A dimeric protein of any one of claims 1-28 produced by the cell of any one of claims 33-36.

38. A dimeric protein of any one of claims 1-28 produced by a CHO cell.

39. The dimeric protein of claim 38, wherein said CHO cell is a modified CHO cell.

40. The dimeric protein of claim 39, wherein said modified CHO cell comprises an inactivated matriptase gene or a knock-out of matriptase gene.

41. Use of A method for treating a disease, the method comprising administering to a subject in need thereof a dimeric protein of any one of claims 1-28 and 37-40 or a composition of any one of claims 31-32 in the manufacture of a medicament for the treatment of disease, wherein

the disease is an NKG2D ligand expressing disease, cancer or an autoimmune disease, and wherein the dimeric protein or the composition is to be administered to a subject in need thereof.

~~42. The method of claim 41, wherein said disease is an NKG2D ligand expressing disease.~~

~~43. The method of claim 41, wherein said disease is cancer or autoimmune disease.~~

~~44~~42. The ~~method~~use of claim ~~43~~41, wherein said cancer is an NKG2D ligand expressing cancer.

~~45~~43. The ~~method~~use of ~~any one of claims 41 or claim 42~~41 or claim 42-44, wherein said subject has an NKG2D ligand expressing cancer.

~~46~~44. The ~~method~~use of claim ~~45~~43, wherein said ligand expressing cancer is lung cancer, ovarian cancer, colon cancer, pancreatic cancer, bladder cancer, breast cancer, hepatocellular carcinoma (HCC), renal cancer, nasopharyngeal carcinoma (NPC), prostate cancer, melanoma, plasma cell cancer, leukemia, lymphoma, glioma, or neuroblastoma.

~~47~~45. The ~~use~~method of claim ~~46~~44, wherein said leukemia is acute myeloid leukemia (AML), chronic myeloid leukemia (CML) or chronic lymphocytic leukemia (CLL).

~~48~~46. The ~~use~~method of any one of claims 41-~~47~~45, further comprising ~~treating the subject with~~an additional anti-cancer therapy or an anti-cancer agent to be administered to the subject.

~~49~~47. The ~~use~~method of claim ~~48~~46, wherein said additional anti-cancer therapy is surgery, radiation therapy, chemotherapy, gene therapy, DNA therapy, viral therapy, RNA therapy, adjuvant therapy, or immunotherapy.

~~50~~48. The ~~use~~method of any one of claims ~~48~~46-4947, wherein said additional cancer therapy is a chemotherapy that damages DNA.

~~51~~42. The ~~use~~method of any one of claims 41-~~50~~48, wherein said NKG2D ligand is MICA, MICB, ULBP1, ULBP2, ULBP3, ULBP4, ULBP5, or ULBP6.

~~52~~50. The ~~use method~~ of claim 41, wherein said autoimmune disease is rheumatoid arthritis, colitis, celiac disease, multiple sclerosis, alopecia areata, type 1 diabetes, chronic obstructive pulmonary disease, atherosclerosis, or metabolic syndrome associated with type 2 diabetes.

~~53~~51. A method for diagnosing an NKG2D ligand expressing disease, the method comprising

- (1) obtaining a biological sample from a subject having an NKG2D ligand expressing disease;
- (2) contacting said biological sample with a binding reagent comprising a dimeric protein of any one of claims 1-28 and 37 or a composition of any one of claims 31-32; and
- (3) detecting a binding of said NKG2D ligand to said binding reagent; wherein a binding of said NKG2D ligand to said binding reagent indicates a diagnosis of said NKG2D ligand expressing disease in said subject.

~~54~~52. The method of claims ~~53~~51, wherein said NKG2D ligand is MICA, MICB, ULBP1, ULBP2, ULBP3, ULBP4, ULBP5, or ULBP6.

~~55~~53. A dimeric protein of any one of claims 1-28 and 37-40, a polynucleotide of claim 29, a vector of claim 30, or a composition of any one of claims 31-32, for use as a medicament.

~~56~~54. A dimeric protein of any one of claims 1-28 and 37-40, a polynucleotide of claim 29, a vector of claim 30, or a composition of any one of claims 31-32, for use in the manufacture of a medicament.

~~57~~55. A dimeric protein of any one of claims 1-28 and 37-40, a polynucleotide of claim 29, a vector of claim 30, or a composition of any one of claims 31-32, for use in the treatment of a disease.

~~58~~56. A dimeric protein of any one of claims 1-28 and 37-40, a polynucleotide of claim 29, a vector of claim 30, or a composition of any one of claims 31-32, for use in the treatment of a disease, wherein said disease is an NKG2D ligand expressing disease.

~~59~~57. A dimeric protein of any one of claims 1-28 and 37-40, a polynucleotide of claim 29, a vector of claim 30, or a composition of any one of claims 31-32, for use in the treatment of a disease, wherein said disease is cancer or autoimmune disease.